A COMPARISON OF CHRONIC PERIODONTITIS BETWEEN HIV-SEROPOSITIVE SUBJECTS AND THE GENERAL POPULATION OF THE GA-RANKUWA AREA, SOUTH AFRICA

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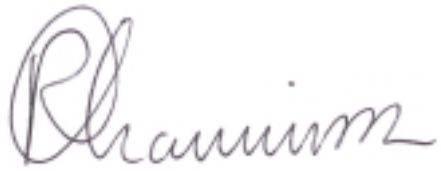
DECLARATION

I, Razia Abdool Gafaar Khammissa declare that this research report is my own work. It is being submitted for the degree of Master of Science in Dentistry to 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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28 day of May, 2008

DEDICATION

I want to dedicate this to my late dad whom I loved very much. Without his guidance, values and wisdom, this Masters degree would not have been accomplished.

Many thanks to my mum and family for the ongoing support and motivation to further accomplish my academic career.

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I would like to thank Professor Feller, Professor Altini and Professor Fatti for their hard work, professionalism and ongoing guidance.

ABSTRACT

Aim

The aim of this study is to compare the degree of severity of chronic periodontitis in HIV-seropositive subjects with chronic periodontitis to control subjects with chronic periodontitis, in the Ga-Rankuwa area in South Africa.

Methods

Two cohorts of subjects with chronic periodontitis were recruited for this study over a period of time: thirty HIV-seropositive subjects; and 30 control subjects presumed to be HIV - seronegative and apparently in good health.

Results

When all the periodontal indices were compared and evaluated there was no association between HIV-serostatus and the periodontal indices. When periodontal indices were compared between HIV-seropositive subjects using highly active anti-retroviral therapy (HAART), and HAART-naïve subjects, there was no statistical differences regarding gingival recession, plaque index and bleeding index. However, the mean pocket depth in HAART-naïve seropositive subjects was slightly greater than in HIV-seropositive subjects using HAART. Correlation coefficient of mean pocket depth in relation to log CD4+ T cell count in the HIV-seropositive HAART-naïve group of subjects showed a significant negative correlation (P = -0.947), but there was no correlation between the mean gingival recession values and the log CD4+ T cell counts in the same group (P=0.303*)*. For the HIV-seropositive subjects using HAART the correlation coefficient test failed to show significant statistical relationships between log CD4+ T cell count and mean pocket depth (P=0.903*)* and mean gingival recession (P=0.312*)* in HIV-seropositive subjects using HAART.

Conclusion

HIV-seropositive subjects with chronic periodontitis show clinical manifestations of similar degree of their periodontal disease to those of healthy control subjects with chronic

periodontitis, with no differences in the mean pocket depth, gingival recession, plaque index and bleeding index.

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CHAPTER 1

1.0 INTRODUCTION

The relationship between chronic periodontitis and HIV infection is not clear and a considerable difference in opinion exists regarding the prevalence of conventional chronic periodontitis among HIV-seropositive subjects (Holmstrup & Glick, 2002; Stanford & Rees, 2003). Microbiological studies have failed to detect any major differences in the subgingival microbial flora of HIV-seropositive subjects with chronic periodontitis compared to HIV-seronegative controls (Zambon, Reynolds & Genco, 1990; Moore, Moore & Riley, 1993) and the humoral immune response to the periodontopathic bacteria is similar in both groups (Yeung, Taylor & Sherson, 2002).

Some authors reported a higher prevalence of periodontal attachment loss and a more rapid progression of periodontal disease over time in HIV-seropositive subjects compared to HIV-seronegative controls (Tomar, Swango & Kleinman, 1995; Robinson, Scheiham & Challacombe, 1996; Robinson, Boulter & Birnbaum, 2000). In addition, it has been reported that a great portion of the periodontal attachment loss seen in HIV-seropositive subjects with chronic periodontitis is expressed by the presence of localised recession, rather than the development of deep periodontal pockets as in conventional chronic periodontitis in HIV-seronegative subjects (Robinson, et al., 1996; Robinson, 1997; McKaig, Patton & Thomas, 2000; Ryder, 2002). However, others failed to document differences between HIV-seropositive and -seronegative subjects with chronic periodontitis regarding the natural course of the periodontal disease (Scheutz, et al., 1997; Matee, Nguvumali & Lembariti, 1999).

This considerable difference of opinion regarding the natural course of chronic periodontitis in HIV-seropositive subjects may be a cause of confusion in respect of the periodontal management of HIV-infected subjects.

CHAPTER 2

2.0 AIMS OF THE STUDY

- The aim of this study is to compare the degree of severity of chronic periodontitis in HIV-seropositive subjects with chronic periodontitis to control subjects with chronic periodontitis, in the Ga-Rankuwa area in South Africa.
- Pocket depth (PD), gingival recession (GR), plaque index (PI) and bleeding index (BI) were compared by HIV-serostatus, the use of HAART, and CD4+ T cell count.

CHAPTER 3

3.0 LITERATURE REVIEW

3.1 Chronic periodontitis

Periodontitis is defined as an "inflammatory disease of the supporting tissues of the teeth caused by specific micro-organisms, resulting in progressive destruction of the periodontal ligament and alveolar bone with pocket formation, recession or both" (Novak, 2006). Chronic periodontitis is the most frequent occurring form of periodontitis, occurs most commonly in adults, may affect a variable number of teeth, is associated with the presence of dentogingival plaque, and has a variable rate of progression (International workshop for the classification of periodontal disease. Consensus Report, 1999).

Chronic periodontitis can be further classified by the extent and severity. Chronic periodontitis is defined as localised when ≤30% of sites are affected and generalised if >30% of sites are affected. The severity of chronic periodontitis is determined by the amount of clinical attachment loss (CAL) and is catergorised into slight (1-2mm CAL), moderate (3-4mm CAL) and severe (≥5mm CAL) (International workshop for the classification of periodontal disease. Consensus Report, 1999).

3.2 Pathogenic mechanisms of chronic periodontitis

3.2.1 Humoral and cellular immunity in chronic periodontitis

While the dentogingival biofilm is critical to the development of chronic periodontitis, the vast majority of the periodontal tissue destruction that occurs in chronic periodontitis is the outcome of immunoinflammatory responses of the host to the dentogingival biofilm challenge rather than the outcome of the direct virulence effect of the periodontopathic bacteria (Marshall, 2004). These responses may act as a double-edged sword: one edge is protective and fighting the periodontopathic micro-organism, while the other edge causes periodontal tissue destruction (Teng, 2003).

Innate immune cells in the periodontium (macrophages, monocytes, natural killer cells, polymorphonuclear leukocytes, Langerhans cells and other dendritic cells, resident mucosal cells) become activated following their encounter with periodontopathic bacteria and produce and release a variety of proinflammatory cytokines (IL-1, IL-6, TNF-α) and inflammatory mediators (PGE_2, matrix metalloproteinases), that singularly or in combination can cause extracellular matrix degradation and alveolar bone resorption (Zambon, 1996; Offenbacher, 1996). The innate immune cells, and the modified tissue environment (through the change in cytokine profile) subsequently activate naïve adaptive immune cells including T and B lymphocytes that generate an adaptive immune response (humoral and cell mediated) towards the periodontopathic bacteria (Zambon, 1996; Baker, 2000; Teng, 2003).

The humoral immune response is mounted parallel to, and immediately after the innate response and is an early event in the response toward the periodontopathic bacteria. Activated

B lymphocytes differentiate into plasma cells producing antibodies. Antigen-specific antibodies are able to trigger complement-mediated lysis, antibody-dependent cytotoxicity, antibody dependent neutralisation and opsonisation. These humoral responses against periodontopathic bacteria in the dentogingival biofilm are protective to the host and it is believed that the humoral arm of the immune system does not participate in periodontal tissue destruction. However, it is not always effective (Kinane, Lappin & Koulouri, 1999, Kinane & Lappin, 2001; Teng, 2003). If it is not successful in eradicating the periodontopathic bacteria, the cell mediated immune response is fully immobilised as a second line of defence to fight the periodontopathic bacteria (Offenbacher, 1996; Teng, 2003).

