EVALUATION OF SALIVARY BASIC FIBROBLAST GROWTH FACTOR (bFGF) IN TOBACCO CHEWERS WITH CHRONIC PERIODONTITIS

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DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation titled **"EVALUATION OF SALIVARY BASIC FIBROBLAST GROWTH FACTOR (bFGF) IN TOBACCO CHEWERS WITH CHRONIC PERIODONTITIS"** is a bonafide and genuine research work carried out by me under the guidance of **Dr. K.V. ARUN, M.D.S.,** Professor and Head, Department of Periodontology, Ragas Dental College and Hospital, Chennai.

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CERTIFICATE

This is to certify that this dissertation titled **"EVALUATION OF SALIVARY BASIC FIBROBLAST GROWTH FACTOR (bFGF) IN TOBACCO CHEWERS WITH CHRONIC PERIODONTITIS"** is a bonafide record of work done by **Dr. M.GANESH KUMAR** under my guidance during the study period of 2014-2017.

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

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LIST OF ABBREVIATIONS

IL-6	-	interleukin 6
TNF-α	-	tumor necrosis factor alpha
GCF	-	gingival crevicular fluid
HIV	-	human immunodeficiency virus
FGF -2	-	fibroblast growth factor type 2
FGFR	-	fibroblast growth factor receptor
ELISA	-	enzyme linked immune sorbent Assay
BOP	-	bleeding on probing
A.a	-	Aggregatibacter actinomycetamcomitans
P.g	-	Porphyromonas gingivalis
Ig	-	Immunoglobulin
PGE2	-	Prostaglandin E2
RNA	-	Ribonucleic Acid
LT	-	Leukotoxin
AST	-	Aspartate amino transferase

AKT	-	alkaline phosphatase
OPG	-	osteoprotegrin
RANKL	-	Receptor Activated Nuclear Kappa Ligand.
ST	-	Smokeless Tobacco.
GFR	-	Growth factor receptor
PTB	-	Phosphotyrosine binding
MAP	-	Mitogen activated protein
IP3	-	inositol triphosphate
DAG	-	DiacylGlycerol.
PDGF	-	Platelet Derived Growth factor
TGF-β	-	transforming growth factor beta
VEGF	-	Vascular endothelial growth factor
GAG	-	Glycosaminoglycans

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Introduction

INTRODUCTION

Periodontitis is a multifactorial disease that is characterised by chronic inflammation of the supporting tissues of the tooth. Although the etiopathogenesis of periodontal disease is not yet fully understood, evidence indicates that periodontitis results from an exaggerated, non-protective host immune response to microorganisms that are present in a sub-gingival biofilm[88]. The loss of periodontal ligament fibres and alveolar bone is thought to be a result of host derived cytokines and proteases rather than bacterial derived factors.

Consequently pro inflammatory cytokines like IL-1β, IL-6, TNF-α and host derived proteases like MMP 8 and 9 have been implicated in the disease process and used as biomarkers to predict disease progression[65]. Host derived breakdown products used as biomarkers are proteins that contribute to the homeostasis of the periodontium under physiological conditions and are released and/or undergo alterations in inflamed states.

Basic fibroblast growth factor (FGF2) originally identified as a protein capable of promoting fibroblast proliferation, plays an important role in endothelial cell proliferation, migration, differentiation, and survival[38]. As both collagen turn over and neovascularisation are key events, it may be expected that bFGF plays an important role in the homeostasis of the periodontium. Periodontitis being a chronic inflammation that is associated

with collagenolysis, it is reasonable to assume that bFGF is involved in the pathogenic process as well.

The role of tobacco as a risk factor in periodontal disease has been well documented. Voluminous literature exists regarding its ability to increase the extent and severity of periodontal disease, affect healing and worsen the prognosis of chronic periodontitis[10]. Several mechanisms have been proposed to explain its role in the etiopathogenesis of periodontal disease, including its influence on the microvasculature and fibroblast microtubular assembly.

Tobacco chewing is a habit that is fairly prevalent in many Asian countries including India[2,3]. Various constituents of smokeless tobacco like nicotine, nitrosamide have been demonstrated not only to have carcinogenic potential but also induce inflammatory changes in the oral mucosa. The effects of tobacco chewing on the periodontium have not been studied as extensively as that of smoking, but several studies have reported increased gingival recession and attachment loss as a result of tobacco chewing[13].

Based on its ability to influence vascularity and to induce fibrosis, it is plausible that some of the effects of tobacco chewing on periodontal disease are mediated through bFGF.

The advantages of using saliva over GCF for identifying biomarkers of interest include ease of collection, non-invasiveness and potential for rapid screening[64]. As the flow rate of saliva is not affected by tobacco chewing, it

may be used as the diagnostic medium to predict progressive periodontal disease.

bFGF and FGFR have been previously identified in saliva and used as markers in oral malignancy. However, its role as a potential salivary biomarker in the etiopathogenesis of periodontitis in a tobacco chewing population is yet to be investigated.[14,15]

Aims and Objectives

AIMS AND OBJECTIVES

The aims of the present study were:

1. To identify and quantify the level of bFGF in saliva of patients with chronic periodontitis with tobacco chewing habit
2. To compare the salivary bFGF levels in periodontal health with that of chronic periodontitis patients with tobacco chewing habit

Review of Literature

REVIEW OF LITERATURE

Periodontitis is defined as an inflammatory disease of the supporting tissues of the teeth caused by specific microorganisms resulting in progressive destruction of the periodontal ligament and alveolar bone with pocket formation, recession or both.

The chronic activity of periodontal diseases progress with cyclic period of exacerbation and remission. The attachment loss associated with periodontitis has been seen either continuously or in episodic bursts of disease activity.

Microbial challenge:

The plaque formation begins with gram positive bacteria which accumulate on the tooth surface and if they continue to grow it results in an environment that favours growth of gram negative bacteria. A non-protective host response further facilitates bacterial colonisation and growth. Predominant bacterial species that have been involved in the disease process include P.gingivalis, A.actinomycetamcomitans, T.denticola, B.forythis, F.nucleatum, P.intermedia, C.rectus, Peptostreptococcus, E.corrodens.[21]

Host response to the microbial challenge:

Bacteria and bacterial products interact with the junctional epithelium and penetrate the underlying connective tissue. The small blood vessels plexus

seen beneath but deep to the junctional epithelium becomes inflamed. Leucocytes exit the post capillary vessels and there is a large increase in the neutrophil count, as it moves through the Junctional epithelium and into the sulcus.

The bacterial normal metabolic products including fatty acids and the peptide N-formylmethionylleucylphenyalanine and LPS of gram negative bacteria, these products activate the junctional epithelial cells to release various inflammatory mediators including IL-8,IL-1α,PGE2 ,MMP-2, MMP and TNF-α.

The tissue response to the early signal by widening of intercellular spaces and found first fluid filling space which some as a medium for diffusion and neutrophil migration.[20]

The inflammatory phase involves a reactive defensins response to the bacterial products. This evidence by the activation of serum proteins into the tissue movement of neutrophil out of the vessels and into the sulcus, epithelial cells proliferation and selective deposition of mononuclear cells in the tissues.[23]

This shapes the local and systemic immune responses. There is an increase in tissue lymphocytes, plasma cells and macrophages which changes the metabolism of the local fibroblasts to favor a reduction in collagen

synthesis and activation of the local and systemic immune responses and production of antibodies.

Biomarker: The term 'biomarker' refers to biologic substances that can be measured and evaluated to serve as indicators of biological health, pathogenic processes, environmental exposure and pharmacologic responses to a therapeutic intervention.[29]

Salivary biomarkers in periodontitis:

MMP -8, OPG, MIP-1α, IL -1β, IL – 8 and TNF -1α were reported to be associated with chronic periodontitis.

