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Literature Review

Human Beta-defensin-1 and Periodontal Disease: Past, Present, and Future

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ABSTRACT

Human beta-defensin-1 (HBD-1), a peptide released by the immune system, has been investigated for its association with periodontal disease. Several studies have found positive findings of its expression related to disease progression, whereas others have reported some tendencies. This review highlights studies associated with HBD-1 and periodontal disease in both primary and clinical investigations, as well as the underlying mechanism and discusses further research possibilities for HBD-1. HBD-1 acts as an innate immune apparatus and mediating the adaptive immune system; therefore, its role in the pathogenesis of a periodontal disease is indisputable. HBD-1 is mainly expressed in the oral stratified epithelium and sulcular epithelium, where the barrier junction properties (e.g., E-cadherin and β-catenin in the adherent junction) of the gingival epithelial cells serve as the first line of defense against periodontal infection. The HBD-1-reinforced epithelium therefore provides both a mechanical and a chemical barrier action. Critical issues that arise in the gingival epithelium can therefore be alleviated by modulation of HBD-1 production to enhance its protective function. The antimicrobial, anti-inflammatory, and wound healing properties of HBD-1 support its use as a promising clinical treatment strategy.

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INTRODUCTION

Human beta-defensins (HBDs), which are peptides secreted by the immune system, are presently under investigation for their association with the balance between healthy and disease states.[1] They exhibit broad-spectrum antimicrobial activity against bacteria by deactivating the lipopolysaccharide (LPS) activity of gram-negative bacteria,[2] and they are effective against fungi and some viruses.[3] Recently, among the 31 known sub-families of HBDs, three (HBD-1, HBD-2, and HBD-3) have been investigated for their association with maintaining homeostasis in humans.[4–6] HBD-1 has a broader antimicrobial action than other HBDs and is active against both gram-negative and gram-positive bacteria.[7] The mechanism of this antibacterial action, as determined by scanning electron microscopy studies, involves entrapment of the bacteria by nets of HBD-1 as observed following incubation of *Escherichia coli* (*E. coli)* with HBD-1.[8] These findings suggest that, in theory, HBD-1 should show a consistent presence and influence. However, clinical investigations of HBD-1 levels and periodontal status have variously reported positive results, a positive trend, and even no association.[7,9–14]

HBD-1 gene polymorphisms are suspected to be confounding factors that could explain these inconsistent results.[11,15] Several studies have therefore focused on 52G>A (rs1799946), -44C>G (rs1800972), and -20G>A (rs11362) polymorphisms.[16–21] The results point to a possible relationship between *DEFB1* (the gene for HBD-1) and the development of periodontal disease.16 However, the effects of these variants of *DEFB1* on HBD-1 secretion or periodontal tissue modification are unknown.

Evidence is presently lacking for an association between HBD-1 and periodontal disease, either *in vitro, in vivo,* or in clinical studies. Some niches and pathways remain in question, so more precise, detailed, and focused studies are needed to explore the influence of HBD-1 on periodontal disease. The aim of this review article is to discuss the presence of HBD-1 and its function in the pathogenesis of periodontal disease based on existing *in vitro, in vivo,* and clinical studies. In particular, studies of *DEFB1* polymorphism are highlighted, and suggestions are made for further investigations that could provide better insight and understanding for researchers and clinicians.

This review follows the Participant Intervention Comparison Outcome (PICO) guidelines.[22] The participants/population are cells, animals, and/or subjects related to periodontal disease with or without intervention, which is inflammation-related stimulation. Non-periodontal disease, as the negative control, is used for comparison. The measured outcome is HBD-1 production (gene expression, protein levels, and location) or *DEFB1* detection. The literature search was conducted using electronic databases, including PubMed, Scopus, and EBSCO. Keywords were "Human Beta Defensin-1," "HBD-1," "Defensin-1," "*DEFB1*," "Periodontitis," and "Periodontal disease." *In vitro, in vivo,* and clinical studies written as full texts in English and published within the past 10 years were included in the review. Duplications and studies limited to abstracts were excluded from this analysis. Manual screening for critical appraisal was achieved from supporting journals. All journal papers included in this review are discussed and elaborated in the form of narratives or paragraphs.

