Salivary TNF-α As A Bone Remodeling Biomarker Before and After Subgingival Mechanical Debridement

Thesis

Submitted in Partial Fulfillment of the Requirements for
Master Degree

Of

Oral Medicine and Periodontology

By

Mohamed Ahmad Al-Shaikh
B.D.S. (October 6 University)

Department of Oral Medicine ,Oral Diagnosis and Periodontology

Faculty of Oral and Dental Medicine
Cairo University

2010

Supervisors

Prof. Dr. Mohamed Sherien Hassan Aly

Professor of Oral Medicine and Periodontology
Faculty of Oral and Dental Medicine
Cairo University

Dr. Weam Ahmed Maher Rashwan

Lecture of Oral Medicine and Periodontology
Faculty of Oral and Dental Medicine
Cairo University

Dr. Laila Rashed

Assist. Professor of Biochemistry
Faculty of Medicine
Cairo University

ACKNOWLEDGMENT

First, and for most, I feel always indebted to God, the most kind and the most merciful.

Second, I would like to express my sincere appreciation and gratitude to Prof. Dr. ***Mohamed Sherien Hassan Aly*** *Professor of Oral Medicine and Periodontology, Faculty of Oral and Dental Medicine, Cairo University, for his generous supervision, sincere advice and great help and it's an honor for any candidate to work under his supervision.*

My appreciation also goes to Dr. ***Weam Ahmed Maher Rashwan*** *lecturer of Oral Medicine and Periodontology, Faculty of Oral and Dental Medicine for her generous supervision, continuous encouragement and never fading support.*

My great thanks also go to Dr. ***Laila Rashed*** *Assist. Professor of Biochemistry, faculty of Medicine, Cairo University, for her endless guidance and great help.*

I would also like to thank Prof. Dr. ***Mahmoud Al-Refaie,*** *Professor and Head of Oral Medicine and Periodontology Dept., Faculty of Oral and Dental Medicine, Cairo University, for his continuous guidance and meticulous follow up for the scientific work activities in the department.*

Last, but not least, I would like to express my gratitude to all staff members of Oral Medicine and Periodontology Department, Faculty of Oral and Dental Medicine, Cairo University, for their generous cooperation and encouragement through out this study.

List of contents

دور التنخر الورمي لعنصر ألفا اللعابي كعامل حيوي مميز للتغير البنائى للعظم قبل و بعد التقليح تحت اللثوى

رسالة مقدمة

من

الطبيب/ محمد أحمد الشيخ

بكالوريوس طب وجراحة الفم والأسنان

توطئة للحصول على
درجة الماجستير فى طب الفم وعلاج اللثة

كلية طب الفم والأسنان
جامعة القاهرة

2010

المشرفون

أ.د/ محمد شرين حسن علي

أستاذ طب الفم وعلاج اللثة
كلية طب الفم والأسنان
جامعة القاهرة

د/ وئام احمد ماهر رشوان

مدرس طب الفم وعلاج اللثة
كلية طب الفم والأسنان
جامعة القاهرة

د/ ليلى راشد

أ.م الكيمياء الحيوية
كلية الطب
جامعة القاهرة

Introduction

Periodontal disease is a chronic microbial infection that triggers inflammation-mediated loss of the periodontal ligament and alveolar bone that supports the teeth. It affects 45% of adults older than 50 years of age in the United States and is a major cause of tooth loss worldwide**.** ***(Albandar et al., 1999; Petersen and Ogawa 2005)***

Because of the increasing prevalence and associated co morbidities, screening and diagnostic modalities for the early identification of periodontitis initiation and progression, as well as objective measures for response to therapy are being sought. ***(Beck and Offenbacher 2005)***

Whole saliva represents a promising diagnostic fluid for the screening of periodontal disease. It is a fluid that contains constituents of exocrine glands in the oral cavity and gingival crevicular fluid (GCF). Saliva is readily available and easily collected without specialized equipment or personnel**.** ***(Frodge et al., 2005)***

Several mediators of chronic inflammation and tissue destruction have been detected in whole saliva of periodontitis patients. ***(American Academy of Periodontology 2003; Lamster et al., 2003)***

