

Evaluation of Levels of Proinflammatory Chemokines MIP-1 α and MIP-1 β in Gingival Crevicular Fluid of Primary, Mixed and Permanent Dentition

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Received : 21-12-18.

Accepted : 28-01-19.

Published : 12-04-19.

INTRODUCTION

Hippocrates famously stated way back in the 4th century, “teething children suffer from itching of the gums, fever, convulsions, and diarrhea.” During the eruption of teeth, many chemical changes occur. Chemokines are vital mediators of cell migration as they recruit precise leukocytes to the infection sites during inflammation, immune surveillance, and development. They have structural relation to cytokines, with the main function of regulating cell trafficking. The cells of periodontium that produce chemokines are fibroblasts, osteoclasts, polymorph nuclear leukocytes, endothelial cells, macrophages, monocytes, lymphocytes, and mast cells. Chemokines are categorized into two main families based on their

ligand structure: CC and CXC. Monocyte chemoattractant protein 1 (MCP-1), RANTES, macrophage inflammatory protein (MIP)-1 α and MIP-1 β belong to CC group.^[1,2]

Earlier studies on chemokines revealed that they are expressed after systemic and local inflammation in several body fluids, such as serum, saliva, and gingival crevicular fluid (GCF). GCF is a composite mix of derivatives from serum, leukocytes, antibodies,

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How to cite this article: Swathi B, Charitha M, Mandava D, Tugaram N, Mudrakola DP, Yelamanchi R. Evaluation of levels of proinflammatory chemokines MIP-1 α and MIP-1 β in gingival crevicular fluid of primary, mixed and permanent dentition. J Int Soc Prevent Communit Dent 2019;9:205-9.

ABSTRACT

Aim: This study aims to find out the proinflammatory chemokines macrophage inflammatory protein (MIP)-1 α and MIP-1 β levels in gingival crevicular fluid (GCF) of primary, mixed, and permanent dentitions.

Materials and Methods: GCF of 160 individuals (4–15 years of age) was collected by the extracrevicular method. They were categorized into four groups (40 per each group). Group I: subjects with primary dentition (4–5 years of age), Group II: 40 subjects in early transition period (6–8 years), Group III: 40 individuals in the late transition period (9–11 years), and Group IV: 40 individuals with permanent dentition (12–15 years). MIP-1 α and MIP-1 β levels were determined in the samples of GCF by ELISA method. Data were analyzed by software SPSS Version 20 (IBM SPSS Statistics for Windows, IBM Corp., Armonk, NY: USA).

Results: MIP-1 α and MIP-1 β were detected in all samples. The highest mean MIP-1 α and MIP-1 β concentrations in GCF were detected in the early transition period, while the lowest concentrations were seen in primary dentition group. The chemokine levels were higher in girls than in boys in Group III. There was a substantial rise of MIP-1 α and MIP-1 β levels during eruption.

Conclusions: Since levels of MIP-1 α and MIP-1 β in GCF are positively associated with tooth eruption, they may perhaps be deemed as novel biomarkers in the eruption process.

KEYWORDS: Chemokines, cytokines, gingival crevicular fluid, teething, tooth eruption

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Quick Response Code:



Website: www.jispcd.org

DOI: 10.4103/jispcd.JISPCD_438_18

enzymes, chemokines, cells of periodontium, and oral bacteria. In healthy individuals, GCF is a transudate, and in conditions such as gingivitis and periodontitis, it gets altered into true inflammatory exudate.^[1-3]

Shapira *et al.* stated that gingival trauma related to active teething may cause release of inflammatory cytokines.^[4] Wise *et al.* established that MCP-1 was secreted by dental follicle (DF) cells to signal mononuclear monocytes to arrive at the developing DF. Here, these cells fuse resulting in osteoclasts, which resorb the alveolar bone and make a pathway for eruption. Hence, the first important event in tooth eruption is initiated by MCP-1 and other chemokines. This knowledge may help us in predicting the eruptive process and taking the necessary steps to improve the overall health of dentition.^[5]

Very few studies are carried regarding chemokines, such as MCP-1, in tooth eruption of animal models. To the best of our knowledge, no study was done in humans regarding chemokines role in tooth eruption in children and adolescents.^[5-8] Hence, we examined the inflammatory cytokines levels in GCF of primary, mixed and permanent dentition, during the eruption.

