

**OCCURRENCE OF ORAL LESIONS ASSOCIATED WITH HIV/AIDS IN PATIENTS
RECEIVING HAART AT THE COMPREHENSIVE CARE CLINIC,
THIKA DISTRICT HOSPITAL, KENYA**

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**Occurrence of oral lesions associated with HIV/AIDS in patients receiving HAART at
the comprehensive care clinic, Thika District Hospital, Kenya**

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**A Thesis Submitted in Partial Fulfilment for the Degree of Master of Science in Public
Health in the Jomo Kenyatta University of Agriculture and Technology**

2010

DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

Signature

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This thesis has been submitted for examination with our approval as university supervisors.

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DEDICATION

To my wife Mercy, my daughter Fiona, my son Kelvin and my parents Peter and Beth for their support, encouragement and understanding during the involving process of preparation of this thesis.

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TABLE OF CONTENTS

DECLARATION	iii
DEDICATION	iv
ACKNOWLEDGEMENTS	v
TABLE OF CONTENTS	vi
LIST OF TABLES	ix
LIST OF FIGURES	x
LIST OF PLATES	xi
LIST OF APPENDICES.....	xii
LIST OF ABBREVIATIONS AND ACRONYMS	xiii
ABSTRACT	xv
CHAPTER ONE: INTRODUCTION	1
1.1 Background	1
1.2 Study Justification	3
1.3 Objectives	4
1.3.1 General Objective	4
1.3.2 Specific Objectives	4
CHAPTER TWO: LITERATURE REVIEW	5
2.1 Overview of Oral Lesions Associated with HIV Infection.	5
2.2 Epidemiology of Candidiasis	8
2.3 Pathogenesis of oral candidiasis	8
2.4 Clinical manifestations of oral candidiasis	9

2.5 Diagnosis of oral candidiasis	11
2.6 Correlation of OPC with HIV infection and its progression	12
2.7 HAART and its impact on OPC.....	12
2.8 HAART Regimens	14
CHAPTER THREE: MATERIALS AND METHODS.....	16
3.1 Study design.....	16
3.2 Study Site.....	16
3.3 Study Population	16
3.3.1 Sample Size Determination	16
3.3.2 Sampling procedure.....	17
3.3.3 Inclusion criteria.....	17
3.3.4 Exclusion criteria.....	18
3.4 Data Collection	18
3.4.1 Administering the Questionnaire.....	18
3.4.2 Clinical Examination of the oral cavity.....	19
3.4.3 Microscopic examination of specimens for <i>Candida</i> pseudo-hyphae.....	19
3.5 Data Management	20
3.5.1 Data storage.....	20
3.5.2 Data analysis.....	20
3.6 Ethical Considerations.....	20
3.7 Study Limitations	21

CHAPTER FOUR: RESULTS	22
4.1 Socio-demographic Characteristics	22
4.2 Clinical characteristics.....	25
4.3 HAART Regimens	26
4.4 Characteristics of HAART usage.....	26
4.5 Prophylaxis and treatment against opportunistic infections	27
4.6 Occurrence and distribution of the oral lesions associated with HIV disease.	29
4.7 Occurrence of oral lesions of HIV/AIDS and immune cell changes	30
4.8 Occurrence of oral lesions and duration of HAART.....	32
CHAPTER FIVE: DISCUSSION	37
CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS	47
6.1 Conclusions.....	47
6.2 Recommendations	47
REFERENCES	49
APPENDICES	59

LIST OF TABLES

Table 1: Prevalence of ‘oral lesions strongly associated with HIV’ in the HAART era; comparison of published studies	7
Table 2: Socio-demographic and clinical profile of the study subjects	23
Table 3: One way ANOVA for mean ages, CD4+ count and duration on HAART between sexes	25
Table 4: Distribution of patients by HAART combination	26
Table 5: Distribution of patients on HAART according to duration on HAART and concurrent medications	29
Table 6: Distribution of individuals with oral lesions by CD4+ count	32
Table 7: Distribution of individuals with oral lesions by duration of HAART	34
Table 8: Odds ratios for occurrence of OPC in subjects over 24 weeks on HAART	35

LIST OF FIGURES

Figure 1:	Distribution of subjects by gender and age groups	24
Figure 2:	Distribution of concurrent medications used by subjects	28
Figure 3:	Distribution of oral lesions by CD4+ count range	31

LIST OF PLATES

Plate 1:	Kaposi's sarcoma on the soft palate in one of the study subjects.....	30
Plate 2:	Pseudo-membranous candidiasis on the dorsum of the tongue in one of the study subjects.....	30

LIST OF APPENDICES

Appendix 1:	Oral manifestations of HIV disease in adults	59
Appendix 2:	List of Some Anti-retroviral Drugs.....	60
Appendix 3:	Consent Information and Consent Form	61
Appendix 4:	Patient Questionnaire	66
Appendix 5:	Comprehensive Care Clinic Patient Card.....	69
Appendix 6:	Adult Data Capture Sheet for Oral Lesions	71
Appendix 7:	Gram Staining Procedure	72
Appendix 8:	KEMRI/National Ethical Review Committee Study Approval.....	73

LIST OF ABBREVIATIONS AND ACRONYMS

AC	Angular Cheilitis
AIDS	Acquired Immunodeficiency Syndrome
ANOVA	Analysis of Variance
ART	Antiretroviral Therapy
CCC	Comprehensive Care Clinic
CD4+	Human T helper Cells Expressing CD4 Antigen (T helper cell)
CDC	Centres for Disease Control and Prevention
CI	Confidence Interval
EEC	European Economic Commission
EC	Erythematous Candidiasis
ELISA	Enzyme-Linked Immunosorbent Assay
HAART	Highly Active Anti-retroviral Therapy
HIV	Human Immunodeficiency Virus
HIV+	Human Immunodeficiency Virus Positive
IRIS	Immune Reconstitution Inflammatory Syndrome
ITROMID	Institute of Tropical Medicine and Infectious Diseases
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KEMRI	Kenya Medical Research Institute
KS	Kaposi's sarcoma
LGE	Linear Gingival Erythema
NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitor

NRTI	Nucleoside Reverse Transcriptase Inhibitor
OHL	Oral Hairy Leukoplakia
OI	Opportunistic Infection
OPC	Oro-pharyngeal Candidiasis
OR	Odds ratio
PI	Protease Inhibitor
RNA	Ribonucleic Acid
STI	Sexually Transmitted Illness
SPSS	Statistical Package for Social Sciences
TB	Tuberculosis
US	United States of America
WHO	World Health Organization

ABSTRACT

The prevalence of some easily detectable oral manifestations of HIV/AIDS decreases with HAART. Their presence may therefore be used as an indicator of the effectiveness of HAART. The objective of this study was to determine the occurrence and patterns of HIV/AIDS-related oral lesions in relationship to HAART usage, with a particular focus on oro-pharyngeal candidiasis (OPC).

In this cross-sectional study, every 5th HIV-positive Comprehensive Care Clinic (CCC) outpatient on HAART was selected. They underwent oral cavity examination for the presence of different clinical forms of OPC and other oral lesions associated with HIV infection. Individual patient medical records were perused for relevant clinical data. Gram Stain smears from OPC lesions were examined under a light microscope for the presence of *Candida* pseudo-hyphae. Data was recorded in structured questionnaires and standard forms, entered into MS Access and then transferred to Statistical Package for Social Sciences (SPSS) for analysis. Chi square was used to analyze the statistical significances of the differences in frequencies and proportions. The data was stratified for periods below and above 24 weeks on HAART. One-way ANOVA was computed comparing the mean ages, mean CD4 count and mean durations on HAART between the sexes. Odds ratios were calculated for the occurrence of OPC in subjects who had been on HAART for 24 weeks or more with adjustments for age, CD4 count, use of antifungal drugs, use of antibiotics and missed HAART doses marital status and employment status.

A total of 404 (63% female) patients were examined. Male: female ratio was 1:1.7. The mean age was 39.8 ± 9.5 years. All the patients examined were on triple HAART therapy. Patients who had been on HAART for less than 24 weeks were 69 (17.1%) while 335 (82.9%) had been on HAART for more than 24 weeks. Oral lesions were observed in 63 (16%) patients. OPC was the commonest (12%), followed by oral hairy leukoplakia (3%). Erythematous candidiasis was the most predominant type of oral candidiasis (6%) followed by pseudo-membranous candidiasis (5%) and angular cheilitis (1%). There was no statistically significant difference in occurrence or type of oral lesions observed between those patients who had been on HAART for more or less than 24 weeks ($p = 0.12$).

Compliance to HAART by the study subjects is satisfactory. The occurrence of oral cavity lesions of HIV/AIDS in patients on HAART at Thika District Hospital is low and shows a gradual decrease over time with HAART usage. Clinicians attending to persons with HIV/AIDS should be capable of diagnosing the oral lesions associated with HIV/AIDS and may use these lesions as pointers for confirming the immune status of the patients using the appropriate laboratory testing. Oral health clinicians should routinely examine for oral opportunistic and neoplastic diseases in patients with HIV/AIDS and offer appropriate treatment to improve the quality of life of these patients. The rate of occurrence recorded in this study may be generalized to populations of persons who know and have accepted their HIV status and are on HAART. More studies, preferably longitudinal, need to be conducted for reasonable periods of time in order to get a better picture on the patterns of occurrence of oral lesions in adults on HAART in Kenya.

CHAPTER ONE

1. INTRODUCTION

1.1 Background

There is a close relationship between general health/disease and oral mucosal reactions. Many diseases show specific and/or non-specific changes which may evolve throughout the course of the disease but the mucosal reaction may also constitute the initial clinical sign of the disease (Axell, 1992). The oro-facial region often reflects the presence of various underlying systemic and generalized disease processes, including various infectious, metabolic and immune-mediated disorders through specific/non-specific lesions (Cleveland, 2003). The mouth and pharynx can be easily examined by clinicians with a wide range of professional training. Many oral lesions are often clearly visible and some can be diagnosed accurately on clinical features alone (Greenspan JS, 1997). The oral lesions associated with HIV/AIDS can be fungal, viral or bacterial in origin. HIV-associated oro-facial lesions have been considered as: clinical indicators of HIV infection in otherwise healthy, undiagnosed individuals; early clinical features of HIV infection; clinical markers for the classification and staging of HIV disease; predictors of HIV disease progression (Coogan *et al.*, 2005). They can therefore be used as entry or end-points in therapy and vaccine trials and can be determinants of opportunistic infection and anti-HIV therapy as well as being useful in staging and classification systems (Coogan *et al.*, 2005; Greenspan *et al.*, 2002). Seven commonly occurring lesions, namely, oral candidiasis

(OPC), oral hairy leukoplakia (OHL), Kaposi's sarcoma (KS), linear gingival erythema (LGE), necrotizing ulcerative gingivitis, necrotizing ulcerative periodontitis and non-Hodgkin lymphoma are strongly associated with HIV infection and have been identified internationally (Appendix 1, EEC-clearing house 1993). These lesions may be present in up to 50% of people with HIV infection and in up to 80% of those with a diagnosis of AIDS (Butt *et al.*, 2001; Wanzala and Pindborg, 1995). The lesions parallel the decline in numbers of CD4+ cells and an increase in viral load, and are independent indicators of disease progression (Greenspan *et al.*, 2002). Thus, in cases where HIV status is unknown and where HIV testing is difficult, for example, in some developing countries, certain oral lesions may provide a strong indication of the presence of HIV infection (Coogan *et al.*, 2005).