It appears that CD8+ T cells do not have a direct role in fighting periodontal pathogens. They may have an indirect protective role through regulating other components of the immune responses and fighting viruses within periodontal tissues, and it is likely that they do not contribute to periodontal tissue destruction during periodontal disease progression (Teng, 2003). On the other hand CD4+ T helper cells have a major role in the chain of events that lead to alveolar bone loss and periodontal disease progression (Theill, Boyle & Penninger, 2002; Taubman, Valverde & Han, 2005).

Following activation by dedicated antigen presenting cells (APC) (macrophages/monocytes, B lymphocytes, dendritic cells, Langerhans cells) which present periodontopathic bacterial antigens in the context of MHC class II molecules, CD4+ T helper (Th) cells can differentiate into two distinct subset phenotype : Th1 cells that express IL-12, IL-2, INF-γ and TNF-α/B (type 1 cytokines) and are primarily involved in cell mediated immunity, and Th2 cells that express IL-4, IL-5, IL-6, IL-10 and IL-13 (type 2 cytokines), that are mainly involved in humoral (antibody-mediated) immune response (Taylor, Preshaw & Donaldson, 2004). The

generation of a specific Th subtype (Th1 versus Th2) is complex and depends on the type of cytokines present in the local micro-environment, the antigen type and dose, the characteristics of the APC presentation, among other factors (Spellberg & Edwards, 2001; Yamazaki & Nakajima, 2004). The balance between Th1/Th2 cells in the periodontium is dynamic and may shift from one predominant subtype to the other depending on the local circumstances. In the pathogenesis of periodontal disease this balance is important and dictates if the mounted immune response will protect the host against periodontal infection or will be pathogenic and contributes toward progressive periodontal tissue destruction (Seymour & Gemmel, 2001; Teng, 2003).

There are conflicting reports in the literature regarding which type of the Th response is associated with a stable periodontal lesion versus a progressive one. A number of studies support the view that Th2 type immune response is associated with a stable lesion while a Th1 response is responsible for periodontal disease progression (Takeichi, Haber & Kawai, 2000; Taubman & Kawai, 2001). However, other reports favour the concept that periodontal disease progression is associated with the predominance of Th2 type immune response (Gemmel & Seymour, 2004; Taylor, et al., 2004).

In principle the immune system is employing Th1 type responses to protect the host against many bacterial infections, and it switches to a Th2 type response when the micro-organisms are cleared in order to re-establish homeostasis (Spellberg, et al., 2001). It is reasonable to assume therefore, that in active periodontal disease, both Th1 and Th2 cytokine profiles will be expressed; Th1 type inflammatory cytokines mediate periodontal tissue destruction while Th2 type cytokines are engaged in tissue repair and homeostasis during the inflammatory process (Roberts, McCaffery & Michalek, 1997; Kinane, et al., 2001).

In any event, gingival lesions from subjects with chronic periodontitis demonstrate highly variable cytokine profile, and the lymphocytic cell population that infiltrate the diseased periodontal tissues changes with the progression of the specific inflammatory diseased sites (Yamazaki, et al., 2004).

Both Th1 and Th2 cells with some of their associated cytokines are involved in alveolar bone loss during periodontal disease progression (Teng, 2002, Teng, 2003). Th1 cell associated cytokines are involved in the production of pro-inflammatory cytokines (IL-1, TNF-α), that can mediate alveolar bone destruction while Th2 cell associated cytokines have anti-inflammatory characteristics and are involved in the biological processes of tissue repair and remodeling (Kinane, et al., 2001; Teng 2003). Furthermore, Th2 cells take part in the induction of B cell-mediated humoral immune response, that are protective against periodontal infection (Teng, 2003). Th1 cell mediated immunity on the other hand, is characterised by a macrophage/T cell response.

Interferon gamma (IFN-γ) secreted by Th1 cells activate macrophages to increase phagocytosis, oxidative burst metabolites, and intracellular killing of micro-organisms. INF-γ also mediates the secretion of inflammatory substances and cytokines such as prostaglandins, TNF-α and IL-1 which perpetuates an inflammatory reaction that causes periodontal tissue damage (Yamazaki, et al., 2004). Alteration in the balance of Th1/Th2 cytokines may shift the immune response away from protection of the host from periodontopathic infection, towards host derived periodontal tissue damage and progression of periodontal disease.

Receptor activator of nuclear factor-κB (RANK) is a protein member of the TNF family. It is present on the surface of osteoclasts and their precursor cells. Its ligand (RANKL) can be

expressed on osteoblasts, T lymphocytes and bone marrow stromal cells. In the presence of colony-stimulating factor-1 (CSF-1), RANK-RANKL interaction on osteoclast precursor generates activation and differentiation of osteoclasts and subsequent alveolar bone resorption (Teng, 2003; Taubman, et al., 2005). Osteoprotegerin (OPG) is a soluble decoy receptor for RANKL, and by binding to RANKL, OPG inhibits osteoclastogenesis (Baker, 2000). RANKL – OPG interaction is regulated by hormones (Vitamin D, estrogen, glucocorticoids) and cytokines (TNF-α, IL-1, IL-4, IL-6), and the balance of this interaction dictates osteoblastogenic versus osteoclastogenic processes (Theill, et al., 2002; Teng, 2003).

Periodontopathic bacteria trigger the expression of RANKL on CD4+ T helper cells that result in osteoclast activation and alveolar bone loss during periodontal disease progression (Teng, 2003; Taubman, et al., 2005). In addition, activated T cells produce cytokines such as TNF-α, IL-11, IL-17 and INF-γ that lead to increased RANKL expression on osteoblasts. This results in T cell-mediated indirect alveolar bone resorption (Taubman, et al., 2005). In addition, INF-γ secreted by CD4+ Th1 cells can upregulate the expressions of MHC class II and other regulatory molecules on APC, leukocytes and mesenchymal cells, which may further recruit other signaling molecules associated with bone remodeling (Teng, 2003).

3.3 Periodontopathic bacteria and chronic periodontitis

The microbial flora of periodontal lesions in HIV-seronegative and -seropositive subjects is similar regarding the periodontopathic bacteria that populate the dentogingival biofilm. The periodontopathic bacteria are mainly gram-negative anaerobes and include *Porphyromonas gingivalis, Fusobacterium nucleatum, Actinobacillus actinomycetemcomitans, Eikenella*

coroddens, Wolinella recta, among other species (Robinson, Adegboye & Rowland, 2002); and HIV-seropositive subjects with chronic periodontitis do not harbour higher counts of periodontopathic bacteria (Ģoncalves, Ferreira & Silva, 2005).

However, HIV-seropositive subjects show elevated levels of opportunistic bacteria in their oral cavities that usually are not implicated in the development and progression of chronic periodontitis, and elevated levels of fungal and viral micro-organisms (Ryder, 2002). Some extra-oral bacterial species, not common in HIV-seronegative subgingival microflora can be found in HIV-seropositive subjects and include *Enterobacteria spp, Pseudomonas aeruginosa, Clostridium difficile, Clostridium clostridiiforme* and M*ycoplasma salivarium* (Robinson, et al., 2002).

The significance of the presence of these extra-oral micro-organisms regarding the pathogenesis and the clinical manifestation of chronic periodontitis in HIV-seropositive subjects is difficult to determine.

3.4 Antigen presenting dendritic cells and periodontitis

Dendritic cells (DC) are dedicated antigen presenting cells which have the capacity to induce and orchestrate primary immune responses, immunological memory, and immunological tolerance (Banchereau, Brier & Caux, 2000). At least three subsets of human DC are known. Myeloid CD34+ progenitors differentiate into CD14+ CD11+ dendritic cell precursor cells in the peripheral blood. These cells give rise to dermal or interstitial dendritic cells. The same myeloid CD34+ progenitors may differentiate also into CD14- CD11+ Langerhan precursors

that give rise to Langerhan cells in response to stimulation by granulocyte/macrophage colony-stimulating factor (GM-CSF), IL-4, and transforming growth factor (TGF)-β. In addition lymphoid CD34+ progenitors can differentiate into CD14- CD11- IL-3R+ precussors in the blood (plasmacytoid T cells) and further differentiate into immature dendritic cells in the presence of IL-3 (Banchereau, et al., 2000; Pulendran, Maraskovsky & Banchereau, 2001).

In principle, immature dendritic cell, following encountering and capturing specific antigens, migrate to lymphoid tissues, where they mature and present the antigen in the context of the MHC to T helper cells and elicit specific immune responses (Banchereau, et al., 2000). Distinct dendritic cell subsets can initiate different Th responses; myeloid CD11+ DC usually promote Th1 type response while CD11- plasmacytoid dendritic cells promote Th2 type response (Pulendran, et al., 2001).