MATERIALS AND METHODS

We carried this prospective study on 160 healthy controls (80 boys and 80 girls; 4–15 years) who attended the CKS Theja Institute of Dental Sciences and Research, Renigunta Road, Tirupathi, Andhra Pradesh, India. The study was carried from January 1 to December 31, 2016. Randomization was achieved by computer draw method, and the sample size was determined from a similar studies using the formula: $n = ([z\alpha + z\beta])^2$ with a confidence interval of 95% and power of 95% for the study. The study has registered Ethical Committee Approval (CKST/E12A/2016).

Parents of all the involved children signed an informed consent and clearance was obtained from the Institutional Ethical Clearance Committee. After taking a thorough medical history, plaque index (Silness and Loe), gingival index (Loe and Silness), decay, missing and filled teeth (DMFT)/deft (<3) scores were recorded for each subject to avoid bias in the results.

Children were categorized into four groups with two subgroups each. Group I; 40 individuals in primary dentition period (4–5 years of age); Group I A (20 males) and Group I B (20 females). Group II had 40 individuals in the early transition period (6–8 years of age), subdivided into Group II A (20 males) and Group II B (20 females). Group III; 40 individuals in late transition period (9–11 years), divided into Group III A (20 males) and Group III B (20 females). Group IV; 40 individuals

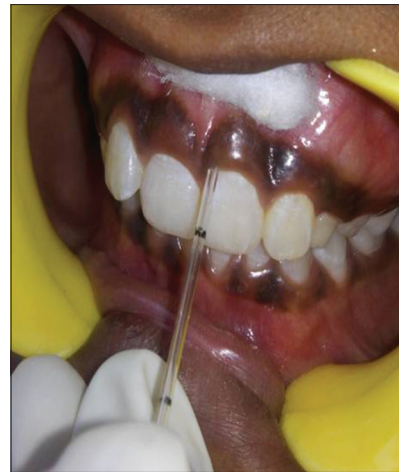


Figure 1: Collection of gingival crevicular fluid samples in Group II patients

with permanent dentition (12–15 years of age), Group IV A (20 males) and Group IV B (20 females).

INCLUSION CRITERIA

Healthy, cooperative controls without any radiographic confirmation of caries/periodontal infections.

EXCLUSION CRITERIA

Patients with a history of systemic diseases (e.g., juvenile diabetes), gingival or periodontal infections, caries, receiving antibiotics, or nonsteroidal anti-inflammatory drugs 6 months before the study were excluded.

GINGIVAL CREVICULAR FLUID SAMPLING

Tooth polishing was performed before the start of the study. With a sterile mouth mirror and a Goldman/Fox Williams periodontal probe, the periodontal status was examined clinically. After isolating the area with cotton rolls, 1–3 μ l microcapillary pipettes (Sigma Aldrich Chemical Company, USA; Catalog No. p0549) were used for GCF collection. By positioning the tip of the pipette extracrevicularly (unstimulated) for 30 s, a standard volume of 3 μ l GCF was collected from each test site, i.e., buccal, mesial, distal, and lingual/palatal sites of matured primary molars in primary dentition, using the graduation marks on the micropipette, as described by Ureles *et al.* The GCF in mixed dentition was collected from sites distal to second primary molar, and in permanent dentition distal sites of permanent first molars as mentioned by Rody *et al.* [Figure 1].^[9] The pipettes were transferred to the laboratory immediately and frozen at -70°C for analysis.

ANALYSIS OF MACROPHAGE INFLAMMATORY PROTEIN-1A AND MACROPHAGE INFLAMMATORY PROTEIN-1B IN GINGIVAL CREVICULAR FLUID SAMPLES BY ELISA

We performed the ELISA using the quantitative sandwich enzyme immunoassay technique. A polyclonal antibody

specific for MIP-1 α and MIP-1 β were precoated onto a microplate. Samples were put into the wells, and any MIP-1 α and MIP-1 β present was bound by the immobilized antibody. After washing, an enzyme-linked polyclonal antibody specific for MIP-1 α and MIP-1 β was added. Then again wash was performed, and a substrate solution was added. It resulted in the development of color as per concentration of total MIP-1 α and MIP-1 β [pro-and/or active: Figure 2]. The color intensity was measured using ELISA reader.

