EVALUATION OF CIRCULATORY AND SALIVARY LEVELS OF IL-17 IN PERIODONTAL HEALTH AND DISEASE

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CERTIFICATE

This is to certify that this dissertation titled **"EVALUATION OF CIRCULATORY AND SALIVARY LEVELS OF IL-17 IN PERIODONTAL HEALTH AND DISEASE "** is a bonafide record of work done by **Dr. NAJUMUDEEN .A** during his postgraduate study period 2008-2011.

This dissertation is submitted to the TAMILNADU DR.MGR MEDICAL UNIVERSITY in partial fulfilment for the award of degree of **MASTER OF DENTAL SURGERY, BRANCH – II PERIODONTOLOGY.** It has not been submitted (partial or full) for any other degree or diploma.

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LIST OF ABBREVIATIONS

BGI - Biofilm Gingival Interface

CAL - Clinical Attachment Level

CD - Cluster Differentiation

CRP - C Reactive Protein

ELISA - Enzyme Linked Immuno Sorbent Assay

GCF - Gingival Crevicular Fluid

IFN - Interferon

Ig - Immunoglobulin

IL - Interleukin

LPS - Lipopolysaccharide

MMP - Matrix Metalloproteinase

OPG - Osteoprotegrin

PPD - Periodontal Probing Depth

RA - Rheumatoid Arthritis

RANKL - Receptor Activator of Nuclear Factor – κ B Ligand

STAT - Signal Transduction and Activation of Transcription

T reg - Regulatory T Cell

TCR - T Cell Receptor

TGF - Transforming Growth Factor

Th - Helper T Cell

TLR - Toll Like Receptor

TNF - Tumor Necrosis Factor

CONTENTS

LIST OF TABLES

LIST OF GRAPHS

ABSTRACT

Background:

This study was performed to evaluate the circulatory and salivary levels of Interleukin-17 (IL-17) in periodontal health and disease and to correlate it to serum high sensitivity- C reactive protein (hs-CRP) levels.

Materials & Methods:

Peripheral blood and saliva samples were collected from forty periodontal healthy and diseased patients who were recruited for the study in Ragas Dental College and Hospital. Serum was separated from patient's blood and whole unstimulated saliva was centrifuged and subjected to sandwich ELISA analysis for quantification of serum and salivary IL-17 levels. Serum hs-CRP level was detected by the immunoturbidimetric method. Statistical analysis to evaluate the serum and salivary levels of IL-17 was done using the independent sample student 't' test and Pearson's correlation analysis to correlate the serum and salivary IL-17 levels to the hs-CRP.

Results:

The serum IL-17 level in the periodontally diseased group was decreased when compared to the healthy group ($D = 23.77 \pm 0.43$; $H = 25.66 \pm 1.06$), which was not statistically significant ($P = 0.67$; $P > 0.05$). However, there was a statistically significant ($P = 0.038$; $P < 0.05$) decrease in the

salivary IL-17 levels in periodontally diseased group when compared to the healthy group (D = 22.24 ± 0.29; H = 24.63 ± 0.93). There was a statistically significant increase (P = 0.02; P < 0.05) in hs-CRP levels in periodontal disease when compared to health (D = 2.25 ± 0.29; H = 1.16 ± 0.03). There was a statistically significant correlation (P = 0.02; P < 0.05) between the serum and salivary levels of IL-17 in periodontal disease (serum = 22.24 ± 0.29; saliva = 23.77 ± 0.43), but there was no statistically significant correlation between the serum and salivary levels of IL-17 to that of hs-CRP, in the periodontal disease group (IL-17 and circulating hs-CRP levels, P = 0.706 and/or salivary IL-17 and circulating hs -CRP levels, P = 0.54).

Conclusion:

Salivary IL-17 was significantly decreased in periodontal disease when compared to serum IL-17 levels did not correlate with hs-CRP levels.

Key words: Th17, IL-17, Periodontitis, periodontal pathogenesis, ELISA.

INTRODUCTION

Periodontal disease is a chronic inflammatory disease, normally the result of a specific bacterial infection associated with a complex biofilm adhering to the tooth surface, leading to progressive loss of attachment and bone[4]. The tissue destruction in periodontal lesions results from the recruitment of the host cells via activation of monocytes/macrophages, lymphocytes, and other cell types. T lymphocytes have been postulated to play a central role in this process[104]. The Th1/Th2 paradigm has often been used to explain periodontal disease progression but there are still a number of unresolved questions regarding this model[26]. The factors that govern the activation and the effector function of the predominant T cell subsets in the chronic inflammatory periodontal tissues are not yet fully elucidated[85].

Recently, a T helper subset, termed the Th17 cells, was identified and has been proposed to explain some of the short comings of the Th1/Th2 model. Th17 cells have been suggested to play an important role in autoimmune and inflammatory disorders[9, 47]. Atkins CJ and Bettelli E et al [5, 9], showed that the Th17 cells arose in the presence of TGF-β, IL-6, IL-1β and were expanded specifically by IL-23. Although the primary effector cytokine of these Th17 cells is IL-17, other cytokines such as IL-12, IL-16 and TNF-α, are also produced.

IL-17 has emerged as a crucial regulator of inflammatory responses[17]. It is known to activate fibroblasts, epithelial cells, endothelial cells and osteoblasts to produce pro inflammatory cytokines such as IL-6, IL-8, GCSF and MMP[102]. It is this ability to mediate inflammatory/immune responses that has implicated IL-17 in the etiopathogenesis of periodontal disease[38].

Elevated levels of IL-17 have been identified in GCF following periodontal disease[18]. Similarly, increased IL-17 level has been reported in the synovial fluid in patients with rheumatoid arthritis and osteoarthritis[13]. Over expression of circulatory IL-17 levels have also been consistently reported in inflammatory / autoimmune conditions such as RA and SLE[98, 17]. Although several investigators have demonstrated increased levels of proinflammatory cytokines such as IL-1, IL-6, TNF-α (Yamasaki et al 2004) [101], it is not yet fully understood if periodontal disease may affect circulating IL-17 levels.

Whole saliva is an important physiologic fluid that provides a non-invasive tool for rapid screening and disease prediction[40]. Renewed interest in salivary diagnostics has resulted from development of point of care, microfluidic systems that provide instantaneous data about recent disease activity[61]. Various inflammatory mediators in saliva such as IL-1, IL-6, TNF-α, IFN-γ, etc, have been identified to correlate with periodontal disease activity. Some of the limitations with salivary diagnostics in periodontal disease are its lack of its site specificity, variations due to circadian rhythmicity and unavailability of one specific disease marker[46]. Saliva has also

been used to evaluate both local and systemic inflammatory status as it contains unique biomarkers that reflect both[84].

In recent years, its ability to reflect systemic disease has been extensively investigated. It is not yet fully known which salivary cytokine or groups of cytokines produce the most accurate predictive information regarding the systemic inflammatory state produced as a result of periodontal disease.

There is scant literature regarding salivary IL-17 activity in periodontal disease and its relation, if any, to the systemic inflammatory state.

ABSTRACT

Background:

This study was performed to evaluate the circulatory and salivary levels of Interleukin-17 (IL-17) in periodontal health and disease and to correlate it to serum high sensitivity- C reactive protein (hs-CRP) levels.

Materials & Methods:

Peripheral blood and saliva samples were collected from forty periodontal healthy and diseased patients who were recruited for the study in Ragas Dental College and Hospital. Serum was separated from patient's blood and whole unstimulated saliva was centrifuged and subjected to sandwich ELISA analysis for quantification of serum and salivary IL-17 levels. Serum hs-CRP level was detected by the immunoturbidimetric method. Statistical analysis to evaluate the serum and salivary levels of IL-17 was done using the independent sample student 't' test and Pearson's correlation analysis to correlate the serum and salivary IL-17 levels to the hs-CRP.

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Conclusion:

Salivary IL-17 was significantly decreased in periodontal disease when compared to serum IL-17 levels did not correlate with hs-CRP levels.

Key words: Th17, IL-17, Periodontitis, periodontal pathogenesis, ELISA.

REVIEW OF LITERATURE

Periodontal disease is a chronic inflammatory disease, normally the result of a specific bacterial infection associated with complex biofilm adhering to the tooth surface, leading to progressive loss of attachment and bone[4]. The tissue destruction in periodontal lesions results from the recruitment of the host cells via activation of monocytes / macrophages, lymphocytes, fibroblast and other cell types[104].

Ivanyi and lehner et al (1970) [36], demonstrated that the central role played by T cells in the pathogenesis of periodontal disease. They are now believed to be involved in the homeostasis of periodontal tissue, modulation of the inflammatory/immune responses and the mediation of the bone loss observed in periodontal disease.

