Using Saliva as a Biomarker for Periodontal Disease-Literature Review

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ABSTRACT:

Saliva is a useful diagnostic fluid for periodontal diseases. Observing salivary biomarkers for oral and systemic diseases could become an important complement to clinical examinations in preventing periodontal disease in early stage. Periodontal diseases are mainly the inflammation of tooth supporting structures which affects gingiva, periodontal ligament, and alveolar bone. Recent findings indicate that it is possible to detect biomarkers for oral diseases within saliva samples. The aim of this research will be to investigate if known salivary biomarkers could be used for detection of periodontal diseases.

Materials and Methods: A randomly selected sample of adults (20–89 years), living in Thunder Bay, will be invited to participate. 100 individuals will be examined clinically using standard examination procedures. Participants will be divided in 2 groups. First group will receive the results of the examination and if any participant will be susceptible for periodontal disease, they will be advised to take professional care according to their results. Second group will be the control group. Stimulated saliva samples will be collected and analysed for concentrations of IL-1b, -6, -8, lysozyme, matrix metalloproteinases (MMP)-8 and tissue inhibitor of metalloproteinase (TIMP)-1 using ELISA, immunofluorometric assay or Luminex assays. The effect of using saliva as a biomarker will be evaluated by measuring clinical attachment loss (CAL) at the beginning of the study and after 5 years. The measurements of CAL will be taken every 6 months during the period of 5 years. The comparison of control group and the test groups will be done by analysis of variance (ANOVA) statistical test.

KEY WORDS: Saliva, Biomarker, Periodontal Disease, ANOVA, Luminex assays

INTRODUCTION:

Periodontal disease affects a large population of Canada. Approximately 16% of adults (20-79 years) have moderate, and 4% of adults have severe inflammatory periodontal disease (Health Canada, 2010). The diagnosis of active phases of periodontal disease, and the identification of patients at risk for active disease, represents a challenge for both clinical investigators and clinicians. 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 assess disease severity (Polson & Goodson, 1985). Occasionally, monitoring of the microbial infection and analysis of the host response in gingival crevicular fluid (GCF) are utilized in an attempt to identify individuals at risk for future breakdown (Curtis et al. 1989).

However, still there is no test which is routinely used for detecting periodontal disease. Clinical and radiographic assessment of periodontal disease remains the basis for patient evaluation. This is true despite the fact that clinical monitoring is time consuming, subject to considerable measurement error, and is often poorly tolerated by patients.
In addition, the frequency of radiographic evaluation is limited. These measures provide information primarily about disease severity, and are not useful measures of disease activity. 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 purpose of this research proposal is to highlight the potential application of saliva for diagnosis of periodontal disease (Kauffman & Lamster, 2000).

**Literature Review**

Periodontitis is a chronic, non-reversible, and multifactorial inflammatory disease which affects the periodontal tissues supporting the teeth. Periodontitis is initiated and proliferated through a complex interaction between periodontal pathogens and the host defense system. It starts with a microbial infection, followed by a host mediated destruction of periodontal tissues caused by hyper activity of leukocytes and generation of cytokines, eicosanoids and matrix-metalloproteinases (Hani, Abdulrahman, Raed, Amrita, & Sukumaran, 2014). Clinically, the disease progresses with loss of attachment to root surface, formation of a deep pocket, alveolar bone resorption, and subsequent loss of tooth. It is the most common disease affecting the oral cavity after dental caries and the major cause of tooth loss, thereby affecting the quality of individual’s life. Because of that reason, early diagnosis and control of the disease is very important. Currently clinicians use probing depths, bleeding on probing, attachment loss, radiographic examination and plaque index as the indicators of periodontal disease. All these signs show us the level or amount of disease already present but they do not show the exact disease activity. Knowing the disease activity might help in early intervention in patients with the disease. This review of the literature focuses the attention on the biochemical markers in saliva that appear to be promising in the future for periodontal diagnosis (Hani, Abdulrahman, Raed, Amrita, & Sukumaran, 2014).

