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Assessing The Effect of Non-Surgical Periodontal Therapy on Levels of Caspase 3 And 7 in Patients With Chronic And Aggressive Periodontitis - A Systematic Review Protocol.

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Abstract--- *Background: Chronic periodontitis (CP) and Aggressive Periodontitis (AgP), are characterized by destruction of periodontal tissues in response to dental plaque. Both have localized and generalized presentations initiated by bacterial plaque, but they differ in the pattern of destruction; familial aggregation and the quantity of dental plaque. Apoptosis has been reported to be one of the mechanisms of tissue destruction in periodontitis, and a family of cysteine proteases, named caspases have been reported to mediate the process. Two mechanisms involved in apoptosis are intrinsic and caspase-dependent extrinsic pathways. These pathway results in the formation of a death-inducing signaling complex (DISC) which in turn causes activation of the executioner caspases-3,6&7 to start the process of cell-death or apoptosis. In subjects with chronic periodontitis, the probability of detection of Caspase-3 has been found to be greater in gingival crevicular fluid compared to serum and saliva. Additionally, detection of caspase-3&7 marked an early event prior to any overt morphological changes characteristic of apoptosis. Therefore, evaluation of Caspase levels may help in early detection of sites with periodontitis as well as probably determine the probability of an inactive site becoming active. Therefore, compositional changes in gingival crevicular fluid of site specific periodontal disease may help in correlating the level of disease activity and tissue destruction.Objectives: To assess the effect of non-surgical periodontal therapy on levels of caspase 3 and 7 in patients with chronic and aggressive periodontitis. Search Methods: We will search PUBMED and CENTRAL, EMBASE without language restrictions up to March 2019. We will also search for ongoing studies in trial registers, perform handsearching, check bibliographic references of relevant articles to seek potentially relevant research. We won't apply any restrictions on language, date, or publication status. Selection Criteria: We will*

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include randomised controlled (parallel-group or cross-over) trials, case control studies, cohort studies, case reports comparing the levels of caspase3 and 7 before and after SRP in patients with AgP and CP.

Keywords--- *Chronic periodontitis, Aggressive Periodontitis, Apoptosis, caspases*

I DATA COLLECTION AND ANALYSIS

Three review authors will independently assess studies for eligibility. Three review authors will then extract data and assess the risk of bias for individual studies using standard Cochrane methodology. The evidence will be assessed using GRADE and create 'Summary of findings' tables.

II INTRODUCTION:

II.I. Description of the condition:

Periodontitis or chronic inflammation of the tooth-supporting structures, results in destruction of periodontal tissues, ultimately leading to loss of teeth. A dysbiosis in the oral microflora causing a shift from normal commensals to a more pathogenic flora is linked to loss of homeostasis between the host response and bacterial challenge. The aberrant inflammatory response initiated by the presence of dental biofilm leads to tissue destruction. Therefore, the immuno-inflammatory responses of the host are thought to be at the heart of the periodontal pathogenesis.[1]

Two forms of Periodontitis that result in destruction of periodontal tissues have been well illustrated.[2]Chronic periodontitis (CP) is a form of periodontitis initiated by bacterial plaque and leads to periodontal tissue destruction. In contrast, Aggressive Periodontitis (AgP), another distinct form of periodontitis, also initiated by the presence of plaque biofilm, causes a more severe destruction of the periodontal tissues.[3]CP and AgP differ in the pattern of destruction seen; the destruction being more severe in AgP, familial aggregation seen in AgP and the quantity of dental plaque.

In addition to the direct bacterial mechanisms, indirect mechanisms involving cascades of cellular events activated by the bacterial products causing destruction of the host connective tissue and bone have been reported. Apoptosis has been reported to be one of the mechanisms of tissue destruction in periodontitis.[4]Periodontitis has been associated with several bacterial species such as the gram negative bacterium Porphyromonas gingivalis (Pg), which has been reported to cause damage to the gingival fibroblasts in severe type of periodontitis.[5] Similarly, apoptosis of gingival epithelial cells has been reported to be caused by Lipopolysaccharides (LPS) of Aggregatibacteractinomycetemcomitans (AaC) and apoptosis inducing activity was seen with Fusobacterium nucleatum (F. nucleatum).

