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ESTIMATON OF NLRP3 INFLAMMASOME LEVELS IN GINGIVAL CREVICULAR FLUID AND SERUM OF HEALTHY AND PATIENTS WITH CHRONIC PERIODONTITIS

Dental Science

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ABSTRACT

Periodontal disease is defined as a plaque-induced inflammation of the periodontal tissues that results in a loss of support around the teeth. It is the leading cause of tooth loss among adults. This process is characterized by destruction of the periodontal attachment apparatus, loss of alveolar bone, apical migration of the epithelial attachment, and formation of periodontal pockets. Although the presence of periodontal pathogens is a prerequisite, the progression of periodontal disease is dependent on the host response to pathogenic bacteria that colonize the tooth surface. Processing of IL-1β and IL-18 is regulated by intracellular innate immune response system, known as NLRP3 (NALP3 or cryopyrin) inflammasome complex. NLRP3 belong to NOD- like receptor family, is a type of pattern
recognition receptor (PRR) which is activated by a number of microbial and host components such as proteoglycans, lipopolysaccharide, extracellular ATP, monosodium urate crystals. Aberrant activation of the innate immune system has been recognized to be an important mechanism of disease pathogenesis which involves formation of NLRP3 inflammasome that secretes pro-inflammatory cytokines such as IL-1β and IL-18 leading to periodontal tissue destruction. Hence the present study is conducted to estimate NLRP3 inflammasome level in GCF and serum of patients with healthy and patients with chronic periodontitis and to correlate with clinical periodontal parameters.

KEYWORDS:

INFLAMMASOME, PERIODONTITIS,IL-1β, INNATE IMMUNITY, ANOVA

INTRODUCTION

Periodontal disease is defined as a plaque-induced inflammation of the periodontal tissues that results in a loss of support around the teeth. It is the leading cause of tooth loss among adults. This process is characterized by destruction of the periodontal attachment apparatus, loss of alveolar bone, apical migration of the epithelial attachment, and formation of periodontal pockets[1]

The innate immune system is the first line of defense against invading pathogens. There are several families of receptors called pattern recognition receptors (PRRs) that recognize highly conserved pathogen associated molecular patterns (PAMPs) as well as host-derived danger signals called damage associated molecular patterns (DAMPs). There are several families of PRRs including Toll like receptors (TLRs), Nod-like receptors (NLRs), C- type lectin receptors (CLRs) and RIG like receptors (RLRs)[2].

Till date 22 NLR Family members have been identified in humans. They are cytosolic receptors, which detect microbial products or stress signals[3]. There are 4 inflammasome known till date involving NLRP1, NLRP3, NLRP4 and absent in melanoma (AIM2)[4]. The best characterized and most studied is the NLRP3 inflammasome. Once the inflammasome complexes have been activated they induce signaling cascades, which leads to the induction of a variety of pro-inflammatory cytokines.

Processing of IL-1β and IL-18 is regulated by intracellular innate immune response system, known as NLRP3 (NALP3 or cryopyrin) inflammasome complex which is activated by a number of microbial and host components such as proteoglycans, lipopolysaccharides, extracellular ATP, monosodium urate crystals[5].

Upon activation NLRP3 inflammasome complex is formed by interaction with adaptor molecule ASC (apoptosis associated speck-like protein containing a CARD) which then recruits pro-caspase-1 that results in generation of active caspase-1 molecule leading to maturation and secretion of IL-1β and IL-18[6]. IL-1β is important in periodontal diseases due to its potency in inhibiting bone formation and enhancing bone resorption stimulating the production of prostaglandin E2, collagenase, and proteinase.

In a study done by N. Bostanci et al, gingival tissues were collected from periodontal diseased patients and healthy patients. Quantitative real time PCR showed increased mRNA expression of NLRP3 inflammasome in periodontal diseased patients as compared to healthy patients[7].

So, with the current understanding of the etiopathogenesis and progression of periodontal disease , until now, there is no published data in the literature estimating NLRP3 inflammasome in gingival crevicular fluid.

