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ORIGINAL ARTICLE *Volume 14 (Issue 4), 2025*

PAKISTAN JOURNAL OF MEDICINE & DENTISTRY

Exploring Antimicrobial Peptides in Preventive Dentistry: A Novel Approach to Oral Microbiome Management

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ABSTRACT

Background: The oral microbiome plays a major role in maintaining oral health. Antimicrobial peptides like AMPs provide strong antifungal and antimicrobial properties. This study evaluated the expression levels of these genes in response to antimicrobial interventions for managing the oral microbiome.

Methods: This cross-sectional study (June 2022 to December 2022) contained 60 subjects: healthy subjects (n = 20) and gingivitis patients (n = 40). OpenEpi 3.0.0. was used for sample size calculation. It was conducted at Baqai Medical University and analysed at Liaquat College of Medicine and ISRA University Karachi. Total RNA was extracted and converted to cDNA for qRT-PCR analysis. The gene expression levels of HTN1 and HTN3, normalized to GAPDH, were analysed. ANOVA and t-tests were used to assess statistical significance ($p < 0.05$). SPSS v20 was utilized to perform these tests.

Results: Demographic variables like age, gender, smoking, and index did not differ significantly. qPCR revealed that chlorhexidine administration resulted in a 2.5 ± 0.31–fold and a 1.9 ± 0.27–fold increase in HTN1 and HTN3 expressions, respectively ($p < 0.001$). Application of fluoride resulted in a 1.8–fold increase in HTN1 ($p < 0.01$) and a 1.6–fold increase in HTN3 ($p < 0.01$), and LL-37 mimetics caused a 2.1–fold rise in HTN1 expression ($p < 0.01$) and a 1.7–fold elevation in HTN3 gene expression ($p < 0.01$).

Conclusion: The administration of antimicrobial agents modifies HTN1 and HTN3 gene activity for potential use in managing oral diseases.

Keywords: Histatins, PCR, Antimicrobial Peptides, Oral Health, Gene Expression.

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Citation: Tasleem F, Rafiq T, Younus A, Ambrin, Aleem A, Imtiaz A Exploring Antimicrobial Peptides in Preventive Dentistry: A Novel Approach to Oral Microbiome Management. Pak J Med Dent. 2025 September;14(4): A-B. Doi: https://doi.org/10.36283/ziun-pjmd14-4/003.

Received: Mon, April 21, 2025 **Accepted:** Fri, August 15, 2025 **Published:** Mon, September 29, 2025

INTRODUCTION

The oral microbiome plays a major role in maintaining oral health and strives for a balance between commensal and pathogenic microorganisms [1]. This imbalance can result in bacterial dysbiosis, which can eventually lead to gingivitis and periodontitis [2]. The Properties of antimicrobial peptides (AMPs), especially histatins (HTN1, HTN3), which exhibit strong antimicrobial, antifungal, and wound healing properties, are considered essential components of innate immunity [3,4]. Their expression, under the influence of antimicrobial interventions, can be used to evaluate their role in oral disease management [5]. Salivary glands secrete histatins, which play a role in regulating microbial colonization in the oral cavity. HTN1 and HTN3 contribute to the host defense mechanism, exhibiting significant antimicrobial activity against Candida albicans and various bacterial species [6,7]. Although these peptides can be expressed at different levels, the expression levels of these peptides may vary in the presence of various pathological conditions like gingivitis and periodontitis [8,9]. Knowledge about the variations caused by antimicrobial treatments, such as natural and synthetic agents, and how they affect histatin expression, can provide useful information for developing novel strategies for oral microbiome management [10].

Therefore, a study was required to evaluate the role of AMPs in detail against antimicrobial and antifungal actions in the oral cavity.

The purpose of this study was to evaluate the levels of HTN1 and HTN3 gene expression following antimicrobial intervention in healthy individuals, gingivitis patients, and periodontitis patients. Using quantitative real-time PCR (qRT-PCR), the gene expression levels of histatins were determined to assess the effect of each treatment, including chlorhexidine, fluoride, and plant AMPs. It compared the intervention and control groups to determine whether antimicrobial intervention could modulate histatin expression and correlate with oral health status. This research delivered molecular information about histatins' regulatory pathways while establishing their potential role in preserving oral health.

METHODS

This cross-sectional study was conducted at Baqai Medical University and analysed at Liaquat College of Medicine and ISRA University Karachi from June 2022 to December 2022. Institutional study approval (EC/06-2023) was obtained, and saliva with gingival epithelial cell samples were obtained from 20 healthy individuals, 40 gingivitis patients using standard oral sampling protocols after informed consent. The sample size was calculated using the OpenEpi 3.0.0 software on the basis of a 1.5-fold expected difference in gene expression with 80% power, and 5% significance level. Passive drool and oral swab techniques were used to collect saliva samples and gingival epithelial cells samples, respectively. Samples were immediately stored at −80°C for preservation of RNA. The subjects were divided into treatment and control groups. Antimicrobial interventions (0.12% chlorhexidine, 1,500 ppm fluoride, and plant-derived LL-37 mimetics) were practiced on the treatment group as per the manufacturer's guidelines. It is not surprising that the control group received no antimicrobial treatment. Histatin gene expression changes were assessed by collecting samples before and after treatment every 48 hours.

