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Effect of Alox-15 Polymorphism on GCF Levels of Lipoxin-A4 in Chronic Periodontitis: A Preliminary Study

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Lipoxins play an important role in periodontal resolution, hence, investigation of genetic polymorphism of lipoxin gene may provide important information on the role of lipoxins in periodontal disease pathogenesis. The aim of this study was to investigate a polymorphism of C-to-T substitution at position c.-292 in ALOX15 (reticulocyte-type 15 lipoxygenase 1) gene in patients with chronic periodontitis and to associate the polymorphism with gingival crevicular fluid (GCF) lipoxin A4 (LXA4) levels. Forty-five chronic periodontitis and 45 periodontally healthy patients were included in this case-control study. Plaque index, calculus index, sulcus bleeding index, full mouth probing depth (PD) and clinical attachment loss (CAL) were recorded. GCF and blood samples were collected. GCF was analyzed for LXA4 levels by enzyme linked immunosorbant assay. Genotyping of ALOX15 polymorphism was studied using PCR. Mean LXA4 was lower in periodontitis group compared to the periodontally healthy group. There was a negative correlation between CAL and LXA4. The CC genotype was higher in the study group than in the control group. In the study group, mean CAL was significantly lower among individuals with the CT genotype. Mean LXA4 was significantly lower in CC genotype (45.0±7.11 ng/mL) compared to CT genotype (50.81±5.81 ng/mL) among the patients with periodontitis. The results suggest that LXA4 and c.-292T allele are associated with periodontal health. Polymorphisms in the ALOX15 gene may influence periodontal disease pathogenesis. Hence, investigation of such polymorphisms could benefit the evaluation of lipoxins role in periodontal disease.

Key Words: lipoxins, resolution, periodontal disease, clinical attachment loss, polymorphism.

Introduction

Chronic periodontitis is the most commonly occurring form of periodontitis. It is initiated by microbial biofilm resulting in immune-inflammatory responses. The interaction between the periodontopathic bacteria and host immune response results in degradation of the periodontal tissues. There is a complex network of anti and pro-inflammatory cytokines, prostaglandins, reactive oxygen species and proteolytic enzymes involved in the immune-inflammatory responses (1).

Endogenous stop signals of inflammation and induction of resolution are necessary since the uncontrolled process of periodontal inflammation may cause tissue destruction. The endogenous anti-inflammatory mechanism that protects against excessive tissue injury and promotes the restoration of tissue structure and function comprises resolution of inflammation. Recent advances in the area of periodontal disease pathogenesis have suggested that the progression of periodontal disease is due to failure of resolutory pathways in the periodontal tissues. This has led to investigations on the role of pro-resolving molecules like lipoxins, resolvins, protectins and maresins on periodontal inflammation (2). One of the main functions of lipoxins is to inhibit PMNs recruitment, chemotaxis and transmigration across the endothelium, which are hallmarks of the anti-inflammatory process (3). A ground-breaking work by Serhan et al. (4) suggested that lipoxins have a protective role on periodontal inflammation and alveolar bone resorption.

The transcriptional activity and functions of lipoxins is controlled by ALOX15 (reticulocyte-type 15 lipoxygenase 1) gene. ALOX15 gene is located on chromosome 17p 13.3. Genetic polymorphism located in promoter region of this gene has shown to influence production of lipoxins and its associated enzymes. A functional single-nucleotide polymorphism (SNP) resulting from C-to-T substitution at position c.-292, can cause higher transcription of the gene in macrophages (5). This may lead to an increase in the production of ALOX15 mediated lipid metabolites, which play a role in inflammation.

The investigation of genetic polymorphism that affects the transcriptional activity of lipoxins may provide important information on the function of lipoxins in periodontal disease pathogenesis. To the best of the authors' knowledge there is no study investigating the

effect of lipoxins or lipoxin gene polymorphism in chronic patients with periodontitis. Since among the various forms of lipoxins, lipoxin-A4 (LXA4) has been extensively studied, this study was designed to investigate the role of ALOX15 polymorphism (C-to-T substitution at position c.-292) and LXA4 in gingival crevicular fluid (GCF) in chronic patients with periodontitis.