Alpha2-macroglobulin, β-glucuronidase, C-reactive protein (CRP), and cathepsin G are host response indicators reported at higher levels in whole saliva of patients who had periodontal disease compared to control subjects. ***(Lamster et al.,2003;Christodoulides et al.,2005)***

Also, the proinflammatory cytokine interleukin (IL)-1β and matrix metalloproteinase (MMP)-8(neutrophil collagenase) were shown to correlate with clinical indices and radiographic evidence of periodontal disease. ***(Miller et al., 2006; Scannapieco et al., 2007)***

Other salivary biomarkers putatively associated with periodontal disease include hepatocyte growth factor, osteonectin, and osteoprotegerin (OPG). ***(Miller et al., 2006; Scannapieco et al., 2007)***

From a biologic perspective, periodontal disease can be considered to consist of three phases: inflammation, destruction of the connective tissue attachment apparatus followed by apical migration of the junctional epithelium, and altered alveolar bone turnover with net loss of bone density and height. Levels of several salivary biomarkers were reported to correlate with two of the phases of periodontal disease (i.e., the inflammatory and connective tissue destruction phases*)*.However, few analysts associated with alveolar bone turnover activity has been identified in saliva. ***(American Academy of Periodontology2003;Miller et al.,2006; Ng et al.,2007; Scannapieco et al.,2007)***

TNF-α represents a key aspect of bone remodeling and could serve in creating a salivary diagnostic panel for periodontitis. ***(Taubman et al., 2005)***

In this report, we are testing the hypothesis that biomolecules involved in bone remodeling especially tumor necrosis factor-alpha [TNF-α], is increased in the saliva of patients with periodontal disease compared to control subjects as well as before and after mechanical subgingival debridement.

Review of literature

Periodontitis is a disease characterized by loss of connective tissue attachment and bone around the teeth in conjunction with the formation of periodontal pockets due to the apical migration of the junctional epithelium. Although bacteria are obviously the initiating agent in periodontitis, the complexity of the associated microflora and the critical role of the host in determining the outcome of the bacterial challenge cause difficulties in defining specific disease markers in periodontal diseases. ***(Champagne et al., 2003)***

Periodontal disease progression is episodic in nature on a tooth site level; however, the risk of developing periodontal disease is principally patient-based rather than site-based, bacterial virulence factors either result directly in degradation of host tissues or cause the release of biologic mediators from host tissue cells that lead to host tissue destruction. Mediators produced as a part of the host response that contribute to tissue destruction include proteinases, cytokines and prostaglandins. ***(Champagne et al., 2003)***

Another important class of molecules in tissue destruction is the variety of enzymes produced by periodontal microorganisms for instance, collagen degrading enzymes, elastase-like enzyme, trypsin-like proteases, aminopeptidases and dipeptidylpeptidases. Locally, bacterial lipopolysaccharide triggers monocytes, polymorphonuclear leukocytes (PMN, neutrophils), macrophages and the other cells to release interleukin-1 (IL-1), tumor necrosis factor (TNF) and prostaglandin E2 (PGE2). IL-1 and TNF appear to have an important role in periodontal tissue destruction and PGE2 appears to partly responsible for the bone loss associated with periodontitis ***(Miyasaki et al., 2003)***

Periodontal diseases have been seen at an increased rate in many countries throughout the world. Associated with this high prevalence of periodontal disease there has been increase in tooth loss despite the availability of several new therapeutic methods and agents over the past two to three decades. Clinical measurements used in the diagnosis of periodontal diseases are often of limited usefulness in that they are indications of previous periodontal disease rather than the present disease activity. Moreover, as various immunopathogenic mechanisms are involved in the disease process, the combinations of indicators are needed to improve the specificity of diagnosis. ***(Ozmeric, 2004)***

Analysis of saliva and gingival crevicular fluid (GCF) may be especially beneficial in the determination of current periodontal status. Many studies have indicated that the determination of inflammatory mediator levels in biologic fluids is a good indicator of inflammatory activity. Therefore, studies related to the pathogenesis of periodontal diseases usually examine whether biochemical and immunological markers in saliva and/or GCF might be a marker to reflect the extent of periodontal destruction and to predict the future disease progression. ***(Ozmeric, 2004)***

Saliva is a valuable oral fluid that often is taken for granted. It is critical to the preservation and maintenance of oral health, yet it receives little attention until quantity or quality is diminished. Saliva also has become useful as a noninvasive systemic sampling measure for medical diagnosis and research. Therefore, it is necessary to have a good knowledge base concerning the norm of salivary flow and function.

