Evaluation of chemokines in gingival crevicular fluid in children with band and loop space maintainers: A clinico-biochemical study

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Abstract

Background: Chemokines are pro-inflammatory cells that can be induced during an immune response to recruit cells of the immune system to a site of infection. **Aim:** This study was conducted to detect the presence of chemokines, macrophage inflammatory protein-1α (MIP-1α), and 1β (MIP-1β) and estimate their levels in gingival crevicular fluid (GCF) in children with band and loop space maintainers. **Materials and Methods:** MIP-1α and MIP-1β levels were estimated in GCF samples from twenty healthy children and twenty children with band and loop space maintainers. Periodontal status was evaluated by measuring gingival index, plaque index, and Russell's periodontal index. The GCF samples were quantified by ELISA, and the levels of MIP-1α and MIP-1β were determined. **Results:** The mean MIP-1α concentrations in healthy children and those with space maintainers were 395.75 pg/μl and 857.85 pg/μl, respectively, and MIP-1β was 342.55 pg/μl and 685.25 pg/μl, respectively. MIP-1α and MIP-1β levels in GCF from children with space maintainers were significantly higher than in the healthy group, and statistically significant difference existed between these two groups. **Conclusion:** MIP-1α and MIP-1β can be considered as novel biomarkers in the biological mechanism underlying the pathogenesis of gingival inflammation in children with space maintainers.

Keywords: Band and loop space maintainers, chemokines, gingival crevicular fluid, inflammation, macrophage inflammatory protein-1α, macrophage inflammatory protein-1β

Introduction

Space maintainers are intraoral appliances used to preserve arch length following the premature loss of primary teeth by guiding the permanent teeth into proper alignment and occlusion.[1] The band and loop appliance is the most commonly used fixed space maintainer in pediatric dentistry. The control of plaque formation which results in inflamed gums around the molar bands is a defending task for a pedodontist.[2] Improper cementation of bands on the tooth leads to local tissue response due to several factors such as plaque accumulation, close proximity to the gingival sulcus, and the increased area of the tooth that is covered, which makes oral hygiene maintenance difficult.[3] Direct injury to the gingiva as a result of overextended bands[4] and mechanical or chemical irritation due to exposed cement also causes a local tissue response that may lead to inflammation with additional difficulty in brushing and flossing.

Chemokines are critical mediators of cell migration and recruitment of specific leukocytes to the sites of infection during immune surveillance, inflammation, and development.[5] Chemokines mediate the recruitment and subsequent activation of specific leukocytes to inflamed tissues,[6] and therefore, play a key role in the immune response. MIP-1α is a cysteine-cysteine (CC) chemokine that was first identified in an Lipopolysaccharide (LPS)-treated monocytic cell line. It attracts monocytes, T lymphocytes, natural killer (NK) cells, dendritic cells, and granulocytes to inflammatory sites.[7] *Porphyromonas gingivalis* and *Actinomyces actinomycetemcomitans* induce high levels of MIP-1α in mononuclear cells.[7] The chemokine MIP-1α (also called CCL3) is considered to be the most abundantly expressed chemokine in periodontal diseases[8] and is a

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ligand for the chemokine receptors CCR1 and CCR5. It is primarily associated with the chemoattraction of monocytes/macrophages, dendritic cells (through binding to CCR1), and lymphocytes (differentiated into the Th1 phenotype through binding to CCR5).[9] Therefore, since macrophages and Th1 cells are typical sources of bone resorptive cytokines such as tumor necrosis factor-α and interferon-γ,[10] MIP-1α could have a potential role in inflammatory bone resorption in periodontal diseases. MIP-1α-positive cells increase in number with increasing severity of periodontal disease[11] and are associated with increased levels of lymphocytes in inflamed tissues.[8] Therefore, due to the increased leukocyte chemoattractant capability by MIP-1α expression, it is considered to have a potential role as a regulator of osteoclast differentiation, and it is also potentially involved in the immune pathogenesis of periodontal diseases.[12]

