ORAL LESIONS IN PAEDIATRIC PATIENTS ON HAART

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DECLARATION

I, Avni Bhula declare that this research report is my own work. It is being submitted for the degree of Master of Science in Dentistry in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

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Avni Bhula

Date

DEDICATION

To my family for their unconditional support throughout this journey.

ABSTRACT

Background

The majority of children on highly active antiretroviral therapy achieve immune reconstitution and virologic control; however, there is a subset of individuals who clinically worsen on recovery. It is important to profile and characterize oral lesions in children on HAART as they may be clinical predictors of HAART failure or syndromes related to HAART.

Method

Two hundred and forty children aged between one month and 14 years were examined for oral lesions at the Rahima Moosa Mother and Child Hospital in Johannesburg, South Africa. All patients were on HAART. The classification for presumptive diagnostic criteria for oral lesions was based on the review of Oral HIV/AIDS Research Alliance: updated case definitions of oral disease endpoints. Age (in months and years) at the time of examination and at diagnosis of HIV, duration of antiretroviral treatment (months), viral load (RNH-HIV-1 per ml), $CD4^+$ T-lymphocyte cell count (cells/mm^3) and HAART regimen were recorded.

Results

Twenty two children out of 240 presented with either active lesions or scarring as a result of previous disease whilst on HAART, making up 9,2% prevalence rate of oral lesions in children on HAART in this cohort. This is significantly lower than prevalence rates reported by other African and International studies in a similar population. Hyperpigmentation due to HAART accounted for 50% of the intra-oral lesions detected. Other intra-oral lesions included focal

epithelial hyperplasia, major aphthous ulcer, recurrent herpetic infection and ulcer of unknown origin. Ten children presented with extra-oral lesions and 80% of these lesions were caused by diseases related to the *Herpesviridae* family. We report no significant association between presence of lesions and $CD4^+$ T-lymphocyte count, viral load, age at diagnosis, duration of HAART and HAART regimen. However, a significant statistical association was found between presence of oral lesions and current age. The average current age of children presenting with oral lesions is 10,7 years. Six children out of 22 presenting with oral lesions, presented with discordant responses to HAART.

Conclusion

The prevalence of oral lesions in paediatric patients on HAART in this cohort was low. The significant association of current age and the presence of lesions may reflect the evolution of prevention of mother to child transmission strategies and more timely diagnosis of HIV and HAART implementation over the last ten years. However, further research needs to be conducted into discordant responses to HAART in the paediatric population.

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LIST OF ABBREVIATIONS

HIV	Human Immunodeficiency Virus
AIDS	Acquired Immunodeficiency Syndrome
$HIV^{+}ve$	HIV-positive
$HIV^{-}ve$	HIV - negative
HAART	Highly active antiretroviral therapy
IRIS	Immune reconstitution inflammatory syndrome
CD4	Cluster of differentiation 4
ART	Antiretroviral therapy
ARV	Antiretroviral
NRTI	Nucleoside reverse transcriptase inhibitor
NNRTI	Non-nucleoside reverse transcriptase inhibitor
PI	Protease inhibitor
DNA	Deoxyribonucleic acid

RNA	Ribonucleic acid
WHO	World Health Organisation
EC-Clearinghouse	European Community- Clearinghouse
OARAC	Office of AIDS Research Advisory Council
OHARA	Oral HIV/AIDS Research Alliance
NHLS	National Health Laboratory Service
TH1	T-helper cells 1
T_{REGS}	Regulatory T-cells
TH17	T-helper cells 17
HPV	Human Papilloma Virus
HSV	Herpes simplex virus
OC	Oral candidiasis
KS	Kaposi Sarcoma
OHL	Oral Hairy Leukoplakia

FEH	Focal epithelial hyperplasia
RHI	Recurrent herpetic infections
VZV	Herpes varicella zoster
HZ	Herpes zoster
MC	Molluscum contagiosum
LGE	Linear gingival erythema
IgG	Immunoglobulin G
ANC	Antenatal care
MTCT	Mother to child transmission
PMTCT	Prevention of mother to child transmission
PMTC	Prevention of mother to child
ABC	Abacavir
3TC	Lamivudine
AZT	Zidovudine

D4T	Stavudine
EFV	Efavirenz
LPV/r (Kaletra)	Lopinavir/ritonavir
RTV	Ritonavir
NVP	Nevirapine
MDR/XDR-TB	Multidrug resistant/ extensively drug resistant tuberculosis

DEFINITIONS

HAART is defined as a combination of ARV drugs; usually two nucleoside reverse transcriptase inhibitors and a protease inhibitor or one nucleoside reverse transcriptase inhibitor, a non-nucleoside reverse transcriptase inhibitor and a protease inhibitor (11).

Initiation of HAART is defined as the first time three or more antiretroviral drugs from at least two different classes are administered within one week (27).

Disease progression is defined as a more advanced disease stage after baseline evaluation (35).

Clinical failure is defined as the appearance or reappearance of WHO clinical stage 3 or stage 4 events after at least 24 weeks on ART in a treatment- adherent child (94).

Immunologic success is defined as an increase from pre- ART $CD4^+$ T-lymphocyte count or an increase in $CD4^+$ T-lymphocyte percentage $\geq$ 15% (95).

Immunologic failure is defined as developing or returning to the following age-related immunological thresholds after at least 24 weeks on ART, in a treatment-adherent child: (1) $CD4^+$ T-lymphocyte count of< 200 cells/µl or percent $CD4^+$ < 10% for a child two years and older but less than five years of age. (2) $CD4^+$ T-lymphocyte count < 100 cells/µl for a child 5 years of age or older (94).

Virologic success is defined as achieving a viral load < 50 copies/ml (95).

Virologic failure is defined as a persistent HIV viral load $\geq$ 5000 copies/ml after at least 24 weeks on ART in a treatment-adherent child (94).

CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

The human immunodeficiency virus (HIV) has had a global impact with an estimated 3, 4 million children living with HIV at the end of 2011. Ninety one percent of these children live in sub-Saharan Africa (1). The Acquired Immunodeficiency Syndrome (AIDS) is the cause of one third of deaths in children under the age of five (2).

The majority of children in developing countries are born into extreme poverty and this is the major challenge to paediatric HIV care. The long history of economic migration in Africa or in the case of orphans with the death of one or both parents, likely due to HIV, means that caregiving roles are often adopted by those other than the biological parents, mainly grandmothers and aunts (3). With the increased responsibility of caring for additional children in the household arise a number of financial, social and educational challenges (4). Adoptive caregivers who may have their own HIV illness, are often elderly and suffering from chronic diseases themselves and are unable to provide proper care to the HIV-positive (HIV+) child. Extended family caregiving is not always feasible, and many children leave school to head households. Furthermore, the stigma related to HIV+ patients and even those who are related to or care for an HIV+ patient, known as secondary stigma (4), presents as a psychosocial impediment to timely HIV diagnosis and/or treatment in the paediatric population.

Vertically acquired HIV infection, that is mother to child transmission, accounts for 85% of all paediatric cases worldwide (5).Vertically infected children are different from infected adults as they have immature immune systems and thus the virus has a shorter incubation period with a more rapid and fulminant disease process (6, 7, 8, 9, 10, 11).The HIV disease course in children has many similarities to that in adults, however, differences in risk factors, modes of transmission, patterns of seroconversion and natural history exist. The spectrum of clinical

lesions found in the HIV+ paediatric population is another considerable difference. The oral cavity is home to numerous fungal, bacterial and viral microorganisms and is particularly susceptible to infection in HIV+ patients (5). Oral manifestations of HIV/AIDS present as important clinical signs of immunosuppression, HIV viral load and disease progression (12). The prevalence of oral lesions in HIV+ paediatric patients varies in different parts of the world, with figures as high as 63% reported in South African children (5). This is in comparison to 61% prevalence rate in Brazil, 55% in Romania and 49% in Thailand (13).

Through quantitative and qualitative restoration of the immune response, highly active antiretroviral therapy (HAART), has altered and decreased morbidity and mortality of HIV-infected individuals (14). Majority of children achieve immune reconstitution and virologic control, however there are a subset of individuals who clinically worsen with recovery of the immune system, which leads to a new spectrum of diseases. This is called the Immune Reconstitution Inflammatory Syndrome (IRIS) (15).

There is an abundance of literature on oral lesions in HIV+ adults but there are comparatively few reports on children, particularly in South Africa (5,12).The significance of this study is that oral lesions in children on HAART, may be clinical predictors of HAART failure or disease syndromes related to HAART. Identification of these lesions is important for both medical and dental practitioners to administer the correct multidisciplinary approach to paediatric HIV care. Therefore, the aim of this study is to profile and characterize oral lesions in paediatric patients on HAART. Objectives include describing the types of clinical oral lesions found in children on HAART ; to assess if CD4+ T-lymphocyte count, viral load and HAART regimens are determinants for the types of lesions found; to report on cases of the immune reconstitution inflammatory syndrome and to compare the findings of this study to other South African, African and international studies in a similar population.

1.2 Literature review

South Africa is home to the world's largest HIV epidemic, with 6,3 million HIV-infected individuals in 2013 (16).According to the UNAIDS HIV and AIDS estimates, 360 000 children, aged between 0 and 14 years were living with HIV/AIDS in South Africa in 2013. Two million and four hundred thousand children, between 0 to 17 years of age, were orphaned by the AIDS pandemic (16).The high coverage of efficient prevention of mother to child transmission (PMTCT) interventions has resulted in a substantial reduction in new paediatric HIV infections at 6 weeks of age from 5.8% in 2009 to 3.5% in 2010 and 2.7% in 2011. However, this still translates into thousands of infants infected annually (17).

Chronic pneumonitis, otitis media (including other recurrent bacterial infections), chronic diarrhea, lymphadenopathy, hepatosplenomegaly, developmental delays and failure to thrive are the major systemic symptoms in paediatric HIV (5). However, oral lesions are often the first symptoms of HIV infection with oral candidiasis being the most common manifestation in this population (5,6,7,8,9,12,18,19,20,21,22).

Antiretroviral therapy (ART) is the treatment indicated for HIV- infected individuals as it successfully suppresses replication of retroviruses and brings about preservation of the immune system. The first antiretroviral treatment was advocated as a single drug therapy. However, it was found that due to the mutative potential of the virus, improvements in clinical and immunologic status of patients were not sustained and resistance to monotherapy followed. This led to the advent of combination therapy with the use of at least 3 drugs from two different classes of drugs for treatment of the HIV infection. This is called highly active antiretroviral therapy (11). Currently, antiretroviral therapy consists of 30 different drugs from 8 different categories including nucleoside and nucleotide reverse transcriptase inhibitors (NRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI), protease inhibitors (PI), entry inhibitors (fusion inhibitors), HIV integrase inhibitors, co-receptor antagonists (also known as CCR5

antagonists) and maturation inhibitors (11).

The mechanism of action of a nucleoside reverse transcriptase inhibitors (NRTI) is through its incorporation into the newly synthesized viral DNA strand and to act as a 'faulty nucleotide,' resulting in DNA chain termination. Non-nucleoside reverse transcriptase inhibitors (NNRTI) bind directly to the enzyme called reverse transcriptase and interfere with its function. Protease inhibitors (PI) target the activity of the enzyme called protease and inhibits the final assembly of new virions (11).

Based on the World Health Organisation (WHO) protocol, the current regimen of HAART in South Africa and that which is advocated at the Rahima Moosa Mother and Child Hospital in Johannesburg, for patients under the age of 3 is: Abacavir (NRTI) + Lamuvidine (NRTI) + Kaletra syrup (Lopinavir/Ritonavir, which are PIs). Paediatric patients over the age of 3 receive: Abacavir (NRTI) + Lamuvidine (NRTI) and Efavirenz (NNRTIs). This regimen follows the protocol of standardized national ART regimens for infant and children as directed by the South African Department of Health (23).

In resource-poor countries such as those in sub-Saharan Africa, providing HIV care to infected patients is challenged with regards to laboratory monitoring. The benefit of antiretroviral therapy may be dampened by long term toxicity and the emergence of drug resistant virus. Routine monitoring of HIV viral load and $CD4^+$ T-lymphocyte count allows for detection of side effects of medications and drug resistant virus. The WHO recommends monitoring $CD4^+$ T-lymphocyte count every six months and viral load testing when the capacity exists. However, in developing countries, cost of laboratory monitoring compounded by the lack of technical expertise and infrastructure means that routine monitoring may not always be feasible. This means that patients may experience prolonged virologic failure and develop drug resistant mutations without detection, which could increase morbidity and mortality (24). After the scaling up of ART programs, the South African National Health Laboratory Service (NHLS) responded by

providing an increased number of laboratories for HIV viral load and CD4 testing services. However, with the increasing numbers of patients eligible for ART, additional capacity of the existing laboratory services is required (25). Hsiao *et al*, studied the linkage of South African infants from HIV diagnosis to ART services and reported that linkage to care is more likely in children in urban areas than in rural areas (Odds ratio (OR) =1.89, 95% CI). The explanation for this in part is mothers not returning to receive results due to the migration patterns of women during pregnancy and postpartum. Women in South Africa are known to migrate to urban centres for antenatal care (ANC) and maternal care but return to rural areas after giving birth (26). In these areas, a further delay is caused by difficulties experienced in conducting HIV testing at primary health care centres, transporting specimens to the laboratories and the return of results back to the centre. This delay may necessitate referral of the infant to another centre, which may increase mortality in this population (26). Furthermore, Glencross *et al*, reported that most clinics in South Africa have reasonable access to laboratory services. However, they acknowledge that resources are limited in South Africa and a geographical difference in distribution of services exists, with the focus of service being on more densely populated areas. West and North West areas of the country were found to have general pathology services but lacked CD4 testing facilities. The study confirmed that patients requiring $CD4^+$ tests from these areas had to be referred to adjacent districts sometimes 100-200 km away; which may cause delay in treatment (25). In the study by Glencross *et al*, the national laboratory service was deemed largely reliable, however, site visits to provincial clinics revealed that many clinics are challenged by regional transport and logistical deficiencies, staffing and skills deficiencies and poor or inconsistent electricity supply(25).

The response markers such as HIV-1 RNA plasma levels and $CD4^+$ T-lymphocyte count vary greatly between adults and children. In children due to the presence of an active thymus gland, immune recovery following HAART is stronger than in adults. However, virologic response is diminished in comparison to adults this is because in children, particularly in infants, the immature immune system allows less efficient control of HIV replication, resulting in high viral

loads. The increase in $CD4^+$ T-lymphocyte count is independent of the rate of virologic response (27).

In a study conducted by Walker *et al,* the authors evaluated the association between age, HAART initiation, $CD4^+$cell percentage and HIV-1 RNA loads. Within the first few years of life in all children, including HIV-uninfected children, there is a natural but considerable decline in $CD4^+$ T –lymphocyte count. Therefore, in this study, immunologic response was measured in $CD4^+$ cell percentage instead of $CD4^+$ T-lymphocyte count. They found that $CD4^+$ cell percentage increased substantially after HAART initiation regardless of pre- HAART HIV-1 RNA or the clinical status of the patient. Short and long term increases were greater and faster in younger children than in older children, even if they were severely immunosuppressed. Once again this may be attributed to an active thymus gland in younger children. In contrast to successful immunologic response, younger children are less likely to achieve HIV-1 RNA <400 copies after initiating HAART (27). This is in agreement with the study conducted by Ramirez-Amador *et al* (14) which suggested that children can restore immune function well without complete viral suppression. However, poor virologic response leads to an increased risk of drug resistance, which is of concern. Older children showed slower increases in $CD4^+$ % but presented with viral loads of HIV-1 RNA< 50 copies/ml. In both younger and older children, pre-HAART viral load was not a significant predictor of virologic response. Therefore, children of the same age, with high pre-HAART viral loads have the same chance of viral suppression as those with lower viral loads. This is in contrast to studies conducted in adults, which show that patients with lower pre-HAART HIV-1 RNA have a better chance of achieving undetectable values (27).

