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Case Study

COMPARITIVE EVALUATION OF TISSUE GLYCOGEN AND SALIVARY PH IN CHRONIC GINGIVITIS AND CHRONIC PERIODONTITIS PATIENTS

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ABSTRACT

BACKGROUND: The inflammation of gingiva is known as gingivitis with microorganisms present in the gingival sulcus, being the major cause for this pathologic condition. Periodontitis is a chronic inflammatory disease which is a successor of gingivitis. The role of bacterial putrefactive processes in the etiology of periodontitis has been discussed in many studies. To explore such possibilities further, investigation of relationship between the saliva and adjacent pocket tissue on a chemical basis is essential. Glycogen is a normal fuel reservoir for most tissues, changes in this constituent should give some indication of the metabolic activity within the tissue. The presence of saliva is vital for the maintenance of healthy oral tissues. Acting as a biological fluid this also acts as a diagnostic fluid in many cases. Salivary PH is has been found to be biologic marker for inflammatory process. It is therefore postulated that salivary ph can be correlated with tissue glycogen levels.

AIM: The aim of the present study is to compare the tissue glycogen level with salivary ph in both chronic gingivitis and chronic periodontitis patients.

MATERIALS AND METHODS: the study sample was collected from patients who attended the department of Periodontics St. Joseph Dental College; patients who were diagnosed with chronic- generalized periodontitis and chronic generalized gingivitis. The patients were allocated to 2 groups of 10 patients each. Saliva sample was collected from all the patients and PH was measured. Biopsy was taken at the deepest pocket for periodontitis patients and at any site in gingivitis patient. The amount of glycogen is measured in all the biopsy samples using PAS staining.

RESULTS: The amount of glycogen in gingivitis is greater than the amount of glycogen in periodontitis. The salivary ph of gingivitis patients is more basic than the salivary ph of periodontitis patients.

CONCLUSION: Correlating the above findings, it can be concluded that when the salivary PH is acidic then there is depletion of glycogen in gingiva and when the salivary PH is basic then the amount of glycogen is elevated in gingiva.

Introduction:

Gingiva is a part of the oral mucous membrane which extends from the dento-gingival junction to alveolar mucosa and is subject to the friction and pressure of mastication. The inflammation of gingiva is known as gingivitis. Nearly 96% of population is affected by gingivitis, which if neglected, may further lead to periodontitis. Microorganisms present in the gingival sulcus are responsible for the pathologic changes seen in gingivitis[1]. Periodontitis is defined as an inflammatory disease of supporting tissues of teeth caused by specific microorganisms or groups of specific microorganisms, resulting in progressive destruction of the periodontal ligament and alveolar bone with periodontal pocket formation, gingival recession or both. The disease is characterized by loss of clinical attachment due to

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destruction of the periodontal ligament and loss of the adjacent supporting bone.

The role of bacterial putrefactive processes in the etiology of periodontitis has been discussed in many studies. To explore such possibilities further, investigation of relationship between the saliva and adjacent pocket tissue on a chemical basis is essential. Since cellular glycogen is the normal fuel reservoir for most tissues, changes in this constituent should give some indication of the metabolic activity within the tissue. Hence the amount of glycogen varies with the severity of gingivitis and periodontitis[2]. Saliva is one of the biological and diagnostic bodily fluids. It is a glandular secretion, which constantly bathes the teeth and the oral mucosa. The presence of saliva is very important for the maintenance of healthy oral tissues[3]. It also acts as a diagnostic fluid in many cases. Here, salivary PH is determined as biologic marker for inflammatory process.

Hence fore, it is estimated that salivary ph can be correlated with tissue glycogen level. The aim of the present short study is to compare tissue glycogen level with salivary ph in both chronic gingivitis and chronic periodontitis patients.

The aim of the study is to evaluate and compare the glycogen level and PH of saliva in both chronic gingivitis and chronic periodontitis patients.