Following the early reports of the oral manifestation of HIV in residents in the developed world, there have now been many reports of the oral lesions of HIV-infected individuals in the developing countries. Despite differences in transmission risk behavior, geographical location, gender distribution, ethnicity, nutritional status and endemic disease, there are few or no differences between the oral disease of HIV infected individuals in the developing world when compared to those of the developed world (Patton *et al.*, 2002), particularly as regards the prevalence and type of common oral lesions observed (EEC-Clearing house 1993).

Highly active antiretroviral therapy (HAART) leads to an increase in CD4+ T-cell count, decrease HIV-RNA viral load, and result in a decreased frequency and severity of opportunistic disease including HIV-related oral disease (Ho, 1995).

1.2 Study Justification

It has been observed that HIV-infected patients are more likely to be colonized by *Candida* than healthy individuals (Mocroft *et al.*, 2003) and that HAART markedly decreases mortality and morbidity as well as the incidence of AIDS-related opportunistic infections (Kaplan *et al.*, 2000). When successful, HAART results in undetectable viral load (< 50 copies/mm³) at 16-24 weeks after initiation of therapy. Virological response to successful HAART shows similar trends with different drug combinations and so far remains the most reliable laboratory measure of a patient's immune status. Since the prevalence of HIV/AIDS-related oral candidiasis decreases with HAART, it has been suggested that OPC might be a clinically useful marker of patients' immune recovery when considered alongside available laboratory markers (Flint *et al.*, 2006). In Kenya, there has been little work done in this area, but its potential value to the clinical management of HIV/AIDS is apparent, especially where numerous and frequent measures of CD4+ lymphocyte cell counts and viral load may not be readily available or affordable in our resource-limited settings.

1.3 Objectives

1.3.1 General Objective

To examine the occurrence and patterns of HIV/AIDS-related oral lesions in relation to HAART usage among HIV positive adults attending the comprehensive care centre at Thika District Hospital.

1.3.2 Specific Objectives

- 1) To determine the characteristics of HAART usage among the study subjects
- 2) To determine the occurrence of oral lesions, including OPC, associated with HIV/AIDS among adults on HAART.
- 3) To determine the relationship between the occurrence of oral candidiasis and HAART usage after stratifying for periods before and after 24 weeks of therapy initiation.

1.3.3 Study Variables

Independent variables: name, age, sex, marital status, employment status, HAART combination, HAART compliance, duration on HAART, concurrent medication, presence or absence of HIV/AIDS related oral lesions

Dependent variables: clinical form and anatomical site of OPC, presence of *Candida* pseudo-hyphae or spores on smears

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Overview of Oral Lesions Associated with HIV Infection.

The prevalence of oral lesions strongly-associated with HIV/AIDS varies according to region of the world and cohort examined. In a review of literature done to ascertain the nature and prevalence of oral lesions in different regions, oral candidiasis was found to be the most commonly reported oral lesion (Hodgson *et al.*, 2002). Oral hairy leukoplakia (OHL) appears to be the second most common lesion, with reported prevalence from 0% to 20% in Africa (Mugaruka *et al.*, 1991; Arendorf *et al.*, 1998; Butt *et al.*, 2001), 3–13% in Asia (Nittayananta and Chungpanich, 1997; Ranganathan *et al.*, 2000), up to 43% in Mexico (Gillispie and Marino 1993), and from 7% to 30% in the US and Europe (Lamster *et al.*, 1994; Schmidt-Westhausen *et al.*, 1997; Schuman *et al.*, 1998), with lower prevalence among women than men. The prevalence of Kaposi's sarcoma (KS) varies from 0% to 13% in Africa (Tukutuku *et al.*, 1990; Arendorf *et al.*, 1997; Butt *et al.*, 2001) and from 0% to 38% in the US and Europe (Schmidt-Westhausen *et al.*, 1997; Schuman *et al.*, 1998).

Linear gingival erythema (LGE) is present in up to 16% of patients in developing nations (Ranganathan *et al.*, 2000) and 22% in the US (Lamster *et al.*, 1994). Necrotizing (ulcerative) gingivitis and necrotizing (ulcerative) periodontitis are reported to be present in up to 16% and 17% of patients in Africa, respectively (Tukutuku *et al.*, 1990), and a 23%

prevalence of necrotizing ulcerative periodontitis has been reported in India (Anil and Challacombe, 1997). In contrast, in the US and Europe, necrotizing (ulcerative) gingivitis and necrotizing (ulcerative) periodontitis are reported in up to 11% and 19% of HIV-infected subjects, respectively (Schuman *et al.*, 1998). All the patients in a study done in Kenya had some form of periodontal disease - gingivitis, necrotizing gingivitis, periodontitis (Butt *et al.*, 2001).

The few studies of oral lesions in patients on HAART have been conducted in developed countries and indicate significant differences in the influence of HAART on types and occurrence of oral lesions (Greenspan *et al.*, 2004; Schmidt-Westhausen *et al.*, 2000). Most studies in African on HIV-associated oral lesions had been done during pre-HAART era (Hodgson *et al.*, 2002; Arendorf *et al.*, 1997; Butt *et al.*, 2001; Adurogbangba *et al.*, 2004; Matee *et al.* 2000). In a study done in Tanzania, adult patients receiving HAART were found to have a significantly lower prevalence of oral lesions, particularly oral candidiasis and oral hairy leukoplakia (Hamza *et al.*, 2006). Table 1 shows the occurrence of oral lesions associated with HIV/AIDS in different parts of the world. The commonly observed oral lesions, OPC and OHL, have been shown in separate columns.

Table 1: Prevalence of ‘oral lesions strongly associated with HIV’ in the HAART era; comparison of published studies.

Reference	Country	Sample size	Any Oral Lesion	OPC (%)	OHL (%)
Margiotta <i>et al.</i> (1999)	Italy	104	36%	10% OPC 6% PMC 4% EC	9.6
Schmidt-Westhausen <i>et al.</i> (1997)	Germany	70	43%	31% OPC 24% PMC 6% EC 4% AC	0
Arendorf <i>et al.</i> (1998)	South Africa	600	60%	38% OPC 16% PMC 16% EC 7% AC	20
Wanzala and Pindborg (1995)	Kenya	337	21%	13% EC 1% PMC 1% AC	3
Matee <i>et al.</i> (2000)	Tanzania	192	----	12% OPC 12% PMC 0% EC 0% AC	6
Hodgson (1997)	Zambia	107	----	25% OPC 19% PMC 6% EC	5
Patton <i>et al.</i> (2000)	USA	299	38%	17% OPC	11
Nittayananta & Chungpanich (1997)	Thailand	124	82%	66% OPC 54% PMC 25% EC 6% AC	13

Key: OPC = oro-pharyngeal candidiasis; PMC = pseudomembranous candidiasis; EC = erythematous candidiasis; AC = angular cheilitis; OHL = oral hairy leukoplakia

2.2 Epidemiology of Candidiasis

Candida is a commensal organism and part of the normal oral flora in about 30% - 50% of the population, and is capable of producing opportunistic infections (superficial mycosis) within the oral cavity when appropriate predisposing factors exist. The most frequently isolated species is *Candida albicans*, although other species have been isolated in HIV-infected patients such as *C. glabrata*, *C. tropicalis*, *C. krusei*, *C. parapsilosis*, *C. guilliermondii* and *C. dubliniensis* (Ranganathan *et al.*, 2006).

2.3 Pathogenesis of oral candidiasis

Various factors trigger pathogenesis, which gives rise to several different clinical forms of candidiasis (Hodgson *et al.*, 2002). General factors that may lead to clinically evident oral candidiasis are the immune status of the host, the oral mucosal environment, and the particular strain of *C. albicans* with the hyphal form being the one most commonly associated with pathogenic infection (Lynch *et al.*, 1994). The oral mucosa is continuously challenged by the resident microbial flora and occasionally by microbial pathogens. It is therefore armed with several cell populations which individually, or in association, can produce a protective innate or acquired immune response. These cells include Langerhans' cells, macrophages, β - and γ -T cells (including CD4+ and CD8+ cells), keratinocytes, and polymorphonuclear lymphocytes. The local response by these cells together with the systemic response to HIV infection, determine the outcome in relation to oral manifestations (Louis *et al.*, 2004).

The natural history of HIV infection is characterized by progressive, insidious impairment of the immune system function, with a widely varying rate of progression between patients. CD4+ lymphocyte cell count and the quantification of viral RNA in blood plasma have been found to be the main laboratory markers of disease progression (Mellors *et al.*, 1996). High viral load is currently considered to be one of the main indicators of the progression of HIV-induced immune suppression (Patton *et al.*, 1999). Advanced clinical stages of HIV infection have been associated with more frequent occurrence of OPC than the early stages. In the early stages, OPC affects mainly the oral mucosa, and only involves the esophageal mucosa in more advanced stages (Imam *et al.*, 1990). The presence of OPC in subjects with HIV infection has been associated with the occurrence of esophageal candidiasis and more frequent progression to AIDS (Scully *et al.*, 1991). OPC is an early oral sign of immunodeficiency and it has been used as a marker of severity in HIV infection. The occurrence of OPC and the progression of early clinical forms (erythematous variant and angular cheilitis) to more severe advanced forms (pseudomembranous variant) are favoured by immune deterioration, as reflected by the drop in CD4+ lymphocyte cell count (Imam *et al.*, 1990; Ceballos *et al.*, 1996).

2.4 Clinical manifestations of oral candidiasis

Acute or chronic erythematous candidiasis is the most commonly encountered clinical form of candidiasis in HIV+ individuals. The clinical features of erythematous candidiasis include reddish areas, mainly located on the back of the tongue and hard palate. This is

more frequent in the early stages of HIV infection, in patients with CD4+ counts greater than 200 cells/ μ l (Ceballos *et al.*, 1996).

Acute or chronic pseudomembranous candidiasis is the most classical form and comprises up to 50% of all *Candida* infections in HIV+ individuals and is significantly more common in patients with CD4+ lymphocyte cell counts of less than 200 cells/ μ l. It is characterized by the presence of white or yellowish-white, soft or gel-like plaques that grow in a centrifugal pattern and can be eliminated by scraping leaving an erythematous lesion that can be painful on occasion. The plaques may appear anywhere inside the oral cavity, although they are most frequent in the buccal mucosa, palate, oro-pharynx and margins of the tongue. Pain, burning sensation or dysphagia may accompany these presenting features (Ceballos *et al.*, 1996).