MMP -8 was reported to be a biomarker indicative of response to therapy (Sexton).[87]

AST and P.gingivalis demonstrated high levels of predictive ability for disease progression.

A novel diagnostic approach was investigated for the combinatorial ability of P.gingivalis, IL -1 β and MMP-8 to detect periodontitis.

Fine DH performed a longitudinal evaluation of salivary cytokines in children who developed localized aggressive periodontitis and reported MIP-1α as an early detection marker.[29]

The peptide, Procalcitonin is an established serum biomarker for inflammation. Increased mean levels of salivary procalcitonin were found for subject with periodontitis compared with healthy control subjects (Bassim CW).

Point –of care diagnostics:

In the absence of one specific biomarker for periodontal diseases, a panel of markers identified through longitudinal analysis may be employed using a lab on chip technology. This technology allows rapid screening of large population and allows results to be obtained immediately.

Personalized medicine in periodontics:

Personalized medicine is a medical model that uses genetic, genomic, environmental and clinical diagnostic testing to individualize patient care. This approach uses clinical assessment and subclinical profiles to improve highly individualized diagnosis, prognosis and treatment algorithm (Giannobile)[33]

Personalized medicine for periodontal diseases may soon involve utilization of saliva to develop subclinical profiles ,identifying and measuring specific genotypes putative pathogen inflammatory marker and collagen degradation biomarkers to make informed clinical decisions about disease susceptibility, site specific –risk and treatment intervention (Kornman KS) .

During the screening phase, the use of saliva to recognize patients at risk for future disease activity makes it possible to employ risk management strategies, preventive care and/or behavior change on the part of the patient to prevent the onset of the disease at the diagnostics stage, identifying the presence of disease at the earliest possible stage may allow for less-invasive treatment. (Taba M)[98]

Smokeless tobacco:

Smokeless tobacco is tobacco that is not burned. It is also known as chewing tobacco, oral tobacco, spit or spitting tobacco, dip, chew and snuff. most people chew or suck (dip) the tobacco in their mouth and spit out the tobacco juices that build up ,although "spitless" smokeless tobacco has been developed. Nicotine in the tobacco is absorbed through the lining of the mouth.

People in many regions and countries including North America, Northern Europe, India and other Asian countries and some parts of Africa have a long history of using Smokeless tobacco products.[70]

There are two main types of smokeless tobacco:

Chewing tobacco which is available as loose leaves plugs (bricks) or twists of ropes, a piece of tobacco is placed between cheek and lower lip,

typically toward the back of the mouth. It is either chewed or held in place. Saliva is spit or swallowed.

Snuff, which is finely cut or powered tobacco, may be sold in different scents and flavours[82]. It is stored moist or dry. It is available loose in dissolvable lozenges or Strips or in small pouches similar to tea bags .the user places a pinch of moist snuff between the cheek and gums or behind the upper or lower lip. Another name for moist Snuff is sinus (pronounced "snooze"). Some people inhale dry snuff into the nose.

Harmful chemicals in smokeless tobacco:

There is no safer form of tobacco, at least 28 chemicals in smokeless tobacco have been found to cause cancer.[50] The most harmful chemicals in smokeless tobacco are tobacco specific, nitrosomine which are formed during the growing ,curing ,fermenting and aging of tobacco .the level of tobacco - specific nitrosomines varies by product.[105]

In addition to a variety of Nitrosomines, other cancer causing substances. tobacco include polonium-210 (a radioactive element found in tobacco fertilizer) and polynuclear aromatic hydrocarbons (also known as polycyclic aromatic hydrocarbon).[49]

Clinical effects of smokeless tobacco:

Oral mucosal disease:

Tobacco use is associated with a range of changes to the oral mucous membrane cells. Oral candidiasis is a mucosal infection caused by the candida albicans fungus.[1]It has been suggested that tobacco use may slow the immune system, making smokeless tobacco users more susceptible to infections.

Leukoplakia:

Oral leukoplakia is the most common form of potentially malignant disorder in the oral cavity .[104] it is estimated that three out of every four users of smokeless tobacco will develop an Oral leukoplakia at the site where they place the tobacco product in their mouth[96]. A case control study of smokeless tobacco use amongst South Asians also found a significantly increased risk of esophageal cancer associated with the use of areca nut and betel Quid when used with chewing tobacco.[77]

Wound Healing:

Tobacco use is known to impair wound healing. Tobacco chewers have decreased level of salivary and serum Immunoglobulin which affects wound healing in the oral cavity and mouth's ability to clear pathogens [81].

Tobacco chewers have decreased levels of blood oxygenation leading to decreased oxygen delivery to the tissues.[95] Loss of blood clot that follows the removal of teeth occurs four times more frequently in smokers than non-smokers.[22]

A significantly higher prevalence of bleeding on probing was found in betel quid chewers than non-chewers among the subjects with higher plaque level, greater gingival inflammation, deeper probing depth or greater attachment loss.[103]

Betel chewing has been associated with an increased gingival bleeding and an increased prevalence of Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis as determined by PCR. Also an increased periodontal progression due to betel chewing has been suggested.

Ling et al reported higher levels of A. actinomycetemcomitans and P.gingivalis in betel chewers using a sensitive but non-quantitative PCR method. The prevalence of the periodontitis associated bacteria observed using the lower detection level of score 1 (>104) was close to 100% in both chewers and non-chewers for all bacteria tested, while the significant differences were found using the higher cut off level (score 3 or higher corresponding to >105).

Studies also reported that periodontitis among the betel chewers was more severe than in non-chewers indicated by both higher mean values of probing pocket depth and attachment loss.

SMOKELESS TOBACCO AND PERIODONTAL DISEASE:

Smokeless Tobacco (ST) contains numerous chemicals, such as nicotine and nitrosamines, which are potential local irritants. Although ST products vary considerably in their composition, nicotine is a common element in all of these products. Plasma levels in ST users and smokers range from 22 to 73 ng/ml and salivary concentrations range from 70 to 1560 µg/ml. 27·28. Irritation from ST components may stimulate the production of inflammatory mediators, thereby leading to the observed tissue alterations seen in ST users.

Robertson et al could not find a relationship between poor oral hygiene and gingivitis in the development of oral ST lesions. They hypothesized that localized tissue destruction following ST usage may be due, in part, to production of inflammatory mediators, such as PGE2 and IL-lß, in response to ST alone or in combination with bacterial lipopolysaccharide (LPS). Because monocytes are the primary target cell for LPS, which is ubiquitous in the periodontium, and major producers of periodontal inflammatory mediators, PGE2 and IL- ß.

Their study revealed that smokeless tobacco is capable of stimulating monocyte secretion of PGE2 and IL-lß. Smokeless tobacco was a more potent stimulator of PGE2 than P.gingivalis LPS and had an effect similar to E. coli LPS. Even though monocytes/macrophages represent only 3.5% of total cell

counts in progressing Periodontitis sites, 25 these cells are capable of producing large amounts of PGE2 and IL-lß.

Studies have established that the macrophage/fibroblast volume density ratios are elevated in habitual ST lesions compared to non-placement sites in the same individual. Elevated secretion of PGE2 and IL-lß has been associated with active periodontitis and is likely to be involved in the localized soft tissue destruction seen at the site of ST placement.

In view of IL-1's role in keratinocyte proliferation and its inflammatory effects, this cytokine may contribute to mucosal and gingival alterations observed in ST users.

ST placement sites exhibit higher levels of gingival inflammation accompanied by an increase in gingival crevicular fluid (GCF) levels of inflammatory mediators Prostaglandin E2 (PGE2) and interleukin-1 (IL-1). Prostaglandin E2 and IL-1 have been directly linked to increased epithelial proliferation in other conditions, as well as to collagen and alveolar bone loss in periodontal diseases. Therefore, these are potential mediators involved in ST induced mucosal changes.