Material and Methods

Study Population

The study population was chosen among male and female patients from outpatient department at M. R. Ambedkar Dental College and Hospital, Bangalore from June 2013 to December 2013. The patients were enrolled after obtaining ethical clearance from the institutional review board. Two hundred patients were screened and stratified into either periodontally healthy (control) group or periodontally diseased (study) group after they satisfied the exclusion and inclusion criteria. Written informed consent was obtained from the patients who agreed to participate in the study.

Exclusion criteria were patients who had any periodontal treatment in past 2 years; history of diabetes; patients with any of the systemic diseases known to have an impact on periodontal diseases; patients on any anti-inflammatory drugs or antibiotics treatment; patients with habit of tobacco use present or past (smoking or chewing); pregnant and lactating females.

Inclusion criteria for the study group were: patients with chronic periodontitis; age ≥35 years; presence of ≥8 teeth having ≥5 mm of periodontal clinical attachment loss; at least three affected teeth other than first molars (6).

Inclusion criteria for control group were: age ≥35 years; clinically healthy periodontium (presence of ≤5% sites with bleeding on probing); sulcus depth up to 3 mm; no or incipient attachment loss (≤2 mm) (6).

Measurement of Periodontal Status

After the demographic factors had been recorded, the following periodontal parameters were measured: plaque index (7); calculus index (8); sulcus bleeding index (9); full mouth probing depth recorded at six sites per tooth using a UNC-15 probe (University of North Carolina); full mouth clinical attachment loss with cementoenamel junction as the reference recorded at six sites per tooth using UNC-15 probe. The examiner recorded all the periodontal parameters on two occasions 24 h apart from each other the and correlation coefficient was calculated. The calibration process was repeated until the examiner had substantial correlation as measured by Cohen's kappa. Kappa coefficient for intra-examiner agreement was in the range of 0.721-0.773.

Collection of GCF

GCF samples were collected a day after the probing depth was recorded. GCF was collected from the deepest probing site after removal of supra-gingival plaque. The site of collection was isolated to prevent contamination of saliva. A 5-µL sample of GCF was collected by placing micro-capillary pipettes (Sigma Chemical Company, St Louis, MO, USA) at the entrance of gingival crevice. The pipettes were placed at the entrance of the sulcus and there was no stimulation. The samples of GCF were stored in plastic vials at -70^0 C until analyzed for LXA4 concentrations.

Method of LXA4 Estimation

The levels of LXA4 were estimated using a commercially available ELISA kit (Neogen^{TM} Corporation, Lansing, MI, USA) in accordance with the manufacturer's instructions. The GCF samples were diluted using phosphate buffered saline to make up to the required volume as per the manufacturer's requirement. The dilution factor was recorded. The diluted GCF samples were then placed in duplicate in two wells. Buffered saline was used as blank in one well and the standards were according to the manufacturer's guidelines. The optical densities as recorded by ELISA reader were converted into concentration using the standard curve. The values were then multiplied by the dilution factor to obtain concentration in the given sample. Two values were obtained for each sample and average was the final measured concentration.

Blood Sampling and DNA Extraction Procedures

Two milliliters of peripheral blood samples were collected by veni-puncture of anticubital vein in EDTA tubes after aseptic precautions. DNA extraction was done by solution based method using the columns, reagents and protocol from Chromous Biotech^{TM}

Designing of the Primer

The nucleotide sequence of ALOX-15 gene in Homosapiens was located on NCBI site (http://www.ncbi.nlm.nih.gov/nuccore/AY505111). The gene sequence was identified with Genebank n^o AY5051111. The link to 'pick primers' on the same page was selected. The software requires parameters like region to be amplified (Range), primer melting temperature, and PCR product size. The parameters that amplify only -292C promoter region with forward primer were selected. Among several primer pairs, the primer pair that amplifies -292C promoter by last nucleotide of forward primer was selected. The forward primer (F1) was TGCTGTACCAGGCGTTGATTC and the reverse primer was TGTTGGAACCGGCATAGAGC Mismatch forward primer (F2) was TGCTGTACCAGGCGTTGATTT, which was designed by replacing last nucleotide 'C' with 'T' in F1.

