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Comparison of the interleukin-33 among the healthy and aggressive periodontitis cases: An original research

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***Abstract*---** Introduction: Interleukin-33 (IL-33) is a novel alarmin that warns immune cells of tissue destruction in injury or infection. Hence in this study we aim to compare the interleukin-3 among the healthy and aggressive periodontitis cases. Materials and Methods: We conducted a cross-sectional, biochemical, genetic study. We divided

the groups to three as healthy group, chronic periodontitis patients, and generalized aggressive periodontitis patients. IL-33 concentration in GCF, as well as plasma, was quantified. Results: A significant difference was found in IL-33 concentration in GCF and plasma between the three groups. GG genotype of IL-33 SNP rs1157505 was associated with the highest GCF and plasma IL-33 concentration and was significantly more in GAgP than healthy or CP groups. IL-33 SNP rs7044343 did not show any such association. All GAgP patients had the highest GCF and plasma concentration of IL-33. Conclusions: IL-33 may be a potential inflammatory marker of periodontitis. GG genotype of SNP rs1157505 may be associated with generalized aggressive periodontitis.

***Keywords*---**Interleukin-33, Chronic Periodontitis, Aggressive Periodontitis.

Introduction

Cytokine immunebiology pivots the development of numerous destructive inflammatory diseases such as autoimmune arthritis, Crohn's disease, and periodontitis. IL-33 is the newest entrant to the IL-1 family.[1] IL-33 (IL-1F11 [IL-1 Family member 11]) is a bi- functional alarmin that not only links innate and adaptive immune responses as a cytokine but is also a nuclear factor.[2-3] Cytokines are the primary mediators of immunoregulation; therefore, the corresponding genetic regulatory factors concerned with cytokine function and periodontitis have been probed. Association between a composite genotype of the IL-1 gene with severe periodontitis has been found.[8] Polymorphisms in the IL-33 gene have been found to be associated with Alzheimer's disease (AD) and rheumatoid arthritis. [4-6] Hence in our study we aim to estimate IL-33 in gingival crevicular fluid (GCF) and plasma and to study the association of IL-33 gene single-nucleotide polymorphisms (SNPs) rs1157505 and rs7044343 in chronic periodontitis (CP) and generalized aggressive periodontitis (GAgP) patients.

Materials and Methods

We conducted a cross-sectional, biochemical, genetic study. We divided the groups to three as healthy group-H, chronic periodontitis patients-CP, and generalized aggressive periodontitis- GAgP patients. IL-33 concentration in GCF, as well as plasma, was quantified. Patient approval and ethics clearance were taken. Only healthy nonsmoking subjects were selected. Periodontal evaluation included gingival index (GI), probing pocket depth (PPD), probing attachment level (PAL), and presence or absence of sulcular bleeding upon probing. PPD and PAL were measured. Different sites were sampled to obtain a suitable volume of GCF in healthy subjects. In the CP and GAgP groups, a single sampling site with the greatest PAL along with radiographic evidence of alveolar bone resorption was chosen per subject. The sample GCF volume in the Periopaper strip was quantified by Periotron 8000 and transferred to microcentrifuge tubes (with identification markings) containing 0.4 mL of phosphate-buffered saline and stored at −80°C till the assay was run.

DNA and plasma extraction
Ten milliliters of blood was obtained from each participant by venipuncture. Two milliliters of the blood was used for plasma separation. The plasma was stored in a plastic vial at −80°C until the time of assay. The remaining 8 ml of the whole blood sample was promptly transported in ethylenediaminetetraacetic acid-coated vacutainers in the molecular genetics laboratory for DNA extraction and subsequent SNP analysis of IL33 gene.
Interleukin-33 analysis
IL-33 concentration in GCF and plasma, obtained from the study subjects, were measured using Human IL-33 ELISA.
Interleukin-33 genotyping was done later by Miller's method[6] and Genotyping was carried out by PCR-restriction fragment length polymorphism as described by Chapuis etal.[7]
The comparison of the groups was done using appropriate statistical tools and $p<0.05$ was kept as significant.