The tissue destruction in periodontal disease mainly results from various host-derived pro-inflammatory mediators, such as interleukin-1, TNF-a, and PGE2.42. ANE significantly increases the production of PGE2 through the activation of COX-2 expression in human PMNs.

In addition, the intracellular calcium levels and p38 and ERK MAPK signalling pathways are involved in the ANE-induced PGE2 synthesis. These findings indicate that the activation of the COX-2/PGE2 pathway may be one of the mechanisms by which the areca nut augments the periodontal destruction in areca quid chewers.

Areca nut extracts increased the levels of IL-1β, IL-6, IL-8, and tumor necrosis factor - alpha (TNF-α), which are responsible for amplifying the inflammatory response in periodontal tissues. IL-1β and IL-8 production and secretion are increased when cells come in contact with nicotine.

In addition, the areca nut, which contains arecoline and nicotine, inhibits the growth and attachment of fibroblasts, inhibits protein synthesis, suppresses the differentiation and functionality of dendritic cells, and might be cytotoxic for periodontal fibroblasts, leading to the progression of the pre-existing periodontal disease and impairment of the periodontal attachment.

Buccal sites usually exhibit a thin alveolar bone causing alveolar dehiscence, and that tobacco chewing is likely to cause chemical injury to the thin gingiva, leading to the loss of marginal gingiva. Like RC, the CAL was significantly higher in gutka chewers. Javed et al, revealed site-specific gingival inflammation at the area of placement.

SMOKELESS TOBACCO AND CLINICAL EFFECTS ON THE PERIODONTIUM:

Epithelial proliferation and down-growth along the root, as well as collagen and bone destruction, are the major structural changes evident in periodontal attachment loss and gingival recession. (Baker DL et al). Increased epithelial thickness is the primary alteration in the ST-induced white lesion, although sub-epithelial inflammation and salivary fibrosis also are present. These changes occur in response to the chemical and/or mechanical irritating effects of ST components. This stimulus is likely to result in the release of inflammatory mediators which may play a role in the development of pathologic alterations.

The higher codes of loss of attachment were observed in pan chewers with tobacco compared with pan chewers without tobacco. It was found that pan chewers with tobacco had 7 times more risk of having loss of attachment when compared with the pan chewers without tobacco, emphasizing the fact that although betel nut has deleterious effects on the periodontium, the addition of tobacco leads to a synergistic effect between betel nut and tobacco on the periodontal tissues.

Kumar et al had shown a significant impact on the severity of periodontal diseases among tobacco users as compared to non-users and the

risk of periodontal pockets increased as the duration and frequency of tobacco consumption increased.

Sood et al also revealed higher prevalence of periodontal disease in different smokeless tobacco users. Peak incidence of smokeless tobacco consumption was observed in the age group between 21 and 30 years (39.4%) in this study. Increasing demand and usage amongst young adults and in older women living in rural southern areas in United States have been reported by many.

Greater gingival inflammation was seen in smokeless tobacco users and alone smokeless tobacco users than non-tobacco users. Attachment loss is an important component of the periodontal disease measure that defines past history of the disease and is especially pertinent in the assessment of current exposure to tobacco. Present study revealed CAL of more than 5 mm in majority of smokeless tobacco users.

ST users tend to have more severe recession (REC) and Clinical Attachment Loss (CAL) and a greater proportion of sites with higher values of REC and CAL compared with never users. The greatest increase in severity of CAL was found to be localized to sites on mandibular teeth, buccal surfaces, anterior and molars, which may be a result of the retention of the ST product in the oral cavity.

Some in vitro studies have shown that areca nut extracts and nicotine suppress the growth of periodontal fibroblast and gingival keratinocytes. Although clinical studies have investigated the influence of habitual Betel Quid (BQ) chewing on periodontal health, the difference in the severity of periodontal disease among individuals chewing BQ with and without tobacco remains unclear. It is therefore hypothesized that individuals chewing BQ with tobacco experience more intense periodontal inflammation as compared with those chewing BQ without tobacco.

BOP was significantly higher among individuals chewing BQ with tobacco as compared with those chewing BQ without tobacco. Habitual tobacco smoking and alcohol consumption are independent risk factors for periodontal inflammation.

Biology of fibroblast growth factor:

Fibroblast growth factor which was first discovered in pituitary extracts in 1973 is broadly expressed in cells and tissues. Acidic fibroblast growth factor (FGF1) and basic fibroblast growth factor 2 (FGF2) were originally isolated from the brain and pituitary gland as growth factors for fibroblast ,since then at least 22 distinct FGF s have been identified or isolated Fibroblast growth factors have been found in both vertebrates and invertebrates. Many fibroblast growth factor genes have been identified in vertebrates including ten Fibroblast growth factor in zebra fish (FGF-2-

4,6,8,10,17a,18,24) six in xenopus (FGF 2-4,8-10) in chicken (FGF1-4,8-10,12,13,16,18-20) in mice (FGF1-18,20-23) and humans (FGF1-14,16-23)whereas only three drosophilia FGF genes and two caenorrhabditis elegans.[45] FGF genes have been observed in invertebrates. Human FGFs a contain 22 members FGF1, FGF2, FGF3, FGF4(INT2), FGF5, FGF6, FGF7, FGF8, FGF9, FGF10, FGF11, FGF12, FGF13, FGF14, FGF15, FGF16, FGF17, FGF18, FGF19, FGF20, FGF21, FGF22 and FGF23 (Katch M)[52]

The Fibroblast growth factor family members comprises 23 members although there are only 18 FGFR ligands. Four family members do not bind with FGFR as FGFR as FGF homologous factors (FGF11, FGF12, FGF13, FGF14) and are more correctly referred to as FGF homologous factors.[25] In addition there is no human FGF-15 gene, the gene orthologous to mouse FGF-15 and FGF -19.[63]

Biological functions of FGFs:

FGFs exert their physiological roles on functions such as cell proliferation, migration, differentiation and angiogenesis in various cells and tissues [109].

Cell proliferation:

Cell proliferation by FGFs have been reported in many cell types including enothelial cell FGF 1 is a proliferative factor for human or

adipocytes[41] that may be important to overall regulation of human adipogenesis in additionFGF1leads to an increase in the proliferation of IEC-6 Caco-and HT-29 cell lines cell lines with FGF2 and FGF7. FGF2 induces cell proliferation after flia specific gene transfer in mice and stimulates the proliferation and survival of neuro-epithelial cells isolated from the telencephalon and mesencephalon of E10. FGF4 knocked mouse embryos experience post implantation lethally owing to the necessity of FGF4 for trophoblast proliferation. FGF7 (called human KGF) is related to the epithelial cell growth. FGF10 play a role in the pathogenesis of prostrate cancer via facilitation epithelial cell proliferation (Thomson A.A).[108] FGF18 has also have been stimulate the proliferation of cultured mouse primary osteoblast, osteoblastic MC373-E1 cells, primary chondrocytes and prechondrocytic ATDC5 cells, although inhibited the differentiation and matrix synthesis of these cells.[40] Interestingly some FGF s stimulate proliferation of cancer cells as well as normal cells .

Cell migration:

Cell migration is a central processes in the development and maintenance of multi cellular organisms. Tissue formation during embryonic development, wound healing, and immune responses all require the orchestrated movement of cells in particular directions to specific locations. Cells often migrate in response to and forward specific external signals in

a process and forward specific external signals in a process known as chemotaxis[11].