Hence the present study is conducted to estimate NLRP3 inflammasome levels in GCF and serum of healthy and patients with chronic periodontitis and the possibility of using NLRP3 inflammasome as a biomarker.

METHODOLOGY:

The study population consisted of subjects belonging to both the sex and all subjects were selected from M.R. Ambedkar Dental College and Hospital, Bangalore, Karnataka, India and B.R.Ambedkar Medical College and Hospital, Bangalore, Karnataka, India. Approval from the Ethical Committee of M.R. Ambedkar Dental College and Hospital was obtained. The nature and purpose of the study was explained to the individuals and written consent was taken from them. Out of 70 subjects, 60 agreed to participate in the study and 30 subjects met the inclusion criteria.

The study population consisted of a total of 30 subjects who were divided into two groups, 15 each group.

Group I: 15 patients - 30 samples (15 GCF and 15 Serum samples) periodontally healthy patients

Group II: 15 patients -30 samples (15 GCF and 15 Serum samples) patients with chronic periodontitis.

All patients underwent full mouth periodontal examination that

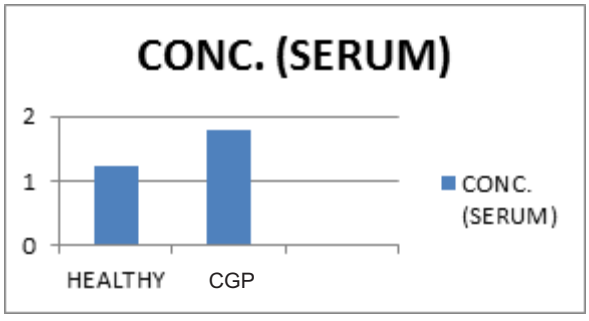
included bleeding index (BI)(Ainamo and Bay), plaque index (Silness and Loe), probing depth (PD) and clinical attachment level (CAL). PD and CAL were recorded at six sites per tooth using UNC-15 graduated probe.

Volumetric micro-pipettes were placed intra-crevicularly at the entrance of the gingival crevice and standardized volume of 3µl GCF samples were collected from each patient. The samples were transferred from the pipettes into eppendorf tubes and immediately stored at -80°C. 2ml of blood was drawn from the median cubital vein from cubital fossa using venipuncture technique. Collected blood was centrifuged at 1000 rpm for 10 minutes to obtain serum that was stored frozen at -80°C, until analyzed for NLRP3 inflammasome with commercially available ELISA kit.

RESULTS

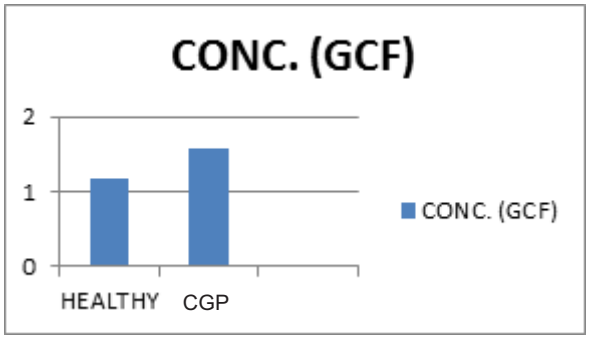
When comparing the Mean concentration of NLRP3 inflammasome in serum of 2 groups, the ANOVA p value of periodontitis subjects was significantly higher than healthy group (p<0.001). In healthy it is 1.24±0.07 ng/ml and in periodontitis it is 1.78±0.16 ng/ml

Figure 1: MEAN COMPARISON OF NLRP3 INFLAMMASOME CONCENTRATION (GCF)



When comparing the Mean concentration of NLRP3 inflammasome in GCF of 2 groups, the periodontitis group has higher mean concentration than healthy group (P<0.001). In healthy it is 1.18±0.07 ng/ml and in periodontitis it is 1.59±0.12 ng/ml

FIGURE 8: MEAN COMPARISON OF NLRP3 INFLAMMASOME CONCENTRATION (GCF)



Multiple linear regression analysis was done in the group I (healthy group), Overall the model was statistically significant with p value <0.05.

