Association of Vitamin D Serum Levels and Periodontal Disease Severity in HIV Seropositive Women

BY

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THESIS

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LIST OF ABBREVIATIONS

ARI	Acute respiratory infections
ARVt	Antiretroviral therapy
BI	Bleeding Index
CAL	Clinical Attachment Loss
CDC/AAP	Center for Disease Control and Prevention/American Academy of Periodontology
CEJ	Cemento-enamel Junction
CVD	Atherosclerosis related cardiovascular disease
FGM	Free Gingival Margin
HAART	Highly Active Antiretroviral Therapy
HIV	Human Immunodeficiency Virus
MI	Multiple Sclerosis
NoT	Number of Teeth
PD	Probing Depth
PI	Plaque Index
S.D.	Standard Deviation
VDR	Vitamin D Receptor
VitD	Vitamin D
VL	Viral Load
WIHS	Women's Interagency HIV Study

SUMMARY

Objectives: Vitamin D deficiency is commonly observed in women and among people living with human immunodeficiency virus (HIV). Abnormally low serum VitD levels are often associated with negative health outcomes including increased prevalence of mucosal infections. Association between VitD status and periodontitis in HIV has yet to be explored. In this study we examine the association of VitD serum levels with the severity of periodontal disease in HIV + women. We hypothesized a positive association between VitD deficiency and poor periodontal health.

Materials and Methods: Serum 25(OH) VitD and mean (m) Periodontal Disease markers (number of teeth (NoT), Clinical Attachment Loss (CAL), Probing Depth (PD), Bleeding Index (BI), Plaque Index (PI) and Periodontal Disease Diagnosis (CDC/AAP definition) were investigated cross-sectionally in 75 HIV+ Chicago Women's Interagency HIV oral substudy participants between 1995 and 2003. VitD deficiency was defined as <20ng/ml. Linear regression was used to determine associations between PDmarkers and VitD deficiency for the total sample (including smoking status) and with stratification by current tobacco smoking status. All available potential covariates (age, race, education, smoking, mPI, VL, CD4 and use of HAART) were entered into each Linear Regression model. Only the variables that were significant on the 0.10 level were retained in the final multivariate model. Association between PDmarkers and VitD Deficiency, after confounder adjustment, was considered statistically significant when P value was <0.05.

Results: Participants were predominantly African Americans (71%) and smokers (52%); mean age was 39.5 y/o; 43% had completed high school. Mean Biomarkers levels were VitD (20.2ng/ml), HIV Viral Load (VL) ($123x10^3$ copies/ml), CD4 count ($351/mm^3$). 12% reported using highly active antiretroviral therapy (HAART). Average NoT was 20.7, mPD (1.7mm), mCAL (1.2mm), mBI (0.2) mPI (0.9); 16% met the criteria for moderate/severe Periodontal Disease. Among HIV+ non smokers, VitD Deficiency was associated with greater mCAL (p=0.049/β-coefficient=0.278) in multivariate models. Among HIV+ smokers, VitD Deficiency was only associated with mBI (p=0.002 β-coefficient=-0.329) in multivariate models.

Conclusion: In HIV+ non smokers, VitD Deficiency was associated with more severe Periodontal Disease.

Clinical Significance: From this study, VitD deficiency seems to be associated with markers of periodontal health in HIV+ individuals. Factors such as smoking seem to affect this association. It is therefore important for future studies investigating the association of Periodontal Disease and VitD to consider stratification for clinically significant factors including tobacco smoking.

I. INTRODUCTION

A. Background

In year 1981, the first case of AIDS was reported by the US Centers for Disease Control and Prevention. Since then, more than 60 million people have been infected with HIV and more than 30 millions have died worldwide[1].The overall growth of the global AIDS epidemic appears to have stabilized with a steady decline in new HIV infections since the late 1990's[1]. However, the levels of new infections overall are still high. In 2009, an estimated 2.6 million people became newly infected, which is approximately one-fifth fewer than in 1999[1]. In sub-Saharan Africa, the estimated 1.8 million newly infected people in 2009 was 20% lower than the estimated number of new cases in 2001[1]. However, that declining trend is not worldwide. According to WHO, Central Asia is experiencing a fast growing HIV epidemic, as a result of multiple socio-economic problems associated with that region[2].

For the last 30 years of HIV epidemic, HIV infection and AIDS have caused devastating global and medical effects and it continues to be one of the leading causes of premature death, especially in the developing world[1]. Since the early 1990s, however, the face of HIV infection has significantly changed. Due to the discovery of newer antiretroviral therapies and improved access to them, the death rate of young adults between 25-44 years old from AIDS has declined significantly in the United States and an overall dramatic reduction in mortality and morbidity rate has been reported[3]. Consequently more people

infected with HIV have a longer life with better health than before. With approximately 30 anti-HIV agents available, the estimated life expectancy of certain HIV-infected individuals is close to that of uninfected individuals[4].

Despite the overall health improvement which includes a reduction in the incidence of oral clinical manifestations of HIV infection, oral and other healthcare providers need to remain cognizant of the oral lesions associated with that disease. A sizable number of HIV infected people are ignorant of their HIV status[5] and historically many oral lesions represent the first signs of HIV infection[5].

Oral candidiasis, oral hairy leukoplakia, Kaposi's sarcoma, linear gingiva erythema and necrotizing periodontal diseases have all been associated with HIV infection[5]. However, with significant progress in anti-viral therapy, a decline in the prevalence and incidence of these oral lesions has been reported[4]. Nowadays, more common forms of periodontal disease, including chronic periodontitis have to be addressed and treated in HIV-infected patients. So far, there is no consensus in the literature about whether HIV infection has a deleterious effect on the periodontium with several studies reporting conflicting results[6,7].

Vitamin D and its role in general health have recently attracted a considerable interest in both research and clinical care. The main function of VitD is the maintenance of bone health throughout life[8]. This is achieved through the enhancement of calcium and phosphorus absorption, osteoclast and osteoblast activity, and by controlling parathyroid hormone levels[8]. Independently of this well recognized role in bone homeostasis, VitD status in

patients has now been shown to be associated with a wide range of physiologic and disease states such as cancer and immune-related diseases[9]. This is believed to be due in-part to the effect VitD has on essential immune functions[9]. Specifically, VitD exerts an indirect antimicrobial and anti-inflammatory effect so that pathologically low levels of VitD may result in infection or immune dysfunction[9]

The potential role VitD plays in the regulation of immune function has drawn the interest of many researchers. This is particularly true in HIV clinical research. Whether Vit D status may contribute to further immune dysfunctions in people living with HIV has been hypothesized by many. It has been reported that VitD deficiency may blunt immune function and exacerbate HIV complications, including opportunistic infections[10]. However, the overall evidence for this finding is relatively weak[10], as the relationship has been reported to be only associative[10]. The aforementioned association of low VitD with defective immune function and inflammation generated also an interest in the potential effects of VitD deficiency in the severity of periodontal disease. Again, even if the existing evidence is inconclusive and weak, there are several studies, reporting an inverse association of VitD serum levels with periodontal disease severity[11,12,13]. In this context, VitD supplementation was enthusiastically embraced and studied by many researchers as a promising solution for improvement of many systemic conditions including HIV infection and periodontal disease[14,15]. The results of these studies, suggesting a beneficial effect of VitD supplementation on periodontal health, while limited

by their scope, generated overall weak evidence to enable safe conclusions[14,15].

Apart from the still questionable role of VitD in periodontal health, there are risk factors that have been consistently shown to have a detrimental effect on the severity of periodontal disease; tobacco smoking being one of them[16]. Smoking has been associated with increased clinical attachment loss, bone loss and PD and decreased number of teeth[16]. Smokers have also been shown to respond less favorably to the treatment of periodontal disease[17,18]. The detrimental effects of smoking have been attributed to multiple factors, including increased number of periodontopathogenic bacteria in smokers[19], decreased PMN function, including chemotaxis and phagocytosis[20], increased numbers of circulating T- and B- lymphocytes[20] and decreased vascularity of the tissues[21,22]. The effects of smoking on the deterioration of the periodontal status are so overwhelming, that many studies examining the effect of other potential risk factors to periodontal disease often exclude smokers or heavy smokers, as these subjects could potentially mask other associations[12].

B. Significance of the study

To our knowledge, this study is the first one which investigates whether there is an association between VitD serum levels and the severity of periodontal disease in an HIV population. At the onset of the HIV epidemic, infection with HIV resulted in fast declining immune status, which quickly culminated in life-threatening opportunistic infections and AIDS[1]. The onset of better antiviral treatment changed the clinical course of the disease for the better[4]. It resulted

in a major increased life expectancy and a dramatic decrease in the incidence of the signature HIV-related opportunistic diseases in people living with HIV[1]. Treating periodontal disease and avoiding losing teeth, can now be an achievable treatment goal meant at improving the quality of life of these patients. What is more, better control over HIV replication and sustained and increased CD4 in people living with HIV has made other factors affecting health outcomes more relevant to HIV care. This includes tobacco usage[4] and potentially VitD status.

The potential role of VitD deficiency in the periodontal disease status of these patients is the subject of interest of this study. If an association between VitD and periodontal disease is supported by the data, it would warrant future studies to test VitD supplementation as a cost-effective, safe, and feasible additional therapeutic approach to ameliorate periodontal health in people living with HIV.

C. Objective and hypothesis of the study

Objective:

To examine the association of serum VitD status and the severity of Periodontal Disease in HIV infected women.

Hypothesis:

A positive association exists between VitD deficiency and the severity of periodontal disease among HIV+ women.

