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Review Article
Existence of Human Beta Defensin-1 Peptide on Periodontal Disease: An Updated Review Based on Case-control and Cross-sectional Studies

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Abstract

Background: Recently, several studies have tried to reveal the existence of Human Beta Defensin-1 (HBD-1) peptide in periodontal disease. However, the result of this interaction is still unclear and erratic. An analysis study from all reports from different sources was performed to evaluate and clarify these inconsistent results. **Materials and Methods:** Electronic search was conducted through several online databases (Google Scholar, Science Direct, PUBMED, NCBI and Cochrane.org). Articles written in english up to October, 2016 were involved in this analysis. Outcome and significant relationship amongst this peptide and periodontal status were analyzed. **Results:** Positive and negative findings data concerning the existence of HBD-1 in periodontal tissue linked to inflammation were found and investigated. Interestingly, localization of HBD-1 was found in oral stratified epithelium, sulcular epithelium but not in junctional epithelium. This issue was critical because junctional epithelium is well-known as port of entry for bacterial invasion and manifestation in gingivitis and periodontitis. **Conclusion:** Its existence was detected in both healthy and inflamed gingival tissue, but the level was varied upon different stage. Both case control and cross-sectional studies revealed conflicting findings. The HBD-1 gene polymorphism may serves as one of the reasons of these inconsistent findings.

Key words: Human beta defensin-1, periodontitis, periodontal disease

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Human Beta Defensins (HBDs) or Beta Defensins (BDs) or defensin beta-1 (DEFB-1) are a family of naturally antimicrobial peptides that produced by epithelial cells and expressed in many organs including gingival epithelium[1,2]. It is a group of small, cationic, cysteine-rich protein, with weight less than 7 kDa[3]. Gingival epithelium is constantly exposed to a variety of microbial challenge. As a part of periodontal apparatus, gingival epithelium was a first line of host defense mechanism to bacterial invasion; further destruction is leading to periodontal disease. Periodontal disease defined as an inflammatory disease (both chronic and acute progression) on dental supporting tissues[4]. In the epithelium, these peptides assumed has provided mechanical as well as chemical barrier activities against bacterial infection during periodontal disease[1]. Its explicitly, gingival epithelium serves a crucial function in local resistance and in inflammatory response during periodontal disease[5]. Defensins are able to improve adaptive immune system mechanism by attracting monocytes, T-lymphocytes, dendritic cells and mast cells to the infection area. It is also reported that the capacity of macrophage phagocytosis can be approved[6]. Immune host response is one of important elements in disease development and progression. It is reported that HBDs are able to deactivate the lipopolysaccharide (LPS) activity of Gram-negative bacteria[7]. Due to its varied benefit, further idea is coming to proposing HBDs for promising tool for clinical application such as local topical antibiotic, anti-inflammatory immune modulator and also wound healer[8].

From total of 31 types of HBDs[9], so far only three HBDs (HBD-1, HBD-2 and HBD-3) that have been analyzed, detected and confirmed in human oral epithelial cells[10-12]. In maintaining homeostasis, HBD-1 could be detected in epithelial cells. Whereas, HBD-2 and HBD-3 were positively elevated during disease process followed by IL-1b, TNFα and IF-γ activities[1]. Amongst other HBDs, it is reported that HBD-1 has a broad spectrum of antimicrobial action including Gram-negative, Gram-positive bacterial, fungal and viruses[2]. Some studies revealed the existence of HBD-1 in both mRNA expression and protein level, but some are not. Other studies also tried to clinically demonstrate the association of HBD-1 level with the severity of periodontitis[13]. Another interesting study concluded that salivary antimicrobial peptides are able to become biomarker for early stage of periodontitis[14]. Moreover, functional polymorphism of HBD-1 or DEFB-1 is potential marker for dental caries[15]. This lack and unsynchronized findings may contribute to hypothesize a new perspective idea of HBD-1 in dentistry especially periodontology. Thus, this study aimed to summarize previous studies of HBD-1 in periodontal disease and describe possible mechanism on how the HBD-1 effects in maintaining periodontal health.