For RNA extraction, the Pure Link RNA Mini Kit (ThermoFisher, AM1931, USA) was used following the manufacturer's instructions. Thus, RNA concentration and purity were measured using NanoDrop (ng/µL, 260/280 ratio) and were subjected to cDNA synthesis using the High-Capacity cDNA Reverse Transcription Kit (ThermoFisher, AM7832, USA). In silico PCR using the UCSC Genome Browser was performed to validate the designed specific primer sets for HTN1, HTN3, and GAPDH (housekeeping gene). Gradient PCR optimization was performed, and qRT-PCR was conducted with the SYBR green master mix (Applied Biosystems) under optimized cycling conditions. Relative gene expression analysis was performed by employing the ΔΔCt method.

Significance testing ($p < 0.05$) was performed using SPSS v20 with ANOVA or t tests. Regression analysis was performed to assess correlations between histatin expression levels and oral health status.

RESULTS

Table 1: Baseline Demographic and Clinical Characteristics

Characteristic	Control Group (n=20)	Treated Group (n=40)	p-Value (ANOVA)
Age (years)	51.2 ± 6.9	52.8 ± 8.1	0.42
Male: Female	10:20	11:19	0.80
Smoking (%)	15%	18%	0.65
Plaque Index (Score)	2.1 ± 0.5	2.3 ± 0.6	0.28

60 samples were collected from the study population (21 male, 39 female) with an average age of 52±7.5 years. Demographic data revealed no significant variation in age, gender, and oral baseline hygiene habits among the two groups. The demographic data is shown in **Table 1**

Table 2: Comparison of HTN1 and HTN3 Gene Expression Levels (MEAN ± SD) Between Control and Antimicrobial-Treated Groups

Variable	Control Group (Mean ± SD)	Treated Group (Mean ± SD)	*p*-Value (ANOVA)
HTN1 Expression	1.00 ± 0.15	2.5 ± 0.31	*< 0.001***
HTN3 Expression	1.00 ± 0.18	1.9 ± 0.27	*< 0.001***

qRT-PCR analysis revealed significant differences in HTN1 and HTN3 expression across the study groups. In untreated gingivitis patients, HTN1 and HTN3 expression was significantly downregulated compared to healthy individuals ($p < 0.01$). The comparison between HTN1 and HTN3 is shown in **Table 2**.

Following antimicrobial treatment, both genes showed upregulation in the treatment groups, with the highest increase observed in the gingivitis patients. The chlorhexidine-treated group showed a 2.5-fold increase in HTN1 expression and a 1.9-fold increase in HTN3 expression relative to baseline (*p*

< 0.001). Fluoride and LL-37-mimetic treatment groups exhibited similar trends, with fluoride leading to a 1.8-fold increase and LL-37-mimetics showing a 2.1-fold increase for HTN1 ($p < 0.05$). A significant positive correlation was found between serum albumin levels and histatin expression ($r = 0.52$, $p = 0.003$), indicating that patients with higher albumin levels showed better histatin gene upregulation following antimicrobial treatment. The fold expression data is shown in **Figure 1.**