The respective polarisation of the Th type response depends on the distinct subset type of the dendritic cell and its maturation stage, the cytokine profile of the micro-environment and the type of pathogen. As the immune response evolves and the infection changes its dynamics, the initial given Th response may change accordingly through modifications in the function of the evolved DC (Pulendran, et al., 2001).

Oral epithelial Langerhans cells are part of the dendritic cell family and are important members of the innate arm of the immune system (Cutler & Jotwani, 2004). They are located in the basal and suprabasal layer of the epithelium and express on their cell membrane the following markers: MHC class II molecules, CD1a and CD4 glycoproteins, among others. Langerhan cells in its immature state, recognise and capture microbial antigens, then migrate

to regional lymph nodes via the lamina propia where they become mature dendritic cells expressing CD83 glycoprotein on the cell membrane. The mature dendritic cells are efficient antigen presenting cells; following intracellular processing of the captured antigens, the resultant peptides are loaded on MHC class II molecules on the cell surface for presentation to CD4+ T cells. As a result of this process, CD4+ T cell undergo activation, priming and antigen-specific clonal expansion (Cutler, et al., 2004).

In immunocompetent subjects with chronic periodontitis there is an increase in the number of Langerhan cells in the pocket epithelium and in CD83 mature dendritic cells in the adjacent lamina propia (Cutler, et al., 2004). In response to the challenge of the periodontopathic bacteria and the subsequent production of pro-inflammatory cytokines by other immunoregulatory cells, Langerhans cells are mobilised to the pocket epithelium, capturing the microbial antigens, migrate to lymph nodes as mature dendritic cells, presenting the captured antigen to naïve CD4+ T cells, and activate them. These activated CD4+ T cells undergo differentiation and clonal expansion and migrate from the lymph node to the lamina propia of the pocket epithelium to take part in the local immune response against the periodontopathic bacteria. These mature dendritic cells and activated T cells form lymphoid foci's in the lamina propia of the periodontal pocket wall (Cutler, et al., 2004). To these lymphoid foci in the lamina propria, Langerhan cells, in the presence of pro-inflammatory cytokines, migrate from the gingival epithelium, mature into CD83+ professional antigen presenting dendritic cells, and interact with memory CD4+ T cells without having first to migrate to the lymph nodes.

3.5 Immunopathogenic mechanisms of HIV infection

The HIV virus is a member of the Retrovirus family. The HIV genome consists of three major (Gag, Pol, Env) and six accessory (Tat, Rev, Vif, Vpr, Nef, Vpu) genes. Pol codes for the major enzymes reverse transcriptase, Gag codes for internal structural proteins and Env codes for the evelope glycoprotein gp120 and transmembrane protein gp41. HIV replication begins when gp120 protein binds to the CD4 receptor on the hosts T lymphocyte. For HIV to fuse to, and enter its target cell efficiently, binding to a co-receptor is required. The two major co-receptors for HIV are CCR5 and CXCR4. HIV strains using CCR5 as a co-receptor are referred to as R5 viruses and strains that use the CXCR4 as a coreceptor are referred to as X4 viruses. R5 viruses infect mainly macrophages and predominate during the initial stages of HIV disease, whereas the synctium inducing X4 variants emerge during a later stage of the infection (Yin, Jay & Grbic, 2007).

HIV disease is a chronic, progressive disease with variable periods of clinical latency. The hallmark of HIV disease is a profound immunodeficiency, resulting primarily from progressive demunition of CD4+ T cells. The HIV-associated direct mechanisms causing depletion of CD4+ T cells include accumulation of unintergrated reverse-transcribed viral DNA in the infected cell, synctium formation, increase in plasma membrane permeability, induction of apoptosis, and budding of viruses from HIV infected cells. Indirect mechanisms of HIV-induced depletion of CD4+ T cells include a CD8+ cytotoxic T cell response, mutation of HIV gene products and HIV-associated thymic dysfunction (Yin, et al., 2007; Feller & Lemmer, 2007b)

Continous budding of viruses from infected cells can cause cell membrane disruption with increased permeability resulting in death of the cell. Specific HIV proteins can induce cell membrane permeability causing cell death. Increased cellular toxicity is induced by the build-up of unintergrated linear viral DNA in the infected cell. HIV specific cytotoxic T-lymphocytes (CTL) recognises the HIV-infected cells in an MHC class I restricted manner. This cell to cell contact activates the mechanisms for lysis of HIV-infected cells. Furthermore, HIV nef protein can induce apoptosis of bystander CD4+ T cells (Paranjape, 2005).

In addition to the CD4+ T-cell depletion, HIV infection is characterised by functional defects of macrophages. Most of the HIV infected macrophages are found in tissues and not in the peripheral blood. Monocytes and macrophages provide a safe vehicle for transport of HIV to various parts of the body, as well as function as a reservoir, whose output remains largely protected from the hosts defence mechanisms. As the CD4+ T cell numbers decline parallel to the HIV disease progression, macrophages may be the major vehicle of continued viral replication (Cotran, Kumar & Collins, 1999).

Advanced HIV disease is characterised by defects of cytotoxic CD8+ T cell response. The profound loss of CD4+ T cells in HIV infection has an impact on the CTL response. For maturation of CD8+ cytotoxic T cells to occur, IL-2 is required. IL-2 is secreted by HIV-specific CD4+ and CD8+ T cells. With the depletion of CD4+ T cells, secretion of IL-2 is also diminished, resulting in an inability of CTL to control HIV replication (Altfeld & Rosenberg, 2000).

HIV progression is characterised by high levels of HIV viremia and a reduction in the number of CD8+ cytotoxic T cells. Reduction of CD8+ cytotoxic T cells is a result of defects in maturation as well as clonal exhaustion (Northfield, Harcourt & Lucas, 2005). CD8+ cytotoxic T cells are chronically activated due to persistent exposure to high HIV levels. Disturbances in the maturation process of memory CD8+ T cells and premature loss of existing effector T cells is a consequence of the chronic activation and excessive proliferation of these cells (Appay & Rowland-Jones, 2002; Northfield, et al., 2005).

As HIV disease advances, in the absence of highly active anti-retroviral treatment (HAART) and in the presence of persistent high plasma viral load, HIV-seropositive subjects experience a shift from T-helper (Th)-1 dominant cellular immunity mediated by interleukin (IL)-2, IL-12 and interferon-gamma to a Th-2 dominant humoral response mediated by IL-4, IL-5 and IL-10, and a suppressed Th-1 cytokine response (Imami, Antonopoulos & Hardy, 1999; Handa & Bingham, 2001). This alteration in the cytokine network during the course of HIV disease promotes the dysregulation of immune responses and is associated with a number of inflammatory conditions.

3.6 HIV disease and highly active antiretroviral treatment (HAART)

With the introduction of highly active antiretroviral therapy (HAART) in 1996 as a standard of care in HIV-infected patients, there has been a decrease in the frequencies of HIV-associated infections as well as a decrease in the incidence and prevalence of HIV-associated neoplasms (Palella, Delaney & Moorman, 1998). Despite the mild toxicity and adverse effects of HAART, the combination of drugs comprising protease inhibitors and reverse

nucleoside inhibitors have turned HIV disease into a chronic, controllable disease (Monini, Sgadari & Barillari, 2003).

HAART improves the immune function by suppressing HIV viral replication leading to an increase in the CD4+ T cell counts (Palella, et al., 1998; Douek, Picker & Koup, 2003) and resulting in an improved quality of life and prolonged survival (Palella, et al., 1998). In patients receiving HAART, the recovery of CD4+ T cell count is associated with the decrease in HIV replication and occurs in two phases. The first phase owing to the redistribution of T cells is associated with an increase in circulating memory T cells previously trapped in lymphoid tissue. The second or the repopulation phase usually occurs a few months after the introduction of HAART and is characterised by the production of naïve T cells (Palella, et al., 1998).