Human saliva is composed of 98% water and 2% of other compounds, such as electrolytes, mucus, antibacterial compounds, salivary proteins and various enzymes (Rathnayake N et al, 2013). Saliva has several functions which includes rinsing and cleaning the mouth, lubricating soft tissues of mouth, bolus formation and therefore in deglutition, speech and facilitation of mastication, for the digestion of some particles, and antibacterial defence (Lee, Wong. 2009). Many inflammatory biomarkers associated with oral diseases have been detected in saliva, such as interleukins-1b, -6, -8 (IL-6, IL-1, IL-8), tumour necrosis factor-alpha (TNF-a) and matrix metalloproteinases (MMP)-8 and -9 (Fox 1993, Kaufman & Lamster 2000, Seymour & Gemmell 2001, Miller et al. 2006). Furthermore, salivary biomarkers have been used to detect periodontitis, to demonstrate distinct biological phases of periodontal disease, and to assess caries risk (Uitto et al. 1990). An increasing number of molecular markers are gradually being identified in saliva and some represent biomarkers for other different diseases, such as cancer, cardiovascular diseases, autoimmune diseases, viral and bacterial diseases and human immunodeficiency virus (Rathnayake et al, 2013).

**Biomarker:** A biologic feature that can be used to measure the presence or progress of disease or the effects of treatment and that can be accurately reproducible.

The following is a list of salivary biomarkers identified through research till now.

**Table 1. Salivary biomarkers for periodontal disease**

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<th>Proteomic Biomarkers</th>
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<th>Microbial biomarkers</th>
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**Table 1. Salivary biomarkers for periodontal disease**
The correlation between salivary biomarkers and clinical features of periodontal disease has been evaluated for three aspects of periodontitis which are inflammation, collagen degradation and bone turnover (Miller et al. 2006). Levels of these biomarkers vary in individuals with the presence of disease. But their amount in individuals without disease is always far less than individuals with the disease.

Barnfather et al. (2005) 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 after 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). Thus incorporation of individualized personal feedback using a point-of-care test for salivary nicotine metabolites in a general practice based smoking cessation program increased cessation rates by 17% at 8 weeks and reduced tobacco use. So, a leading cause for periodontal disease can also be stopped with the help of saliva test. A clinical study used the hand-held Integrated Microfluidic Platform for Oral Diagnostics instrument to rapidly (3–10 min) measure the concentrations of MMP-8 and other biomarkers in small amounts (10 ml) of saliva. Another study reported application of a lab-on-a-chip system for the concomitant measurement of the salivary biomarkers C-reactive protein, MMP-8 and IL-1b as related to the clinical expression of periodontitis. This study demonstrated 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-1b and MMP-8 increases in whole saliva of periodontitis patients. Furthermore, the lab-on-a-chip approach has a limit of detection that is five orders of magnitude lower than that achieved using the high-sensitivity C-reactive protein (hsCRP) enzyme-linked immunosorbent assay. 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 lab-on-a-chip assay procedure demonstrates a detection limit at 5 fg/ml and a useful range between 10 fg/ml and 10 pg/ml (Bradley, S. H., David, T. W., Lei, Z., & Paulo, M. C. 2009).

Using saliva as a biomarker is a non-invasive technology and these diagnostic methods look really striking. Saliva collection is a very easy process and it can be done by any individual with very little training. Salivary biomarkers, whether produced by healthy individuals or by individuals affected by specific diseases, are sentinel molecules that could be used to examine health and perform disease surveillance. Ongoing research has developed very simple tools which can be used even at home and that give results instantly. Analysis of saliva can offer a cost-effective approach for the screening of large populations. The development of microchips and microfluidic platforms for salivary components has great potential in the use of oral fluid for point of care testing (Christodoulides, N., Dharshan, P., Ebersole, JL., Florino, PN., Miller, CS., Mohanty, S. et al, 2007).
Summing it up, we get the partial answer for the research question which was, “Is it beneficial to use saliva for detecting periodontal diseases?” Using saliva as a biomarker for periodontal disease provides reliable and fast results before the signs and symptoms of periodontal diseases even start appearing. Therefore, if we diagnose early, we can prevent this widely spread disease of the soft tissues of mouth. In the end, further research is necessary to make it more practically acceptable.

**Research Method**

**Rationale**

Chronic Periodontitis is a microbial disease that can be largely prevented and effectively treated if diagnosed early. Gingivitis is a reversible form of gum disease, which can be transformed to periodontitis if not treated in time. Once a client develops periodontitis, the condition becomes irreversible. It can be controlled by regular professional care and oral self care at home. So, it is better if the disease is detected in the early stages, so that it can be treated in time and periodontitis can be prevented.