A relationship between the virologic response and the plasma drug concentration exists. Inadequate plasma concentrations of, in particular, protease inhibitors, may be accountable for the differences in virologic response rates (14). In South Africa, children < 3 years of age, are prescribed regimens that include PI's. As reported by van Zyl *et al*, Lopinavir (LPV), co-

formulated with low dose Ritonavir (LPV/r) is the preferred drug of choice as it has a high resistance barrier. Furthermore, many infants in the South African situation are co-infected with tuberculosis and require Rifampin therapy. Rifampin reduces the metabolism of LPV and reduces plasma concentration. Ritonavir (RTV)-only PI's have been found to be independently associated with major PI resistance and mutations. Therefore combination LPV/r at correct dosages is used in our protocols (28).Furthermore, a study conducted by Meyers *et al*, reports that Rifampin therapy is common in the South African setting as many children are co-infected with TB. They report that Rifampin causes accelerated clearance of both PI's and NNRTI's and therefore reduces plasma concentrations (29). Walker *et al*, found that children who received lower than recommended doses had a lower chance of suppressing viral load. They found that under-dosed children were able to suppress viral loads to HIV-1 RNA < 400 copies/ml but not to HIV-1 RNA < 50 copies/ml. However, dosing had no effect on the immunologic response (27). This is further supported by a study conducted in South African children by Orrell *et al*, who reported that poor adherence or adherence to a suboptimal regimen or dosing can lead to first and second line treatment failure (30). ABC and 3TC currently form part of the first line of treatment under the South African National ART guidelines. These guidelines followed WHO recommendations in 2010 to replace d4T with ABC as there were growing concerns related to d4T toxicity. A study conducted by Technau *et al*, reported that children treated with ABC/3TC have a lower chance of achieving viral suppression and a higher chance of viral rebound than children treated with d4T/3TC combinations. Therefore concerns about viral failure related to NRTI'S also exists and is not only related to PI's (31). The difficulty faced in administering HAART to paediatric patients is lack of paediatric formulations, limited options of drugs available for children, reliance on caregivers to administer the medication and toxicity of HAART in long term therapy (14,31). Early initiation of HAART for children decreases mortality and disease progression. It is important that timely initiation of HAART occurs and an attempt is made to balance progression of HIV disease, complicated long term side effects and development of resistance (17). Furthermore, in the South African setting where treatment options are limited and the potential toxicity associated with lifelong ART is a reality, Cotton *et*

al, investigated the outcomes of early time-limited ART and deferred ART, in the children with HIV early antiretroviral treatment (CHER) trial (32).The concept of early time-limited ART, which is initiated at diagnosis of primary infection, is to prevent disease progression but also safely allow a subsequent planned period off ART, thus reducing resistance and preserving future treatment options. The results of this trial found that early time-limited ART had better clinical outcomes than deferring ART to a later age as an attempt to prevent long term toxicity or drug resistance. The reason for this may be in part due to the fact that initiating ART early limits the HIV reservoir and preserves the immune response (32).

Eligibility for ART initiation as guided by the South African Department of Health is as follows (23):

Table 1.2.1 Criteria for eligibility to start ART (23)

Eligible to Start ART • All children less than 5 years of age, irrespective of $CD4^+$ T-lymphocyte count. • Children 5-15 years of age with WHO clinical stage 3 or 4 of $CD4^+$ T-lymphocyte count $\leq$ 350 µl
Require Fast-Track (start ART within 7 days of being eligible) • Children less than 1 year of age • WHO clinical stage 4 • MDR or XDR-TB • $CD4^+$ T-lymphocyte count <200 cells/µl

Current WHO recommendations include initiation of HAART to all children younger than 12-24 months regardless of the $CD4^+$ T- lymphocyte count or viral load (23). WHO antiretroviral

guidelines are updated every two years. The above mentioned protocol is adopted from the 2013 consolidated guidelines, which were supplemented in March 2014. The latest guidelines recommend ART to be initiated for all patients with $CD4^+$ T-lymphocyte counts ≤ 500 cells/mm^3. The guidelines also state that it should be initiated immediately, regardless of $CD4^+$ T-lymphocyte count, in children up to the age of five years, individuals with active tuberculosis, individuals co-infected with HBV with severe chronic liver disease and individuals living in sero-discordant partnerships. All pregnant and breast feeding women should be offered lifelong ART (33).The 2013 guidelines raised the threshold to 500 cells/mm^3, thus attempting to accelerate the trend of early initiation. Speculations of future 2015 guidelines may be administration of ART to all patients , regardless of $CD4^+$ T-lymphocyte counts as is recommended in developed countries such as France and the United States of America. This is not true for all developed countries as the most recent United Kingdom guidelines still recommend starting ART ≤ 350 cells/mm^3 and to only " consider" initiating ART ≤ 500 cells/mm^3 (34).Children who initiate HAART at a time of mild disease are reported to show reduced progression to moderate disease. When progression does occur it is delayed by 3 years (35). HAART use in children with moderate disease at initiation is associated with a decreased proportion of children progressing to severe disease and death. Therefore, HAART, is associated with reduced and delayed progression of HIV regardless of the disease stage at initiation. Furthermore, for children who show progression it is primarily based on laboratory immunosuppression rather than clinical disease (35).

It can therefore be concluded that immune recovery occurs rapidly in HIV-infected children after the initiation of antiretroviral therapy and majority of children benefit greatly from therapy. However, perinatal transmission of HIV is often associated with neglected HIV prevention measures and so there is a delay in diagnosis and thus a delay in initiation of treatment.

Studies conducted by Egger *et al*, suggested that $CD4^+$ T-lymphocyte counts, in particular,

baseline $CD4^+$ T-lymphocyte count is more strongly associated with the probability of disease progression to AIDS or death in patients on HAART. They concluded from their findings that baseline viral load does play a role in disease progression but only in individuals with baseline viral loads above 100 000 copies/ml (36). Furthermore, Helleberg *et al* observed that $CD4^+$ T-lymphocyte count is also a marker of inflammation or unstable atherosclerotic plaques. In this study, it was also shown that in the absence of immunosuppressive drugs, radiotherapy and chemotherapy, virally-suppressed patients showed a considerable drop in $CD4^+$ T-lymphocyte count and this was associated with an increased risk of death, emphasizing the importance of regular monitoring of $CD4^+$ T-lymphocyte counts in patients receiving ART (37). These findings were further supported by a study conducted by Lapadula *et al,* who reported that patients who showed a positive virologic response to treatment coupled to immunologic failure, had a higher risk of progression to AIDS. Furthermore, the inability of the immune system to detect and destroy neoplastic cells, compounded by persistent immune activation also influences the risk of developing non-AIDS malignancies and cardiovascular disease. Hence, the authors suggested monitoring $CD4^+$ T-lymphocyte counts every 6 months even after achieving viral suppression. The findings of this study also support the protocol to administer ART to patients with $CD4^+$ T-lymphocyte counts $\leq$ 500 cells/mm^3 as mortality amongst patients with $CD4^+$ T-lymphocyte> 500 cells/mm^3 with prolonged viral suppression is the same as that of the general population (38). Despite these findings, protocols developed by the Office of AIDS Research Advisory Council (OARAC), which were updated in May 2014, state that measurements of $CD4^+$ T-lymphocyte counts is useful prior to ART initiation and viral load should be used to monitor the effectiveness of therapy after initiation. The viral load is the established surrogate marker for treatment response (39). These guidelines have taken lead from much prior studies conducted by Mellors *et al,* who concluded that viral loads strongly predict that rate of decrease in $CD4^+$ T-lymphocytes and the progression to AIDS and death. However, women, children and members of different ethnicities and racial minority groups were not included in this study (40). The OARAC protocol states that $CD4^+$ T-lymphocyte count monitoring has no clinical benefits in virally-suppressed patients who have $CD4^+$ T-lymphocyte

$\geq$ 200 cells/mm^3 after 48 weeks on therapy (39). This is supported by more recent studies conducted by Girard *et al,* who agree that there is little clinical benefit in continued CD4$^+$ T-lymphocyte count monitoring in patients who have viral loads $\leq$ 50 copies/ml and CD4$^+$ T-lymphocyte counts $\geq$ 200 cells/mm^3 (41).It states that although a decline in CD4$^+$ T-lymphocyte count may occur in patients with successful viral suppression, it is uncommon. Furthermore, the costs of monitoring CD4$^+$ T-lymphocyte counts from 6 months to 12 months will result in a significant amount of annual savings. However, patients who fail to maintain viral suppression whilst receiving treatment, should be monitored for CD4$^+$ T-lymphocyte counts every 3 to 6 months (39). It has been well established that disease progression is marked by a decrease in the laboratory marker of CD4$^+$ T-lymphocyte count and an increase in plasma viral load. However, in developing countries, the tools and/or resources to consistently monitor progression of disease may not be available. Therefore, clinicians rely more on clinical parameters such as weight and height to monitor progress, particularly in the paediatric HIV+ population (42).

Weight and height of HIV+ paediatric patients tends to lag behind HIV-uninfected children of the same age (43). Growth failure, which is not only in the form of stunting and weight loss but also failure to thrive, is attributed to inadequate calorie intake, gastrointestinal infections, opportunistic infections and endocrine abnormalities (40). HAART results in improvement in weight-for-age and height-for-age in comparison to baseline values. Increases in weight and height are seen in patients who are viral responders more significantly than non-responders and therefore successful HAART should have a positive impact on growth (43). There are, however, studies conducted by Yotebieng *et al,* that found antiretroviral treatment to be associated with improved weight-for-age and height-for-age but not associated to viral suppression or treatment failure (44). In a study conducted by Musoke *et al,* it was found that children who were on HAART but not showing a significant increase in weight or height were also experiencing immunologic failure (45). Therefore, these basic growth parameters, which are inexpensive to measure by primary health care workers in resource-limited settings, are associated with successful HIV treatment outcomes and form good predictors of clinical disease progression and

are useful markers for high –risk children (45). Paediatric HIV+ patients are routinely monitored for height and weight in follow up appointments because poor nutritional status and stunting at the time of ART initiation is associated with a poor prognosis (44). Furthermore, other studies have confirmed that low $CD4^+$ T-lymphocyte counts, anemia, chronic diarrhea, reduced weight-for-age and WHO clinical stage 4 conditions at the time of ART initiation contribute significantly to paediatric mortality and disease progressions, despite treatment with ART (46).

Anemia is defined as having a haemoglobin level ≤ 10 mg/dl (47). Severe anemia is defined as having a haemoglobin level ≤ 6,5mg/dl (42). Studies conducted by Koye *et al*, found low haemoglobin levels at initiation of ART to be a significant predictor of mortality in children on treatment. Furthermore, it is commonly associated with disease progression and death in paediatric patients with HIV(47). Children with lower $CD4^+$ T-lymphocyte counts at initiation are at a greater risk of opportunistic infections, which can impair the positive effects of ART. Chronic diarrhea decreases drug absorption and impairs metabolism (48). The WHO has four clinical stages to represent the clinical forms of disease in patients not receiving treatment. Stage 4 is advanced HIV/AIDS disease. Clinical staging allows children at risk of rapid disease progression to be recognized and in severely limited settings, it allows resources to the prioritized to patients that need them the most (49).

Oral manifestations of HIV in children are classified according to the European Community – Clearinghouse (EC-Clearinghouse) and WHO. Existing diagnostic criteria have been updated by the Oral HIV/AIDS Research Alliance (OHARA)(50).

Table 1.2.2 OHARA oral manifestations of paediatric HIV (50)

Fungal	**Viral**	**Bacterial**	**Salivary gland disease**	**Neoplasms**	**Idiopathic**
Pseudomembranous Candidiasis	Hairy leukoplakia	Necrotizing gingivitis/ periodontitis	Parotid enlargement	Oral Kaposi Sarcoma	Recurrent aphthous stomatitis
Angular cheilitis	Herpes labialis		Salivary hypofunction	Oral non-Hodgkins lymphoma	Ulcerations
	Recurrent intra-oral herpes simplex			Oral squamous cell carcinoma	Necrotizing stomatitis
	Oral Warts				

IRIS, also known as the immune reconstitution syndrome or restoration disease (14), is defined as a collection of signs and symptoms resulting from the ability to mount an immune response to antigens or organisms, associated with immune recovery, that is, an increase in $CD4^+$ T-lymphocyte count and/or a rapid decrease in viral load, in patients on antiretroviral therapy (52). Immune reconstitution is defined as a $CD4^+$ T- lymphocyte cell count of >200 cells/mm^3 or an increase of $\geq$100 cells/mm^3 over baseline any time since the initiation of HAART (53). It is a consequence of antigen-specific exaggerated inflammatory response in which the normal regulatory mechanisms, that limit the degree of inflammation, are absent (14).

There are two clinical syndromes associated with IRIS; the "unmasking" and the "paradoxical" syndromes. In unmasking IRIS, there is a subclinical, previously unrecognized infection which flares up after HAART initiation. Paradoxical IRIS is the exacerbation of a past known and often treated disease (9). A third syndrome of IRIS possibly exists. In addition to the paradoxical and unmasking syndromes, an autoimmune syndrome is also thought to exist.

This is not a well studied phenomenon but has been seen with regards to Hashimoto's thyroiditis. Further research still needs to be conducted for this syndrome to be officiated (54).
French also reported autoimmune diseases in HIV+ patients who responded favourably to antiretroviral treatment. He reported evidence on Graves disease as a complication of immune restoration seen in patients who recovered from severe $CD4^+$ T-lymphocyte deficiency (55). This was supported by Dhasmana *et al*, who reported that Graves disease has a tendency to occur approximately two years after ART initiation (56). Although the aetiology of this possible third syndrome of IRIS has not yet been identified; it may be due to an increased susceptibility to autoimmune diseases as a result of an acquired defect in immune tolerance (55). Sarcoidosis has also been reported to be a potential autoimmune disease related to the restoration of the immune system(55). The manifestations of re-occurrence of sarcoidosis include lymphadenopathy, pulmonary involvement, erythema nodosum and other skin eruptions (56).

Currently, there are no confirmatory tests for IRIS and it remains a diagnosis of exclusion (51). IRIS is considered in patients who have an emergence or clinical worsening of opportunistic infections. These conditions may occur with an unusual presentation or disease course wherein the possibility of HAART failure and adverse drug reactions have been excluded. The appearance of symptoms has a temporal relationship with the initiation of therapy and associated immune recovery (52). Ramirez-Amador *et al*, concurred with this statement and described IRIS as a rapid, dysregulated restoration of antigen-specific immune response during antiretroviral therapy that most often occurs within 3 months of therapy initiation (14).