Antiretroviral therapy is recommended for all patients with symptomatic or asymptomatic HIV infection with CD4+ T cell counts of ≤ 200 cells/mm^3. Potentially life-threatening conditions such as lymphoma or tuberculosis may be decreased with the initiation of antiretroviral therapy at this stage (Yeni, Hammer & Hirsch, 2004). Generally, antiretroviral therapy is not recommended for patients having a CD4+ T cell count of >350 cells/mm^3 due to significant drug toxicity and the development of metabolic disorders and cardiovascular diseases. However, antiretroviral therapy is recommended in patients with a high viral load or a rapid diminution in CD4+ T cell counts even if their CD4+ T cell count is above 200 cells/mm^3 (Yeni, et al., 2004).

The currently available antiretroviral regimens cannot eradicate the HIV infection. This is due to the pool of latently infected CD4+ T cells established early in the course of HIV

infection and persists despite HAART-associated prolonged suppression of HIV replication (Chun, Stuyver & Mizell, 1997; Finzi, Hermankova & Pierson, 1997; Wong, Hezareh & Gunthard, 1997). The goals of HAART include suppression of viral load, reconstitution of immune function, reduction of HIV-related morbidity and mortality and improvement in the quality of life (Finzi, et al., 1997; Wong, et al., 1997).

Following the initiation of HAART, the immune system is partially reconstituted: there is a gain in the CD4+ T cell counts, an improvement in the functional competence of the immunoregulatory cells, and a shift from a predominant Th-2 type cytokine profile to a more normal balanced Th1/Th2 ratio (Finzi, et al., 1997).

3.7 Chronic periodontitis and HIV infection

The relationship between chronic periodontitis and HIV infection is not clear (Holmstrup, et al., 2002; Stanford, et al., 2003). Some authors reported a higher prevalence of periodontal attachment loss and a more rapid progression of periodontal disease over time in HIV-seropositive subjects compared to HIV-seronegative controls (Robinson, et al., 1996; Roberts, et al., 1997; McKaig, Thomas & Patton, 1998; Robinson, et al, 2000). In addition it has been reported that a great portion of the periodontal attachment loss seen in HIV-seropositive subjects with chronic periodontitis is expressed in the form of localised recession, rather than the development of deep periodontal pockets as in chronic periodontitis in HIV- seronegative subjects (Robinson, et al., 1996; Robinson, 1998; McKaig, et al., 2000; Ryder, 2002). However, others failed to document differences between HIV-seropositive and -seronegative

subjects with chronic periodontitis regarding the natural course of the periodontal disease (Scheutz, et al., 1997; Matee, et al., 1999).

Microbiological studies have failed to detect any major differences in the subgingival microbial flora of HIV-seropositive subjects compared to HIV-seronegative controls (Zambon, et al., 1990; Moore, et al., 1993) and the humoral immune response to the periodontopathic bacteria is similar in both groups of subjects (Yeung, et al., 2002).

In HIV-seropositive subjects with chronic periodontitis there is a significant reduction in Langerhan cell numbers in the pocket epithelium, compared with HIV-seronegative subjects with chronic periodontitis (Myint, Yuan & Schenck, 2000). It is therefore possible that the localised depletion of Langerhan cells in the oral epithelium of HIV-seropositive subjects may alter the local host immune response towards periodontopathic bacteria, and increase the susceptibility of the HIV-seropositive subjects to the development and progression of periodontal diseases (Myint, et al., 2000; Chou, Epstein & Cassol, 2000). The oral mucosal Langerhan cells are the target of HIV, and HIV p17 structural protein can be detected in oral Langerhan cells of HIV-seropositive subjects, particularly in patients with high HIV viral load. Such HIV-infected dendritic cells could be a target for a cytotoxic CD8+ T-cell response, resulting in the depletion of Langerhan cells in the HIV affected areas of the oral epithelium (Chou, et al., 2000). However, there is no evidence that this theoretical chain of events in fact promotes periodontal tissue destruction in HIV-seropositive subjects with chronic periodontitis.

It is important to note that HIV-infected epithelial cells and HIV-infected Langerhan cells (Chou, et al., 2000) might represent a possible significant risk for HIV transmission through

oral fluids, especially in the presence of active periodontal disease. Oral fluids contain saliva, epithelial and mononuclear cells, gingival crevicular serum transudate, and blood products (from active periodontal sites) which all might contain HIV. This might have significant implications for public health issues related to transmission of HIV infection (Chou, et al., 2000).

HIV-seropositive subjects with periodontal disease have greater number of herpesviruses in periodontal pockets than HIV-seronegative control subjects with similar pocket depth. Chronic periodontitis in HIV-seropositive subjects is associated with a higher prevalence of single or combined infection of HHV 6, 7, 8 and EBV than chronic periodontitis in HIV-seronegative subjects. Moreover, HHV-8 is found solely in periodontal lesions of HIV-seropositive subjects with chronic periodontitis. However these findings do not imply that viruses have a specific role in the initiation and progression of chronic periodontitis in HIV-seropositive subjects (Feller, Meyerov & Lemmer, 2007a; Slots, 2005).

It is a well-known fact, that HIV infected subjects have increased frequencies of viral infections due to their immuno-suppression. Therefore, it is not surprising that periodontal soft tissues of HIV-seropositive subjects harbour greater numbers of herpesviruses compared to periodontal soft tissues of HIV-seronegative subjects. However, it is difficult to determine if the increase in number of herpesviruses in periodontal pockets of HIV-seropositive subjects with chronic periodontitis has any clinical or pathological significance regarding the development and progression of periodontal tissue breakdown (Feller, et al., 2007a).

Candidal infection is associated with medical conditions characterised by suppressed cell mediated immunity including HIV infection, diabetes mellitus, immunosuppressive and

corticosteroid therapies among others (Feller, Buskin & Blignaut, 2005). HIV-seropositive subjects with chronic periodontitis demonstrate a higher prevalence of candida in their subgingival microbial flora obtained from periodontal pockets compared to immunocompetent subjects with chronic periodontitis. In one study (Jabra-Rizk, Ferreira & Sabet, 2001), 82% of HIV-seropositive examined subjects with periodontitis were positive for oral yeasts, and 66% of them harboured candida species in their subgingival samples (periodontal pockets >5mm). However the authors did not specify what type of periodontal disease was involved.

One can assume that the increased number of candida species in the periodontal pockets of HIV-seropositive subjects with chronic periodontitis has the potential to promote the local inflammatory process and to interfere with the local host immune response in the periodontal tissues. The outcome of such a process might be an exacerbation of the existing inflammatory process caused by the periodontopathic bacteria resulting in accelerated periodontal tissue breakdown. However there is no direct evidence to substantiate such an assumption (Feller, et al., 2005).

3.8 Wound healing and HIV infection

Wound healing is a dynamic continium process, comprising several overlapping phases that encompass coagulation, inflammation (early and late stages), parenchymal and mesenchymal cell proliferation (granulation tissue phase), epithelisation, maturation and remodeling. This process is clearly a multifactorial one involving complex interaction between immunoinflammatory cells (macrophages, monocytes, lymphocytes, dendritic cells, polymorphonuclear leukocytes, etc), mucosal resident cells (epithelial cells, fibroblasts,

osteoblasts, etc), and is orchestrated by cytokines, growth factors and other biological mediators in the micro-environment (Sedlarik, 1997; Monaco & Lawrence, 2003).

Clinical studies have failed to demonstrate that HIV-associated immune suppression leads to an increased incidence of impairment in wound healing (Luck, Logan & Benson, 1996). This can be explained by the fact that CD4+ T cells do not play a critical rôle (unlike monocytes/macrophages) in wound healing. In principle lymphocytes are observed in the wound healing process in the granulation tissue phase, approximately at the fifth day following injury (Park & Barbul, 2004). Animal studies on wound healing suggest that depletion of CD8+ T cells result in greater wound-breaking strengths and collagen synthesis, while depletion of CD4+ T cells has no effect on either mechanical strength or collagen formation. However, an overall depletion of T cells result in reduction in mechanical strength and collagen content of the wound (Park, et al., 2004).

In HIV-seropositive subjects free of pre-operative contamination, the incidence of wound infection following orthopaedic implant surgery is similar to that of HIV-seronegative controls, and low CD4 + T cell count does not seem to affect the incidence of post operative infection. However, when there is pre-operative contamination, the incidence of infection in HIV-seropositive subjects increases markedly compared to HIV-seronegative subjects with pre-operative contamination (Harrison, Lewis & Lavy, 2002).