MATERIALS AND METHODS

STUDY DESIGN:

A total of 20 patients were selected who attended the department of Periodontics St. Joseph Dental College, Eluru in which 10 were patients with chronic generalized gingivitis (group a) and 10 patients were with chronic- generalized periodontitis (group b).

INCLUSION CRITERIA FOR CHRONIC PERIODONTITIS PATIENTS:

All the patients with chronic generalized periodontitis were selected according to the following criteria. All the patients should have 1) poor oral hygiene; 2) At least eight teeth with a probing depth of > 5 mm and attachment loss of > 2 mm. The selection criteria for chronic gingivitis patients is 1) Gingival index score ≥ 2, 2) Plaque index score ≥2.

EXCLUSION CRITERIA FOR THE STUDY:

The exclusion criteria for the study is as follows 1)patients with systemic diseases,2)Patients with smoking habit, 3)pregnant patients,4)lactating mothers, and 5)diabetic patients.

PROCEDURE:

Approval of the study was obtained from the ethical committee of St. Joseph Dental College and an informed consent was taken from all participants before performing the study. Plaque indices, gingival indices and probing depth are measured for all the patients.

COLLECTION OF SALIVA:

The armamentarium for measurement of salivary ph is Saliva collecting jar and digital ph meter shown in Fig 2. The patients were asked to report early in the morning and the saliva samples were obtained during which subjects were requested not to drink any beverages except water. Drinking water was given to the subjects and asked to rinse the mouth well. After 5min, the subject was asked to spit whole saliva. The subjects were asked to refrain from talking and drop down the head and let the saliva run naturally to the front of the mouth (Fig 3). The subjects spit into the collection tube about once a minute for up to 10 min. A total of 5 ml of saliva was collected in sterile 10 ml beakers. The pH of the saliva was immediately measured in order to prevent any deterioration of the sample.

SALIVARY ANALYSIS:

Salivary pH was measured with the help of a single electrode digital pH meter (Fig 1). The pH meter was calibrated daily. The electrode was dipped in hydrochloric acid of 0.1 N overnight. Before dipping the electrode in the sample, a filter paper is used to dry it completely every time. After analysis of pH, the electrode tip was washed with distilled water. The liquids and chemicals were freshly prepared every day. The PH of the saliva is recorded for both chronic gingivitis and chronic periodontitis patients. The results are shown in table 1.

COLLECTION OF BIOPSY:

Armamentarium for collection of biopsy is No 15 sized blade, Bp handle, Local anesthesia (0.2%lignocaine), (Fig 2).The deepest pocket of the

mouth is selected. After obtaining adequate local anesthesia with 2% lignocaine, the interdental papilla involving the pocket epithelium is excised with no 15 size blade (Fig 4). The tissue excised should be 2×2mm size. It is thoroughly rinsed in water to remove the saliva as it contains amylase which breakdown the glycogen present in the tissue. It is later fixed in 10%formaline for 24 hours before processing for staining. The sample is sent for histopathology analysis. The bleeding is controlled at the biopsy site and periodontal dressing is given. Post operative instructions are given and medication is prescribed for the next 3 days.

HISTOPATHOLOGICAL STAINING AND ANALYSIS:

Hematoxiline, eosin and PAS (periodic acid Schiff) staining are done for the tissue after processing of the biopsy into sections. Hematoxyline and eosin assessed the presence of inflammation and PAS staining was used for study of presence or absence of glycogen (Fig 5, 6).