Angular cheilitis is a disease of the lip that presents with reddening, cracks or splitting and scabs at the corners of the mouth and it tends to be bilateral. It is frequently a mixed infection caused by gram-positive *Cocci* and *Candida*. In HIV+ patients it is typically chronic or recurrent (Ceballos *et al.*, 1996). Linear gingival erythema (LGE) is an intensely erythematous condition that clinically manifests as a 2- or 3-mm band, located along the gingival margin. It appears to be closely related to the sub-gingival colonization by various species pertaining to the *Candida* genus (Aguirre *et al.*, 2004). Immunosuppression should be suspected (and amongst possible causes, HIV infection), when faced with any clinical form of candidiasis that is both severe and chronic in young people and/ or in individuals

with uncommon clinical features without an apparent cause to justify them (Ranganathan *et al.*, 2006; Hodgson *et al.*, 2002).

2.5 Diagnosis of oral candidiasis

Candidiasis has identifiable clinical characteristics that make its diagnosis easy on visual examination (Samaranayake, 1992). In addition, candidiasis may be confirmed by a positive potassium hydroxide (10% KOH) wet mount, Gram-stained smear, culture or biopsy. A swab of the oral lesions is placed in saline and the evaluation for yeast is carried out immediately by mixing a drop of this solution with a drop of potassium hydroxide (KOH) solution on a glass slide. The potassium hydroxide lyses the patient's cells, making the yeast easier to see. Alternatively, the swab is rolled onto a slide, fixed (by passing over a flame) and Gram stained. This slide does not have to be stained or evaluated immediately therefore making Gram stain a more convenient procedure. Specimens are examined for the presence of small, round to oval, thin-walled, clusters of budding yeast cells and branching pseudo-hyphae. The demonstration of pseudo-hyphae in scrapings or smears from oral and esophageal lesions should be considered significant, provided the clinical manifestations support the diagnosis (WHO, 1999).

Fungal cultures can also be done but are rarely necessary to make a diagnosis of oral candidiasis. They are, however, helpful in identification of *Candida* species or when colony counts are required. A swab of the oral lesions or an aliquot of a mouth rinse is inoculated into Sabouraud's dextrose agar within a few hours of collection and incubated

for up to two days at 37° C. Colonies are confirmed as yeast by performing a Gram stain. In culture, colonies are typically white to cream colored with a smooth wax-like surface (WHO, 1999). Biopsy specimens of oral lesions may also be used to distinguish certain forms of leukoplakia and oral herpetic ulcers similar in appearance to certain *Candida* variants (Chaisson *et al.*, 1995).

2.6 Correlation of OPC with HIV infection and its progression

The reported prevalence of oral candidiasis varied from 56% to 81% in Asia (Ranganathan *et al.*, 2000), 0–94% in Africa (Tukutuku *et al.*, 1990) and 5–92% in the US (Lamster *et al.*, 1994), largely depending on the prevalence of AIDS diagnoses or individuals with low CD4+ counts among those studied. Between 20 and 94% of all HIV-infected patients present with oral candidiasis. In more than 75% of these individuals, some form of mycosis has been observed at some point during the course of illness (Fine *et al.*, 2003). Erythematous candidiasis, pseudomembranous candidiasis and angular cheilitis are the most common types of oral candidiasis amongst the HIV-infected patient population and are significantly predictive of progression to AIDS in these individuals (Schuman *et al.*, 1998)

2.7 HAART and its impact on OPC

HAART has changed the lives and the course of disease in those infected with HIV (Appendix 2). Since its introduction, there has been a marked reduction in AIDS deaths in those HIV-infected individuals who accept their status and avail themselves for treatment (Ho, 1995). The aims of HAART are maximal and durable suppression of the viral load in

the peripheral blood; restoration and preservation of immunological function (immune reconstitution); improvement in quality of life, and reduction in HIV-related morbidity and mortality. Successful treatment causes a sustained suppression of the virus, resulting in an undetectable viral load (< 50 copies/mm³) at 16 to 24 weeks after initiation of therapy. A concomitant rise in the CD4+ lymphocyte cell count is also observed. Increases in CD4+ cell counts in response to HAART confer immunologic reconstitution and a decreased incidence of opportunistic infections. Treatment failure or HAART failure has been ascribed to the development of resistance by the virus to the combination of drugs used, non-compliance with the drug regimen, or suboptimal potency or blood levels of the drug combination. Studies have shown that viral load falls and stabilizes to less than 500 copies/ml in over 80% of the patients 24 weeks after successful initiation of HAART regardless of the drug combination or pre-HAART viral loads (Lepria *et al.*, 2001; Philips *et al.*, 2001).

There is a close association between a patient's immune state and the presence of OPC; with an increase in OPC frequency as CD4+ lymphocyte cell counts decreases (Imam *et al.*, 1990). In HIV-infected patients, OPC has been associated with a less favourable immune status and more accelerated deterioration of this situation (Ceballos *et al.*, 1996). Phelan *et al.*, (1997) found that a CD4+ count of less than 200 cells/ μ l or the presence of OPC at the time of her data collection increased the risk of death or development of AIDS, and such prognosis was much worse if both factors were present at the onset of evaluation. This relationship between OPC and low CD4+ lymphocyte counts may be used to interpret the

presence of OPC as an indirect marker of the immune status of HIV-infected patients when a CD4+ lymphocyte cell count is unavailable (Phelan *et al.*, 1997)

Oral co-morbidities in HIV infection were some of the first manifestations of the disease to be documented. Classification and staging of HIV with oral lesions included as part of the criteria has been published (Appendix 1). HAART has been shown to decrease the prevalence of oral candidiasis in patients with AIDS (Patton *et al.*, 2000). Oral manifestations of HIV infection might therefore serve as good markers for monitoring not only restoration of immune function (along with the numerical parameters of viral load and CD4+ counts), but also HAART failure (Ceballos *et al.*, 1996).

2.8 HAART Regimens

Since HAART is administered for long periods of time, three drug combinations are used in an attempt to minimize viral resistance to the drugs. According to the 'Guidelines on antiretroviral drug therapy in Kenya, 2005' HIV/AIDS patient are treated on a combination of three classes of drugs (see Appendix 2); namely nucleoside reverse transcriptase inhibitor (NRTI), a non-nucleoside reverse transcriptase inhibitor (NNRTI) and a protease inhibitor (PI). In general, a minimum combination of three drugs from at least two different classes as in the following combinations is preferred; 2 NRTIs + NNRTI; 2 NRTIs + PI/r (ritonavir boosted PI). The first line therapy includes four different combinations of drugs: Stavudine or Zidovudine + Lamivudine + Nevirapine or Efavirenz. The second line regimen is used in cases of treatment failure and includes the following drug combination:

Didanosine + Abacavir + Lopinavir/ritonavir (Kaletra) or Tenofovir + Abacavir +
Liponavir/ritonavir (Kaletra).

CHAPTER THREE

3. MATERIALS AND METHODS

3.1 Study design

A cross-sectional study involving HIV/AIDS adult patients on HAART attending a comprehensive care clinic was carried out.

3.2 Study Site

The study site was Thika District Hospital HIV Comprehensive Care Clinic (CCC). Thika District is one of the seven districts in central province of Kenya. The district has a population of about 680,000. HIV/AIDS continues to have a devastating impact in this district, which has the highest disease burden in central province. The HIV prevalence rate in the district by end of 2007 was 3.6%. Thika District borders highly populated parts of the adjacent districts whose residents seek health services in its health facilities.

3.3 Study Population

Thika District Hospital is considered a high volume district hospital due to the high numbers of in- and out-patients. It serves an average of 750 out-patients daily. The CCC was serving an average of 67 active patients daily by the end of December 2007.

3.3.1 Sample Size Determination

An average prevalence of HIV/AIDS-related oral candidiasis of 50% was assumed (Butt *et al.*, 2001). The sample size was based on the formula below (Fisher, 1998)

$$N = \frac{Z^2 pq}{t^2}$$

Where Z = Confidence interval (1.96 for 95% CI)

p = prevalence of HIV/AIDS related oral candidiasis in a previous study in Kenya.

q = (1- p)

t = precision (5% = 0.05)

Data collection was done over a period of 4 months, from September through December 2008. Since the study population was less than 10,000, the finite population correction factor $N_{f=n/(1+(n/N))}$ was applied. This gave a minimum sample size of 362 patients.

3.3.2 Sampling procedure

Systematic sampling was applied. Every 5th HIV-positive outpatient at CCC who was on HAART and satisfied the inclusion and exclusion criteria was selected. The patients were referred to the investigator by the attending clinicians. Where the 5th patient did not qualify the next patient was selected.

3.3.3 Inclusion criteria

- Adults over the age of 18 years who have been tested and confirmed to be HIV positive
- Consistency and compliance to HAART.
- Willingness to participate in the study and give consent.

3.3.4 Exclusion criteria

- Those not tested and confirmed to be HIV positive.
- Those with poor compliance for HAART
- Those unwilling to participate in the study or give consent.

3.4 Data Collection

3.4.1 Administering the Questionnaire

All participants were requested to provide voluntary written informed consent before enrollment. The researcher asked for consent from the patients after explaining to them the nature of the procedures to be carried out in a simple fashion (Appendix 3).

Socio-demographic data, patients' medical details including information on other illnesses and other medication, and the observations of oral cavity examinations were recorded in a structured questionnaire (Appendix 4) administered by the researcher. Each subject's medical records (Appendix 5) were reviewed by the researcher for relevant information about the date confirmed HIV+, date he/she was started on HAART, HAART combination and the most recent recording of CD4+ cell count. HIV testing for the subjects had been done and confirmed at the hospital laboratory using ELISA (Murex HIV Ag/Ab Combination, Murex Biotech Limited, UK). The CD4+ lymphocyte cell count used had been carried out in the hospital laboratory through flow-cytometry using fluorescence-activated cell sorter (FACSCount™, Becton Dickinson WW Inc).

3.4.2 Clinical Examination of the oral cavity

Oral cavity examination was carried out by the researcher, a dental surgeon by training, using established WHO clinical diagnostic criteria for oral pathologic conditions associated with HIV infection (Appendix 1) and recorded in a standard data capture sheet (Appendix 6). Examination was done in the CCC consultation office using natural daylight and, where necessary, a headlamp was used. Wooden tongue depressors were used to retract the lips and depress the tongue for a careful oral and pharyngeal examination. Oral lesions associated with HIV infection, the different clinical forms of OPC and their anatomic location was noted. Intra-oral photographs of lesions were taken occasionally with a Cannon Ixy55 digital camera. Diagnosis of OPC and LGE was based on both clinical manifestations and detection of organisms on smears. Diagnosis of the Kaposi's sarcoma lesion was confirmed through histological examination of an incision biopsy while that of OHL was confirmed through clinical appearance.