HIV-seropositive subjects with CD4+ T cell counts lower than 100 cells/mm³ receiving emergency (palliative) and elective (curative) surgery will heal properly, but with 3-4 weeks delay, as long as opportunistic infection and underlying HIV-related malignancies are controlled. HIV-seropositive subjects who undergo anorectal surgery and laparotomy have

higher frequencies of impairment of wound healing than HIV-seronegative control subjects. It seems that surgical procedures performed on sites that harbour high bacterial loads (anorectal surgery, internal fixation of open fracture, laparotomy), may have higher risk for wound infection and delayed wound healing (Harrison, et al., 2002).

Clinical studies of HIV-seropositive subjects undergoing oral surgery, failed to identify any significant deficit in wound healing. Post-tooth extraction complications are uncommon in HIV-seropositive subjects, and these subjects do not experience an increased incidence of delayed healing when compared to HIV-seronegative subjects (Porter, Scully & Luker, 1993), and post operative antibiotics are not associated with less complications (Dodson, 1997).

One can deduct from these findings that the healing capacity of the periodontium remains functionally normal during HIV infection. Therefore it is probable that during chronic periodontitis, the healing potential of the diseased periodontal tissues remain very much unchanged in HIV-seropositive subjects, compared to immunocompetent subjects with chronic periodontitis. Moreover, following periodontal treatment, including different types of periodontal surgery, the response to treatment is the same in HIV-seropositive subjects and immunocompetent subjects (Dodson, 1997).

CHAPTER 4

4.0 MATERIALS AND METHODS

4.1 Subject population

Ethical approval was obtained from the University of Limpopo and University of Witwatersrand, Johannesburg. After obtaining an informed consent, 60 subjects, 30 HIV-seropositive and 30 healthy control subjects (presumed HIV-seronegative) with chronic periodontitis were recruited for this study. All participants were black persons from the Ga-Rankuwa area in South Africa (34 females, 26 males) between the ages of 18 and 45 years belonging to a similar socioeconomic background. The gender of the participants is shown in Table 1.

The groups of HIV-seropositive subjects were composed of 16 subjects receiving highly active anti-retroviral therapy (HAART) and 14 HAART-naïve subjects at the time of diagnosis of their chronic periodontitis. The HIV-serostatus was confirmed by enzyme-linked immunosorbent assay (ELISA) and western blot. The CD4+ T cell counts were performed for 13 subjects that had given informed consent. Six HIV-seropositive subjects had CD4+ T cell counts below 200 cells/mm^3.

4.2 Periodontal health status

Chronic periodontitis was diagnosed by a clinical examination and radiographic evidence of periodontal attachment loss by a single clinician (RK). Subjects were diagnosed with chronic periodontitis when at least three sites had pockets with probing depth ≥ 5mm and/or had gingival recession and there was radiographic evidence of alveolar bone loss (Figure 1). The periodontal indices measured were pocket depth (PD) (Figure 2), gingival recession (GR) (Figure 3), plaque index (PI) and bleeding index (BI). Periodontal PD measurements were taken at six sites per tooth (mesio-buccal, mid-buccal, disto-buccal, disto-lingual, mid-lingual and mesio-lingual) and the GR measurement were taken at two tooth sites (buccal or lingual) around the erupted teeth, excluding third molars and residual roots. The periodontal examination forms of all participants in this study are shown in appendix 2. The periodontal pocket depth and gingival recession measurements were done with a periodontal probe with Williams markings. Bleeding index per mouth was calculated by summing the measurements of the gingival tooth sites bleeding on periodontal probing (one possible measurement per tooth: bleeding or not bleeding) and dividing it by the number of teeth present in the mouth (appendix 1). The plaque index per mouth was calculated (after using disclosing solution) by summing the measurements of plaque present at four tooth sites (mesial, buccal, distal and lingual) and dividing it, by the number of teeth multiplied by four (appendix 2).

In this study, pocket depth (PD), gingival recession (GR), plaque index (PI) and bleeding index (BI) were compared by HIV-serostatus, the use of HAART and CD4+ T cell counts. CD4+ T cell counts were stratified into the following groups; CD4+ T cell count < 200 cells/mm^3, CD4+ T cell counts 200 – 500 cells/mm^3 and CD4+ T cell count > 500 cells/mm^3. HAART was defined as the use of at least two nucleoside reverse transcriptase inhibitors with

either a non-nucleoside reverse transcriptase inhibitor or a "boosted" protease inhibitor (Mortality and causes of death, 2005). CD4+ T cell counts were available for eight HIV-seropositive subjects using HAART and for five HAART-naïve subjects. There were only eight subjects that admitted to smoking, and because of this small number, smoking was not correlated to the periodontal indices.

No attempt was made to differentiate between active chronic periodontitis sites (suppuration from pockets, bleeding on probing, continuous loss of periodontal attachment) and stable chronic periodontitis sites with evidence of previous loss of periodontal attachment.

For the purpose of this study a mean pocket depth value per mouth and a mean gingival recession value per mouth were calculated in order to enable an easy comparison of these two important periodontal indices between the studied and the control group of patients.

The mean pocket depth per tooth was calculated by summing the measurements per tooth and dividing by the number of measurements. The mean pocket depth per mouth (per participant) was calculated by summing all the mean pocket depth per tooth in the mouth and dividing it by the number of teeth. The mean gingival recession per mouth was calculated by summing the measurements of the recessions of the involved teeth and dividing it by the number of teeth affected by recession.

Fig. 1 Pan x-ray showing moderate alveolar bone loss present in chronic periodontitis

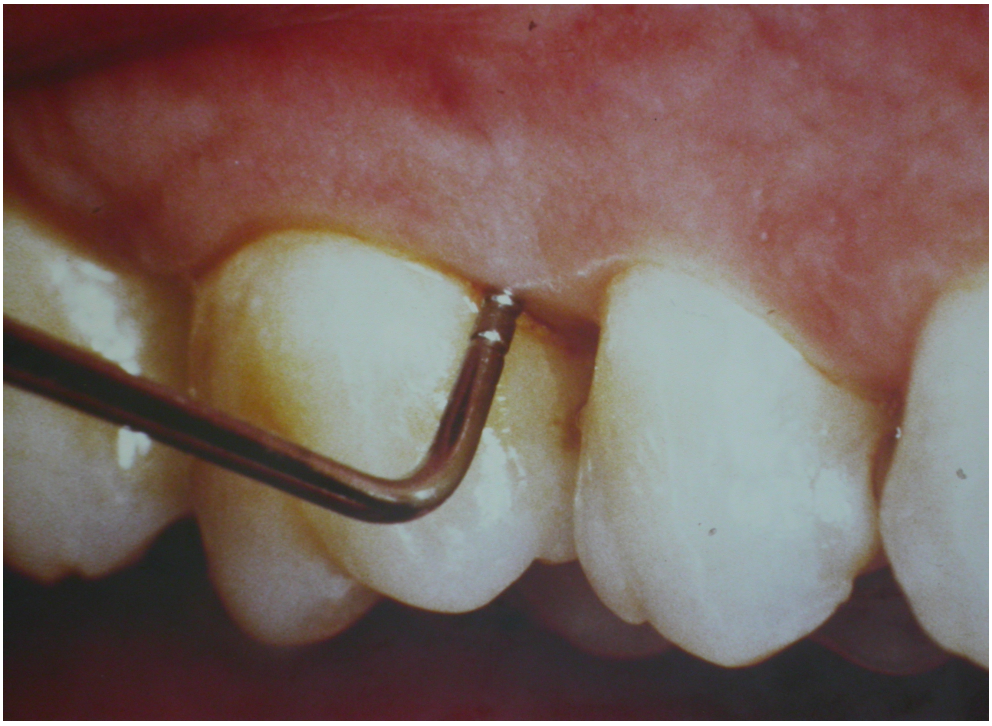

Fig. 2 Periodontal Probing

Fig. 3 Photograph showing gingival recession on mandibular 31, 32 and 41

4.3 Statistical analysis

The statistical test in the present study was used in other similar studies reported in the literature and recommended by a statistic expert (Prof Fatti). The statistical analysis was performed by the researcher (RK), and supervised by the statistician. The results of the statistical tests were analysed by the researcher and her supervisors and approved by the statistician.