Classification of T cells:

T cells are classified based on their function into various categories such as helper T (Th) cells, cytotoxic T (Tc) cells and regulatory T (Treg) cells.

T Helper cell subsets:

Brostoff J et al (1999) [11] reported that T helper cells were so designated because they played a crucial role in the process of antibody production by the B cells. Originally the presence of two different subsets of T

helper cells Th1 & Th2 was described by **Parish & Liew et al (1972) [72]. Later, Mossman & Coffman et al (1989) [63]**, delineated two distinct cytokine profiles associated with the Th1 and Th2 cells and subsequently, the Th1/Th2 paradigm has been used to explain the pathogenic mechanisms involved in several inflammatory / immune disorders including periodontal disease[98].

Mossmann and Coffman et al (1989) [63] found that the T cells have been greatly facilitated by their division into functional subsets. The basis for this division was the identification of distinct cytokine production profiles among T cell clones, giving rise to T helper Th1 and Th2 subsets. The developmental and functional relationship between these prototypic Th subsets was subject to intense study and provided the framework for classifying T cell responses for almost two decades. These 'classical' subsets exemplify the characteristics required to claim subset status. They can be differentiated from naive T cells under the influence of exogenous cytokines and each express unique transcription factors which confer subset-specific expression profiles of cytokine production and effector function.

Sakaguchi F et al (1985) [83], identified a minor subpopulation of $CD4^+$ cells capable of preventing the development of autoimmunity which revolutionized the concept of T cell regulation. Identification of forkhead box P3 (FoxP3) as the lineage-specific transcriptional regulator determining this suppressive phenotype confirmed the status of FoxP3+ **regulatory T cells (Tregs)** as distinct from previously described effector subsets[37].

A newer subset of $CD4^+$ cells termed, the Th17 were then identified[28]. The existence of IL-17 was known previously but it was only with the discovery of the distinct subset of Th17 cells that it was recognized as the primary source of this cytokine. The Th17 responses are protective against certain extracellular bacterial and fungal infections. Dysregulated Th2 responses promote the development of allergy and asthma, while uncontrolled Th1 and Th17 responses can result in autoimmune inflammation; therefore, the actions of these effector $CD4^+$ cells need to be controlled strictly.

Th1/Th2 paradigm/controversies/limitations:

Mosmann and Sad (1996) [64] reported that most cloned lines of murine $CD4^+$ T cells can be classified into two groups, Th1 and Th2, based on their cytokine profiles and their related functional activities. The differentiation of $CD4^+$ T cells into Th1 or Th2 is the crucial event in determining whether humoral or cell-mediated immunity will predominate. As $CD4^+$ T cells differentiate, they are thought to go through an intermediate stage, known as ThO. ThO cells express some differentiated effector functions that are characteristic of both the inflammatory and the helper T cells. Several lines of evidence have demonstrated that IL-4 stimulates differentiation into Th2 cells, where as IFN-γ, IL-12, and TGF-β enhance Th1 development[64]. Mouse Th1 cells produce interleukin 2 (IL-2), interferon-γ (IFN-γ), and lymphotoxin (LT), whereas Th2 cells produce IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13. Human Th1 and Th2 cells produce similar patterns, although the synthesis of IL-2, IL-

6, IL-10, and IL-13 is not as tightly restricted to a single subset as in mouse T cells. Several other proteins are secreted by both Th1 and Th2 cells, including IL-3, TNF-α, granulocyte-macrophage colony stimulating factor (GM-CSF), and members of the chemokine (CK) families.

The characteristic cytokine products of Th1 and Th2 cells are mutually inhibitory for the differentiation and effector functions of the reciprocal phenotype. In several cases, alteration of these patterns by cytokine or anti-cytokine reagents reverses host resistance or susceptibility to infection. **Mosmann and Sad (1996)** [64] showed that there is ample evidence that these cytokine patterns are important in mediating resistance to several infectious agents. **Liblau et al (1995)** [48] suggested that Th1 cells contribute to the pathogenesis of organ-specific autoimmune diseases, while Th2 cells prevent them and protect the host from intracellular and extracellular pathogens and/or toxins, respectively.

Seymour & co-workers (1979) [85] have suggested that the early stable periodontal lesion was associated with a robust infiltration of Th1 cells. On the contrary **Ebersole et al (1994)**[19] have proposed that a Th2 cytokine profile was associated with a stable lesion, while Th1 profile was associated with a progressive lesion**.**

The Th1/Th2 paradigm elegantly explained much of the T cell mediated immunity: indeed for 20 years, nearly all diseases were pigeonholed

into one or the other category in many cases regardless of how poorly they actually fit into these models[26]. However it has long been considered that there are nagging discrepancies with respect to the model. In the last few years, the discovery of a new $CD4^+$ subset has resolved many of these controversies and has simultaneously raised a host of new questions and research directions [97].

The role of Th17 cells and their signature cytokine in periodontal disease is only beginning to be addressed.

Development of Th17 cell:

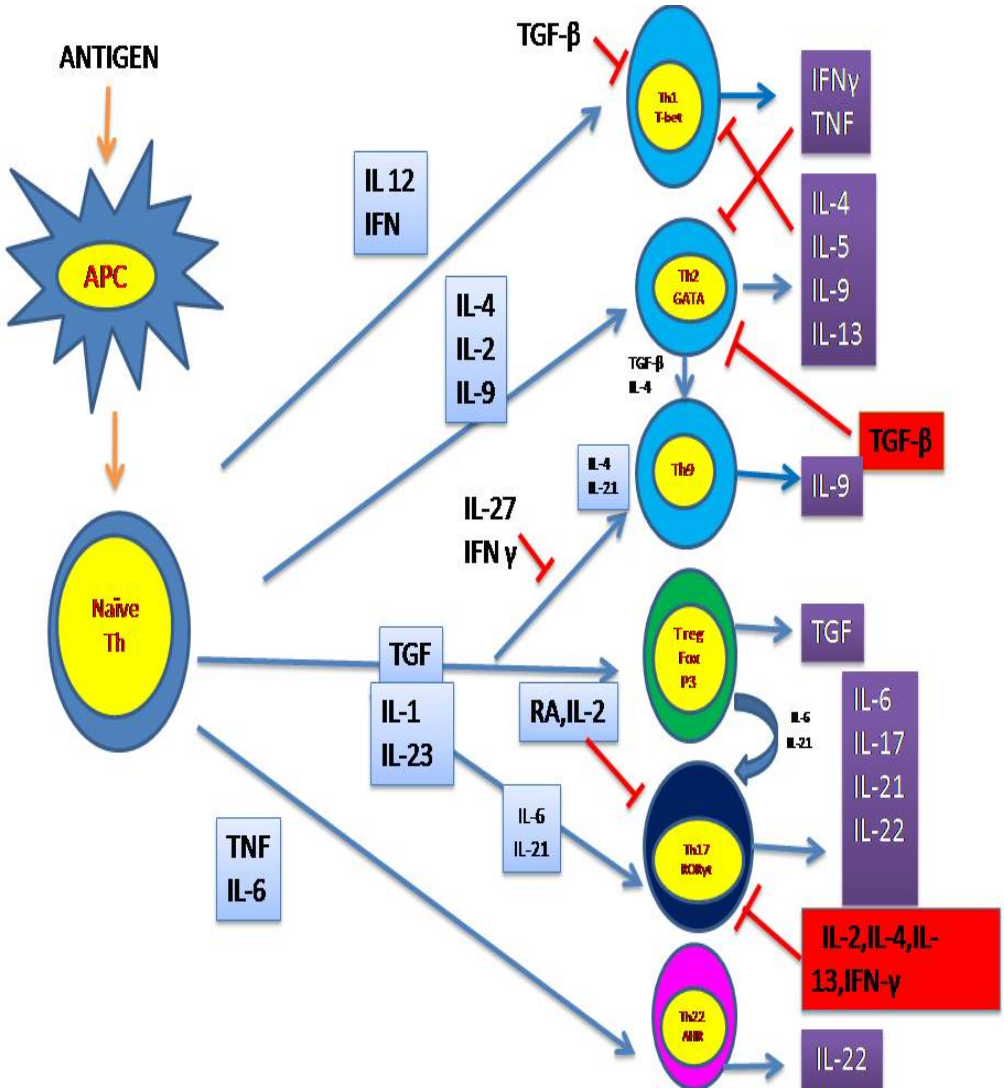
Chen Y et al (2006) [15] suggested that T cell biology has been enriched and enlivened by the description of two further subsets. IL-17 producing T cells were identified as important drivers of autoimmune pathology, forcing the re-evaluation of the role of Th1 cells in models of autoimmunity.