1. ORIGIN AND ANATOMY OF SALIVARY GLAND

Saliva is a clear, slightly acidic mucoserous exocrine secretion. Whole saliva is a complex mix of fluids from major and minor salivary glands and from gingival crevicular fluid, which contains oral bacteria and food debris. ***(Roth and Calmes, 1981; Edgar, 1992)***

The major salivary glands include the paired parotid glands, which are located opposite the maxillary first molars, and the submandibular and sublingual glands, which are found in the floor of the mouth. Minor glands that produce saliva are found in the lower lip, tongue, palate, cheeks, and pharynx. ***(Calmes, 1981)***

The terms *major* and *minor* refer to the anatomic size of the glands. The average daily flow of whole saliva varies in health between 1 and 1.5 Litres Percentage contributions of the different salivary glands during unstimulated flow are as follows: 20% from parotid, 65% from submandibular, 7% to 8% from sublingual, and less than 10% from numerous minor glands. Stimulated high flow rates drastically change percentage contributions from each gland, with the parotid contributing more than 50% of total salivary secretions. ***(Edgar, 1990)***

The types of cells found in the salivary glands are acinar cells, various duct system cells, and myoepithelial cells. Acinar cells, in which saliva is first secreted, determine the type of secretion produced from the different glands. Secretion can be classified as serous, mucous, or mixed; serous secretions are produced mainly from the parotid gland, mucous secretions from the minor glands, and mixed serous and mucous secretions from the sublingual and submandibular glands. ***(Roth et al., 1981)***

Duct system cells found in the salivary ducts are classified as intercalated, striated, and excretory. Intercalated duct cells are the first duct network

connecting acinar secretions to the rest of the gland. These cells are not involved in the modification of electrolytes, as are the remaining duct cells. Striated cells are second in the network, functioning as electrolyte regulation in resorbing sodium. The final duct cells, the excretory duct cells, contribute by continuing sodium resorption and secreting potassium. Excretory duct cells are the last part of the duct network before saliva reaches the oral cavity. Myoepithelial cells, which are long cell processes wrapped around acinar cells, contract on stimulation to constrict the acinar cell. This function, secreting or "squeezing out" accumulating fluid, is the result of a purely neural process. ***(Roth et al., 1981; Garrett 1987; Edgar 1992)***

2. COMPOSITION

Saliva is composed of a variety of electrolytes, including sodium, potassium, calcium, magnesium, bicarbonate, and phosphates. Also found in saliva are immunoglobulins, proteins, enzymes, mucins, and nitrogenous products, such as urea and ammonia. These components interact in related function in the following general areas: (1) bicarbonates, phosphates, and urea act to modulate pH and the buffering capacity of saliva; (2) macromolecule proteins and mucins serve to cleanse, aggregate, and/or attach oral microorganisms and contribute to dental plaque metabolism; (3) calcium, phosphate, and proteins work together as an antisolubility factor and modulate demineralization and remineralization; and (4) immunoglobulins, proteins, and enzymes provide antibacterial action. The listed components generally occur in small amounts, varying with changes in flow, yet they continually provide an array of important functions. ***(Dowd, 1999)***

Saliva is a very dilute fluid, composed of more than 99% water. Saliva is not considered an ultrafiltrate of plasma initially, saliva is isotonic, as it is formed in the acini, but it becomes hypotonic as it travels through the duct

network. The hypotonicity of unstimulated saliva allows the taste buds to perceive different tastes without being masked by normal plasma sodium levels. ***(Grant et al., 1988)***

Hypotonicity, especially during low flow periods, also allows for expansion and hydration of mucin glycoproteins, which protectively blanket tissues of the mouth lower levels of glucose, bicarbonate, and urea in unstimulated saliva augment the hypotonic environment to enhance taste. ***(Tabak et al., 1982)***

The normal pH of saliva is 6 to 7, meaning that it is slightly acidic. The pH in salivary flow can range from 5.3 (low flow) to 7.8 (peak flow). Major salivary glands contribute most of the secretion volume and electrolyte content to saliva, whereas minor salivary glands contribute little secretion volume and most of the blood-group substances. ***(Edgar, 1990)***