II. CONCEPTUAL FRAMEWORK AND RELATED LITERATURE

A. Vitamin D Physiology

Solar Ultraviolet B (UV-B) irradiation (wavelengths of 290-315nm) is the primary source of metabolic activation of VitD for most people[23]. Dietary sources of VitD are also available, but limited in general. VitD comes in two forms: $VitD_2$ (ergocalciferol) and $VitD_3$ (cholecalciferol). $VitD_2$ and D_3 are regarded as equivalent and interchangeable[23]. $VitD_2$ comes from irradiation of the yeast, fungi and plant sterol ergosterol whereas $VitD_3$ is mainly synthetized in the skin but it can also be found in dietary intakes such as in oily fish and cod liver oil. Active $VitD_3$ derives from the UV-B irradiation of the skin, which triggers the photolysis of 7-dehydrocholesterol to previtamin D_3 in the plasma membrane of human skin keratinocytes and dermal fibroblast. Once formed, previtamin D_3, entrapped in the plasma membrane, undergoes re-arrangement of its double bonds to form the more thermodynamically stable $VitD_3$. Then, it is released from the plasma membrane lipid bilayer into the extracellular space and into circulation after binding to the VitD binding protein in the dermal capillary bed[23]. Excessive solar UV-irradiation will not result in VitD intoxication because excess $VitD_3$ and previtamin D_3 are photolyzed to biologically inert photoproducts[23].

$VitD_3$ from the diet or the skin undergoes 2 sequential hydroxylations, first in the liver to $25(OH)D_3$ by D-25-hydroxylase enzyme (CYP2R1) to form the pro-hormone 25-hydroxyvitamin D and then in the kidney to its biological active form, 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) by 1a-hydroxylase

(CYP27B1)[23]. In the healthy state this renal synthesis has been confirmed as the sole source of $1,25(OH)_2D_3$ [24]. This metabolite has the highest affinity for the nuclear VitD Receptor (VDR), through which, it exerts its biologic actions[25].

There are many factors that alter the cutaneous production of $VitD_3$, either by influencing the number of solar UVB photons that penetrate the skin or alter the amount of 7-dehydrocholesterol in the skin. The amount of 7-dehydrocholesterol in the epidermis is relatively constant and begins to decline later in life[26]. Time of day, season and latitude also significantly influence the production of $VitD_3$ [23]. During winter time, less $VitD_3$ synthesis is taking place and the reason being is that the sun's rays are entering at a more oblique angle. That causes the UVB photons to pass through the ozone layer for a greater distance, resulting in greater absorption[23].

$VitD_3$ is fat soluble and any excess that is produced, can be stored in the body fat and used during the winter. However, for obese individuals, the fat can be an irreversible sink for VitD, resulting in VitD deficiency[27]. When obese individuals were exposed to the same dosage of $VitD_2$ orally or exposed to simulated sunlight for the same period of time as non-obese individuals, they exhibited increases in blood VitD concentrations of no more than 50%, compared with the non-obese subjects[28,29].

B. <u>Regulation of Vitamin D levels</u>

$1,25(OH)_2D_3$ is tightly regulated in view of its important biological role of in calcium homeostasis. Many factors, including serum calcium, serum

phosphorus and PTH regulate its renal production[23]. When VitD levels are adequate, intestinal absorption of dietary calcium is approximately 30-40%, which is double compared to the calcium absorption in an inadequate VitD state. Thus, when $1,25(OH)_2D_3$ are low, resulting in low serum calcium levels, the body increases the production and release of PTH into the circulation[23]. PTH increases tubular reabsorption of calcium in the kidney, increases calcium mobilization from the bone and most importantly enhances the production of $1,25(OH)_2D_3$. More specifically, PTH stimulates 1a-hydroxylase (CYP27B1 gene) in proximal renal tubular cells, resulting in an increase in the synthesis of $1,25(OH)_2D_3$ in the kidney, which then by negative feedback reduces PTH levels by decreasing parathyroid gland activity and increasing serum calcium[23]. $1,25(OH)_2D_3$ may also regulate keratinocyte differentiation, melanocyte apoptosis and melanin production and these functions could be another mechanism of regulating the cutaneous synthesis of $VitD_3$ by negative feedback[23]. Due to its wide range of effect, presently VitD is not identified anymore as a vitamin, but as a steroid hormone that regulates an astonishingly complex system of functions.

C. <u>Vitamin D bioactivity</u>

The most prominent role of VitD in the human body is the regulation of calcium and phosphate homeostasis, through its action on at least three organs, the kidney, the small intestine and the bone[30]. This action is mediated through the expression of VitD Receptor (VDR) in intestinal, renal and bone tissues. Plasma levels of $1,25VitD_3$ have demonstrated a positive association

with intestinal absorption[30] and with the renal tubular reabsorption of calcium[31].

VitD increases the ability of the small intestine to absorb dietary calcium and phosphate by binding and activating the VitD receptors found in intestinal epithelial cells. The activation of VDRs results in the upregulation in the expression of epithelial calcium channels (in particular ECaC2), of cytosolic calcium-binding proteins (calbindin-D_{9k}) and of plasma membrane proteins (PMCA and NCX), through which intestinal uptake of both calcium and phosphorus is enhanced[32].

In the kidneys, 1,25$(OH)_2D_3$ increases the mRNA levels of calbindin 28K which codes for a protein with key role for calcium reabsorption[31]. In an animal study, global-VDR (-/-) mice were showed to have an increased renal excretion of calcium irrespective of the PTH levels or their calcium and phosphate diet[33].

Plasma 1,25$(OH)_2D_3$ has both anabolic and catabolic activities in bone and its specific action depends on the adequacy or inadequacy of dietary calcium intake. In vitro and in vivo studies have shown that VitD activity can either promote or inhibit bone formation and stimulate or inhibit bone mineral catabolism, in order to maintain plasma calcium homeostasis under varying physiological circumstances[34].

In case of hypocalcaemia, 1,25$(OH)_2D_3$ through VitD receptors in osteoblasts, increases the RANKL expression by osteoblast cells in bone, which results in osteoclastic differentiation and bone resorption[23]. This action results in an

increase in plasma calcium levels, contributing to the maintenance of calcium plasma homeostasis.

The importance of $1,25(OH)_2D_3$/VDR in RANKL expression is shown by studies where VDR (-/-) mice, even if they developed hypocalcaemia and hypophosphatemia with significantly raised PTH levels, they failed to increase osteoclastic activity, probably due to insufficient levels of RANKL. Even when VDR (-/-) mice were fed a diet, high in calcium and phosphate, they demonstrated marked osteopenia despite normalization of serum calcium and phosphate levels. The decreased bone volume resulted from impaired mineral apposition and not increased bone resorption[33].

In contrast to the catabolic effects of $1,25(OH)_2D_3$ in cases of hypocalcaemia, under conditions of adequate dietary calcium intake, $1,25(OH)_2D_3$/VDR has been suggested to mediate anabolic activity within the bone, with however controversial reports in the literature. In an animal study, overexpression of the VDR, in mature osteoblastic lineage cells, resulted in an increased mineral apposition and decreased bone resorption activity, with increased cortical and trabecular bone volumes[35]. However, the increased bone mineral density was lost in mice fed with low dietary calcium, suggesting that the underlying mechanism of the anabolic effects of $1,25(OH)_2D_3$/VDR in the bone, depends on the adequacy of dietary calcium[36].

An area of controversy in the VitD field is the extent to which the actions of $1,25(OH)_2D_3$ on bone cells affect bone mineral homeostasis. VDR is expressed by the three major bone cell types: Osteoblasts, osteoclasts and osteocytes. Ablation of the VDR gene produces hereditary VitD-resistant

rickets (HVDRR), which is a rare autosomal recessive disorder, characterized by hypocalcaemia, hypophosphatemia and rickets, a bone tissue mineralization abnormality in children[37]. Severe VitD deficiency due to malnutrition or lack of sun exposure gives also rise to rickets in children and osteomalacia in adults, as a result of reduced calcium absorption from the intestine and subsequent defective mineralization of the bones. Symptoms of these diseases include among others, bone tenderness, muscle weakness, increased tendency for fracturing and skeletal deformities in children[38]. Thus, it has long been thought that VitD enhances bone mineralization through its actions. However, since rickets can be treated with normalizing plasma calcium and phosphorus in patients with VitD Deficiency, there is evidence that the essential action of VitD in this condition is on the level of intestinal calcium and phosphate absorption and not at the bone[39]. In an animal study, global-VDR (-/-) mouse models, demonstrated rachitic bone changes when fed a standard diet; however when they were fed with high calcium, phosphorus and lactose diet until 10 weeks of age, plasma calcium and phosphate levels were corrected and normal bone volume and strength were achieved[40]. Ablation of VDR in the intestine with concomitantly reduced calcium absorption, increased cortical bone porosity in such a level to initiate spontaneous bone fractures[41]. When an intestinal- specific transgene for VDR was expressed in the global-VDR (-/-) mouse, calcium absorption was restored preventing the rachitic changes of the VDR knockout mice[42]. These data show that bone defects are dependent on the availability of plasma calcium and phosphate the level of which are regulated by VDR mediated

intestinal absorption. Thus, the role of VitD in bone mineralization is more so through its action in intestinal calcium absorption, than on direct effects in bone cells.

As it will be discussed in detail later, the role of VitD against osteoporosis and its sequelae, the most severe one being hip fractures, has also been controversial. Studies have showed the beneficial effects of VitD and calcium supplementation on the prevention of hip fractures[43,44], however few studies have analyzed the individual effect of VitD supplementation; an important parameter as Calcium supplementation has been known to have an independent protective effect on fractures[45]. A recent meta-analysis concluded that neither higher nor lower dose VitD supplementation prevents hip fracture[46].