Atkins CJ et al (2006) [5] elucidated the factors that promote the development of the Th17 cells [transforming growth factor (TGF)-β, IL-6 and IL-21] and the regulators of their transcriptional profile (RORγt and RORα) established Th17 cells as a third effector T cell subset. The three effector subsets appear to have evolved to cope with the threat posed by distinct classes of pathogen.

Infante Duarte et al (2000)[33] demonstrated that lipopeptides from Borrelia burgdorferi triggered T cell to produce IL-17, TNF-α and GM-CSF, cytokines not associated with either the Th1/Th2 lineage. These findings were

consistent with reports that IL-17 is not made by Th1/Th2 cells, despite being $CD4^+$ cell derived. **Agarwal et al 2002** [2] demonstrated that IL-23 stimulates murine $CD4^+$ T cells to secrete IL-17 following stimulation of the TCR.

Harrington et al (2005) [28]**,** suggested that the lineage separation between Th1 and Th2 cells was solidified with findings that IL-17 secreting CD4+ T cells arise in the absence of Th1 and Th2 induced transcription factors and cytokines. Thus began an explosion of interest in the generation and function of Th17 cells. They are thought to develop in the presence of TGF-β, however in synergy with IL-6 / IL-1. In addition, IL-21 and IL-23 signals are necessary for expansion and stabilization of their phenotype[29]**.** Of these conductive signals, IL-23 (a member of IL-12 family) has been identified in the inflamed tissues as part of the early chemokine / cytokine infiltrate[66]**.** The presence of IL-6, IL-1 and TGF-β in the chronically inflamed gingival tissues is well documented and would seem to create an environment conductive to Th17 development[79]**.**



Ivanov II et al (2006) [35] demonstrated that for Th17 cells, the master transcription factor retinoic acid-related orphan receptor gamma (RORγ), is required for IL-17 production. RORγ belongs to the super family of steroid nuclear receptors and is most closely related to a retinoic acid receptor (RAR) subfamily of transcription factors.

Studies have demonstrated that RORγt regulation of IL-17 production is not known, nor has it been defined whether RORγt directly or indirectly regulates IL-17a gene transcription. One possibility is that RORγt directly binds to IL-17a promoter. While a potential ROR binding site is present, it has not been demonstrated to be functional. One important means of regulating RORγt expression is through cytokines, especially IL-6 and related cytokines. IL-6 regulation of RORγt is mediated by STAT3, but it has not been established that STAT3 binds the Rorc gene.

IL-17:

Chabaud M et al (1999) [13] stated that IL-17 is a pro inflammatory cytokine derived from T cells and produced mainly by cells of Tho/Th1 phenotype but not cells of Th2 phenotype.

Abbas et al AK (1996)[1] during the past years identified a distinct subset of $CD4^+$ T cells that secrete IL-17 and the closely related cytokine IL-17F as well as other inflammatory cytokines such as IL-6 and IL-22. Currently the IL-17 cytokines include a family of six members (IL-17 A-F), with at least two of them having potent pro-inflammatory properties, IL-17A or CTLA-8 and IL-17F. Both are produced by the recently described Th17 cell subset, are localized at the same chromosomal locus (1A4), share a 55% of homology at the protein level, and seem to have similar function. IL-17A and IL-17F work mostly as homodimers, but IL-17A/F heterodimers have been recently

described in several independent reports **(Harrington LE et al 2007)**[29] suggesting a role in inflammatory response for regulation of such IL-17 complexes.

Kolls J.K and Rose et al (2004)[42] found that since IL-17, also called IL-17A, was identified a decade ago from activated T cells, other cytokines, including IL-17B, C, D, E (also called IL-25) and F have been reported and constitute the IL-17 cytokine family. IL-17, the best-studied member in this family, is a pro-inflammatory cytokine that was historically associated with many inflammatory diseases, such as rheumatoid arthritis, asthma, lupus, and allograft rejection.

Park H et al (2005)[73] in his invitro study has shown that the mRNA expression of genes encoding several chemokines (CCL2, CCL7, CXCL1, and CCL20) and matrix metalloproteinases 3 (MMP3) MMP13 were significantly upregulated after IL-17 treatment. Moreover, IL-17 and TNF-α exhibited strong synergy in promoting inflammatory gene expression. In vivo, overexpression of IL-17 in the lungs resulted in chemokine upregulation and tissue infiltration by leukocytes. IL-17 binds to and signals through IL-17RA, a member of the IL-17R family[102]. Though IL-17 is mainly secreted by activated T cells, the IL-17 receptor is ubiquitously present in all cells.

Effect of IL-17 on individual cell types:

Chabaud et al (2000)[14], reported that T cells are involved in bone destruction via IL-17 production in rheumatoid arthritis. IL-17 has been shown to stimulate epithelial, endothelial, fibroblastic cells to produce IL-6, IL-8 and PGE_2**.** In addition, IL-17 induces RANKL production by osteoblasts[44]. As IL-17 shares properties with IL-1 and TNF-α, it may affect osteoclast mediated bone resorption. It was also hypothesized that T cells in periodontal lesion produce IL-17 and exacerbate inflammatory periodontal disease by activating gingival fibroblasts to produce inflammatory mediators. It has also been reported that T cells can be directly involved in bone metabolism via T cell derived cytokines, receptor activator of NF-κB ligand (RANKL), and RANK[92]**.**

Gingival fibroblasts:

In chronic periodontal lesions, IL-17 has been detected in gingival tissue biopsies and gingival crevicular fluid[69, 96]**.** IL-17 treatment of HGFs has also been observed to lead to IL-6 production, which may contribute to local tissue inflammation[91]**.** Even so, the underlying mechanisms through which IL-17 influences development and severity of periodontal disease remain unclear. It has been hypothesized that IL-17, especially when combined with IFN-γ, may modulate the responses of HGFs [94].

Yongvanichit et al 2008[103] tested the hypothesis that IL-17, especially when combined with IFN-γ, may modulate the responses of human gingival fibroblasts (HGFs). IL-17 induced IL-8 and minimal intercellular adhesion molecule ICAM-1 expression. It had no effect on expression of HLA-DR, CD40, or the immune-suppressive enzyme indoleamine 2,3-dioxygenase (IDO). The effects of IL-17 on HGFs were compared with those of IFN-γ. Unlike IL-17, IFN-γ augmented the expression of HLA-DR, ICAM-1, and IDO, but not IL-8. Thus, IL-17 and IFN-γ induce different HGF responses when administered separately. Interestingly, when IL-17 and IFN-γ were combined, marked enhancement of ICAM-1, IL-8, and IDO expression by HGFs was observed.

Sorsa et al 2007[89] hypothesized that the IL-17 has been reported to up-regulate IL-1β and TNF-α and tissue-destructive matrix metalloproteinases (MMP) in local migrant and resident cells. Immunocytochemistry disclosed elevated IL-1β, TNF-α, and IL-17 levels in periodontitis. These cytokines induced proMMP-1 and especially MMP-3 in gingival fibroblasts, whereas MMP-8 and MMP-9 were not induced. IL-17 was less potent as a direct MMP inducer than IL-1β and TNF-α, but it induced IL-1β and TNF-α production from macrophages, and IL-6 and IL-8 from gingival fibroblasts. In accordance with these findings, immunocytochemistry disclosed that MMP-1 and MMP-3 were increased in periodontitis. Gingival fibroblasts may play an important

role in tissue destruction in periodontitis via cytokine-inducible MMP-1 and MMP-3 production, in which IL-17 plays a role as a key regulatory cytokine.

Epithelial cells:

1. Studies by **Linden A et al (2001)** [49] demonstrated that Interleukin IL-17 may contribute to neutrophilic airway inflammation by inducing the release of neutrophil-mobilizing cytokines from airway cells. The study reported that mitogen activated protein kinases were involved in IL-17 induced release of IL-8 and IL-6 in bronchial epithelial cells.
2. **Kawaguchi et al (2001)** [41] in a study investigated the role of IL-17, in the modulation of primary bronchial epithelial cells, the expression of IL-6, IL-8, and intercellular adhesion molecule 1 (ICAM-1) and the potential involvement of mitogen-activated protein (MAP) kinases in IL-17 mediated signaling. The result showed that IL-17 induced time-dependent expression of IL-6 and IL-8.

Osteoblasts:

Fang Shen et al (2005)[21] demonstrated the spectrum of genes controlled by IL-17 and TNF-α in the pre osteoblast cell line MC3T3-E1. The genes included pro-inflammatory chemokines and cytokines, inflammatory genes, transcriptional regulators, bone remodeling genes, signal transducers, cytokine genes and those involved in apoptosis. The CXC family chemokines were the most dramatically induced by IL-17, which suggested that IL-17

activation of bone cells influenced neutrophil recruitment and subsequent amplification of inflammation.