Results

The GAgP group had the highest mean IL-33 concentration in GCF and plasma. The GCF concentration of IL-33 differed significantly between the three groups. The plasma concentration of IL-33 differed significantly between groups H and GAgP ($P < 0.001$) and the groups CP and GAgP ($P < 0.001$). GCF and plasma concentration of IL-33 was found to be statistically independent of GI, PPD, or PAL in any group. TABLE 1. The frequency of IL33 SNP rs1157505 GG allele was greatest in GAgP (26.7%) compared to other groups. IL33 SNP rs7044343 TC genotype correlated with the lowest PPD in the healthy group ($P = 0.030$). The GG genotype subjects had the highest levels of IL-33 in GCF and plasma. IL33 rs7044343 TT genotype subjects of the healthy group had the highest plasma concentration of IL-33 [Tables 2–5]. The CC and CG variants of rs1157505 SNP, when divided into individual groups, mottled significantly in the levels of GCF and plasma IL-33, whereas the GG variant did not show a statistically significant inter-group difference in IL-33 concentration in GCF and plasma. The CC, TC, and TT variants of rs7044343 SNP, when divided into individual groups, showed a statistically significant difference in the levels of GCF IL-33 and plasma IL-33 between the groups.

Table 1
Various gingival crevicular fluid and plasma interleukin-33 concentration

Parameters	Group H (n=30)	Group CP (n=30)	Group GAgP (n=30)	$F^{\dagger}/\chi^2$	P
Age (years)	26.30±5.421	39.87±9.486	27.43±6.709	31.018	<0.001*
Sex					
Females (n)	14	14	11	4.727	0.094
Males (n)	16	16	19		
GI	NA	1.92±0.317	2.40±0.255	42.146	<0.001*
PPD (mm)	1.93±0.740	6.30±1.264	6.63±1.189	174.066	<0.001*
PAL (mm)	NA	7.33±1.626	7.57±1.165	0.408	0.525
GCF IL-33 concentration (pg/ml)	417.13±43.244	469.27±50.803	556.65±82.737	51.262	<0.001*
Plasma IL-33 concentration (pg/ml)	13.86±4.452	16.41±8.225	204.32±147.938	58.961	<0.001*

*Statistically significant at P<0.05; $^{\dagger}F$ – Kruskal–Wallis test. All values except sex have been expressed as mean±SD. SD – Standard deviation; NA – Not applicable; H – Healthy; CP – Chronic periodontitis; GAgP – Generalized aggressive periodontitis; n – Number of subjects; mm – Millimeter; pg/ml – Picogram per milliliter; GI – Gingival index; PPD – Probing pocket depth; PAL – Probing attachment level; GCF – Gingival crevicular fluid; IL – Interleukin; P – Probability; χ^2 – Chi-square

Table 2
Association of genotype rs1157505 (C/C, C/G, G/G) with respect to gingival crevicular fluid and plasma interleukin-33

Group	rs1157505	*n*	GCF IL-33 concentration (pg/ml), mean±SD	χ^2*	*P*	Plasma IL-33 concentration (pg/ml), mean±SD	χ^2*	*P*
Group H	CC	13	410.32±43.93	0.954	0.621	14.57±3.242	3.045	0.218
	CG	16	423.88±44.248			13.57±5.285		
	GG	1	397.8			9.19		
Group CP	CC	19	471.89±56.851	0.275	0.872	17.26±9.48	1.431	0.489
	CG	10	462.64±41.934			15.39±5.616		
	GG	1	485.8			10.39		
Group GAgP	CC	7	523.01±57.688	2.247	0.325	199.36±185.592	1.509	0.47
	CG	15	559.12±72.785			175.28±131.091		
	GG	8	581.46±114.299			263.13±144.885		

*Kruskal–Wallis test, $P<0.05$ considered as statistically significant. - NA – Not applicable; IL – Interleukin; *n* – Number of subjects; pg/ml – Picogram per milliliter; GCF – Gingival crevicular fluid; SD – Standard deviation; H – Healthy; CP – Chronic periodontitis; GAgP – Generalized aggressive periodontitis; CC – Homozygous dominant genotype; CG – Heterozygous genotype; GG – Homozygous recessive genotype; *P* – Probability; χ^2 – Chi-square

Table 3
Association of genotype rs7044343 (C/C, T/C, T/T) with respect to gingival crevicular fluid and plasma interleukin-33

Group	rs7044343	*n*	GCF IL-33 concentration (pg/ml), mean±SD	χ^2*	*P*	Plasma IL-33 concentration (pg/ml), mean±SD	χ^2*	*P*
Group H	CC	3	431.57±42.725	1.018	0.601	11.52±2.183	8.953	0.011†
	TC	18	416.24±31.347			12.37±3.695		
	TT	9	414.11±64.377			17.61±4.336		
Group CP	CC	2	463.43±31.643	0.949	0.622	9.72±0.936	4.064	0.131
	TC	19	462.42±49.749			15.91±7.881		
	TT	9	485.03±57.118			18.95±9.293		
Group GAgP	CC	9	563.38±119.275	0.07	0.966	214.36±139.742	0.302	0.86
	TC	14	542.63±37.042			210.62±155.36		
	TT	7	576.06±100.887			178.83±162.622		

