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Assessment and comparison of Periostin levels in peri-implant sulcular fluid as a biomarker in healthy peri-implant sites and sites with peri-implant mucositis and peri-implantitis.

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Abstract: Background – Peri-implant diseases need early diagnosis and intervention in order to prevent implant failure. Periostin is an enzyme imperative for periodontal health which decreases with increasing severity of inflammation and levels revert back to normal after treatment. Alterations in the levels of periostin with relation to peri-implant diseases is not yet been investigated. This study aims to explore the role of periostin in peri-implant health and disease. Objectives: To evaluate and compare the levels of Periostin in peri implant sulcular fluid (PISF) in healthy peri-implant sites and sites with peri-implant mucositis and peri-implantitis with its clinical correlation. Method and design: Total of 66 implant sites loaded at least one year earlier will be included in this study and divided into three groups. Group I (healthy peri-implant tissues), group II (peri-implant mucositis), and group III (peri-implantitis). Levels of periostin in peri-implant sulcus fluid (PISF) acquired by micro-capillary pipettes acquired by micro-capillary pipettes will be determined from each group using human periostin ELISA kit. Results: The results will be analyzed as levels of periostin in peri-implant sulcular fluid recorded by ELISA in all the three groups and will be subjected to intergroup comparison and will be presented after statistical analysis. Conclusion: Levels of periostin in peri-implant sulcular fluid may prove to be a magnificent tool in early and prompt diagnosis of peri-implant diseases which will help in planning and execution of appropriate treatment, hence preventing further destruction of supporting tissues increasing longevity of dental implants.

Keywords: Periostin, Peri-implant mucositis, Peri-implantitis, PISF

Background and rationale:

As with periodontal diseases, the biofilm is contemplated as the principal etiological factor for inception and advancement of destruction of peri-implant tissues and mediators of inflammation stimulated through microbial invasion are accountable for the development of peri-implant diseases such as peri-implant mucositis and peri-implantitis.[1,2]

Diverse well established clinical measurements are defined for assessment of dental implants; although; prompt recognition of the inflammatory response before the instance of clinical manifestations is imperative for timely analysis and impeding additional tissue destruction. PISF is the inflammatory exudate emanating from the vascular channels of gingival.[3,4]

PISF may benefit to delineate early peri-implant inflammatory changes with inflammatory mediators, tissue degradation components and volumetric alterations during inflammatory process. Through assessment of the PISF constituents and their correlation with clinical signs, peri-implant disorders can be diagnosed and rectified prior the clinical signs are evident.[5] Several constituents of PISF have been researched such as inflammatory mediators (cytokines, prostaglandins), tissue degradation products (MMPs, acute phase proteins), bone turnover markers and mineralized tissue components.[6-9]

Periostin, formerly termed as OSF-2 which was later referred as periostin, expresses in the PDL and periosteum. It is a matrix protein primarily expressed in the PDL, periosteum, and lying on the alveolar bone surfaces in patients.[10] Periostin performs pivotal roles in tooth morphogenesis, bone and tooth remodeling, wound repair, and cardiovascular diseases.[11,12] The role of Periostin is preservation of PDL integrity when subjected to motorized loading[13] including orthodontic tooth movements.[14] Periostin secretion by human PDL fibroblasts has been reported to be decreased in GCF, determining its possible role in periodontal disease progression.[15]

Many research studies have assessed the influence of periodontal disease on range of periostin in GCF, saliva and serum in chronic gingivitis, chronic periodontitis[16] and aggressive periodontitis[17] patients and also in healthy peri-implant sites.[18]

Till date no studies exist in the literature investigating PISF periostin levels and their correlation with peri-implant mucositis and peri-implantitis. Thus this will be the first study till date to assess and compare the range of periostin in healthy peri-implant sites and sites with peri-implant diseases.

Study goals & objectives:

The goal of the present study is to evaluate levels of Periostin as the biomarkers of inflammation in healthy peri-implant sites and sites with peri-implant mucositis and peri-implantitis and to correlate the levels of Periostin in PISF with clinical parameters in peri-implant diseases.