STATISTICAL ANALYSIS

The results were averaged (mean \pm standard deviation) for each parameter. Intergroup comparisons were performed using ANOVA, $P < 0.05$ was considered statistically significant. Statistical Package for the Social Sciences (SPSS) software, version 20.0 (IBM SPSS Statistics for Windows, IBM Corp., Armonk, NY: USA) was used for Data analysis.

RESULTS

CLINICAL PARAMETERS

The clinical parameters, such as gingival index, plaque index, and DMFT/deft, recorded for all 160 individuals and analysis revealed the difference being insignificant [Table 1].

MACROPHAGE INFLAMMATORY PROTEIN-1A AND MACROPHAGE INFLAMMATORY PROTEIN-1B LEVELS

All the samples in each group showed positive for MIP-1 α and MIP-1 β [Graphs 1 and 2]. The mean values of MIP-1 α and MIP-1 β were highest in Group II and least in Group I [Table 2; $P = 0.001$].

Intracomparisons between the genders in each group were made by Games-Howell test, and it revealed that MIP-1 α and MIP-1 β values were more in girls than boys [Table 2 and Graph 3].



Figure 2: Development of yellow color after addition of 2N sulfuric acid

DISCUSSION

Tooth eruption is an intricate and an extremely regulated process that results from the eruptive forces that come from DF cells. Studies in dogs revealed that DF plays a main role in eruption, with teeth unable to erupt in situations where it was removed. Moreover, if tooth was replaced with amalgam, keeping DF intact, the artificial tooth erupted.^[8-10]

To date, few studies on animal models did report the role of MCP-15 in tooth eruption. Our study was undertaken to elucidate the role of chemokines in tooth eruption. GCF collection was made by microcapillary pipettes to avoid tissue trauma, so as to attain an undiluted GCF sample, thereby enabling precise measurement of variables.^[7,8]

The sites used for GCF collection were mesial, buccal, distal, and palatal gingival sulci of primary molars. Following the methodology of Ureles *et al.*, a larger volume of GCF could be retrieved. In mixed dentition periods, GCF was obtained from the distal side of permanent first molar and deciduous second molar to prevent contamination, as illustrated by Rody *et al.*^[8,9] In individuals in the first transition period, GCF was also collected from mesial, distal, labial and palatal sulci of permanent incisors since they were the first teeth to erupt and with significant gingival and mucosal changes. In permanent dentition individuals, GCF was obtained only from the distal side of first molars to prevent salivary contamination, as described by Rody *et al.*^[9]

Studies showed that cytokines undergo rapid degradation *in vitro* if proper storage and handling measures are not taken. Therefore, samples were stored at -70°C . To prevail over these demerits, ELISA method was used in our study for accurate quantitative determinations of MIP-1 α and MIP-1 β with high sensitivity and specificity. The commercially available, sensitive ELISA kit can detect the smallest amount of MIP-1 α and MIP-1 β in the samples.^[6,7]

Our results suggest that chemokines are demonstrable in GCF of primary dentition, which is similar to Wise *et al.*, who also observed a rise in the levels of interleukin-1 β (IL-1 β), IL-8, and tumor necrosis factor (TNF) α at the time of the eruption.^[5]

Table 1: Mean values of plaque index, gingival index, Decay, Missing, and Filled Teeth/deft for the four groups