3.4.3 Microscopic examination of specimens for *Candida pseudo-hyphae*

Specimens from OPC lesions were taken by gently drawing a wooden tongue depressor across the lesion. The specimens were then transferred onto a clean glass slide, dried in air and fixed by passing the slide over a flame at the chair-side. The slides were then labeled, placed in a covered slide rack and later forwarded to the laboratory ready for Gram staining (Appendix 7) and examination under a light microscope to confirm the presence of *Candida pseudo-hyphae*.

3.5 Data Management

3.5.1 Data storage

Data was stored in the computer being used, flash disks, compact disks (CDs) and on the internet. Completed data collection forms and questionnaires were retained as hard copy back up.

3.5.2 Data analysis

Data was coded and double entry done in MS Access. Data was then transferred from MS Access to Statistical Package for Social Sciences version 12.0 (SPSS Inc, Chicago, Illinois, USA) for cleaning and statistical analysis. Data cleaning was done to exclude repeated and incomplete data by running frequencies in SPSS. Chi-squared test and one way ANOVA were used to compare differences in proportions of oral lesions between age groups, sexes and CD4+ ranges. Odds ratios were calculated to examine the relationship between the occurrence of oral lesions and age, CD4+ count and duration on HAART. Statistical significance for all analyses was at $p \leq 0.05$.

3.6 Ethical Considerations

Approval to conduct this study was granted by KEMRI/National Ethical Review Committee (Appendix 8), JKUAT Scientific Committee and the Medical Superintendent, Thika District Hospital. All participants were requested to provide voluntary written informed consent before enrollment. Participants were guaranteed of confidentiality by ensuring that no identifying information was made available to anyone who was not directly involved in the study at any time during or after the study. Patients were given

appropriate advice and/or medication on complaints relating to oral/dental conditions and referred for health problems that could not be addressed at the center. All procedures done for the purposes of this study were free of charge to the participants. The results of the study will be made available to the participating institutions and the individual participants as appropriate.

3.7 Study Limitations

The CD4+ cell count measurements are done every 6 months for the patients attending Thika District Hospital CCC. Some of the records available for CD4+ count were 3 or 4 months old and therefore may not have accurately reflected the patient's immune status at the time of examination. The data obtained in this cross-sectional study could not be analyzed to conclusively address issues related to changes in observations in a time-dependent manner. Additional aspects of risk for these HIV-associated oral lesions may not have been conceptualized or assessed in this study design.

CHAPTER FOUR

4. RESULTS

4.1 Socio-demographic Characteristics

A total of 404 HIV-positive subjects were enrolled into the study, comprising 256 (63.4%) females and 148 (36.6%) males. The mean age for this study sample was 39.8 ± 9.5 years (range 21–79years). Majority of the study subjects 213 (52.7%) were married with more of the males 113 (53.1%) being married compared to the females, 100 (46.9%). Most of the subjects 289 (71.6%) were in their fourth or fifth decades of life. Majority of the study subjects were either in part time employment 144 (35.6%) or were unemployed 136 (33.7%). Over two thirds of the study subjects had been on HAART for duration of more than 24 weeks (Table 2).

Table 2: Socio-demographic and clinical profile of the study subjects

Characteristic & Category	n =	Number (Percentage) of subjects	
		Males	Females
Age			
20 – 29	54	10 (18.5%)	44 (81.5%)
30 – 39	157	52 (33.1%)	105 (66.9%)
40 – 49	132	56 (42.4%)	76 (57.6%)
50 +	61	30 (49.2%)	31 (50.8%)
Totals	404	148 (36.6%)	256 (63.4%)
Marital Status			
Single	61	8 (13.1%)	53 (86.9%)
Married	213	113 (53.1%)	100 (46.9%)
Others (separated, divorced, widowed)	130	27 (20.8%)	103 (79.2%)
Employment Status			
Full time	124	63 (50.8%)	61 (49.2%)
Part time	144	58 (40.3%)	86 (59.7%)
Unemployed	136	27 (19.9%)	109 (80.1%)
CD4+ count (cells/μl)			
0 -200	135	57 (42.2%)	78 (57.8%)
201 – 500	216	73 (33.8%)	143 (66.2%)
501 +	53	18 (34.0%)	35 (66.0%)
Duration on HAART			
Below 24 weeks	69	18 (26.1%)	51 (73.9%)
24 weeks and above	335	130 (38.8%)	205 (61.2%)
Totals			

Percentages may not add up to exactly 100 due to rounding off

Most of the subjects in this study were in their fourth and fifth decades of life with males having a higher mean age than females. Among the males the peak age group was 40-49 years while the age group 30-39 years was the peak age among the females (Figure 1).

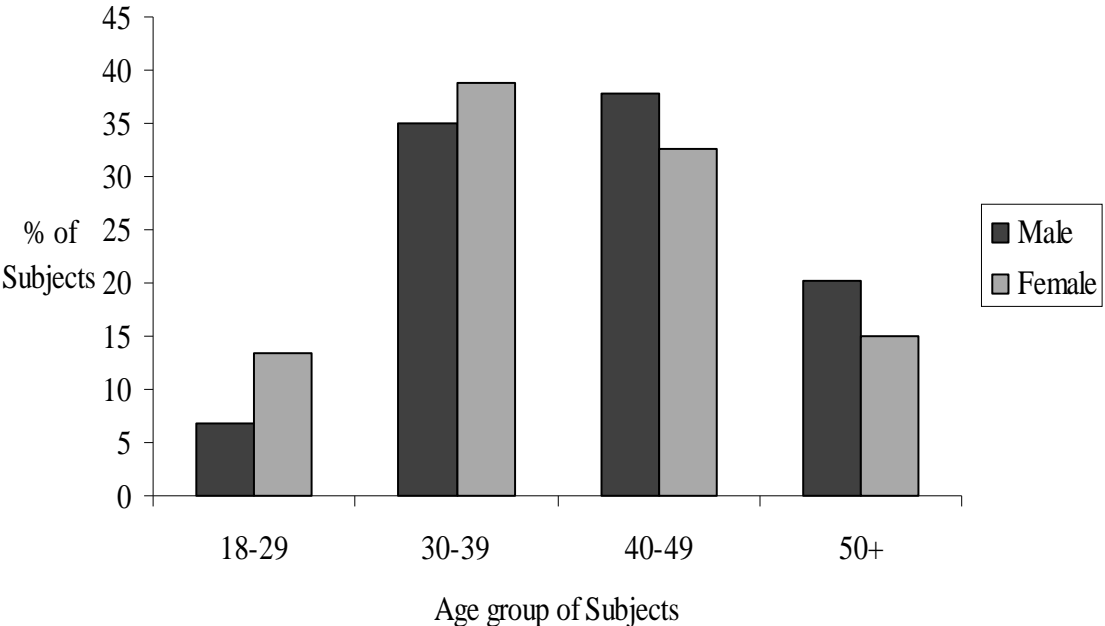


Figure 1: Distribution of subjects by gender and age groups

One-way ANOVA showed a significant difference ($p = 0.01$) between the mean ages of males (42.28 ± 9.9) and females (38.43 ± 9.0). Other results for one-way ANOVA are shown in Table 3.

Table 3: One way ANOVA for mean ages, CD4+ count and duration on HAART between sexes

Characteristic	Mean		p-value
	Male	Female	
Age	42.3 ± 10.0	38.4 ± 9.0	0.01
CD4+ cell count	287.5 ± 190.5	317.4 ± 226.3	0.18
Duration on HAART	86.4 ± 59.6	79.8 ± 60.7	0.29

4.2 Clinical characteristics

On the basis of the most recent absolute CD4+ cell count, 135 (33.4%) of the subjects had a count less than 200 cells/ μ l, 216 (53.5%) had a count between 200 and 500 cells/ μ l and 53 (13.1%) had a count greater than 500 cells/ μ l. The mean CD4+ count was 306.5 ± 214 cells/ μ l (range; 12 –1416 cells/ μ l). Males had a lower mean CD4+ cell count compared to females (Table 3). Participants who had been on HAART for 24 weeks or more had higher mean CD4+ cell count (331.3 ± 211.8 cells/ μ l) compared to those who had been on HAART for less than 24 weeks (185 ± 183.0 cells/ μ l).

4.3 HAART Regimens

Eleven different HAART combinations were recorded in this study. Among those examined 312 (77.2%) patients were on the first line drug combination: stavudine + lamivudine + nevirapine. Only three subjects were on a HAART regime containing a protease inhibitor. The most commonly used classes of drug combination were 2NRTIs + NNRTI (Table 4).

Table 4: Distribution of patients by HAART combination

HAART combination	Class of drugs	Number of patients	Proportion (%)
stavudine + lamivudine + nevirapine	2NRTIs + NNRTI	312	77.2
stavudine + lamivudine + effavirenz	2NRTIs + NNRTI	57	14.1
stavudine + lamivudine + tenofovir	2NRTIs + NNRTI	20	5.0
Other Combinations	Various classes	15	3.7

NRTI – nucleoside reverse transcriptase inhibitor; NNRTI – non- nucleoside reverse transcriptase inhibitor

4.4 Characteristics of HAART usage

Majority 335 (82.9%) of the study participants had been on HAART for more than 24 weeks while 69 (17.1%) had been on HAART for less than 24 weeks. There was fair adherence to HAART regimen with 400 (99 %) participants not having missed any doses of

HAART drugs in the previous 24 hours. Those who had missed at least one dose in the previous 3 days were 43 (10.6%). The single most common reason for missing doses was forgetting in 27 (62.7 %) patients, with 16 (37.2%) patients missing doses for reasons such as side effects, being away from home and hospitalization. A higher proportion of men (12.2%) had missed at least one dose of HAART in the previous 3 days compared to women (9.8%).

4.5 Prophylaxis and treatment against opportunistic infections

The most commonly documented medication concurrent to HAART in this study was prophylaxis against opportunistic infections (OIs) using co-trimoxazole 336 (83.2%). Other medications were additional antibiotics 68 (16.8%), pain relievers 63 (15.6%) and antifungal drugs 41 (10.1%) as illustrated in Figure 2. Fourteen (3.5%) patients were on TB treatment, one was suspected to have TB but had not been confirmed and the rest had no signs of TB. Only 2 (14.3%) patients of those on TB treatment had oral lesions.