Cell migration of FGF's varies with subfamilies, apparently both FGF1 and FGF2 play important roles in the migration of cochlear ganglion neurons in mice. FGF2 induces cell migration after flia specific gene transfer in mice, and stimulates cell migration of mouse embryonic limb myogenic cells such as FGF4(Webb .S)[110]. FGF7 is known to stimulate migration and plasminogen activity (PA) of normal human keratinocytes similar to FGF2, FGF8 is a potent chemoattractant in the migration of mesencephalic neural crest cells (Kubota Y).[113]

Cell differentiation:

In development biology, cellular differentiation is the process by which a less specialized cell becomes a more specialized cell type differentiation occurs numerous times during the development of multicellular organisms as they change from a single zygote to a complex system of tissues and cell types.[26] Differentiation is common in adults as well .specifically adult stem cells divide and create fully differentiated daughter cells during tissue repair and normal cell turnover .differentiation dramatically changes the size, shape, membrane potential, metabolic activity and responsiveness of a cell signal.[115]

Cell differentiation of FGF also varies with subfamilies[47]. FGF1 and FGF2 play important roles in the initial differentiation of cochlear ganglion neurons in mice (29), moreover FGF2 stimulates the differentiation of neuro-epithelial Cells into mature neurons and glial cells FGF7 is essential for the morphogenesis of subrabasal keratinocytes and establishment of the normal program of keratinocyte differentiation .exogenous FGF20 stimulates the differentiation of monkey stem cells into dominogeric neurons after treatment in vitro.[114]

Angiogenesis:

Angiogenesis is the process of the formation of new blood vessels from pre-existing vessels. The process plays a key role in various physiological and pathological conditions such as embryonic development, wound repair, inflammation and tumor growth angiogenesis is a multistep process that begins with the degradation of the basement membrane by activated endothelial cell sprouts into the stromal space.

Numerous indicators of angiogenesis have been identified, including members of the VEGF family angiopoietins, TGF –alpha and beta, PDGF TNF-α, interleukins, chemokines and members of fibroblast growth factor (FGF) family. However only a limited number of the 22 members of the FGF family have been investigated for their angiogenic potential in vitro and in vivo.[9] the angiogenic properties of FGF1 and FGF2 are well known.

Specifically FGF1 and FGF2 induce the promotion of endothelial cell proliferation and the physical organization of endothelial cell proliferation and the physical organization of endothelial cells into tube like structures .thus they promote angiogenesis FGF1 and FGF2 are more potent angiogenic factor than VEGF or PDGF [41].FGF4 also has angiogenic properties[16] .

Delivery system for FGF:

Due to their specific biologic functions and roles, FGF have the potential for application to induce the regeneration of a wide spectrum of tissuses, including skin, blood vessels, muscle, adipose tendon /ligament, cartilage, bone, tooth, and nervous tissuses[46]. Indeed many previous studies have evaluated the administration of FGF's directly to the sites of wound, similar to that of other growth factors. However, when free FGF solutions are injected in vivo, they rapidly lose their biological functional activity, primarily due to diffusional loss and /or enzymatic inactivation/degradation[48]. Therefore gain satisfactory performance, a large amount of FGF's with a continuous dose for the determined period is required.

Conversely when FGF's are adsorbed onto or encapsulated within materials their degradation risk can be largely protected while securing the biological activity .therefore to make full use of the FGF 's .It is essential to develop appropriate materials and substrates to contain and deliver them to defective regions after which following and substrates to contain and deliver

them to defective regions ,after which allowing their release at a controllable and sustainable rate . A wide range of biomaterials and substrate to contain and deliver them defective regions ,after which allowing their release at a controllable rate and sustainable rate .A wide range of biomaterials including synthetic and natural polymers and even tissue matrices have been studied as candidate materials to carry FGF's and elicit their therapeutic efficacy in vitro nd /or in vivo.

While their functional activity has been demonstrated in different types of cells in vitro and /or target tissues in vivo there have been relatively few ,if any reports addressing the delivery mechanism .In this part ,we review the application of FGF's in concert with medical material for tissue regeneration .specific targets include skin, cartilage, bone, blood vessel muscle, tendon /ligament and nerve.[58] The materials are developed to specifically conjugate with FGF's or encapsulate with the structure and are engineered in the form of hydrogels or porous scaffolds or nano and microparticles.

Porous scaffolds:

Generally the most common scaffold materials range polymers (synthetic or natural) and ceramics to their composites ,which can be chosen depending on the tissues of concern .many natural polymers such as collagen ,alginate ,fibrin ,silk, chitosan and GAG's are biologically well defined,

tissue compatible and degradable,[8] therefore they regarded as feasible materials for the intake of growth factors within the structures such polymers are easily engineered into porous folds by dissolution in water based solutions and subsequent freeze drying .because they dissolve well in water ,the incorporation of FGF's is possible during the fabrication step[17]. when incorporated within the structure, growth factor are released through the scaffold when they come into contact with the fluid .In most cases ,bulk diffusion is the dominant method of releasing growth factors ,which is led by water permeation and can be accelerated by collapse of the polymer network

Due to their comprising a class of proteins or polysaccharides natural polymers contain a large number of ionic groups ,apart from chitosan ,which is highly positively charged ,all of other proteins mentioned above preserve a large number of negatively charged groups .therefore depending on their charge characteristics (basic or acidic) FGFs can form charge –charge interactions with natural polymers[54] .in such cases the FGF –incorporated scaffolds show sustained and long term delivery of FGF 's if their structure is maintained without collapse . More specific biochemical interactions between the FGF's and natural proteins are favoured in maintenance of the stability of FGF's.

FGF in inflammatory conditions

Michael J cross et al reported that Fibroblast growth factor (FGF) & vascular Endothelial Growth factor (VEGF) can inhibit & deregulate blood vessel formation and causes cancer. FGF and VEGF bind to cell surface receptor with tyrosine kinase activity, and activation of receptor kinase activity allows coupling to downstream signal transduction pathways that causes proliferation, migration and differentiation of endothelial cells.

Michael Jeffers et al examined the activity of the protein FGF-20 in 2 animal models of acute intestinal inflammation and in mechanistic studies in vitro, and reported that prophylactic administration of FGF-20 significantly reduced the severity and extent of mucosal damage, distal colonic edema, histologic inflammation, and epithelial cell loss relative to animals administered vehicle control. No toxicity was noted during administration of FGF-20 to normal controls. They concluded that FGF-20, having demonstrated therapeutic activity in 2 experimental models of intestinal inflammation, represents a promising new candidate for the treatment of human inflammatory bowel disease.

Kuo Yuan et al reported that the etiology of pyogenic granuloma was due to the imbalance between angiogenesis enhancers like VEGF and bFGF, and inhibitors like estrogen receptor, and detected abundant levels of bFGF in the extracellular matrix of pyogenic granuloma.

Lynn K. Chang et al conducted spatio-temporal studies on the growth of capillary blood vessels and capillary lymphatic vessels in tissue remodelling, and

have suggested that lymphangiogenesis is angiogenesis-dependent. They revisited this concept by using fibroblast growth factor 2 (FGF-2) to stimulate the growth of both vessel types in the mouse cornea, and demonstrated that there is a dose-dependent response of FGF-2 for lymphangiogenesis, which can occur in the absence of a pre-existing or developing vascular bed, i.e., in the absence of angiogenesis, in the mouse cornea.

Yoshio Shimabukuro et al examined the effect of FGF-2 on the production of hyaluronan (HA), an extracellular matrix playing important roles in homeostasis and inflammatory/wound healing responses, by human periodontal ligament (HPDL) cells. An inhibition binding-protein assay revealed that FGF-2 significantly increased HA production by HPDL cells in a dose dependent manner. Their results provided new evidence for the possible involvement of FGF-2 in the regulation of HA production and its appreciable roles in not only homeostasis but also regeneration of periodontal tissues.

Michele Rossini et al examined the expression of FGF-1 and its receptor specifically in various inflammatory renal diseases with interstitial inflammation, including lupus nephritis (LN), acute interstitial nephritis (AIN), and acute rejection superimposed on CAN, and examined any linkage to type of inflammatory infiltrate and fibrosis. They reported that the expression of FGF-1 and FGFR-1 in infiltrating lymphocytes and macrophages, and of FGFR-1 in tubules, is supportive, but does not prove causality, of the possibility that FGF-1 might have both autocrine and paracrine functions in renal inflammation.