Genotyping of ALOX15

Genotyping for the ALOX15 polymorphism was performed using a PCR fragment amplified by the forward primer TGCTGTACCAGGCGTTGATTC and the reverse primer TGTTGGAACCGGCATAGAGC. The forward primer contained one nucleotide mismatch and hence the PCR amplification was conducted for every sample using both F1 and F2 primers. Conditions for genotyping were: PCR in a 20 mL reaction mixture containing 1 mL genomic DNA (extracted DNA), 1 mL of the primer (F1 or F2), 1 mL reverse primer, 0.5 mL Taq polymerase enzyme, 2.5 mL of Taq polymerase Buffer, 2 mL of dNTP mixture and 12 mL deionized water. This mixture was prepared and aliquoted into each tube. The tubes were placed in the thermal cycler and DNA amplification was carried out using standard conditions. The reaction mixtures were incubated for 5 min at 95 °C to cause initial denaturation, followed by 35 cycles of denaturation at 95 °C for 45 s, annealing for 45 s (at 56 °C for F1 and at 54 °C for F2) and extension at 72 °C for 45 s. The incubation continued for 5 min at 72 °C for final extension. PCR amplified products were subjected to electrophoresis through 2% agarose gel containing 1x TAE, 20 mL of each amplified product was mixed with 5 mL of bromophenol blue loading dye. Electrophoresis was performed at 25 V for 2 h. The gel was visualized under UV light illuminator after staining with ethidium bromide.

Statistical Analysis

A piori power analysis was carried out to calculate sample size. Sample size was determined as a function of power and level of significance. The sample was estimated to be 28 in each group for an 80% statistical power and 5% level of significance. Descriptive statistical analysis was carried out in the present study. Results on continuous measurements are presented as mean ± SD and categorical measurements are presented in number (%). Student's t test was used to find the significance of study parameters on a continuous scale between the two groups. Pearson's correlation was used to find the relationship between concentration of GCF markers and periodontal parameters. Multiple regression analysis was conducted to evaluate the effect of various periodontal parameters, and GCF-LXA4 and ALOX15 polymorphism on CAL.

Results

This was a case-control study with 21 females and 24 males in the study group (n=45) and 27 females and 18 males in the control group (n=45). The flow chart in Figure 1 depicts the screening and selection of patients. The demographic factor distribution of the two groups is shown in Table 1. There was no significant difference between the mean age and gender distribution between the study and control groups. Table 2 shows the means of various periodontal parameters between the study and control group. All the periodontal parameters were significantly higher in the study group.

Four samples of study group and five samples of control group did not have a measurable amount of LXA4. Means were calculated for 41 samples in study group and 40 samples in control group. Mean LXA4 in study group was 47.79±12.69 ng/mL and 53.14±6.89 ng/mL in control group. Mean LXA4 was higher in the control group and the difference was statistically significant. Correlation analysis was conducted to evaluate the association between the clinical periodontal parameters and LXA4 (Table 3). The results showed that there was a negative correlation between GCF-LXA4 and all the periodontal parameters, i.e., with increase in the values of periodontal parameters there was decrease in the values of LXA4.

Table 4 shows the genotype distribution allele frequencies, and allele carriage between the study and control groups. The frequencies of the ALOX15, c.-292C/T genotypes in the study and control groups were found to be in agreement with the Hardy-Weinberg equilibrium ($p>0.05$, $\chi^2<3.84$).