The pathogenesis of IRIS is not well understood. HIV infection depletes $CD4^+$ T lymphocyte cells including T-helper cells 1 (TH1) and regulatory T-cells (T_{REGS}). TH1 cells mediate the predominant host defense against extracellular pathogens and T_{REGS} are responsible for immunoregulation. Following initiation of HAART, there is a rapid recovery of TH1 and a slower reconstitution of $T_{REGS.}$ IRIS may be attributed to an exaggerated, uncontrolled inflammatory immune response due to TH1 cells in the absence or deficiency of normal T-cell

regulatory mechanisms. Furthermore, there has been a discovery of a new lineage of T-helper cells called T-helper 17 cells (TH17). The T-helper 17 plays a critical role in maintaining inflammatory response and is known to produce an accelerated memory response to antigens. T_{REGS} and TH17 have a reciprocal relationship and in the absence of $T_{REGS,}$ the restoration of TH17 may further contribute to the cytokine imbalance in favour of pro-inflammatory signals (14).

A review done by dos Santos Pinheiro *et al,* reported a reduction in the frequency and severity of HIV associated oral disease after using HAART (57). However, they also found an association between treatment with HAART and increased presentation of salivary gland disease and oral warts caused by the Human Papilloma Virus (HPV) in children. However, this study conducted by dos Santos Pinheiro *et al*, was a study based on a literature review. This means that direct comparisons between the studies cannot be made to obtain concluding results as each study viewed had different subject groups and methodologies. Furthermore, case-control studies were also included in the literature search and it is difficult, once again, to base conclusions on single case-study reports. The prevalence of oral diseases such as oral candidiasis (OC), Kaposi Sarcoma (KS) and oral hairy leukoplakia (OHL) have decreased since the introduction of HAART. However, conditions such as oral hyperpigmentation was significantly increased in the paediatric population receiving HAART (57). A study conducted by Wang *et al,* found Herpes viruses to be commonly associated with IRIS in both adults and children, with the varicella zoster virus (VZV) and herpes simplex labialis virus occurring most commonly in children (15). Various studies have reported different prevalence rates of IRIS in the paediatric population. Studies in Thailand, India and Peru have reported prevalence rates of 18,9%, 19% and 20% respectively (15,52). Studies conducted by Boulware *et al*, reported an incidence rate of 10-19% (51). However, little is known about oral lesions in paediatric patients on HAART and the incidence of IRIS with most studies concluding that further research should be conducted in this field.

CHAPTER 2: METHODS

2.1 Study design and population

The study was a cross-sectional, descriptive investigation. It was conducted on HIV+ paediatric patients attending the Empilweni Clinic, at the Rahima Moosa Mother and Child Hospital, Johannesburg, Gauteng, South Africa from March 2014 to April 2014. The WHO classifies age groups as neonates (less than one month old), infants (one years old or younger but older than one month), children (older than 1 years but under the age of ten), adolescents (individuals between the ages of ten and nineteen) and adults (nineteen years and older) (1).

2.1.1 Inclusion criteria

Paediatric patients with a confirmed HIV^+ status, $CD4^+$ T- lymphocyte cell counts and viral loads, aged between one month -14 years old. All patients are on HAART.

2.1.2 Exclusion criteria

Children not on HAART, outside of the age group (neonates) or those with incomplete records such as missing $CD4^+$ T-lymphocyte counts and viral loads, were not included in the study.

This was a convenient sample as, due to ethical reasons, it is difficult to determine the HIV status of children in a general population.

2.2 Ethical considerations

Ethical approval (Appendix A) for this study was obtained from the Committee for Research in Human Subjects at the University of the Witwatersrand. The clearance certificate number is M131045. Information on the study was made available in two languages, English and Zulu, to the patients' parents, guardians and caregivers as applicable, after which informed consent was sought.Informed consent (Appendix B) was attained from the patients' parents, guardians or caregivers before examinations commenced. Consent was obtained for clinical examinations and any photographs to be taken. Informed consent forms were made available in two languages, English and Zulu. Children over the age of 7 years signed a minor assent form (Appendix C).

2.3 Clinical Examination

A total of 240 HIV infected children, aged 1 month to 14 years, of which 128 were male and 112 were female, were examined at the Empilweni Clinic between March 2014 and April 2014. The classification for presumptive diagnostic criteria for oral lesions was based on the review of Oral HIV/AIDS Research Alliance: updated case definitions of oral disease endpoints (18). The examinations were conducted by the investigator, who was calibrated prior to commencement of clinical examinations, according to the case definitions mentioned above. Calibration was done by diagnosis of 34 clinical photographs of oral lesions under test circumstances. The test was repeated twice thereafter, at intervals 1 day and 3 days until 90% accuracy was achieved. Unfortunately, intra-examiner calibration could not be ensured by re-examining a small percentage of children due to the unwillingness of parents and caretakers to take time off work to bring the children to the clinic for a non-medical all-research purpose examination. Children at the Empilweni clinic are seen on a monthly basis, as this study was completed in a month no child was examined twice. Returning for re-examination would mean parents/ caregivers have to take a day off work and children miss a day of school. Furthermore, an extra trip to the clinic incurs additional transport cost. For similar reasons on telephonic follow- up with parents, no

children clinically diagnosed with oral lesions presented for biopsy of lesions. Therefore, re-examinations and biopsies of lesions could not be performed and serves as a limitation for this study.

Oral examinations were conducted with children sitting on a chair or on the lap of a caregiver. Examinations consisted of palpation and visual inspection of the mouth and facial structures, using a wooden spatula and hand-held light. If a lesion was detected and required biopsy and treatment, the patient was referred to the Department of Oral Medicine and Periodontology at the University of the Witwatersrand. A letter was also written and placed in the file to inform the medical practitioner of the presence of the oral lesion, suggesting blood tests to check CD 4^+ T-lymphocyte counts and viral loads, to be repeated if deemed necessary. Blood tests were recommended in all patients presenting with oral lesions excluding hyperpigmentation due to HAART therapy. Photographs of the lesion were taken, without revealing the identity of the patient. These were used to facilitate profiling and characterization of lesions, but also as an aide for quality/accuracy control. Photographs were used as aides in accuracy control by visually comparing diagnoses made by the examiner with that of an experienced clinician. No discrepancy was detected during comparison, and a 100% agreement on diagnoses was recorded. Informed consent was obtained prior to taking photographs. Age (in months and years) currently and at the time of diagnosis of HIV, duration of antiretroviral treatment (months), viral load (RNH-HIV-1 per ml) and $CD4^+$ T-lymphocyte cell count (cells/mm^3) were recorded from the patient's records following examination.

2.4 Data Analysis

Data was transferred from the data collection sheets onto an Excel© spread sheet and then imported into the statistical software, Statistical Package for the Social Sciences (SPSS). Descriptive statistics with the use of case processing summary tables and cross tabulations for gender was used. Significance of association between age, CD4+ T-lymphocyte count currently

and at baseline, viral load currently and at baseline and duration of HAART with the presence of oral lesions was determined using t-tests. A *P*- value <0.05 was considered significant. Given the skewed distribution of the data and the unbalanced samples of the two groups we were comparing, we could not assume normality of the underlying sampling distributions. Therefore we had to revert to conducting non-parametric, Mann Whitney tests and not t-tests. Pearsons correlation test was used to determine the association between CD4+ T-lymphocyte count and viral load.

CHAPTER 3: RESULTS

The total sample for this study constituted 240 valid cases made up of 128 males and 112 females. . Table 3.1 shows the distribution of the sample by lesions group (Yes - positive for "have oral lesions" or No – negative for "have oral lesions"). Twenty two out of 240 cases had oral lesions (9,2%).

Table 3.1 "Have lesions"

		n	%
Have lesions	Yes	22	9.2%
	No	218	90.8%
	Total	240	100.0%

Of the twenty two cases with oral lesions, 41% were female and 59 % were male (table 3.2).

Table 3.2 "Have lesions" by gender

			Gender		
			Female	Male	Total
Have lesions	Yes	n	9	13	22
		%	8.0%	10.2%	9.2%
	No	n	103	115	218
		%	92.0%	89.8%	90.8%
	Total	n	112	128	240
		%	100.0%	100.0%	100.0%

Of the lesions detected, ten children presented with extra-oral lesions and twelve children presented with intra-oral lesions (Table 3.3). HAART-induced hyperpigmentation constituted majority of the intra-oral lesions detected. This is a difficult lesion to diagnose and criteria included asymmetric, localized areas of pigmentation on oral mucosae but more importantly no reports of pigmentation in the medical history prior to therapy. Other lesions included focal epithelial hyperplasia, major aphthous ulcer, ulcer of unknown origin and recurrent herpetic infections. Amongst those presenting with extra-oral lesions, three children presented with scarring as a result of previous herpes varicella zoster (chicken pox) and one child presented with active herpes varicella zoster at the time of examination. One child presented with scarring as a result of herpes zoster. Two children presented with scarring as a result of previous herpes simplex and one child presented with active herpes simplex at the time of examination. History obtained from parents/ caregivers confirmed that those children who presented with scarring were on HAART at the time of active disease. This was corroborated with the medical history from the files and confirmed by checking treatment previously administered for the lesions where possible. However, cases where the child was receiving treatment at another clinic or in a different region of South Africa did occur, as well as cases where at the time of the lesion, patients did not report to the clinic for treatment and so there is no record of it in the files. Other extra-oral lesions detected included molluscum contagiosum and skin eruptions due to hypersensitivity to Bactrim. No cases of

candidiasis, parotid enlargement, Kaposi sarcoma, oral hairy leukoplakia, linear gingival erythema or cervical lymphadenopathy were found.

Table 3.3 Description of intra-oral and extra-oral lesions

		n	%
Intra oral disease presence: Description	Focal epithelial hyperplasia	3	25.0%
	Hyperpigmentation	6	50.0%
	Major apthous ulcer	1	8.3%
	Recurrent herpetic infections	1	8.3%
	Ulcer of unknown origin	1	8.3%
	Total	12	100.0%

		n	%
Extra oral disease presence: Description	Herpes simplex	3	30.0%
	Herpes varicella zoster (chicken pox)	4	40.0%
	Herpes zoster	1	10.0%
	Hypersensitivity to Bactrim	1	10.0%
	Molluscum contagiosum	1	10.0%
	Total	10	100.0%

Figure 3.1 illustrates the current age distribution of the sample. An overall average age of 8,7 years was reported (sd = 3,3). While the distribution of current might not be perfectly normal, inspection of the distribution did not reveal any notable outliers.

Figure 3.1 Histogram of current age

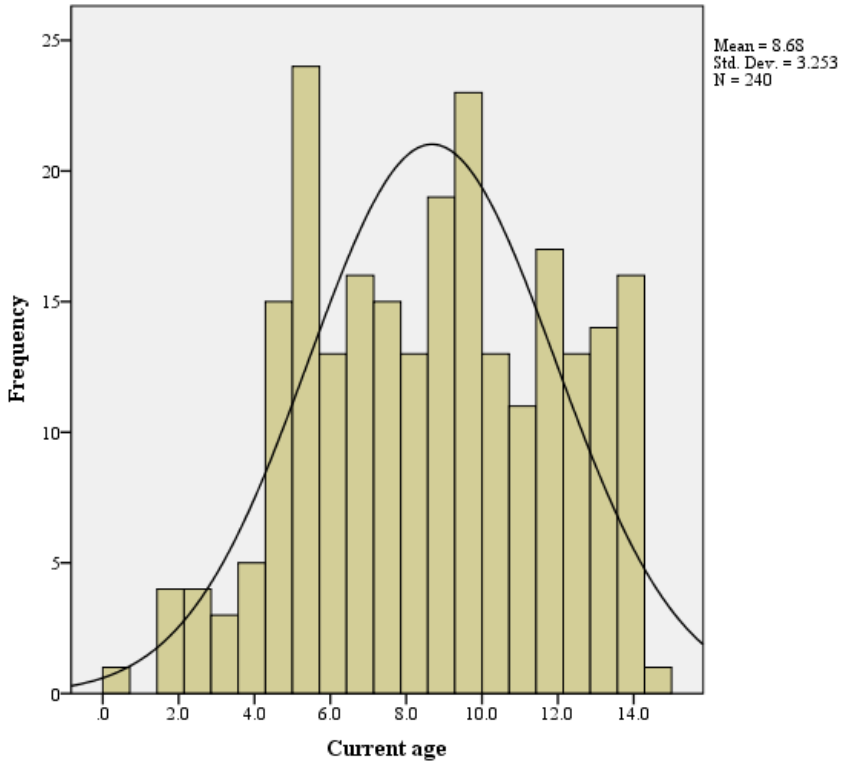


Table 3.4 refers to a statistically significant difference in the average current ages of children with and without oral lesions group was identified (p = 0,002). Those with oral lesions reported an average current age of 10,7 years (sd = 3,4). This in comparison to a lower average current age of 8,5 years amongst those without oral lesions (sd = 3,2).

Table 3.4 Average current age by lesions group

		Have lesions		
		Yes	No	Total
Current age	Mean	10.7	8.5	8.7
	Standard Deviation	3.4	3.2	3.3
	Median	12.3	8.5	8.8
	Valid n	22	218	240

The age distribution at time of HIV diagnosis (refer to figure 3.2) indicates a positively skewed distribution. Seventy-eight of the 240 cases (32,5%) were diagnosed with HIV at age one and younger, thus during infancy. While an average age of 3,1 years are reported, this statistic is skewed by the long positive tail of the distribution. A more representative value would therefore be the median age of two years. Therefore this study finds no correlation between the age at diagnosis of HIV and the presence of oral lesions.

Figure 3.2 Histogram of age at time of HIV diagnosis

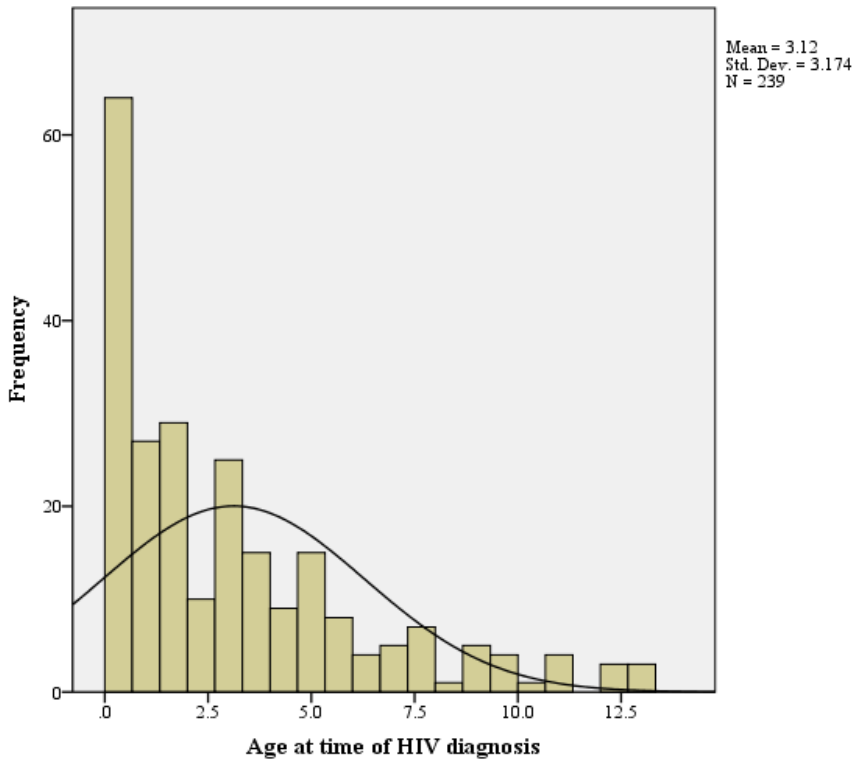


Table 3.5 reports comparative summary statistics for the variable age at time of HIV diagnosis by lesions group. For both the average and median values the average age at time of HIV diagnosis were observably higher for the Yes-group (4,7 and 3,1) than the No-group (3,0 and 2,0). Due to the significantly skewed distribution evident for the dependent variable, a Mann-Whitney U test was employed to test whether the two samples of values could be considered different. The p-value of 0,080 suggests no significant difference between the Yes group and the No group based on the age at time of HIV diagnosis.

