Influence of Periodontitis on Injectable Platelet Rich Fibrin (iPRF) Levels of Beta Defensin -1 in Periodontal/ Peri-implant therapy. Case Control Study

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Abstract

Aim & Background
Periodontitis is an inflammatory condition initiated by polymicrobial flora but mediated by host immune response. This local inflammatory response is further shown to bring about alterations in expression of various cytokines and proteins at systemic level. Injectable platelet rich fibrin (i-PRF) which is a derivative of centrifuged blood is widely used in periodontal and peri-implant therapy. It exhibits its anti-inflammatory effect through various proteins especially beta defensin. This study aims to evaluate the influence of periodontitis on human beta defensin-1 (hBD-1) levels in i-PRF.

Methods
This case control study evaluated 50 subjects 25 each in case (periodontitis) group and control (healthy) group for their periodontal status and i-PRF levels of beta defensin (hBD-1).

Results
There was a significantly decreased i-PRF levels of hBD-1 in the periodontitis group compared to the healthy group. The correlation analysis showed there was a relationship between probing depth and hBD-1 levels.

Conclusion
Thus, it can be concluded that periodontal health status influences the iPRF levels of beta defensin, which can further influence the clinical outcomes of iPRF applications in wound healing and regeneration.
Clinical Significance: iPRF form periodontally diseased subjects might have a reduced levels of anti-inflammatory cytokines (hBD-1) thereby predisposing to a compromised or incomplete healing potential at the site of application.

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Keywords

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INTRODUCTION

Periodontitis is an inflammatory disease of the supporting structures of the teeth which is considered as a multifactorial origin(1). However, polymicrobial biofilm is a prerequisite for the initiation of the disease resulting in a host-microbial interaction leading to destruction of the host tissue including alveolar bone, cementum, and periodontal ligament(1,2). Conventional periodontal therapy is targeted at eliminating these polymicrobial biofilms by the process of scaling and root planing that removes the plaque and calculus on the tooth root(3). However this does not completely remove the etiologic agents, because of the infiltration capacity of periodontal pathogens like P Gingivalis into the periodontal tissues(4). Even after the mechanical periodontal therapy it has been found that the periodontal pathogens are still present in the connective tissue of the periodontal pockets adjacent to deceased root surfaces(4). These can repopulate the debrided area at a later stage and can prevent complete healing or might lead to recurrence of the disease(5). Adjunct periodontal therapies like use of systemic as well as locally delivered antibiotics were later inducted to eliminate these periodontal pathogens from the periodontal tissues(5,6). Evidence on the adjunctive use of antibiotics has shown that the reduction of periodontal pathogens and improved clinical parameters extended to a maximum of 6 months compared to mechanical periodontal therapy alone(7). Nevertheless, the possibility of antibiotic resistance and the side effects expected from the high dosage needed to attain the required concentration of drugs in the periodontal tissues should also be weighed over the benefits(8).

In this context, modulating the host response of the periodontal disease process either by reducing the excessive inflammatory process or by enhancing the anti-inflammatory response has been an emerging treatment option(9). Host modulation therapy in periodontal disease management is an adjunctive to and follows conventional mechanical therapy that almost eliminates the bacterial load from the periodontal sites(10), which can be both systemically as well as locally delivered(9,11). Enamel matrix proteins (Emdogain, Straumann AG, Germany) is one of the commercially available locally delivered host modulating agents that enhances the regeneration process within the periodontium(12,13). Other locally delivered host-modulating against are growth factors (GF) that are available as recombinant human or bovine derived both accounting for possible immunogenic reactions and high cost(14). This is overcome by the advent of Platelet-rich fibrin which is autologous in nature with abundance of growth factors that eliminate the immunogenic reactions(15).

Platelet-rich fibrin (PRF) is a second-generation platelet concentrate that has been introduced by Choukroun J et al in 2001(16). Since then it has been widely used in dental, oral and periodontal applications for its accelerated healing capacity(17). Injectable Platelet-rich fibrin (iPRF) is a recent development in PRF family which is available in a liquid consistency fibrin network for a period of 10 - 15 mins and gets clotted after that(18). It can be injected into tissue for therapeutic purposes and has reported extended benefits(18). Its beneficial effects are due to its 3-dimensional fibrin matrix that has platelets, leukocytes entrapped within it and an enormous amount of growth factors (GFs) (platelet derive growth factor (PDGF-AA, PDGF-AB, PDGF-BB), transforming growth factor beta 1 (TGFB1), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), and insulin-like growth factor (IGF)) released from these cells(19). These GFs stimulate collagen synthesis in PDL cells and gingival fibroblasts, induce cell proliferation and mineralization potential in osteoblasts, and increase endothelial cell activity in vitro(20). Apart from these growth factors, the fibrin matrix also contains some anti-inflammatory proteins that are present in the plasma as well that are released from the activated platelets and white blood cells (monocytes, macrophages and dendritic cells)(21). Some of the anti-inflammatory cytokines reported are IL-4, TGF, human beta-defensins etc, which along with the other cytokines contribute to the anti-inflammatory effect of PRF that are shown in studies(21).