Sandra I. Zittermann and Andrew C. Issekutz investigated the effect of bFGF on acute dermal inflammation and the recruitment of monocytes, T-cells, and neutrophils, and reported that bFGF synergistically potentiates inflammatory mediator-induced leukocyte recruitment, at least in part, by enhancing CAM up-regulation on endothelium.

Young-Suk Kim et al studied the effects of Fibroblast Growth Factor-2 on the expression and regulation of chemokines in human dental pulp cells, and suggested that FGF-2 plays a role not only as a differentiation inducing factor in the injury repair processes of pulpal tissue but also as a positive regulator of chemokine expression, which may help in tissue engineering and pulp regeneration using HDPCs

Jeong Goo Leea et al described the molecular mechanism of endothelial mesenchymal transformation (EMT) mediated by fibroblast growth factor-2 (FGF-2) in corneal endothelial cells (CECs). They reported that Interleukin-1β (IL-1β) greatly upregulates FGF-2 production in CECs, thus leading to FGF-2-mediated EMT.

Masoomeh et al identified and quantified the marrow stem cells (MSCs) derived from blood and bone marrow recruited and migrated to the wound site, and showed that the synergistic effects of transforming growth factor-beta (TGF-β) and basic fibroblast growth factor (b-FGF) lead to a significant increase in migration and recruitment of both PBMSCs and BMSCs to the wound site, with more potent effects on PBMSCs as compared with BMSCs. Reverse transcription polymerase chain reaction of collagen type I (COL1A1) transcripts (348 bp) confirmed that TGF-β and b-FGF activate collagen I (production in marrow stem cells at higher transcription

levels), with more vigorous effects of TGF-β on PBMSCs as compared with the same condition on BMSCs.

Materials and Methods

MATERIALS AND METHODS

A total of 52 subjects were included in the study based on the following criteria:

INCLUSION CRITERIA:

52 Patients who reported to the department of periodontology at Ragas dental college and hospital, Uthandi, Chennai were divided into 2 groups according to the 1999 AAP criteria based on their periodontal status

1. **GROUP A:**

   Chronic periodontitis and tobacco chewers -27 systemically healthy subjects between 20-60 years of age with Chronic periodontitis and who consume tobacco in forms of Pan masala, Gutka, Hans and Snuff products etc for the past 6 months and above diagnosis of generalized chronic periodontitis was made based on Clinical parameters of PPD >/5mm ,CAL>/3mm in > 30 sites .

2. **GROUP B :**

   Periodontally healthy patients -25 systemically healthy subjects between 20 -60 years of age with periodontal health. Periodontal Health was determined by absence of bleeding on probing, PPD <3mm and no evidence of clinical attachment loss.

Subjects who could fulfill the Scheduled appointment and who could give written informed consent will be included in the study. In addition the patients should exhibit a **Normal salivary flow rate** of at least >/0.5ml/min following a 5 minute salivary flow rate.

The study was undertaken following approval from the institutional review board. All the subjects were informed that this research work was in no way directly related to the therapy or cure of the disease. Informed consent was obtained from the subjects who accepted to participate in the study.

EXCLUSION CRITERIA:

- Patients with any systemic diseases that influence salivary gland behaviour.
- Patients who are under any medications that affect salivary flow.
- Pregnant women and lactating mothers.
- Patients with OSMF or any other mucosal lesions that relate to tobacco chewing

METHOD:

PERIODONTAL EXAMINATION:

Subjects enrolled in the study were examined with a mouth mirror and Williams Periodontal probe. Clinical diagnosis of generalized chronic periodontitis was based on measurements of Pocket probing depth, Clinical

attachment loss , Gingival bleeding Index (Full mouth gingival bleeding score FMBS), Plaque Index (full mouth Plaque score –FMPS), Full mouth plaque and bleeding scores were recorded by examining four surfaces per Tooth : Labial/buccal, lingual/Palatal, mesial and distal surfaces.

Saliva Collection:

The technique proposed by **Navazesh et al. (2008)** was used for saliva collection. 5ml of unstimulated saliva was collected in a sterile disposable plastic container. The subjects were asked to refrain from intake of any food or beverage (except water) one hour before the test session. They were advised to rinse their mouth several times with plain water and to relax for five minutes before sample collection. The subjects were then asked to lean the head forward over the container with the mouth slightly open and allow the saliva to drain into the container with eyes open. The time given for saliva sample collection was 5-10 minutes. The saliva samples were centrifuged at 2800rpm and stored at -20 degree Celsius till further analysis was done.

ARMAMENTARIUM

DIAGNOSTIC INSTRUMENTS

1. Mouth mirrors
2. Graduated William's probe.
3. Tweezers

ARMAMENTARIUM - II:

1. Vaccutainers (clot activator)
2. Disposable containers for saliva collection,
3. Centrifuge tubes,
4. Laboratory Centrifuge
5. Eppendorf tubes,
6. Ice pack (for transfer)
7. Refrigerator (-21),
8. adjustable single channel micropipettes
9. disposable tips for use with single channel micropipettes
10. Microtitre plate reader

11. Microplate washer,

12. Centrifuge

13. Immunoassay analyzer

14. Distilled water,

15. Miscellaneous laboratory items

    i. Beaker

    ii. Tissue paper

    iii. Gloves

    iv. Tin foil

16. Sandwich ELISA kits

a. **Human bFGF Kit: Ray Biotech** which contains

1. bFGF-A Microplate (A)
2. Wash Buffer Concentrate (20X) (B)
3. Standard Protein (C)
4. Detection Antibody bFGF (F)
5. HRP-Streptavidin Concentrate (G)
6. TMB- One step substrate Reagent (H)
7. Stop Solution (I)

8. Sample diluent buffer(D2)
9. Assay Diluent B (E2)
10. cell lysate buffer (J)

Additional Materials Required:

1. Microplate reader capable of measuring absorbance at 450nm.
2. Precision pipettes to deliver 2ul to 1 ml volumes.
3. Adjustable 1-25ml pipettes for reagent preparation
4. 100ml and 1 liter graduated cylinders
5. Absorbent paper.
6. Distilled or deionized water
7. Log-log graph paper or computer and software for ELISA data analysis
8. Tubes to prepare standard or sample dilutions.

REAGENT PREPARATION:

1. Reagents and samples were brought to room temperature (18-25'C) before using the sample diluent Buffer (D2) and assay diluent (E2) were diluted 5-fold with deionized or distilled water before use. cell lysate buffer (J) was diluted 2-fold with deionised or distilled water

2. Sample Dilution: tissue lysate and cell lysate was diluted atleast 5 fold with 1X sample diluent buffer (D2). as recommend a minimum of 1mg of protein per 1ml of original lysate solution standard protein (C) vial
3. To prepare a100ng/ml standard. Dissolve the powder thoroughly by a gentle mix. Add100µl bFGF standard from the vial of standard protein (C), into a tube with 900µl sample Diluent buffer to prepare a 10000 pg/ml standard solution. Pipette 300µl 1X sample
4. Diluent Buffer was eluted into each tube and the stock standard solution was as per manufacturer's instructions.
5. 100ul of 1X Assay Diluent was added into the vial to prepare a detection antibody concentrate, Pipetted up and down and mixed gently.
6. The detection antibody concentrate WAS diluted 5 fold with 1X Assay Diluent (E2) before use.
7. HRP-Streptavidin concentrate was diluted 120-fold with 1X Assay Diluent

ASSAY PROCEDURE:

Samples were brought to room temperature and all reagents were prepared according to manufacturer's instructions.