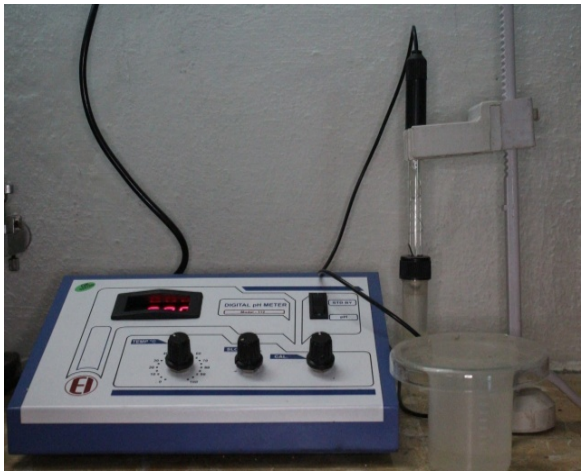


Figure 1: DIGITAL PH METER

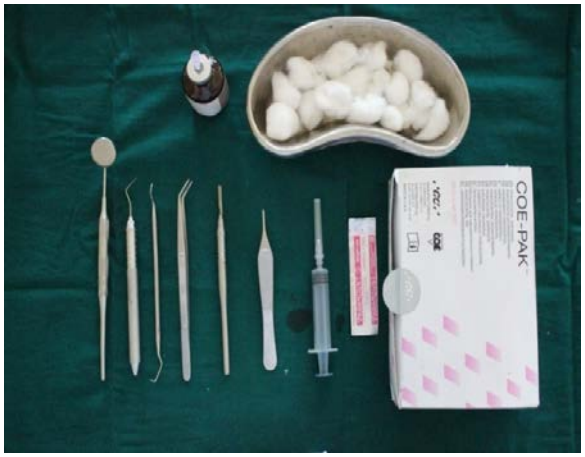


Fig 2: ARMAMENTARIUM FOR COLLECTION OF BIOPSY

Fig 3: COLLECTION OF SALIVA IN A 10 ML CONTAINER

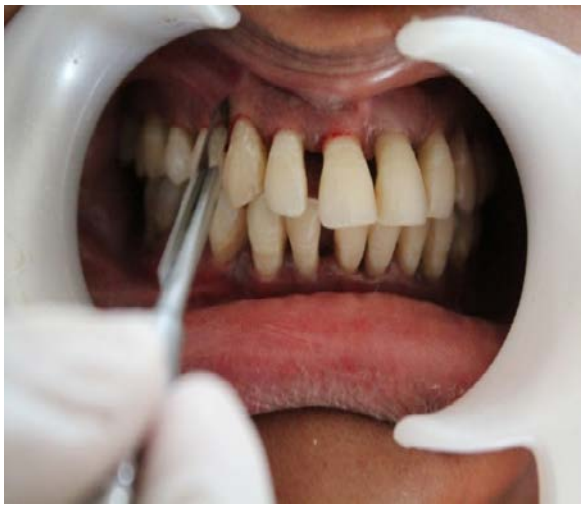


Fig 4: INCISION FOR TAKING BIOPSY AT THE DEEPEST POCKET OF THE MOUTH

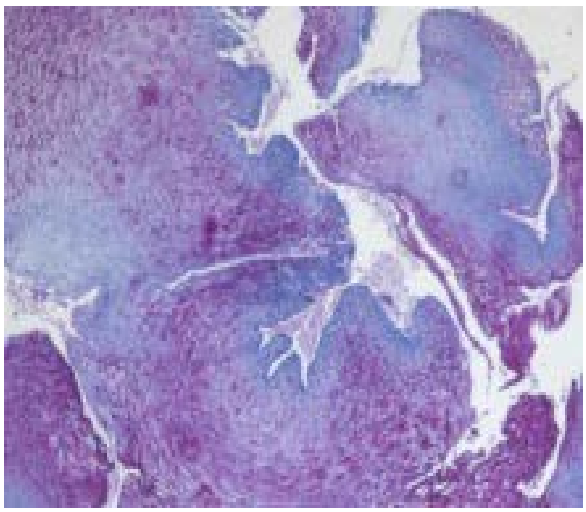


Fig 5: PAS STAINED GINGIVITIS TISSUE

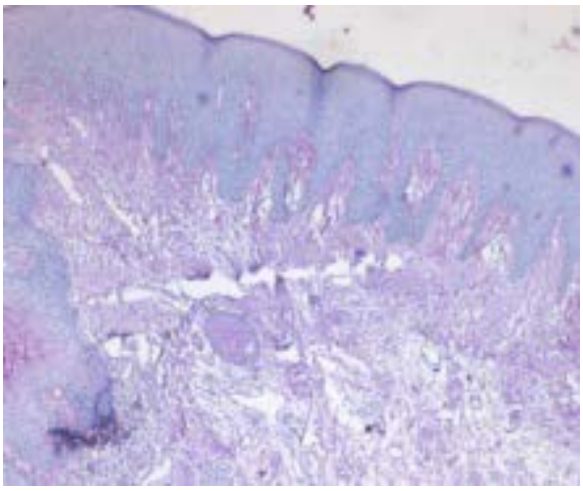


Fig 6: PAS STAINED PERIODONTITIS TISSUE

STASTICAL ANALYSIS:

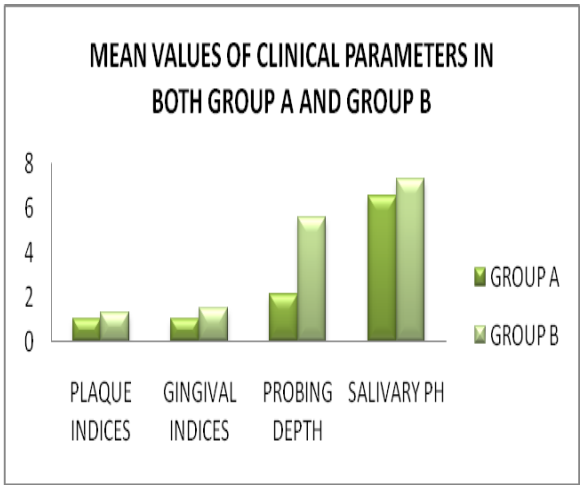
The mean values, standard deviations and standard error of plaque index, gingival index, probing depth, and PH of saliva are measured using paired t test (graph pad prism version 7.2) and the p value is recorded (table 1).Chi square test (SPSS version 20) is used to record the glycogen content in the gingival epithelium (table 2). The mean values of the clinical parameters were depicted in a bar diagram (Graph 1).

Table 1: PAIRED T TEST

CLINICAL PARAMETERS	MEAN VALUES		STANDARD DEVIATION		P VALUE
	GROUP A	GROUP B	GROUP A	GROUP B	
PLAQUE INDICES	1.02	1.32	±0.01	±0.06	<0.0001
GINGIVAL INDICES	1.02	1.47	±0.013	±0.08	<0.0001
PROBING DEPTH	2.1	5.6	±0.1	±0.26	0.0074
SALIVARY PH	6.51	7.29	± 0.06	± 0.02	0.0101