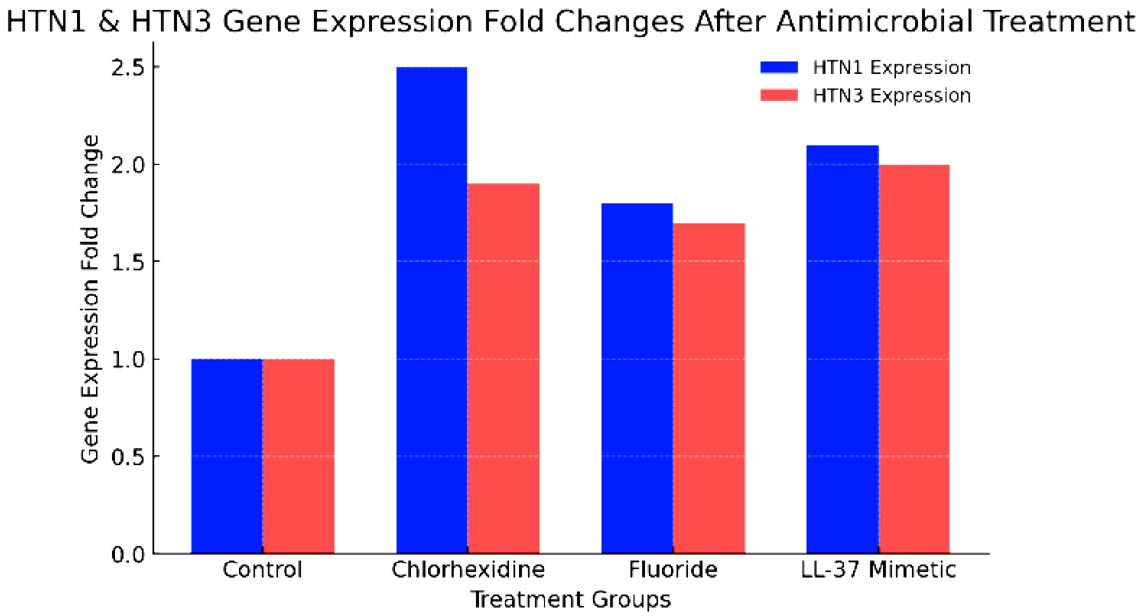

Figure 1: Expression Profiling of Histatins

DISCUSSION

This study aimed to identify the expression levels of Histatin (HTN1, HTN3) genes as a result of antimicrobial interventions used for the management of the oral microbiome [11]. This study's findings showed that antimicrobial treatments have a major influence on the gene expression in patients with gingivitis and periodontitis. The cationic peptides, salivary histatins, played an important role in oral homeostasis due to their antimicrobial, antifungal, and tissue healing functions [12]. There have been several studies to demonstrate that histatins, particularly HTN1 and HTN3, play a role in biofilm disruption and inhibition of microorganisms, which are of great importance for oral health. Histatin levels decreased in periodontitis and gingivitis as bacterial disease load and tissue inflammation increased [13].

The fact that histatin expression was downregulated in individuals with chronic periodontal inflammation corroborates previous research that indicated the same. A researcher found a reduction in salivary histatin levels in periodontitis patients compared to healthy individuals, emphasizing the use of histatins as indicators of disease severity [14]. Building on this understanding, the present study went further to show that particular antimicrobial interventions could modulate the expression of histatin genes, providing a rational new therapeutic approach for restorative dentistry [15]. Of the AMPs derived from natural sources, those that with the most significant effect on increasing HTN1 and HTN3 expression indicated that they had the potential to stimulate endogenous host defense mechanisms [16].

An important finding was the surprising increase in histatins in response to plant-derived peptides, as such natural antimicrobials had garnered interest for targeting pathogenic bacteria while maintaining commensal microbiota [17]. It was demonstrated in previous work that plant-derived AMPs, such as LL-37 mimetics, not only possessed antibacterial activity but also conferred epithelial barrier function. A similar mechanism was indicated by our findings, as AMPs could cause cellular pathways that initiate an increase in histatin production, subsequently enhancing the host's natural defense system against oral pathogens [18].

Fluoride and chlorhexidine also upregulated histatin expression, though to a lesser extent than AMPs. Chlorhexidine was still the gold standard as an antimicrobial in dentistry, used for purposes such as plaque control and periodontal therapy [19,20]. As recently demonstrated by a researcher, chlorhexidine cytotoxicity in oral epithelial cells might limit its ability to increase histatin production [21,22]. These findings were corroborated by these results, which indicated that chlorhexidine killed microbes, but it might not significantly affect host protective factors such as histatins.

Fluoride, on the other hand, showed moderate elevation of histatin expression [23]. This was consistent with existing literature suggesting that fluoride could alter salivary protein composition and enamel remineralization [24,25].

However, several limitations must be noted. Although the sample size was relatively small, which hampered the generalizability of the results, the short study duration did not account for possible changes in histatin expression over prolonged periods of time. In addition, protein level validation was not carried out, thus preventing direct correlation between gene expression outcomes and histatin activity. These results should be confirmed, and the mechanisms determining histatin regulation by antimicrobial agents should be investigated using proteomic approaches and longitudinal monitoring of animals.

CONCLUSION

Overall, this study demonstrated that modulation of histatin expression through antimicrobial interventions represented a novel strategy to manage the oral microbiome. The results confirmed histatins as major players in innate immunity and particularly suggested that plant peptides might offer therapeutic advantages over standard agents such as chlorhexidine and fluoride. The mechanisms by which histatins were regulated required further exploration, and optimized treatment protocols for periodontal disease prevention should be developed.

LIST OF ABBREVIATIONS

CHX: Chlorhexidine

FL: Fluoride

LL-37: Cathelicidin antimicrobial peptide

HTN1: Histatin-1

HTN3: Histatin-3

mRNA: Messenger ribonucleic acid

qRT-PCR: Quantitative reverse-transcription polymerase chain reaction

GEC: Gingival epithelial cells

ACKNOWLEDGMENT

None

FUNDING

None

CONFLICT OF INTEREST

None

ETHICAL APPROVAL

The Certificate for Study was obtained and conducted at Baqai Medical University, Karachi (ERC-22-06-2022).

AUTHORS' CONTRIBUTION

All authors have equal contributions as per ICMJE.

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