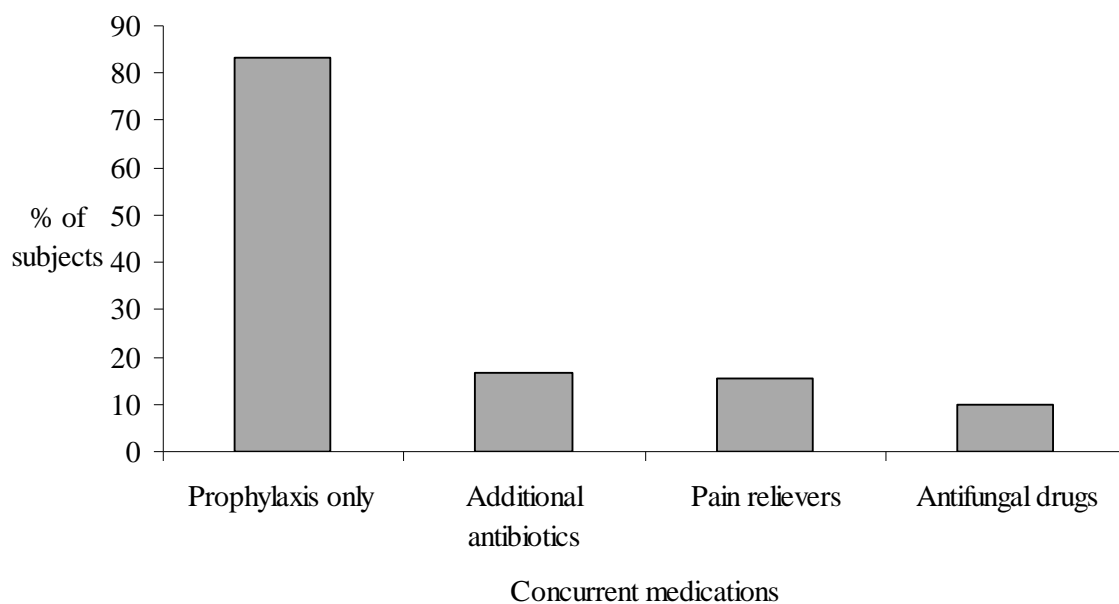


Figure 2: Distribution of concurrent medications used by subjects

The proportion of patients taking antifungal medicines and other antibiotics in addition to co-trimoxazole prophylaxis were higher in study subjects who had been on HAART for less than 24 weeks compared to those who had been on HAART for more than 24 weeks (Table 5). Patients who had been on HAART for less than 24 weeks were more than twice as likely to be on antifungal medication compared to those who had been on HAART for 24 weeks or more (OR = 2.055, 95% CI [1.235 – 3.422], versus OR = 0.807, 95% CI [0.652 – 0.999], $p = 0.009$). Patients who had been on HAART for less than 24 weeks were more likely to be on additional antibiotics compared to those who had been on HAART for 24 weeks or more (OR = 1.492, 95% CI [0.910 – 2.446] versus OR = 0.908, 95% CI [0.790 – 1.044], $p = 0.121$).

Table 5: Distribution of patients on HAART according to duration on HAART and concurrent medications

Characteristic	Category	Duration on HAART	
		Below 24 weeks (n = 69)	24 weeks and above (n = 335)
Subjects taking antifungal drugs	Yes	13 (18.8%)	28 (8.4%)
	No	56 (81.2%)	307 (91.6%)
Subjects taking antibiotics	Prophylaxis only	53 (76.8%)	283 (84.5%)
	Prophylaxis and additional antibiotics	16 (23.2%)	52 (15.5%)
Subjects on other treatment	Pain relievers, anti-TB drugs	17 (24.6%)	60 (17.9%)

4.6 Occurrence and distribution of the oral lesions associated with HIV disease.

A total of 63 (15.5%) patients had oral lesions. Oral candidiasis was the most commonly observed oral lesion in 44 (10.9%) patients, followed by oral hairy leukoplakia in 12 (3%) patients, gingivitis in 8 (2%) patients and Kaposi's sarcoma (Plate 1) in one patient (0.2%). Among the clinical variants of oral candidiasis, erythematous candidiasis was the most frequently observed lesion in 21 (5.7%) patients followed by pseudomembranous candidiasis (Plate 2) in 23 (5.2%) patients, and angular cheilitis in 3 (0.7%) patients.

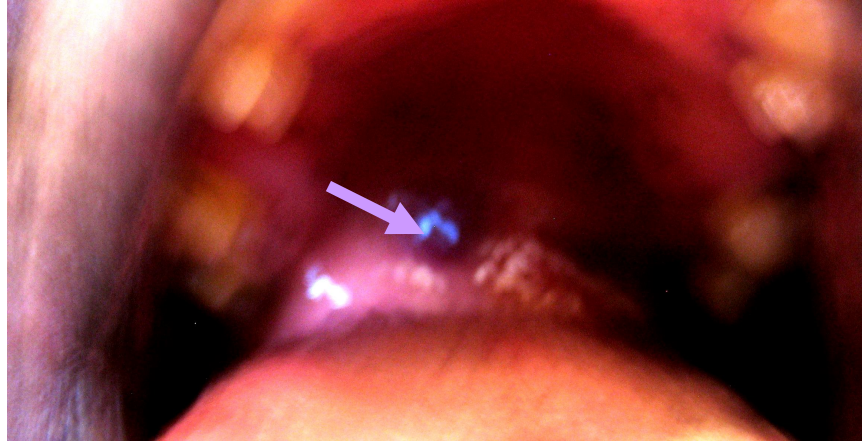


Plate 1: Kaposi's sarcoma on the soft palate in one of the study subjects.

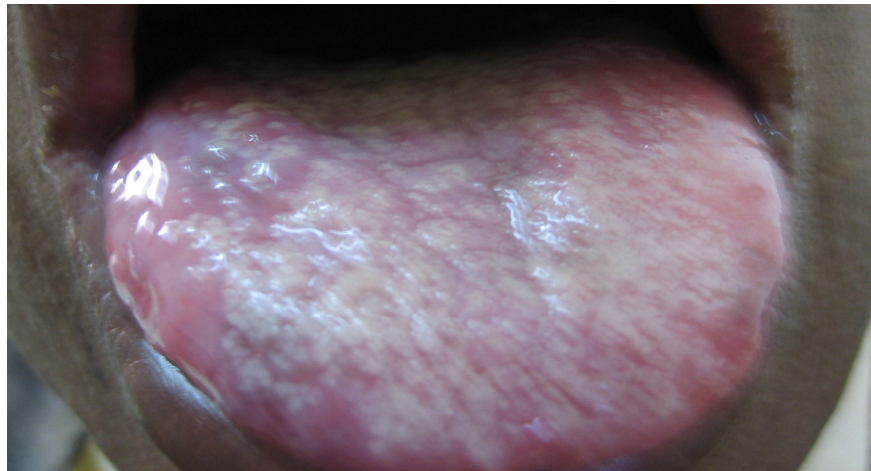


Plate 2: Pseudo-membranous candidiasis on the dorsum of the tongue in one of the study subjects.

4.7 Occurrence of oral lesions of HIV/AIDS and immune cell changes

Having one or more oral lesions was more common among those with CD4+ counts <200 cells/ μ l at 26 (19.3%), with 35 (16.2%) of those having CD4+ counts from 200 to 500 cells/ μ l and 7 (13.2%) of those with CD4+ counts >500 cells/ μ l presenting with lesions. A general decline in occurrence of oral lesions was observed as the CD4+ count increased

(Figure 3). The odds of having an oral lesion were lower (OR = 0.918) at CD4+ count above 200 cells/ μ l than at CD4+ count below 200 cells/ μ l (OR = 1.170). The odds were however not statistically significant at 95% CI.

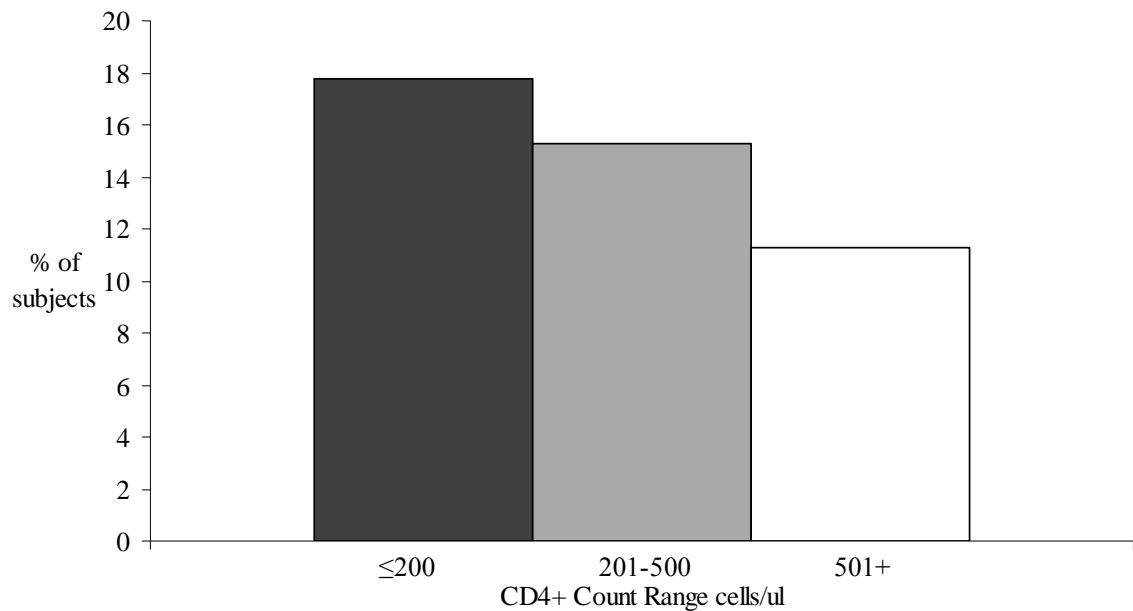


Figure 3: Distribution of oral lesions by CD4+ count range

Having any of the clinical variants of OPC was more common 18 (13.3%) in those with CD4+ counts < 200 cells/ μ l; followed by 24(11.1%) in those with CD4+ count between 200 and 500 cells/ μ l; and 5 (9.4%) in those with CD4+counts >500 cells/ μ l. The differences were, however, not statistically significant ($p = 0.585$). Higher proportions of PMC, EC, AC, and OHL were observed in the subjects with absolute CD4+ cell count below 500 cells/ μ l (Table 6).

Table 6: Distribution of individuals with oral lesions by CD4+ count

Oral Lesions	<200 cells/μl	200-500 cells/μl	>500 cells/μl
PMC	6(4.4%)	13(6.0%)	2(3.8%)
EC	10(7.4%)	10(4.6%)	3(5.6%)
AC	2(1.5%)	1(0.5%)	0
OHL	5(3.7%)	5(2.3%)	2(3.8%)
KS	1(0.7%)	0	0
LGE	2(1.5%)	6(2.8%)	0
No Lesion	109(80.7%)	181(83.8%)	46(86.8%)
Total	135(100%)	216(100%)	53(100%)

Percentages within CD4+ range as a proportion of the sample within that range

PMC – pseudomembranous candidiasis; EC- Erythematous candididasis; AC-Angular cheilitis,

OHL- Oral Hairy Leukoplakia; LGE- Linear Gingival Erythema; KS- Kaposi’s sarcoma

4.8 Occurrence of oral lesions and duration of HAART

Duration on HAART in this study refers to the period from the initiation of the HAART regimen up to the time of examination during this study. A higher proportion 17 (24.5%) of

oral lesions were observed in those on HAART for duration less than 24 weeks than those on HAART for more than 24 weeks 51 (15.1%) as illustrated in Table 7. A Chi-squared test was conducted which showed no statistical significance ($p = 0.122$) in the occurrence of oral lesions with duration on HAART. There was also no statistical significance in the occurrence of any OPC lesion with duration on HAART ($p = 0.139$). The risk of oral lesions was less likely in those who had been on HAART for 24 weeks or more (OR = 0.905, 95% CI [0.783 – 1.047]) than in those who had been on HAART for less than 24 weeks (OR = 1.504, 95% CI [0.907 – 2.491]) with $p = 0.122$.