A family of cysteine proteases, named Caspases (Casps) have been reported to mediate the process of Apoptosis. An intrinsic mechanism involving the mitochondria, and another mitochondrial-independent (extrinsic) mechanism is involved in apoptosis. The extrinsic pathways are induced by an external stimuli, whereby a death-inducing signalling complex (DISC) is formed. This complex activates the initiator Casp-8 which in turn causes activation of the executioner Casp-3 to start the process of cell-death or apoptosis. The intrinsic pathway involves activation of

Casp-9 through formation of an apoptosome. Activated Casp-9 induces apoptosis by enabling the proteolysis maturation of Casp-3, the executioner protease.

Clinical parameters and radiographic evaluation are commonly relied on to determine the history of periodontal disease; however they cannot determine the present status of the disease or indicate future sites of periodontal destruction.[6] Compositional changes have been reported in the fluid exuding from the gingival crevice, the 'Gingival Crevicular Fluid' (GCF),[6–8] in response to local disease activity. GCF has widely been utilized as an index, reflecting changes that take place, locally, in inflammation. Since most of the periodontal diseases are site specific,[8] a correlation of the measured biochemical markers present in the GCF with levels of periodontal disease activity and destruction of supporting tissues may provide information of the current destruction. Furthermore, there is a need to develop advanced diagnostic methods which would predict not only the presence of severe inflammation but also help in determining the potential sites with risk of conversion from inactive to active. [9]

III DESCRIPTION OF THE INTERVENTION

Apoptotic factors especially the executionary caspases – caspase 3 and caspase 7.

Casp-3 has been detected in cells of different lineages[10] and owing to its regulatory role in cleaving critical cellular substrates, it has been considered to be a main- player in actual destruction of the cell. Furthermore, an increased activation of Casp-3 has been demonstrated through immunoblot assays and substrate assays in gingival tissue sections from CP patients compared to no activation in the healthy ones.[11] Activated Casps in increased numbers were seen in the inflamed gingival epithelium and connective tissue, in contrast to relatively no Casp activation found in healthy tissues.[12] Furthermore, the single nucleotide polymorphisms involving CASP3 gene were found to be significantly associated with CP.[13] Interestingly, loss of attachment considered to be the hallmark of periodontitis has been associated with apoptosis of gingival fibroblasts.[14] Activation of Casp-3 was found to be associated with stimulation of host response in lytic lesions of the bone. This indicated that mechanism associated was through apoptosis of periosteal cells by the stimulated host-response.[15] Furthermore, the activation of Casp in the apoptotic process was considered to occur as an early event,[12] and hence may open up newer diagnostic possibilities for early detection of periodontitis sites. Therefore, the present study will be carried out to evaluate the changes in GCF levels of Casp-3 in patients with Aggressive Periodontitis and Chronic Periodontitis.

How the intervention might work

Gingival crevicular fluid (GCF) is an exudate present in the gingival sulcus and its compositional pattern has been employed as an index of local inflammation (Khalaf 2018). The levels of caspase-3, as an effector caspase, were found higher in the GCF of patients with CP.Detection of increased levels of caspase 3&7 may serve as an index of apoptosis taking place in periodontitis and therefore, may help in early detection of sites with periodontitis.Hence, this review is focused on the changes in the levels of caspase-3&7 following non-surgical periodontal therapy in subjects with chronic and aggressive periodontitis.

Why is it important to do this review

A considerable number of cells in the gingival epithelium and connective tissue revealed active caspases, whereas in healthy tissues almost no caspase activation was observed. These results, therefore, suggest that caspase activation may be functionally involved in periodontitis-associated tissue damage. Role of conventional treatment for periodontal diseases affecting the levels of caspases is not yet clear. Hence, the objective of this review will be to evaluate the levels of caspase3 and 7(executioner caspases) in GCF of patients with CP and AgP after non surgical periodontal therapy.