Table 3.5 Average age at time of HIV diagnosis by lesions group

		Have lesions		
		Yes	No	Total
Age at time of HIV diagnosis	Mean	4.7	3.0	3.1
	Standard Deviation	4.3	3.0	3.2
	Median	3.1	2.0	2.0
	Valid n	22	217	239

The distribution of CD4+ baseline count for the sample group is shown in figure 3.3. The distribution reveals a positively skewed tail, indicating the highest frequencies of CD4+ occuring at lower CD4+ baselines. The median is 651,5 cells per mm^3 and is reported as a more valid measure of central tendency (refer to table 3.6).

Figure 3.3 Histogram of CD4+ T-lymphocyte baseline count

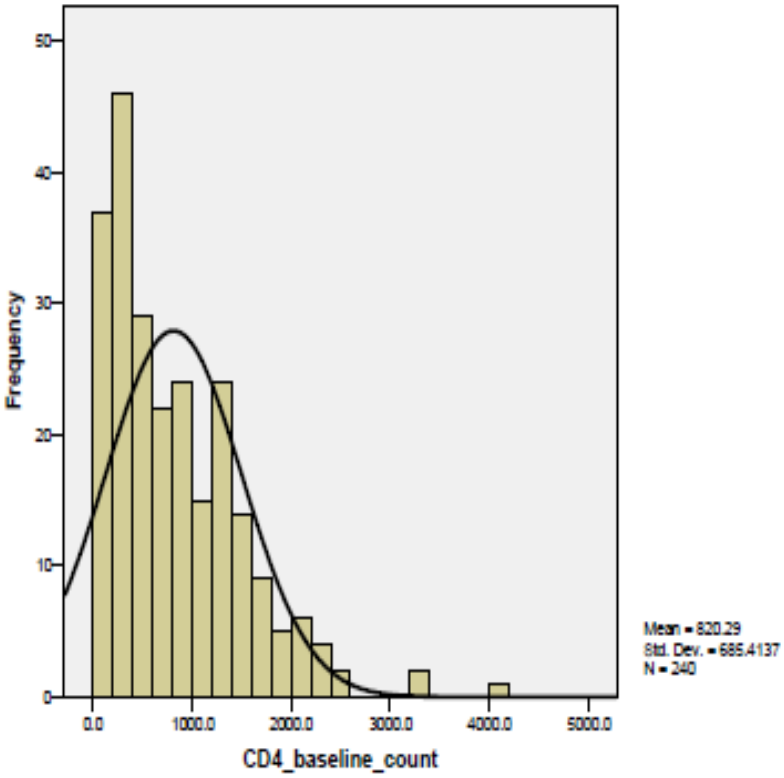


Table 3.6 reports the average, standard deviation and median values by lesions group. While lower CD4 values are reported for the Yes-group (both the average and median values), the p-value of 0,619 from the Mann-Whitney U test suggests no significant difference between the Yes-group and the No-group. Therefore there is no significance of baseline $CD4^+$ T-lymphocyte counts to the presence of oral lesions.

Table 3.6 Average CD4+ T-lymphocyte baseline count by lesions group

			Have lesions		
			Yes	No	Total
CD4 count	baseline	Mean	756.3	826.7	820.3
		Standard Deviation	669.8	688.1	685.4
		Median	566.5	659.5	651.5
		Valid n	22	218	240

The distribution of CD4+ latest count for the sample group is shown in figure 3.4. As in the case of the CD4+ baseline count, the distribution reveals a positively skewed tail. The average case reported a count of 944 cells per mm^3 (refer to table 3.7).

Figure 3.4 Histogram of CD4+ T-lymphocyte latest count

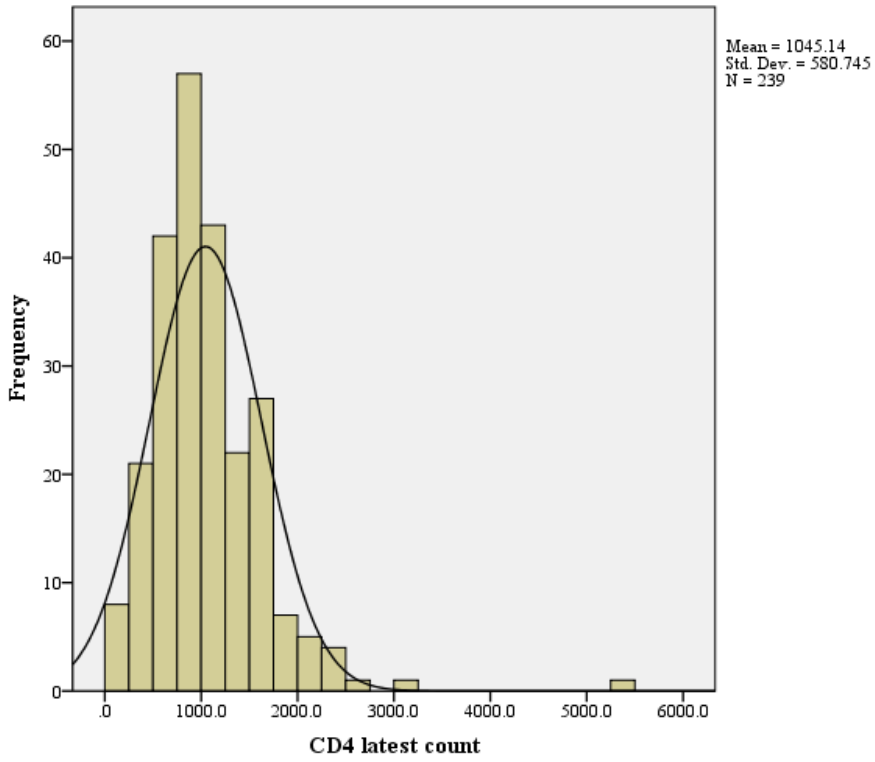


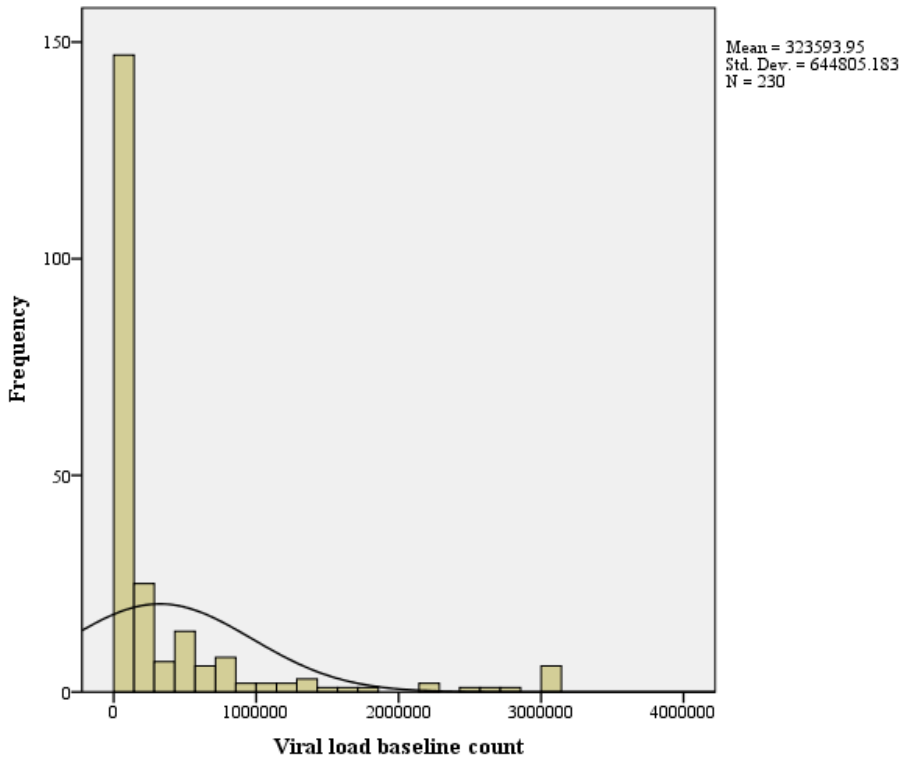
Table 3.7 reports the average, standard deviation and median values by lesions group. Lower CD4 values are reported for the Yes-group (both the average and median values), however, the p-value of 0,096 from the Mann-Whitney U test suggests no significant difference between the Yes-group and the No-group. Therefore, $CD4^+$ T-lymphocyte count in this study did not prove to be a predictor for oral lesions or have any association with the presence of oral lesions.

Table 3.7 Average CD4+ T-lymphocyte latest count by lesions group

			Have lesions		
			Yes	No	Total
CD4 count	latest	Mean	888.1	1 060.3	1 045.1
		Standard Deviation	587.7	579.2	580.7
		Median	779.0	971.0	944.0
		Valid n	21	218	239

Figure 3.5 shows the distribution of viral load baseline count for the sample group. The distribution reveals a positively skewed tail. A median viral load baseline count of 49 416,5 copies/ml is reported (refer to table 3.8).

Figure 3.5 Histogram of viral load baseline count



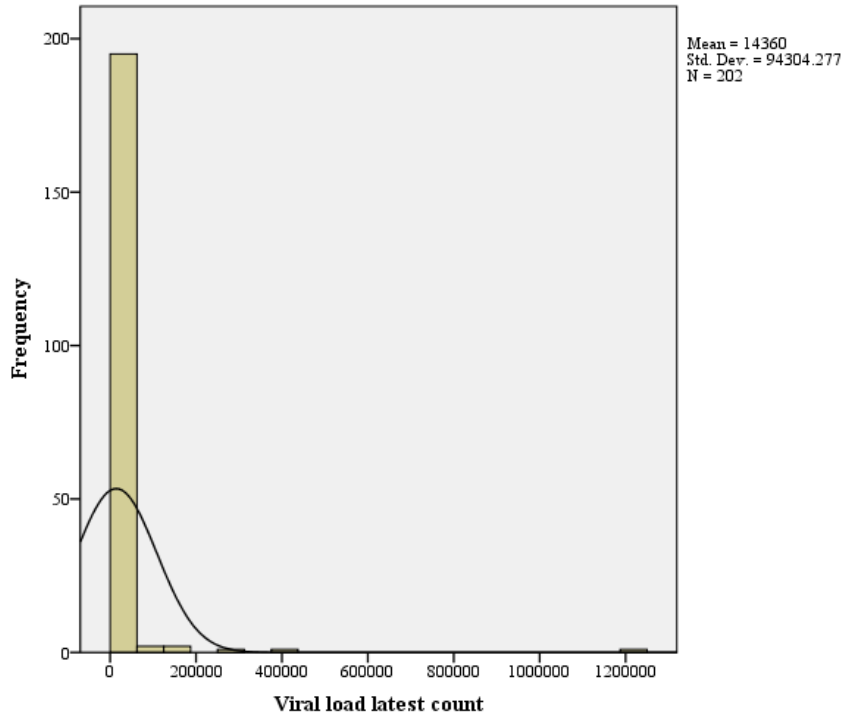
The Mann-Whitney U test (p = 0.272) indicates no significant difference between the Yes- and No-groups for viral load baseline counts.

Table 3.8 Average viral load baseline count by lesions group

		Have lesions		
		Yes	No	Total
Viral load baseline count	Mean	278 144.6	328 401.1	323 594.0
	Standard Deviation	344 050.3	669 112.0	644 805.2
	Median	100 000.0	41 514.5	49 416.5
	Valid n	22	208	230

Figure 3.6 shows the distribution of viral load latest count. The distribution reveals a positively skewed tail. A median viral load baseline count of 294 copies/ml is reported (refer to table 3.9).

Figure 3.6 Histogram of viral load latest count



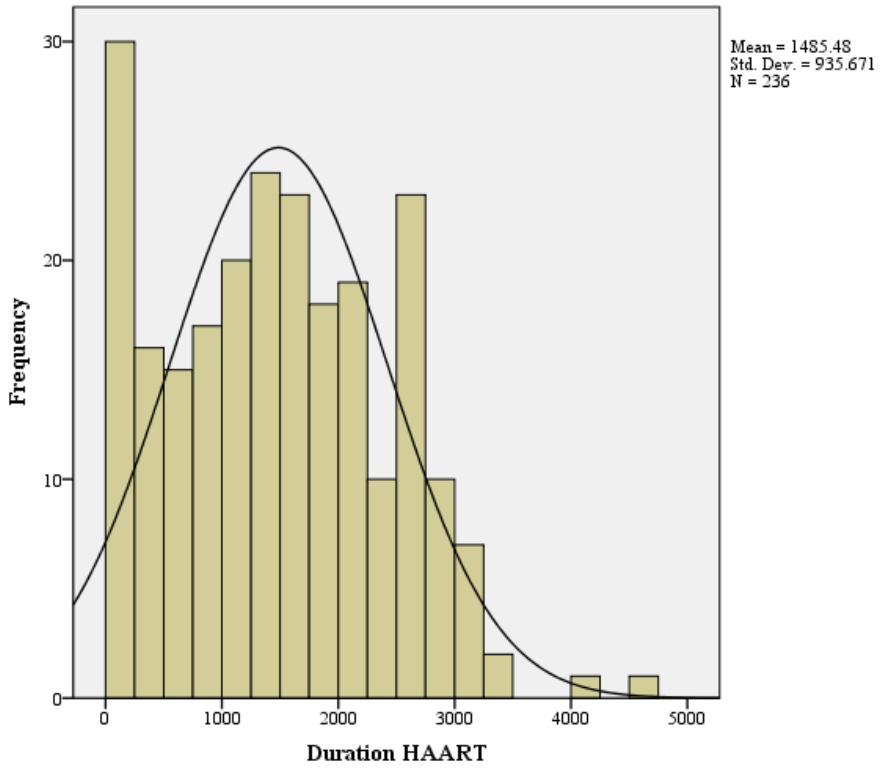
The Mann-Whitney U test reveals a p-value of 0.052. While this suggests no significant difference between the Yes- and No-group group for viral load latest counts based on a 95% level of confidence, it is only marginally higher than the 0,05 level.

Table 3.9 Average viral load latest count by lesions group

		Have lesions		
		Yes	No	Total
Viral load latest count	Mean	14 497.1	14 345.8	14 360.0
	Standard Deviation	37 595.4	98 396.8	94 304.3
	Median	1 148.0	288.0	294.0
	Valid n	19	183	202

Inspection of the distribution of number of days on HAART reveals a significant proportion of cases (30 cases) that were on HAART for less than 250 days (refer to figure 3.7).

Figure 3.7 Histogram of days on HAART



The average case have been on HAART for 1 444,5 days. No significant difference was evident between the lesion groups (p = 0,533) (refer to table 3.10).

Table 3.10 Average days on HAART by lesions group

		Have lesions		
		Yes	No	Total
Duration HAART	Mean	1 599.4	1 474.9	1 485.5
	Standard Deviation	1 002.3	931.0	935.7
	Median	1 583.5	1 435.5	1 444.5
	Valid n	20	216	236

While the Spearman correlation between CD4 latest count and viral load latest count indicates a negative coefficient of -0,106, the coefficient is, however, not considered significantly different from zero (p = 0,132). Therefore no linear relationship exists between the two variables.