MIP-1β belongs to the CC chemokine subfamily. It is considered to be the most abundantly expressed chemokine in periodontium in correspondence to MIP-1α.[12] Both of these chemokines exert similar effects on monocytes, but their effects on lymphocytes differ: MIP-1α selectively attracts $CD8^+$ lymphocytes and MIP-1β selectively attracts $CD4^+$ cells.[13] MIP-1β was initially characterized as a chemoattractant for activated $CD4^+$ cells and has been shown to selectively attract Th1 cells, as opposed to Th2 and effector cells. This observed selectivity for Th1 cells most likely results from the preferential expression of CCR5 (MIP-1β receptor) on Th1 cells and suggests a potential role forMIP-1β in directing the host pro-inflammatory responses.[13]

Till date, studies have been undertaken to assess the gingival condition clinically using plaque and gingival index after placement of bands in orthodontic volunteers. However, no study had been done to evaluate the levels of chemokines in the gingival crevicular fluid (GCF) of children with space maintainers. Therefore, the present study was designed to assess the levels of MIP-1α and MIP-1β in such volunteers to obtain more accurate and to better understand the underlying factors.

Materials and Methods

Children were selected from OPD, Department of Pedodontics, Institute of Dental Sciences and Research. Healthy male and female children of 6–9 years age with band and loop space maintainers for at least 6 months and deft scores ≤3 were included in the study. Volunteers with other infections (intraoral and systemic), having received periodontal or antibiotic therapies 6 months before testing, using mouth rinses containing antimicrobials preceding 2 months from the study, with diabetes, or with other orthodontic appliances, were excluded from the study. All eligible volunteers were thoroughly informed about the nature, methods, risks, and benefits of the study. Their participation was made by obtaining written consent. The study was carried out after approval of the Institute's Ethical Committee.

Criteria for participant grouping

The selected children were categorized into two groups (twenty children each):

- Group I (healthy controls): Twenty children, 6–9 years of age, with clinically healthy gingiva and deft score ≤3
- Group II (space maintainers): Twenty children, 6–9 years of age with band and loop space maintainers.

Gingival index, plaque index (PI), and Russell's periodontal index[14-16] were assessed. In Group I, GCF was collected from the distal sites of permanent first molar and deciduous second molar regions as described by Rody *et al*.[17] [Figure 1]. For Group II, GCF was collected from the site around bands with the most severe signs of inflammation [Figure 2]. Without touching the marginal gingiva, supragingival plaque was removed to avoid contamination and blocking of the microcapillary pipette. A standardized volume of 3 ml GCF was collected from each test site by placing the tip of a 1–3 ml calibrated volumetric microcapillary pipette (Sigma Aldrich Chemical Company, USA; catalog number p0549) extracrevicularly (unstimulated) for 5–20 min. The test sites that did not express the standard volume (3 ml) of GCF and micropipettes contaminated with blood or saliva were excluded. The collected GCF was immediately aliquoted and stored at −70°C until the time of the assay. An ELISA was performed on the stored samples to determine the chemokines present. ELISA was performed using the quantitative sandwich enzyme immunoassay technique (R and D Systems; catalog numbers DMP300 and DTM100). Data analysis was carried out using the Statistical Package for Social Sciences (SPSS version 20). Unpaired *t*-test was applied for the analysis of the data.

Results

The data were analyzed using the SPSS software program (version 11.5, SPSS Inc., Chicago, IL, USA). As shown in