Role of IL-17 in systemic/autoimmune diseases:

Rheumatoid arthritis (RA) is a chronic systemic disorder that is characterized by autoimmunity, infiltration of joint synovium by activated inflammatory cells, synovial hyperplasia, neoangiogenesis and progressive destruction of cartilage and bone. RA is considered to be a systemic Th1 associated inflammatory joint disease, and T cell comprises of the large proportion of inflammatory cells invading the synovial tissue. T cell activation and migration into the synovium occur as early consequences of the disease, and these cells adopt a pro-inflammatory phenotype. Considerable evidence now supports a role for T cells in the initiation and perpetuation of chronic inflammatory process prevalent in RA. Although T cells represent a large proportion of the inflammatory cells during inflammation, T cell derived cytokines are much abundant. However, because the vast majority of these are memory T cells, IL-17 is up regulated in early disease.

Johnson et al (2004) [38] found, that IL-17 was significantly elevated in the synovial fluid of patients with RA, and was present in osteoarthritic joints as well. Bone erosion mediated by over expression of IL-17 has been shown to occur through alterations in the RANKL/OPG ratio[52]**.** Furthermore, IL-17 knockout mice are highly resistant to collagen induced arthritis **(Nambu A et**

al 2003) [67] and blocking IL-17 reduces inflammatory symptoms and bone loss with CIA[95]**.** Conversely, excess IL-17, as provided by adeno-virus-mediated gene vectors, exacerbates disease[51]**.**

Katz Y et al (2000) [39] showed that apart from the role of IL-17 in autoimmune arthritis, IL-17 and its family member exhibit a potential role in other inflammatory diseases, such as lung, gut, and skin inflammation. IL-17 regulated the gene expression and protein synthesis of a complement system. It has a regulatory role on C3 expression and synthesis and an amplifying effect on TNF induced factor B synthesis. In addition, IL-17 stimulates granulopoesis and was a strong inducer of neutrophil recruitment through chemokine release[60]**.**

Antonysamy MA in (1999)[3] demonstrated that IL-17 promoted angiogenesis and a role for IL-17 was suggested in allogenic T cell proliferation that might be mediated in part through a maturation inducing effect on dentritic cells. Studies revealed that T cell cytokine had an important function in the activation of T cells in allergen specific T cell mediated immune responses. IL-17 induced neutrophil accumulation in infected lungs. Greatly diminished recruitment of neutrophils into lungs was found in mice with homozygous deletion of the IL-17 receptor in response to a challenge with a gram negative pathogen. IL-17E transgenic mice showed growth retardation, jaundice, a Th2-biased response and a multi organ inflammation[75]**.** Furthermore, IL-17 expression was observed in ovarian, endometrial and

cervical cancers exhibiting an angiogenic effect[23]. However, further studies are needed to unravel the role of IL-17 in the pathogenesis of these autoimmune inflammations.

The role of IL-17 in the bone microenvironment was that it stimulated the proliferation of human mesenchymal stem cells (hMSCs). MSCs are pluripotent progenitor cells that contribute to the regeneration of mesenchymal tissue, including bone, adipose tissue, cartilage, muscle, ligament, tendon, and stroma[76]. IL-17 was shown to increase the formation of fibroblast like colonies from bone marrow cells[32]. The IL-17 dependent signalling pathway that leads to increased proliferation of hMSC and showed that generation of reactive oxygen species (ROS) is crucial for increased proliferation. Furthermore, the results showed that IL-17 also stimulated the migration, motility, and osteoblastic differentiation of hMSCs. In addition, IL-17 induces the expression of M-CSF and RANKL on hMSCs, thereby stimulating osteoclastogenesis.

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease which is in part mediated by the migration of monocytes from blood to RA synovial tissue, where they differentiate into macrophages and secrete inflammatory cytokines and chemokines.

Shahrara S et al (2009)[88] in his study has shown that in vivo, IL-17 mediates monocyte migration into sponges implanted into SCID mice. In vitro, IL-17 was chemotactic, not chemokinetic, for monocytes at the concentrations detected in the RA synovial fluid. Further, IL-17-induced monocyte migration was mediated by ligation to IL-17RA and RC expressed on monocytes and was mediated through p38MAPK signaling. Finally, neutralization of IL-17 in RA synovial fluid or its receptors on monocytes significantly reduced monocyte migration mediated by RA synovial fluid. These observations suggest that IL-17 may be important in recruiting monocytes into the joints of patients with RA, supporting IL-17 as a therapeutic target in RA.

IL-17 in periodontal disease:

The immune cell activation has been long considered to occur dominantly as a Th1 or Th2 response. **Rose NR et al (2003)** [81] found that however, numerous diseases cannot be characterized unambiguously as Th1 or Th2. An important addition to the Th development model came with the re-emerging recognition of the T suppressor or Treg cell subset. Treg cells as $CD4^+$ $CD25^+$ cells that antagonize Th1 and Th2 immune response, in part by secreting suppressive cytokines. Treg cells arise in the presence of immunosuppressive cytokine TGF-β and secrete IL-10 and TGF-β[80]**.** The Treg population mediates resolution of inflammation and can prevent autoimmunity in number of animal models. **Mottet C et al (2003)** [65] showed that Treg cells

are believed to play a significant role in preventing chronic, unresolved inflammation that is characterized of autoimmune diseases, such as RA, MS and colitis.

Seymour GJ (1993) [86] discussed the role of Th1 / Th2 subsets in the progression of periodontal disease. It was proposed that Th1 response is characteristic of a stable lesion, whereas a Th2 response is typical of progressive lesion. Alternatively, Th1 cells mediate tissue destruction, whereas Th2 cells play a protective role. **Dutzan N et al (2005)** [18]**,** demonstrated that there is little consensus regarding the predominant Th subset in periodontal disease, the presence of inflammatory cytokines is well established and these cytokines fall into Th1 and Th17 categories. Specifically, TNF-α, IFN-γ and IL-1β, IL-2, IL-6, IL-11 and IL-17 were identified reproducibly in periodontal pockets and GCF.

Gingival inflammation begins when endotoxin or bacterial LPS induce synthesis of proinflammatory cytokines by gingival cells. Initially cell mediated immune response occurs in response to those bacteria. **Ellis SD et al (1998**)[20] which results in release of Th1 type cytokine into the gingiva, modulating the features and potential spread of the inflammation. The inflammation resolves coincident to antibody synthesis by Th2 cell type. There is evidence suggesting a defective transition from a Th1 to Th2 cytokine profile in the gingiva within periodontitis. There is also evidence for the

altered balance between concentrations of pro-inflammatory and anti-inflammatory cytokines within the microenvironment at several non oral sites.

Mitnick MA (2001)[62] demonstrated that IL-17 significantly upregulated IL-6 expression and in turn IL-6 negatively regulated IL-11 by gingival cells suggesting that IL-11 and IL-17 might have significantly different function in the pathogenesis of periodontitis. IL-17 is produced by activated $CD4^+$ T cells. IL-17 has been suggested to be a fine tuning cytokine since it activates both fibroblast and endothelial cells to increase the secretion of IL-6 in the presence of TNF-α. The cells of the Th1/Th0 phenotype produce IL-17, but not cells of the Th2 phenotype. IL-11 has been reported to have anti-inflammatory properties due to its inhibition of the expression of proinflammatory cytokine and nitric oxide by macrophages[13].

Studies on GCF levels of IL-17:

Dutzan N et al (2005)[18] determined the presence of IL-17 in gingival crevicular fluid (GCF) samples and in the culture supernatants of gingival cells from patients with chronic periodontitis. The results showed that the total amount of cytokine IL-17 was significantly higher in the periodontitis group than the control group. Significantly higher GCF volume and amount of total proteins were obtained from periodontitis patients as compared with control subjects. A higher concentration of IL-17 was detected in culture supernatants from periodontitis patients compared with healthy subjects, either with or

without phytohaemagglutinin (PHA) stimulation. Treatment with PHA induced a significant increase in the production of IL-17 in healthy subjects and periodontitis patients. Therefore the total amount of cytokine IL-17 in GCF samples and in the culture supernatants of gingival cells were significantly increased in periodontal disease.

Takashi (2002)[93] demonstrated that the role of interleukin-17 (IL-17) in the progression of periodontitis focusing on the presence of mRNA expression of IL-17 evaluated using RT-PCR and direct sequencing, IL-17 production in gingival crevicular fluid (GCF) by ELISA and cultured periodontitis tissues by Western blot analysis; and whether recombinant human IL-17 (rhIL-17) and culture supernatants of periodontitis tissues induced IL-6 production from cultured human gingival fibroblasts (HGF). The IL-17 mRNA expression in periodontitis tissues were detected, whereas IL-17 in GCF sample was not detectable.