MATERIALS AND METHODS

This study followed the preferred reporting items for systematical reviews and meta-analyses (PRISMA) statement guidelines. Screening protocol: Independent reviewer searched an electronic or online report from Google Scholar, Science Direct, PUBMED, NCBI and Cochrane.org. Only articles within up to October, 2016 were involved. These following key words or phrases were used: (Human Beta Defensin-1 or Beta Defensin-1 or Defensin-1 or HBD-1 or BD-1 or DEFB-1) and (Periodontitis or chronic periodontitis or aggressive periodontitis or periodontal diseases). Only full text-articles written in english up to October, 2016 were involved in this analysis. Titles and abstracts of papers were screened. Reviewers decided an article that meets the inclusion criteria by following the guidelines (Fig. 1).

Inclusion and exclusion criteria: The inclusion criteria for this analysis are: (1) A case-control study, cross-sectional study, randomized or not clinical trial study, prospective or retrospective studies, (2) Studies that analyzing the level HBD-1, expression of HBD-1 and periodontal status, (3) Sample collection from Gingival Crevicular Fluid (GCF), subgingival plaque and gingival biopsy, (4) Methods of sample analysis were: The enzyme-linked immunosorbent assay (ELISA), Polymerase Chain Reaction (PCR), real-time PCR and (5) No systemic diseases on subject. Whereas, the exclusion criteria are: (1) A case report/case studies, (2) Animal studies and (3) Duplicate publication.

Data compilation: Information data gathered from articles were compiled by reviewer. The data included: (1) First author's name, (2) Publication year, (3) Publication journal, (4) Country of population, (5) Study design, (6) Number of sample, (7) Sample collection, (8) Methods of analysis and (9) Outcome.

RESULTS AND DISCUSSION

Expression of HBD-1 in healthy periodontal tissue: Periodontal disease was begun with growth of plaque biofilm on the teeth surfaces or gingiva. Generally, numerous of oral bacteria in subgingival plaque are divided into pathogen and commensal bacteria. *Porphyromonas gingivalis*,

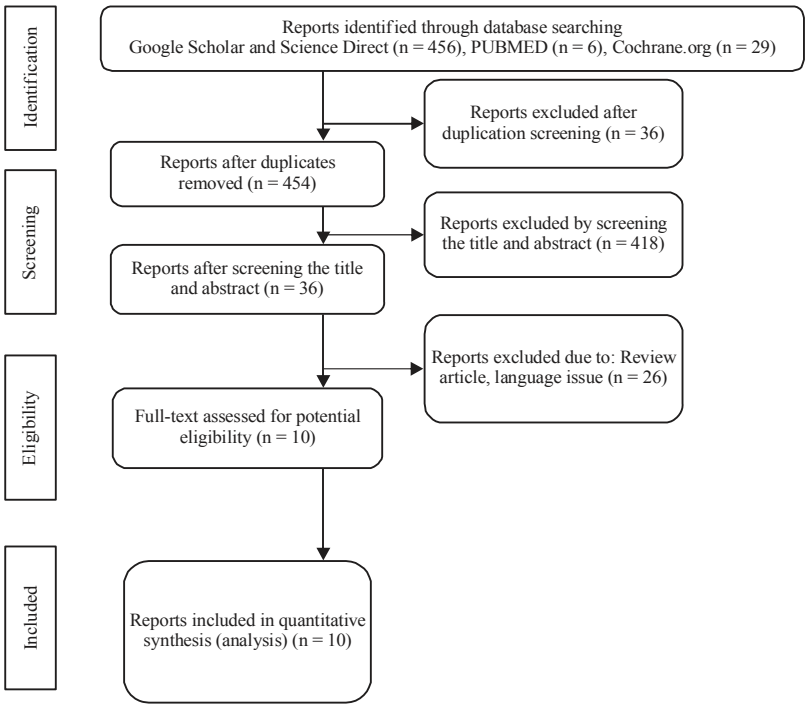

Fig. 1: PRISMA flowchart for screening protocol

Tannerella forsythia and *Treponema denticola* were classified to red complex pathogenic periodontal bacteria. *Aggregatibacter actinomycetemcomitans* also known as periodontal pathogenic bacteria. Besides, even though *Fusobacterium nucleatum* was not considered as a major perio pathogen bacteria, but it is reported that it plays crucial role in the development of biofilm. *Fusobacterium nucleatum* was found in both healthy and disease site, but its level was different[16]. Dale[10] reported in using immunohistochemistry that HBD-1 was detected in both healthy and inflamed gingival tissue induced by *Fusobacterium nucleatum*[10]. Localization of HBD-1 was found in oral stratified epithelium, sulcular epithelium but not in junctional epithelium[10,17]. This issue was critical because junctional epithelium is well-known as port of entry for bacterial invasion and occurrence in gingivitis and periodontitis. Figure 2 shows the location of HBD-1[18].