D. Role of Vitamin D in the immune function

1. Antimicrobial effects of Vitamin D

The initial defense against the periodontal pathogens includes the expression of a number of host defense peptides, such as β-defensins and cathelicidins from oral epithelial cells[47]. The only human cathelicidin, LL-37, is a multifunctional peptide, with antimicrobial activity against both Gram-positive and Gram-negative bacteria, as well as some viruses[48]. It also exhibits chemotactic properties and plays a role in dendritic cell maturation[49]. It has been shown that the $1,25(OH)_2D_3$, can induce, in vitro, the expression the

cathelicidin LL-37, and increase its antibacterial activity against Aggregatibacter actinomycemetcomitans; suggesting thus, that VitD could have a beneficial effect in periodontal health[50]. Another mechanism that has been reported is that in the presence of pathogens, Toll-like receptors on human monocytes and macrophages activate genes in the VitD pathways, including the CYP27B1 gene (which encodes for 1,25-a hydroxylase) and the VDR[51]. The subsequent increased production of 1,25-a hydroxylase resulted in an increase of the production of cathelicidin and enhanced antimicrobial effects[51]. However, most of the antimicrobial effects of VitD and VitD associated enzymes against oral pathogens have been shown in studies conducted in–vitro only. One human study retrospective study has investigated the association of 25(OH)D plasma concentrations and periodontopathogenic bacteria. It found no association after examining for presence or absence of P. Gingivalis, T. Forsythia, F. Nucleatum, P. Intermedia, C. Rectus[52]. The authors concluded that these results could be explained either by the characteristics and limitations of the study or by a true absence of association.

2. <u>Vitamin D role in the adaptive immune response and inflammation</u>

Beyond the well known role of VitD in the calcium and bone metabolism, several clinical studies have reported that VitD plays a significant role in the regulation of the immune response and possesses anti-inflammatory effects[23,54-58,60,61,66]. As aforementioned, VitD_3 is metabolized to 25(OH)D_3 in the liver and then further hydroxylated by the enzyme 25 hydroxyl VitD_3-1a

hydroxylase (CYP27B1) to the active form 1,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$). This active form then binds to VDR, which acts as a ligand-activated transcription factor[23]. It is now well reported that CYP27B1 and VDR are expressed in cells of the immune/inflammatory system in the human body, including macrophages, dendritic cells and T- cells[53], which provides the biological basis for the role of VitD in inflammation and associated diseases. In a recent study, VitD inhibited p38 phosphorylation and pro-inflammatory cytokine IL-6 and TNF-a production in LPS-stimulated human monocytes[54]. VitD has been also demonstrated to suppress prostaglandin pathways via inhibition of cyclooxygenase-2 production[55]. VitD has also been shown to interfere with NF-kB activation and signaling, by increasing IkBa expression in the cells, thus reducing the nuclear translocation of the activated NF-kB subunits[56]. Several studies have also shown that $1,25(OH)_2D_3$ inhibits the differentiation and maturation of human Dendritic cells (DCs), which are the most potent antigen-presenting cells[57]. This effect of VitD on DCs, results in the induction of T-regulatory cells, an important event for suppressing the inflammatory response of T-effector cells[58]. VitD inhibits proliferation of T-cells in a VDR-dependent manner[66] and production of pro-inflammatory cytokines, including IFNγ, IL-17 and IL-21 in CD4+ T-cells[59]. Moreover, VitD has been showed to promote development of T-regulatory cells expressing cytotoxic T-lymphocyte antigen 4 and FOXP3[60]. $25(OH)D_3$ levels below 21ng/ml have also been found to have an inverse relationship with CRP concentration, indicating a potential link of Vit D with inflammatory markers[61].

E. Vitamin D deficiency

1. Definition and Epidemiology

Throughout the literature there is no consensus about the optimal levels of serum 25(OH)D. VitD deficiency is defined by most recent researchers as 25(OH)D level of less than 20ng/ml (50nmol per liter). VitD insufficiency is considered as 25(OH)D between 21 to 29ng/ml and VitD sufficiency as serum 25(OH) levels of equal or more than 30ng/ml[62,63,64]. As shown by recent NHANES data, it is estimated that 25-30% of the US population is VitD deficient and only 20-25% of the population has serum VitD levels of at least 30ng/ml[65,66].

2. Causes of Vitamin D deficiency

There are many causes for VitD deficiency, including reduced synthesis and absorption as well as heritable and acquired disorders of VitD metabolism[67-69]. Reduced skin synthesis of VitD could be due to sunscreen use, skin pigmentation, aging and season, latitude and time of day[67-69]. It has been reported that from November to February, above 35 degrees north latitude, little or no VitD can be produced[67]. Decreased absorption could be due to malabsorption resulting from cystic fibrosis, celiac disease, Whipple's disease, Crohn's disease and other causes[67-69]. Obesity reduces also availability of VitD, due to sequestration in body fat with an inverse association present between serum VitD levels and BMI. Decreased synthesis of VitD can be caused by liver failure or chronic kidney disease too[67-69]. The most common cause of VitD deficiency due to heritable disorders is rickets (autosomal

dominant or X-linked) which causes from reduction or no renal synthesis of 1,25$(OH)_2$D to partial or complete resistance to its action[67-69]. Acquired disorders causing VitD deficiency have been reported to be tumor-induced osteomalacia, primary hyperparathyroidism, granulomatous disorders including sarcoidosis, tuberculosis or lymphomas and hyperthyroidism[67-69].

3. Association with systemic diseases

a. Osteoporosis and Fracture

As previously mentioned, lower levels of VitD leads to an increase in the PTH secretion, resulting in bone resorption; this occurs in order to maintain adequate calcium and phosphorus levels[70]. Thus, VitD sufficiency is important for physiologic skeletal development and bone health in childhood[67] and in adults[71]. More specifically, in children VitD levels of <15ng/ml result in bone deformities, skeletal mineralization defects and short stature, all characteristics of Vitamin D deficiency rickets[67]. In adults, VitD deficiency and increased PTH levels, result in osteomalacia; a decreased mineralization of collagen matrix resulting in a reduction of structural support[71], which has been associated with increased risk of fracture[71]. Also, since low levels of VitD lead to an increase of osteoclastogenesis and bone resorption through PTH secretion[70], VitD deficiency can precipitate and worsen osteopenia and osteoporosis[72]. VitD levels of over 30ng/ml have been associated with a lower risk of fracture[73]. Few studies have been conducted about the potential effects of VitD supplementation on lowering the risk of fracture, reporting either beneficial effects or no effects whatsoever[74,75]. However, most of the

studies that have shown a benefit of Vit D supplementation on reduction of fractures, supplemented patients with both calcium and VitD. This is important as calcium has been shown to have an independent effect on reducing fractures[45]. A meta analysis of seven RCT's examining the effects of VitD supplementation on the prevention of hip fractures showed no significant differences in those randomized to VitD supplementation versus placebo/control, with dose significant differences between those supplemented with <800 IU/day > or = 800 IU/day[45].

b. <u>Acute respiratory infections (ARI)</u>

At least one epidemiological study has suggested an association between VitD insufficiency due to less sun exposure and ARI[76]. Other clinical studies, have suggested an inverse association between VitD levels and ARI[77,78]. In a large retrospective study, each 10nmol/L increase in 25(OH)D_3 was associated with a 7% lower risk of respiratory infection and increased lung function in British adults[79]. VitD supplementation has also been found to be beneficial in reducing the risk of ARIs. Camargo et al. reported that children with VitD supplementation of 300IU/daily had significantly fewer ARIs compared to controls during the study period[80]. The anti-infective mechanisms of VitD have been linked with the induction of formation of potent antimicrobial peptides such as cathelicidins and also in its ability to modulate inflammatory factor levels in ARI patients[80].

c. Atherosclerosis related cardiovascular disease

Numerous studies have reported VitD deficiency (<20ng/ml) as one of the new risk factors for coronary heart disease (CHD)[81,82]. Three large retrospective studies demonstrated that VitD deficiency was associated with increased risk for CHD, including hypertension, diabetes mellitus, obesity, high serum LDL and Triglyceride levels and low serum levels of HDL[83,84,85]. Despite however these findings from observational studies, RCTs designed to assess the impact of VitD supplementation on CVD outcomes are conflicting and even more largely unknown. Most evidence shows that VitD supplementation has no effect on vascular disease mortality[86,87], however, some RCT results have shown that a higher intake of VitD is associated with a lower risk of CVD, an effect attributed to an improvement of vascular endothelial function and decrease in inflammation [88,89]. The anti-atherogenic effect of VitD has been attributed to the regulation of the immune/inflammatory response. VitD has been found to inhibit COX-1 /COX-2 expression, promote prostaglandin catabolism and reduction of ROS production, as well as suppression of proinflammatory cytokines, all mechanisms involved in endothelial dysfunction[90]. Despite that evidence and proposed mechanisms of action, the role of VitD in CVD remains to be elucidated.

d. Asthma

Epidemiological and clinical studies have reported an association between asthma and VitD deficiency, with the majority of asthmatic children to be VitD

deficient[91,92]. VitD deficiency has been also found to increase the risk of severe asthma exacerbations[93]. The mechanisms however, of VitD deficiency in the pathophysiology of asthma are not completely understood, with most researchers, attributing it to the potential effect of VitD in the inflammatory response. VitD has been found to increase the production of IL-10, which is an anti-inflammatory cytokine, and decrease the proinflammatory cytokine levels in airway smooth muscle cells[94,95]. Also, VitD supplementation reduces the levels of TNFa, which could be responsible for reducing allergic airway inflammation[95].

e. Multiple Sclerosis

MS is a chronic inflammatory disease of the central nervous system (CNS). There is some evidence that VitD deficiency might be one of the most important environmental factors for the prevalence and progression of MS[96,97]. Although a potential link between reduced risk for MS and VitD supplementation has been widely assumed, VitD-repletion therapies have not shown an effect on the progress or the risk of relapse of MS[98]. However, in a study with high Dose VitD supplementation (20,000 IU/day for 12 weeks), VitD was found to increase proportion of IL-10 CD4+ T-cells and decrease the ratio between IFNγ and IL-4+CD4+ cells[99].

f. Other inflammation/immune related disorders

Inflammation has also been found to play an important role in other chronic diseases, including hypertension, diabetes and congestive heart failure.