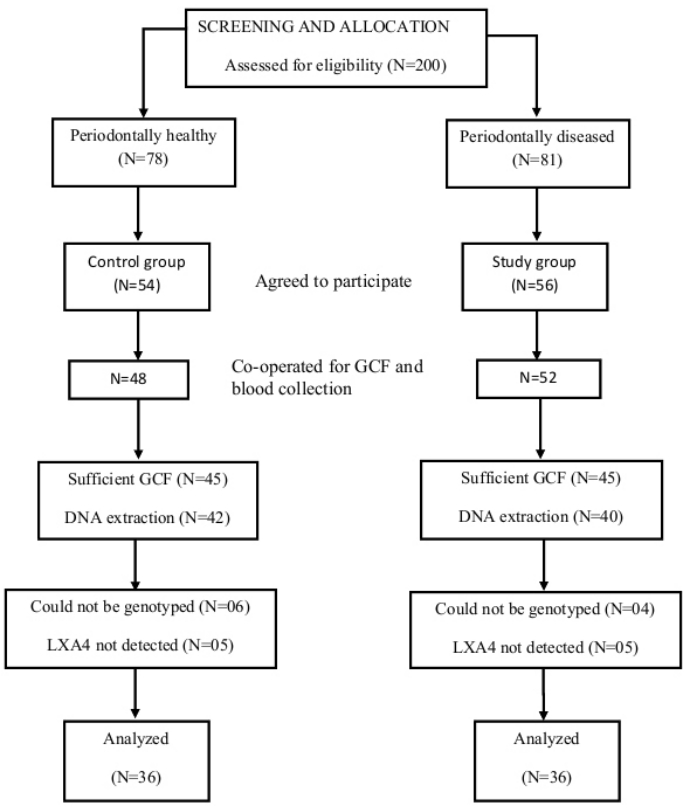

Figure 1. Flow chart showing selection of patients.

Distribution of the CC, CT and TT genotypes in the control and study groups are significantly different. 64% of patients with periodontitis and 36% of healthy patients were homozygous for genotype CC. The genotype CT was present in 58% patients in healthy group and 22% patients in periodontitis group. The genotype CC was higher in study group whereas genotype CT was higher in control group. The analysis did not reveal any significant influence of gender on genotype distribution and allele frequency. The power for the difference detected in genotype distribution among the two groups was 66.1% for CC and 89.5% for CT and TT. The occurrence of -292C allele carriage was similar in the study and control groups but the occurrence of -292T carriage was significantly higher in the control group (63.88%) compared to study group (36.1%). The results showed that -292C allele frequency was higher in the study group whereas in the control group -292T allele frequency was higher.

In order to investigate whether carriage of the C/T alleles is associated with periodontal parameters, differences in various periodontal parameters and GCF-LXA4 were compared for different genotypes in the periodontitis group. Patients were classified according to genotype CC, CT and TT. Table 5 shows the means of periodontal parameters and GCF-LXA4 in the three genotype groups. For an easier comparison, the genotypes were divided as high risk and low risk groups and then the parameters were compared. Table 6 shows the means for the high risk and low risk groups. Mean GCF-LXA4 was significantly (p=0.05) lower in high risk group (45.0±7.11 ng/mL) compared to low risk group (50.81±5.81 ng/mL). Power analysis showed that the power for the difference in mean LXA4 levels among the low risk and high risk group was 51%. Mean CAL was 5.45±0.57 mm in the CC group and 4.98±0.76 in the CT and TT group. Mean CAL was significantly higher in the high-risk group (p=0.03). Means of BI and PD were also higher in the CC group but the difference was not statistically significant.

Multiple regression analysis was used to evaluate the effect of the CC genotype, GCF-LXA4, CI, BI, and PD on CAL. Table 7 shows the multiple regression analysis parameters in the study group. All the predictors had a significant effect on CAL as interpreted by the elasticity. The effects of CI, PI, and BI were not significant, but the effect of PD, GCF-LXA4 and CC genotype was statistically significant as implied by the β coefficient value. Adjusted R squared value was 0.507, which suggests that 50% of change in CAL is explained by the predictors. The F test value is statistically significant (p=0.001) implying that there is a significant relationship between the set of predictors and CAL.