Table 3.11 HAART Regimen by lesions group

			Have lesions		
			Yes	No	Total
HAART regimen	ABC 3TC KALETRA	n	11	26	37
		%	50.0%	11.9%	15.4%
	AZT ALLUVIA	n	1	0	1
		%	4.5%	0.0%	0.4%
	ABC 3TC EFV	n	5	79	84
		%	22.7%	36.2%	35.0%
	ABC 3TC STOCRIN	n	1	1	2
		%	4.5%	0.5%	0.8%
	D4T 3TC EFV	n	1	33	34
		%	4.5%	15.1%	14.2%
	AZT 3TC EFV	n	0	7	7
		%	0.0%	3.2%	2.9%
	AZT 3TC KALETRA	n	1	1	2
		%	4.5%	0.5%	0.8%
	D4T KALETRA 3TC	n	1	28	29
		%	4.5%	12.8%	12.1%
	D4T 3TC ALLUVIA	n	1	9	10
		%	4.5%	4.1%	4.2%
	AZT DDI ALLUVIA	n	0	3	3
		%	0.0%	1.4%	1.3%
	AZT 3TC NVP	n	0	5	5
		%	0.0%	2.3%	2.1%
	ABC EFV AZT	n	0	1	1
		%	0.0%	0.5%	0.4%
	ABC 3TC ALLUVIA	n	0	13	13
		%	0.0%	6.0%	5.4%
	AZT 3TC ALLUVIA	n	0	4	4
		%	0.0%	1.8%	1.7%
	ABC D4T EFV	n	0	1	1
		%	0.0%	0.5%	0.4%
	ABC AZT KALETRA	n	0	1	1
		%	0.0%	0.5%	0.4%
	3TC MONOTHERAPY	n	0	4	4
		%	0.0%	1.8%	1.7%
	AZT EFV KALETRA	n	0	1	1
		%	0.0%	0.5%	0.4%
	ABC 3TC NVP	n	0	1	1
		%	0.0%	0.5%	0.4%
	Total	n	22	218	240
		%	100.0%	100.0%	100.0%

It can be seen from Table 3.11 that 35% of children on treatment were on the ABC, 3TC, EFV regimen but 50% of children who presented with lesions were on the ABC, 3TC, Kaletra regimen. However, due to a small set of responses that is unbalanced and responds to mostly single categories, means that we were unable to test for differences with HAART regimen and the presence of oral lesions.

CHAPTER 4: DISCUSSION

It has been well established that oral lesions are often the earliest signs of HIV disease and are clinical markers for immunosuppression, HIV viral load and disease progression (3,12,14). Disease progression is monitored by serum CD4 $^{+}$ T-lymphocyte counts, which is used to assess immune competence; and HIV-RNA levels, which is used to assess viral replication. Therefore they are also used as indicators of immune and virologic failure or success in patients on antiretroviral therapy (58). Therefore, the presence of oral lesions in children on HAART, may present as clinical predictors of HAART failure and disease progression to AIDS.

Studies conducted by Duggal *et al*, reported significant associations between $CD4^{+}$ T-lymphocyte counts, viral loads and oral lesions. They, found that disease progression is most accurately measured by increasing viral loads, however, a lower $CD4^{+}$ T – lymphocyte count has a more significant association with the presence of oral lesions (6). They also found a strong statistical association between the presence of oral lesions and children with low CD 4^{+} T-lymphocyte counts (12). In contrast to this study, a study conducted by Gaitan-Cepeda *et al,* reported a significant association between oral lesions, in particular oral candidiasis and higher viral loads. They concluded that oral lesions may be associated with virologic failure in adolescents on HAART (7). In this study, the association of $CD4^{+}$ T-lymphocyte count and viral load at baseline to the presence of oral lesions was assessed and no significant association was found. Rwenyonyi *et al* (8) and Nabbanja *et al* (21), also found no influence of $CD4^{+}$ T-lymphocyte count on the presence of oral lesions but they experienced the same limitation as we did in our current study and that is that $CD4^{+}$ T-lymphocyte counts were obtained from the medical files retrospectively and could have changed drastically at the time of examination and diagnosis of lesions, therefore these findings need to be interpreted with caution. Our study found a weak, negligible association between $CD4^{+}$ T-lymphocyte count and viral load. This is in agreement with studies conducted by Sturt *et al* and Ramirez –Amador *et al,* who reported that increases in $CD4^{+}$ T-lymphocyte count is independent of the rate of virologic response (14,35).

In our study, 27% of children who presented with either intra-oral or extra-oral lesions, showed a decrease in the latest $CD4^+$ T-lymphocyte count from baseline and a significant improvement in reduction of the viral load with the introduction of HAART. One child presented with a decrease in $CD4^+$ T-lymphocyte count and an increase in viral load. The latter case may be deemed as HAART failure as there had never been a positive response to therapy. The other cases however, can neither be categorized as HAART failure nor IRIS; as in the case of IRIS patients need to show disease at a time of immunologic and virologic success. Signs and symptoms after HAART initiation that are indicative of an IRIS event includes prolonged fever, dyspnoea, abdominal pain, diarrhea, headache, weakness, lymphadenitis, subcutaneous nodules and abnormal skin lesions (59). HIV+ve individuals with or without IRIS, but presenting with the same infection or inflammatory condition, have similar clinical symptoms and laboratory results. Therefore, prior to making a diagnosis of IRIS, the clinician must rule out the possibility of delayed recovery of immune function, inadequate antimicrobial therapy for a pre-existing condition, drug resistance, drug toxicity and superinfection by other pathogens (60).

In a study conducted in Thailand by Puthanakit *et al*, the incidence of IRIS amongst HIV+ve children was 19%. Twenty nine patients experienced thirty two episodes of IRIS. Of these, 43.7% were caused by *Mycobacterium species,* 21, 9% by the varicella zoster virus and 18.7 % were manifestations of herpes simplex labialis. The remainder of the cases comprised of cases of *Cryptococcus neoformans,* and one case of Guillan-Barré syndrome (59). In this study, a nine year old female patient presented with HSV labialis at week 4 (post HAART initiation), herpes dermatomal zoster at week 12 and *Mycobacterium avium complex* (MAC) pulmonary infection at week 26. Her baseline $CD4^+$ T-lymphocyte count was 44 cells/mm^3, the reading, closest to the time of IRIS events, was 214 cells/$mm^{3.}$ The viral load at baseline was 5.57 $\log_{10}$ copies/ml and had decreased to 1.70 $\log_{10}$ copies/ml at the time of IRIS events. Despite, showing immunological and viral recovery, this patient died during the IRIS episode. This study also concluded that patients who developed IRIS had lower baseline $CD4^+$ T-lymphocyte percentages than those who did not develop IRIS (59). This is in agreement with a study conducted by

Tangsinmankong *et al,* who concluded that severe immunodeficiency at baseline and rapid immunologic and virologic response to HAART are risk factors for the development of IRIS. All the children in their study, presented with herpes zoster infection, with significant increases in $CD4^+$ T-lymphocyte numbers and percentages. Six out of seven children at the time of the IRIS event had viral loads < 400 copies/ml(61). On the other hand, a study conducted by Wang *et al*, found that children who developed IRIS, had a higher baseline viral load and tested positive for indicators of malnutrition. They found no association between baseline $CD4^+$ T-lymphocyte counts and IRIS. The IRIS events included mycobacterium tuberculosis, Bacillus Calmette Guerin lymphadenitis, VZV and herpes labialis. They found a prevalence rate of 20% (15), which is similar to the prevalence rate found in the study conducted by Puthanakit *et al* (59).The differences in association of $CD4^+$ T-lymphocyte counts and IRIS may be due to the smaller sample size in the study conducted by Wang *et al* (15).

The cases in our study represent rather, discordant responses to therapy. Both forms of discordant responses that is immunologic or virologic, are predictive of mortality in HIV+ patients on HAART (24). Ineffective immune restoration, that is, patients who present with decreased viral loads but no improvement or decreasing $CD4^+$ T-lymphocyte counts, is associated with progression to AIDS and non-AIDS diseases and malignancies (38). In a study conducted by Lapadula *et al*, the authors explain that a persistent state of immunosuppression increases susceptibility to opportunistic infections. Furthermore, there is a lack of immune surveillance to detect and destroy neoplastic cells, therefore, these patients are also more susceptible to malignancies (38). Those patients who are virologic non-responders are also at risk of increased mortality as sustained virologic suppression is necessary to maximize $CD4^+$ T-lymphocyte count and function (62). In a study conducted by Moore *et al*, immunological non-responders had a relative hazard (RH) of 1,87 and viral non-responders had an RH of 3,48 for mortality. Lack of adherence to therapy was found to be associated with both types of discordant responses (62). Patients with incomplete adherence or those who are on suboptimal regimens are

more likely to have higher rates of mortality as they develop incomplete immunologic and virologic responses (63).This is indicated by the fact that patients who show > 95% adherence to therapy have an RH of 0.56 for mortality (62).In our study, the number of children presenting with lesions is low, however of these children with lesions, those presenting with concomitant discordant responses to therapy is high. This may account for the lack of association found between $CD4^+$ T-lymphocyte count, viral loads and the presence of lesions. Other factors such as the small sample size, the time lag between clinical examination with diagnosis of lesion and the latest $CD4^+$ T-lymphocyte count and viral load readings and duration of HAART; may also play a role in this.

Therefore, the findings of this study must be interpreted with caution.

The findings of this study are in contrast to a study conducted in a similar South African population by Duggal *et al* in 2010. Duggal et al found oral lesions to be associated with a lower $CD4^+$ T –lymphocyte count and high viral loads, reporting a prevalence of 52% of oral lesions in children receiving antiretroviral therapy (12).Comparatively, our study reports a prevalence rate of 9,2% in 2014. It must be noted that the Duggal *et al* study had a significantly smaller sample size than our study (n=56). They reported OC, in particular pseudomembranous candidiasis, as the most frequently encountered oral lesion.

Oral candidiasis has been recognized as having an association with decreased CD 4^+ T-lymphocyte counts and an increased risk of developing AIDS (12).This was also shown in a study conducted by Gaitan-Cepeda *et al,* who reported a 20% prevalence rate of oral lesions in adolescents on HAART and 30,6% prevalence rate in children on HAART. They reported oral candidiasis as the most frequent oral opportunistic infection with a prevalence rate of 21,8% in their study (7). A study conducted by Pomarico *et al* is in agreement with the findings of these previous studies reported that HIV infected children had a higher prevalence of oral lesions; particularly oral candidiasis; than non-infected children. They also report a significant decrease in the prevalence of oral candidiasis in children on HAART (64). Two mechanisms were proposed: HAART results in reduced infection with immune reconstitution and

secondly medications included in the HAART regimen have direct anti-yeast effects. The latter statement relates in particular to protease inhibitors which inhibit candida- secreted aspartic proteinases (SAPs),which interfere with the growth and pathogenicity of the microorganism (64).Most children at the Empilweni clinic are on HAART regimens containing PI's and this may explain why no OC was detected in our study.

Oral candidiasis was also the most prevalent lesion in a study conducted by Kumar *et al*, who reported a prevalence of 61.65% of oral lesions in children on HAART. Oral candidiasis accounted for 20, 86% of lesions, with 16,56% for angular cheilitis, 8,28% for necrotizing ulcerative gingivitis, 7,66% for necrotizing ulcerative periodontitis, 5,53% for linear gingival erythema and 2,76% for aphthous ulcers (9). Rwenyonyi *et al*, found oral lesions in 67,8% of children on HAART but in contrast to the previous study, they found no linear gingival erythema, NUG, KS or atypical oral ulcerations (8).Similarly, we report no cases of KS. As reported in a study conducted by Cox *et al*, KS represents only 0.4% of malignancies related to HIV in children in the United States. This, however, is not true for the prevalence of KS in sub-Saharan Africa where previous reports show a 30% prevalence of HHV-8 in Botswana and 35% in Uganda (65).This indicates that even within sub-Saharan Africa, geographical differences in the prevalence of HHV-8 exists and although previous reports from South Africa have reported a much lower prevalence rate of 9% in comparison to other African countries, it may explain why we report no cases of KS. However it is possible that no cases of KS were detected in our study due to the small number of children presenting with lesions.

Our study reports no cases of OHL, LGE, parotid enlargement and cervical lymphadenopathy but does report cases of MC (Appendix E), scarring as a result of RHI and FEH.

Molluscum Contagiosum (MC) is produced by a member of the DNA poxvirus group and in children it is transmitted by nonsexual contact. It is a common finding in children regardless of their HIV status. This therefore makes it difficult to draw any correlations due to the ability of

the virus to spread among HIV negative populations that live in close proximity to each other (66). Diagnosis of recurrent herpetic infection was made by the visible scarring and history offered by the child's parent/guardian. We reported on those cases that presented with scarring as a result of previous disease as the lesions still occurred whilst the child was on HAART.

FEH is strongly associated with HPV; and although HIV is not the causative agent for FEH, HIV infected patients are more likely to develop oral HPV infections, than non-infected patients (67). This is further supported by the findings of a study conducted by Pinheiro *et al*, who examined 50 HIV+ve children and 50 HIV-ve children between the ages of 3 and 13 years old. HPV DNA was detected in 12% of HIV+ve children and in 6% of uninfected children. They also reported that HPV DNA may present early in HIV infection (68). The primary mode of HPV transmission in adults is sexual intercourse (68). In children, HPV is transmitted directly by skin or mucosal contact; or indirectly from fomites, perinatally from an infected mother or by sexual abuse (69,70).Children born to HIV+ve mothers have a higher exposure to HPV during birth, as HIV+ve mothers have a 2-3 times higher prevalence rate with a higher HPV viral load than HIV-ve women (26). This is supported by studies conducted by Mammas *et al*, that also suggested that maternal HPV viral load is a determinant for HPV transmission to the neonate (70). HIV infected individuals show high risk sexual behaviour and due to multiple partners and exposures, they frequently acquire HPV. Another explanation for the high HPV detection in HIV+ve individuals could be the increased replication of HPV and/or persistence of HPV (68). This statement is further supported by Moscicki *et al*, who reported the rate of persistence of both high and low risk HPV types to be higher amongst HIV infected women than HIV uninfected women(71). Syrjänen *et al*, reported that contamination of a newborn's oral cavity with genital HPV from the mother's cervix appears to occur commonly at birth or during the perinatal period. They also report that detection of HPV in mothers was strongly associated with HIV seropositivity (69).Furthermore, exposure to HPV may continue from the mother/parents during child care (71). Other studies have reported a concordance of HPV types detected in newborn babies and their mothers in the range of 57% to 69%. Therefore, these infants may

acquire HPV postnatally from kissing, siblings and fomites (72).