HUMAN BETA-DEFENSIN-1

Three beta-defensins have been identified in humans, namely human beta-defensin-1 (HBD-1), HBD-2, and HBD-3.[23] Both HBD-1 and HBD-3 exhibit antimicrobial effect against gram-positive and gram-negative bacteria, as well as adenoviruses,[24,25] whereas HBD-2 specifically targets gram-negative bacteria and Candida albicans.26 In addition, HBD-2 is tenfold more effective than HBD-1 against *E. coli*.[26] HBD-1 is a small cysteine-rich protein weighing less than seven kDa and containing three disulfide bonds.[27] It is naturally produced by epithelial cells[7,14,28] and is constitutively expressed in skin keratinocytes and in urinary and respiratory tract epithelial cells, as well in gingival epithelial cells.[29–32] Its expression can also be detected in saliva and gingival crevicular fluid.[33–35] Given its location, HBD-1 represents the first line of defense against invading periodontal pathogens.

In their association with systemic disease, defensins are known to maintain homeostasis by restraining bacterial overgrowth and fending off commensals and pathogens.[36,37] HBD-1 reportedly exerts its antimicrobial activity by modulating the human intestinal microbiota.[38] In vivo studies on the human colon have localized HBD-1 in the mucus,[39] while HBD-1 is also expressed in the urinary system, including the bladder, distal nephrons of the kidney,[40] distal ureter,[41] and urothelium.[42] The finding that the mRNA level for HBD-1 was ten times higher in the kidney than in the ureter and bladder[41] has been considered to reflect an abundant expression of HBD-1 by the epithelial lining of the nephron and secretion of HBD-1 into the urinary tract.[40] HBD-1 levels are increased in pyelonephritis patients,[43] and HBD-1 shows elevated antibacterial activity against Escherichia coli in an in vitro inflammatory disease model of the urinary tract.[43] *In vivo* investigation using Beta Defensin-1 (*DEFB-1*) knockout ($DEFB1^{-/-}$) mice also revealed an increased risk of bacteriuria in the absence of HBD-1 expression.[42] An *in vivo* study of the protective role of

HBD-1 in a mouse model of urinary tract disease showed that HBD-1 prevented spontaneous infection based on the bacterial inoculation level. HBD-1 prevented infection against inoculations of 10^4 to 10^6 CFU of *E. coli*, but not against an inoculation of 10^8 CFU,[44] suggesting that HBD-1 secretion via mucus and HBD-1 expression in the urinary tract resolved the infection and aided the immune system in maintaining homeostasis.

ROLE OF HUMAN BETA-DEFENSIN-1 IN THE PATHOGENESIS OF PERIODONTAL DISEASE

Several clinical investigations have attempted to confirm the function of HBD-1 and to find the link between its level and periodontal disease severity. Patients with periodontitis showed higher HBD-1 mRNA expression than healthy subjects, and HBD-1 showed the highest expression among all HBD forms.[13] The association between HBD-1 and inflammation was also determined to be mediated by tumor necrosis factor α (TNFα). In young children, mRNA expression of HBD-1 was correlated with the expression level of TNFα mRNA ($r = 0.668$, $p<0.0025$).[28]

However, other clinical studies failed to find an association between HBD-1 level in diseased versus healthy subjects.[14,45] The HBD-1 protein level was also higher in patients with periodontitis than in patients with gingivitis,[11] as well as in healthy controls.[46] By contrast, patients with mild chronic periodontitis exhibited a tendency toward higher expression of HBD-1 compared to the other groups.[10] One possible explanation for these inconsistent findings is that HBD-1 contributes to the innate immune system. However, its involvement during the periodontal disease process remains unclear due to the strong induction of pro-inflammatory cytokines.[14,47] Nonetheless, polymorphism of the HBD-1 gene seems to be another reason.