Multiple R	0.987
R-squared	0.975
Adjusted R-squared	0.961
F-TEST	71.196
p-value	<0.05

Multiple linear regression analysis was done in the group II (periodontitis group), Overall the model was highly statistically significant for both PD and CAL with p value <0.0001.

Multiple R	0.985
R-squared	0.971
Adjusted R-squared	0.955
F-TEST	60.655
p-value	<0.001

DISCUSSION

Periodontitis is a complex disease in which disease expression involves intricate interaction of microorganism with host immune-inflammatory response and subsequent alteration in bone and connective tissue homeostasis. It is now understood that immune and inflammatory responses are critical to pathogenesis of periodontitis and are shaped by a number of host related factors, both intrinsic and extrinsic. If left untreated periodontitis results in soft tissue and progressive bone destruction and leads to tooth loss[1].

The innate immune system is critical in the initial defense against pathogenic microorganisms. As the first line of host defense, the innate immune system relies heavily on the presence of evolutionarily conserved pattern recognition receptors(PRR), the best characterized of which include membrane-bound Toll-like receptors(TLR), retinoid- inducible gene 1-like receptors (RLR), C-type lectin receptors (CLR) and nucleotide-binding-domain-like receptors(NLR), to recognize various pathogens or their components[8,9].

Activation of PRRs initiates signaling cascades that result in formation of large multiprotein signaling platforms, so-called inflammasomes. The term 'inflammasome' was coined by the late Jurg Tschopp and his research team in 2002. Inflammasomes are nucleotide-binding-domain-like receptors, a family of cytosolic pattern recognition receptors containing multiprotein complexes functioning as a molecular platform which trigger the maturation and secretion of pro-inflammatory cytokines, such as interleukin-1 and interleukin-18 which cause a wide variety of biological effects associated with infection, inflammation and autoimmune processes[10].

In this present study 70 subjects were screened, 60 agreed to participate in that 30 subjects met the criteria and study population was categorized as periodontally healthy group (n=15) and periodontitis group (n=15). Periodontal parameters like plaque index (PI), bleeding index (BI), probing depth (PD), clinical attachment level (CAL), were recorded. Serum samples were collected as it is an effective and sensitive method to quantify the change in body. GCF samples were collected as GCF possess a great potential for serving as an indicators of periodontal disease and collection of GCF is a non-invasive and simple procedure.

Mean concentration of NLRP3 inflammasome in both GCF and serum was significantly higher periodontitis group as compared to healthy group at p<0.0001. Evaluation of the result obtained from this study, can be suggested that increased inflammation and periodontal destruction has shown to increase the NLRP3 inflammasome levels which is in accordance with the study by N. Bostanci et al which showed increased mRNA expression of NLRP3 inflammasome in periodontal disease patient as compared to healthy patient using PCR technique.

Thus it could be stated from the present study that NLRP3 inflammasome levels might be considered as potential markers for periodontal disease. Other reported studies have taken either serum or gingival tissue samples and have used PCR technique to estimate the levels of NLRP3 inflammasome. Our study is the first to estimate the levels of NLRP3 inflammasome in GCF using ELISA method.

Further longitudinal studies with larger sample size and interventional studies are needed to study the role of NLRP3 inflammasome in periodontal disease progression and use it as "novel biomarker" using GCF.

CONCLUSIONS

The present study was conducted to estimate GCF and serum levels of NLRP3 inflammasome in healthy and patients with chronic periodontitis and to correlate its level with clinical periodontal parameters. Results of our study suggested that the mean NLRP3 inflammasome levels of both GCF and serum was more in periodontitis patients compared to healthy group.

In addition, there was a positive correlation between NLRP3 inflammasome levels of GCF and serum with clinical periodontal parameters which indicates that with increase in the clinical periodontal parameters there is increase in the GCF and serum levels of NLRP3 inflammasome.

In conclusion the present study throws light on the emerging role of NLRP3 inflammasome in chronic periodontitis which will pave way for future host modulation therapy. However further case control interventional studies with larger sample size are required.

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