Table 2: Mann Whitney U test

	GLYCOGEN CONTENT IN GINGIVAL EPITHELIUM			P-VALUE
	PRESENT	ABSENT	TOTAL	
GINGIVITIS GROUP (GROUP A)	2	8	10	0.002
PERIODONTITIS GROUP (GROUP B)	8	2	10	



GRAPH 1: GRAPHICAL REPRESENTATIONS OF CLINICAL PARAMETERS

RESULTS:

The amount of glycogen in gingivitis is greater than the amount of glycogen in periodontitis and the salivary ph of gingivitis patients is more basic than the salivary ph of periodontitis patients.

The comparison between the two groups was showed in the above table. The amount of glycogen was shown in the table 2.

DISCUSSION:

Saliva is a dilute fluid, with over 99% of it being made up of water. Whole saliva collected from the mouth is a complex mixture of both organic and inorganic components. Submandibular gland (4) secretes about 60% of the total 750 ml of saliva secreted daily. The parotid gland secretes about 30%, sublingual about 5% and less than 7% is secreted by minor salivary glands(5). The saliva in the oral cavity is either resting or pooled saliva. Saliva has a pH normal range of 6.2‑7.6 with 6.7 being the average resting PH. It should be noted here that the PH of mouth does not fall below 6.3. As a buffer, it maintains the ph of oral cavity at neutrality i.e. at 6.7-7.3.