Gingival keratinocyte cells collected from patients with periodontitis and healthy subjects showed a significant upregulation of mRNA expression of HBD-1 in healthy cells and downregulation in periodontitis cells in response to stimulation with TNFα (100, 150, and 200 ng/ml).[12] Wide variations in constitutive expression of HBD-1 have also been reported in response to different stimuli.[48] One recent study clarified the mechanism by which a microbial metabolite, 10-Hydroxy-cis-12-octadecenoic acid (HYA), reduced alveolar bone loss in a mouse model of periodontitis by finding that HYA induced a 1.4-fold increase in defensin (DEFB4A) expression and improved the epithelial barrier junction component in the epithelial cell, especially E-cadherin.[49] These findings might support the idea that HBD-1 maintains homeostasis in both keratinocytes and epithelial cells.

A critical issue that has attracted attention is the detection of HBD-1 mainly in the oral stratified epithelium and sulcular epithelium, but its absence in the junctional epithelium.[6] This finding somehow opposes the proposed theory of a defense role for HBD-1, since the port of entry for pathogens is the junctional epithelium.[47] However, other studies have clarified that defensins derived from neutrophils might be directed to protect against periodontal disease since the neutrophil defensins are expressed in the junctional region.[5] As shown in Figure 1, the colonization of bacteria and even the physiological response could promote the production of HBD-1. Although some periodontal pathogens, such as *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia*, can survive and invade gingival tissue,[13] it is investigated that defensins are also able to deactivate the lipopolysaccharide activity of Gram-negative bacteria.[2]

HBD-1 stimulates the production of chemo-attractant chemokines, including IL-8 and CCL2, in dendritic cells, thereby recruiting phagocytes and lymphocytes to the infection site.[13] The epithelial cells and neutrophils in inflamed tissue also produce defensin in response to physiological and pathological conditions, thus HBD-1 plays roles in both innate and adaptive immune system responses. It also triggers the attraction of monocytes, T-lymphocytes, dendritic cells, and mast cells to the infection site,[50] as summarized in Figure 2.

The mechanism by which HBD-1 entrapped bacteria was also revealed by scanning electron microscopy images showing abundant HBD-1 within net-like structures surrounding the *E. coli* cells.[8] Incubation of *E. coli* cells with HBD-1 resulted in the formation of these nets, supporting a previous hypothesis of an interaction between HBD-1 and the bacterial cell envelope.[51,52] Transmigration assays confirmed that this entrapment system inhibited the translocation of *Klebsiella pneumoniae* from the upper to the lower compartment.[8] The net formation was also seen under physiological conditions in duodenal fluid, which contains high levels of proteases.[8] Thioredoxin-modified HBD-1, also known as disulfide bond-modified HBD-1, showed an even more potent antimicrobial activity.[37] These findings verified that net formation is an additional protective action of HBD-1.

HBD-1 GENE POLYMORPHISM

Polymorphism of HBD-1 has been proposed as the main reason for the conflicting genotype results seen in patients with periodontitis.[10,11,15,46] Three polymorphisms in the *DEFB1* 5' untranslated region (UTR), at position g. -52G>A (rs1799946), g. -44C>G (rs1800972), and g. -20G>A (rs11362), appear to be

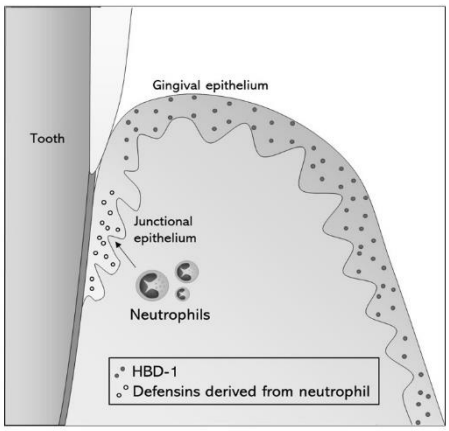

Figure 1. The distribution of human beta-defensin-1 (HBD-1) in periodontal tissue. HBD-1 is found in the oral stratified epithelium and sulcular epithelium; while defensins derived from neutrophils are detected in the junctional epithelium.[1,45,47]

