Short Communication

Comparative Evaluation of Periodontal Ligament-associated Protein-1/ Asporin Levels in Periodontal Tissue in Health and Disease

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Abstract

Background: Periodontal ligament-associated protein-1 (PLAP-1)/asporin is an extracellular matrix protein that plays a protective role in the pathogenesis of periodontitis. There is a paucity of information about the association between PLAP-1/asporin and periodontitis in human PDL. Thus, in this study, PLAP-1/asporin levels between participants with healthy periodontium and chronic periodontitis were compared and correlated with periodontal parameters. **Materials and Methods:** Fifty participants were recruited and divided into 25 in each group: Group 1 (control) and Group 2 (test). Probing pocket depth (PPD) and clinical attachment level (CAL) were recorded. Periodontal ligament (PDL) samples were collected from extracted teeth for estimating PLAP-1/asporin levels using the Human Asporin Enzyme-Linked Immunoassay Kit. **Results:** A statistically significant difference ($P = 0.001$) in the PLAP-1/asporin levels was observed between Group 1 and Group 2. A weak negative correlation was observed between PLAP-1/asporin levels and periodontal parameters (PPD and CAL) in both groups. **Conclusion:** In this study, higher PLAP-1/asporin levels in participants with healthy periodontium highlight the protective role of PLAP-1/asporin in maintaining periodontal homeostasis.

Keywords: *Chronic periodontitis, periodontal ligament, periodontal ligament-associated protein-1/ asporin*

Introduction

Periodontal ligament (PDL) is a specialized fibrous tissue that surrounds and attaches the roots of the tooth to the alveolar bone. Its principal function is to support the teeth in their sockets and permit them to withstand masticatory forces to an extent. PDL is a reservoir for periodontal tissue homeostasis.[1] Periodontal ligament-associated protein-1/ asporin (PLAP-1/asporin) is an extracellular matrix protein expressed in human PDL that functions to maintain periodontal homeostasis.

Asporin belongs to the small leucine-rich proteoglycan gene of the class I subfamily. Yamada *et al*. were the first to explore the presence and regulation of PLAP-1/asporin in PDL.[2] Apart from periodontal tissues, asporin is present in smooth muscle cells such as human articular cartilage, aorta, uterus, heart, and liver.[3]

PLAP-1/asporin inhibits bone morphogenetic protein-2 and prevents the PDL cells from undergoing osteogenic and cementogenic processes.[4,5] It also suppresses the signaling of bone formation in PDL, preventing ankylosis.[6,7] Overexpression of PLAP-1/asporin in the PDL cells downregulates the signaling of toll-like receptors (TLR2) and TLR4 by direct molecular interactions and it thereby regulates periodontal immune inflammatory response.[8] PLAP-1/asporin is believed to have a protective role in periodontitis and maintains periodontal homeostasis.[8,9]

There are a few reports discussing the association of PLAP-1/asporin and periodontitis. An *in vitro* study using cloned mouse PDL cells demonstrated the effect of PLAP-1/asporin on periodontitis.[8] Another *in vivo* animal study identified the expression of PLAP-1/ asporin in experimental periodontitis.[9] To the best of our knowledge, there are no human studies quantitatively estimating the PLAP-1/asporin levels in PDL tissues and correlating it with periodontal parameters. Due to the paucity in existing literature, the present study intends to quantitatively

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assess the PLAP-1/asporin levels in participants with healthy periodontium and chronic periodontitis, and correlate it with periodontal parameters, probing pocket depth (PPD), and clinical attachment level (CAL).

Materials and Methods

A cross-sectional study was carried out in the Department of Periodontology, Indira Gandhi Institute of Dental Sciences, after getting approval from the Institutional Ethics Committee (IGIDSIEC2021NRP26PGMPPAI). The sample size was calculated to be 50 using the mean value of PLAP-1/asporin levels from an earlier article.[10] After obtaining informed consent, the 50 participants were equally divided into a control group (Group 1) of participants undergoing orthodontic treatment and needing therapeutic extraction and a test group (Group 2) of participants with chronic periodontitis or stage IV periodontitis and undergoing extraction of teeth with grade III mobility. Participants with systemic diseases, diabetes, smoking habits, bone-related metabolic diseases, pregnant and lactating women, and participants who had undergone periodontal therapy in the past 6 months were excluded.