Evidence has been reported to support an association between low plasma levels of 25(OH)D_3 and hypertension or diabetes[100,101]. Clinical trials have also confirmed the effect of VitD supplementation on blood pressure lowering and insulin sensitivity increasing effect in patents[102,103].

Apart from inflammatory disorders, VitD deficiency has been associated with immune related disorders, such as rheumatoid arthritis and systemic lupus erythematosous[104,105].

F. Vitamin D and HIV Disease

The prevalence of hypovitaminosis D in HIV seropositive individuals is quite high. According to large prospective US cohort study, 70% of HIV(+) patients had 25(OH)D levels <30ng/ml[106]. Another observational study on a cohort of HIV (+) individuals from 31 European countries reported that 89% of patients had 25(OH)D levels <30ng/ml[107].

There is an increased number of complications that have been reported to be associated with low VitD levels in HIV infected patients including among others CVD[108], type II Diabetes Mellitus[109], bacterial vaginosis[110] and oral candidiasis[111]. Several studies have examined the potential effects of low VitD levels and the immune function of HIV(+) individuals. VitD deficiency has been shown to affect HIV disease progression and mortality [107,112], with patients with higher VitD levels having a smaller risk for disease progression and AIDS defining events, including pulmonary tuberculosis[113]. Conflicting data have been reported however for the effects of VitD status on CD4+count. A positive association has been reported between increased 25(OH)D levels

and higher CD4 numbers with immune reconstitution[114,115], whereas, two small RCTs reported that VitD supplementation did not change CD4+ counts, during a period of 6 months to a year[116,117]. Currently, there is no strong evidence that increased 25(OH)D levels, through VitD supplementation, could improve CD4 restoration and decrease the deleterious long term effects of HIV infection.

Whether ARVt affects directly VitD levels is still under investigation. A longitudinal study suggests that this may be the case[118]. More specifically, the non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs), efavirenz was shown to have the most consistent and profound effects of VitD levels, whereas limited evidence suggests that nevarapine, etravirine and rilpivirine may have less or no impact[118]. On the other hand, HIV Protease Inhibitors (PIs) and Nucleoside Reverse Transcriptase Inhibitors (NRTIs) have not been apparently been associated with VitD deficiency with the possible exception of zidovudine[118].

G. Vitamin D and Periodontal Disease

There is an abundance of data in the literature investigating the association of VitD with periodontal disease.

A 5 year prospective study (Buffalo OsteoPerio study), the longest and largest conducted so far, examined the association of VitD plasma levels and the progression of periodontal disease in 655 postmenopausal women. Disease progression was assessed using changes in alveolar crest height, CAL, PD and percent of gingival sites that bled upon assessment. The authors found

no association between VitD levels and periodontal disease progression after adjusting for a number of confounding factors including age, education, frequency of dental visits, smoking status, self-reported history of diabetes, current use of osteoporosis related medications or bone therapies, BMI, recreational physical activity and baseline measures of periodontal disease. They concluded that supplementation of VitD for prevention of periodontal disease progression is not warranted at this time[119].

Hiremath et al. in a randomized controlled trial, studied the anti-inflammatory effects of VitD in 88 patients initially diagnosed with gingivitis. After VitD supplementation for a period of 3 months with a different dosage for each of the 4 groups, authors found a dose dependent gingival anti-inflammatory effect of Vitamin D. Patients on 2000IU of VitD showed reduction of gingivitis faster than the patients in the 500IU group. Serum VitD concentration of >30-35ng/ml was found to be the level over which VitD exerts its anti-inflammatory action[120].

Antonoglou et al. in a prospective study showed a positive association between the serum 1,25(OH)D and periodontal health in type I diabetic patients. More specifically, patients with no or mild chronic periodontitis at baseline had higher mean 1,25(OH)D levels compared to patients with moderate or severe chronic Periodontitis. Interestingly, periodontal therapy and elimination of inflammation resulted in an increase in serum 1,25(OH)D levels with no conclusions drawn however for the possible underlying mechanism[121].

Alshouibi et al. assessed the association between Vitamin D and periodontal health in 562 older men. Periodontal health was assessed through alveolar bone loss, PD and CAL, whereas VitD was assessed as a total VitD intake calculated by questionnaires completed by the individuals and taking into consideration foods, supplements and multivitamins. They reported that after adjustment for a number of co-variables, total VitD intake was inversely associated with odds of severe periodontitis and moderate to severe alveolar bone loss. Each 100 IU increment in daily total VitD intake was associated with reduced odds of severe periodontitis, with the lower odds for disease severity among men consuming >800 IU per day. A major limitation of the study was that VitD was assessed by questionnaires, which is not as accurate as the serum VitD levels which measures both endogenous production and diet[122].

Bastos et al. studied the association of VitD serum levels and chronic periodontitis in patients with chronic kidney disease (CKD). They showed that patients with CKD and chronic periodontitis had lower serum levels of vitamin D and were most often insufficient/deficient in 25(OH)D in relation to CKD patients without chronic periodontitis[123].

Dietrich et al used the NHANES III data to evaluate the association between the serum 25(OH)D and CAL. They found no association for patients younger than 50 years old. However, for men and women older than 50y/o, they found an inverse association between mean CAL and serum 25(OH)D levels after correction for a number of confounding variables including age, race, ethnicity, socioeconomic status, estrogen use among the women, smoking,

diabetes, BMI and gingival bleeding. The BMI was found to have no association with CAL and did not attenuate the association of VitD levels with CAL, thus they concluded that their findings were more likely to be explained by the anti-inflammatory effects of VitD[124].

In another study, Dietrich et al examined the association between serum 25(OH)D concentration and gingival inflammation. They found an inverse association between serum concentrations of $25(OH)D_3$ and prevalence of bleeding on probing (BOP) after correction for a number of confounding variables. This association was attributed to the anti-inflammatory effect of vitamin D and the authors suggested that the higher serum 25(OH)D levels may be beneficial in regards to gingival health[125].

Jabbar et al compared 370 postmenopausal women with and without periodontal disease (active or past) and observed that the serum concentrations of $25(OH)D_3$ were significantly lower in those with either active or past periodontal disease[126].

Additionally, in a case-control study, pregnant women with periodontal disease had lower median serum $25(OH)D_3$ levels and an increased likelihood for VitD insufficiency (<75 ng/ml), compared to periodontally healthy women[127].

Krall et al demonstrated that increased intake levels of calcium and VitD had a beneficial effect on tooth retention. However, only total calcium supplementation and not VitD was inversely associated with risk for tooth loss. Moreover, tooth loss was self reported by the patients through questionnaires, with no specifications for the etiology for the loss of teeth[128].

Miley DD et al reported that subjects enrolled in a periodontal maintenance program who took oral VitD and calcium supplementation presented a trend for better periodontal health as indicated by clinical and radiographic measurements, compared to those who did not[129].

H. Periodontal Disease and HIV Disease

HIV has been associated with the presence of periodontal lesions including linear gingival erythema and necrotizing periodontal diseases, subclassified as necrotizing ulcerative gingivitis and periodontitis. The association of HIV infections with more common forms of periodontal disease including chronic periodontitis is less clear and controversial with different studies reporting conflicting results. Before the use of HAART, several studies reported that periodontal attachment loss was greater in HIV infected patients when compared with healthy controls. Worse periodontal outcomes correlated with declining CD4 counts[130,131]. Barr et al. reported that periodontitis progressed more rapidly in HIV-seropositive men who had CD4 lymphocyte counts of <200/mm^3 compared to those with >=200/mm^3 CD4 counts[130]. Moreover, higher levels of viral loads in gingival crevicular fluid have been linked with increased clinical attachment loss[132]. It has also been suggested that the infection of periodontal tissue cells by HIV could create a direct lytic effect on cells of gingival epithelium, accentuating periodontal disease destruction[133]. However, when ARVt was introduced, some studies reported no difference in periodontal disease severity and progression between HIV-positive and HIV-negative patients[7]. Goncalves et al. reported that the long term use of HAART

in a Brazilian HIV-infected population resulted in improvement of periodontal disease status[134]. McKaig et al. in a cross-sectional study of 326 HIV – infected adults, found that people taking antiretroviral medications were one-fifth as likely to suffer from periodontal disease when compared with patients not taking these medications[135]. Alves at al. evaluated periodontal disease progression by using Probing Depths (PD), Clinical attachment loss (CAL) and tooth loss as periodontal markers in HIV(+) and HIV(-) women from 1995 and 2002. They found that periodontitis progression did not differ when comparing HIV-infected and non-infected women. Alves et al. also found no association between CD4 counts/viral load and PD/CAL. However a 10-fold increase in viral load was associated with an increase in tooth loss. The use of HAART resulted in marginally less pocket depth among seropositive individuals[7]. On the contrary, Vernon et al. reported a high level and extent of periodontal disease, even if most of the patients were being treated with HAART. The patients with CD4+ T-Cell counts of <200cells/mm^3 were at greater risk for periodontal disease [136]. Fricke et al also found no difference on the periodontal status of HIV-infected individuals with or without antiretroviral therapy[137]. In another recent study, no association was observed between PD/CAL and CD4+ levels in HIV patients on HAART therapy[138]. Martinez Canut et al. also did not find any correlation between CD4 count levels and the severity of periodontal disease[139].