Human beta-defensins (hBD) are antimicrobial polypeptides that are majorly(22) secreted by keratinocytes, but also by monocytes, macrophages and dendritic cells(22). These proteins possess potent host modulating effects, through various mechanisms like antimicrobial, antinflammatory activity, angiogenesis and tissue regeneration capacity. These along with alpha-defensins, cathelicidin are components of the innate immunity system that regulate host response at times by initiation and progression of immune response(23) or by limiting it. Thus, the presence of this antimicrobial protein peptide contributes to the beneficial effect of iPRF.

There are many factors that may influence the beta-defensin levels in serum like presence of chronic inflammation, hyperglycaemia etc. Even though periodontitis which is a chronic inflammatory state has been proven to alter the local levels of various proinflammatory or anti-inflammatory cytokines(24), it is also strongly reported that periodontitis, has an effect on the levels of various proinflammatory or anti-inflammatory cytokines in the systemic levels too(25–27). Specifically recent evidences on influence of periodontitis on salivary and gingival crevicular fluid (GCF) levels of beta-defensin have shown that there is a decreased levels in periodontitis subject when compared to periodontally healthy subjects(28). Interestingly all these above evidence raises the doubt on the influence

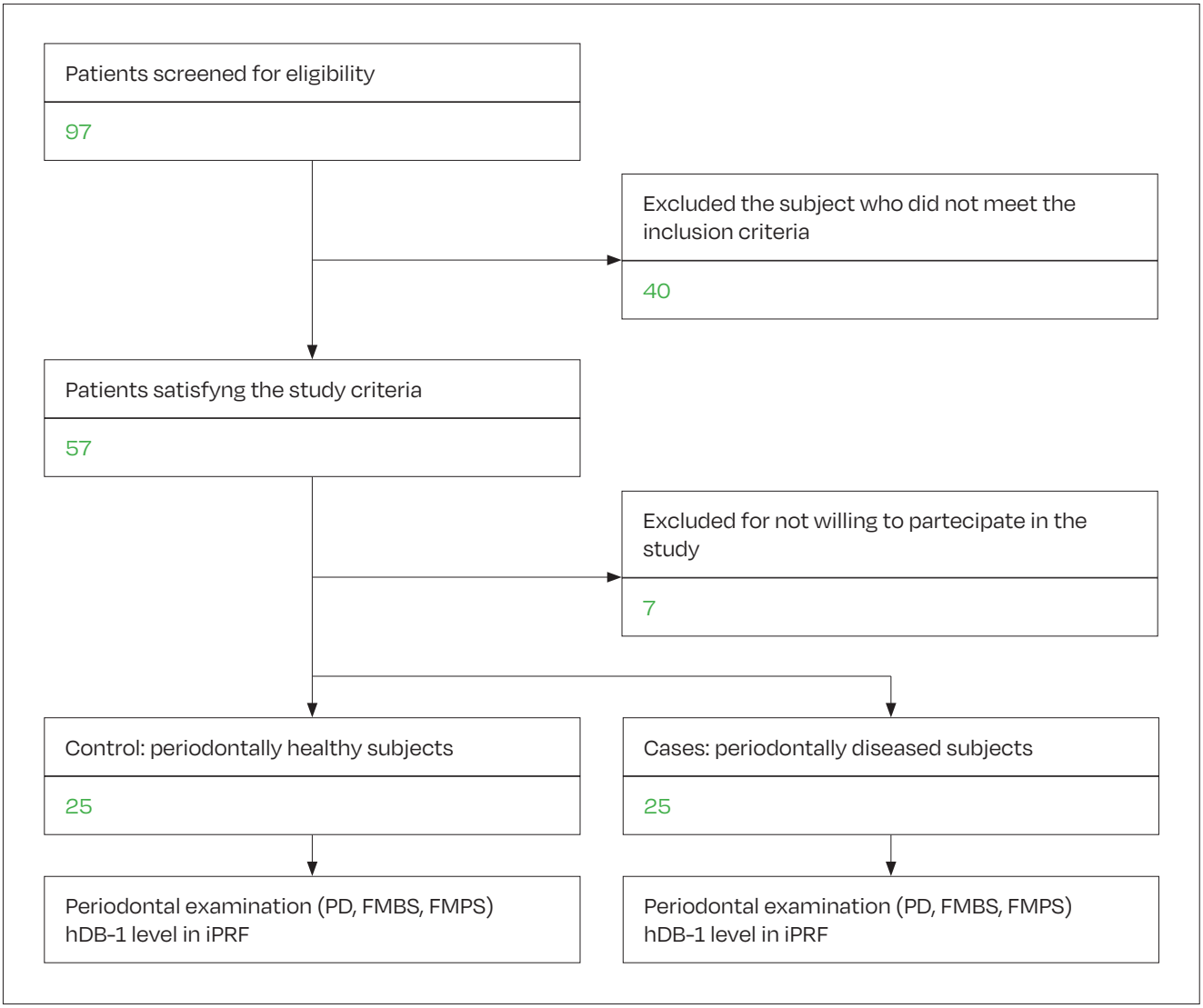

Fig. 1 Flowchart showing the study design.

of periodontal status on iPRF beta-defensin 1 (hBD-1) levels, which is a derivative of centrifuged blood. Any difference in iPRF hBD-1 levels in periodontitis subject might influence the periodontal healing as well. Thus, the aim of the present study is to evaluate the influence of periodontitis on the iPRF levels of hBD-1.

MATERIALS AND METHODS

The present study was designed as a case-control study and was approved by the institutional ethical committee at Saveetha Dental College and Hospitals, Chennai, India.

Sample size

Sample size was calculated based on an earlier study (29) reported with 95% confidence interval and 80% power of the test and showed a sample size of 5 subjects in each group (case versus control) is sufficient to detect a difference in beta-defensin levels. Considering the low sample size, we increased it to 25 subjects in each group (case versus control).

Study Population

The study population was recruited from the subjects reporting to the outpatient department of periodontics, Saveetha Dental College & Hospitals, based on the following inclusion and exclusion criteria.