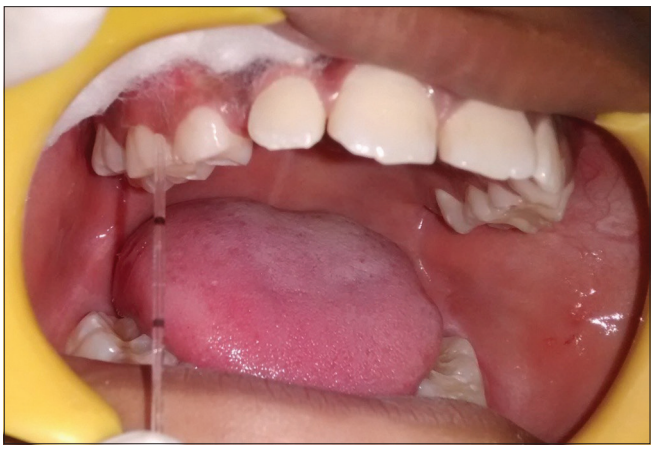


Figure 1: Collection of gingival crevicular fluid from Group I

Table 1, the mean PI for Group I was 0.215 ± 0.152 and for Group II was 1.618 ± 0.341. The mean gingival index (GI) was 0.256 ± 0.084 for Group I and 1.554 ± 0.319 for Group II [Table 2]. The mean Russell's periodontal index for Group I was 0.199 ± 0.136 and for Group II was 0.825 ± 0.223 [Table 3]. The differences in Tables 1-3 were highly statistically significant ($P < 0.001$). All the samples in each group tested positive for the presence of MIP-1α and MIP-1β. The mean total GCF concentration of MIP-1 in Group I was 395.75 ± 15.46 pg/μl and was 857.85 ± 67.02 pg/μl in Group II [Table 4 and Graph 1]. The mean concentration of MIP-1β in the GCF from Group I was 342.55 ± 31.90 pg/μl and in Group II was 685.25 ± 103.50 pg/μl [Table 5 and Graph 2]. The mean MIP-1α and MIP-1β concentrations in the GCF were significantly higher in Group II, which was statistically significant ($P = 0.001$).

Table 1: Mean plaque index for Groups I and II

	Number of samples	Mean	SD	SE	*t*-test	*P*
Group I	20	0.215	0.152	0.021	17.731	<0.001
Group II	20	1.618	0.341	0.076		

SD: Standard deviation; SE: Standard error

Table 2: Mean gingival index for Groups I and II

	Number of samples	Mean	SD	SE	*t*	*P*
Group I	20	0.256	0.084	0.071	17.610	<0.001
Group II	20	1.554	0.319	0.019		

SD: Standard deviation; SE: Standard error

Table 3: Mean Russell's periodontal Index for Groups I and II

	Number of samples	Mean	SD	SE	*t*	*P*
Group I	20	0.199	0.136	0.071	10.743	<0.001
Group II	20	0.825	0.223	0.050		

SD: Standard deviation; SE: Standard error

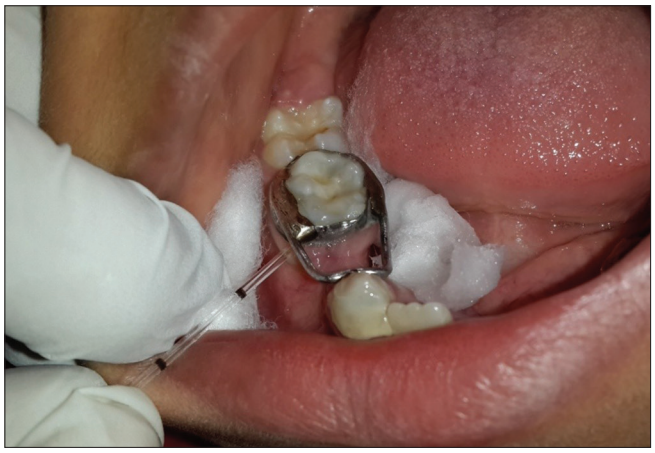


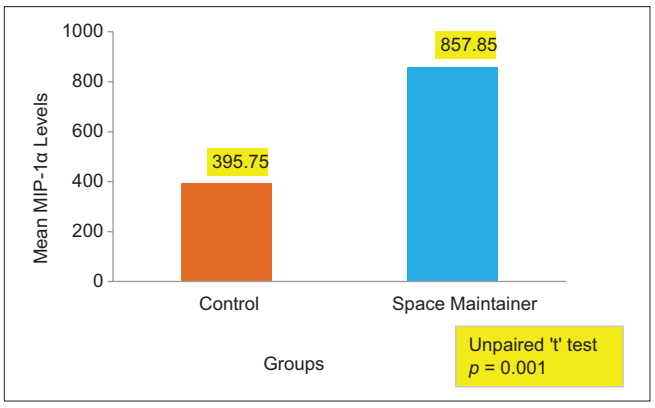
Figure 2: Collection of gingival crevicular fluid from Group II