All data were entered into the Microsoft Excel program and analysed using its data analysis package. Programs for performing ANOVA, *t*-tests, Pearson Correlation coefficients and histograms were performed to conduct statistical hypothesis tests and explore associations. P values of < 0.05 were regarded as statistically significant.

Single factor analysis of variance was used to test for differences in the periodontal indices between the healthy control subjects and the HIV-seropositive subjects with chronic periodontitis. The two-sample *t*-test was employed to test the differences in the periodontal indices between the HIV-seropositive HAART-naïve subjects and the HIV-seropositive subjects using HAART. The Pearson Correlation coefficient was used to test if there were significant relationships between the periodontal indices of the HIV-seropositive subjects to the log CD4+ T cell count.

A log transformation of the CD4+ T cell count was calculated to correct for its skew distribution, and for the different scales of measurement between these counts and the periodontal indices.

CHAPTER 5

5.0 RESULTS

When all participants were evaluated, there was no association between HIV-serostatus and periodontal indices. HIV-seropositive and control subjects with chronic periodontitis had similar mean PD, GR, PI and BI (Table 1), and gender had no influence on the results (data not shown). The mean periodontal indices were compared between HIV-seropositive and control subjects (Figures 4-7) using ANOVA and the mean P-values were as follows: P = 0.23 for PD, P = 0.82 for GR, P = 0.65 for PI and P = 0.59 for BI. Clearly none of them indicate significance at the $P < 0.05$ level (Table 2).

HIV-seropositive subjects using HAART with chronic periodontitis, and HAART-naïve subjects with chronic periodontitis had similar mean GR, PI and BI (Table 3). When periodontal indices were analysed using the *t*-test comparing HIV-seropositive subjects using HAART, and HAART-naïve subjects (Table 4), there was no statistical differences regarding mean GR (P=0.92), PI (P=0.32) and BI (P=0.53). However, the mean PD in HAART-naïve seropositive subjects was slightly greater than in HIV-seropositive subjects using HAART (P=0.01). The mean PD in the HAART-naïve seropositive subjects was 3.36mm vs 3.07mm in HIV-seropositive subjects using HAART.

Pearson Correlation coefficient of mean PD in relation to CD4+ T cell count in the HIV-seropositive HAART-naïve group of subjects showed a significant negative correlation (P = -0.947), but there was no correlation between the mean GR values and the CD4+ T cell counts in the same group (P=0.303*)* (Appendix 2).

For the HIV-seropositive subjects using HAART the Pearson Correlation coefficient test failed to show significant statistical relationships between CD4+ T cell count and mean PD (P=0.903*)* and mean GR (P=0.312*)* in HIV-seropositive subjects using HAART.

Unexpectedly, the mean CD4+ T cell count was higher in the HAART-naïve group of subjects than HIV-seropositive subjects using HAART. However, the CD4+ T cell counts were available only for a small number of subjects, eight subjects in the HAART-naïve group and five subjects in the HIV-seropositive subjects using HAART (Table 5), and the late introduction of HAART in HIV-seropositive subjects in South Africa could be responsible for these mean CD4+ T cell counts (Table 5).

Table 1 Epidemiological features and periodontal indices of HIV-seropositive subjects with chronic periodontitis, and control subjects with chronic periodontitis

	Presumably HIV-seronegative control subjects with chronic periodontitis	**HIV-seropositive subjects with chronic periodontitis**
Females	14	20
Males	16	10
Smokers	5	3
Pocket depths Mean STDEV*	3.196mm 0.58	3.205mm 0.32
Gingival recession Mean STDEV*	1.53mm 0.89	1.66mm 0.71
Number of gingival recessions STDEV*	195 5.11	202 5.46
Plaque Index Mean STDEV*	75.21% 23.6	75.59% 20.16
Bleeding Index Mean STDEV*	50.03% 19.4	47.3% 23.25

*STDEV – standard deviation

Table 2 Mean P-values for the different periodontal indices compared between HIV-seropositive and healthy control subjects using ANOVA

Periodontal indices	**P-value**
Pocket depth (PD)	0.23
Gingival recession (GR)	0.82
Plaque index (PI)	0.65
Bleeding index (BI)	0.59

None of them indicate significance at the $P<0.05$ level.

Table 3 Epidemiological features, periodontal indices and immunological indices of HIV-seropositive subjects using HAART, of HAART-naïve HIV-seropositive subjects, and of control subjects. All subjects had chronic periodontitis

	Presumably HIV-seronegative control subjects with chronic periodontitis	**HIV-seropositive subjects using HAART with chronic periodontitis**	**HAART-näive HIV-seropositive subjects with chronic periodontitis**
Females	14	12	8
Males	16	4	6
Smokers	5	2	1
Pocket depths Mean STDEV*	 3.196 0.58	 3.069 0.28	 3.359 0.30
Gingival recession Mean STDEV*	 1.53 0.89	 1.67 0.79	 1.64 0.64
Number of gingival recessions STDEV*	195 5.11	100 4.14	102 3.56
Plaque index Mean STDEV*	 75.21% 23.6	 71.85% 20.4	 79.34% 26.1
Bleeding index Mean STDEV*	 50.03% 23.25	 42.53% 18.3	 52.05% 22.1
CD4+ Count Mean STDEV*	Not available	171.63cell/mm³ 108.94	257.44cell/mm³ 171.25

*STDEV – standard deviation

Table 4 Mean P-values for the different periodontal indices compared between HIV-seropositive subjects using HAART and HAART-naïve seropositive subjects using the *t*-test

Periodontal indices	**P-value**
Pocket depth (PD)	0.01*
Gingival recession (GR)	0.92
Plaque index (PI)	0.32
Bleeding index (BI)	0.53

*Significant at the P<0.05 level. The mean PD in HAART-naïve seropositive subjects was slightly greater than in HIV-seropositive subjects using HAART.

Table 5 The categories of $CD4^+$ T cell levels of the HIV-seropostive subjects using HAART, and HIV-seropositive HAART-naïve subjects.

	HIV-seropositive subjects using HAART	**HIV-seropositive HAART-naïve subjects**
CD4+ count < 200	4	2
CD4+ count 200 - 500	4	2
CD4+ count > 500	0	1
Mean CD4+ Count	172cell/mm³	257cell/mm³