The control group subjects were recruited base on the following criteria

1. Systemically healthy subjects within the age range of 30 - 60 years.
2. Periodontally healthy subjects with following criteria
   a. Presence of at least 20 teeth at the time of initial examination.
   b. Subjects with periodontal condition showing probing depth (PD) of less than or equal to 3 mm

at all sites.
c. Subject with full mouth bleeding score showing less than 10 percent of the sites involved.
d. Subjects with a history of tooth loss due to periodontal reason were excluded.
e. Subjects with a history of any non-surgical or surgical periodontal therapy were excluded.

The case group subjects were recruited based on the following criteria.

Inclusion Criteria

1. Age group of 30 to 60 years.
2. Subjects who are diagnosed with periodontitis based on the 2018 classification(2).
3. Subjects with at least 20 teeth present at the time of initial examination.
4. Subjects with periodontal condition showing probing depth (PD) of ⩾ 5mm in at least 20 percent of sites.
5. Subjects with periodontal condition showing clinical attachment loss (CAL) of ⩾2mm in at least 20 percent of sites.

Exclusion Criteria

1. Pregnant and lactating women.
2. Subjects with systemic conditions like diabetes mellitus, anaemic (<11 g/dl) are known to alter beta-defensin levels.
3. Smokers.
4. Use of immunosuppressive medications, insulin injections, consumption of antibiotics and any antioxidants and anti-inflammatory agents in the last 3 months.
5. History of periodontal therapy in the preceding 1 year.
6. Vitamins A/C/E or any forms of antioxidant therapy within the last 6 months.
7. Subject participating in any other clinical trials.

A total of 57 subjects satisfied the above criteria and were informed of the study design and protocol, from which 50 subjects gave a written informed consent to participate in the study and were included in the study (Fig. 1). The study population was age and gender matched between the case and control groups. The control and case groups had 25 subjects each with 13 males and 12 females to reduce the selection bias. The demographic details like age, sex, socio-economic data were collected and recorded in data sheets (Table 1).

Periodontal evaluation

For all the recruited subjects a baseline full mouth periodontal examination was done by a single trained calibrated examiner and the following clinical parameters were recorded using a William's probe (Hu-Friedy,Chicago, USA).