Table 7: Distribution of individuals with oral lesions by duration of HAART

Oral Lesions	Duration on HAART	
	Below 24 weeks	24 weeks and above
PMC	4 (5.8%)	17 (5.0%)
EC	6 (8.7%)	17 (5.0%)
AC	1 (1.4%)	2 (0.6%)
OHL	5 (7.2%)	7 (2.1%)
KS	1 (1.4%)	0
Gingivitis	0	8 (2.4%)
Sub-Total (Any oral lesion)	17 (24.6%)	51 (15.2%)
No Oral Lesion	52 (75.4%)	284 (84.8%)
Total	69 (100%)	335 (100%)

Five subjects had more than one type of oral lesion.

A general decline in the proportions of the different clinical variants of OPC was observed, with lower values in the group with over 24 weeks of HAART administration (9.9% vs. 15.9%). The odds of having OPC were lower at HAART duration 24 weeks and above (OR = 0.894, 95% CI [0.749 – 1.067]) compared to the duration below 24 weeks (OR = 1.552, 95% CI [0.883 – 2.726]) with $p = 0.139$.

Odds ratios were calculated for the occurrence of OPC in subjects with 24 weeks or more of HAART use with adjustment for age, CD4+ count, use of additional antibiotics, use of antifungal drugs, missed HAART doses, marital status and employment status. The

unadjusted ratio was 0.576, 95% CI (0.275 – 1.205). The ratios were not statistically significant at 95% CI. The characteristics analyzed showed different effects among the different strata and probably act as effect modifiers. The stratum specific odds ratio for the CD4+ count above 500 cells/ μ l was statistically significant (OR = 0.918, 95% CI [0.845 – 0.998]).

Table 8: Odds ratios for occurrence of OPC in subjects over 24 weeks on HAART

Adjustment		Odds Ratio [95% CI]	Mantel-Haenszel Common Odds Ratio [95% CI]
Factor	Strata		
Age	Below 40 years	0.557 [0.213 – 1.461]	0.589 [0.259 – 1.338]
	Above 40 years	0.563 [0.172 – 1.837]	
CD4+ cell count	0 – 200 cells/ μ l	0.456 [0.163 – 1.272]	0.559 [0.265 – 1.181]
	201 - 500 cells/ μ l	0.821 [0.174 – 3.865]	
	501+ cells/ μ l	0.918 [0.845 – 0.998]	
Additional antibiotics	Used	0.788 [0.182 – 3.406]	0.603 [0.288 – 1.266]
	Not used	0.545 [0.231 – 1.284]	

Antifungal drugs	Used	0.743 [0.199 – 2.779]	0.883 [0.358 – 1.938]
	Not used	0.906 [0.298 – 2.758]	
Missed at least one dose of HAART in the previous 3 days	Yes	0.625 [0.273 – 1.433]	0.547 [0.256 – 1.166]
	No	0.919 [0.767 – 1.101]	
Marital Status	Single	1.008 0.760 – 1.337	0.138 [0.275 – 1.197]
	Married	0.959 0.765 – 1.202	
	Others	0.713 0.461 – 1.102	
Employment Status	Full time	0.970 0.704 – 1.337	0.187 [0.285 – 1.278]
	Part-time	0.946 0.710 – 1.260	
	Unemployed	0.845 0.637 – 1.122	

Unadjusted Odds Ratio: - 0.576, 95% CI [0.275 – 1.205].

CHAPTER FIVE

5. DISCUSSION

Following the introduction of specific anti-HIV therapies, there have been well-documented changes in the frequency and character of the oral complications of HIV disease. Various studies have reported that HAART has a marked effect on the prevalence and clinical appearance of oral lesions (Schmidt-Westhausen, 2000; Greenspan *et al.*, 2004, Powderly *et al.*, 1998). This study showed a higher proportion of HIV-infected females compared to males similar to other studies in Africa (Butt *et al.*, 2001; Adurogbangba *et al.*, 2004). The Kenya AIDS Indicator Survey (2007) also showed a higher prevalence of HIV infection in females (9.2%) as compared to males (5.8%) perhaps because women are known to be more vulnerable in communities where HIV is spread mainly through heterosexual transmission (UNAIDS, 2002).

The majority of the subjects in this study were in their fourth and fifth decades of life. This is in contrast to other studies (Adurogbangba *et al.*, 2004; Chidzonga, 2003) where the age distribution of the HIV subjects showed a high prevalence in the third and fourth decades of life. This may be due to fear of social stigma, low uptake of testing and consequently low uptake of treatment by the lower age groups. A one way ANOVA conducted for mean ages between sexes showed that male subjects had a higher mean age than female subjects probably because more females get infected with HIV at younger ages (UNAIDS, 2002).

Oral disease and other opportunistic infections may affect the quality of life in HIV-infected individuals but treatment usually results in a measurable symptomatic improvement. Adherence to HAART is an essential component of treatment success. A high degree of adherence to ARV drugs is necessary for optimal virological suppression. Imperfect adherence is common and results from the present study showed that 43 (10.6%) of the study subjects had missed at least one dose of HAART in the previous three days. One survey indicated that one-third of patients missed doses within 3 days of the survey (Ickovics *et al.*, 1997). Another study has shown that 90–95% of the doses should be taken for optimal suppression because lesser degrees of adherence are more often associated with virological failure. This means a patient should not miss HAART doses more than three times a month in the case of a twice-daily regimen (Peterson, 2000).

Factors associated with poor adherence include a poor adherence counseling, high pill burden, forgetfulness, mental depression, lack of patient education, inability of patients to identify their medications, drug toxicity and being too ill (Chesney, 2000). The fairly good adherence reported in this study may be explained by the measures that have been taken in the healthcare system and at Thika District Hospital CCC to optimize adherence which include: provision of affordable and simplified treatment with uninterrupted supply of HAART; a user-friendly health facility, respecting confidentiality, overcoming stigma and discrimination; provision for social support by promoting and facilitating peer support and formation of groups for people living with HIV/AIDS through day care centres and other

mechanisms; development of individual treatment plans that fit HAART into patients lifestyles/daily events and identification of treatment reminders.

This study excluded patients with documented poor compliance to HAART such as those who had defaulted for long intervals of time. These patients are usually attended separately to facilitate adherence counseling and would therefore add a bias in sampling. Data on missed doses of HAART in this study may therefore not accurately represent overall HAART compliance. Questions were, however, still asked to the study subjects to gauge their adherence to the drug regimes. Among those who had missed at least a single dose of HAART the most common reason was forgetting 27 (62.7%) thus the need to intensify adherence support and counseling in co-ordination with treatment support persons/organizations. Sixteen patients (37.2%) missed doses for reasons such as side effects, being away from home and hospitalization. Most of the subjects are part time workers or unemployed and expected that they would be back to their houses in time to take their drugs. A higher proportion of men (12.2%) had missed at least one dose of HAART in the previous 3 days compared to women (9.8%). This may be attributed to the generally poor health seeking behaviour in men, their traditional role as bread winners occupying most of their time and the feeling of discomfort taking pills around others. More than two thirds of the patients were on the same HAART combination; stavudine + lamivudine + nevirapine. This has been reported to be an effective first line combination well within the financial capability of resource-limited settings as in Kenya. It is also available in fixed dose combinations that reduce the pill burden to the patient (Ministry of Health, Guidelines on antiretroviral drug therapy in Kenya, 2006).

The proportion of patients taking antifungal medicines and other antibiotics in addition to co-trimoxazole prophylaxis in this study were higher in subjects who had been on HAART for less than 24 weeks compared to those who had been on HAART for more than 24 weeks. This compares favourably with, and can be explained by, the finding that HAART leads to an increase in CD4+ T-cell count, decreased HIV-RNA viral load, and result in decreased frequency and severity of opportunistic disease including HIV-related oral disease (Ho, 1995). Viral load has been shown to fall and stabilize to less than 500 copies/ml in over 80% of the patients 24 weeks after successful initiation of HAART regardless of the drug combination and pre-HAART viral loads (Lepria *et al.*, 2001; Philips *et al.*, 2001).

In the current study the occurrence of oral cavity lesions of HIV/AIDS in patients on HAART appears to be limited to candidiasis, oral hairy leukoplakia, gingivitis and one case of Kaposi's sarcoma out of the seven commonly reported oral lesions of HIV/AIDS (Appendix 1). The observed overall occurrence (15.5%) of oral lesions in this study is in agreement with the overall proportion of 15.6% reported for Kenyan commercial sex workers (Wanzala *et al.*, 1989) but far much lower than the 90% overall prevalence reported for Zairean patients (Tukutuku *et al.*, 1990) and the 60.4% reported in South Africa (Arendorf *et al.*, 1998). This is probably due to the fact that this study and that by Wanzala *et al.*, (1989) were carried out among out-patients while the other studies used in-patients. Oro-pharyngeal candidiasis and OHL are the two most common oral diseases associated with HIV/AIDS. In the current study the occurrence of OPC and OHL were

around 10.9% and 3% respectively similar to the findings of other studies that reported higher prevalence of OPC than OHL (Eyeson *et al.*, 2002; Patton *et al.*, 2000). Having one or more oral lesions and any of the clinical variants of OPC appears to be more common among those with low CD4+ counts compared to those with higher counts. The difference in occurrence of oral lesions within the different CD4+ count ranges was, however, not statistically significant ($p = 0.585$). A lower occurrence of oral lesions was observed in patients who had been on HAART for 24 weeks or more than in those who had been on treatment for less than 24 weeks. This probably reflects the improved immune status conferred by HAART beyond the critical period of 24 weeks. The 3% ($n = 404$) occurrence of OHL in the current study is in agreement with 3% ($n = 203$) in a black population in a London study involving outpatients on HAART (Eyeson *et al.*, 2002). Patton *et al.*, (2000) also found a decrease in oral OHL from 26% to 11% after protease inhibitor therapy. Similar to another study (Patton, 2000), data from this study showed an association between OHL and poor immune status, CD4 <200 cells/ μ l, but showed a higher occurrence of OHL in female sex in contrast to a study by Nittayananta *et al.*, (2001). The seemingly higher occurrence of OHL in females in this study may be attributed to the possible exclusion of male homosexuals by Nittayananta *et al.* (2001) who concentrated on HIV-infected hetero-sexual subjects and drug abusers. The 0.2% occurrence of Kaposi's sarcoma observed in this study falls within the range of 0% to 13% observed in Africa (Tukutuku *et al.*, 1990; Arendorf *et al.*, 1997; Butt *et al.*, 2001) and from 0% to 38% in the US and Europe (Schmidt-Westhausen *et al.*, 1997; Schuman *et al.*, 1998).