Periodontal parameters such as PPD and CAL were recorded using a mouth mirror and UNC-15 probe. Recordings were made at the mesiobuccal, mid-buccal, distobuccal, mesiolingual, mid-lingual, and distolingual surfaces around each tooth by a single calibrated investigator.

Human PDL tissues were isolated from healthy and diseased periodontium in accordance with the method described previously.[2] After extraction of the tooth, human PDL tissues were removed by scraping the tissues from the center of the root surface using universal curettes. The extracted tissue samples were rinsed and stored in an Eppendorf tube containing phosphate-buffered saline (PBS) solution at 80°C until further processing with the Human Asporin Enzyme-Linked Immunoassay (ELISA) Kit (GENLISA™, Krishgen Biosystems) as per manual instructions.

The extracted tissue sample was weighed, minced, and homogenized in PBS (pH 7.4) with a plastic pestle homogenizer. The homogenized tissue was thawed at 2°C–8°C and centrifuged at 2000–3000 revolutions/min for approximately 20 min and the supernatant was collected carefully.

The sandwich ELISA technique was used for analysis. Monoclonal antibodies were precoated onto the microwells. Samples and standards were pipetted into microwells, and human PLAP-1/asporin present in the sample was bound by the antibodies. Biotin-labeled antibody was added and streptavidin-horseradish peroxide (HRP) was pipetted and incubated to form a complex. After washing the microwells, the substrate solution tetramethylbenzidine (TMB) was added to it, and the color was developed proportionally to the amount of human asporin (ASPN) present in the sample. Color development was then stopped by the addition of a stop solution, and the absorbance was measured at 450 nm [Figures 1-4].

The variables of the study are presented as the median values and interquartile ranges. The intergroup comparison between groups 1 and 2 was analyzed using the Mann–Whitney *U* test. Spearman's rank correlation was used to observe the correlation of PLAP-1/asporin levels with PPD and CAL.

Results

The demographic and clinical parameters of the participants in groups 1 and 2 are presented in Table 1. The results are presented as the median value and interquartile range.

Group 1 consisted of participants with an average age group of 19 (18–22.75) and healthy periodontium. PPD and CAL were found to be 1.80 (1.60–1.90). PLAP-1/asporin levels were estimated to be 0.42 (0.35–0.65) ng/mL [Table 1]. A negative correlation was present between PLAP-1/asporin levels and clinical parameters with $r = -0.237$ for PPD and CAL [Table 2].

Table 1: Comparison of the age category, probing pocket depth, clinical attachment level, and periodontal ligament-associated protein-1/asporin levels between Group 1 and Group 2

Group	Age category	PPD	CAL	PLAP-1/asporin
Group 1, *n*=25 (control group)	19 (18–22.75)	1.80 (1.60–1.90)	1.80 (1.60–1.90)	0.42 (0.35–0.65)
Group 2, *n*=25 (test group)	49 (45–62)	4.20 (3.65–4.57)	5.25 (4.50–6.27)	0.12 (0.06–0.20)
P	0.001*	0.001*	0.001*	0.001*

PPD: Probing pocket depth; CAL: Clinical attachment level; PLAP-1: Periodontal ligament-associated protein-1

Table 2: Correlation of periodontal ligament-associated protein-1/asporin levels and clinical parameters between Group 1 and Group 2

	Group 1 (control group)		Group 2 (test group)	
	Correlation coefficient	Significant (two-tailed)	Correlation coefficient	Significant (two-tailed)
PLAP-1/asporin with PPD	−0.237	0.315	−0.328	0.158
PLAP-1/asporin with CAL	−0.237	0.315	−0.363	0.116

PPD: Probing pocket depth; CAL: Clinical attachment level; PLAP-1: Periodontal ligament-associated protein-1