S.No	ASSAY STEPS	PROCEDURE
1	Addition	A serial dilution of the stock standard was prepared to obtain a standard curve
2	Addition	100µl of each, sample and zero, was added in appropriate number of wells
3	Incubation	The wells were covered with the plastic cover plate and incubated at room temperature for 2 hours
4	Wash	The cover was removed and wells washed as follows a. Aspiration of contents from each well b. Addition of 0.3ml of 1x wash buffer into each well c. Aspiration of the contents again d. Repetition of steps b and c 2 more times
5	Addition	50µl of diluted biotinylated anti-IL-17A was added to all wells
6	Incubation	Wells were covered with plastic plate cover and incubated at room temperature for 1 hour
7	Wash	Step 4 was repeated
8	Addition	100µl of Streptavidin-HRP solution was added to all wells
9	Incubation	Wells were covered with plastic plate cover and incubated at room temperature for 30 mins
10	Wash	Step 4 was repeated
11	Addition	100µl of TMB substrate solution was added to all wells
12	Incubation	Wells were covered with plastic plate cover and incubated at room temperature in the dark for 15 mins
13	Addition	100µl of stop reagent was added to all wells

The micro plate reader was used at 450nm to read the absorbance value in each well and quantify the bFGF levels.

STATISTICAL ANALYSIS:

The mean values of bFGF in both test and control groups were used for analysis. Both paired t test and the Mann Whitney U test were used to compare the health versus disease results and significance was set at $p<0.05$.

PROFORMA

Evaluation of salivary basic fibroblast growth factor (bFGF) levels in tobacco chewers with chronic periodontitis

CASE NO: A/B DATE:

NAME:

AGE:

SEX:

CHIEF COMPLAINT:

PAST MEDICAL HISTORY:

PAST DENTAL HISTORY:

PERSONAL HISTORY:

ORAL HYGIENE STATUS:

TOBACCO USAGE: SMOKING/NON –SMOKING

DURATION:

FREQUENCY:

TYPE:

IT USED IN COMBINATION:

ALCOHOL USE: YES/NO

INTRA-ORAL SOFT TISSUE EXAMINATION

BUCCAL MUCOSA:

TONGUE:

PALATE:

ALVEOLAR MUCOSA:

GINIGIVAL EXAMINATION

PHENOTYPE:

PIGMENTATION:

CONSISTENCY:

RADOIGRAPHIC FINDINGS

Consent Form

I…….S/o,d/o,w/o……. .agedabout……..years…….residing at……do solemnly

And state as follows.

I have been explained about the nature and purpose of the study in which I have been asked to participate.

I give my consent after knowing full consequence of the dissertation/thesis/study and I undertake to cooperate with the doctor for the study.

I have been given the opportunity to ask questions about the procedure.

I also authorize the Doctor to proceed with the study and I will cooperate with the doctor.

I have also agreed to come for regular follow up for a period of atleast one year.

I am also aware that I am free to withdraw the consent given at any time during the study in writing.

The doctor has explained the procedure to me and I have understood the same and signed my consent in (English/Tamil/Hindi/Telugu.....).

SIGNATURE OF THE PG STUDENT **SIGNATURE OF THE PATIENT**

SIGNATURE OF THE GUIDE: **SIGNATURE OF THE HOD**

Counselling Form:

Tobacco usage…….. packets per year

I was advised regarding ill effects of tobacco use and motivated for tobacco cessation.

SIGNATURE OF THE PG STUDENT **SIGNATURE OF THE PATIENT**

Photographs

FIG .1. DISPOSABLE SALIVA CONTAINERS

FIG.2. EPPENDORF TUBES

FIG .3. SAMPLE COLLECTION

FIG.4. CENTRIFUGE

FIG.5. MICRO PIPETTE AND TIPS

FIG.6 AUTO WASHER

FIG. 7 .MICRO PLATE READER

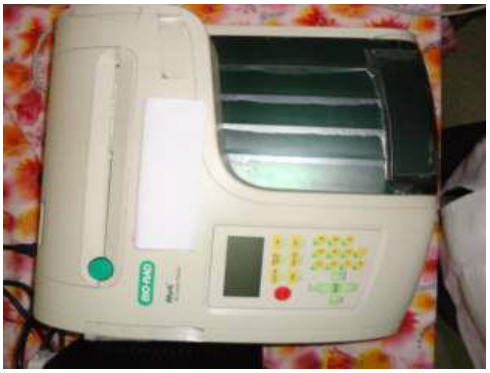


FIG.8 REFRIGERATOR

FIG.9.COMPUTER

FIG 10 IMMUNOANALYZER

PROCEDURE

FIG 11 ***MICROPLATE WELL DURING PROCEDURE***

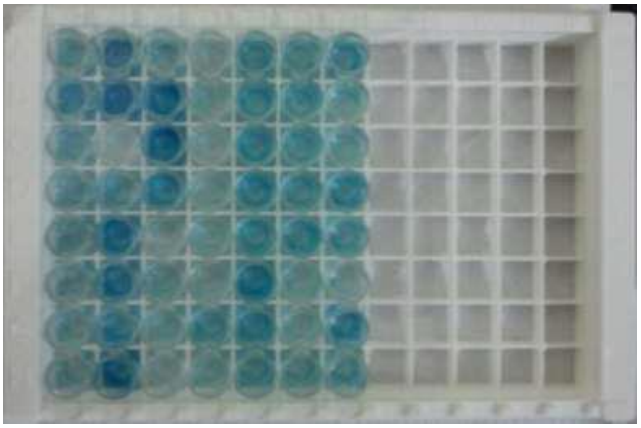


FIG 12 ***MICROPLATE WELL AFTER STOP REAGENT***

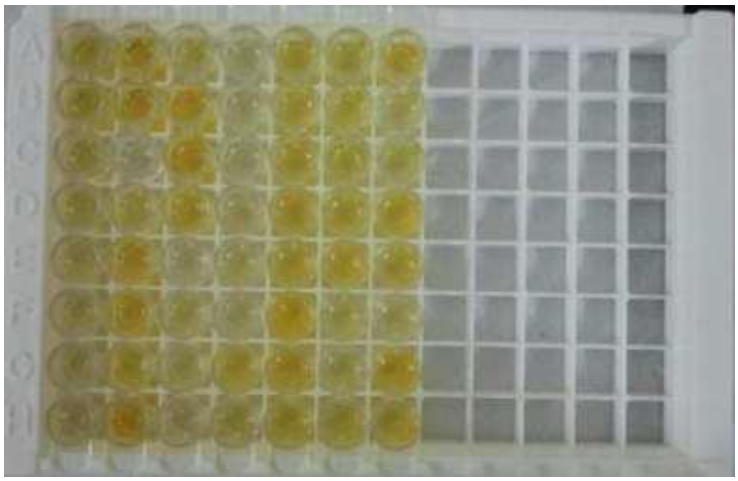


FIG 13 ***RESULTS PRINTED OUT BY THE IMMUNO ASSAY ANALYZER***

Results

RESULTS

The study is aimed at comparison of bFGF in saliva of subjects with chronic periodontitis and those with periodontal health.

27 patients with chronic periodontitis associated with tobacco chewing and 25 healthy subjects were included in the study. Saliva samples were collected from each group. FGF levels in saliva were assessed using sandwich ELISA technique.

Saliva was collected in sputum container and stored in refrigerator at 25˚C. Fibroblast Growth Factor levels were assessed using sandwich ELISA technique. Absorbance was measured at 450 nm as primary wavelength and as reference wavelength in terms of 650 nm.

The raw data from all the samples examined are represented in table 1 and table 2.

The ELISA results followed the standard curve, indicating the reproducibility and the accuracy of the results obtained. The standard curve in represented in .