Several investigators have studied the periodontal microbiota associated with chronic Periodontitis in HIV-infected patients reporting conflicting results. Some studies concluded that HIV-infected and non-infected individuals with

CP have a similar composition of subgingival microbiota [140,141]. On the contrary, other studies, detected a greater prevalence of common periodontopathogenic bacteria such as A. actinomycetemcomitans, F. Nucleatum, P. Gingivalis, P. Intermedia, T. Forsythia and T. Denticola, in HIV-infected compared to non-infected individuals[142,143]. Moreover, Staphylococcus epidermidis, Candida albicans and some species of Clostridia have been detected in the subgingival microbiota of HIV patients[143]. E. faecalis has been found to be significantly more prevalent in HIV-infected patients with <200CD4/mm^3 suggesting that HIV-induced immunosuppression could create conditions for colonization and growth of uncommon opportunistic bacteria[134]. Despite their presence, the role of these opportunistic microorganisms in the pathogenesis of periodontal disease is not clear. The presence of C. Albicans has been linked with the possibility for worsening periodontal disease through damage to junctional and crevicular epithelium, facilitating the invasion of more common periodontopathogenic bacteria to the gingival connective tissue[144]. It has also been found that Candida species trigger an increased response of pro-inflammatory cytokines, which could contribute to more attachment loss in HIV– infected patients[144]. The presence of other viruses in the periodontium of HIV-infected individuals such as CMV, EBV and HSV may also contribute to the pathogenesis of periodontal disease[145]. It has been suggested that increased levels of these viruses could suppress host defense mechanisms and increase secretion of pro-inflammatory cytokines as IL-1 and TNF-a, providing the growth conditions for other periodontal pathogens[146]. A recent study has investigated

the composition of subgingival microbiota of HIV-infected patients undergoing HAART therapy in comparison to non-HIV infected individuals and found higher levels and prevalence of periodontopathogenic bacteria in the latter group; suggesting a potential protector effect of HAART[134]. The fact that studies report conflicting results with no consensus about whether there is a difference in the composition of subgingival microbiota in HIV-infected and non-infected individuals could be explained by different methods for analysis of subgingival plaque including culture, PCR, DNA probe and dark field microscopy. The quantity of biofilm samples analyzed and the immunological characteristics of subjects could also be responsible for these differences[145].

From the review of the literature presented above, it can be concluded that the true impact of HIV-infection on periodontal disease is not entirely clear. Conflicting results regarding whether HIV-infection has a deleterious effect on PD severity and progression have been reported. There is no consensus about whether severe HIV–induced immunodeficiency expressed by viral load and CD4+ level status has any effect on the periodontium, as well as what is the real effect of HAART therapy on the periodontal status, if any.

I. <u>Periodontal Disease and Tobacco Smoking</u>

Cigarette smoking has long been associated with periodontal disease and tooth loss, with smokers being characterized by a greater periodontal disease severity and progression[147]. According to a meta-analysis of six studies with a total of 2,361 subjects, smokers had an odds ratio of 2.82 of having periodontal disease compared to non smokers[148]. A dose response has also

been reported, with the number of pack years being positively correlated with amount of attachment loss and alveolar crest height loss[149]. These findings have been consistent among diverse populations (Europe, Asia, USA, South America, Australia)[147]. Smokers have been reported to respond less well to non surgical[150] and surgical periodontal therapy[151], with wound healing impairment being the causative factor. Also, smokers have been reported to have a greater risk for periodontal disease recurrence and need for retreatment[152]. An extensive body of evidence has indicated a number of pathologic mechanisms regarding the effects of smoking on periodontal health an disease. More specifically, smoking has been associated with increased number of periodontopathogenic bacteria[153], reduced gingival blood flow, due to peripheral vasoconstriction[154], altered PMN function, including chemotaxis and phagocytosis[155], increased cytokine production (IL-1b, IL-6, TNF-a)[156] and increased number of CD3, CD4 and $CD8^+$ T-cell subsets[157]. All of the above mechanisms have been reported to explain the increased disease severity in smokers. There is, however no clear evidence that shows that one mechanism is of greater importance than any other.

J. <u>Women's Interagency Human Immunodeficiency Virus Study</u>

The WIHS is an ongoing multicenter, prospective, observational study of women who are either HIV infected or at risk for HIV acquisition, enrolled at six sites: Chicago, San Francisco Bay Area (SF), Brooklyn and Bronx/Manhattan, New York, Washington, DC (DC), and Los Angeles (LA)[158]. The WIHS cohort is designed in a way to reflect the demographics of the HIV

infected women in the USA and it is the largest US cohort of HIV seropositive women being studied to date[158].

The study was originally established in 1993. The objectives were (i) to investigate the clinical manifestations of HIV infection in women, (ii) determine the pattern of decline of CD4+ cells, (iii) associate the CD4+ cell changes to other immunologic and virologic parameters and to the clinical manifestations of HIV and (iv) study potential risk factors that may be related to the rate and type of HIV disease progression[158].

The WIHS protocol includes a baseline visit and follow up visits every 6 months. The baseline visit and the follow ups consist of an interview, physical and gynecologic examinations and collection of blood and genital specimens[158].

From 1995 through 2004, a subset of WIHS participants was enrolled in a study of oral health, in order to investigate its relationship with HIV infection[159]. Oral visits were scheduled within 2 weeks of core visits, in order for the oral health data to be able to be used in conjunction with core visit data. Oral health examiners were trained and calibrated to a gold standard examiner and to each other prior to clinical examinations[159].

The components of the oral protocol exam were (i) questionnaire for oral health habits, including frequency of dental check ups (ii) stimulated and unstimulated saliva volume produced (iii) salivary gland examination (visual examination and palpation), (iv) oral mucosa tissue exam for presence of lesions (v) smears (putative candida and herpes lesions), (vi) coronal and root caries exam, (vii) tooth count, (viii) periodontal examination including plaque

index, gingival banding score, papillary assessment score, gingival bleeding index, clinical attachment loss, (ix) collection of subgingival plaque and microbiologic profile and (x) dental prosthesis assessment[159].

For periodontal examination, one maxillary and one mandibular quadrant on each patient was randomly selected, unless the individual had less than ten teeth, in which case, all of the teeth and surrounding periodontal tissues were examined. The quadrants selected remained the same for each of the follow up visits[159].

Plaque assessment was a modification of the Silness and Loe plaque index[160] (Table I) and was assessed in four sites per tooth (distobuccal, straightbuccal, mesiobuccal and lingual)[159]. It should be noted that categories “2” and “3” from the original index were collapsed into a single category “2” so that examiners only have to distinguish between visible plaque and plaque that cannot be seen, but is detectable with a probe[159] (Table II).

TABLE I. PLAQUE INDEX (SILNESS AND LOE)

Plaque index Silness and Loe[161]	Criteria
0	No plaque in the gingival area
1	A film of plaque adhering to the free gingival margin and adjacent area of the tooth. The plaque may only be recognized by running a probe across the tooth surface
2	Moderate accumulation of soft deposits within the gingival pocket, on the gingival margin and/or adjacent tooth surface, which can be seen by the naked eye
3	Abundance of soft matter within the gingival pocket and/or on the gingival margin and adjacent tooth surface.

TABLE II. PLAQUE INDEX IN WIHS

Plaque index (PI) in WIHS	Criteria
0	No plaque in the gingival area
1	A film of plaque adheres to the free gingival margin and adjacent area of the tooth. The plaque may be recognized only by running a probe across the tooth surface
2	There is an accumulation of soft deposits within the gingival pocket, on the gingival margin and/or adjacent tooth surface which can be seen by the naked eye.

The gingival bleeding index was also a modification of the Gingival index proposed by Loe and Silness[161] (Table III). After teeth were dried, the periodontal probe was inserted no more than 2mm into the gingival sulcus, at the distal of each tooth and moved gently into the mesial interproximal area[159]. The bleeding points in four sites per tooth (distobuccal, straightbuccal, mesiobuccal and lingual) were recorded for each tooth of the quadrant as present or absent[159] (Table IV)

TABLE III. GINGIVAL INDEX (SILNESS AND LOE)

Gingival Index Silness and Loe[161]	Criteria
0	Normal Gingiva
1	Mild inflammation-slight change in color, slight edema. No bleeding on probing
2	Moderate inflammation-redness, edema and glazing. Bleeding on probing
3	Severe inflammation-marked redness and edema. Ulceration Tendency on spontaneous bleeding.

TABLE IV. GINGIVAL BLEEDING IN WIHS

Gingival Bleeding (B.I.) in WIHS	Criteria
0	No Bleeding
1	Bleeding

CAL was assessed in the same four sites as the plaque and gingival bleeding index[159]. The distance from the free gingival margin (FGM) to the CEJ and the distance from the FGM to the bottom of the sulcus (probing depth/PD) were measured using a periodontal probe[159].