Discussion

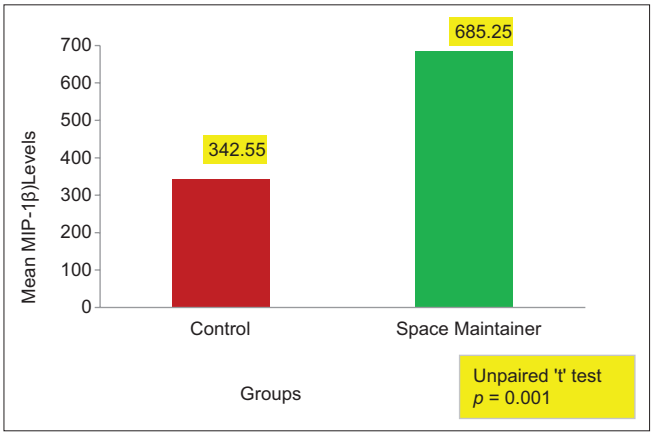
In the present study, GI, PI, and mean concentrations of MIP-1α and MIP-1β in GCF were found to be increased proportionately from Group I to Group II and showed positive correlations with clinical parameters. A possible reason for the increased levels of MIP-1α in GCF may be due to the combined actions of adhesion molecules and their effects on leukocyte migration. In this study, the concentrations of MIP-1α in GCF were compared between Groups I and II, which were statistically significant ($P = 0.001$). This clearly suggests that MIP-1α concentrations in GCF increased progressively from Group I to Group II. The mean concentrations of MIP-1β in GCF were lower in Group I when compared to Group II. These levels increased proportionately from Group I to Group II and showed a positive correlation with clinical parameters. The possible reasons for the increased GCF levels of MIP-1β could be because of recruitment and retention of specific leukocyte subsets into the gingival crevice in response to plaque accumulation, periodontal pathogens, their bacterial components (e.g. LPS), and the release of chemokines at the site of injury. When the GCF concentrations of MIP-1β in Groups I and II were compared, statistically significant differences were noticed ($P = 0.001$). This clearly suggests that MIP-1β levels in the GCF increased progressively from Group I to Group II.

The variability of MIP-1α and MIP-1β concentrations within children of each group could be attributed to their role in different stages of disease process at the time of GCF collection. High concentrations of MIP-1α (800 pg/μl and 765 pg/μl) and MIP-1β (580 pg/μl and 620 pg/μl) were present in two volunteers in Group I, which could be due to subclinical inflammation, allergy, or any infection not reported by the children. Low concentrations of MIP-1α (400 pg/μl and 380 pg/ml) in two participants and MIP-1β (350 pg/μl) in one volunteer were observed in Group II, due to stability of the diseased sites.

MIP-1α is an inflammatory mediator that facilitates infiltration of selective leukocyte subsets into local sites



Graph 1: Mean gingival crevicular fluid concentration of macrophage inflammatory protein-1α in Group I and II



Graph 2: Mean gingival crevicular fluid concentration of macrophage inflammatory protein-1β in Group I and II

Table 4: Mean macrophage inflammatory protein-1α concentrations in gingival crevicular fluid for Groups I and II

	Mean±SD (pg/µl)	Mean difference	*t*	*P*
Group I	395.75±15.46	462.10	30.04	0.001*
Group II	857.85±67.02			

*P<0.05 (significant), SD: Standard Deviation

Table 5: Mean macrophage inflammatory protein-1β concentrations in gingival crevicular fluid for Groups I and II

	Mean±SD (pg/µl)	Mean difference	*t*	*P*
Group I	342.55±31.9	342.70	14.15	0.001*
Group II	685.25±103.5			