The mean concentration of bFGF in chronic periodontitis patients with tobacco chewing habit is 329.012 +/ 178.643 pg, while that of healthy persons is 92.261 pg. There is a statistically significant difference between the disease and health samples at $p<0.000$.

Intergroup comparison of calculated concentration in tobacco chewers with chronic periodontitis and healthy persons using Mann –Whitney U test:

When the Mann Whitney U test was used there was a statistically significant increase ($p<0.000$) of bFGF in chronic periodontititis patients when compared to healthy group .

These results are represented in table 5.

Tables and Graphs

Table : 1

Saliva samples			
Sample No	Name of the patient	Absorbance	Calculated concentration (pg/ml)
	Tobacco chewers with chronic periodontitis		
1	T1	0.084	**237.367**
2	T2	0.102	**401.019**
3	T3	0.092	**310.101**
4	T4	0.088	**273.734**
5	T5	0.098	**364.652**
6	T6	0.072	**128.265**
7	T7	0.123	**591.947**
8	T8	0.099	**373.744**
9	T9	0.080	**201.000**
10	T10	0.140	**746.507**
11	T11	0.069	**100.990**
12	T12	0.101	**391.927**
13	T13	0.071	**119.173**
14	T14	0.118	**546.488**
15	T15	0.077	**173.724**
16	T16	0.085	**246.459**
17	T17	0.125	**610.130**
18	T18	0.091	**301.009**
19	T19	0.071	**119.173**
20	T20	0.072	**128.265**
21	T21	0.082	**219.183**
22	T22	0.105	**428.294**
23	T23	0.125	**610.130**
24	T24	0.089	**282.826**
25	T25	0.093	**319.193**
26	T26	0.070	**110.082**
27	T27	0.108	**455.570**

Table :2

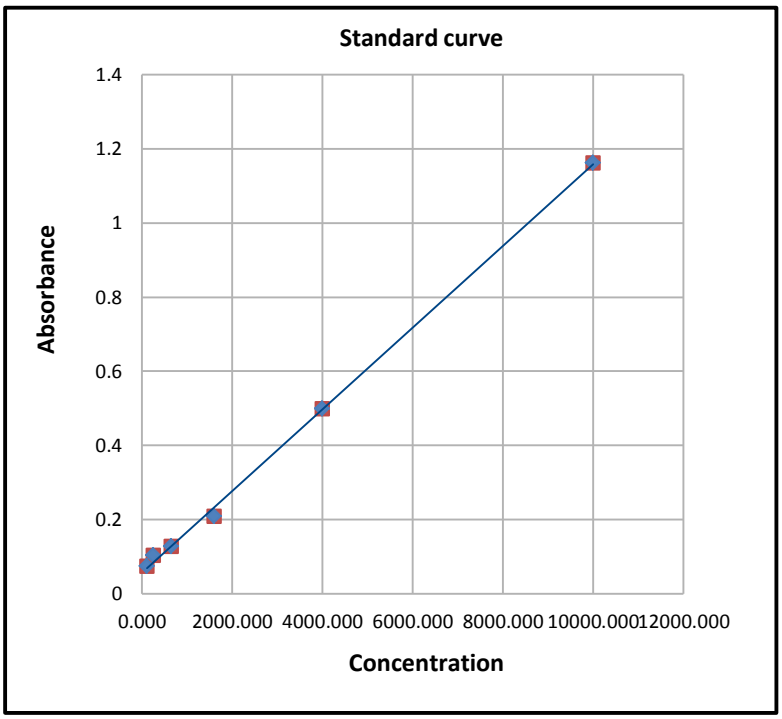
	Healthy subjects		
1	H1	0.083	**228.275**
2	H2	0.081	**210.091**
3	H3	0.064	**55.531**
4	H4	0.065	**64.623**
5	H5	0.069	**100.990**
6	H6	0.076	**164.632**
7	H7	0.074	**146.449**
8	H8	0.062	**37.347**
9	H9	0.066	**73.714**
10	H10	0.065	**64.623**
11	H11	0.061	**28.256**
12	H12	0.062	**37.347**
13	H13	0.065	**64.623**
14	H14	0.074	**146.449**
15	H15	0.061	**28.256**
16	H16	0.065	**64.623**
17	H17	0.068	**91.898**
18	H18	0.060	**19.164**
19	H19	0.065	**64.623**
20	H20	0.067	**82.806**
21	H21	0.065	**64.623**
22	H22	0.086	**255.550**
23	H23	0.066	**73.714**
24	H24	0.069	**100.990**
25	H25	0.062	**37.347**

Table : 3

STANDARD CURVE

Standard reading

Standards	Absorbance
102.4	0.074
256	0.103
640	0.128
1600	0.208
4000	0.499
10000	1.161

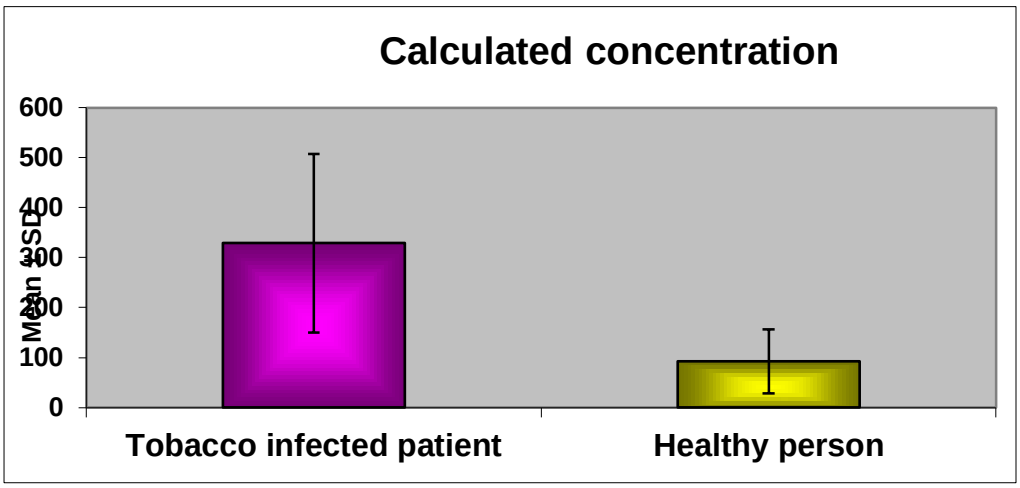


R^2 = 0.9989
slope= 0.0001
intercept= 0.0579

Blank 0.051

Graph : 1

	Mean	S.D
Tobacco Chewers with: chronic periodontitis	329.012	178.6431
Healthy subjects :	92.26176	64.4213.



Discussion

DISCUSSION

Periodontitis is a chronic inflammatory disease that is initiated as gingival inflammation in response to plaque microorganisms. The exact mechanisms for the transition from gingivitis to periodontitis are not yet known but individual disease susceptibility seems to play a major role.[111] The various factors that affect disease susceptibility include genetic and environmental factors.[75]

Environmental factors such as lifestyle dietary habits and tobacco related habits.[37] There is voluminous literature linking smoking increased severity of periodontal diseases, worsening of prognosis and delayed wound healing. Tobacco chewing is an habit that is prevalent in certain cultures of Asian subcontinent, including India.[2] Although not as well documented as smoking the effects of tobacco chewing on the oral cavity and the periodontium is also well recognised.[35]

Existing literature has reported a variety of oral lesions such as mucosal lesions, premalignant lesions like leukoplakia and malignancies like Squamous cell carcinoma, to be associated with tobacco chewing habit[66]. In relation to the periodontium, the most commonly reported symptoms include burning sensation in the gingiva, halitosis, ulcerations in the oral cavity and gingiva and discolouration of teeth[76].