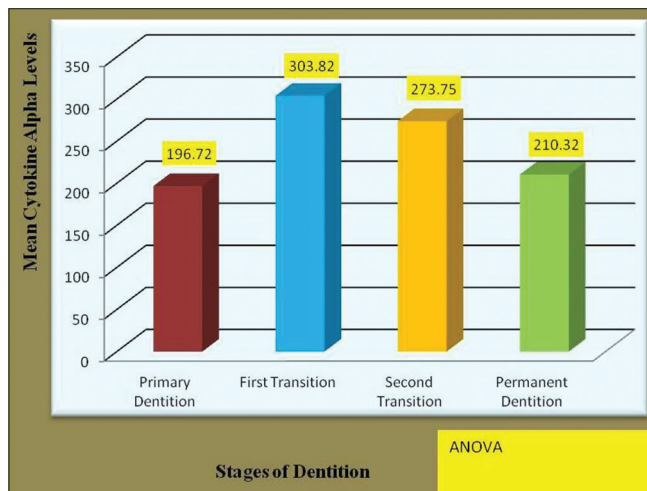
Group	Plaque index	Gingival index	DMFT/deft	P
Primary dentition	0.265 \pm 0.098	0.242 \pm 0.087	0.30 \pm 0.46	>0.05 (NS)
First transition	0.215 \pm 0.093	0.256 \pm 0.087	0.45 \pm 0.59	
Second transition	0.305 \pm 0.094	0.280 \pm 0.089	0.52 \pm 0.64	
Permanent dentition	0.310 \pm 0.085	0.298 \pm 0.084	0.35 \pm 0.57	

NS=Not significant, DMFT=Decay, Missing and Filled Teeth

Table 2: Mean concentrations of macrophage inflammatory protein-1 α and macrophage inflammatory protein-1 β for the subgroups

Group	MIP-1 α		MIP-1 β		P
	Boys	Girls	Boys	Girls	
Primary dentition	194 \pm 10.63	199.4 \pm 8.19	283.2 \pm 23.46	291.2 \pm 19.0	0.001 (S)
First transition	300.7 \pm 10.65	306.95 \pm 9.95	383.2 \pm 24.0	395.7 \pm 15.46	
Second transition	267.9 \pm 10.20	279.5 \pm 11.15	346.6 \pm 17.3	358.6 \pm 8.12	
Permanent dentition	203.7 \pm 52.00	216.9 \pm 57.28	315.1 \pm 81.21	332.3 \pm 89.43	

S=Significant, MIP=Macrophage inflammatory protein

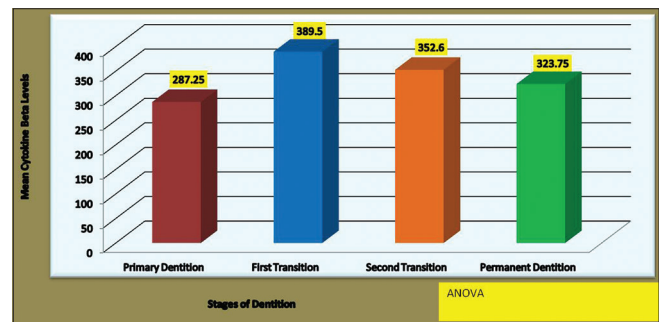


Graph 1: Mean chemokine alpha levels in all groups

Group II showed maximum concentration of chemokines, which agrees with the results of Cohen and Carpenter who stated that epidermal growth factors and other inflammatory mediators are necessary for eruption and that their administration into rodents hastened eruption in them.^[11] Wise *et al.* reported aberrated eruption in mice that lacked Type I receptor for IL-1 α (IL-1R). Tooth eruption was delayed for 2 days for molars and 1 day for incisors, and administration of IL-1 α hastened eruption by 2 days. In Group IV, the MIP-1 α and MIP-1 β levels were lower than that in Groups II and III. This could be due to the establishment of occlusion.^[5]

Intergroup comparisons showed that low values in primary dentition could be owing to the completion of eruption process by 4 years of age. Shapira *et al.* also reported cytokines in GCF of erupting primary teeth.^[4] Memarpour *et al.* stated that the release of these cytokines, like TNF, is more likely due to systemic symptoms such as increased temperature, during teething. When there is inflammation, IL-1 β is the main endogenous pyrogen which causes tissue damage by stimulating neutral metalloproteinases release from fibroblasts and other mesenchymal cells. It also stimulates the production of many other cytokines.^[12]

The maximum levels of MIP-1 α and MIP-1 β were seen in the early transition group and could be owing to the active