In a study conducted in a similar Tanzanian population, Hamza *et al* in 2006, found that parotid gland enlargement was the most common lesion (19,6%), followed by OC (11,8%), KS (3,9%), OHL (3,9%), herpes simplex (2,7%) and oral warts (2%) (27). Although the methods of examination in this study were similar to those in our study, their findings are different to our reported results. We report no cases of parotid enlargement, OC, KS, OHL or oral warts (18). Hamza *et al*, also report that they found no significant change in the occurrence of oral lesions in children receiving HAART compared to those not receiving HAART (Odds ratio =0.35). We report a significantly low prevalence of oral lesions in children on HAART (18). This may be due to a few differences in the study. Firstly, in the Tanzanian study, both adults and children were examined. Only 51 children between the ages of 2 and 17 years old were examined, of these 51 children, only 22 were on HAART. We examined 240 patients aged between one month to 14 years of age, all of whom were receiving HAART and therefore had a much larger sample size. Hamza *et al*, were also able to draw a conclusion that there was a significant association between the presence of oral lesions and $CD4^+$ T-lymphocyte count. This is an accurate finding in comparison to our finding of no association of $CD4^+$ T-lymphocyte count to the presence of oral lesions as they were able to carry out haematological tests at the time of clinical examination and lesion diagnosis. Due to a lack of funding at the Empilweni clinic we were unable to do this and had to rely on retrospective records which were at times taken > 6 months prior to examination. Another reason for the differences in findings may be the duration of HAART. In our study, the average patient had been on HAART for 1444,5 days (4 years), whereas in the Hamza *et al* study, majority of patients had only been on HAART for one to six months. In a study conducted by Meless *et al*, the patients had been on HAART for a similar median duration of 4,6 years and also report a much lower prevalence of oral lesions at 8,3% in HIV+ children on HAART in West Africa (22).Similarly to our study they reported that oral lesions in this population still occurred, but rarely. In contrast to our study and the Hamza *et al* study, they reported no cases of herpes simples.

The Human Herpes Simplex Virus I (HSV) is usually associated with labial lesions. One child presented with scarring as a result of previous herpes labialis (Appendix E) and one child presented with active herpes simplex involving the skin in the peri-oral and submental regions as well as an inoculation lesion on the tragus of the right ear. The prevalence of HSV-1 infection is known to increase progressively from childhood and is inversely related to the child's socio-economic background (19,57). However, with involvement of herpes viruses in the oral cavity and surrounding facial skin, it is difficult to ascertain HIV or HAART as the cause, as other contributing factors such as the geographic occurrence of pathogens, climatic conditions, psychological stress and infectious diseases amongst HIV-uninfected peers do play a role.

Our study, however, is in agreement with a study conducted by dos Santos Pinheiro *et al* in 2012, which reported a high prevalence of infection caused by the *Herpesviridae* family in this population but has no association with immune suppression, viral load and HAART (19). Ten out of twenty two cases presented with extra-oral lesions and 80% of these lesions were caused by diseases related to the *Herpesviridae* family of viruses. Four children presented with either active lesions or scarring as a result of VZV (chicken pox) (Appendix E). The Human Herpes Virus 3(HHV 3) causes primary varicella, mainly in children. The distinct characteristic of persistence in latency and stimulus-based reactivation of herpes viruses, is the cause of Herpes Zoster (HZ), and may occur in cases of immunosuppression promoted by HIV infection (19). One child presented with scarring as a result previous of HZ (Appendix E). During the time of primary varicella infection, HAART is protective against developing HZ and is even less prevalent in those with varicella-specific IgG seroprotection (19). HZ has been found to occur in children with IRIS. However, in this study the child presented with virologic suppression with a baseline count of 90 000 copies/ml to a latest count of 24 034 copies/ml but presented with a drop in $CD4^+$ T lymphocyte-count from 1300 cells/mm^3 at baseline to 449 cells/mm^3 at the latest reading. Therefore, it is difficult to classify the child as having IRIS but rather a discordant response to antiretroviral therapy.

In a Nigerian study conducted by Adebola *et al* in 2012, the authors concluded that paediatric patients receiving HAART had a significantly lower prevalence of oral lesions. They also found OC; in particular angular cheilitis; to be the most prevalent lesion (20). This is in contrast to our study, where no OC nor angular cheilitis was detected. This difference in findings may be attributed to the differences in the HAART regimen. The HAART regimen in this study comprised of Stavudine, Lamivudine and Nevirapine only, with exclusion of PI's.
Although HAART regimens at the Empilweni clinic are dictated by protocols, we found a lot of variations in regimen but most of the regimens included a PI. As discussed previously, it has been reported in the literature that PI's exhibit an anti-yeast effect and may account for the absence of fungal infections detected in our study. Adebola *et al*, found an increased prevalence of gingival/periodontal disease and reported that these lesions are more prevalent in Africa and Asia due to poor nutrition and inadequate oral hygiene measures (20). They found that 3, 8% of the population presented with salivary gland disease and 14,3% presented with aphthous ulcerations (20). Our study, reports two cases of oral ulceration, one was diagnosed as a major aphthous ulcer and the other as an ulcer of unknown origin. Aphthous ulcers are common among HIV-infected children and tend to be larger and more painful than in HIV uninfected children. The aetiology of these ulcers is unknown but may be attributed to the adverse effects of antiretroviral drugs or deficiency of vital nutrients (73). We were unable to determine the type of ulcer in the ulcer of unknown origin case as at the time of examination, the ulcer was in the healing stage and no obvious history could be provided by the patient or guardian.
Adebola *et al* also reported two cases of KS and no cases of OHL. In comparison to other studies at that time, they reported a decreased prevalence of oral lesions, in particular OC and HIV-associated gingivitis (20). HIV-associated gingivitis was not reported in our study. The differences in these findings may be attributed to different social and geographical variations in the disease presentations. Furthermore, Adebola et al based their diagnosis of oral manifestations on the diagnostic criteria described by the USA Oral AIDS Collaborative Group (1992), whereas our diagnoses were based on the review of OHARA: updated case definitions

of oral disease endpoints (2009).

In a study conducted in India by Ponnam *et al* in 2012, a prevalence rate of 43% of children on HAART presenting with oral lesions was reported. Lesions included OC, ulcerative stomatitis, dental caries, gingivitis, periodontitis, HAART induced pigmentation and one case of mucocoele (73). This study found six cases of children presenting with hyperpigmentation in the oral cavity and accounts for 50% of the intra-oral lesions detected. The clinical appearance of oral hyperpigmentation is single or diffuse multifocal brown/black melanotic macules that appears in the mucosa, particularly buccal mucosa (74). This manifestation is difficult to diagnose and other causes such as racial pigmentation need to be excluded on the basis of a recent onset as described by the patient or a care-giver and the site of occurrence (74). It is also important to take a thorough history to exclude other clinical causes of pigmentation such as smokers melanosis, Albright's disease and neurofibromatosis (75).

Baghirath *et al* , found oral hyperpigmentation to be the second most prevalent oral manifestation, after oral candidiasis, amongst paediatric patients on ART. In this study, they found prevalences of 5,6% of children on short term ART and 12,5% of children on long term ART. No hyperpigmentation was observed in HIV+ve children who were not on ART but presented with other lesions and no hyperpigmentation was detected in HIV-ve children (76). Taiwo *et al*, conducted a study on the impact of HAART on oral lesions in adult patients and also found oral hyperpigmentation to be the second most common lesion following oral candidiasis, with a prevalence of 18,3% (77). Oral hyperpigmentation constituted 50% of intra-oral lesions or manifestations detected in our study. This pigmentation is thought to be drug induced, particularly in patients on regimens containing Zidovudine (27,31). However, studies conducted by Sontakke *et al*, found oral hyperpigmentation in HIV +ve individuals, who were not on any medications suggesting that medications that form part of ART/HAART regimens are not the only causative factor (78).

Oral melanin hyperpigmentation may be caused by antiretroviral medications such as Zidovudine or antifungals such as Ketoconazole, but it may also be caused by HIV-induced cytokine dysregulation, adrenocortical deficiency, which is common in HIV+ve individuals or it may be idiopathic (6, 18, 75).

Feller *et al*, also explained that HIV-induced upregulation of IL-1, IL-6 and TNF-α promotes the production of α-melanocyte-stimulating hormone (α-MSH) by oral melanocytes with an increased production of melanin resulting in HIV-induced oral melanosis. Cytokine dysregulation may follow path with decreasing $CD4^+$ T-lymphocyte counts and is frequently observed in patients with $CD4^+$ T-lymphocyte counts < 200 cells/mm^3. This is supported by studies conducted in ART-naïve patients by Namakoola *et al*, who also found that oral hyperpigmentation was significantly associated with $CD4^+$ T-lymphocyte counts < 200 cells/mm^3 (79). However, other studies conducted by Umadevi *et al*, report that increased oral hyperpigmentation occurred in patients with $CD4^+$ T-lymphocyte counts > 200 cells/mm^3 in both HAART (43,8%) and non-HAART (14,8%) groups of adults, suggesting, as in prior studies, that it may be due to Zidovudine therapy (74).Another proposed etiology is that mucosal inflammation can promote melanin pigmentation. There are more immune-inflammatory cells in subepithelial connective tissue of HIV+ve individuals, even in clinically healthy mucosa, and these inflammatory or post-inflammatory states may contribute to hyperpigmentation of the oral mucosa (75). We attribute the varying prevalences and findings from our study and other studies, to the fact that the occurrence of this manifestation may vary according to different geographical locations (79), race – pigmentation is more commonly seen in dark-skinned people (75) and that it is not only drug-induced but may be due to other factors (75).

Oral hyperpigmentation is not included in the case definitions of oral disease endpoints by the OHARA group. At the 7^{th} World Workshop on Oral Disease in HIV/AIDS in Hyderabad, 2014; oral hyperpigmentation was a controversial topic. Some clinicians/ researchers are of the belief

that hyperpigmentation is merely a side effect of the drug and should not be described as an oral manifestation of HIV/AIDS. However, half of the children in our study who presented with an intra-oral lesion presented with hyperpigmentation and the authors of this report deem it necessary to report on this finding. Side effects or adverse drug reactions that alter a patient's appearance or aesthetic appeal, are not only cosmetically and psychologically challenging, but may also decrease adherence to therapy. This in turn can cause potential drug resistance and treatment failure(63). Sharma *et al*, report 71,1% of adverse drug reactions in their study with nail hyperpigmentation being the most common adverse reaction detected (80). Studies conducted by Borrás-Blasco *et al,* concur with this and report that 80% of HIV+ individuals experience adverse drug reactions such as rashes, hyperpigmentation, hair loss and hypersensitivity reactions at some point in therapy. They report that adverse drug reactions rather than treatment failure causes patients to discontinue HAART within the first year of treatment (81).

Although mucocutaneous hyperpigmentation is not life threatening, it has a negative effect on the quality of life of HIV+ve individuals, which can lead to compromised adherence to drugs, suboptimal drug levels, which causes incomplete viral suppression and decreased $CD4^+$ T-lymphocyte counts, and the development of drug resistance. All of these factors lead to failure of ART regimens and compromises future therapy for these patients (63).This is particularly important in the population we have studied. The way in which a patient views the severity of this side effect/ manifestation is based on different characteristics, one of which is age. Many of the patients in our study were adolescents or children soon to approach adolescence, a time when appearance becomes of increasing importance to the individual. Therefore, to dismiss findings of oral hyperpigmentation in our study would be an underestimation of its potential harmful effects not only on the quality of life of the patient but also on treatment.

Furthermore, one child presented with a hypersensitivity reaction to Bactrim. This reaction is due to the drug and is unrelated to the HIV or immune status of the patient. However, HIV-infected

patients are predisposed to developing drug-induced eruptions due to their altered immune function and defective metabolism of drugs (82).

This study found no association with age at diagnosis of HIV but a significant statistical association with current age and the presence of lesions. Furthermore, an assessment of the correlation between presence of oral lesions and duration of HAART revealed that the average number of days on HAART was 1444,5 days (4 years), however, no significant association could be made between the length of time on HAART and the presence of oral lesions. The mean age of children presenting with lesions was 10,7 years. Only children under the age of 14 were assessed in the study as this allows us to compare our findings to other studies in a similar age group. Up to the age of 14, no difference in HIV prevalence by gender is observed suggesting vertical transmission. Children older than the age of 14, show a higher prevalence of HIV in females compared to males, reflecting the increased risk of infection in females via horizontal, sexual transmission (83). However, no route of transmission can be assumed as non-vertical (horizontal) routes of transmission by blood transfusion, surgery, exposure to sexual abuse and the use of unsterilized sharp objects for example during circumcision, do occur (84). In our study, there was no evidence in the patient's files of a history of sexual abuse, sexual activity and blood transfusions; which leaves vertical transmission as the most likely route of transmission. Mother to child transmission (MTCT), may occur in utero with a transmission rate of 5-10%; intrapartum at 10-20% or through breastfeeding at 5-15%. In the absence of antiretroviral prophylaxis, majority of HIV transmission occurs at the time of delivery (10).

There is an age-dependent difference in the progression of HIV disease between the adult and paediatric population (5, 7, 8, 910, 11). This is because perinatally acquired infection occurs during an important time of immune development and disrupts this dynamic process. The infant is reliant on the maternal immune system for protection and immune guidance as transplacental antibodies and breastmilk contain immunomodulatory molecules that stimulate growth and guide the child's immune development. These early maternal influences have lifelong effects on

immune response but in the case of HIV infection, is perturbed (10). Disease progression in infants, due to the immature immune system, is faster due to a shorter incubation period. Without antiretroviral therapy there is a 50% probability of death by the age of 2 years (3,83,85,). Regardless of $CD4^+$ T- lymphocyte count or plasma HIV-RNA levels, younger children are at a greater risk of death and disease progression than older children (35). Many studies have reported that oral lesions are predictors of disease progression. Gaitan-Cepeda *et al*, report that oral lesions, in particular oral candidiasis, have a significant association to viral failure in HIV+ children and adolescents on HAART (7).In contrast to this, Hamza et al, found the presence of oral lesions in patients receiving HAART and those not receiving HAART, correlates with of $CD4^+$ T- lymphocyte count < 200 cells/mm^3 (18). Miziara *et al*, reported that oral lesions had moderate sensitivity, high specificity and positive predictive value to predict immune failure. It also has low sensitivity, high specificity and positive predictive value to predict virologic failure. They concluded that oral manifestations of HIV are important markers of immune and virologic failure in children on HAART (58).However, Patton *et al*, argue that the usefulness of oral lesions as signs of disease progression or ART failure in compounded by IRIS, adverse reactions to medications that are part of the HAART regimen and the direct effect of some medications on oral lesions e.g. the anti-candidal effect exhibited by PI's (86).Although lesions such as OC, KS and OHL, are indicators of a failing immune system, identification of oral lesions should not replace repeated $CD4^+$ T- lymphocyte count and regular viral load assessments to monitor treatment (86). Oral lesions were detected in older children in this study. This may be due to the evolution of prevention of mother to child (PMTC) strategies over the past ten years (26,87).

Widespread implementation of highly effective HIV interventions began in the 1990's in developed countries. However, antiretroviral prophylaxis for prevention of mother to child (PMTC) HIV transmission only became available in Africa around 2004.South Africa began a free ART national roll out in the same year (88). Thereafter, protocols were developed for improved implementation of PMTC strategies and paediatric antiretroviral drugs became more

widely available (83). In South Africa, by 2011, PMTC HIV programs had reduced perinatal transmission to 2,7% (83). Patients who failed to benefit from PMTC programs but had early maternal and infant diagnosis are at an advantage because effective early antiretroviral therapy is associated with early virologic suppression which is associated with normalization of B and T cell numbers and function (10). Also, increased thymic function in HIV-infected children enhances their immune recovery and they are able to fight infections better than those who are diagnosed later in life with delayed initiation of HAART (10). Furthermore, latent viral reservoirs are established early in infection but early initiation of therapy minimizes the extent of these viral reservoirs. HIV+ paediatric patients who initiate HAART within the first few months of life have lower levels of proviral HIV DNA and lower levels of replication-competent virus in comparison to children who received treatment later in life (10). This may be significant to this study as it may account for the prevalence of lesions in children around the age of 10, who at the time of initial infection may not have benefitted from early initiation of HAART, whereas the younger children in the study did benefit from early maternal diagnosis due to PMTC programs and early implementation of HAART. However, due to the small number of patients presenting with lesions with no evidence of association to CD4+ T-lymphocyte count and viral loads, this deduction should be interpreted with caution.