Studies of HBD-1 related to periodontal disease: From 36 eligible articles from screening process, 10 articles were met the criteria and analyzed. The summary was given in Table 1.

Dale postulated that different HBDs act in region-specific, moreover other α-defensin and LL37, derived from neutrophils may play a crucial role in junctional region[10]. Wang *et al.*[19] reported that HBD-1 mRNA expression in healthy and periodontitis were not significantly different ($p<0.05$), but interestingly HBD-1 was found to be the highest expression amongst other HBDs[19].

Saitoh *et al.*[1] revealed that mRNA of HBD-1 expression has a statistically correlated with mRNA TNFα expression ($r = 0.668$, $p<0.0025$)[1]. This finding might suggests that HBD-1 was induced by inflammatory cytokine such as TNFα. Higher expression of TNFα in tissue might serves as indicator for an active and severely inflammation process. In contrast with Dale[10] and Saitoh *et al.*[1] it is concluded that HBD-1 strongly expressed in inflammatory tissue that other beta defensins[1]. Li *et al.*[3] found no significant difference of HBD-1 both in mRNA gene expression and protein level amongst healthy and inflamed groups. It has been clarified that HBDs contribute in innate immune system but do not involved with cytokines interaction during periodontal disease process[3]. Bissell *et al.*[20] also reported that no differences were detected in mRNA expression of HBD-1 between healthy and inflamed subjects[20].

Liu *et al.*[5] did experimental study on gingival keratinocyte cells collected from healthy and periodontitis tissues. Researchers treated cells using different concentration of TNFα (100, 150 and 200 ng mL^{-1}). Researchers found the significant up-regulated mRNA HBD-1 expression in healthy tissue and down-regulated mRNA HBD-1 expression in

Table 1: Summary of articles related to the existence of human β-defensin-1 (HBD-1) and periodontitis

References	Country	Study design	Sample size-sample collection	Assessment method	Measuring unit	Outcome (p-value)
Dale and Krisanaprakornkit[11]	United State of America	CS	>15 (Inflamed GT and control)	IHC	Relative expression (%)	HBD-1 peptides were detected in all gingival samples
Lu *et al.*[17]	Hongkong	CS	29 GT (22 periodontitis, 7 control)	IHC	Relative expression (%)	The mean expression levels of HBD-1 in periodontitis tissues (18.12±16.71%) were significantly higher than in healthy tissues (8.79±7.04%) ($p<0.05$)
Saitoh *et al.*[1]	Japan	CS	20 GT (3 hyperplasia gingiva, 10 gingival abscesses, 7 fenestration)	RT-PCR	Relative expression (%)	The expression of TNF-a mRNA was associated with HBD-1 mRNA ($r = 0.668$, $p<0.0025$)
Bissell *et al.*[20]	United State of America	CS	49 GT (29 periodontal disease, 20 control)	RT-PCR	Relative expression (%)	No statistically significant different between HBD-1 expression in healthy (65%) and diseases group (75.9%) ($p>0.05$)
Vardar-Sengul *et al.*[2]	Turkey	CC	55 GT (15 gingivitis, 15 aggressive periodontitis, 15 chronic periodontitis)	RT-PCR	Relative expression (%)	Expression of HBD-1 gene was significantly lower in gingivitis and higher in the chronic periodontitis group, than in the control group ($p<0.001$)
Ertugrul *et al.*[22]	Turkey	CC	80 GCF of 20 gingivitis (HG), 20 periodontitis (HP), 20 diabetes type 2-gingivitis (DM2G) and 20 diabetes type 2-periodontitis (DM2P)	ELISA	pg mL^{-1}	HP group had significantly higher levels of HBD-1 compared to HG group ($p<0.05$)
Liu *et al.*[5]	China	CS	20 GT (10 chronic periodontitis, 10 control)	RT-PCR	Relative expression (%)	Significantly lower mRNA expression of HBD-1 were found in chronic periodontitis lesions compared to healthy tissues ($p \leq 0.05$)
Wang *et al.*[19]	China	CS	54 GT (25 chronic periodontitis, 29 control)	RT-PCR	Relative expression (%)	The HBD-1 gene-expression levels were slightly lower in chronic periodontitis compared to healthy group. But, no significant differences were found ($p>0.05$)
Li *et al.*[3]	China	CS	96 GT (46 chronic periodontitis, 50 control)	IHC and RT-PCR	Relative expression (%)	No significant mRNA expression of HBD-1 in healthy and periodontitis samples ($p<0.05$)
Sulijaya *et al.*[13]	Indonesia	CS	104 (GCF) of chronic periodontitis patients	ELISA	pg mL^{-1}	The levels of HBD-1 in mild periodontitis group were tended to be higher than severe periodontitis group ($p = 0.087$). But, the difference was not statistically significant ($p<0.05$)

