Assessment and Comparison of Ghrelin and Chemerin Levels in Gingival Crevicular Fluid and Serum as Predictive Biomarkers in Aggressive Periodontitis Patients: A Study Protocol

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Abstract: Background: Ghrelin is a recently described peptide hormone that is secreted predominantly by the stomach. It induces appetite and thereby controls food intake and energy balance. It has been also observed modulatory effects on the immune system and bone metabolism. Chemerin binds to an orphan G coupled receptor which modulates the innate immune system and chemotaxis of immature dendritic cells and macrophages. Objective: Comparative evaluation of GCF & Serum "levels of Ghrelin and Chemerin" as the (Predictive) biomarkers of inflammation in aggressive periodontitis. Methodology: Total 80 samples will be included and divided into two groups. Group I includes 40 GCF samples from 40 subjects with aggressive and Group II includes 40 Serum samples from 40 subjects with aggressive periodontitis group. The GCF will be collected from the site with deepest probing depth. Gingival index, probing pocket depth and clinical attachment level will be measured one day before GCF collection to avoid stimulation of the sample and its contamination with blood. The Ghrelin and Chemerin levels in GCF and serum samples will be measured using an ELISA kit. Expected Result: The present study will have found correlation the GCF and

serum levels of ghrelin and chemerin with the severity of disease process so as to obtain an insight into the probable roles of ghrelin and chemerin in immune pathogenesis of periodontal diseases. Conclusion: This will further broaden the possibility of using Ghrelin and Chemerin as an marker of inflammation in periodontal diseases.

Keywords: Ghrelin, Chemerin, Biomarkers, Inflammatory markers

Introduction:

Periodontitis is an inflammatory disease characterized by the destruction of the supporting tissues. To destruct the periodontium, microorganisms and inflammatory mediators are primarily accountable.[1]It is mediated by various inflammatory mediators like: cytokines (IL 1α, TNF α, IL 8), prostaglandins and several other enzymes. These inflammatory mediators are increased in periodontitis and co relate with the disease activity. Pro-inflammatory cytokines leads to increase in RANKL expression and suppresses OPG expression which causes bone loss.[2]Therefore from literature it has been observed that periodontitis causes destruction of periodontal tissues along with affects individuals health.[3,4]Many such mediators have been identified in serum and saliva and have served as various markers.

Ghrelin is a peptide hormone which is principally secreted by the stomach, however it is also present in lesser amount in the other tissues of the body including salivary glands and teeth.[5]literature has shown that ghrelin have favorable effect on endocrine function, also authors have investigated the modulatory effect of ghrelin on the immune system &bone metabolism.[6,7]

Ghrelin have down regulation activity of the lipopolysaccharide (LPS) which promotes proinflammatory cytokine production such as interleukin IL-1b and tumor necrosis factor (TNF-α). They have effect on differentiation and proliferation of osteoblastic cells and bone formation through the anti-inflammatory activity.[8]

Deng et al. in their study have observed that ghrelin significantly increases the expression of various markers of bone metabolism like alkaline phosphatase (ALP), osteocalcin (OSC), and collagen type-1.[9]Ghrelin levels have also shown to have a positive relation with osteoprotegerin (OPG) levels and negatively relation with the levels of sRANKL.[10]

A newly described adipokine, chemerin is secreted in liver and adipocytes that is co-related with diabetes, central obesity &metabolic syndrome risk factors.[11]Chemerin binds to the receptors such as CMKLR- 1, ChemR 23andmodulates the immune system, chemotaxis of immature dendritic cells &macrophages.[12]Chemerinleads to attract ChemR 23 which exposes thedefense cells to adhesion molecules and extracellular matrix proteins.[13]

So the current analytical study will be carried out with basic aim of comparing the GCF and Serum levels of Ghrelin and Chemerin in individuals with aggressive periodontitis.

Aim:

Comparative evaluation of GCF & Serum "levels of Ghrelin and Chemerin" as the (Predictive) biomarkers of inflammation in aggressive periodontitis.

Objectives:
1. To evaluate "levels of Ghrelin and Chemerin" in GCF and serum of aggressive periodontitis patients.
2. To compare "levels of Ghrelin and Chemerin" in GCF and serum of aggressive periodontitis patients

3. To correlate the "levels of Ghrelin and Chemerin" in GCF and serum with periodontal parameters in aggressive periodontitis patients

Material and Methods:
Sources of the Data:

The subjects to be studied will be selected from the outpatient section, Department of Periodontics, Sharad Pawar dental college and hospital, Sawangi.

Statistical Analysis:

The sample size was calculated using;

σ is population Standard Deviation(SD)

d is the difference to be detected

From the previous article it was proved that

After applying the formula calculation are as follows,

$$n = \frac{(Z\alpha/2 + \beta/2)^2 \times 2 \times \sigma^2}{2}$$

So, by above formula sample size will be 80 . Thus each group will have a sample of 40.

Study Design
In this analytical study, a total of 80 samples from 40 subjects divided into two groups: The groups are as follows:

Group I: 40 GCF samples from 40 subjects with aggressive periodontitis group: Involving 2 or more permanent teeth other than incisor and 1st molars.

Group II: 40 Serum samples from 40 subjects with aggressive periodontitis group: Involving 2 or more permanent teeth other than incisor and 1st molars

Inclusion criteria

- Age range between 35 -60 years
- Generalised Aggressive periodontitis Patients according to American Academy of Periodontology
- Subjects should have at least 20 natural teeth.