South African PMTC guidelines were aligned with WHO guidelines of PMTC in 2010 and the program is reported to have 98.9% coverage for antenatal HIV testing, 78,3% $CD4^+$ T-lymphocyte count testing, 91,8% antiretroviral prophylaxis to mother and/or baby and 89% feeding advice (17). Despite great improvements in PMTCT in South Africa, the prevalence of HIV amongst pregnant woman is still high. This may be due to a few factors; the first is that most women present for their first antenatal visit after 14-16 weeks gestation. However, 12-16 weeks of maternal antiretroviral therapy is required, depending on the viral load, to achieve viral suppression at the time of delivery. This results in suboptimal prevention of transmission (17). Furthermore, HIV may be acquired during the pregnancy and postpartum, which will not be detected unless repeat testing is done. Lack of retesting misses the opportunity of maternal

diagnosis and has increased risk of mother to child transmission (MTCT) because of high HIV viral loads during primary infection (89). In South Africa, 4% of women who test HIV – ve initially, become HIV+ve later in pregnancy. Therefore re-testing is essential to detect any seroconversion during pregnancy and breastfeeding (90).Social issues such as perceived poor quality of services at clinics, financial constraints and distance from local clinics are all contributing factors to women not seeking antenatal care (11). A study conducted in Malawi by Braun *et al*, suggests that PMTCT, early infant diagnosis and paediatric ART are not sufficiently integrated. The loss of follow up or delay in ART initiation may be due to the fact that HIV-infected mothers receive ANC and maternal care at one location but have to attend a different location for paediatric care. The cost of transport impedes infant HIV test follow up (91). Silal *et al*, report that in South Africa, over 90% of women utilize maternal health services. However, barriers to access of obstetric care remain. Besides the location of facility and costs of treatment, the attitudes of care-givers plays a large role in determining the influx of women seeking care. Health care for pregnant women and infants is free, which is a positive for women; however the increased workload linked to free services impacts negatively on the staff morale. This results in poor staff engagement and contributes to women not wanting to return to a facility for delivery or future treatment (92). Late initiation of HAART in children may also be due to maternal factors such as feelings of guilt, denial and "lack of readiness" (17). As reported by Technau *et al* in 2014, 91.8% of infected mothers and their children received PMTC, however, in 52% of this population, prophylaxis was started late, not started according to the guidelines or there was poor adherence to therapy (17). Similarly, this study found discrepancies between age of diagnosis of HIV and initiation of HAART. Age of diagnosis in this context may not be of significance due to the psychological and socio-economic stigma related to HIV as explained above. Furthermore, Hsiao *et al*, also report on a delay between the first HIV polymerase chain reaction (PCR) and the first viral load testing date (done as part of the ART work-up). They reported a median delay of 146 in 2005, 81 days in 2007, 39 days in 2008 and 33 days in 2010 (26).

The authors attribute this delay to a few reasons. Firstly, PCR testing is expensive, time

consuming and requires specialized laboratory equipment. Secondly, issues of conducting HIV testing in primary health care centres, transporting specimens to these laboratories and the return of results back to the centre, at which stage may require referral of the infant to another centre also causes delay.

Thirdly, in this study, only 57% of mothers returned to the facility to receive the results (26). A report by Creek *et a*l, also states that in resource-limited settings, difficulty in returning results quickly to a health centre may cause results to go unclaimed (93).The National consolidated guidelines for PMTCT of HIV and the management of HIV in children, adolescents and adults, which was updated in December 2014, also recognizes linkage of HIV+ individuals after testing positive to continuous care as a problem. The guidelines explain two main time periods when patients are lost: 1) Between testing HIV+ve and the first CD 4^+ T-lymphocyte count test and 2) Between the first CD 4^+ T-lymphocyte count test and the return visit to obtain results (90).They attribute this loss of patients to barriers including having to travel long distances to the clinic, long waiting times at the clinic, clinic staff shortages, inability to take time off work, lack of full understanding of the treatment plan ,and fear of stigma and discrimination (90).Hsiao *et al*, explain the non- return of mothers to receive results to be partly due to the migratory patterns of women during pregnancy and postpartum. Women, in South Africa, are known to migrate to urban centres for ANC and maternal care but return to rural areas after giving birth (26).

Therefore timely diagnosis of HIV in children does not necessarily incur timely linkage to treatment and the implementation of HAART. We would expect children who are diagnosed early to receive treatment early and thus experience less mortality including oral lesions. However, in our study we did not find an association between age at diagnosis of HIV and the presence of oral lesions, as HAART; which is responsible for reducing mortality, is not necessarily implemented early, even in cases of early HIV diagnosis. This observation, whilst true for some, may not be true for all subjects. This may however, explain the failure to find an

association between age at diagnosis of HIV and presence of oral lesions in our study.

Our study has several limitations. Firstly, none of the patients who were referred for biopsy presented for biopsies and so diagnoses of lesions were based on clinical appearance and history offered by the child's guardian/parent. At times, the guardians/parents could not give exact timings as to when the lesions occurred but confirmed that children were on antiretroviral treatment when these lesions occurred. This means that the lesions seen could not be attributed to immunologic or viral response to HAART. Furthermore, lesions like 'chicken pox' and MC occur in uninfected children of the same age and may not necessarily be attributed to HIV or HAART. Data available for the latest $CD4^+$ T-lymphocyte count or viral load was at times over 6 months old and did not reflect the child's immunologic and virologic status at the time of the lesion as the clinic has limited funding available for repeat laboratory testing. This made it difficult to identify possible cases of IRIS. Furthermore, correlation analyses was difficult and not possible due to the low prevalence or low sample size (n=22) of children presenting with intra/extra- oral lesions. With these limitations, the results of this study should be interpreted with caution.

CHAPTER 5: CONCLUSION

This study, in comparison to other African and International studies, reported a relatively low prevalence of 9,2% of oral lesions in paediatric patients on HAART. This might highlight the success of HAART administration in HIV+ children. Oral hyperpigmentation accounted for 50% of the intra-oral lesions detected. Other intra-oral lesions found included focal epithelial hyperplasia (25%), recurrent herpetic infections (8,3%) , major aphthous ulcer (8,3%) and ulcer of unknown origin (8,3%). Extra-oral lesions were also detected and 80% of these lesions were caused by the *Herpesviridae* family of viruses including herpes simplex, herpes varicella zoster, and herpes zoster. The study also reports no significant association between $CD4^+$ T- lymphocyte count, viral load, HAART regimen or duration of HAART and the presence of oral lesions. Due to the small number of lesions and many HAART regimens, statistical associations could not be made. No significant association was detected between age at diagnosis of HAART and the presence of oral lesions; however, a significant association was detected between current age and the presence of lesions. In this study, the age of diagnosis often did not match the time of HAART initiation and this may be related to maternal influences which include the fear of discrimination and stigmatization; and feelings of guilt or denial. The significant association between current age and the presence of oral lesions could be an indicator of the progress made in PMTCT strategies, early maternal and infant diagnosis and early implementation of HAART over the last ten years in South Africa. However, further research should be conducted on discordant responses to therapy in the paediatric population. We also recommend oral examinations to be conducted regularly by clinicians with improved record keeping.

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APPENDIX A

R14/49 Dr Avni Bhula

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M131045

NAME: (Principal Investigator)	Dr Avni Bhula
DEPARTMENT:	Oral Medicine and Periodontology Rahima Moosa Mother & Child Hospital Empilweni Clinic
PROJECT TITLE:	Oral Lesions in Paediatric Patients on Highly Active Antiretroviral Therapy
DATE CONSIDERED:	25/10/2013
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof S Shangase/Prof N Wood
APPROVED BY:	Professor Charles Feldman, Co-Chairperson, HREC (Medical)
DATE OF APPROVAL:	17/12/2013

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

DECLARATION OF INVESTIGATORS

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

I/~~we~~ fully understand the conditions under which I am/~~we~~ are authorized to carry out the above-mentioned research and I/~~we~~ undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/~~we~~ undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**.

Principal Investigator Signature

Date 14/01/2014

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

APPENDIX B

Information Sheet in English

Hello,

My name is Dr A Bhula from the Department of Oral Medicine and Periodontology at the University of the Witwatersrand. You are receiving this information sheet as a parent/ legal guardian to a child that is HIV positive. We are doing a study to find out what types of lesions occur in children on HAART. As part of the study we will also record the viral load and the CD4 count. Oral lesions associated with HIV infection are common and can be caused by many factors e.g. bacterial, viral and fungal infections and can affect various parts of the mouth for example, the tongue, the gums, the palate or the lips. They can also present as lumps or ulcers. However, these lesions are less commonly seen in patients on ARV's (HAART), and if they are seen in patients on HAART it should raise concerns. The results of this study could enable the medical workers to pick up these problems earlier, and might form a helpful diagnostic aid in resource poor areas where facilities are lacking.

Being in this study will not negatively affect the child in any way. The child's name will not be recorded on the data sheet, and the information provided by you and that taken from the child's file will remain strictly confidential. Therefore no one will be made aware of your child's HIV status or be able to tie him / her to the completed data sheet.

If you do agree to allow your child to participate in the study, the oral examination will be done first thing on the morning of the routine clinic visit.The oral examination will include taking photographs of the lesions detected in the child's mouth without revealing his/her face and identity. This will ensure that the child does not lose his/her place in the queue and his/her treatment from the HIV clinic is not negatively affected in anyway. You will also have to sign the informed consent form, answer a few questions asked by myself and allow me to access information in the child's file. You will also have to allow me (Avni Bhula) to examine his/her mouth, which will take about 5min.

If the child presents with HIV-associated oral lesions we may have to draw blood to assess for CD4+ T cell counts and viral loads, should these not be on file or too old. No more than 5ml of

blood will be drawn, as is done with regular CD4+ T cell counts and viral load testing. 5ml is equal to one teaspoon of blood. There is little to no risk associated with drawing blood, but there is a chance that the area where we draw blood from may be tender afterwards. The child will then be referred to the Oral medicine clinic at Wits to have the oral lesions managed. If required, a small piece of the lesion will be taken and sent to the lab for testing (biopsy) to aid the personnel at the clinic in the diagnosis. The biopsy will be performed by the doctors at the Wits Oral medicine clinic, and there may be some discomfort and mild swelling after the procedure, but painkillers will be provided. If tooth decay or other lesions affecting the skin are detected, the child will be referred to the dentist or to the Dermatology clinic respectively for treatment.

The oral examination will be of great benefit to the child as it will allow us to detect and refer for treatmentof oral lesions if we find any, thus improving comfort and function. Participation in the study is not compulsory. If you do not wish your child to participate in this study, the treatment he / she is receiving from the HIV clinic will not be affected at all, and an oral examination can still be conducted if you wish. Should you have any questions regarding the study you can contact me (Dr Avni Bhula) at 0823144299 or by email to bhula_a@yahoo.com. If you are unhappy about the way your child has been treated at any point during this study you can contact the chairperson of the Human Ethics Research Committee, Professor P Cleaton-Jones at 011 7172301.

Thank you for your assistance

Dr A Bhula

Informed Consent Form

I have been informed about the study being conducted and agree to have my child participate in this study.

Name: _____

Signature: _____

Date: _____

Information sheet in Zulu - Iphephalesaziso

Sawubona

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Uma udingaukwazikancononomaimininingwanemayelananocwaningo, ungesabiukufonaubuzekuDokotela A. Bhula – 0823144299, nomai-email bhula_a@yahoo.com. Uma udingaukusitshelaukungakuphathangakahlekulolucwaningoungafonelaiREC

administratorusihlalouNjingalwazi P. Cleaton-Jones 011 717 2635.

Ngiyabongangosizolwenu

Dokotela A. Bhula

Imvume

Ngichazelwengocwaningoolwenziwayo, futhingiyavumaukubaingxenyeyalolucwaningo.

Igama: _____

Ukusayina:_____

Usuku:_____

APPENDIX C

Minor Assent Form

Hello,

My name is Avni Bhula, I am a dentist. Dentists take care of teeth and any other problems in the mouth. You are taking medication to help you feel good and we are studying how well these medications are working and if they affect your mouth.

We would like to look into your mouth and check if there are any sores or ulcers. This will take 5 minutes and we will be using a light and a wooden spatula. There are no injections or anything else that will make you sore. Your parents or the adult you are here with will be with you during the time I look into your mouth. We will also be explaining to them and asking them for permission.

Your age and results from your blood tests will be taken from your file but we will not take your name. This means nobody will be able to know it was you we have examined. If you do have a sore in your mouth, we will be asking permission to take a photograph.

If we do find something wrong in your mouth, we will send you to the doctors and dentists who will be able to help with this so that you can eat and speak comfortably again. This will also help your medical doctor know if the medications you are taking is helping you. If your doctor feels the medication is not helping then he/she may take a blood test, as you have had before.

You do not have to take part in the study if you do not want to. It is not compulsory and will not affect your treatment that you have been receiving from the clinic.

Yours Sincerely,

Dr. A. Bhula

Do you allow me to check your mouth?

Please tick:

YES	NO

Initials or name: _____

APPENDIX D

DATA COLLECTION SHEET

Candidate study number: **Date:**

- Gender : Male Female

- Current Age (months, years) : _____
  Age at diagnosis of HIV (months,years) : _____

- $CD4^+$ ($cells/mm^3$) : Baseline reading _____
  Date of baseline reading _____

  Latest reading _____
  Date of latest reading_____

- Viral load (RNH-HIV-1 per ml) : Baseline reading _____
  Date of baseline reading _____

  Latest reading _____
  Date of latest reading _____

- Duration of treatment with HAART (months) _____

- HAART regimen: Drugs and dosages used _____
  _____
  _____

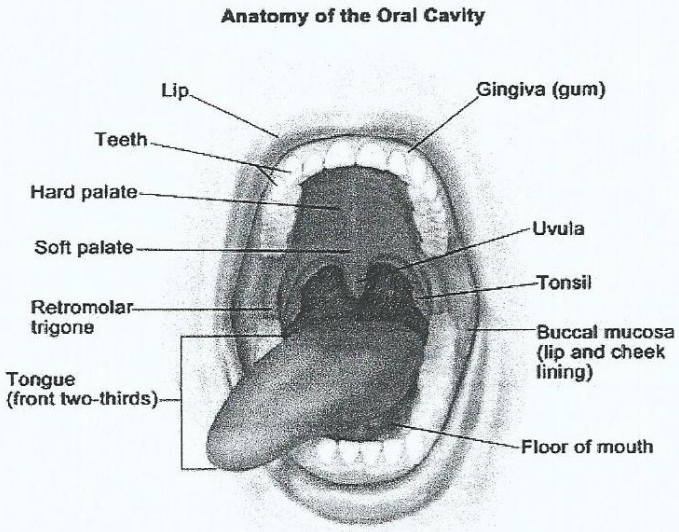
- Presence of intra-oral disease : Yes No
  If yes, see attached sheet.

- Presence of extra-oral disease: Yes No
  If yes,see attached sheet.