Discussion

Chronic periodontitis is one of the most prevalent diseases affecting mankind. Though various risk factors have been implicated for the manifestation and progression of periodontitis, the selective susceptibility of individuals for

Table 1. Demographic factors: mean±SD for age and gender

	Study group N=36	Control group N=36	p value
Age	45.92 .87	42.72±8.15	0.09
Gender	F=17, M=19	F=19, M=17	0.12

Table 2. Results for periodontal parameters expressed as mean±SD

	Study group N=36	Control group N=36	p value
Calculus index	1.77±0.39	0.50±0.23	<0.001*
Plaque index	1.53±0.39	0.55±0.21	<0.001*
Bleeding index	92.20±14.86	3.54±1.32	<0.001*
Probing depth (mm)	4.71±0.99	1.87±0.24	<0.001*
Clinical attachment level (mm)	5.33±0.61	0.65±0.46	<0.001*
Lipoxin-A4 (ng/ml)	47.16±13.34	52.28±7.09	0.01*

*statistically significant.

Table 3. Correlations between periodontal parameters and LXA4

Correlations	Healthy		Periodontitis	
	r value	p value	r value	p value
LXA4:BI	-0.006	0.48	-0.043	0.41
LXA-4: PD	-0.052	0.38	-0.067	0.35
LXA-4: CAL	-0.008	0.48	-0.036	0.42

Table 4. Genotype distribution in the study and control group

	Control group N (%)	Study group N (%)	p value
Genotype			
CC	13 (36.12)	23 (63.90)	0.039*
CT	21 (58.34)	08 (22.20)	0.025*
TT	02 (5.54)	05 (13.90)	0.058*
Allele carriage			
T	23 (63.88)	12 (36.10)	0.045*
C	31 (94.46)	31 (86.10)	0.071
Allele frequency			
C	47 (65.29)	54 (75.00)	0.062
T	25 (34.71)	16 (25.00)	0.054

*statistically significant.

periodontitis has remained an enigma for the researchers. Genetic factors are a new addition to the list of risk factors for periodontitis (10,11). As the immune system plays a crucial role in the pathogenesis of periodontitis, the focus has been on identification of genetic polymorphisms in several aspects of periodontitis immune-regulation. The present study investigated ALOX15 c.-292C/T polymorphism in patients affected with chronic periodontitis among the Indian population. The association between this polymorphism and GCF-LXA4 levels was also evaluated.

ALOX15 gene encodes 12/15 LOX (lipoxygenase) and is highly expressed in macrophages and fibroblasts. 12/15-LOX is a key enzyme involved in the formation of lipoxins, protectins and resolvins, which demonstrated anti-inflammatory and/or pro-resolving bio-actions (12). ALOX15 is the reticulocyte-type 15 lipoxygenase1, a non-heme iron-containing dioxygenase that inserts molecular oxygen into polyunsaturated fatty acids to produce lipoxins. Lipoxins are known to antagonize pro-inflammatory effects and provide mechanisms for resolution of inflammation (13).

Resolution of periodontal inflammation has been in the forefront of periodontal research in the recent years. There is ample evidence to suggest that the destruction of tissues in chronic inflammatory periodontal disease is due to failure of resolution pathways to restore homeostasis (2). The process of resolution is orchestrated by various endogenous lipid mediators namely lipoxins, resolvins, protectins and maresins. Lipoxins were the first identified endogenous anti-inflammatory lipid mediators, which function as "braking signals" for neutrophils or chalones in inflammation (3). Though the role of lipoxins in other systemic diseases has been extensively studied, their role in pathogenesis of periodontal disease is yet to be explored. Currently, there are no studies evaluating the association of lipoxins and/or lipoxin gene polymorphism with chronic periodontitis.

This study included a study group comprised by periodontally diseased patients and a control group comprising periodontically healthy patients. According to Garlet et al., it is suggested to include patients with gingivitis as controls because the inclusion of periodontally healthy subjects does not allow the determination of the 'susceptible' or 'resistant' nature of the included patients

Table 5. Comparison of periodontal parameters among different genotypes in the periodontitis group

	CC	CT	TT	p value (ANOVA)
LXA4	45.00±7.11	51.04±4.06	50.58±5.21	0.041*
BI	93.35±12.57	87.56±8.34	89.40±9.26	0.43
PD	4.71±0.93	4.32±1.08	4.58±1.95	0.705
CAL	5.45±0.57	4.81±0.52	5.05±0.86	0.036*

*statistically significant.