In a study by **Avani R Pradeep et al (2009)**[6] the role of IL-17 and IL-18 in periodontal health and disease and the effect of periodontal treatment on its concentration were collected from both the groups. ELISA analysis was done to estimate the levels of IL-17 and IL-18. IL-18 levels in GCF increased proportionately with the severity of periodontal disease and decreased after treatment. IL-17 level in GCF was nearly undetectable. The study concluded that absence of IL-17 in GCF indicated that it cannot be considered as a biomarker of periodontal disease progression.

Buduneli et al (2009)[12] in a study evaluated the effects of initial periodontal treatment on GCF levels of IL-17, RANKL and OPG in smoking and non-smoking patients with chronic periodontitis. The study showed that smoking does not affect the GCF levels of IL-17, RANKL and OPG in patients with chronic periodontitis. Both smokers and non-smokers were affected similarly by the initial periodontal treatment with regard to GCF concentration of IL-17 and OPG.

Berker E et al (2009)[8] in a study determined the total amount, concentration and ratio of interleukin IL-11 and IL-17 in gingival crevicular fluid (GCF) of chronic periodontitis (CP) patients were analyzed by enzyme-linked immunosorbent assays. The total amount and concentration of IL-11 and IL-17 were significantly lower in the Chronic Periodontitis, especially in areas with deeper pockets (probing depth ≥ 5mm). The data confirmed that the decreased ratio of IL-11:IL-17 may be a factor, which has shown this imbalance between the cytokines activities in deeper pockets.

Studies on circulatory levels of IL-17:

Brooks CN et al (2010)[10] in view of the inflammatory nature and severity of aggressive periodontitis hypothesized that IL-17 might be detected in sera from patients with aggressive periodontitis. The results of this study showed that IL-17 was barely detectable in sera from periodontally healthy individuals, but was present at significantly higher concentrations in sera from

those with LAgP and GAgP. Multivariate analyses demonstrated associations of IL-17 concentrations with periodontal attachment loss, but not with current smoking. Therefore, Th17 responses may be characteristic of AgP, and IL-17 may play a role in the pathogenesis of aggressive periodontitis.

Luciene Cristina Figueiredo et al (2010)[53] evaluated the serum levels of tumor necrosis factor-alpha (TNF-α), interferon-γ, and interleukin IL-4, IL-17 and IL-23 in subjects with generalized chronic periodontitis (GCP) and generalized aggressive periodontitis (GAgP) before and after non-surgical periodontal therapy. The results demonstrated a significant improvement in clinical periodontal status. At baseline, concentrations of TNF-α and IL-17 were significantly higher in the GAgP group compared to the other groups. There was a significant decrease in serum concentrations of TNF-α and IL-17 at 6 months post-therapy in the GAgP group. The concentration of TNF-α remained elevated in the GAgP group compared to the GCP group at 6 months post-therapy.

Salivary diagnostics:

As a non-invasive technology, saliva diagnostic methods are highly attractive. Salivary biomarkers, whether produced by healthy or diseased individuals, are sentinel molecules that could be used to scrutinize health and perform disease surveillance.

In general, clinical parameters including probing depth, attachment level, bleeding on probing (BOP) plaque index (PI) and radiographic loss of alveolar bone are used to access disease severity[77]**.** Occasionally, monitoring of the microbial infection **(Listgarten in 1992 and Greenstein in 1988)** [50, 27] and analysis of the host response in gingival crevicular fluid (GCF) are utilized in an attempt to identify individuals at risk for future breakdown[16, 45]**.** Recently, genetic analysis has also been suggested as a means to identify individuals who are at high risk for more severe periodontitis[43]**.** However, as of yet, no clinical or laboratory test is routinely employed in the monitoring of patients with periodontal disease. It has long been realized that a rapid and simple diagnostic test that can provide a reliable evaluation of periodontal disease and identify patients at risk for active disease would be of value to both clinicians and patients.

Saliva is a fluid that can be easily collected, contains locally-derived and systemically derived markers of periodontal disease, and hence may offer the basis for a patient specific diagnostic test for periodontitis. The fluid samples can be considered as whole saliva, and saliva can be collected from a specific salivary glands parotid, submandibular and sublingual, as well as minor salivary glands[54]**.** It can be collected with or without stimulation.

Whole saliva consists of a mixture of oral fluids, and includes the secretion of the major and minor salivary glands, in addition to constituents of non salivary origin derived from GCF, expectorated bronchial secretions,

serum and blood cells from oral wounds, as well as bacteria and bacterial products, viruses and fungi, desquamated epithelial cells and food debris [54].

Saliva has been used as a diagnostic fluid in medicine[55, 22]. The use of saliva for periodontal diagnosis has been the subject of considerable research activity, and proposed marker for disease include proteins of host origin (i.e. enzymes, immunoglobulins), phenotypic markers (epithelial keratins), host cells, hormones (cortisol), bacteria and bacterial products, volatile compounds and ions[56].

Mansheim.B.J (1980) [57] measured Antibodies against a capsular antigen of Bact asaccharolyticus in the serum (IgG) and saliva (IgA) by an enzyme-linked immuno-sorbent assay, ELISA. The levels of IgG were present in a group of 8 patients with adult, rapidly advancing periodontitis and in 3 patients with juvenile periodontitis. Antibody levels in periodontitis were not significantly different from those of age matched normal subjects. Levels of salivary IgA against Bacteria asaccharolyticus determined in healthy volunteers and in adult and juvenile periodontitis patients were not statistically different among the three groups.

Ingman T et al (1993) [34] studied the profile of salivary proteases and their cellular origin, with special reference to polymorphonuclear leukocytes and bacteria, was studied in localized juvenile periodontitis and compared with adult periodontitis and healthy controls. General proteolytic activity in

saliva as well as collagenase, elastase-like and trypsin-like activity and patients with localized juvenile periodontitis to doxycycline inhibition was studied. The saliva of localized juvenile periodontitis patients contained low amounts of collagenase compared with adult periodontitis saliva, and almost all salivary collagenase was found to exist in endogenously active form, as was found to be the case also in adult periodontitis patients and healthy controls.

Over C et al (1993)[71] determined the mean levels of myeloperoxidase (MPO) in samples of gingival crevicular fluid (GCF), whole saliva and peripheral blood neutrophils from patients with rapidly progressive periodontitis (RPP) and adult periodontitis (AP) using a spectrophotometric method. MPO activity detected in the samples was significantly increased in the patient groups when compared to the healthy subjects. The highest MPO activity was found in the RPP group. Also the increased MPO activity in periodontally diseased patients can be attributed to the increased number of neutrophils, the degranulation of these cells and also their hyperactive state in the presence of chronic antigenic stimulation.

Markkanen H (1986)[58] Studied the concentrations of IgA, lysozyme and beta 2-microglobulin (beta 2-m) in wax-stimulated mixed saliva from 28 patients with severe periodontitis and from 28 healthy controls. The correlation between IgA and beta 2-m concentrations was highly significant in both groups studied (P less than 0.0001, and P less than 0.002), whereas beta

2-m and lysozyme concentrations were positively correlated in patients but not in controls. A significant correlation between IgA and lysozyme was found only in periodontal patients (P less than 0.001).

Sandholm L et al (1987) [82] measured the concentration of salivary IgG and IgA and the levels of salivary IgG and IgA antibodies to Actinobacillus actinomycetemcomitans Y4 by ELISA in 205 persons including patients with juvenile and adult periodontitis as well as healthy subjects. Compared to the concentration observed in subjects with a healthy periodontium, a significantly increased concentration of salivary IgG was found in 34% of the patients with moderate adult periodontitis and in 57% of the patients with severe adult periodontitis. The level of salivary IgA was less influenced by the periodontal condition. The level of salivary IgG antibody to A. actinomycetemcomitans was significantly elevated in 55% of the patients with untreated juvenile periodontitis and in 28% of the patients treated for JP.

Recent advances:

As a non-invasive technology, saliva diagnostic methods are highly attractive. Salivary biomarkers, whether produced by healthy individuals or by individuals affected by specific diseases, are sentinel molecules that could be used to scrutinize health and perform disease surveillance.

Wong DT et al (2006)[99] demonstrated that the development of microchips and microfluidic platforms for salivary components has great potential in the

use of oral fluid for point-of-care testing. Microfluidic and microelectromechanical systems are integrated arrangements comprising mechanical elements, sensors, actuators and electronics on a common silicon substrate developed through microfabrication technology. Researchers are designing "lab-on-a-chip" prototypes. These handheld, automated, easy-to-use and integrated systems will enable simultaneous and rapid detection of multiple salivary protein and nucleic acid targets.