Exclusion criteria:

- Pregnant/lactating women
- Smokers/tobacco in any form
- Patients with prior history of periodontal disease
- Patients with bleeding disorders or under immunosuppressive chemotherapy
- Disorders affecting bone
- The post-menopausal women
- Any systemic disease Subjects should not be on any medication.

Information regarding their dietary status, oral hygiene habits, gingival and periodontal status along with other routine clinical data will be noted in the specially designed chart. The information about the need of study will be clarified and signed informed consent will be taken from patients. Study protocol is approved by Institutional Ethical committee of DMIMS, Sawangi (Meghe), Wardha.

Patients will be evaluated under good illumination through mouth mirror, tweezers, and Williams graduated periodontal probe and with the pellets of cotton.

Method for collection of GCF

Maxillary sites will be selected for the collection of GCF samples to avoid contamination from saliva. The sampling areas will be isolated to avoid contamination. GCF collection [2µl] will be done using micro capillary pipettes of length 125mm and bore size of 0.01mm. The micropipette will be placed at the entrance of the gingival sulcus and unstimulated GCF will be collected. All the samples will be stored at -70°C till the assay procedure is carried out.

Method for collection of Serum

2 ml blood will be collected from the antecubital fossa through 20-guage needle with the help of syringe. Further sent for the laboratory investigations. Serum will be extracted from blood and kept at -70°C till the assay procedure.

Clinical measurements

At first visit the following clinical parameters will be recorded for all the patients: Gingival Index (Loe and Silness), Probing pocket depth in mm and Clinical attachment level (CAL) in mm. PPD and CAL measurements will be performed at six sites per tooth. All measurements will be performed by a single calibrated examiner (PB).The radiographs will be taken as adjuvants to confirm the site assessment. The GCF will be collected from the site with deepest probing depth. Gingival index, probing pocket depth and clinical attachment level will be measured one day before GCF collection to avoid stimulation of the sample and its contamination with blood.

Measurement of Ghrelin and Chemerin in GCF & Serum

The Ghrelin and Chemerin levels in GCF and serum samples will be measured using an ELISA kit according to the protocol explained by the manufacturer.

Statistical analysis:

All the results will be tabulated and statistically analyzed using " SPSS software (version 16© SPSS, Chicago, IL)". Data will be presented as mean and standard deviation. The comparison between the two studies groups will be determined by using ANOVA test.Pearson's correlation coefficient will also be used for the intragroup correlation of Ghrelin and chemerin GCF and serum levels and clinical parameters (GI, PPD and CAL). Values will be considered significant when $P < 0.05$.

Expected Outcome:

Due to the effect of systemic health on periodontitis, it will be observed that periodontitis will influence the ghrelin and chemerin levels similar to other chronic inflammatory diseases. Ghrelin and Chemerin level are useful in assessing future disease susceptibility and prognosis and thereby evolving cost effectiveness. Ghrelin and Chemerin levels in the GCF might be related

with periodontal disease. So, our study will have found correlation the GCF and serum levels of ghrelin and chemerin with the severity of disease process so as to obtain an insight into the probable roles of ghrelin and chemerin in immune pathogenesis of periodontal diseases. This will evaluate the range of level of GCF and serum Ghrelin and Chemerin for aggressive periodontitis. This will further evaluate the possibility of using Ghrelin and Chemerinas an marker of inflammation in periodontal diseases.

Discussion

The study is intended to correlate the expression levels of Ghrelin and Chemerin with the intensity of disease progress to find the possible role of Ghrelin and Chemerin in immune pathogenesis of periodontal diseases as well as assessment of future the disease sites. Furthermore, studies observed that periodontitis affects the periodontium with respect to the systemic health of an individual. Till date, there are no studies evaluating and comparing the circulating ghrelin and chemerin levels in Gingival Crevicular Fluid (GCF) and serum as Predictive biomarkers of aggressive Periodontitis.

Jentsch et al evaluated the levels of ghrelin and chemerinin relation with dietary update and obesity in periodontally healthy and diseased patients who differs in their body mass. The author reported that the amount of ghrelin found in GCF was lower in chronic periodontitis and obese subjects. The author concluded that lesser ghrelin and higher chemerin levels in the GCF might be co-related with the periodontal disease and obese individuals.[16]

Yılmaz et al assessed the chronic periodontitis patients to determine the plasma levels of ghrelin. The author evaluated 35 chronic periodontitis & systemically healthy individuals. The parameters used for correlation were serum cytokines and bone turnover markers. The author concluded that no significant correlation were found in ghrelin levels and periodontal parameters.[17]

Patnaik et al evaluated chronic periodontitis (CP) with and without type-2 diabetes mellitus (DM) cases to detect the levels of chemerin between GCF and tear fluid. The author concluded that both these levels of chemerin can be used as inflammatory markers in CP and DM.[18]

Dogan et al conducted the study to estimate the levels of chemerin and IL-6 levels. The author concluded that periodontitis and type 2 diabetes mellitus increases the release of levels of chemerin. Conversely, non-surgical therapy reduces the chemerin levels in chronic periodontitis with and without type 2 diabetes mellitus.[19]

Studies by Khatib et al emphasize on use of Ghrelin for the Management of Cachexia Associated with Cancer [20-21-22].

Conclusion

The study found that periodontitis can play possible role in circulating ghrelin and chemerin levels. This will further broaden the possibility of using Ghrelin and Chemerinas an marker of inflammation in periodontal diseases.

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