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ORIGINAL ARTICLE

Interleukin Expression as a Diagnostic Tool in Chronic and Aggressive Periodontitis

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ABSTRACT

Introduction: Periodontitis is an inflammatory, complex, multifactorial disease of supporting teeth, which may be classified into two main types, chronic periodontitis (CP) and aggressive periodontitis (AgP), among other subclasses as done in the 1999 world workshop classification of periodontal diseases and conditions. Several risk and susceptibility factors have been proposed for it. Periodontal disease results when balance between host factors and etiologic agents is disrupted. Bacteria have a primary role in the initiation of periodontal disease, and a range of host-related factors influence the clinical presentation and rate of progression of disease. Genetic variations that modify immunological reactions identify the disease susceptibility in various individuals.

Aim: The aim of the study is to assess specific interleukins (ILs) and their presence in CP and AgP, which could be further utilized as a diagnostic modality.

Material and methods: A cross-sectional, pilot study was carried out.Subjects were assigned to two groups: group I for CP and group II for AgP. Gingival crevicular fluid (GCF) samples were taken using the micropipette method and were analyzed using enzyme-linked immu nosorbent assay (ELISA) for individual ILs. Clinical, microbiological, and radiographic parameters were also analyzed.

Results: Studies have reported prevalence of IL-1 and IL-10 in the CP group, and IL-1, IL-4, and IL-6 in the AgP group.

Conclusion: Many studies have proved the effect of various single or composite nucleotide polymorphisms on susceptibility, progression, or severity of periodontal diseases. Despite these studies, the association between periodontal disease and candidate genes is still not clear. The reports of the familial nature of CP are less frequent as compared with AgP. The striking familial aggregation of trait in AgP is consistent with significant genetic etiology. Interleukins are a group of cytokines that have complex immunological functions including proliferation, migration, growth, and differentiation of cells.

Keywords: Aggressive periodontitis, Chronic periodontitis, Cytokines, Gingival crevicular fluid, Interleukins.

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INTRODUCTION

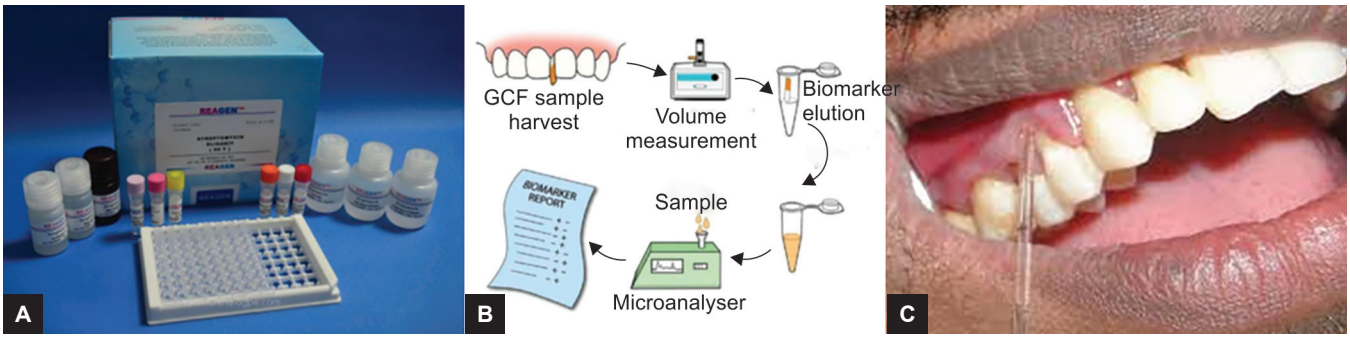
Periodontitis is a complex multifactorial disease, which can be further divisible into CP, AgP, and other forms based on clinical, microbiological, and radiographical findings. Periodontal disease results when balance between host factors and etiologic agents is disrupted.

Bacteria have a primary role in the initiation of periodontal disease, and a range of host-related factors influence the clinical presentation and rate of progression of disease. Genetic variations that modify immunological reactions identify the disease susceptibility in various individuals. Interleukins are a group of cytokines that have complex immunological functions including proliferation, migration, growth, and differentiation of cells. They can be pro- or anti-inflammatory and some ILs also function as chemokines or chemoattractants for other cells. They are believed to be a vital component of host response as they decide the pathogenesis. The present study focuses on expression and prevalence of IL-1, 4, 6, and 10 in CP and AgP patients.