Barnfather et al (2005)[7] investigated the effect of immediate feedback from a point-of-care test for salivary nicotine metabolites in promoting smoking cessation and reduction in tobacco use. Saliva samples were analyzed at presentation and at 8 weeks for salivary nicotine metabolites using a 10 min semi-quantitative point-of-care test. They found that a higher smoking cessation rate was achieved with the point-of-care test (23% of cases vs. 7% of controls; $P < 0.039$), and overall tobacco use also decreased (68% of cases vs. 28% of controls; $P < 0.001$).

Herr et al (2005)[30] reported that microfluidic method facilitates hands-free saliva analysis by integrating sample pretreatment (filtering, enrichment, mixing) with electrophoretic immunoassays to quickly measure analyte concentrations in minimally pretreated saliva samples. Rapid (<10 min) measurement of levels of the collagen-cleaving enzyme MMP-8 in saliva from healthy and periodontally diseased subjects can be achieved using 20 µl of saliva. Based on this, a portable diagnostic device called the "Integrated

Microfluidic Platform for Oral Diagnostics" was developed. An early clinical study in which the hand-held Integrated Microfluidic Platform for Oral Diagnostics was used to rapidly (3–10 min) measure the concentrations of MMP-8 and other biomarkers in small amounts (10 ml) of saliva has been reported[31].

Miller et al (2007)[61] reported application of a lab-on-a-chip system for the concomitant measurement of the salivary biomarkers C-reactive protein, MMP-8 and IL-1β as related to the clinical expression of periodontitis and he demonstrates that the results achieved by the lab-on-a-chip approach are in agreement with those acquired by standard enzyme-linked immunosorbent assay, with significant IL-1β and MMP-8 increases in whole saliva of periodontitis patients. The results of the lab-on-a-chip assay were linear for three orders of magnitude, whereas those of the enzyme-linked immunosorbent assay were only linear for two orders of magnitude. The availability of more sophisticated analytic techniques gives cause for optimism that saliva will eventually be the biomedium of choice in clinical diagnostics. These salivary biomarker detectors can be used in the office of a dentist or another health care provider for point-of-care disease screening and detection.

Studies on salivary levels in IL-17:

Stewart C et al (2008)[90] studied that the Th1/Th2 paradigm has been expanded by the discovery of Th17 cells, a subset of $CD4^+$ memory T cells

characterized by their unique ability to secrete interleukin-17 (IL-17) family cytokines. Importantly, Th17 cells appear to be intimately involved in autoimmunity. The study was made to investigate whether the Th17/IL-23 system is up-regulated in (SS). Sera, saliva, and salivary glands from C57BL/6.NOD-Aec1Aec2 mice (a model for primary SS), as well as sera, saliva, and salivary gland biopsy specimens obtained from patients with primary SS, were evaluated for IL-17 and IL-23 expression by immunohistochemistry, real-time polymerase chain reaction, and the Luminex system. The results suggested that the Th17/IL-23 system is up-regulated in C57BL/6.NOD-Aec1Aec2 mice and SS patients at the time of disease. A correlation between up-regulated IL-17/IL-23 expression and specific clinical manifestations of Sjogren's syndrome has yet to be identified.

MATERIALS AND METHODS

Study Population:

40 patients who attended the out patient Department of Ragas Dental College and Hospitals, Chennai were enrolled in the study. Patients were divided into two groups based on their periodontal health status. Informed consent was obtained from all the patients. The study was undertaken following approval from the institutional review board.

Selection criteria:

Group A: Healthy Gingiva - Patients exhibiting no signs of periodontal disease, determined by the absence of clinical attachment loss, absence of bleeding on probing in < 10% of sites. Probing Pocket Depth of <3mm.

Group B: Comprises of Chronic Periodontitis Patients with teeth exhibiting PPD ≥ 5 mm and CAL ≥ 3mm and radiographic evidence of bone loss in atleast 6 teeth, who were otherwise systemically healthy on general examination in Ragas General Hospital.

Chronic periodontitis patients were categorised according the BGI index (Offenbacher et al 2007)[70]. Five levels of disease were defined based upon shallow (PD ≤ 3mm) or deep (PD ≥ 4mm) and Probing Depths in combination with low or high bleeding scores. The BGI-H was defined as all

PDs ≤ 3mm and < 10% BOP; BGI-gingivitis (BGI-G) was defined as individuals with all PDs ≤ 3 mm and ≥ 10% BOP. Three BGI deep lesion (DL) groups were created based upon full mouth PD and BOP scores. BGI-deep lesion/low bleeding (BGI-DL/LB) was defined as one or more sites with PD ≥ 4 mm and BOP extent scores < 10%; BGI-DL/moderate bleeding (BGI-DL/MB) was defined as one or more sites with PD ≥ 4 mm and BOP extent scores between 10% and 50%; and BGI-DL/severe bleeding (BGI-DL/SB) included subjects with one or more sites with PD ≥4 mm and BOP extent scores ≥50%. These five BGI categories created a gradient of disease severity while creating categories that differed by biologic phenotype at site-based and patient-based levels.

Exclusion criteria:

- Patients with history of periodontal therapy or antibiotic therapy and other medication in the last 6 months
- Patients with history of systemic diseases that may affect the periodontal status
- Pregnancy and Lactation
- Smokers
- Evidence of any other active oral infections eg: pulpal pathology.

SAMPLE COLLECTION:

SERUM SAMPLE:

3ml of Peripheral blood was drawn from patients using venepuncture from the antecubital fossa and transferred using sterile test tubes and used for ELISA analysis.

Peripheral blood was drawn prior to onset of Phase I periodontal therapy in periodontitis patients. All patients underwent complete hematological investigation to rule out systemic diseases and conditions.

SALIVA SAMPLE:

Salivary samples were collected according to the technique described by Navazesh Mahvash et al 2008[68].

The patients were advised to refrain from intake of any food or beverage (water exempted) at least one hour before the test session. Smoking, chewing gum and intake of coffee were also prohibited during this hour. The subjects were advised to rinse his or her mouth several times with distilled water and then to relax for five minutes.

During collection of saliva the patient were advised to make every effort to minimize movement, particularly movements of the mouth. Patients were asked to swallow to void the mouth of saliva. Then the patients were asked to lean the head forward over the container with the mouth slightly open and allow the saliva to drain into the container with the eyes open.

ARMAMENTARIUM

1. Disposable needle and syringe
2. Vaccutainers
3. Test Tubes
4. Centrifuge Tubes
5. Micropipettes
6. Micropipette tips
7. Pasteur pipette
8. Laboratory Centrifuge
9. Refridgerator
10. Ice pack (for transfer)
11. Sterile saliva containers

Serum sample preparation:-

2ml of the 3ml blood that was drawn was allowed to clot at room temperature for 30 mins and centrifuged at 3000 rpm for 10 mins. Serum was then transferred to a labelled poly propylene tube and stored at -70^{o} c.

Saliva preparation:

The collected saliva is centrifuged at 3000 rpm at 4°C for 5min. The supernatant fraction is then separated and then stored at −80°C.

REAGENT PREPARATION

All reagents were brought to room temperature before use.

Wash Buffer:

The crystals in the concentrate were warmed to room temperature and mixed gently until the crystals have dissolved completely. 20 ml of Wash Buffer Concentrate were diluted into deionised or distilled water to prepare 500 ml of Wash Buffer.

Substrate Solution:

Color Reagents A and B were mixed together in equal volumes within 15 minutes of use and was protected from light. 200 µL of the resultant mixture was required per well.

IL-17 Standard:

IL-17 Standard was reconstituted with 1.0 ml of deionised or distilled water. This reconstitution produced a stock solution of 20000 pg/ml. The standard was allowed to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. 900 µl of the Calibrator Diluent RD6-21 was pippetted (for serum/plasma samples) into the 2000 pg/ml tube. 500 µl of Calibrator Diluent was pippetted into the remaining tubes. The stock solution was used to produce a dilution series (below). Each tube was thoroughly mixed before the

next transfer. The 2000 pg/ml standard served as the high standard. The appropriate Calibrator Diluent serves at the zero standards (0 pg/ml).

ASSAY PROCEDURE:

All reagents and samples were brought to room temperature before use.

1. Excess microplate strips from the plate frame were removed and returned to the foil pouch containing the desiccant pack, and resealed.

2. 100 µl of Assay Diluent RD1-36 was added to each well.