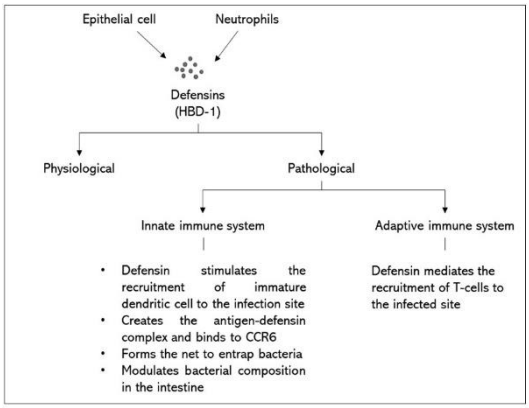

Figure 2. Mechanism of HBD-1 suppression of periodontal disease. Epithelial cells and neutrophils produce defensin (HBD-1) in both physiological and pathological processes. In the pathological process, defensin will stimulate the recruitment of immature dendritic cells, create the antigen-defensin complex, form a net that entraps bacteria, and thereby modulate bacterial composition. Defensin also mediates the migration of T-cells to the infected site.[8,13]

involved in the secretion of HBD-1,[52] as shown in Table 1. These single nucleotide polymorphisms (SNPs) were significantly associated with the susceptibility of having periodontitis among Japanese[18] and European patients,[16,19,20] but not in Americans,[17] suggesting ethnic differences.

In brief, the association between DEFB1 g. -20G>A and periodontal conditions is inconsistent, with some studies claiming no association.[16,17,53] By contrast, the g. -20G/G genotype was detected more frequently in periodontitis patients,[20] and the g. -20A allele was confirmed to decrease *DEFB1* gene expression.[54,55] Patients with diabetes mellitus (DM) also show genetic variations in the *DEFB1* gene (SNP -20: G allele, GA, and GA genotypes) and the *DEFB1* 5'UTR haplotypes (GCG and ACG), which might be associated with the known susceptibility of patients with DM to periodontitis; patients with chronic periodontitis but without DM also show these variations.[21] Therefore, the g. -20G polymorphism may be related to a greater production of HBD-1, which would lead to an immune system over-reaction and increase the risk of developing periodontitis.

An association of the g. -44G/C *DEFB1* SNP (rs1800972) with periodontitis susceptibility was also reported in Japanese patients with severe and moderate chronic periodontitis. The g. -44CC genotype was correlated with periodontitis (OD 2.510), severe chronic periodontitis (OR 4.154), and moderate-to-severe periodontitis (OR 4.038).[18] Another study found no association of g. -44 C/G with early-onset periodontitis,[56] also known as juvenile or aggressive periodontitis. A further study demonstrated that the position -44 was influenced by the putative nuclear factor kappa beta (NFκB) binding site such that a change would alter the NFκB affinity and HBD-1 regulation at the transcription step.[57]

The rare *rs1800972* allele has been proposed to be a protective allele.[58] By contrast, the rs1047031 variant alters the function of HBD-1 in the maintenance of the epithelial barrier.16 The rs1047031 SNP has five bp located inside the 3' UTR and the sequence that includes the 5' and 3' UTRs shows no significant changes in the polymorphism.[16] Therefore, SNP rs1047031 itself is assumed to increase the susceptibility to periodontal disease.