Previous literature has reported that in spite of no significant difference between quid chewers and non chewers with respect to oral hygiene, there was a greater severity of periodontal disease.[5]

CPI-code -4 with a probing depth of > 6mm was seen in 30% of pan chewers in tobacco compared with 7.3% of pan chewers without tobacco. It was found that Pan chewers with tobacco had 4.7 times more risk of having periodontal pockets than Pan chewrs without tobacco.[30]

Smokeless tobacco users tend to have more severe REC and CAL and a greater proportion of site with higher values of REC and CAL compared with never users[6]. The greatest increase in severity of CAL was found to be localised to sites on mandibular ,anterior teeth and molar which may be the result of retention of the smokeless tobacco (ST) product in the oral cavity.

The present study was undertaken with the aim to obtain a better understanding of the etiopathogenic mechanisms underlying the periodontal diseases that is associated with tobacco chewing habit.

In the present study only patients who exhibited Tobacco chewing habit (Pan chewers) were included. Patients with concomitant tobacco smoking habit were excluded from the study to avoid counfounding of the results. Patients included in the study exhibited clinical signs of periodontitis but no other mucosal / malignant or premalignant lesions. This was done to ensure that the marker used in the study (bFGF) would show changes relating

only to Periodontal inflammation and not due to any other inflammation/ malignancy in the oral cavity. Previous studies have documented increased bFGF presence in saliva in patients with oral malignancy[36].

The pathogenic mechanisms underlying the disease process had been documented. Arecoline the major alkaloid of arecanut , inhibit cell attachment cell spreading and cell migration and decreased cell growth and collagen synthesis. Arecanut extract induce the production of PGE2,[59] the activation of intracellular calcium, concentrate P-38 mitogen activated protein kinase [62] and the extracellular signal regulated protein kinase inhibitor. All these findings suggest that areca nut chewing may induce an inflammatory response[117].

Fibroblast growth factor is a multipotent growth factor that produces different effects depending on the cell types it acts[7]. b Fibroblast growth factor is thought is thought to be the most biologically active among this family of proteins. It is a potent angiogenic factor[19] and it also involved in collagen synthesis[18] and matrix turnover by the fibroblast[39]. As both neovascularisation and fibrosis are commonly associated with tobacco chewers, bFGF may be implicated in the pathogenesis of periodontal disease that is associated with tobacco chewing habit [12].

The present study was undertaken to investigate if FGF levels were altered in periodontal disease when compared to health.

Saliva was chosen as the medium because of the following reasons

1. Ease of collection, non invasiveness and rapid screening

2. Pan chewing habit has been linked with pathologically changes in the oral cavity as a whole without any direct influence on the salivary glands, making saliva a more suitable candidate when compared to GCF .

The ELISA method was chosen for the estimation of FGF because of its predictability and reproducibility of results.[43] The other methods of protein estimation such as NMR tend to be much more extensive in nature and the clinical applicability of these techniques is somewhat limited by their cost. PCR dependent methods although more accurate, estimate only the mRNA in a given sample and may not give a complete representation of the functional protein available[55].

The results of the study indicate that there was a significantly higher expression of bFGF in the test group when compared to the controls

Due to the previous explained exclusion criteria, it can be hypothetised that the significantly higher salivary bFGF values related to periodontal disease.

These results are in agreement with the results of Kuo Yuan et al who studied FGF levels in in gingiva of chronic periodontitis sample.

As salivary bFGF levels are obtained from tissue, it can be fypothesized that our results are in agreement with these results.

There are no previous results of bFGF in periodontal disease with which these results can be compared. However, Yuan et al, have studied the role of bFGF in pyogenic granuloma and have reported increased expression of FGF in the inflammatory/ angiogenic lesion.

Nicotine and arecoline present in pan are known to significantly affect vascularity and increase inflammation.

bFGF levels may be upregulated through the following mechanisms

1. Nicotine may directly induce upregulation of bFGF through its effect on neovascularisation as it is absorbed through the mucosal tissues.
2. Nicotine, nitrosamine, arecholine induce inflammatory changes by upregulation of pro inflammatory mediators such as IL-1. IL6 and PGE2. These mediators in turn, affect angiogenesis through its effects on the angioblasts, thereby indirectly upregulating Bfgf

It is possible that the greater bFGF presence in saliva is a result of the increased vascular tissue response to these agents.

Additionally, the role of pan in inducing fibrosis is well established. The results of this study indicate that the increase in FGF is related to the fibrotic changes that occur in the oral cavity as a whole including the periodontium.

Nicotine and other products are absorbed through the oral mucosa and thereby gain access to the fibroblasts of the oral cavity, including the gingival fibroblasts.[83] On stimulation, directly by the areca nut products or indirectly through inflammatory mediators, fibroblasts produce increased levels of bFGF, the primary response being matrix turnover.

These changes have been previously reported by Robertson et al. and our results only reinforce the above results.

Saliva is a second hand marker in periodontal disease, all its constituents are probably released from the GCF. In turn, GCF is thought to reflect the environment of the periodontal tissues that it is closely associated with.

The clinical implications of the study are as follows:

1. FGF may be used as a marker for predicting progression of periodontal disease in tobacco chewers. Long term studies with a greater sample size could help establish its reliability as a marker.
2. FGF or its signalling pathways may be used as a therapeutic target to treat fibrotic changes that occur as a result of tobacco chewing. Further studies are required to elucidate the molecular mechanisms that regulate the process.
3. FGF has already been used for regenerative therapy in animal models for furcation defects; its use has been approved for human

use as well. These results suggest that one must excise caution in using FGF for regenerative purpose in tobacco chewers because of its strong association with fibrosis as fibrosis in itself may be detrimental to periodontal regeneration.

This study has the following limitations:

1. The sample size is reasonably small considering the population and the prevalence of tobacco use. Therefore the power of the study is not high.
2. The cross-sectional nature of the study means that the progression of the disease is not determined which would require longitudinal studies.

Summary and Conclusion

SUMMARY AND CONCLUSION

The study titled "Evaluation of salivary basic Fibroblast Growth Factor (bFGF) in tobacco chewers with chronic periodontitis" using sandwich ELISA technique enrolled fifty-two patients seeking periodontal treatment with tobacco usage assessed as using any form of smokeless tobacco for a period of least 6 months.

After oral prophylaxis, unstimulated pooled saliva was collected as per established protocol. The technique proposed by **Navazesh et al (2008)** was used for saliva collection. 5ml of unstimulated saliva was collected in a sterile container, by asking the subjects to lean the head forward over the container with the mouth slightly open and allow the saliva to drain into the container. The time given for saliva sample collection was 5-10 minutes. The saliva samples were centrifuged at 2800 rpm and stored at -20 degree Celsius, and results were statistically analyzed.

Within the limits of this study, the following conclusions have been elucidated:

The patients who consume tobacco showed higher salivary bFGF (329.012 +/ 178.643 pg) in chronic periodontitis than healthy patients (92.261 pg) with $p<0.000$, suggesting that bFGF may play an important role in the disease pathogenesis and progression.

However, further controlled clinical trials with a larger sample size should be executed to evaluate the effectiveness of this marker.

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Annexure

ANNEXURE

RAGAS DENTAL COLLEGE & HOSPITAL
(Unit of Ragas Educational Society)
Recognized by the Dental Council of India, New Delhi
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TO WHOMSOEVER IT MAY CONCERN

Date: 06/01/2017

From

The Institutional Ethics Board,

Ragas Dental College and Hospital,

Uthandi,

Chennai- 600119

The dissertation topic titled **"Evaluation of Salivary Basic Fibroblast Growth Factor (bFGF) in Tobacco Chewers with Chronic Periodontitis"** submitted by **Dr. M.Ganesh Kumar,** has been approved by the Institutional Ethics Board of Ragas dental college and hospital.

Dr. N.S.Azhagarasan,MDS,

Member secretary,

Institutional Ethics Board,

Ragas Dental College and Hospital.

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