Table 6. Comparison of periodontal parameters in high risk and low risk patients in the periodontitis group

	High risk of periodontitis (CC)	Low risk of periodontitis (CT and TT)	p value
LXA4	45.00±7.11	50.81±5.81	0.05*
BI	93.35±12.57	88.48±9.74	0.08
PD	4.71±0.93	4.45±1.18	0.26
CAL	5.45±0.57	4.98±0.76	0.03*

*statistically significant.

Table 7. Multiple regression analysis in periodontitis group (CAL as predictor)

Variable	Elasticity	S.E	p-value	β	S.E.	p-value	95% CI for β
AGE	0.115	0.116	0	0.148	0.150	0.166	-0.159 to 0.455
CI	0.012	0.109	0	0.023	0.211	0.455	-0.409 to 0.455
PI	0.111	0.072	0	0.250	0.162	0.069	-0.081 to 0.581
BI	0.084	0.169	0	0.118	0.238	0.311	-0.369 to 0.605
PD	0.287	0.096	0	0.508	0.170	0.003*	0.159 to 0.856
GCF-LXA4	-0.118	0.055	0	-0.299	0.138	0.021*	-0.581 to 0.016
GENOTYPE CC	0.065	0.028	0	0.324	0.142	0.016*	0.033 to 0.614
Constant	0.329	0.137	0	0	0	0.5	
Regression statistics	R-squared		0.638	F-TEST		4.864	
	Adjusted R-squared		0.507	p-value (F test)		0.001*	

*statistically significant.

(14). Since there is evidence to suggest that all cases of gingivitis do not progress to periodontitis, the present study has included periodontally healthy patients as controls (15). Periodontal status was recorded along with estimation of GCF-LXA4 concentration and analysis of ALOX15 c.-292C>T polymorphism. Patients were selected according to guidelines to rule out the confounding effects. Patients chosen to the study had not taken antibiotics, immune-modulatory or anti-inflammatory drugs for six months. Also not included in the study were those individuals who were smokers, or who had any systemic disease and patients who have received any periodontal treatment in past two years.

There was no significant difference in the age and gender distribution between the two groups suggesting age and gender matching. Means of various periodontal parameters were significantly higher in the study group. Further, evaluating the relationship between periodontal disease status and LXA4, the results of this study suggested that the mean GCF-LXA4 concentration was significantly lower in the periodontitis group as compared to periodontally healthy group. Also there was a negative correlation between LXA4 and bleeding index. The association between periodontal disease severity and LXA4 was evaluated by correlation of LXA4 with PD and CAL and the results revealed that there was a negative correlation of LXA4 with PD and CAL.

LXA4 is a pro-resolving and anti-inflammatory mediator and there is sufficient evidence to implicate the role of lipoxins in the resolution of periodontal disease (2). The results of the present study are also suggestive of a protective role of lipoxins in periodontal inflammation. Though at present there are no studies reporting the association between chronic periodontitis and LXA4; the role of LXA4 has been investigated in localized aggressive periodontitis (16). Neutrophils from localized aggressive patients with periodontitis generated LXA4, suggesting a role for this molecule in periodontal disease. The administration of lipoxins potently blocked neutrophil transport and lowered PGE2 levels within exudates when *Porphyromonas gingivalis* was introduced in murine dorsal air pouches.

Another study aimed at investigating the effect of LXA_4 on activation of neutrophils demonstrated that generation of reactive oxygen species stimulated by Porphyromonas gingivalis was inhibited by LXA4. The results suggest that a supplement of exogenous LXA4 could facilitate anti-inflammatory actions that can decelerate progression of chronic inflammatory disorders, such as periodontitis and atherosclerosis (17).

LXA4 decreases production of MMP-3 and increases TIMP production (13). Lipoxin A4 inhibits the expression of IL-1β, IL-6, and TNF-α meanwhile it improves the expression of IL-10 (18). LXA4 receptor expression enhances fibroblast proliferation, migration and wound closure. Collagen type I and III deposition were also enhanced by LXA4 (19). All these facts concur with the notion that LXA4 is anti-inflammatory and pro-resolutory in action.