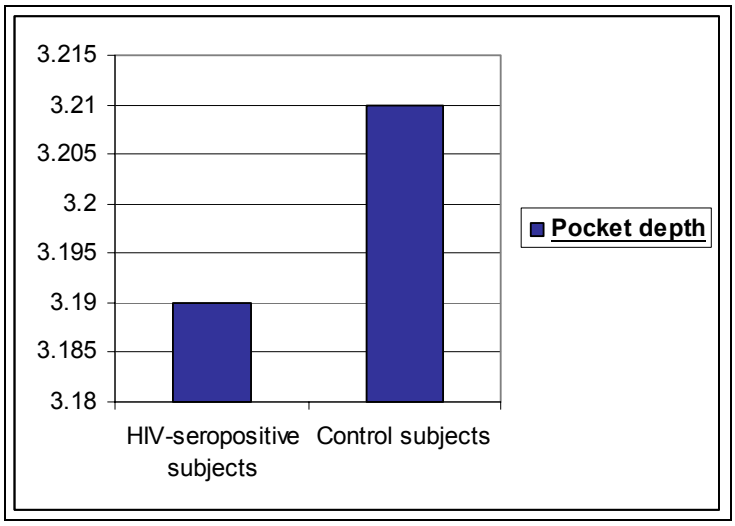

Fig. 4 Mean pocket depth in mm in HIV-serpositive and control subjects

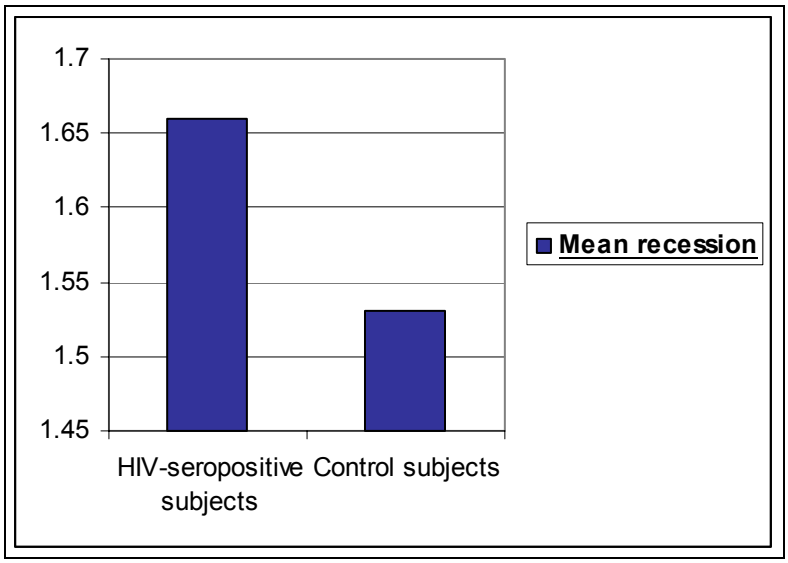

Fig. 5 Mean gingival recession sites in HIV-seropositive and control subjects

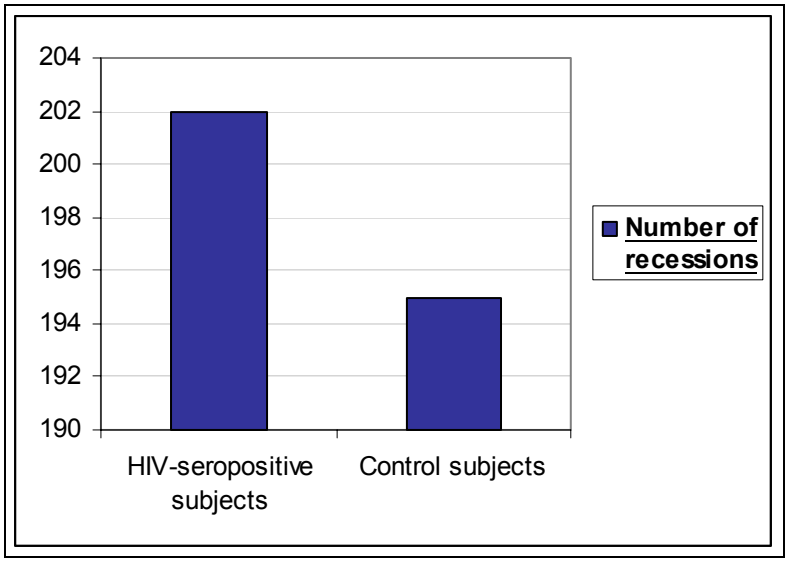

Fig. 6 Mean number of gingival recession sites in HIV-seropositive and control subjects

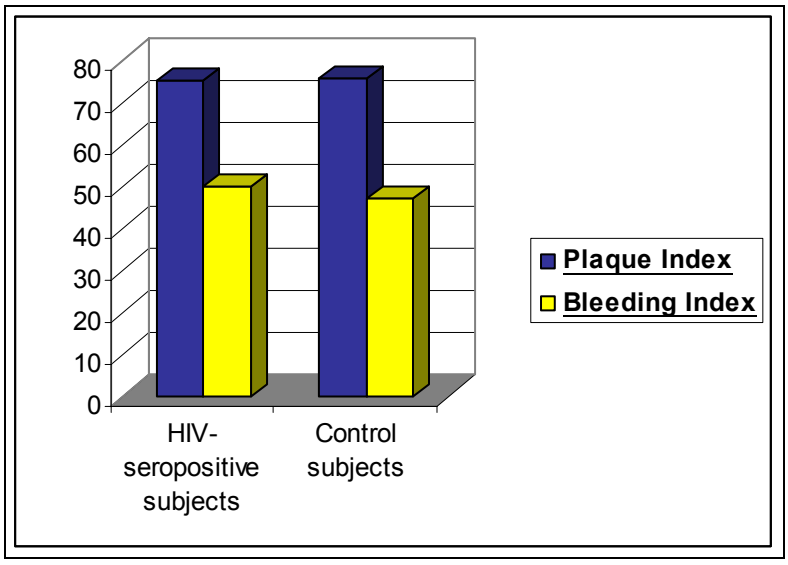

Fig. 7 Mean plaque index and bleeding index in HIV-seropositive and control subjects

CHAPTER 6

6.0 DISCUSSION

Some investigators reported that periodontal disease in HIV-seropositive subjects is characterised by increased periodontal attachment loss and accelerated periodontal disease progression compared to periodontal disease in HIV-seronegative subjects (Robinson, et al., 1996; Robinson, et al., 2000). It was also suggested that the attachment loss in HIV-seropositive subjects with chronic periodontitis is associated with GR rather than deep periodontal pocket formation (Robinson, et al., 1996; Robinson, 1998; McKaig, et al., 2000). This study fails to document any differences in the mean PD, GR, PI and BI between HIV-seropositive and control subjects with chronic periodontitis. This conforms with other studies that documented similiar clinical manifestations and natural course of chronic periodontitis in HIV-seropositive and –seronegative subjects (Holmstrup & Westergaard, 1994; Scheutz, et al., 1997; Persson, Hollender & Persson, 1998; Matee, et al., 1999).

In this study the mean GR was similiar in HIV-seropositive and control subjects and between HIV-seropositive HAART-näive and HIV-seropositive subjects using HAART. Moreover, the mean GR values were not associated with low CD4+ T cell counts. This is in disagreement with the findings of McKaig, et al., (2000) and Lamster, et al., (1997) that found a negative correlation between CD4+ T cell count and increased frequency of recession. McKaig, et al., (2000) reported that recession in HIV-seropositive patients with chronic periodontitis is more likely to occur with CD4+ T-cell count <200 cells/mm³ than with CD4+ T-cell count between 200-499 cells/mm³, is associated with co-existing oral candidiasis, and is not related to traumatic tooth-brushing. From our clinical experience, and supported by the

results of this study, HIV-seropositive subjects with chronic periodontitis do not have an increase in the number of gingival sites and/or severity of gingival recession, and low CD4+ T cell counts is not a risk factor associated with increased frequency and severity of GR.

This study demonstrates that both HIV-seropositive and control subjects with chronic periodontitis, have similar mean PD, GR, PI and BI measurements, and that HIV-infection dose not pose a greater risk for accelerated periodontal attachment loss. This is supported by other studies (Stanford, et al., 2003; Khongkunthian, Grote & Isaratanan, 2001). Therefore the periodontal treatment modalities for chronic periodontitis in HIV-seropositive subjects should be same as for chronic periodontitis in immuno-competent subjects. If indeed some HIV-seropositive subjects demonstrate increased levels of attachment loss, it may be the result of periodontal disease activity that took place before they contracted the HIV disease. In order to determine the timing of the initiation and progression of the periodontal attachment loss in relation to HIV infection one would need reliable clinical and radiological records of the periodontium documenting the alveolar bone loss before and after HIV contraction (Feller, Wood & Raubenheimer, 2006).

The profound HIV-associated immune suppression does not seem to increase the risk for the development of chronic periodontitis. The diminution in the CD4+ T cell counts, the dysregulation in the cytokine network and the qualitative defects of macrophages, monocytes, polymorphonuclear leukocytes, dendritic cells and T lymphocytes do not predispose the HIV-seropositive subject to increased colonisation of periodontopathic bacteria (Gonçalves, Ferreira & Silva, 2004; Gonçalves, Ferreira & Souza, 2007), to delayed wound healing of the oral tissues (Porter, et al., 1993) to increased incidence of wound infection following oral

surgery and periodontal treatment (Dodson, 1997), and to periodontal tissue destruction (Schuetz, et al., 1997; Holmstrup, et al., 1994; Matee, et al., 1999).

Since the periodontal tissue destruction in chronic periodontitis is mediated mainly by host derived cellular immune responses (Offenbacher, 1996), and since these mechanisms are to a great extent suppressed in HIV-infection, HIV-seropositive subjects with chronic periodontitis may show reduced rather than exaggerated periodontal tissue destruction, compared to immuno-competent subjects with chronic periodontitis. Moreover, active periodontal disease associated with periodontal attachment loss is related mainly to a Th1 cytokine profile (Takeichi, et al., 2000; Taubman, et al., 2001). However, in advanced HIV disease, in the absence of HAART, there is a dysregulation in the cytokine network characterised by a shift from Th1 predominant cytokines to a Th2 cytokine profile that is less associated with periodontal tissue destruction. This only reinforces the concept that HIV-associated immune dysregulation may not contribute to the development of chronic periodontitis.