Safety considerations:

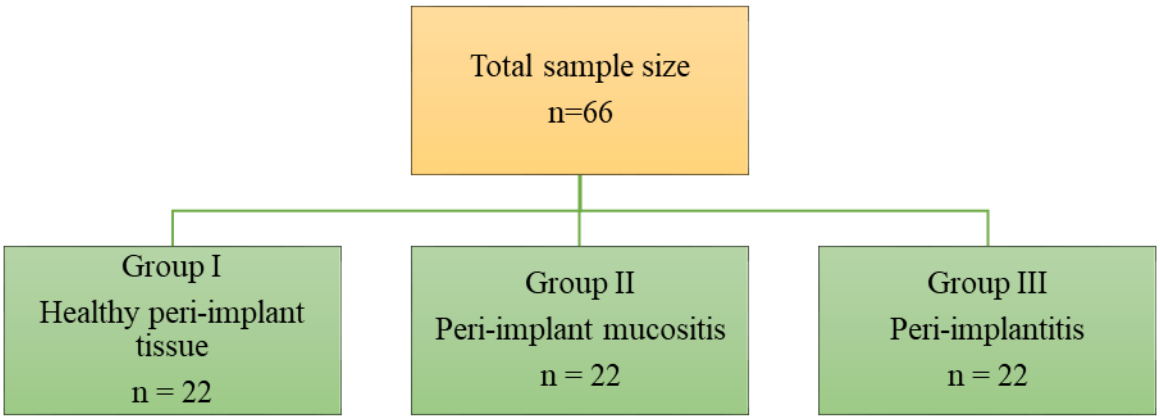
All the safety measures for the study participants will be taken care of and clinical, radiographic records and sample collections required for this study will be done with utmost care not to cause any harm to the participants and peri-implant tissues.

Follow up protocol:

This being an observational cross-sectional study no follow visit protocol is planned and all clinical parameters will be recorded and sample collection will be performed on two consecutive days.

Methods/Design:

This is an observational cross sectional study design. Sample size for this study was calculated using the formula $n = (Z(1- \alpha/2)^2 SD^2/d2$. A total of 66 samples from 66 peri-implant sites will be divided into three groups.



It will be made clear to the potential subjects that participation will be voluntary and written and informed consent will be collected from those who agree to participate.

The protocol for this study has been approved by "Institutional Ethics Committee Ref. No; DMIMS(DU)/IEC/2018-19/7357, the present study will be conducted in 'Dept of Periodontics & Implantology, SPDC, Sawangi, Wardha'." Each patient's detailed case history will be recorded.

To be eligible to join this study subject should be 35-60 years of age with implant placed and loaded at least one year before assessment. Healthy implant sites are defined as individual unattached clinically immobile dental implant with < 0.2 mm per year of radiographic evidence of bone loss when compared to baseline (at the time of implant loading) without any clinical sign of infection and inflammation[19]. Peri-implant mucositis is referred to an inflamed mucosa with a bleeding index of 2 and/or suppuration but without any evidence of bone loss.[29] Peri-implant bleeding will be assessed using the mBI. Peri-implantitis is referred to the existence of inflamed mucosa with a positive BOP, PPD ≥5 mm, and bone loss of more than or equal to 2 mm and/or more than 3 threads of implant.[20] Subjects will be excluded if they use tobacco in any form, pregnant or lactating women, patients who received periodontal treatment, antibiotic or antiseptic therapy 6 months prior to the study, patients with bleeding disorders or under immunosuppressive chemotherapy, subjects with any systemic disease which can alter the course of periodontal disease or on medication like bisphosphonates, steroids, cyclosporine A, calcium, vitamin A or hormone replacement therapy (HRT).

Clinical parameters:

a. Modified Plaque Index (mPlI)[21]

Mombeli et al. modified Plaque Index by Silness and Löe to evaluate formation in the marginal plaque around implants

b. Modified Bleeding Index (mBI)[21]

The modified bleeding index (mGI) is a modification of gingival index for application around oral implants.

c. **Probing pocket depth (PD)**[22]
d. **Bleeding on probing (BOP)**[23]
e. **Suppuration:** suppuration will be assessed as purulent discharge from gingival sulcus on probing or digital pressure along the gingiva of associated dental implant.[23]
f. . **Radiographic examination:** radiographic bone assessment will be calculated as follows.[24]

i. Bone Level (%) = 100 – D1 x 100/RL
ii. Radiographic Bone Loss (%) = Final bone level (%) – Initial bone level (%)

Method for collection of PISF:

At first visit clinical parameters will be recorded by a single calibrated examiner for all the patients: mBI by Mombelli and co-workers, and mPlI by Mombelli and co-workers BOP, Probing pocket depth in mm and CAL in mm. The radiographs will be taken as adjuvants to confirm the site assessment. The PISF will be collected from the site with deepest probing depth. Clinical parameters will be recorded one day before GCF collection to avoid stimulation of the sample and its contamination with blood.

The sampling areas will be secluded with cotton rolls to keep away from sample infection by saliva. PISF collection [4µl] will be done using micro capillary pipettes of length 125mm and bore size of 0.01mm. The micropipette will be placed at the orifice of the gingival sulcus and unstimulated GCF will be collected. All the samples will be stored at -70^{o}C till the assay procedure is carried out.