*P<0.05 (significant), SD: Standard Deviation

of inflammation.[11] Levels of MIP-1α are reported to be constitutively low or absent in uninflamed and healthy periodontal tissues but are increased in gingival biopsies in both chronic and aggressive periodontitis lesions.[18] This has been confirmed by many studies.[11,18,19] Kabashima *et al*.[11] suggested that cells expressing chemokines such as MIP-1α may modulate the pathogenesis of periodontitis and may be responsible for stimulating the destruction of tissue and resorption of alveolar bone. Among inflamed gingival tissues, MIP-1α expression was abundant in the basal epithelium.[19] It was reported that the MIP-1α levels were higher than that of other chemokines (IP-10, RANTES, and MCP-1) at sites of microbial-induced inflammation.[11] The ability of gingival epithelial cells to produce MIP-1α may provide a sustained source of this chemokine, thus modulating the host response to inflammation in the gingival sulcus and in the surrounding gingival epithelium.[19] The ability of MIP-1α to induce osteoclast formation suggests a role for MIP-1α in later stages of inflammatory bone destruction that is characteristic of periodontal lesions. In this manner, MIP-1α levels may modulate the course of periodontal disease. Its production by epithelial cells may function in the acute inflammatory response, whereas its potential role in osteoclast formation may be more important in late aspects of the disease. PMNs also function in acute and chronic stages of the disease. Inhibition of cytokines such as MIP-1α may provide opportunities for therapeutic intervention strategies to prevent tissue destruction in aggressive forms of periodontitis.[20]

Syndergaard *et al*. reported that salivary concentrations of interleukin (IL)-1β, IL-6, MMP-8, MIP-1α, and PGE2 were higher in individuals with gingivitis when compared to healthy participants.[21] Similar results were obtained by others who reported that salivary concentrations of these cytokines were significantly higher in children with periodontal disease than in healthy individuals.[22,23] The results of the current study agree with Garlet *et al*.,[18] who reported that MIP-1β was more prevalent and highly expressed in children with chronic periodontitis than in control participants ($P < 0.05$). The results of the present study contrast with Emingil *et al*.[24] and Fokkema *et al*.,[25] who reported that children with generalized aggressive periodontitis and chronic periodontitis have similar MIP-1β levels in GCF as compared to children with gingivitis and healthy periodontal tissue. Mohamed *et al*.[26] reported higher levels of IL-8 and MIP-1β in the GCF of children with diabetes.

Many studies have been undertaken to determine the gingival condition following band placement. In congruence with the current study, Boyd R *et al*.[2] reported that banded molars showed significantly greater gingival inflammation and plaque accumulation than bonded molars during treatment. The present study is comparable with Sadiq and Badea[27] who concluded that banded molars in both adults and adolescents had significantly more plaque accumulation and gingival inflammation than bonded molars. Arikan *et al*.[28] investigated the effect of fixed and removable space maintainers on periodontal status in children. They reported that PI, pocket depth, and bleeding index scores were significantly greater in follow-ups in the fixed space maintainers groups and concluded that fixed space maintainers can result in inflammation of periodontal tissues in children. The present study correlates with Schei *et al*.,[29] who studied the effects of bands that are used in fixed orthodontic appliances on dental plaque accumulation. They reported that the colonization of *Streptococcus mutans* is increased in most of the children, with increased pocket depth and bleeding on probing due to increased plaque among the fixed appliance groups. Paschos *et al*.[30] compared the severity of clinical inflammatory parameters and the level of the inflammatory mediator IL-1 during orthodontic treatment using brackets and bands. In accordance with their study, the current study reported significantly higher values for pocket depth and GI for teeth with bands in comparison with teeth treated with brackets. The results of the present study agree with those of Naga Sri and Sosa,[3] who reported a significant increase in plaque scores, gingival scores, and

pocket probing depths in the experimental group following tooth banding.

Conclusion

Increased GCF MIP-1α and MIP-1β levels suggest that these CC chemokines are important mediators in the pathogenesis of periodontal damage around the molar bands of space maintainers. Their potential role in osteoclast formation may be more important in chronic and late aspects of the disease. Inhibition of cytokines such as MIP-1α and MIP-1β may provide opportunities for therapeutic intervention strategies to prevent tissue destruction of periodontitis in children.

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Conflicts of interest
There are no conflicts of interest.

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