The occurrence of periodontal diseases in this study (2%) is considered remarkably low compared with other studies reporting levels as high as 78.3% and 100% (Ceballos *et al.*, 1996; Butt *et al.*, 2001). This may be attributed to the improved immunological status conferred by HAART usage and the different characteristics of the study samples in various studies among other reasons. Numerous reports have indicated high prevalence values of periodontal diseases with HIV infections. The periodontal diseases in HIV/AIDS patients span a wide spectrum of lesions ranging from conventional gingivitis and periodontitis to more severe necrotizing ulcerative gingivitis and necrotizing ulcerative periodontitis (EEC-Clearing House, 1993). The occurrence of periodontal diseases is influenced by some systemic diseases and an individual patient's level of oral hygiene. In addition some of the earlier studies were carried out on patients who were not on HAART while others were on monotherapy (Chattopadhyay *et al.*, 2005).

This study found a general decline in the occurrence of oral lesions as the CD4+ count increased. The observed occurrence of oral lesions was 17.8% for 0-200 cells/ μ l, 15.3% for 200-500 cells/ μ l and 11.3% for over 500 cells/ μ l. This is in agreement with other findings (Imam *et al.*, 1990; Ceballos *et al.*, 1996; Adurogbangba *et al.*, 2004) that the occurrence of oral lesions, including OPC, is favoured by immune deterioration, as reflected by the drop in CD4+ lymphocyte cell count. Antiretroviral therapies have a variable effect on plasma viral load owing to differences in bioactivity, tissue penetration, half-life, ease of developing resistant strains, tolerance, toxicity and regimen complexity that influence adherence. Thus, HAART regimens are often not equally successful in reducing viral load

and their effect on CD4+ cell count would therefore be variable and indirect (Centres for Disease Control and Prevention, 1998). It has been reported that low CD4+ cell count is an important risk factor for OPC and OHL and antiretroviral medications may be protective for OPC (Greenspan *et al.*, 2001).

Candidiasis is the most common HIV-related oral condition in various populations world wide including sub-Saharan Africa (Hodgson, 1997; Arendorf *et al.*, 1998). Even in the absence of clinically evident OPC, the colonization of the oral mucosa by *C. albicans* in patients with HIV/AIDS is usually significantly higher than in healthy subjects; this suggests that the mucosal immune system influences the numbers of *C. albicans* in the mouth. In addition, some antiretroviral medications are secreted in the saliva, suggesting that systemic anti-HIV medications may alter the oral flora through either direct antimicrobial effects on oral pathogens or a common side effect such as reduced saliva production (Chattopadhyay *et al.*, 2005). There was no statistically significant difference in the risk of occurrence of OPC with use or no use of antibiotics and antifungal drugs. In Kenya all patients on HAART take co-trimoxazole unless contraindicated. This has been found to be an effective prophylactic agent against bacterial infections and malaria (Guidelines on antiretroviral drug therapy in Kenya, 2006). The use of co-trimoxazole (as prophylaxis) in almost all the patients and the improved immunity following use of HAART may have masked some of the changes associated with the use of additional antibiotics and antifungal drugs.

In this study OPC was observed in 44 (10.9%) patients. Patients on HAART for the duration of 24 weeks or more had a lower proportion of OPC compared with those on HAART for a duration of under 24 weeks (9.9% vs. 15.9%). This finding is consistent with observations in other studies that have shown significant reduction in these oral lesions associated with HAART usage and linked to the improved immune status of patients (Schmidt-Westhausen *et al.*, 2000; Patton *et al.*, 2000; Nicolatou-Galitis *et al.*, 2004). Schmidt-Westhausen *et al.*, (2000) also evaluated the oral lesions of 61 patients pre-introduction and 24 weeks post-introduction of HAART and showed a drop in the prevalence of oral lesions from 21.3% to 8.2%. This more pronounced drop may be due to the fact that they compared the prevalence before and after being initiated on HAART while in the current study all the subjects were on HAART but for different periods of time. Among the clinical variants of oral candidiasis, erythematous candidiasis was the most frequently observed lesion in 21 (5.7 %) patients, pseudomembranous candidiasis in 23 (5.2 %) patients, and angular cheilitis in 3 (0.7 %) patients. A similar pattern was observed by Umadevi *et al.*, (2007) in a group of patients on HAART, where OPC was present in 8%, pseudomembranous candidiasis was observed in 4%, one case (2%) had erythematous candidiasis and one had angular cheilitis. Schmidt-Westhausen *et al.*, (2000) found oral candidiasis in 66% of patients examined pre- HAART therapy, 10% after 4 weeks of HAART and no cases of oral candidiasis after 24 weeks of HAART therapy indicating a progressive decrease in prevalence with treatment. Greenspan *et al.*, (2001), in their prospective study on 1280 patients over a period of 12 years reported that, after adjusting

for CD4+ cell count and HIV viral load, the odds of having OPC were lower in individuals on HAART (OR = 0.28 [0.12–0.63]) than in individuals who were not on HAART.

In the current study, the odds of having OPC lesions were lower (OR = 0.576, 95% CI [0.275- 1.205]) for those who had been on HAART for 24 weeks or more compared with the ones who had been on HAART for less than 24 weeks (OR = 1.736, 95% CI [0.830- 3.630]). This indicates the reduced likelihood of having OPC lesions in those who have been on HAART for more than 24 weeks. Odds ratios, for OPC and periods above 24 weeks adjusted for age, use of additional antibiotics, use of antifungal drugs and missing at least one dose of HAART in the previous three days did not show statistical significance at 95% level. This concurs with the observation that exposure to antifungal drugs has little or no effect on the level of yeast carriage in HIV-positive patients (Mrudula *et al.*, 2006). While factors such as diminishing cellular immunity, drug interactions, or decreased drug absorption may account for some of the failure to respond, increasing evidence suggests that *Candida* organisms are developing drug resistance (Sangeorzan *et al.*, 1994). Oral mucosal immunity also interacts with the systemic response to HIV infection to determine the final outcome in relation to oral manifestations (Louis *et al.*, 2004). Evidence has also been presented that HAART has an early, immune reconstitution-independent inhibitory effect on *C. albicans* secretory aspartyl proteinases in the oral cavities of HIV-infected patients (Cassone *et al.*, 2002). The low risk of having OPC with a CD4+ count above 500 cell/ μ l was statistically significant (OR = 0.918, 95% CI [0.845 – 0.998]) in this study suggesting that immunity at this level of CD4+ count may be significantly protective of

oral candidiasis. The number of HIV positive adults on ARVs in Kenya was approximately 140,000 by the end of 2007 (National AIDS and STI Control Programme, Ministry of Health, Kenya, 2008). If the figures for the occurrence of OPC recorded in this study were applied, 10.9% or 15, 260 of the adults on ARVs would have OPC at any one time. This shows the public health significance of clinical OPC.

Data in the current study showed no statistically significant association between the type of HAART regimen and presence of OPC, most probably due to the fact that most of the patients 312 (77.2%) were on one type of HAART consisting of stavudine, lamivudine and nevirapine, and that only three patients had a HAART combination containing PI. These study settings and findings are similar to those of a study by Hamza *et al.*, (2006). All the three patients on a HAART combination with a protease inhibitor (PI) in this study had oral lesions. This may be attributed to the fact that in the current study setting PI – HAART drugs were used as second line drugs for those who had exhibited poor response to first line drugs.

CHAPTER SIX

6. CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

- i. The compliance to HAART by patients is satisfactory but continued efforts are required to maintain this trend since the success of treatment is related to strict adherence.
- ii. The occurrence of oral cavity lesions in patients with HIV/AIDS on HAART attending Thika District Hospital CCC is low being limited mainly to candidiasis.
- iii. The occurrence of and the morbidity arising from oral lesions associated with HIV/AIDS decreases over time with proper HAART adherence.

6.2 Recommendations

- i. HIV-associated oral lesions have demonstrated usefulness as indicators of disease progression and as a tool for monitoring HIV infection in conjunction with CD4+ lymphocyte count and plasma viral load. Results from this study, though not statistically significant, suggest that oral lesions especially OPC, may be considered to be clinical markers of the effectiveness of HAART by the attending clinicians. The onset of HIV-associated oral lesions, particularly oral candidiasis, should alert the clinician to confirm virological failure with the appropriate laboratory testing. Clinical evaluation should not be a substitute for periodic viral load measurements;

however, it could be an additional tool for the clinician, especially in resource-limited settings, where viral load assays may not be widely available.

- ii. Dental care of patients with HIV/AIDS by oral health clinicians should include periodic oral examinations to monitor their disease progression and to alleviate symptoms of oral opportunistic and neoplastic diseases, to improve the quality of life of patients infected with HIV.
- iii. Clinicians caring for the HIV-infected persons, often predominantly physicians should be capable of diagnosing the documented oral lesions associated with HIV/AIDS, and they should be knowledgeable in the application of that information for the benefit of their patients.
- iv. The interrelationship of oral disease to the entire biologic and social environment in varying populations must be considered by clinicians to allow for the prediction of infection and disease progression and to help design strategies to prevent or treat the varied oral consequences of HIV/AIDS within the limitations of our knowledge and resources.
- v. The rate of occurrence of oral lesions recorded in this study may be generalized to populations of persons who know their HIV status and who seek care at government hospitals in Kenya. However, further studies, preferably longitudinal, need to be conducted for reasonable periods of time in order to get a better picture of the occurrence of oral lesions in adults on HAART in Kenya.

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APPENDICES

Appendix 1: Oral manifestations of HIV disease in adults

Lesions strongly associated with HIV infection	Lesions less commonly associated with HIV infection	Lesions seen in HIV infection
Candidiasis Erythematous Pseudomembranous Hairy leukoplakia Kaposi's sarcoma Non-Hodgkin's lymphoma Periodontal disease Linear gingival erythema Necrotizing (ulcerative) gingivitis Necrotizing (ulcerative) periodontitis	Bacterial infections Mycobacterium avium-intracellulare Mycobacterium tuberculosis Melanotic hyperpigmentation Necrotizing (ulcerative) stomatitis Salivary gland disease Dry mouth due to decreased salivary flow rate Unilateral or bilateral swelling of the major salivary glands Thrombocytopenic purpura Ulceration NOS (not otherwise specified) Viral infections Herpes simplex virus Human papillomavirus (wart-like lesions) Condyloma acuminatum Focal epithelial hyperplasia Verruca vulgaris Varicella zoster virus Herpes zoster Varicella	Bacterial infections Actinomyces Israel Escherichia coli Klebsiella pneumoniae Cat-scratch disease Drug reactions (ulcerative, erythema multiforme, lichenoid, toxic epidermolysis) Epithelioid (bacillary) angiomatosis Neurologic disturbances Facial palsy Trigeminal neuralgia Fungal infection other than candidiasis Cryptococcus neoformans Geotrichum candidum Histoplasma capsulatum Mucoraceae (mucormycosis/zygomycosis) Aspergillus flavus Recurrent aphthous stomatitis Viral infections Cytomegalovirus Molluscum contagiosum

Source: EC-Clearinghouse classification, 1993.