Study design; CS: Cross-sectional, CC: Case control, Sample collection; GT: Gingival Tissue, GCF: Gingival crevicular fluid, Assessment methods; IHC: Immunohistochemistry, RT-PCR: Real time-polymerase chain reaction, ELISA: Enzyme-linked immunosorbent assay

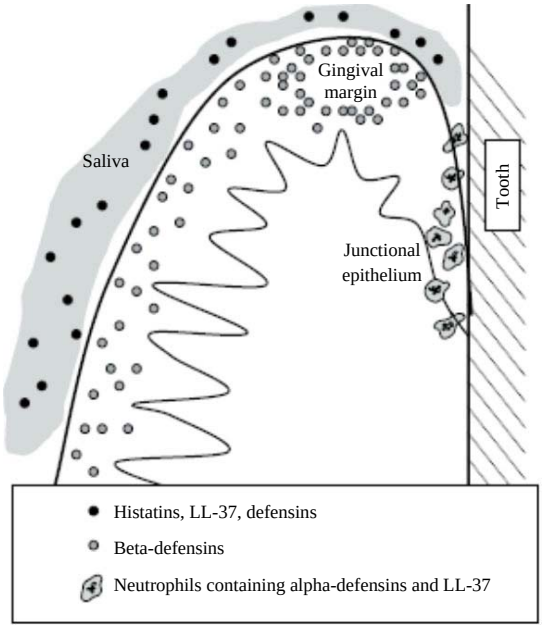

Fig. 2: Defensins distribution in periodontal tissue[18]. Localization of HBD-1 was found in oral stratified epithelium and sulcular epithelium in gingival margin

periodontitis tissue ($p<0.01$)[5]. Other study supported this idea that HBD-1 constitutively expressed and its expression during induction was highly varied based on different stimuli[21]. Moreover, Liu *et al.*[5] clearly demonstrated both NF-kB and MAPK signaling pathways of HBD inducted with TNFα, same as signaling pathway in responses to inflammatory cytokine[5].

Related to the severity of disease, some study conducted by Ertugrul *et al.*[22] found that significantly higher protein level of HBD-1 from chronic periodontitis than gingivitis subjects ($p<0.05$)[22]. Sulijaya *et al.*[13] revealed that HBD-1 protein level was tend to be higher in severe chronic periodontitis than other[13]. Vardar-Sengul *et al.*[2] reported up-regulated mRNA gene expression of HBD-1 in chronic periodontitis and down-regulated in gingivitis and aggressive periodontitis groups ($p<0.001$)[2]. These findings might be happened due to higher activity of pro-inflammatory cytokines induction during more severe infection process and moreover an immune deficiency occurred in aggressive periodontitis. Understanding of concentration of HBD-1 in different stages of disease will help us to clarify the host mechanism responses and treatment prognosis. Interesting statement was made by Krisanaprakornkit *et al.*[23] that *F. nucleatum* may help to retain gingival epithelial cells stimulated and will produce HBD continuously to maintain host defense[23].