3. 100 µl of Standard, control, or sample were added per well. Reagent addition was uninterrupted and completed within 15 minutes. It was covered with the adhesive strip provided and incubated for 3 hours at room temperature.

4. Each well was aspirated and washed, repeating the process twice for a total of three washes. Each well was filled with Wash Buffer (400 µl) using a squirt bottle. After the last wash, any remaining Wash Buffer was removed by aspirating or decanting. The plate was inverted and blotted it against clean paper towels.

5. 200 µL of IL-17 Conjugate was added to each well. It was covered with a new adhesive strip and incubated for 1 hour at room temperature.

6. The aspiration/wash was repeated as in step 4.

7. 200 µl of Substrate Solution was added to each well and incubate for 30 minutes at room temperature. It was protected from light.

8. 50 µL of Stop Solution was added to each well. The colour in the wells changed from blue to yellow. If the colour in the wells turned green or the colour change did not appear uniform, the plate was gently tapped to ensure thorough mixing.

9. The optical density of each well was determined within 30 minutes, using a microplate reader set to 450 nm. Wavelength correction was made by subtracting readings at 540 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. This procedure was followed as readings made directly at 450 nm without correction may be higher and less accurate.

Measurement of High Sensitivity C-Reactive Protein (hs-CRP):-

Measurement of hs-CRP was performed using a immunoturbidimetric assay performed on a Randox Daytoner analyzer (Randex Laboratories, Crumlin Co., Autrium, UK). The assay range was 0.1 to 20 mg/l and detection limit was 0.03 mg/l.

CALCULATION OF RESULTS

The duplicate readings for each standard, control, and sample were averaged and subtracted from the average zero standard optical density.

The optical density of the standard versus the concentration of the standards was plotted and the curve was drawn. The data was linearized by using log/log paper and regression analysis was applied to the log transformation.

The IL-17 concentration for each samples were determined using the standard graph /curve plotted.

Statistical analysis:-

The serum and salivary levels of IL-17 levels in periodontally healthy and diseased patients was compared by calculating the mean and standard deviation for each group. Student 't' test was used for statistical analysis and p value was calculated. Correlation coefficient of serum and saliva in periodontal health and disease was analysed using Pearson's correlation. $p < 0.05$ was considered to be statistically significant at the 5% level.

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Measurement of High Sensitivity C-Reactive Protein (hs-CRP):-

Measurement of hs-CRP was performed using a immunoturbidimetric assay performed on a Randox Daytoner analyzer (Randex Laboratories, Crumlin Co., Autrium, UK). The assay range was 0.1 to 20 mg/l and detection limit was 0.03 mg/l.

CALCULATION OF RESULTS

The duplicate readings for each standard, control, and sample were averaged and subtracted from the average zero standard optical density.

The optical density of the standard versus the concentration of the standards was plotted and the curve was drawn. The data was linearized by using log/log paper and regression analysis was applied to the log transformation.

The IL-17 concentration for each samples were determined using the standard graph /curve plotted.

Statistical analysis:-

The serum and salivary levels of IL-17 levels in periodontally healthy and diseased patients was compared by calculating the mean and standard deviation for each group. Student 't' test was used for statistical analysis and p value was calculated. Correlation coefficient of serum and saliva in periodontal health and disease was analysed using Pearson's correlation. $p < 0.05$ was considered to be statistically significant at the 5% level.

RAGAS DENTAL COLLEGE AND HOSPITAL, CHENNAI.

DEPT OF PERIODONTICS

PATIENT PROFORMA

1. NAME :
2. AGE/SEX :
3. OCCUPATION :
4. ADDRESS :
5. PHONE :
6. CHIEF COMPLAINT :
7. PAST DENTAL HISTORY :
8. MEDICAL HISTORY :
9. PERIODONTAL EXAMINATION :

Gingiva

a. Colour

b. Contour

c. Consistency

d. Position

e. Surface texture

Recession:

Furcation Involvement:

Mobility:

Plaque Index

8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8

Bleeding Scores

8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8

PERIODONTAL PROBING DEPTH & CLINICAL ATTACHMENT LEVEL

CAL																
PPD																
	8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8
PPD																
CAL																

CAL																
PPD																
	8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8
PPD																
CAL																

BGI Index

BGI –H

BGI-G

BGI-DL/LB

BGI-DL/MB

BGI-DL/SB

RADIOGRAPHIC INVESTIGATION:

IOPA:

OPG:

BLOOD INVESTIGATION:

Complete Haemogram

DIAGNOSIS:

TREATMENT:

INFORMED CONSENT

Patient Name:

Age/Sex:

I have been explained about the nature and purpose of the study in which I have been a asked to participate. I understand that, I am free to withdraw my consent and discontinue at any time without prejudice to me or any effect on my treatment.

I have been given the opportunity to ask questions about the procedure. I have been informed that this research work is in no way directly related to the therapy or cure of the disease. I have also given consent for taking blood and saliva samples for the study purpose. I have fully agreed to participate in this study.

I hereby give consent to be included in “EVALUATION OF IL-17 LEVELS IN PERIODONTALLY HEALTHY AND DISEASED PATIENTS”

Date:

Signature of Patient Signature of the H.O.D

RAGAS DENTAL COLLEGE AND HOSPITAL, CHENNAI.

DEPT OF PERIODONTICS

PATIENT PROFORMA

1. NAME :
2. AGE/SEX :
3. OCCUPATION :
4. ADDRESS :
5. PHONE :
6. CHIEF COMPLAINT :
7. PAST DENTAL HISTORY :
8. MEDICAL HISTORY :
9. PERIODONTAL EXAMINATION :

Gingiva

a. Colour

b. Contour

c. Consistency

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e. Surface texture

Recession:

Furcation Involvement:

Mobility:

Plaque Index

8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8

Bleeding Scores

8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8

PERIODONTAL PROBING DEPTH & CLINICAL ATTACHMENT LEVEL

CAL																
PPD																
	8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8
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CAL																
PPD																
	8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8
PPD																
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BGI Index

BGI –H

BGI-G

BGI-DL/LB

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RADIOGRAPHIC INVESTIGATION:

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I hereby give consent to be included in "EVALUATION OF IL-17 LEVELS IN PERIODONTALLY HEALTHY AND DISEASED PATIENTS"

Date:

Signature of Patient Signature of the H.O.D

DISCUSSION

Chronic periodontitis is a chronic inflammatory disease that affects the attachment apparatus of the teeth. Although periodontopathic bacteria are the primary etiological agents in periodontal disease, the ultimate determinant of disease progression and clinical outcome is the host's immune response[87].

It is generally accepted that the periodontal disease results from an imbalance between the Th1/ Th2 cytokines resulting in excessive pro-inflammatory activity. These pro-inflammatory cytokines exhibit a systemic spill over and are in this manner; thought to be capable of mediating the course of systemic disease such as Rheumatoid arthritis, CVD, Diabetes etc[74, 24, 59]. The more recently identified Th17 cells and their signature cytokine IL-17 has been postulated to be involved in autoimmune and inflammatory disorders. It is not yet known if circulatory IL-17 levels are affected by periodontal disease.

Saliva is an important physiologic fluid that has been established as a simple and non invasive diagnostic tool that allows rapid screening, provides accurate and predictive information and enables reliable evaluation of the local and systemic inflammatory status, as it contains unique biomarkers that reflects both[84]. Although several cytokines in saliva such as

IL-1β have been used as disease markers; IL-17 has not yet been extensively investigated. This study was undertaken to determine the circulatory and salivary levels of IL-17 in periodontally healthy and diseased patients and to correlate it with the systemic inflammatory marker, hs CRP.

All patients were categorized into periodontally healthy and disease groups according to well established guidelines (Armitage et al 1999) [4]. The BGI index was used to further categorize the periodontitis group as this has been reported to be a sensitive indicator of systemic inflammation associated with periodontal disease only those which belonged to the BGI-DL category was included. In this study, the patients examined on the BGI category belonged either to the Moderate Bleeding (MB) and Severe Bleeding (SB) group; there were no patients in the Low Bleeding (LB) category.

Blood and salivary samples were obtained from both the groups. Serum was obtained from centrifugation of blood sample. Saliva was collected according to Navezesh et al [68] method of collection, after which serum and salivary IL-17 levels were analyzed using ELISA analysis. hs CRP levels in serum was assessed using the immunoturbidity method.