1. Full mouth bleeding score (FMBS)
2. Full mouth plaque score (FMPS)
3. Probing depth (PD) - measured from gingival margin to the base of the pocket
4. Clinical Attachment Loss (CAL) - measured from the cemento-enamel junction to the base of the pocket.

Collection of iPRF

Subjects from both groups donated blood for i-PRF collection. The i-PRF was prepared according to the protocol developed by Miron & Choukron in 2017(16). Briefly it involves collection of 5 ml intravenous blood from each subject using venipuncture under sterile conditions. The collected blood is transferred to a plain sterile test tube without any anticoagulant and immediately subjected to centrifugation at 70 g force, 700 rpm for 3 minutes. After centrifugation the blood separates into 2 parts, the bottom layer consisting of a red blood cell compartment and top layer as Platelet-rich fibrin plasma which is still in liquid consistency. One ml of the top Platelet-rich fibrin layer is aspirated in a 2 ml syringe and transferred to microtubes. The collected samples maintain a liquid consistency for about 3 - 5 minutes until it clots by slow polymerisation of fibrin formation. The collected samples were labelled and stored at − 80 °C until further analysis.

Quantitative evaluation of beta-defensin levels in iPRF

The beta-defensin 1 (hBD-1) levels in iPRF were measured using a commercially available ELISA kit Human beta - DF ELISA kit® (Bioassay Technology Laboratory, Shanghai Korain Biotech Co., Shanghai,

Characteristics	n=50	Cases (n=25)	Controls (n=25)	p value
Sex				
Male	26	13	13	> 0.05
Female	24	12	12	> 0.05
Age		48.25 ± 10.52	49.02 ± 12.89	> 0.05
Values are presented as Mean ± SD				

Tab. 1 Descriptive statistics of Demographic data.

Variables	Control (Healthy)	Case (Periodontitis)	p value
Periodontal Parameters			
PD (mm)	2.17 ± 0.72	5.32 ± 1.21	< 0.05
FMBS (%)	9.21 ± 2.03	72.54 ± 19.27	< 0.05
FMPS (%)	8.75 ± 2.12	69.27 ± 21.27	< 0.05
CAL (mm)	0	2.72 ± 0.34	
Laboratory assay			
Beta-defensin (hBD-1) (pg/ml)	183.24892 ± 27.85	109.414862 ±19.34	< 0.05
Values are presented as Mean ± SD			

Tab. 2 Shows the intergroup comparison between the control and case group.

	Cases		Control	
	PD	CAL	PD	CAL
Beta-defensin level (pg/ml)	- 0.702	- 0.709	0.017	-
p value	<0.005 (0.002)	<0.005 (0.019)	>0.005 (0.919)	-

Tab. 2 Pearson correlation between clinical periodontal parameters and beta-defensin - 1 levels.

China) following manufacturers instruction; minimum assay sensitivity was 3.8 pg/mL; with an intra-assay coefficient of variation (CV) of 4.6% and inter- assay CV of 6.5%. Biochemical evaluations were performed by an investigator blinded to clinical data. Clinical investigators were unaware of the laboratory results until the study had ended.

STATISTICAL ANALYSIS

All the values were expressed as mean ± standard deviation (SD) since the values showed a normal distribution. The statistical test used was independent t test to compare the clinical parameter and hBD-1 levels between control and case groups. The correlation between clinical parameters and hBD-1 level was assessed using Pearson correlation. All tests were carried out at a significance level of α=0.05 using IBM SPSS Statistics (version 24.0, IBM, Armonk, NY, USA).

RESULTS

The study results showed the mean age of the study population was 48.25 ± 10.52 and 49.02 ± 12.89 in the control and case group respectively. When compared between the group there was no statistically significant difference(Table 1).
The periodontal examination revealed a mean PD of 2.17 ± 0.72 and 5.32 ± 1.21 in the control and case group respectively with a statistically significant difference when compared between the group. Similarly, the mean FMPS also showed a statistically significant difference between the control (8.75 ± 2.12) and case group (69.27 ± 21.27). Further the mean FMBS was 9.21 ± 2.03 and 72.54 ± 19.27 in control and case groups respectively with statistically significant difference(Table 2).
The mean levels of hBD-1 were 183.24 ± 27.85 pg/ml and 109.41 ± 19.34 in the control and case group respectively. When compared between the groups there was a statistically significant difference(Table 2).
The Pearson correlation showed there was strong negative correlation between clinical parameters and hBD-1 levels (<0.05)(Table 3).