Measurement of Periostin in PISF:

The **Periostin** levels in PISF samples will be evaluated by means of an ELISA kit as given by the manufacturer guiding principle.

Data management & Statistical analysis:

All the results will be tabulated and statistically assessed by means of 'SPSS software (version 20© SPSS, Chicago, IL)'. Data will be offered as mean and standard deviation. ANOVA with post–hoc Tukey test will be used to analyze the difference between groups if the data will be normally distributed and if data will be not normally scattered then Kruskal wallis test with post-hoc Bonferroni test will be used.Values will be considered significant when $P < 0.05$.

Problems anticipated:

1. Collection of PISF in healthy pei-implant sites as volumes are very less as compared to inflamed sites
2. Storage of collected PISF samples as required conditions are quite stringent.
3. Cost involved with ELISA diagnostic test.

Benefits and risks of participation

All patients participating in this study will be treated free of charge.

There is no risk of participating in this study, as we already mention the inclusion and exclusion criteria. This is a noninvasive diagnostic procedure which has no deleterious effects on dental

implants or patient's health. No aggressive procedures will be involved. If any adverse events occur, then it will be recorded.

Expected Outcome:

PISF Periostin levels are useful in assessing future disease susceptibility and prognosis and thereby evolving cost effectiveness.

Scope:

1. To set the range of level of PISF **Periostin** for healthy peri-implant sites and sites with peri-implant mucositis and peri-implantitis.
2. To evaluate the possibility of using Periostin as an marker of inflammation in peri-implant diseases.

Limitations:

1. Influence of other anti- inflammatory markers cannot be ruled out.

2. Unidentified systemic conditions may modify results.

Discussion:

GCF is an exudate that contains a myriad of inflammatory mediators that delineate the disease status of local tissue. Similar to GCF, PISF is an exudate from peri-implant tissues which is advantageous in early analysis of peri-implant diseases.[18] Periostin is an enzyme that performs a pivotal role in remodeling and wound healing post surgical and non-surgical periodontal treatment. Periostin is observed to be down-regulated during various periodontal diseases. [25]

This is an observational cross-sectional study to assess the role of periostin in early analysis of peri-implant diseases. To best of our knowledge, there is no research available reporting the function of periostin in the diagnosis of peri-implant diseases whereas many studies authenticate its involvement during the cause of periodontal diseases and also in the course of healing and reconstruction of periodontal tissues post nonsurgical and surgical treatment.

Dental plaque or microbial biofilm forms on intraoral area in an aqueous or wet surroundings. Dental implants provide artificial surfaces that harbor bacteria from saliva and ecologic niches such as periodontal pockets, crypts of the tongue, and tonsils. Several microbial characteristics of the subgingival biofilm around dental implants have been congruent with the occurrence of dental plaque.[26] Peri-implant health compels the lack of bleeding on probing and other clinical signs of inflammation.[27]

Clinical parameters routinely used for assessment of periodontal diseases are not readily related to the tissues around dental implant fixtures and thus standard indices used for the evaluating periodontal health and diseases can't be defined for depicting peri-implant tissues. Hence indices modified specifically for peri-implant tissues will be recorded in this study. Plaque scores will be recorded using the mPlI. The bleeding tendency of the marginal peri‐implant tissues will be evaluated using the mBI.[21]

Peri-implants probing is more unpleasant and also the peri-implant pocket depth measurements are more sensitive as compared to periodontal probing thus probing depth will be assessed by UNC-15 with a tip diameter of 0.5 mm using a probing force of 0.5N which is lesser than periodontal probing.[5]

The hallmark difference in diagnosis of peri-implant mucositis and peri-implantitis is radiographically evident crestal bone loss after initial healing and bone remodeling around dental implants over time along with the existence of inflammatory changes and BOP.[23] In this study, intraoral peri-apical radiographs will be taken at the time of implant placement and before PISF sample collection to evaluate the bone loss and diagnosis of peri-implantitis.

Levels of periostin decrease with increased severity of periodontal disease and at these levels get back to normal with nonsurgical and surgical treatment and resolution of inflammation. Periostin levels were reported to increase in chronic and aggressive periodontitis cases following surgical procedures and these levels returned to baseline levels as the wound healed.[25,26] There is no study available in the literature evaluating alterations in the periostin levels concerning peri-implant health and diseases. Hence this study aims to evaluate the role of periostin in early diagnosis of peri-implant diseases so as prompt treatment can be provided to prevent the destruction of supporting tissues of dental implant and subsequent failure.

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