Appendix 2: List of Some Anti-retroviral Drugs

CLASS OF DRUG	MODE OF ACTION, GENERIC AND OTHER NAMES
Fusion Inhibitor (FI)	Binds to active site of viral protease enzyme, preventing processing of viral proteins into functional forms
	Enfuvirtide (Fuzeon, T20)
Nucleoside Reverse-transcriptase Inhibitor (NRTI)	False nucleotides that, when incorporated by reverse transcriptase, prevent addition of further nucleotides
	Abacavir (Ziagen, ABC)* Abacavir + lamivudine (Epzicom) Abacavir + lamivudine + zidovudine (Trizivir) Didanosine (Videx, ddI)* Emtricitabine (Emtriva, FTC, Coviracil) Zidovudine (Retrovir, AZT, ZDV)* Lamivudine (EpiVir, 3TC)* Lamivudine + zidovudine (Combivir) Stavudine (Zerit, d4T)* Tenofovir (Viread, TDF) Zalcitabine (Hivid, ddC) Emtricitabine + tenofovir DF (Truvada)
Non-nucleoside Reverse-transcriptase Inhibitor (NNRTI)	Binds to reverse transcriptase and inhibit its activity
	Delavirdine (Rescriptor, DLV) Nevirapine (Viramune, NVP)* Efavirenz (Sustiva, EFV)*
Protease Inhibitor (PI)	Binds to active site of viral protease enzyme, preventing processing of viral proteins into functional forms
	Amprenavir (Agenerase, APV) Atazanavir (Reyataz, ATV) Fosamprenavir (Lexiva, FPV) Indinavir (Crixivan, IDV) Lopinavir/ritonavir (Kaletra, LPV/r)* Saquinavir (Fortovase, SQV, Invirase) Ritonavir (Norvir, RTV) Nelfinavir (Viracept, NFV)

*Available at Thika District Hospital CCC.

Appendix 3: Consent Information and Consent Form

Title of Study: Oral Lesions Associated with HIV/AIDS in Patients Receiving HAART at the Comprehensive Care Centre at Thika District Hospital, Kenya.

You are invited to participate in this research study of the relationship between HIV/AIDS, ARV therapy and HIV-related diseases of the mouth (oral thrush). You were selected as a possible participant because you have been tested and confirmed HIV positive. We ask that you read this form and ask any questions you may have before agreeing to be in the study.

This study is being conducted by Dr. John Kihama from the Institute of Tropical Medicine and Infectious Diseases, Jomo Kenyatta University of Agriculture and Technology.

Study Purpose

The purpose of the study is to find out if the HIV-related diseases of the mouth can be used to determine someone's response to ART. If this turns out to be a good sign of response then it can be used as a cheaper and less painful way of monitoring patients instead of using blood.

Study Procedures

If you agree to take part in this study,

- 1) We shall ask you a few questions whose answers we shall note on paper.
- 2) We shall briefly examine your mouth for diseases related to HIV infection and then request to take a photograph of the inside of your mouth without including your face.
- 3) We shall gently wipe any diseased part in the mouth with a wooden spatula for laboratory testing.

- 4) Finally we shall look at your hospital file to get information on date confirmed HIV positive, date started on HAART, HAART combination, other concurrent medications and illnesses treated in the near past.

Risks of Study Participation

This study has few known risks. Although we shall write your details on paper no other person will be allowed to read this information except the researcher. Information likely to be accessed by other persons directly involved in this study will be coded to make sure such people cannot identify you.

I wish to confirm that all materials and instruments we shall use for examination in this study are new, sterile and clean, and that they shall only be used on one person.

Benefits of Participating in our study

You will not be charged for the tests that we shall do although their results can be released to you on request. Any illness we find in the mouth that needs treatment will be treated free of charge. You will be referred for treatment of conditions that we are unable to treat in this facility.

The researcher will also give you advice on maintenance of good mouth hygiene.

Study Costs

Taking part in this study will not involve any payment for those procedures we perform.

Research Related Injury

There are almost no chances of you getting an injury in the course of our study. Examination of the mouth shall be done gently so as to avoid inducing vomiting or injuring your mouth. However, in the event that this research activity results in an injury, for

example through the our use of the tongue depressor, treatment will be available, including first aid, emergency treatment and follow-up care as needed at no cost to you.

Confidentiality

The records of this study will be kept private. Any publications or presentations arising from this study will not include any information that will make it possible to identify you as a subject. Samples collected for use in the laboratory will be kept under lock and key by the researcher himself and will not have any identifying information. Although the photographs of the mouth will be kept, they will only be used by the researcher himself. Your record for the study may, however, be reviewed by officials from the Institute of Tropical Medicine and Infectious Diseases (ITROMID, KEMRI) or Jomo Kenyatta University of Agriculture and Technology. If the records are reviewed, the officials will protect your privacy.

Voluntary Nature of the Study

Participation in this study is voluntary. Your decision whether or not to participate in this study will not affect your current or future relations with this hospital or the other institutions involved. If you decide to participate, you are free to withdraw at any time without affecting those relationships.

Contacts and Questions

The researcher conducting this study is Dr. John Kihama. You may ask any questions you have now, or if you have questions later, you are encouraged to contact him through telephone number 0722-360 646, E-mail: jmkihama@yahoo.com

If you have any questions or concerns regarding the study and would like to talk to someone other than the researcher(s), you are encouraged to contact the following;

The Director, Institute of tropical medicine and infectious diseases (ITROMID),
Jomo Kenyatta University of Agriculture and Technology,
P. O. Box 62000-00200 Nairobi.

Tel. 067 – 52711,

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OR

The Secretary/ Chairman
KEMRI Ethical Review Committee
P. O. Box 54840 – 00200 Nairobi
Tel 020- 2722541, 0722- 205901
E-mail: director@kemri.org

You will be given a copy of this form to keep for your records.

Statement of Consent

The above information has been read and explained to me. I have asked questions and have received answers. I consent to participate in the study.

Subject Names

Signature or left thumb print Date

Signature of person taking consent Date

Signature of Investigator Date

Appendix 4: Patient Questionnaire

PART 1

Serial number: ___ __ _

Date (DD/MM/YYYY) ___/___/___

PART 2

Socio-demographic profile

Participant's Name

Participant's Code

Date of Birth (dd/ mm/ yyyy) ___/___/___

Sex Male []

 Female []

Marital status:

 Married []

 Single []

 Other (specify)

Place of residence

Employment status

 Full time []

 Part time []

 Casual []

 Unemployed []

 Retired []

PART 3

HAART Compliance

How many doses have you missed in the last 24 hours?

None [] One [] Two [] Three []

How many doses have you missed in the last 3 days?

None [] One [] Two [] Three []

If you answered yes to the two questions above which of the following best describes your reason(s) for missing doses?

Side effects []

Forgot []

Inconveniencing frequency/ too many drugs []

Financial constraints []

Others (specify)

Information from Treatment Records

Date diagnosed HIV+ (dd/ mm/ yyyy).....

Date started on HAART (dd/ mm/ yyyy).....

Most recent CD4+ count cells/ μ l

HAART combination

Drug Name	once	twice	thrice
1	[]	[]	[]
2	[]	[]	[]
3	[]	[]	[]

Other medications

Pain relievers 1).....
2).....

Antibiotics (other than for TB) 1).....
2).....
3).....

Antifungal medication 1).....
2).....
3).....

Sleeping pills 1).....
2).....

TB Status No signs of TB []
TB suspected []
On TB Treatment []

Multivitamins 1).....
2).....

Haematinics 1).....
2).....

Other medication (specify)

Appendix 6: Adult Data Capture Sheet for Oral Lesions

CODE	CONDITION	CODE	LOCATION
1	No abnormal condition	A	mucosa of upper lip
2	Pseudomembraneous Candidiasis	B	mucosa of lower lip
3	Erythematous Candidiasis	C	mucosa around corner of mouth on R side
4	Angular Cheilitis	D	mucosa around corner of mouth on L side
5	Oral Hairy Leukoplakia	E	cheek mucosa on R side of patient
6	Kaposi's Sarcoma	F	cheek mucosa on L side of patient
7	Linear Gingival Erythema(LGE)	G	mucosa of upper jaw, between lip/cheek & gums
		H	mucosa of lower jaw, between lip/cheek & gums
		I	mucosa of gums of upper teeth
		J	mucosa of gums of lower teeth
		K	top surface of tongue
		L	sides of tongue
		M	under surface of tongue
		N	floor of the mouth
		O	mucosa of hard palate
		P	mucosa of soft palate
		Q	Other

Modified version from University of Western Cape

Condition	Location

	Yes	No
Photograph taken?		

Candida pseudo-hyphae detection with Gram stain Positive

Negative

Appendix 7: Gram Staining Procedure

A thin smear was made on a clean glass slide, dried in air and fixed by passing through flame of a burner.

- 1) The smear was then covered with crystal violet stain, kept for one minute.
- 2) The slide was washed with water, then covered with Gram iodine and left to stand for one minute.
- 3) The slide was then washed with water.
- 4) Decolouring was done with acetone/alcohol, rocking the slide gently for 10-15 seconds till the violet colour comes off the slide.
- 5) Washed with water immediately.
- 6) Counterstaining was done with saffranin. Counter-stain stands for 30 seconds.
- 7) Washed with water, blotted dry and examined under the oil immersion lens of a microscope.

Appendix 8: KEMRI/National Ethical Review Committee Study Approval.



KENYA MEDICAL RESEARCH INSTITUTE

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KEMRI/RES/7/3/1

26 AUGUST 2008

FROM: SECRETARY, KEMRI/NATIONAL ETHICAL REVIEW COMMITTEE

**THROUGH: DR.Y. KOMBE (PRINCIPAL INVESTIGATOR),
THE DIRECTOR, CPHR,
NAIROBI**

ForWARDED
[Signature]
25/08/08

TO: MR. JOHN M. KIHAMA (PRINCIPAL INVESTIGATOR)

**RE: SSC PROTOCOL No. 1410 (*REVISED*): CLINICAL OROPHARYNGEAL
CANDIDIASIS AND HAART IN HIV/AIDS PATIENTS ATTENDING
COMPREHENSIVE CARE CENTRE AT THIKA DISTRICT, HOSPITAL,
KENYA**

Dear Sir,

This is to inform you that during the 157th meeting of KEMRI/National Ethical Review Committee held on 26th August 2008, the provisional approval for the implementation of the research study by the ERC Chair on Wednesday, 6 August 2008, was ratified by the full Committee.

You may continue with the study.

Sincerely,

CRAW

**C. WASUNNA,
FOR: SECRETARY,
KEMRI/NATIONAL ETHICAL REVIEW COMMITTEE**