**Purpose**

The primary objective of this study is to assess whether using saliva as a biomarker for periodontal disease is effective and efficient way in diagnosing periodontal disease than routine clinical examination. When used as a biomarker, saliva can notify about the presence of disease before the actual signs and symptoms start appearing clinically. This study will determine if using saliva as a biomarker help in detecting periodontal disease early and therefore produce statistically significant results by comparing pre- and post-study periodontal pocket depths and the amount of difference in CAL between control and test group.

**Study Design**

To evaluate the effectiveness of using saliva as a biomarker for periodontal disease, a randomly selected sample of 100 adults living in Thunder Bay will be invited to participate in a clinical study of oral health. Individuals will be of age 29 to 89 years old. All the necessary data collection for this quantitative study will be done by periodontal examination of each patient and documented on the periodontal assessment form (Appendix A). The collected data will be analysed using Statistical Package for Social Science (SPSS). Non-parametric tests will be applied to calculate the characteristics and clinical variables between groups. One-way ANOVA and general linear model will be used to compare the biomarkers between the groups of periodontal health

**Population and Sample.**

The population of interest will be selected by convenient sampling. Patients will be gathered by organising oral health camps across various places in the city of Thunder Bay. The sample will be selected based on predetermined inclusion and exclusion criteria.

**Inclusion Criteria.**

- 29 to 79 years of age with no systemic disease and good overall health
- Presence of $\geq 18$ scorable teeth (not including third molars and teeth with crown and bridge or implants)

**Exclusion Criteria.**

- Presence of systemic illnesses like diabetes mellitus, cancer, human immunodeficiency syndrome, bone metabolic diseases, or disorders that compromise wound healing, radiation, or immunosuppressive therapy
- Presence of smoking
- Presence of advanced chronic periodontitis (defined as the presence of $\geq 6$ teeth with probing depth $\geq 6\text{mm}$, loss of clinical attachment $\geq 4\text{mm}$, and radiographic evidence of bone loss more than one-third of the root length.
- Confirmed of suspected intolerance or allergy to aspirin
- History of receiving periodontal therapy within the previous year

A total sample of 100 subjects will be selected and randomly divided in to two groups of 50 subjects. The study will be explained to each subject, including the benefits, risks, and alternative treatments. After the patients sign the informed consent form (Appendix B) indicating their agreement to participate in the study, each patient will be assigned a patient number in order to maintain the masking of evaluators.

**Investigative techniques.**

The study plans to make a periodontal assessment of the patient’s oral cavity and carry out the experiment based on the comparison and analysis of pre- and post-study data. Stimulated saliva was collected during

5 min. chewing on 0.5 g of paraffin into a graded test-tube. The collected amount was determined,
excluding the foam, and the secretion rate per minute was recorded. Saliva sampling and scoring of results was performed by trained dental assistants. Collected samples were immediately frozen at _20°C until processing. Once the sampling of saliva were completed, each vial with saliva was centrifuged, the supernatants were aliquoted into 1.5 ml Eppendorf tubes (Eppendorf, Hauppauge, NY, USA), and stored at _80°C. Each saliva aliquot was used only once for the determination of cytokines, lysosome or MMP-8 levels.

**Instrumentation.**
A standard calibrated UNC-periodontal probe will be used throughout the trial to make measurements. It has millimeter marking from 1mm to 15mm which is adequate for the purpose of the study. To maximize the validity and reliability of the study, only two evaluators will carry out the whole study and will be examining the same patients throughout the study. Both evaluators will be calibrated to various variables like pressure and angulation of insertion of probe into the sulcus or pocket to minimize the examiner’s bias.

**Sources and Collection of Data.**
The selected participants will be subjected to thorough periodontal examination. Gingival margin level (amount of gingival recession) and periodontal probing depths will be measured around six areas (mesiobuccal, buccal, distobuccal, mesiolingual, lingual, and distolingual) of each tooth and will be documented on the periodontal assessment chart. Based on these two values, clinical attachment level will be determined for each of the six areas of teeth by adding the gingival recession and periodontal probing depth of a specific area. The periodontal assessment will be done for a total of ten times, first time before the saliva sampling will be taken and at the intervals of 6 months after the deliverance of periodontal therapy to the group which will receive the therapy if detected susceptible to periodontal disease.