Presence of intra-oral disease:

Clinical description (size, colour, texture):

Indicate site of lesion:



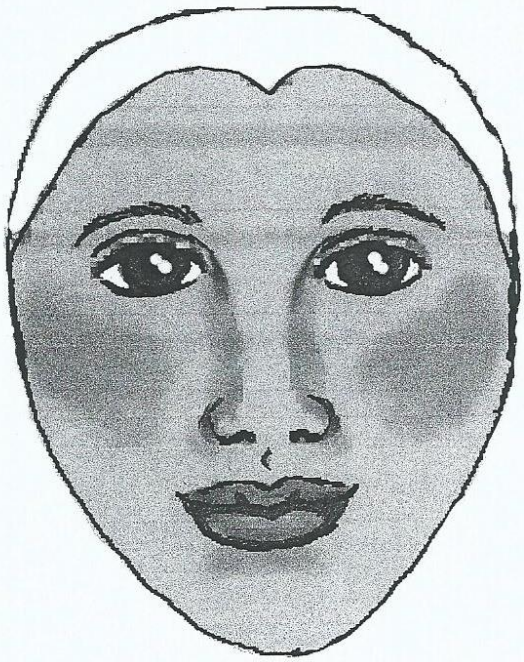
Other notes:

Clinical diagnosis:

Presence of extra-oral disease:

Clinical description (size, colour, texture):

Indicate site of lesion:

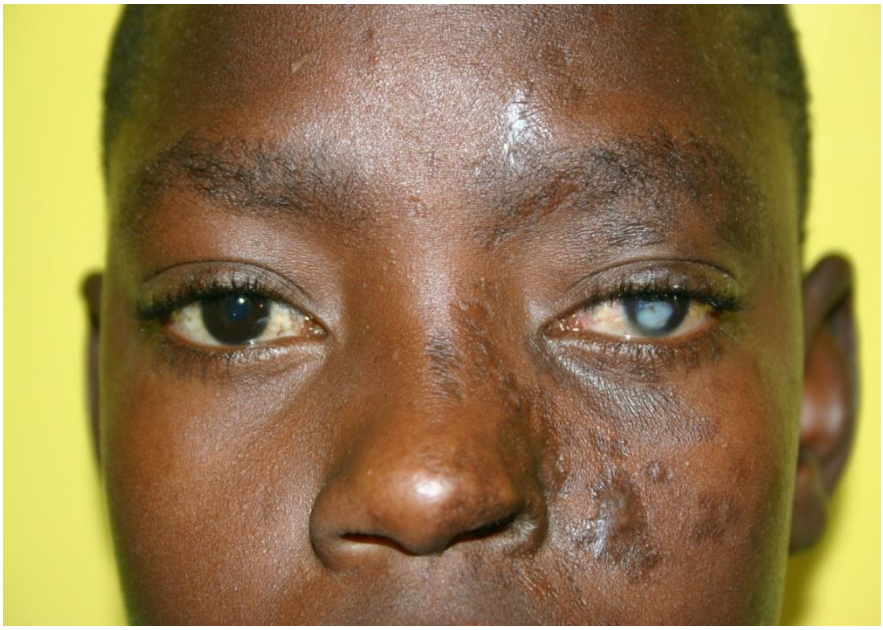


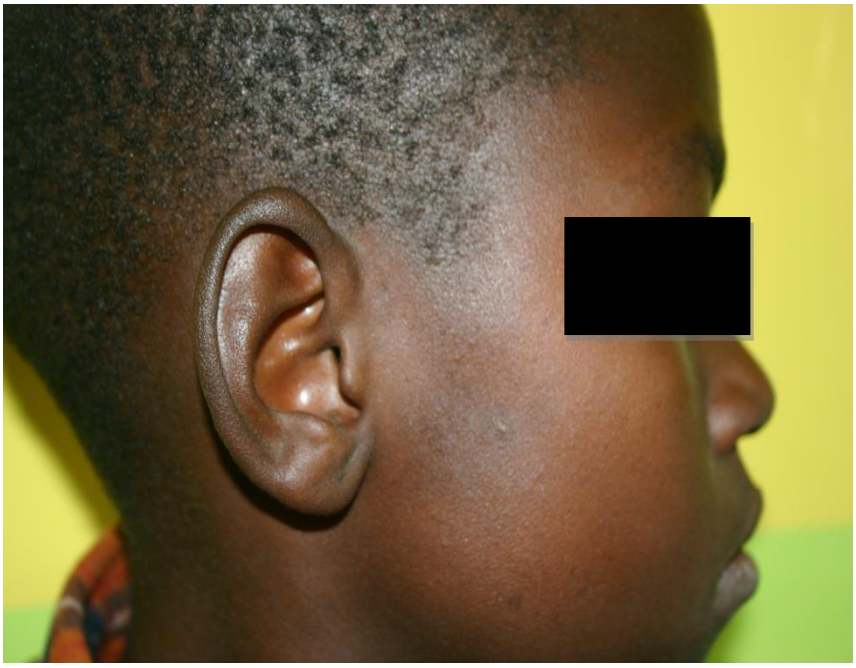
Other notes:

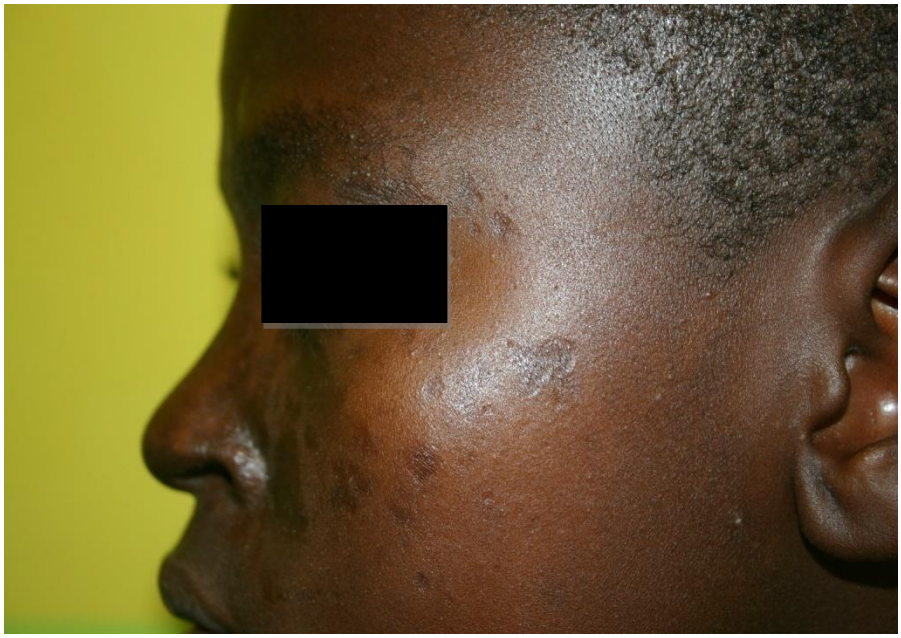
Clinical diagnosis:

APPENDIX E

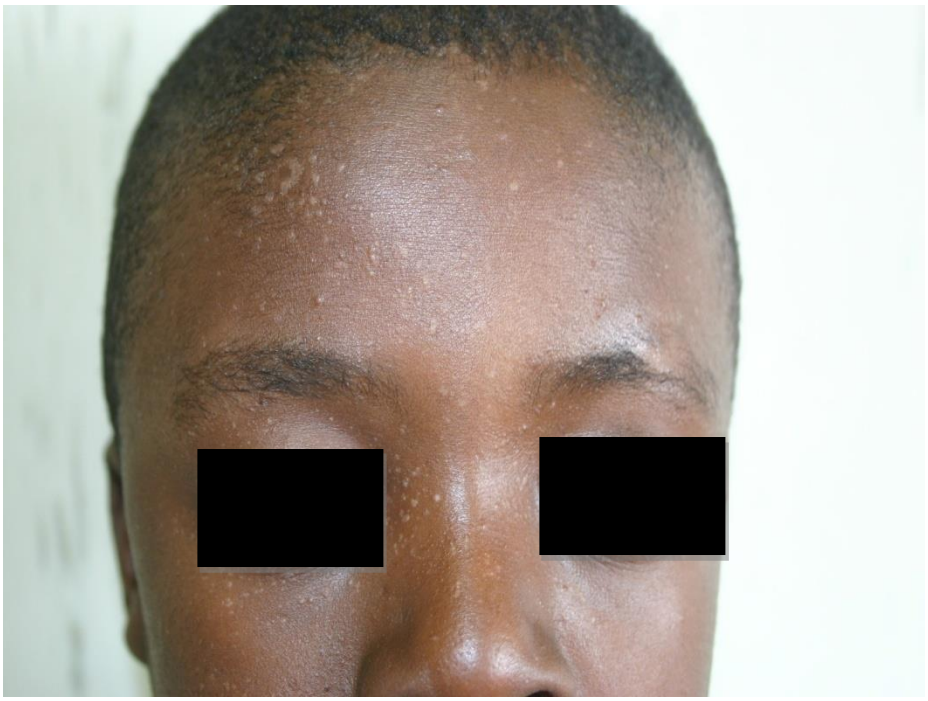
Photographs of child presenting with scarring as a result of Herpes Zoster with ophthalmic involvement of left eye:

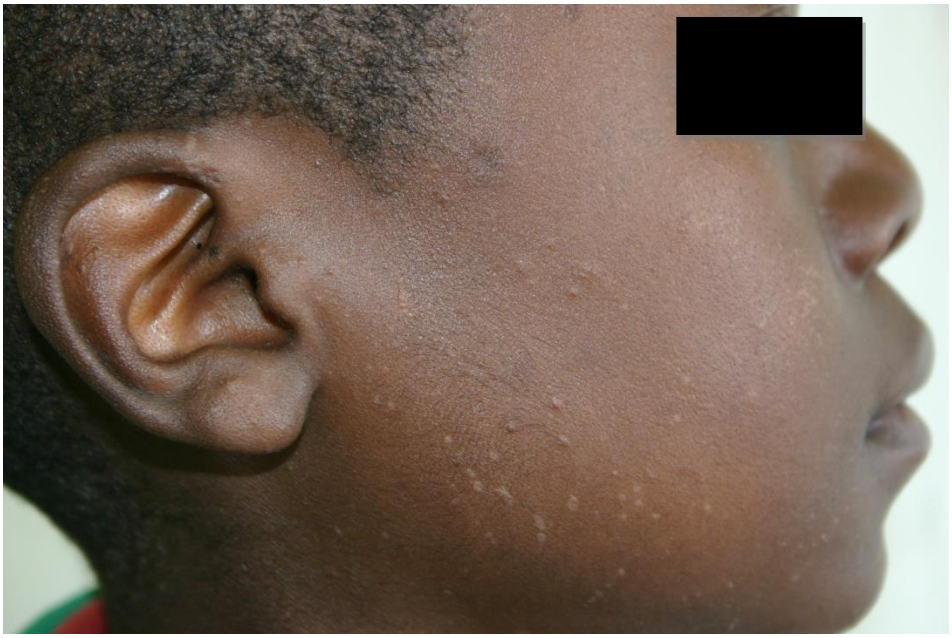


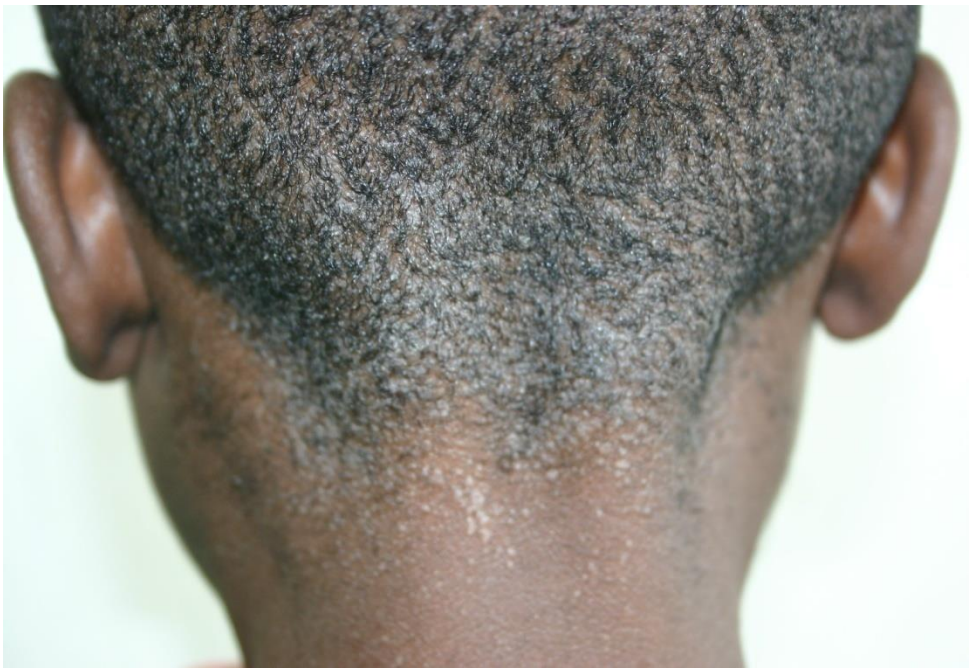




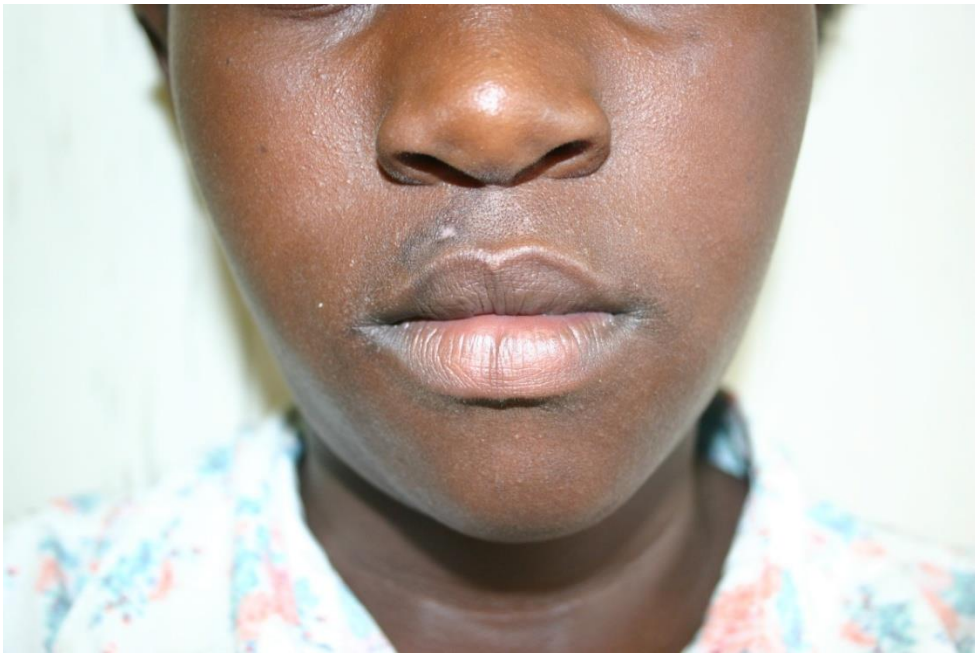
Photographs of child presenting with molluscumcontagiosum:







Photograph of child presenting with scarring as a result of herpes labialis:



Photograph of child presenting with active Herpes Varicella Zoster (healing stage):