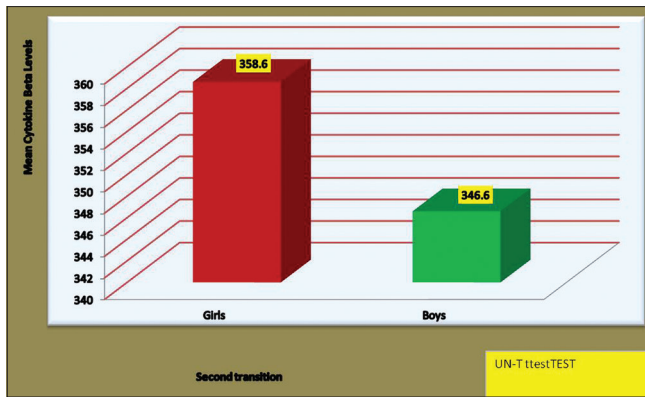
Graph 2: Mean chemokine beta levels in all groups

phase of the eruption. This may be due to stimulation of DF cells thereby osteoblasts and osteoclasts creating a pathway of eruption pathway by release of signaling molecules such as MIP1- α and MIP1- β , into the periodontium of the surrounding mucosa through the junctional epithelium.^[6-8] According to Wise *et al.*, MCP-1 secreted by DF cells stimulate the mononuclear cells, and this is the first significant cellular event of eruption.^[5]

The intragroup comparison showed that the values of MIP-1 α and MIP-1 β were more in girls than boys. This could be owing to hormonal changes since estrogen alters inflammatory response by modifying chemokine expression by periodontal ligament cells. Cielinski *et al.* observed that the maximum influx of mononuclear cells into DF occurs by day three after eruption of mandibular molar in animal models. In turn, these cells combine and form osteoclasts which erode alveolar bone thus creating an eruption pathway.^[13] We noticed that such osteoclastic and osteoblastic activities are required for creating a pathway for eruption.

Group IV exhibited lesser peak values than Groups II and III. This is similar to Kamma *et al.*, who reported that levels of cytokines decreased from adolescents to adults. However, they could not explicate the reason for such variations in adults and adolescents.^[14]

Eruption disturbances are most frequently ascribed to mechanical interferences due to either supernumerary teeth, crowding, odontogenic cysts, or tumors. Ankylosis characteristically is seen after partial eruption of the tooth, and the tooth appears to be submerged in comparison to



Graph 3: Mean chemokine beta levels in girls and boys of late transition period

adjacent teeth, which continue to erupt. The failure of eruption and delay in the eruption is seen in association with conditions such as craniofacial dysostosis, hypopituitarism, and hypothyroidism delayed eruption seems to occur most frequently than the failure of eruption.^[11-14]

Most of the cases with a defect in eruption process result from a genetic disorder, but can also be nonfamilial due to sporadic mutation, like in case of primary failure of eruption (PFE). In PFE, there is a localized failure of eruption in the absence of any systemic involvement. This is due to a primary defect in the eruption mechanism itself. Zhelnin and Zukow^[15] observed reduced levels of proinflammatory chemokines in patients with the obstructed eruption of wisdom teeth.^[14,15]

SUMMARY OF KEY FINDINGS

1. All the samples in each group showed positive for MIP-1 α and MIP-1 β
2. MIP-1 α and MIP-1 β were highest in Group II and least in Group I
3. MIP-1 α and MIP-1 β values were more in girls than boys.

LIMITATIONS OF THE STUDY

1. Parameters such as gingival health, subclinical gingival inflammation, and dental caries were not considered
2. Oral hygiene practice was not considered
3. Unknown systemic factors (teething accompanying systemic disturbances) which might affect the chemokines levels were not considered.

Since eruption is an intricate process involving many molecules/gene factors for creating an eruption pathway, future research should include long-term studies exploring the effects of other molecules necessary for the eruption. Assessing markers such as chemokines MIP-1 α and MIP-1 β can aid in predicting eruption disturbances, thereby preventing malocclusion and other related phenomenon.

CONCLUSIONS

We found that the chemokines MIP-1 α and MIP-1 β were detected in all primary, mixed and permanent dentitions. Their levels were elevated during the active phases of eruption (i.e., first transition period). Hence, determining the role of chemokines will require comprehensive studies performed on several pathological conditions related to primary failures in tooth eruption.

FINANCIAL SUPPORT AND SPONSORSHIP

Nil.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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