Gingival banding score aimed to assess the presence or absence of a continuous band of erythema at the gingival margin at least 1mm in width that extended from the mesial to the distal part of the tooth[159]. The papillary assessment score evaluated and scored the interdental papilla based on the presence of erythema, edema, ulceration, necrosis or pseudomembranous exudate, cratering and alveolar bone exposed[159].

III. MATERIALS AND METHODS

A. Participants and data availability

Our study design is a secondary analysis of cross-sectional data. The data that were used come from the WIHS. Our study sample consisted of 75 individuals coming from the Chicago site. These individuals were selected based on the availability of the VitD serum levels and periodontal disease markers from the same visit.

For each individual in our study, available data from WIHS included (i) age at visit, (ii) race (African-Americans, Hispanics and Caucasians), (iii) current smoking status (yes/no), but not the number of cigarettes for the smokers, (iv) education, stratified as high school completion versus. no high school completion, (v) number of CD4 cells and (vi) HIV Viral Load (VL).

B. Periodontal Examination and markers

From the periodontal disease markers that were measured as part of the WIHS protocol, the data that were requested from the WIHS database for our cohort were the Plaque index (PI), the Gingival bleeding score (BI), the probing depth (PD) and the distance from FGM to CEJ from each tooth site that were calculated as well as the number of teeth. After obtaining the data, for each individual we calculated the Clinical attachment loss for each site by subtracting the recorded distance from the FGM to CEJ from the probing depth (PD). Then, we calculated the mean CAL (mCAL), mean Probing Depth

(mPD), mean Plaque Index (mPI) and mean Bleeding Index (mBI) for each individual and then for the total group, the smokers and the non-smokers. We also diagnosed each individual based on these periodontal markers as having none/mild, moderate or severe periodontal disease using the CDC/AAP definition[162] (Table 5).

TABLE V. CDC/ AAP DEFINITION

CDC/AAP Definition	
None/mild	Neither moderate nor severe periodontitis
Moderate	≥2 interproximal sites with CAL≥4mm (not on the same tooth) or ≥2 interproximal sites with PD>=5mm (not on the same tooth)
Severe	≥2 interproximal sites with CAL≥6mm (not on the same tooth and ≥1 interproximal site with PD≥5mm

Thus the final periodontal markers used in our study were mCAL, mPD, mPI, mBI, tooth count (NoT) and periodontal disease diagnosis.

C. <u>Vitamin D serum levels assessment</u>

Vitamin D serum levels for our cohort were available from a previous study.[110] Vitamin D levels were measured using two methods: the liquid chromatography, tandem mass spectrometry (LC-MS/MS) method and the high performance liquid chromatography (HPLC) using ultraviolet. The two methods are equally sensitive for detecting both forms of 25-hydroxyvitamin D (OH)D(25(OH)D_2 and 25(OH)D_3[110].

Normal serum Vitamin D levels were considered over 31ng/ml, insufficient between 20 and 30ng/ml and deficient less than 20mg/ml. That classification

was based on previously published literature on serum VitD deficiency[62,63,64]. Thus, VitD deficiency was defined as VitD levels of <20ng/ml and patients were stratified based on that (Deficiency versus normal/insufficient).

D. Statistical methods

Dependent variables in our cohort were the periodontal disease markers (mCAL, mPD, mBI, NoT and DIAGNOSIS) and independent variables were the age, current smoking status, education, mPI, mVL, mCD4, use of HAART and Vitamin D deficiency. The following variables were assessed as potential confounders of the association between periodontal disease and VitD deficiency: age, race, smoking, education, mPI, mVL, mCD4 and HAART use. Because, smoking is a very strong risk factor for periodontal disease that can significantly affect its severity and mask the association, if any, of the disease extent with the VitD status, we divided the group in smokers and non-smokers and we run the same statistical analyses for the two subgroups.

Statistical tests available on IBM SPSS statistics version 22 were used for all statistical analyses that were performed. Initially, we compared the aforementioned dependent and independent variables between the smokers and non smokers using the Pearson's chi-squared test for the categorical variables (race, education, CD4 by category, VL by category, HAART, VitD deficiency and diagnosis) and the independent student's *t*-test for the quantitative variables (age, mean serum VitD, mVL, mCD4, mCAL, mPD, mBI, mPI and mNoT) (Table 6). We then stratified the individuals based on VitD deficiency and compared them on age, race, education, mCD4, mVL,

HAART and mPI. These comparisons were made for the total group, the smokers and non-smokers, calculating the p-value using again Pearson's chi-squared and independent t-test for the categorical and quantitative variables respectively (Table 7). Finally, we calculated the mean periodontal disease markers for the VitD deficient, insufficient and sufficient groups in the total group (Table 8), the non-smokers (Table 9) and the smokers (Table 10).

Linear regression was used to determine associations between Periodontal Disease markers (PDmarkers) and VitD Deficiency for the total sample (including smoking status) and with stratification by current tobacco smoking status. All available potential covariates (age, race, education, smoking, mPI, mVL, mCD4 and use of HAART) were entered into each Linear Regression model. Only the variables that were significant on the 0.10 level were retained in the final multivariate model. Association between PDmarkers and VitD Def, after confounder adjustment, was considered statistically significant when P value was <0.05 (Table 11).

The study was approved by UIC-IRB/ACCC: 2011-0516). The study was financially supported by an NIH/NIAID grant. (UO1-AI-034993)

IV. RESULTS

The summary of the characteristics of the study cohort are presented in Table VI. Participants were predominantly African Americans (71%) and smokers (52%); mean age was 39.5 y/o; 43% had completed high school. Mean Biomarkers levels were VitD (20.2ng/ml), mVL ($123x10^3$ copies/ml), mCD4 count ($351/mm^3$). 12% reported using highly active antiretroviral therapy (HAART). Average NoT was 20.7, mPD (1.7mm), mCAL (1.2mm), mBI (0.2) and mPI (0.9); 16% met the criteria for moderate/severe PD.

As shown in Table VI, smokers were more likely to be African-Americans (p=0.018) and less likely to have completed high school education (p=0.002) compared to non smokers. No other differences were noted between these two subgroups, including periodontal disease markers. The smokers had a greater mCAL (1.4±0.8mm) and less mNoT (18.8±6.7) compared to non-smokers (mCAL: 1.01±0.8mm and mNoT: 22.8±6.3) indicative of more severe periodontal disease; however, these differences were not statistically significant. The smokers had also lower mean Serum VitD levels and a greater percentage of VitD deficient individuals compared to non smokers; however, again the differences were not statistically significant.

TABLE VI: TOTAL CHARACTERISTICS AND PDMARKERS FOR TOTAL GROUP, NON-SMOKERS AND SMOKERS

Characteristic	Total Group	Smokers	Non-smokers	p-value
N	75	39 (52%)	35 (48%)	
Race				
African-Americans	53 (71%)	29 (74%)	23 (66%)	**0.018**
Hispanic	8 (11%)	3 (7%)	5 (14%)	
White	14 (18%)	7 (19%)	7 (20%)	
Mean Age (S.D)	39.5 (7.1)	40.2 (7.2)	38.8 (7.2)	0.605
Education (High School Completion)	32 (43%)	10 (26%)	21 (60%)	**0.002**
VitD Category				
Sufficient ≥30ng/ml	13 (17%)	5 (13%)	8 (23%)	
Insufficient 21-29ng/ml	19 (25%)	8 (21%)	11 (31%)	
Deficient ≤20ng/ml	43 (58%)	26 (66%)	16 (46%)	0.069
Mean serum VitD (S.D.)	20.2 (12.8)	18.4 (13.8)	22.5 (11.6)	0.713
CD4 Category				
≤200	25 (33%)	13 (34%)	12 (34%)	
200-499	29 (39%)	12 (32%)	17 (48%)	
≥500	20 (28%)	13 (34%)	6 (18%)	
mCD4 (S.D.)	351 (288)	380 (312)	313(263)	0.354
Viral Load Category				
≤4,000	29 (39%)	15 (38%)	14 (40%)	
>4,000-≤50,000	26 (35%)	12 (31%)	14 (40%)	
>50,000	19 (26%)	11 (31%)	7 (20%)	
mVL ($x10^3$) (S.D.)	123 (374)	123 (306)	127 (445)	0.712
HAART	9 (12%)	5 (13%)	4 (11%)	0.855

Periodontal Indexes				
mCAL (S.D.)	1.2 (0.8)	1.4 (0.8)	1.01 (0.8)	0.418
mPD (S.D.)	1.7(0.4)	1.7 (0.4)	1.7 (0.4)	0.782
mBI (S.D.)	0.2 (0.1)	0.2 (0.1)	0.2 (0.1)	0.662
mPI (S.D.)	0.9 (0.5)	1.09 (0.5)	0.8 (0.4)	0.255
mNoT (S.D.)	20.7 (6.7)	18.8 (6.7)	22.8 (6.3)	0.392
Diagnosis				0.838
None / mild	63 (84%)	33 (84%)	29 (83%)	
Moderate / severe	12 (16%)	6 (16%)	6 (17%)	

As shown in table VII, VIII and IX, when we compared the characteristics of our cohort after stratification by VitD status (Deficient vs. non-Deficient), VitD deficient individuals were more likely to be African-Americans compared to the non-Deficient individuals (p=0.01). Also, VitD deficient patients had significantly lower mVL (p=0.01). The same findings were noted in the non-smokers group but not in the smokers, where no difference was noted in any of the characteristics examined.