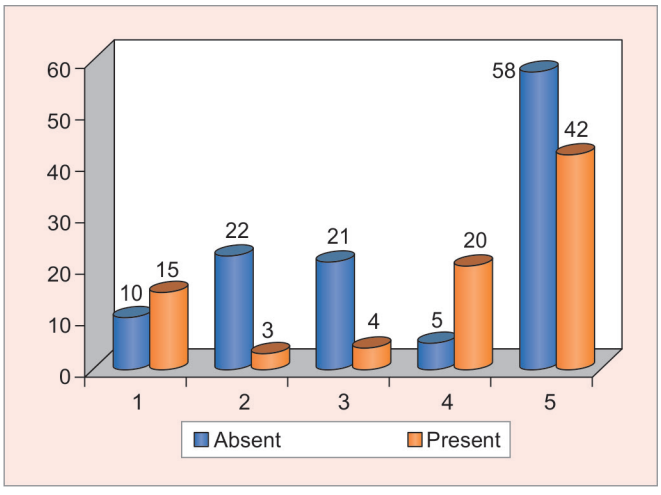
The aim of study was to evaluate the expression of IL-1/4/6/10 in CP and AgP groups and assess prevalence of IL-1/4/6/10 in CP and AgP groups.

MATERIALS AND METHODS

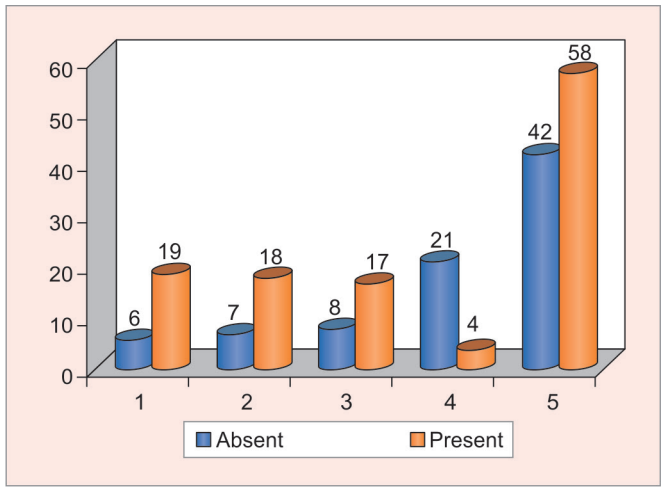
A cross-sectional, pilot study was conducted at the Department of Periodontics and Oral Implantology, Jaipur Dental College, Jaipur, India. The AgP patients were classified according to the clinical criteria proposed by the American Academy of Periodontology.[1] The CP patients were also classified according to the same criteria.[2-4] Subjects were assigned to two groups: group I for CP and group II for AgP. Patients in the age range of 18 to 45 years with at least 24 teeth present were included in the study. Smokers, medically compromised patients, and pregnant and lactating females were excluded from the study.



Figs 1A to C: (A) ELISA kit; (B) Overview of processing of GCF samples; and (C) GCF sampling from a patient



Graph 1: Distribution of CP among study variables



Graph 2: Distribution of AgP among study variables

Gingival crevicular fluid (GCF) samples were taken using the micropipette method and sent to lab. Samples were analyzed using enzyme-linked immunosorbent assay (ELISA) for individual ILs. Clinical, microbiological, and radiographic parameters were analyzed (Fig. 1).

Statistical analysis

Data were subjected to statistical analysis using chi-squared test and *post hoc* Bonferroni test.

RESULTS

Studies have reported prevalence of IL-1 and IL-10 in the CP group, and IL-1, IL-4, and IL-6 in the AgP group. Intragroup comparison shows significant relevance except IL-1 *vs* IL-10 and IL-4 *vs* IL-6 in the CP group and IL-1 *vs* IL-4 and IL-6 in the AgP group (Graphs 1 and 2; Table 1).

Table 1: Distribution of presence of CP and AgP among study variables

	IL-1	*IL-4*	*IL-6*	*IL-10*
CP	15	3	4	20
AgP	19	18	17	4
p-value	0.23	0 (S)	0 (S)	0 (S)

Test applied: Chi-squared test; S: Significant

DISCUSSION

- Periodontitis is considered a complex disease[5] since the pathophysiology presents various biological pathways. Complex diseases are associated with variations in multiple genes, each of which has a small overall contribution and relative risk for the disease process.[6] In periodontal disease, the phenotype is determined by both the genetic makeup and the environmental influences on the affected individual.[5] Most of the genetic research in periodontitis has focused on gene polymorphisms that play a role in immunoregulation or metabolism.[5]
- The present study was carried out to study prevalence of various ILs (IL-1, 4, 6, 10) in both CP and AgP groups and their intragroup association.

In the CP group, expression of IL-10 and IL-1 was found to be statistically significant. The IL-10 is associated with downregulation of immune response following inflammation and IL-1 is strongly associated with bone resorption and release of matrix metalloproteinases. The results are in agreement with earlier studies.