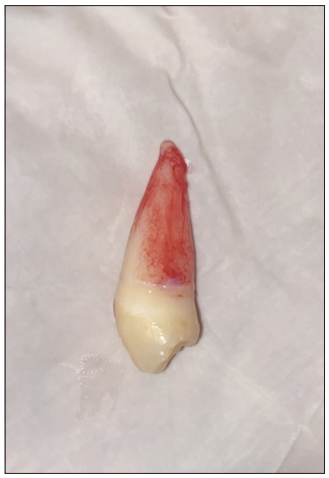

Figure 1: Extracted tooth for sample collection

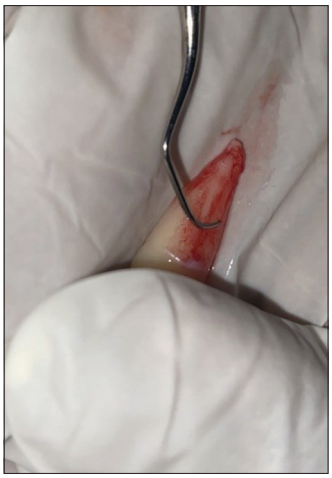

Figure 2: Collection of PDL tissue sample using universal curette

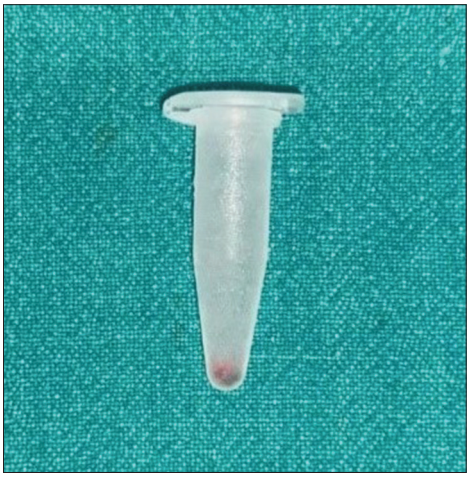

Figure 3: Eppendorf tube containing collected sample

Figure 4: Absorbance reading at 450nm

Group 2 included participants with an average age group of 49 (45–62) and chronic periodontitis. PPD and CAL were found to be 4.20 (3.65–4.57) and 5.25 (4.50–6.27), respectively. PLAP-1/asporin levels were estimated to be 0.12 (0.06–0.20) ng/mL [Table 1]. A negative correlation was present between PLAP-1/asporin levels and clinical parameters with $r = -0.328$ for PPD and −0.363 for CAL [Table 2].

The PLAP-1/asporin levels were lower in Group 1 when compared to Group 2 with a statistically significant $P = 0.001$. A weak negative correlation was observed between PLAP-1/asporin levels and periodontal parameters in both Group 1 and Group 2, and the correlation was not statistically significant [Table 2].

Discussion

PLAP-1/asporin levels in PDL tissues were quantitatively analyzed in human participants with healthy periodontium and chronic periodontitis and correlated with periodontal parameters. In a study done by Yu *et al*. using Wistar rats, PLAP-1 expression was reduced in experimental periodontitis compared to normal periodontal tissues. The authors reported that the reduction in the PLAP-1 expression could have caused an increase in collagen fiber degradation.[9] Similarly, Yamada *et al*. observed an increase in the PLAP-1/asporin expression in cloned mouse PDL, which significantly downregulated TLR2 and TLR4. Furthermore, they reported that PLAP-1/asporin had a defensive role in periodontitis lesions.[8]

In accordance with the above *in vivo* and *in vitro* experiments, the PLAP-1/asporin levels were lower in the PDL tissues of the test group obtained from participants with chronic periodontitis. This probably indicates that the protective role of the protein in the PDL is compromised which led to the disturbance in the ability of periodontal tissues to maintain homeostasis, further leading to periodontitis.

Subsequent to the quantification of PLAP-1/asporin levels, in this study, we observed a negative correlation between PLAP-1/asporin levels and periodontal parameters such as PPD ($r = -0.328$, $P = 0.158$) and CAL ($r = -0.363$, $P = 0.116$) in the test group. This indicates an inverse

relationship of the periodontal parameters with PLAP-1/asporin levels. However, this correlation was weak and did not approach statistical significance. This may be because of the limited sample size which is a definite limitation. We were unable to compare the correlation of PLAP-1/asporin levels with periodontal parameters because to the best of our knowledge, there are no available reports. Age matching of the participants between tests and controls was not possible in this study as PDL tissue was collected from elderly participants with advanced periodontitis where teeth extraction was indicated and young participants seeking orthodontic treatment with the need of therapeutic extraction.

Within the limitations of our study and based on the results obtained, we report the protective role of PLAP-1/asporin in maintaining periodontal homeostasis. However, further research with a large sample size needs to be conducted to estimate PLAP-1/asporin levels before and after nonsurgical and surgical periodontal therapy for external validation.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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