DISCUSSION

Periodontal disease is one of the most prevalent oral diseases affecting most of the population(30). Current evidence has reported a strong link between periodontal disease with many systemic conditions like diabetes mellitus, cardiovascular diseases, and preterm low birth weight etc. (31,32). Most of the research regarding this has pointed out that the mechanism by which periodontal disease contributes as a risk factor for the various systemic conditions is through extension of the local inflammatory burden into the systemic circulation resulting in initiation and triggering of systemic inflammatory response(33–35). In this context the role of periodontitis on iPRF beta-defensin 1 level needs to

be evaluated, since it is one of the major components of it contributing to the anti-inflammatory effect.
This study was conducted as a case-control study, to know whether the periodontitis group had any difference in iPRF hBD-1 levels. The study population was age and gender matched between the case and control group to eliminate selection bias. The periodontal examination revealed that the case group showed a significant increase in PD, FMPS, FMBS compared to the control group. This is because of the inclusion of subjects with presence of periodontitis which had severe plaque, gingival inflammation and periodontal pockets ranging from 4 mm to 6 mm.
Our study results showed presence of hBD-1 in both the groups. This concurs well with earlier reports stating the presence of hBD in PRF that contributes to the antimicrobial and anti-inflammatory action(36). The source of beta-defensin in these PRF mostly would be from the leukocytes as well as platelets entrapped in the fibrin matrix(36).
Further, our study data showed that the iPRF hBD-1 levels were significantly lower in the periodontitis group compared to the healthy subjects group. This is in accordance with earlier data, where it was reported that periodontitis has a negative influence on salivary, GCF and serum levels of beta-defensin(24,28,37). Also it was reported there is an increased level of beta-defensin in early stages of inflammation (gingivitis) subjects but reduces with further progression of diseases (Periodontitis)(36,38). This reduced level of beta-defensin in periodontitis subjects may be due to the predominance of destructive nature of the periodontal lesion that might have damaged the gingival epithelial cells which are the major source of the human beta-defensin 1 - 3. Also studies report that presence of intracellular glucose and insulin transcriptional activities are needed for optimal expression of human beta-defensin - 1 (hBD-1) expression(37). Another possible reason for the reduced levels could be the highly proteolytic environment from host or bacteria origin in the periodontally diseased tissue that could destruct the hBD-1 enzyme(39). The reduced levels of hBD - 1 should be considered clinically significant since it is a multifunctional antimicrobial peptide which is a potent regulator of gingival host response by initiating and at some level limiting the immune response like suppressing pro inflammatory cytokine, regulating complement system(37,40). There is accumulating evidence that it possesses antimicrobial activity against both gram positive and gram negative bacteria like Escherichia coli, Escherichia faecalis, B. megaterium, P. mirabilis etc.(36) by destroying their cell membrane without the need for an adaptive immune system(36). They are characterised by a group of 4 - 5 kDa open ended cysteine rich cationic peptides. Furthermore it plays a vital role in wound healing through the chemotaxis of $\alpha V\beta 3$ receptors that send signals for endogenous secretion of vascular endothelial growth factor (VEGF) which further helps in proliferation and migration of endothelial cells(36,41). This results in angiogenic stimulation which is a crucial step in angiogenesis. It also helps in migration of fibroblast through protein kinase C activation(42).
Thus, considering these biological regulatory roles of beta-defensins, an altered level of it in iPRF seems to bring about less desired effect at the wound site. However further research is recommended to establish whether an altered level of beta-defensin in iPRF really reduces the beneficial effects considering the presence of other antimicrobial protein peptides. Some of the limitations of the study are the smaller sample size and the cross-sectional design of the study with lack of randomisation of the study samples.

CONCLUSION

Thus, it can be concluded that periodontal health status influences the iPRF levels of beta-defensin, which can further influence the clinical outcomes of iPRF applications in wound healing and regeneration..

Clinical significance

iPRF form periodontally diseased subjects might have a reduced levels of anti-inflammatory cytokines (hBD-1) thereby predisposing to a compromised or incomplete healing potential at the site of application.

List of abbreviations

- Human beta-defensin – 1: hbD-1
- Injectable Platelet-rich Fibrin: iPRF
- Gingival crevicular Fluid: GCF
- Probing Depth: PD
- Full mouth plaque score: FMPS
- Full mouth bleeding score: FMBS
- Growth factors: GFs
- Platelet derived growth factor: PDGF-AA, PDGF-AB, PDGF-BB
- Transforming growth factor beta 1: TGFB1
- Vascular endothelial growth factor: VEGF
- Epidermal growth factor: EGF
- Insulin-like growth factor: IGF

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