Alveolar bone resorption is the hallmark of periodontal disease. In an *in-vitro* calvarial bone model it was shown that LXA4 had an inhibitory effect on bone resorption in a dose-dependent manner (20). When LXA4 generation was blocked with 15-LO short interfering RNA, the pro-inflammatory cytokines were elevated and bone resorption was accelerated. The receptor activator of nuclear factor kB-ligand (RANKL): osteoprotegerin (OPG) ratio is considered an important marker for periodontal disease destruction. Application of LXA4 in surgically created defects in mandible showed increase in the number of cells expressing both OPG and RANKL (21). Vandyke et al. study showed that bone degradation and tissue injury in periodontitis was prevented by topical administration of the lipoxin stable analog (4). The above-mentioned studies suggest that lipoxins and their associated enzymes have a protective role in periodontal inflammation and on alveolar bone resorption.

To investigate whether carriage of the C/T alleles in ALOX15 is associated with periodontal disease status, genotype distribution, allele frequency and allele carriage of ALOX15, c.-292C>T gene polymorphism was compared between the study and control groups. The CC genotype was higher in study group whereas genotype CT was higher in the control group. The results also showed that -292C allele frequency was higher in the study group whereas in the control group -292T allele frequency was higher. Mean CAL was compared between different genotypes in the periodontitis group to assess whether carriage of the C/T alleles is associated with periodontal disease severity. Mean CAL was significantly lower in the CT group. From these findings it may be hypothesized that ALOX15 c.-292T allele is protective for periodontal inflammation.

To date there are no studies evaluating the association between ALOX-15 c.-292C>T gene polymorphism and chronic periodontitis. Lipoxins have a wide range of actions on myeloid cells (22) and since myeloid cells play an important role in periodontal disease pathogenesis, the investigation of genetic polymorphism that affects transcriptional activity of myeloid cells may provide information on its role in periodontal disease pathogenesis. The findings of a molecular level study suggested that the up-regulation of ALOX15 c.-292T allele in human myeloid cells may attenuate inflammation and ameliorate inflammatory disease (5). The polymorphism C-to-T substitution at position c._292 leads to higher ALOX15 mRNA levels in macrophages. CT heterozygous carriers had

three times higher ALOX15 mRNA levels in macrophages than CC homozygous carriers. This implies that c.292T allele in ALOX promoter results in higher transcription of the gene, which may lead to an increase in ALOX15-mediated lipid metabolites.

Another fact that was deduced from the findings of this study is that mean GCF-LXA4 was significantly lower in CC genotypes as compared to CT genotypes in the patients with periodontitis. Since ALOX15 is a key enzyme to synthesize LXA4 (13), any alteration in the gene encoding ALOX15 should affect the levels of LXA4. Difference between GCF-LXA4 in different genotypes suggests that carriage of the C/T alleles affects the levels of LXA4 in the periodontal milieu. This finding of the study also concurs with the findings of the above mentioned molecular study which proved that c.-292T allele results in higher ALOX15 mRNA levels. There are no studies investigating the role of ALOX15 polymorphism on LXA4 levels, however the anti-inflammatory role of ALOX15 gene has been extensively studied in animals.

Transgenic and knock out gene animal models provide a powerful approach for studying gene expression and regulation and allows evaluating the cause and effect association of genes in pathological processes. It was found that inflammation associated with excessive PMN recruitment to the periodontium was sharply diminished in TG rabbits overexpressing 15-LO (4). Prolonged 15-LO overexpression enhanced lipoxin generation, thereby reducing local inflammatory response *in vivo*. The study indicated that overexpression of the arachidonate 15-LO type I in TG rabbits lead to enhanced endogenous anti-inflammation. Isolated PMNs from 15-LO TG rabbits were approximately two to four times greater LXA4 generation.

The notion that 15-LOX is a protective pathway in inflammatory responses is supported by studies that demonstrate that over expression of ALOX15 provides functional protection against immune-mediated injury. Transgenic rabbits which selectively express ALOX15 in macrophages and PMNs demonstrated functional protection against neutrophil-mediated tissue injury and microbial inflammation (23). In another study, ALOX15-deficient mice showed delayed wound healing which was associated with a significant decrease in endogenous LXA4 formation (24). There is evidence to support a link between 12/15 LOX and expression of various inflammatory cytokines (25,26). The above-mentioned findings suggest that ALOX15 gene could be a potential area for research on role of resolution in periodontal disease pathogenesis.