POSSIBLE FURTHER STUDIES

A recent classification of periodontal disease has been published, and both chronic and aggressive periodontitis are now combined into a single periodontitis group.[59] For this reason, further clinical investigations of HBD-1 expression or levels should be focused on the staging and grading of periodontitis rather than the concept of chronic or aggressive disease. HBD-1 is predominantly produced by epithelial cells that are distributed in the oral and sulcular epithelium; therefore, a mucosal swab is recommended to provide an epithelial sample of the marginal and sulcular gingiva. Further investigations on gene polymorphisms should also be conducted on larger numbers of racially matched subjects. Overall, the influence of the -52G>A (rs1799946), -44C>G (rs1800972), and -20G>A (rs11362) *DEFB1* genes polymorphisms point to a clear pattern that is linked to the susceptibility to periodontitis.

Table 1. Recent studies of HBD-1 gene polymorphism associated to periodontitis

Author (year)	Population	Number of subjects	Gene polymorphism	Result
Schaefer et al. (2010) [16]	Germany and the Netherlands	Total 3128 (German with CP (675), CP controls (262); German with AP (368), AP controls (1104); Dutch with CP (130), CP control (57); Dutch with AP (164), AP controls (368))	(G/A) rs11362 (C/G) rs1800972 (G/A) rs1799946	The rs1800972 SNP was slightly associated in CP and AP groups. In details, it was driven by the low frequency of the rare G allele seen in AP, but not in CP
Oztruk et al. (2010) [17]	USA	Total 101 (CP and AP)	-52G>A (rs1799946) -44C>G (rs1800972) -20G>A (rs11362)	No association between DEFB1 gene markers and periodontal disease was found
Ikuta et al. (2013) [18]	Japan	Total 105 (28 Severe CP, 13 Moderate-mild CP, 21 AP, and 43H)	-52G>A (rs1799946) -44C>G (rs1800972) -20G>A (rs11362)	The DEFB1 -44C>G was associated with CP (OR 2.51), particularly with severe CP (4.15) and with combined severe and moderate CP (4.04). -44 CC genotype may be associated with susceptibility to CP in Japanese
Sarkisyan et al. (2016) [19]	Russia	Total 142 (P, URTD, H)	-44C>G (rs1800972) -20G>A (rs11362)	Association was found of URTD and P with the marker of DEFB1 -44C>G
Zupin et al. (2017) [20]	Italy	Total 594 (439 CP and 155 H)	-52G>A (rs1799946) -44C>G (rs1800972) -20G>A (rs11362) c*5G>A (rs1047031) c*87A>G (rs1800971)	DEFB1 -20G>A and -44C>G were found to be associated with CP
Rayanne et al. (2018) [21]	Brazil	Total 280 (116 DM2+CP, 95CP, and 69H)	-52G>A (rs1799946) -44C>G (rs1800972) -20G>A (rs11362)	Association found for the DEFB1 -20G>A (rs11362); G allele, GA and GG genotypes were significantly more frequent in DM2+CP (59.5%, 50% and 34%, respectively) and CP (61%, 44.2%, and 38.9%, respectively) than in healthy subject (26.8%, 36.2%, and 8.7%, respectively)

In vitro and in vivo studies are still needed that focus on the antimicrobial effect of HBD-1 against periodontal bacteria, and many opportunities exist for exploration of the action of HBD-1 by selecting different study designs. The idea that HBD-1 acts as an immune-modulator is also promising because the current trends in periodontal therapy approaches are pointing toward host modulation therapy. Therefore, further investigations on the intracellular HBD-1 signaling pathways and HBD-1 interactions with inflammatory mediators are also recommended.

CONCLUSION

These investigations and studies show that the role of HBD-1 in maintaining homeostasis is undeniable, especially in periodontal tissue. The potential shown by HBD-1 against periodontal pathogens varies due to differences in the severity of the disease and in the patient's genetic background, also known as polymorphism. Further investigations are needed to provide a better understanding of the involvement and interaction of HBD-1 in periodontal disease.

CONFLICT OF INTEREST

Authors declare no interest in businesses regarding the content of this manuscript.

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