**Data Analysis Plan.**
The primary objective of the study is to assess whether aspirin is effective in increasing the clinical attachment gain after periodontal therapy or not. Hence, the clinical attachment gain of control group and the test group will be compared by analysis of variance (ANOVA) and found if statistically significant or not. The level of significance will be set at p≤0.05.

Also, the secondary objective of the study is to find out the optimal dosage of aspirin at which the most beneficial effect on the periodontium would be exerted. Hence, the results of the clinical attachment gain of group taking 81mg of aspirin per day and 325mg of aspirin per day will be compared with a one-way-ANOVA. The level of significance will be set at p≤0.05.

**Time Schedule.**
The study will be commenced at four different time intervals. All patients will be oriented about the study. The preliminary evaluation of every patient will be done within the period of 3 weeks starting from patient no.1 to 90. Then the patients will receive thorough periodontal debridement and root planing over the period of next 3 weeks in the same order and will be given thorough oral hygiene instructions. Patients will be given aspirin tablets or placebo every week. After one month of periodontal therapy, the patients will be reassessed in the same manner. The procedures will be repeated at 3 months and 6 months interval for individual patient.

**Ethical Considerations (Human Subject Protections).**
An expedited review application (Appendix C) will be filled and submitted to Research Ethics Board (REB) division of Health Canada. There will be no children in the study. The patients will be oriented about the study and will know the potential benefits and harm that can happen by the study. After explaining the study, an informed consent letter (Appendix B) will be given to each patient to sign. As the study involves manipulation of the gingival tissue and administration of pharmacological agent, both aspects are thoroughly explained to the patients. There is no risk of morbidity or any other physical or mental harm if the patients adhere to the instructions given by the examiners.

**Validity and Reliability.**
The study is such designed from every aspect that the results obtained from it have high validity and reliability. The evaluators are calibrated through meticulous calibration procedures and pocket depth measurements will be obtained from every aspect of teeth and accurate collection of saliva and it’s components will be analysed for biomarkers. The study spans over a duration of 5 years, which can give us
reliable data about the effectiveness of the using saliva to detect periodontal disease.

**Bias.**
There are many potential biases involved in the study:

*Selection Bias.*
The convenient sampling method may be biased and may not represent the overall population of the city. (For example, advanced chronic periodontitis is more common in lower socioeconomic group of people who may not represent overall population)

*Examiners’ Bias.*
In spite of meticulous calibration, examiners are subject to make errors while measuring periodontal pocket depths and assessing periodontal tissue and collecting stimulated saliva samples.

*Subject Bias.*
In spite of thorough instructions to the patients, they may skip the dosage of aspirin or take double dose in a day if they forget to take aspirin the day before. Also, they may not follow strict oral self-care measures which may inadvertently affect results of the study.

In order to reduce the bias, the study will be double-blinded, meaning that neither the patients nor the examiners would know if the patient is getting a placebo, a low dose, or a high dose of aspirin during the course of the study.

**Assumptions.**
The assumptions made in the study are as follows:

- The selected sample of population will be representative of whole adult populations of the city of Thunder Bay
- The information provided by the patients is accurate.
- The evaluation done by the evaluators will be accurate and similar for every patient.
- The patients will take their prescribed dosage of aspirin or placebo regularly without missing it on any day.
- The patients will follow the oral hygiene instructions given by the evaluators.

**Limitations.**
- The study will be conducted over a period of 5 years of time which may not be adequate to assess the long-term results of benefits done by detecting periodontal disease in early stage as periodontal disease is a chronic disease.
- The study will only assess clinical attachment gain which is just one parameter of improvement in periodontal health. There may be other parameters that can be evaluated in the study.

**Implications of the study.**
If aspirin is found to be beneficial, it can help many clients with periodontal diseases. Aspirin is not very expensive and can be administered relatively safely in adults unless they have an allergy to it. This study also tries to find an optimum dose of aspirin at which it would exert maximum benefits.

A progression of this study can involve a more detailed study with a computerized calibrated probe and taking other periodontal parameters like gingival index (GI), periodontal index (PI), bleeding on probing (BOP) etc. into considerations

**Publishing of Study.**
An application will be submitted to The Canadian Dental Hygienists Association (CDHA) for publishing the study and the results in Canadian Journal of Dental Hygiene (CJDH). Upon approval, the study, the findings, and appropriate credits will be published in the journal.

**References**