Additional confounding factor that might influence in this inconsistent result is because of variable copied gene number of HBD-1 polymorphism[24,22]. Growth factor also reported can contribute to these different results[25]. Activity of keratinocytes cells in junctional epithelium and pocket epithelium was hypothesized become another reasons for different findings[17]. Others have assumed that from healthy gingiva toward to disease formation, innate immune was replaced with adaptive immune mechanism overtime[26]. Moreover, mRNA expressions does not always equally to protein concentration. It means that higher mRNA expression of HBD-1 does not always perform higher protein level[16].

Some mechanisms tried to link the existence of HBD-1 on periodontal disease, such as: Colonization of bacteria enhances the production of HBD-1 which acts as an inhibitor. But some periodontopathogenic bacteria such as *A. actinomycetemcomitans*, *P. gingivalis*, *T. denticola*, *T. forsythia* can survive and invade gingival tissue. The HBD-1 stimulates the production of chemokines (Interleukin-8 and CCL2) from dendritic cells and become chemo-attractants. Thus, phagocytes and lymphocytes were brought to the infection site[19].

In inflamed tissue, defensins were predominantly secreted by epithelial cells (Ep), keratinocytes and infiltrating neutrophils (N). Figure 3 shows that (a) Defensin will stimulate the recruitment of immature dendritic cells (iDCs) to the sites of infection and (b) Assist antigen (Ag) uptake by developing a 'Defensin-Ag' complex, (c) Defensins stimulate the maturation of iDCs to mature (mDCs) through Tumor

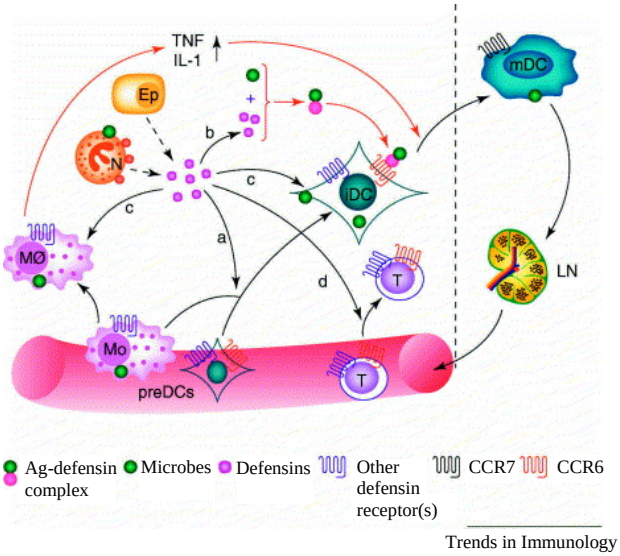

Fig. 3: Possible mechanisms of defensins during infection[19], (a) Defensin will stimulate the recruitment of immature dendritic cells (iDCs) to the sites of infection, (b) Assist antigen (Ag) uptake by developing a 'Defensin-Ag' complex, (c) Defensins stimulate the maturation of iDCs to mature (mDCs) through Tumor Necrosis Factor (TNF) and interleukin 1 (IL-1) induction and (d) Defensins also contribute to as mediator for adaptive antimicrobial immunity by facilitating the recruitment of T-cells to infected site

Necrosis Factor (TNF) and interleukin-1 (IL-1) induction and (d) Defensins also contribute to as mediator for adaptive antimicrobial immunity by facilitating the recruitment of T-cells to infected site[27-29].

CONCLUSION

The expression of HBD-1 in healthy tissue may function as preventing agent to the onset and or progression of disease. Its existence was detected in both healthy and inflamed gingival tissue, but the level was varied upon different stage. The HBD-1 was expressed in oral stratified epithelium, sulcular epithelium but not in junctional epithelium. The HBD-1 gene polymorphism becomes one of the reasons of inconsistent findings.

SIGNIFICANCE STATEMENTS

The existence of Human Beta Defensin-1 (HBD-1) in periodontal disease is important to be investigated. Since it is reported that HBD-1 has a potential of antimicrobial activity against Gram-negative, Gram-positive bacterial, fungal and viruses. Several studies have tried to reveal the role of this peptide. However, the results are inconsistent. In this study, author found the localization of HBD-1 was primarily expressed in oral stratified epithelium, sulcular epithelium but not in junctional epithelium. The idea of HBD-1 in defense mechanism during periodontal inflammation was suggested not correlated. This issue becomes critical because junctional epithelium is port of entry for bacterial invasion in gingivitis and periodontitis.

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