Taking into consideration the profound immune suppression associated with HIV infection one would have expected that HIV-seropositive subjects with low CD4+ T cell counts would be infected with higher frequencies of periodontopathic bacteria and will show an increase incidence of wound infection following oral surgery, compared to HIV-seropositive subjects. However, this is not the case. HIV-seropositive and –seronegative subjects show similar types and quantities of periodontopathic bacteria in their subgingival microbiota, similar incidence of wound healing infection and similar wound healing capacity (Feller, et al., 2006).

HIV-seropositive subjects with periodontal disease harbour similiar periodontopathic bacteria in their subgingival plaque to that of immunocompetent subjects with periodontal disease

(Moore, et al., 1993). However, HIV-seropositive subjects demonstrate bacterial species in their subgingival microbiota that are usually not associated with periodontal disease. These opportunistic micro-organisms include *E. faecalis, A. baumannii* and *Pseudomonas aeruginosa*, and most probably are associated with HIV-related immunosuppression; and with the circumstances that HIV-seropositive subjects are frequently treated in a hospital environment; and with the regular use of antimicrobials to prevent opportunistic infections (Gonçalves, et al., 2005).

HIV-seropositive subjects do not demonstrate impaired wound healing capacity (Luck, et al., 1996) or increase incidence of wound infection following oral surgery (Porter, et al., 1993) compared to HIV-seronegative subjects. The wound healing process is complex and involves interaction between multiple factors including macrophages, dendritic cells, polymorphonuclear leukocytes and mucosal resident cells (epithelial cells, fibroblasts and osteoblasts), cytokines and growth factors (Monaco, et al., 2003). While macrophages and growth factors are the driving force behind the wound healing process (Monaco, et al., 2003; Sedlarik, 1997), CD4+ T cells do not play a critical role in this process (Park, et al., 2004). Therefore, in spite of some impairment in the function of macrophages and the alteration in the cytokine network in HIV-infection, this does not seem to influence the healing capacity of the periodontium.

There is an inverse association between CD4+ T cell counts and the frequencies of HIV-associated oral lesions, in particular when the CD4+ T cell count is lower than 200 cells/mm^3, regardless of the use of HAART. However, HIV-seropositive subjects using HAART have a significant lower prevalence of HIV-associated oral lesions compared to HAART-naïve HIV-seropositive subjects (Tappuni & Fleming, 2001).

The use of HAART in HIV-seropositive subjects has brought about a decrease in the prevalence and severity of chronic periodontitis (Tappuni, et al., 2001; Ryder, 2002). HIV-seropositive subjects with chronic periodontitis using HAART demonstrate reduced counts of periodontopathic bacteria in their subgingival plaque; and the periodontal tissues of such subjects show reduced inflammation and periodontal attachment loss, compared to HIV-seronegative subjects with chronic periodontitis (Gonçalves, et al., 2007).

Even severely compromised HIV-seropositive subjects using HAART with low CD4+ T cell counts (AIDS) and having chronic periodontitis demonstrate similar compositions of periodontopathic bacteria in their subgingival microbiota compared to HIV-seronegative subjects with chronic periodontitis (Gonçalves, et al., 2004). It is possible that HAART-associated reconstitution of parts of the immune responses is successful in controlling the colonisation of the periodontopathic pathogens, even in the presence of low CD4+ T cell counts (Gonçalves, et al., 2004; Gonçalves, et al., 2007). It is also possible that some components of the anti-retroviral drugs have an anti-inflammatory and anti-bacterial properties therefore further facilitating the host to control the periodontal tissue destruction.

This study has a few limitations. Firstly, the control group was composed of healthy subjects, with unconfirmed HIV-serostatus, presumably HIV-seronegative. For various reasons, people in the Ga-Rankuwa area in South Africa are reluctant to have voluntary HIV testing and most probably some of the control subjects were asymptomatic HIV-seropositive unaware of their HIV-serostatus. This is a realistic possibility bringing into consideration that it is estimated that 16.2% of South Africans between the ages of 15 and 49 years are HIV-seropositive (Mortality and causes of death, 2005). However, since there were no differences in the periodontal indices between HIV-seropositive subjects and control subjects with chronic

periodontitis, the unconfirmed HIV-status of the control group does not pose a significant shortcoming.

Secondly, the HIV-seropositive sample size with known CD4+ T cell counts was small. The CD4+ T cell counts were available for only 13 HIV-seropositive subjects, eight subjects using HAART and five HAART-naïve subjects. With such a small examined sample, the results of the statistical analysis regarding CD4+ T cell counts and the periodontal indices could have been biased. In this study the correlation coefficient test between PD and CD4+ T cell count in the HIV-seropositive HAART-naïve group of subjects showed a significant negative correlation ($P<0.01$). This differs from other studies that documented a positive correlation between CD4+ T cell counts and PD measurements in HIV-seropositive subjects; and that HIV-seropositive subjects with a healthy periodontium that are using HAART have a lower CD4+ T cell counts, while HIV-seropositive subjects using HAART with chronic periodontitis have higher CD4+ T cell counts (Vastardis, Yukna & Fidel, 2003). These differences between the results of our study and the other might be owing to our small sample of subjects with CD4+ T cell counts available.

In this study the mean CD4+ T cell counts found in HAART-naïve group of subjects (257cells/mm³) was higher than in HIV-seropositive using HAART group of subjects (172cells/mm³). On first thought this is a paradoxical finding that should be attributed to biased statistical results associated with the small number of subjects with available CD4+ T cell counts. However, on second consideration, this finding might be real. In South Africa, a considerable number of people with suggestive HIV-associated conditions refuse to undergo serological testing for HIV, and at times prefer to receive treatment by traditional healers. This leads to the eventual diagnosis of HIV infection and the initiation of HAART late in the

natural course of HIV disease when the CD4+ T cell count is already very low (<200cells/mm³). In addition, in the South African context, HIV-seropositive subjects from low socioeconomic backgrounds that are dependant on government health department services, are entitled to start HAART only when their CD4+ T cell counts is below 200cells/mm³. This circumstance often leads to the initiation of HAART when the CD4+ T cell count dropped already substantially below the 200 cells/mm³ mark; and it is a well established fact that starting HAART when the CD4+ T cell count is very low, will lead to a reduced reconstitution of CD4+ T cell numbers compared to starting HAART at higher levels of CD4+ T cell. Therefore, the HIV-seropositive subjects using HAART in this study had a lower CD4+ T cell count compared to the CD4+ T cell count of HIV-seropositive HAART-naïve subjects.

In contrast, three of the five HIV-seropositive HAART-naïve subjects with known CD4+ T cell counts had CD4+ T cells above 200 cells/mm³ and therefore not entitled to HAART while the other two subjects had CD4+ T cells below 200cells/mm³, were recently diagnosed with HIV, and were in the process of starting HAART or being treated by a traditional healer. Of interest, the other seven HIV-seropositive subjects with unknown CD4+ T cell counts did not consent to a blood test to check their CD4+ T cell count, quite a frequent behavioural phenomenon experienced in the Ga-Rankuwa area in South Africa.

In this study the mean PD in the HAART-naïve HIV-seropositive subjects with chronic periodontitis was slightly greater than in the HIV-seropositive subjects using HAART (P=0.01). This differs from other studies that show that the use of HAART is associated with reduced severity of periodontal disease (Gonçalves, et al., 2007; Ryder, 2002; Patton, Phelan & Ramos-Gomez, 2002). However, if one brings into consideration that in this study

the mean CD4+ T cell count in the group of HIV-seropositive subjects using HAART was lower than in the group of HIV-seropositive HAART-naïve subjects, it conforms to other studies that report a positive correlation between PD measurements and CD4+ T cell counts (Vastardis, et al., 2003).

In order to have a more reliable result one needs to have a larger sample size with available CD4+ T cell counts and definitive information regarding the HIV-serostatus of the control group.

CHAPTER 7

7.0 CONCLUSION

In this somewhat limited study, HIV-seropositive subjects with chronic periodontitis show similar clinical manifestations of periodontal disease to those of the control subjects with chronic periodontitis with no differences in the mean PD, GR, PI and BI, and a low CD4+ T cell count is not a risk factor for increased frequency and severity of gingival recession. The conclusion is that the treatment of chronic periodontitis in HIV-seropositive and HIV-seronegative subjects should be the same.

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