The saliva has 2 methods for maintaining PH of the oral cavity. Firstly its flow eliminates the carbohydrates which can be metabolized by the bacteria and hence reduces the production of acids by the bacteria. Second, it neutralizes the acidity from drinks and foods, as well as from bacterial activity. Inflammation of the gingival is known as gingivitis, which if not resolved may lead to inflammation of the periodontium called as periodontitis.[6] .The periodontal tissue destruction is a complex process involving plaque accumulation, release of bacterial substances and host inflammatory response. It is manifested by pocket formation and bone loss. Microorganisms and their products cause pockets, which leads to pathologic tissue changes and deepening of the gingival sulcus. A saliva pH of 7.0 usually indicates a healthy dental and periodontal situation. At the neutral pH, there is a low incidence of dental decay combined with little or no local factors (plaque and calculus). Therefore, stable conditions should be found in this environment. If the salivary pH is below 7.0 then usually it indicates acidemia (abnormal acidity of the blood). And in chronic cases, it is susceptible to dental decay, halitosis and periodontitis. A saliva pH above 7.0 usually indicates alkalinity. Excessive alkalinity can bring about the same anaerobic conditions as acidemia, but it is much rarer condition.

There are two key factors to induce plaque formation. First, there must be oral bacteria to attack food particles and elevate the pH. Second the pH must elevate above 7.6 to grow dental plaque crystals that cause periodontal disease. Thus, alkaline pH is essential for plaque growth suggesting the mildly alkaline pH of the saliva obtained from the subjects with generalized chronic gingivitis. As the gingival crevice deepens; the environmental factors in the sub gingival site become more stable, i.e., neutral pH and anaerobic. Under these conditions, asaccharolytic anaerobic and/or proteolytic bacteria such as Fusobacterium, Campylobacter, Prevotella and Porphyromonas are found. Fusobacterium species also utilize glutamic acid as a nutrient and produce acetic and butyric acids. P. gingivalis, P. intermedia and *C.* rectus metabolizes aspartic acid to succinic acid but requires formic acid as a reducing agent. [7]

Takahashi *et al*. (2005)[8, 9] concluded in their study that the periodontopathogens grow in a mildly acidic pH. This is in accordance to our result for pH of chronic periodontitis. The present study is in correlation with Sharmila et.al, in which they concluded that Salivary pH in patients with chronic generalized gingivitis was more alkaline than in patients with clinically healthy gingiva. In patients with chronic generalized periodontitis, the salivary pH was more acidic than the control group.

Glycogen is synthesized from glucose by glucose-6 phosphate and glucose-1-phosphate. Glycogen is stored in injured cells when their metabolism is impaired.It is reasonable to assume that the tissue glycogen is synthesized in situ from hexose and/ or hexose phosphates, and that under adverse conditions; this activity of the cells might be affected. The present study suggested that since putrefaction and glycolysis are mutually antagonistic processes, a depletion of tissue glycogen may predispose gingival tissue to periodontitis and similarly an increase in putrefaction may inhibit glycolytic processes and thus lead to a depletion of tissue glycogen.these

findings were in correlation with Forscher et al[2](1953).

The significance of glycogen accumulation in developing tissues generally has been explained on the basis that it was a source of potential energy for cell differentiation (Chen & Harwick 1977) or a reflection of an anaerobic condition (Dawes & Shelley 1968).

CONCLUSION:

Correlating the above results, the study concluded that the when the salivary PH is acidic then there is depletion of glycogen in the gingiva and when the salivary PH is basic then the amount of glycogen is evident in gingiva. Further longitudinal studies with larger sample size are required for the confirmation and generalization of the present study results.

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