In the case of AgP group, IL-1 and IL-4 were found to be statistically significant, as IL-1is associated with microbial immune response cascade, including guiding inflammatory cells into infection sites, stimulating

Table 2: Shows group wise comparison of various interleukins in AgP group. IL-1,4,6 were at almost similar results so that showed non-significant results

		Mean Difference	*Significance*
IL - 1	IL-4	0.04	1.0
	IL-6	0.08	1.0
	IL-10	0.6	0 (S)
IL-4	IL-6	0.04	1.0
	IL-10	0.56	0 (S)
IL -6	IL-10	0.52	0 (S)

Test applied: *post hoc* Bonferroni test; S: Significant

Table 3: shows group wise comparison of various interleukins in CGP group. Various level of Interleukins showed statistically significant result (p≤0.00) except With IL -1 v/s IL-10 and IL-4 v/s IL-6

		Mean Difference	*Significance*
IL-1	IL-4	0.48	0 (S)
	IL-6	0.44	0.001 (S)
	IL-10	–0.2	0.51
IL-4	IL-6	–0.04	1.0
	IL-10	–0.68	0 (S)
IL-6	IL-10	–0.64	0 (S)

Test applied: *post hoc* Bonferroni test; S: Significant

monocyte and fibroblast eicosanoid release, while IL-4 is associated with humoral immunity and downregulating macrophage function. The results are in agreement with Diehl et al.[7]

On intragroup comparison, prevalence of IL-4, IL-6 expression was greater in the AgP group, (Table 2) which was found to be statistically significant. Prevalence of IL-10 expression was greater in the CP group (Table 3), which was found to be statistically significant. The IL-1 was found to be not statistically significant.

The genetic variations in the cytokine production appear to explain some of the differences in the rates of progression of periodontitis in different adults. The IL-1b has been shown to be present and elevated in the tissues and crevicular fluid from patients with periodontal disease[8] and is a key mediator of the host inflammatory and tissue regulatory pathways in certain chronic inflammatory disorders. The biologic premise of the genetic susceptibility test is that the presence of allele 2 of IL-1B+3954 is associated with an increased propensity to secrete IL-1b on microbial lipopolysaccharide stimulation.[9] This assumption is based on the *in vitro* work of Pociot et al,[10] who reported that people with the allele 2 at the IL-1B+3953 locus (now renumbered as IL-1B+3954) produced increased amounts of IL-1b. In similar studies, peripheral white blood cells[11,12] incubated in the laboratory with bacterial products from Gram-negative bacteria produced more IL-1b if the white blood cells coming from a person who had a specific variation in IL-1 genes (genotype positive).[13]

In linkage analysis that IL-1 genetic variation contributes to an important influence on disease risk.[7] The progression of gingivitis to established periodontal diseases is of primary clinical importance and elucidation of the immunopathogenesis is central to our understanding. In the early stages of inflammation, proinflammatory cytokines secreted by inflammatory cells will predominate. Cytokine genes play a very significant role in orchestrating the immune response. The inflammatory immune response and AgP appears to be genetically determined.[14]

Despite the large number of studies showing a relationship between genetic polymorphism and AgP susceptibility, few studies have proved that AgP may not be associated with genetic polymorphism. Walker et al[15] found that IL-1a allele at +3953 position provides little diagnostic or predictive information for localized juvenile periodontitis. In a cross-sectional study of Caucasians (European origin) with 56 generalized early onset periodontitis patients and 56 healthy controls, a lack of association between IL-1 polymorphism and Gen EOP was observed and no significant differences were found when smoking was included as a covariate or not.[16]

Interleukin-10 is an anti-inflammatory cytokine that plays a role in controlling the inflammatory response.[6,17] Periodontitis lesions presented a significantly higher expression of IL-10 than that in autologous peripheral blood mononuclear cells.[18] Therefore, IL-10 has been considered a candidate susceptibility gene in periodontitis.[18] In a longitudinal study,[19] with a 5-year follow-up, the *IL10* genotype contributed to the progression of periodontal disease. This may reflect the fine balance between IL-10 and disease expression and progression, and the need for prospective longitudinal studies to elucidate the relative contribution of various factors in multifactorial diseases.[19]

CONCLUSION

Interleukin expression can be used as a diagnostic marker for studying various forms of periodontitis. The present study shows prevalence of expression of IL-1 and IL-10 in the CP group and IL-1 and IL-4 in AgP group. Although the results of the present study are comparable with those reported in the literature, prevalence of ILs in disease groups may vary accordingly. Future studies looking at combinations of polymorphisms within specific haplotypes and also correlating host genotype with microbial profiles can provide important information regarding the pathogenesis of this complex disease. Further studies with larger sample populations and with different ethnicities are needed in order to better evaluate the potential association between periodontal disease and polymorphisms in the *IL10*, *ESR2,* and *NOS2A* genes. We can speculate that, in the future, when the role of these genetic factors is finally

elucidated, it will be possible to apply this information for the identification of patients at risk, especially for AgP. This will allow for early treatment, thus reducing the functional and esthetic sequelae associated with the disease.[11,17]

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