Polymorphisms in the ALOX15 gene that alter transcription of inflammatory proteins may influence periodontal disease pathogenesis. Such polymorphisms could be investigated for association with manifestation, severity and progression of periodontal disease. In summary, the results of this study demonstrate that resident LXA4 circuits play an important role in periodontal inflammation and resolution. However, the study has a few limitations and hence the results should be cautiously interpreted. Indeed lipoxins have an important role in resolution of periodontal inflammation but the role of other endogenous molecules cannot be overlooked. Release of other products of arachidonic acid metabolism like 12-(S)-HpETE and 12(S)-HETE, which have pro-inflammatory activities, is also controlled by ALOX15 gene expression. This study has estimated only one molecule from a complex network of different molecules. Also the sample size is small for conclusive remarks. The role of epigenetics in pathogenesis of periodontal diseases is an emerging concept. This study did not consider the role of epigenetics. Studies with genetic deletion of LXA4 biosynthetic pathways or amplification with exogenous LXA4 may provide better evidence that this protective circuit controls key regulators of the periodontal inflammatory response. In view of the prominent expression of 15-LOX and the ALOX receptors in macrophages, which are the chief cells in determining the immune response in periodontal diseases, this hypothesis warrants further studies.

This study demonstrated that ALOX15 gene polymorphism is associated with susceptibility to chronic periodontitis in Indian population and this polymorphism alters the levels of LXA4 in GCF. Since the polymorphism has an effect on the release of lipoxins and lipoxins play an important role in periodontal resolution, different genotypes of the lipoxin gene may show different responses to periodontal treatment. Further studies to evaluate the effect of different genotypes on periodontal treatment may show whether this polymorphism plays any role in the pathogenesis and response to treatment of periodontal diseases. If this hypothesis is proved, then further studies to evaluate the role of lipoxin gene alterations as a therapeutic modality could be conducted.

Resumo

Lipoxinas desempenham um papel importante na recuperação periodonta, portanto, a investigação do polimorfismo genético do gene da lipoxina pode fornecer informações importantes sobre o papel das lipoxinas na patogênese da doença periodontal. O objetivo deste estudo foi investigar um polimorfismo de substituição C-to-T na posição c-292 no gene ALOX15 (reticulócito-tipo 15 lipoxigenase 1) em pacientes com periodontite crônica e associar o polimorfismo com a lipoxina A4 (LXA4) do fluido gengival crevicular (FGC). Quarenta e cinco pacientes com periodontite crônica e 45 pacientes periodonalmente saudáveis foram incluídos neste estudo caso-controle. Índice de placa, índice de cálculo, índice de sangramento do sulco, profundidade de sondagem (PS) da boca toda e perda de inserção clínica (PIC) foram registrados. Amostras do FGC e de sangue foram coletadas. O FGC foi analisado quanto aos níveis de LXA4 por ensaio imunoadsorvente ligado à enzima (ELISA). A genotipagem do polimorfismo ALOX15 foi estudada por PCR. A média de LXA4 foi menor no grupo de periodontite

em comparação com o grupo periodontalmente saudável. Houve uma correlação negativa entre PIC e LXA4. O genótipo CC foi maior no grupo de estudo do que no grupo controle. No grupo de estudo, a média de PIC foi significativamente menor entre os indivíduos com o genótipo CT. A média de LXA4 foi significativamente menor no genótipo CC (45,0 ± 7,11 ng / mL) em comparação com o genótipo CT (50,81 ± 5,81 ng / mL) entre os pacientes com periodontite. Os resultados sugerem que o alelo LXA4 e o alelo c-292T estão associados à saúde periodontal. Polimorfismos no gene ALOX15 podem influenciar a patogênese da doença periodontal. Assim, a investigação de tais polimorfismos pode beneficiar a avaliação do papel das lipoxinas na doença periodontal.

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