3. FLOW

There is great variability in individual salivary flow rates. The accepted range of normal flow for unstimulated saliva is anything above 0.1 mL/min. For stimulated saliva, the minimum volume for the accepted norm increases to 0.2 mL/min. These numbers have been projected from research on general populations. Salivary flow is, however, a very individualized measurement and ideally should be recorded as a base reference after the age of 15. ***(Edgar, 1990)***

Any unstimulated flow rate below 0.1 mL/min is considered hypofunction. ***(Screebny et al., 1987)***

4. FUNCTION

Salivary components work in concert in over lapping, multifunctioning roles, which can be simultaneously beneficial and detrimental. Salivary function can be organized into 5 major categories that serve to maintain oral health and

create an appropriate ecologic balance: (1) lubrication and protection, (2) buffering action and clearance, (3) maintenance of tooth integrity, (4) antibacterial activity, and (5) taste and digestion. ***(Mandel, 1987; Moss, 1995)***

(1) Lubrication and protection

As a seromucous coating, saliva lubricates and protects oral tissues, acting as a barrier against irritants e.g. proteolytic and hydrolytic enzymes. The best lubricating components of saliva are mucins that are secreted from minor salivary glands. ***(Grant et al., 1988)***

(2) Buffering action and clearance

Buffering action and clearance are a second function of saliva through the following components: bicarbonate, phosphate, urea, and amphoteric proteins and enzymes. Bicarbonate is the most important buffering system. It diffuses into plaque and acts as a buffer by neutralizing acids Moreover; it generates ammonia to form amines, which also serve as a buffer by neutralizing acids. More than 90% of the nonbicarbonate buffering ability of saliva is attributed to low-molecular-weight, histidine-rich peptides. ***(Mandel, 1989)***

Urea, another buffer present in saliva, releases ammonia after being metabolized by plaque and thus increases plaque pH. The buffering action of saliva works more efficiently during stimulated high flow rates but is almost ineffective during periods of low flow with unstimulated saliva. Phosphate is likely to be important as a buffer only during unstimulated flow. ***(Roth et al., 1981; Johnson, 1987; Edgar, 1990; Lagerlo and Oliveby; 1994)***

(3)Maintenance of tooth integrity

Maintaining tooth integrity is a third function of saliva, one that facilitates the demineralization and remineralization process. Demineralization occurs when acids diffuse through plaque and the pellicle into the liquid phase of

enamel between enamel crystals. Resulting crystalline dissolution occurs at a pH of 5 to 5.5, which is the critical pH range for the development of caries. ***(Edgar, 1990)***

Dissolved minerals subsequently diffuse out of the tooth structure and into the saliva surrounding the tooth. The buffering capacity of saliva greatly influences the pH of plaque surrounding the enamel, thereby inhibiting caries progression. ***(Stephan, 1944)***

Remineralization is the process of replacing lost minerals through the organic matrix of the enamel to the crystals. Supersaturation of minerals in saliva is critical to this process. The high salivary concentrations of calcium and phosphate, which are maintained by salivary proteins, may account for the maturation and remineralization of enamel. ***(Roth and Calmes, 1981)***

Statherin, a salivary peptide, contributes to the stabilization of calcium and phosphate salts solution, serves as a lubricant to protect the tooth from wear, and may initiate the formation of the protective pellicle by binding to hydroxyapatite. ***(Edgar, 1990; Dowd, 1999)***

Proteins in the protective pellicle, such as statherin, histatins, cystatins, and proline-rich proteins, are too large to penetrate enamel pores. Therefore, they remain on the surface, bound to hydroxyapatite, to aid in controlling crystalline growth of the enamel by allowing the penetration of minerals into the enamel for remineralization and by limiting mineral egress. ***(Scannapieco et al., 1993; Dowd, 1999)***

This control of precipitation and mineral egress enhances the stability of hydroxyapatite in the outer tooth structure. ***(Richardson et al., 1993)***

Low-molecular-weight protein fractions, thought to be derived from the proteolytic processing of larger proteins, are likely to be in exchange with dental plaque fluid. These protein fractions help adjust and augment remineralization,