TABLE VII: TOTAL CHARACTERISTICS BY VITD STATUS FOR TOTAL GROUP

	Total Group		
	VitD Def	Non VitD Def	p
N	43	32	
Race /AA	81%	56%	**0.01**
mAge (S.D.)	40.7(7.2)	38(6.9)	0.73
Education*	38%	50%	0.30
mCD4 (S.D.)	321(287)	390(289)	0.73
mVL ($x10^3$) (S.D.)	81(193)	182(531)	**0.01**
HAART	16%	6.3%	0.18
mPI (S.D.)	1.02(0.5)	0.89(0.5)	0.43

* High School Completion

TABLE VIII: TOTAL CHARACTERISTICS BY VITD STATUS FOR NON-SMOKERS

	Non-Smokers		
	Vit D Def	Non VitD Def	p
N	16	19	
Race /AA	88%	47%	**0.01**
mAge (S.D.)	41(8.7)	37.1(5.3)	0.43
Education *	60%	63%	0.85
mCD4 (S.D.)	236 (183)	379(305)	0.26
mVL ($x10^3$) (S.D.)	40(68)	201(598)	**0.02**
HAART	19%	5%	0.21
mPI (S.D.)	0.86 (0.5)	0.82 (0.4)	0.14

* High School Completion

TABLE IX: TOTAL CHARACTERISTICS BY VITD STATUS FOR SMOKERS

	Smokers		
	Vit D Def	Non VitD Def	p
N	26	13	
Race /AA	77%	69%	0.6
mAge (S.D.)	40.7(6.4)	39.4 (8.7)	0.12
Education*	23%	31%	0.6
mCD4 (S.D.)	366(333)	407(275)	0.9
mVL ($x10^3$) (S.D.)	109 (240)	153 (427)	0.33
HAART	15%	8%	0.49
mPI (S.D.)	1.13(0.51)	0.99(0.6)	0.66

* High School Completion

As shown in tables X, XI and XII, mCAL was greater and mNoT was lower for VitD deficient individuals in the total group and the non-smokers compared to non VitD deficient patients. In smokers, mCAL was greater in the VitD deficient individuals. There was also a trend for lower mBI in VitD deficient patients in all groups examined.

TABLE X: PD MARKERS FOR TOTAL GROUP BY VITD STATUS

	VitD Deficiency	Non VitD deficiency
mCAL (S.D.)	1.4 (0.95)	1.02 (0.73)
mPD (S.D.)	1.72 (0.49)	1.69 (0.37)
mBI (S.D.)	0.19 (0.13)	0.26 (0.21)
mPI (S.D.)	1.02 (0.53)	0.82 (0.46)
mNoT (S.D.)	19.72 (6.8)	22.22 (6.46)

TABLE XI: PD MARKERS FOR NON-SMOKERS BY VITD STATUS

	VitD Deficiency	Non VitD Deficiency
mCAL (S.D.)	1.35 (1.03)	0.72 (0.40)
mPD (S.D.)	1.81 (0.60)	1.64 (0.36)
mBI (S.D.)	0.20 (0.14)	0.24 (0.19)
mPI (S.D.)	0.86 (0.54)	0.82 (0.40)
NoT (S.D.)	20.69 (6.98)	24.74 (5.23)

TABLE XII: PD MARKERS FOR SMOKERS BY VITD STATUS

	VitD Deficiency	Non VitD deficiency
mCAL (S.D)	1.47 (0.91)	1.46 (0.89)
mPD (S.D)	1.67 (0.43)	1.76 (0.39)
mBI (S.D)	0.18 (0.14)	0.28 (0.27)
mPI (S.D)	1.13 (0.51)	0.99 (0.6)
NoT (S.D)	18.96 (6.93)	18.54 (6.5)

The results of the final multivariate linear regression models are presented in Table XIII, XIV and XV. As shown, mCAL was associated with VitD deficiency (p=0.049) only on the non-smoker group, suggesting that VitD deficient patients had greater mCAL compared to the non-deficient individuals. In the total group (p=0.002) and the smokers (p=0.003), mBI was significantly associated with VitD deficiency, suggesting that in these groups, VitD Deficient patients had lower mBI compared to Non-Deficient patients. No other statistically significant associations were observed.

TABLE XIII: ASSOCIATION OF PD MARKERS AND VITD STATUS FROM FINAL MULTIVARIATE LINEAR REGRESSION MODELS WITH RETAINED SIGNIFICANT CHARACTERISTICS BY TOTAL GROUP

	Total Group	
	VitD Deficiency	
	β-coefficient	**p-value**
mCAL	0.119	0.239
adjusted for:		
Age	0.323	0.004
Smoking	0.193	0.098
mPI	0.386	0.001
mPD	0.074	0.526
adjusted for:		
(-)		
mBI	-0.291	**0.002**
adjusted for:		
mPI	0.626	0.000
mNoT	0.013	0.890
adjusted for:		
Age	-0.405	0.000
Smoking	-0.201	0.051
HAART	-0.203	0.037
mPI	-0.351	0.001
Diagnosis	0.186	0.117
adjusted for:		
(-)		

TABLE XIV: ASSOCIATION OF PD MARKERS AND VITD STATUS FROM FINAL MULTIVARIATE LINEAR REGRESSION MODELS WITH RETAINED SIGNIFICANT CHARACTERISTICS BY NON-SMOKERS

	Non-smokers	
	VitD Deficiency	
	β-coefficient	**p-value**
mCAL	0.278	**0.049**
adjusted for:		
Age	0.515	0.004
HAART	-0.262	0.098
mPI	0.504	0.001
mPD	0.175	0.316
adjusted for:		
(-)		
mBI	-0.147	0.336
adjusted for:		
mPI	0.516	0.005
mNoT	-0.132	0.336
adjusted for:		
Age	0.515	0.004
HAART	-0.262	0.098
mPI	0.504	0.001
Diagnosis	0.191	0.271
adjusted for:		
mPI	0.313	0.095

TABLE XV: ASSOCIATION OF PD MARKERS AND VITD STATUS FROM FINAL MULTIVARIATE LINEAR REGRESSION MODELS WITH RETAINED SIGNIFICANT CHARACTERISTICS BY SMOKERS

	Smokers	
	VitD Deficiency	
	β-coefficient	**p-value**
mCAL	-0.046	0.736
adjusted for:		
Race	0.452	0.006
mPI	0.426	0.018
mPD	-0.079	0.623
adjusted for:		
Age	-0.403	0.032
mBI	-0.329	**0.003**
adjusted for:		
mPI	0.615	0.000
mNoT	0.077	0.586
adjusted for:		
Age	-0.467	0.005
Diagnosis	0.151	0.360
adjusted for:		
(-)		

V. DISCUSSION

This cross-sectional, retrospective study aimed to investigate the association of VitD serum levels with the severity of periodontal disease in HIV seropositive women. We hypothesized a positive association of VitD deficiency and periodontal disease severity. Our hypothesis was based on evidence from published literature which reported a protective effect of VitD on periodontal health[54-60].

We found that the hypothesized association between VitD status and periodontal health existed only in non-smokers, with VitD deficient individuals having greater CAL compared to non deficient patients. That association was present after adjustment for potentially confounding variables known to have an effect on CAL.

Our results support the evidence linking VitD status and periodontal disease[120-127]. Millen et al, in a cross sectional study, found that individuals with >50ngr/ml had 33% lower odds of periodontal disease (CDC/AAP definition) compared to individuals with <50nmoL/ml[163]. Jimenez et al. reported that individuals with a VitD levels in the highest quintile had a 14% lower risk tooth loss than those in the lowest quintile, linking these results with the effects of Vitamin D in periodontal disease[164]. VitD status has also been found to play a role in healing after periodontal surgery. Bashutski et al. reported that VitD deficient patients responded less favorably to periodontal surgery compared to VitD sufficient patients and VitD supplementation did not have any effect on outcomes[165].

However, among smokers, an association between VitD deficiency and periodontal disease severity was not found. The most likely explanation is that smoking has such a strong effect on the periodontal health[147], that it could potentially mask the effects of VitD deficiency. In other words, if a patient smokes, levels of serum VitD may not have a significant impact on periodontal health. The potential beneficial effects of VitD in the periodontium most probably cannot outweigh the harmful effects of smoking, thus VitD deficiency or sufficiency becomes less significant to periodontal health and disease smokers. On the contrary, in non smokers, an association with periodontal disease severity was found. It could be concluded that VitD should be considered a factor associated with periodontal health only if more established risk factors such as smoking have been addressed first. In multifactorial diseases, such as periodontitis, primary risk factors may need to be taken into account first in order to unmask weaker associations such as that of VitD.

An unexpected outcome was that in smokers, there was a strong association (β-coefficient: -0.329, p=0.003) between VitD deficiency and lower mBI, indicative of less periodontal inflammation. This comes in contrast with other studies[124,125,], in which VitD serum levels were inversely associated with Bleeding on Probing (BOP). A sound biologic explanation for our unexpected finding cannot be given. It could be hypothesized that this was a statistical error. It is however true that VitD deficiency has been associated with a significant increase in oral epithelial proliferation and keratinization[166]. It could be speculated that this increase may result in the gingival tissue being more

resistant to bleeding[167], after the introduction of a periodontal probe inside the sulcus. This explanation however, is speculative and cannot be verified with the data available in our study. An additional limitation of our study Is that we did not have no information about the number of cigarettes and smoking years for each subject, factors that could all affect the extent of gingival inflammation, as represented by BI[149].