IV METHODOLOGY-

Criteria for considering studies for this review

Types of studies

We are planning to include randomised controlled trials (RCTs) with open or blinded assessment of outcomes. We will require full journal publication with the exception of extended abstracts of otherwise unpublished clinical trials. We will exclude short abstracts (usually meeting reports), non-randomised studies, studies of experimental pain, studies done on animal models, case reports, and clinical observational studies.

Types of participants

Systemically healthy patients aged 30- 50 years with clinical diagnosis of either chronic or aggressive periodontitis will be included irrespective of age, gender and race.

TYPES OF OUTCOME MEASURES

Primary outcomes

Primary outcome will comprise-

- Periodontal Pocket Depth (PPD):Distance measured from gingival margin to base of pocket.
- Clinical attachment loss (CAL): Distance from cemento-enamel junction to the base of pocket.
- Gingival Crevicular Fluid (GCF) Samples: Detection of caspase-3, caspase-7 in the collected sample.

Timing and effect measures

Mean difference with 95% confidence interval post therapy.

Secondary outcomes-

- Mean of Gingival Index (GI): An index for assessing the severity and quantity of gingival inflammation in individual patients or among subjects in large population irrespective of type of index
- Mean of Plaque Index (PI): An index for estimating the status of oral hygiene by measuring dental plaque that occurs in the areas adjacent to the gingival margin irrespective of type of index.

Timing and effect measures

Mean difference with 95% confidence interval post therapy.

Search methods for identification of studies

We will search PUBMED, CENTRAL, EMBASE database without language restrictions. We will use Medical subject headings (MeSH) or equivalent and textword terms. We will search the metaRegister of controlled trials (mRCT) (www.controlled-trials.com/mrct), National clinicaltrials.gov (www.clinicaltrials.gov). Additionally, we will check the reference lists of reviews, retrieved articles for additional studies, and performed citation searches on key articles.

Electronic searches-

MEDLINE (from inception till date)

CENTRAL(from inception till date)

www.ClinicalTrials.gov

Conference proceedings and abstracts

Other searches-

Bibliography of the relevant references

Manual searches of journals, conference abstracts and books

Contacting experts in the field

Data collection and analysis

Selection of studies-

Three review authors (VB,RO,VS) will independently screen the articles retrieved from the searches using the Rayyan online screening tool (18) and will determine the eligibility by reading the abstract of each study identified by the search. Review authors will eliminate studies that won't clearly satisfy inclusion criteria, and will obtain full copies of the remaining studies. Three review authors (RO,VS,SB) will screen full texts of these studies independently to select relevant studies. We will contact study authors by telephone or email, if there is missing information will hamper the study selection, to clarify the necessary information. In the event of disagreement, a fourth author will be adjudicated (SB). We will not anonymise the studies in any way before assessment. We won't apply any language restrictions in the selection of studies. We will include a PRISMA flow chart in the full review that shows the status of identified studies [(19)] as recommended in Part 2, Section 11.2.1 of the *CochraneHandbook for Systematic Reviews of Interventions* [(20)]We will include studies in the review irrespective of whether measured outcome data are reported.

Data extraction (selection and coding)

Three reviewers (RO, VS, SB) will extract data from included studies using a pre-defined data extraction form and will be presented in "Characteristics of Studies Table". Data will be extracted in terms of type of study, details of

participants, details of intervention, outcomes reported. Third reviewer (VB) will resolve the discrepancy amongst the primary reviewers. The discrepancy in risk of bias assessment will be resolved by fourth reviewer (MNK)

Risk of bias (quality) assessment

Three reviewers (VB, RO, VS) will independently assess the risk of bias (RoB) of each included study using the Cochrane domain based, two part tool as described in Chapter 8 of the Cochrane Handbook for Systematic Reviews of Interventions [21]The discrepancy among the primary reviewers will be resolved by fourth reviewer (MNK). We will assess the RoB under following domains.