*Kruskal–Wallis test, †Statistically significant at $P<0.05$. IL – Interleukin; *n* – Number of subjects; pg/ml – Picogram per milliliter; GCF – Gingival crevicular fluid; SD – Standard deviation; H – Healthy; CP – Chronic periodontitis; GAgP – Generalized aggressive periodontitis; CC – Homozygous dominant genotype; TC – Heterozygous genotype; TT – Homozygous recessive genotype; *P* – Probability; χ^2 – Chi-square

Table 4
Association of genotype rs1157505 (C/C, C/G, G/G) with respect to gingival crevicular fluid and plasma interleukin-33 levels irrespective of groups

Allele	*n*	GCF IL-33 levels (pg/ml), mean±SD	χ^2	*P***	Plasma IL-33 levels (pg/ml), mean±SD	χ^2	*P**
CC	39	460.54±65.666	7.631	0.022†	49.05±102.754	7.572	0.023†
CG	41	482.81±81.603			73.18±110.442		
GG	10	553.53±118.569			212.46±166.544		

**P* values of Chi-square test, †Statistically significant at $P<0.05$. *n* – Number of subjects; GCF – Gingival crevicular fluid; IL – Interleukin, pg/ml – Picogram per milliliter; SD – Standard deviation; CC – Homozygous dominant genotype; CG – Heterozygous genotype; GG – Homozygous recessive genotype; *P* – Probability; χ^2 – Chi-square

Table 5
Association of genotype rs7044343 (C/C, T/C, T/T) with respect to gingival crevicular fluid and plasma interleukin -33 levels irrespective of groups

Allele	*n*	GCF IL-33 levels (pg/ml), mean±SD	χ^2	*P**	Plasma IL-33 levels (pg/ml), mean±SD	χ^2	*P**
CC	14	520.85±112.746	2.574	0.276	141.66±149.209	3.376	0.155
TC	51	468.14±64.167			68.11±118.923		
TT	25	484.99±96.524			63.23±109.818		

Discussion

The results of previous studies, by various researchers who have quantified IL-33 in periodontal disease, have been quite contradictory. In the current study, IL-33 was detected in GCF and plasma, which may be due to a newer, more sensitive ELISA kit. Studies that detected IL-33 in GCF, saliva, and plasma have reported that IL-33 levels cannot discern chronic periodontitis patients from healthy individuals. The higher concentration of IL-33 in GCF compared to plasma in our study may be the result of increase in local production of IL-33 from the increased vasculature in diseased periodontal tissues or from periodontal tissue destruction.[8-11] Since we have included systemically healthy subjects, the higher IL-33 plasma concentration in periodontitis groups might be due to a spill-over from the periodontal tissues into the systemic circulation. In our study, PPD and PAL measurements were greater in CP and GAgP groups as anticipated. The IL-33 concentration did not correlate with clinical parameters of inflammation. Analyses of GCF components give an accurate indication of the link between specific metabolic variation and disease status.[12] Hence, the concentration of IL-33 might be a true indicator of the periodontal disease activity in the absence of evident changes in the soft tissue parameters. Mendonça et al. have reported increased IL-33 levels in the saliva of systemic lupus erythematosus patients with chronic periodontitis.[13] Gümüş et al. found that salivary levels of IL-33 are higher in chronic periodontitis groups.[14] Nizam et al. have quantified and noted increased salivary cytokines, including IL-33 in sleep apnea patients as compared to controls.[15] IL33 SNPs rs7044343 and rs11792633 have been studied in various diseases such as Behçet's disease, systemic sclerosis, and rheumatoid arthritis,[10,42] whereas rs1157505 is associated with the risk of developing AD.[16-18] A similarity was seen our study also.

Conclusion

We found a significant difference between the distribution of the rare allelic variant (G/G) of rs1157505 SNP between the three study groups. It was seen that the rare allele variant GG genotype was associated with the highest concentrations of IL-33 in GCF and plasma and GAgP patients exhibited the highest concentrations of IL-33, irrespective of the genotype. IL-33 appears to be a promising biomarker for periodontal health and disease and future

interventional studies may help comprehend its exact role in periodontitis opening new avenues for periodontal therapy. Further studies with larger samples are advocated.

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