Major additional limitations need to be acknowledged before drawing final conclusions about the association of VitD with periodontal disease. First, it is a retrospective, cross sectional study that was performed on HIV seropositive women. Thus, no conclusions can be drawn on causality between VitD deficiency and periodontal disease. The sample size was small and the majority of individuals were African-Americans. Moreover, the data collection was based on a split mouth design, thus they may have not been 100% representative of the periodontal status of each patient[168].

The data collection in this study was between 1995 and 2003, with a small percentage of individuals receiving HAART therapy. A decade ago, HAART was different compared to what it is now[4]. Extrapolation of these results to HIV seropositive individuals of today is problematic, as the profile of HIV infected patients has changed significantly since then including years of infection and available medications[4].

Another limitation of the study is that the individuals examined were under regular dental care. This can be seen by the low mCAL, mPD, mBI and mPI as well as the low percentage of individuals with a diagnosis of moderate / severe Periodontal disease. Consequently, this group of patients may not be

representative of the general HIV+ population, due to the frequency of dental care received[169]. Finally, the true periodontal condition of these individuals may also not be well represented by the periodontal disease markers. The reason is that part of the regular dental care may have been periodontal treatment and extractions of hopeless or questionable teeth. Thus, the severity of periodontal disease may have been underestimated, as by extracting teeth with severe periodontal disease, the mPD and mCAL for each individual also reduce. Ideally, we would like to have information about the periodontal status of these individuals at the initial visit, before any dental treatment.

Moreover, CAL is a periodontal marker that represents the history of periodontal disease and the severity of the periodontal tissue loss on an individual. However, it does not provide information about the current periodontal status and the extent of inflammation of the periodontal tissues. For example, two individuals may have the same mCAL, but one may have active periodontal disease and the other may have a history of periodontal disease, but currently treated and under control. The bleeding index and the periodontal probing depth are markers that more accurately represent the inflammatory status and the periodontal disease activity. In our study, in the non-smokers group, where the association between VitD and mCAL was found, no association was observed between VitD and mPD or mBI.

The results of this study are therefore not universal and should only be considered In the context of the limitations discussed above.

This study reports that VitD serum levels may be a factor associated with periodontal health. Thus, the question arises about whether an oral health provider, dentist or periodontist, need to be assessing VitD serum levels as part of his clinical examination. It is quite far-fetching and unsupported by evidence so far to suggest that evaluation of VitD serum levels should be part of a standard periodontal examination and periodontal risk assessment. Plaque control, oral hygiene, smoking and diabetes control are well established factors, in close relationship and with direct effect to the periodontal tissues and periodontal health. These variables should be the first to be assessed and addressed as part of periodontal treatment. What this study suggests, is that after these factors have been addressed, there may be validity in evaluating secondary factors with VitD serum levels being one of them. An oral health provider could potentially evaluate the presence of VitD deficiency, after the aforementioned factors have been addressed and periodontal disease progression has not been arrested or recurrence is being noted. It is therefore, too early to incorporate VitD supplementation as part of periodontal therapy, in case of VitD deficiency. The evidence so far is scarce and inconclusive[127,128,153].

Future research should aim towards (i) assessing the effects of VitD deficiency in periodontal disease and (ii) evaluating the effects of VitD supplementation on periodontal health in prospective controlled clinical trials. The optimal dosage of VitD supplementation has to be determined and its impact on prevention of comorbidities among HIV-infected people needs to be

assessed. It is important however for future studies to consider stratification of the cohort for clinically significant factors including tobacco smoking.

The implications of potential beneficial effects of VitD supplementation in periodontal treatment could be very significant. In our study, our cohort was HIV seropositive women. In the first years of HIV infection, HIV-seropositive individuals were affected by more severe forms of periodontal disease, such as necrotizing ulcerative gingivitis and periodontitis[5]. After the development of new medications and new treatment modalities, AIDS related morbidity and mortality have been dramatically reduced and the life expectancy and quality of life of HIV-seropositive individuals has significantly increased[4], exposing these patients to more age-related diseases, including among others chronic periodontitis[5]. Many HIV patients are now characterized by undetectable viral load levels, normal CD4+ numbers and restored immune function[4]. Recent literature reports no difference in periodontal disease severity and progression between HIV-positive and HIV-negative patients[7]. Thus, in these individuals other factors, rather than immunosuppression, start to emerge as potential modifiers of periodontal health and disease including smoking[7] and as suggested in this study, VitD. If VitD supplementation is effective in periodontal health, HIV-seropositive individuals could be a group of patients that might benefit from such treatment modality. It is true, that certain populations do not have access or the financial means to ideal periodontal treatment and compliance to periodontal maintenance after active treatment is also low[170]. Maybe these are the populations that could benefit more from

VitD supplementation, compared to individuals that are able to afford periodontal treatment and regular dental care.

VI. CONCLUSION

According to this study, VitD deficiency seems to be associated with markers of worse periodontal health in HIV+ non-smokers.

As the treatment of HIV has improved, new factors have emerged as clinically relevant to the management of oral health of HIV+ individuals. Tobacco smoking has long been recognized as such a factor, this study supports the continued study of VitD status as one of these important emerging factors.

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VIII. VITA

PANAGIOTIS DRAGONAS

First Name	Panagiotis	**Address**	In Greece: Priamou St. 105, Voula
Last Name	Dragonas		Athens, Greece, 16673
Gender	Male		In the U.S.A: 1100 N Dearborn St,
Date of Birth	13 Dec 1986		Chicago, IL 60610 (apt#1305)
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Nationality	Greek	**Phone numbers:** (cell) 312-646-8295	
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- **EDUCATION**

06/2012- Present — **University of Illinois at Chicago (U.S.A.),** Department of Periodontics, College of Dentistry.
Candidate for Certificate in Periodontics
Candidate for Masters in Science
Expected completion date May 2015

09/2004- 08/2010 — **University of Athens (Greece)**, School of Dentistry, **Doctor of Dental Surgery**
(G.P.A. 7.54 out of 10.00) – Ranking: 12th among 143 graduates in total for the academic year 2009-2010

09/2009-11/2009 — **University of Gothenburg (Sweden),** Sahlgrenska Academy, Institute of Odontology, Department of Periodontics, Student Externship Program.

06/2004 1st Arsakeion High School in Palaio Psychiko, Athens, High School Diploma (G.P.A. 19.6 out of 20.0).

- **PROFESSIONAL EXPERIENCE**

11/2010-11/2011 General Dentist in the Division of Dental and Oral Surgery of Salamina Naval and Veterans Hospital,
Head: Commander, Dr. I. Militsis

01/2010-06/2010 Dental Assistant in the Department of Oral and Maxillofacial Surgery of Hippocratio General Hospital of Athens.
Head: Dr. Ch. Dendrinos

09/2008-08/2009 Dental Assistant in a Periodontics Private Practice,
Dr. Spyros Sylvestros, DDS Periodontics clinic

- **SCHOLARSHIPS AND AWARDS**

10/2014 5,000$ Scholarship from the Gerondelis Foundation for Academic Excellence during Post-Graduate Studies

07/2014 15,000$ Scholarship from the Onassis Foundation for Academic Excellence during Post-Graduate Studies

09/2004 Award from the School of Dentistry, University of Athens, for achieving high ranking (3rd) in the general examinations for Admission

09/2004 Honorary Scholarship from the State Scholarships Foundation, IKY, Athens for achieving high ranking (3rd) in the general examinations for Admission at the School of Dentistry, University of Athens.

09/2009-11/2009 Erasmus/Socrates Scholarship for studying abroad as an undergraduate student.

▪ RESEARCH EXPERIENCE

- Master's Research Project: ***Association of Vitamin D serum levels and the severity of Periodontal disease in HIV seropositive women***, University of Illinois at Chicago, Department of Periodontics *(ongoing).*
- "Vitamin D and its association with Periodontal Disease: A Literature Review", Dragonas P., Bobetsis YA, Madianos P.N. *(ongoing)*
- Dragonas P., Drakopoulos T., Kontopoulos K., Mantalias D., Mpistolakis K.: "Association of Periodontal disease with Diabetes mellitus". 12th Student Scientific Congress, University of Athens School of Dentistry, May 2008.
- Dragonas P., Drakopoulos T., Kontopoulos K., Mantalias D., Mpistolakis K.: "Post Removal Techniques". 12th Student Scientific Congress, University of Athens School of Dentistry, May 2008.
- S.N. Zanakis, S. Kyriakou, P. Dragonas, J. Aggelidis, G. Giamarelos, Ch. Dendrinos: "Management of Osteonecrosis in Patients on Bisphosphonates. The answer is still missing". 2nd Meeting of the Hellenic Association of Supportive Care of the Oral Cavity in Cancer, June 2010.

▪ CONTINUING EDUCATION

- AAP Annual meeting: Los Angeles (2012), Philadelphia (2013), San Francisco (2014)
- AAP Spring Conference: Chicago (2013)
- ITI Congress North America: Chicago (2013)
- Klavan Lecture - University of Illinois at Chicago (2012, 2014)
    - Speakers: Yvan Fortin, Richard Sullivan
- Midwest Society and Illinois Society of Periodontology (2012, 2013, 2014)
    - Speakers: Giovanni Zucchelli, Maurizio Tonetti, Brian Mealey, Stewart Froum, Mark Nevins, Ziv Mazor

- **SKILLS AND QUALIFICATIONS**

- **Languages:** Proficient in Greek, English and French
- **ACLS and BLS certification**
- **Graduate Record Examination** (**G.R.E**): *Verbal (500/800)*
  Quantitative (720/800)
  Analytical Writing (3.0/6.0)

- **ACTIVITIES**

- **Swimming** (ranked 6th in the Pan-Hellenic Championship of 1998 in 50m Butterfly)