Circulatory hs CRP levels were significantly elevated in periodontal disease when compared to health, suggesting that periodontal disease does indeed lead to systemic inflammation. These results are in accordance with

the results of Renvert, Nakajima et al [78]. Our results showed that there was no statistically significant difference in the circulatory levels of IL-17 between periodontal health and disease groups. These results are in agreement with those obtained by Infant Duarte et al [33]. These authors reported elevated IL-17 levels only in aggressive periodontitis patients who were not included in our study. It has been postulated that the difference in etiopathogenic mechanism between chronic periodontitis and aggressive periodontitis may be responsible for the differing circulating IL-17 observed in both the conditions. Although a systemic spill over of proinflammatory cytokines is extensively documented; there is no universal agreement about the contribution of these cytokines, either individually or collectively, to the systemic inflammatory status, (Genco et al 2002) [25].

Circulatory IL-17 levels did not correlate significantly with hs CRP. The result suggests that IL-17 may not be an important contributory factor to the systemic inflammatory state caused by periodontal disease. There were no previous reports of this nature with which we could compare our results.

Previous studies have reported that the circulatory IL-17 was significantly elevated and closely related to disease activity in inflammatory bone disorders such as rheumatoid arthritis (RA)[13]. RA is however an autoimmune disorder and therefore the pathogenic mechanisms may

involve IL-17 to a greater extent when compared to periodontal disease. The circulating IL-17 levels did not show any significant correlation with the BGI status, but the small sample size presented a meaningful analysis.

When the salivary IL-17 levels were examined; there was a significant decrease in IL-17 in periodontal disease when compared to health. This was some what an contrary result as previous studies have mostly reported an increased level of this proinflammatory cytokine in exudates such as GCF[18, 96]. Most salivary markers of periodontal disease are thought to be derived from GCF and it may be thus postulated that GCF levels could closely correspond to the salivary levels. Previous studies on GCF may thus be used as a basis of comparison.

Our results may not be attributed to experimental error due to the following reasons;

1. The results were consistent in both the health and disease groups, with only a small SD around the mean. This may be interpreted to mean that the values obtained were not error prone.

2. All experimental procedures starting from the collection of saliva were performed in the same standardized conditions.

Our results are more in agreement with the results of Berker et al [8], who demonstrated decreased IL-17 levels in chronic periodontitis

especially when pockets are deeper than ≥ 5mm. It is possible that the clinical status of the periodontitis patients in our study matched closely with that of Berker et al[8], than that included in the Dutzan et al[18, 96] studies. However, Avani Pradeep et al[6], reported that IL-17 was undetectable in GCF. As we used saliva in this study; the greater volume of fluid obtained could have resulted in above detection limits in our study. This could be postulated to be the reason for our ability to detect IL-17 in saliva although we used the same methodology. The results of our study are thus in agreement with a few previous reports but contrary to most others.

We hypothesize that the reduction in IL-17 could be due to the following reasons;

1) Although considered to be predominantly pro-inflammatory in nature, a dichotomous role for IL-17 has been suggested for in both auto immune and inflammatory diseases[38].

2) Th17 cells have been postulated to be involved in host defense against extra cellular bacteria primarily through influencing neutrophil recruitment to the site of infection[71]. IL-17 may be involved in regulating this early inflammatory event and may potentially play other roles in the later stages of established periodontal disease. This may be the reason for its reduction in the periodontitis group.

3) IL-17 is capable of exerting its effect on several cell types of the periodontal tissues such as epithelial cells, fibroblast, osteoblast etc[14]. It is therefore plausible that most of the cytokine produced could have been used up in the tissue itself to sustain the inflammatory process. Therefore even if there had been an upregulation of the proinflammatory cytokine; this excessive production may have resulted in increased utilization within the periodontal tissues thereby perpetuating the inflammatory process. Consequently, there may have been a decrease in IL-17 level in tissue exudates and therefore saliva.

There is however very little previous literature supporting this hypothesis. There are few reports on IL-17 mRNA expression or the receptor IL-17R expression in gingival tissue or indeed IL-17^{+} cells in tissues in existing literature.

Regardless of the mechanism involved, it is an interesting finding that salivary IL-17 was reduced in periodontal disease when compared to health. No single cytokine has been shown to be effective in disease prediction. It has been suggested that considering the complex etiopathogenic mechanisms involved a cocktail of markers may be required to accurately assess the periodontal disease. Our results suggest that periodontal disease may not be always associated with upregulation

of inflammatory markers; IL-17 and perhaps other cytokines may be down regulated in fluids such as saliva.

Salivary IL-17 could not be correlated with the BGI status, but the small sample size of the sub groups could have interfered with the statistical analysis. These findings certainly warrant further investigation. The salivary IL-17 did not correspond to the systemic inflammatory marker hs CRP, indicating that it cannot be used as a surrogate marker of inflammation.

Another interesting finding in this study was that the salivary and circulating IL-17 significantly correlated with each other in periodontal disease, but not in health. These results suggest that a dysregulation of IL-17 had occurred in periodontal disease. However, its exact role in the etiopathogenic process is yet to be fully understood.

The overall analysis of our result suggests that IL-17 is involved primarily as a mediator of local rather than systemic inflammation.

Limitations of our study include:

1. A small sample size.

2. Cross sectional nature of the study, longitudinal studies are thought to be more effective in identifying disease markers.

3. IL-17^{+} cells or IL-17 mRNA in tissue was not detected. They could have perhaps given a more complete exact picture.

RESULTS

Blood and saliva samples were collected from 40 periodontally healthy and diseased subjects. Serum was obtained from centrifugation of blood. Sandwich ELISA was carried out to evaluate the circulatory and salivary levels of IL-17 in periodontal health and disease. High sensitivity CRP (hs-CRP) was evaluated by an immunoturbidity assay using an automated analyzer.

Evaluation of circulatory levels of IL-17:

The mean serum IL-17 levels in the periodontal health group was 25.66 ± 1.05 pg/ml, while the mean IL-17 levels in the periodontitis group was 23.77 ± 0.43 pg/ml. There was no statistically significant difference in the serum IL-17 levels of the periodontal health and disease groups $P = 0.677$, ($P > 0.05$).

Evaluation of salivary levels of IL-17:

The mean salivary IL-17 levels in periodontal health group was 24.63 ± 0.93 pg/ml, while the mean IL-17 levels in periodontitis group was 22.24 ± 0.29 pg/ml. There was statistically significant decrease in periodontal disease when compared to health $P = 0.03$, ($P < 0.05$).

Measurement of serum of hs-CRP in periodontal health and disease:

There was a statistically significant increase in hs-CRP levels in periodontal disease groups when compared to the healthy groups, [Health = 1.16 ± 0.033 pg/ml; Disease = 2.25 ± 0.29 pg/ml, P = 0.008, (P < 0.05)].

Correlation analysis of the serum and salivary IL-17 levels in periodontal disease:

The Pearsons correlation analysis was used to correlate the serum and salivary levels of IL-17 in periodontal disease. There was a statistical significant correlation between the circulatory and salivary levels of IL-17 in periodontal disease P = 0.0248 (P < 0.05), while there was no significant correlation between the circulatory and salivary levels of IL-17 in periodontal health p = 0.8974 (P > 0.05).

Correlation analysis of the serum and salivary levels of IL-17 with hs-CRP in periodontal disease:

There was no statistically significant correlation between circulating IL-17 and hs CRP levels (P = 0.706). Similarly, there was no significant correlation between salivary circulating IL-17 and hs CRP levels (P = 0.538).

SUMMARY AND CONCLUSION

It is generally accepted that periodontal disease results from an imbalance of the T helper subset cytokines resulting in excessive pro-inflammatory activity in active periodontal disease. The role of IL-17 a signature cytokine of Th17 subset in periodontal disease and its contribution to systemic inflammation has not yet been fully elucidated. So this study was undertaken to

1. Evaluate the circulatory and salivary levels of IL-17 in periodontal disease.
2. Investigate if the circulatory IL-17 levels correspond to the salivary IL-17 levels and to hs CRP, a known marker of systemic inflammation.

The circulatory and salivary IL-17 levels were analyzed by ELISA and hs CRP by immunoturbidity method. The results of all study showed that there was no statistically significant difference in the circulatory IL-17 levels between periodontal health and disease $p = 0.6779$ ($p > 0.05$), while the salivary IL-17 levels was showed a statistically significantly reduction in periodontal disease when compared to health $p = 0.0385$ ($p < 0.05$). Although the circulatory IL-17 levels correlated significantly with salivary IL-17 levels $p = 0.0248$ ($p < 0.05$), both showed a poor correlation to hs

CRP (circulating IL-17; hs CRP p = 0.706) and (salivary IL-17; circulatory hs CRP p = 0.538).

From these result we may conclude that;

1. There was no significant difference in circulating IL-17 levels in periodontal health and disease.
2. Salivary IL-17 level was significantly decreased in periodontal disease when compared to health.
3. Salivary and circulating IL-17 correlated with each other in periodontal disease but not with hs CRP.

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