- Sequence generation.
- Allocation concealment.
- Blinding of participants and personnel.
- Blinding of outcome assessment.
- Incomplete outcome data.
- Selective outcome reporting.
- Other bias, for example, baseline imbalance.

Strategy for data synthesis

We will do meta-analysis only if participants, interventions, comparisons and outcomes are sufficiently similar. We will use RevMan 2014, the statistical package provided by the Cochrane Collaboration for metaanalysis. We will undertake meta-analysis using a fixed-effect model only if the data is sufficient and homogeneous with comparable outcomes. If statistical heterogeneity (I^2 greater than, or equal to 70%) is detected, we intend to identify the sources of heterogeneity, will explore the cause of heterogeneity through subgroup analysis, and undertake subsequent meta-analysis using a random-effects model. When metaanalysis seems inappropriate, we will not pool the results of the included studies, but present a qualitative description of these studies with supporting tables.

Analysis of subgroups or subsets

We will take the subgroups on the basis of type and duration of the local drug agent given.

Unit of analysis issues

In parallel-group RCTs, we will consider an individual participant as the unit of analysis. When incorporating cross-over trials into a meta-analysis, we will follow the approach suggested by Elbourne [22] We will incorporate these trials by taking measurements from experimental intervention periods and measurements from control intervention periods and analysing these as if the trial are a parallel group trial of intervention versus control.

Dealing with missing data

If enough studies will be available; we will carry out an intention-to-treat analysis. We will ask for further information from the authors or manufacturers when published data were missing, incomplete or inconsistent with

RCT protocols. We will contact authors by email if studies will not report the outcome measures of interest, not describe randomisation or intention-to-treat analysis or had missing data.

Assessment of heterogeneity

We plan to assess clinical heterogeneity by using the Chi2 test (P value < 0.10 for statistical significance) and use the I2 statistic to quantify heterogeneity. We will regard heterogeneity as considerable if I2 was more than 75%; substantial if it is between 50% and 90%; moderate if it was between 30% and 60% and mild if less than 40%(23) If we identify statistical heterogeneity (I^2 greater than, or equal to 50%); we will report it and explore possible causes by prespecified subgroup analysis, and will apply a random-effects model.

Assessment of reporting biases

If there would be 10 or more included studies; we plan to conduct a funnel plot test for asymmetry to assess for any evidence of reporting bias.

Data synthesis

We plan to undertake a meta-analysis only if participants, interventions, comparisons and outcomes were judged to be sufficiently similar to ensure an answer that is clinically meaningful and relevant. For analysis, we planned to use RevMan 2014, statistical package provided by the Cochrane Collaboration. If statistical heterogeneity (I^2 greater than, or equal to 50%) will be detected, we will identifiy the sources of the heterogeneity and will perform subsequent meta-analysis using a random-effects model.

‘**Summary of findings’ tables**

We will include ’Summary of findings’ (SoF) tables as set out in the PaPaS author guide [(24)] and recommended in the *Cochrane* Handbook *for Systematic Reviews of Interventions* [(20)].We will present the SoF tables under the following comparisons for all of the primary outcomes:

- Mean of the indices [Plaque index (PI): An index for estimating the status of oral hygiene by measuring
- dental plaque that occurs in the areas adjacent to the gingival margin irrespective of type of index.
- Mean of Gingival index (GI): An index for assessing the severity and quantity of gingival inflammation in individual patients or among subjects in large population irrespective of type of index.
- Mean of relative vertical clinical attachment loss (RVCAL): Distance from cemento-enamel junction to the base of pocket measured using a fixed reference point.
- Mean of relative horizontal clinical attachment loss (RHCAL): Distance from cemento-enamel junction to the base of pocket measured using a fixed reference point.

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