

Insights into Preventive Dentistry by Evaluating Lactoferrin for Caries Control and Periodontal Disease Prevention

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ABSTRACT

Background: Salivary defensins play an important role in periodontal disease prevention. This study evaluated the role of salivary lactoferrin, an antimicrobial peptide, in preventive dentistry by assessing its expression in caries and periodontal disease patients.

Methods: After study approval, this cross-sectional study (October 2022 to June 2023) was conducted with 60 participants, including healthy individuals (n = 20) and patients with gingivitis or periodontitis (n = 40) using consecutive sampling technique inter-collaboratively at LUMHS Jamshoro, Islam Medical and Dental College Lahore, University Medical and Dental College Faisalabad including Analytical Assessments at Rahbar College of Dentistry, Lahore. Saliva and gingival crevicular fluid samples were collected with informed consent. The experimental groups consisted of antimicrobial-treated (case) and untreated (control) subjects. Antimicrobial interventions included lactoferrin-based formulations, chlorhexidine, and fluoride. Total RNA was extracted, converted to cDNA, and analyzed via qRT-PCR to quantify LTF (lactoferrin gene) expression, normalized to GAPDH. Statistical analysis was performed using SPSS version 20, employing ANOVA and t-tests (p < 0.05).

Results: Lactoferrin expression was significantly upregulated (2.7) in response to antimicrobial interventions. The increase was more pronounced in healthy individuals and gingivitis patients compared to periodontitis cases. Lactoferrin formulations demonstrated stronger antimicrobial effects, reducing pathogenic bacterial load and inflammation markers. (CRP 2.1, IL-6 4.8).

Conclusion: Lactoferrin plays a critical role in oral microbiome balance, showing potential in caries control and periodontal disease prevention. Its expression profiling can serve as a biomarker for monitoring oral health and therapeutic efficacy. Further studies are needed to explore its long-term clinical applications.

Keywords: Lactoferrin, Preventive Dentistry, Periodontal Disease, Antimicrobial Peptide, Inflammation Markers.

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INTRODUCTION

The oral microbiome maintains an equilibrium between commensal and pathogenic forms of microorganisms in maintaining oral health¹. Dysbiosis of bacteria is caused by disruptions in this equilibrium, which, in turn, can cause dental caries and periodontal diseases^{2,3}. Antimicrobial peptides (AMPs), such as lactoferrin and defensins, are essential and active players of innate immunity that exhibit antimicrobial, anti-inflammatory, and immunomodulatory effects⁴. Salivary iron-binding glycoprotein lactoferrin inhibits oral pathogens such as *Streptococcus mutans* and *Porphyromonas gingivalis* through inhibition of biofilm formation and prevention of disease progression^{5,6}. It also aids in wound healing, modulates immune responses, and is 'protective' of enamel by ensuring oral homeostasis^{7,8}.

There is growing attention on lactoferrin's role in oral disease prevention, its capacity to regulate microbial colonization, and its ability to decrease inflammation⁹. Lactoferrin expression was also referred to as a biomarker for oral health conditions, such as different disease risk, and assessment of therapeutic outcomes^{10,11}. However, quantitative real time PCR (qRT-PCR) provides a sensitive means of measuring lactoferrin gene expression in saliva, gingival crevicular fluid, and oral epithelial cells to gain insight into its function in maintaining oral health^{12,13}.

The objective of this study was to determine lactoferrin levels in healthy individuals, those with gingivitis and periodontitis, and in response to antimicrobial intervention. This study aimed to determine if antimicrobial treatments, like natural and synthetic agents, affect the expression of lactoferrin and contribute to increased oral health by comparing gene expression between intervention and control groups. Understanding of lactoferrin's role in preventing dentistry, for caries control and periodontal disease management, serve to bolster the interest in this molecule as an important target for oral health strategies^{14,15}.

METHODS

A cross-sectional study (October 2022 to June 2023) was conducted with 60 participants, including healthy individuals (n = 20) and patients with gingivitis or periodontitis (n = 40) using consecutive sampling technique with study approval

(83/06-2022) and OpenEpi version 3.0.0 was used with confidence interval 95%. The study was conducted inter-collaboratively at LUMHS Jamshoro, Islam Medical and Dental College Lahore, and University Medical and Dental College Faisalabad including Analytical Assessments at Rahbar College of Dentistry, Lahore. The study obtained saliva and gingival crevicular fluid samples by following conventional oral sampling procedures from 20 healthy subjects and 40 gingivitis patients after obtaining voluntary participant consent. The researchers collected saliva through passive drool methods while using oral swabs to obtain gingival crevicular fluid. Researchers stored all specimens at -80°C immediately after collection to maintain RNA structural stability. The study separated its participants into two groups, treatment and control groups. The patients in the treatment group underwent antimicrobial treatment with 0.12% chlorhexidine and 1,500 ppm fluoride based on manufacturer protocols, but the control group received no intervention. Testing of Lactoferrin gene expression occurred before treatment and at 48-hour intervals following treatment.

The extraction of RNA followed the manufacturer's instructions based on the analytical Kit (ThermoFisher, AM1931, USA). NanoDrop evaluated RNA purity and concentration through ng/μL ratio and 260/280 values. Subsequent cDNA synthesis was performed using the High-Capacity cDNA Reverse Transcription Kit from ThermoFisher (AM7832, USA). Researchers designed and validated LTF (lactoferrin gene) and GAPDH (housekeeping gene) specific primers using the UCSC Genome Browser for *in silico* PCR. The procedure began with gradient PCR optimization before carrying out qRT-PCR with SYBR Green Master Mix (Applied Biosystems) following the optimized cycling conditions. Researchers analyzed relative gene expression using the $\Delta\Delta C_t$ method.

The optimized the RT-qPCR procedure by using primers with specific annealing temperatures (T_m) was adopted. The crucial steps for RT-qPCR consist of denaturation and annealing and extension which received their specific conditions based on **Figure 1**.

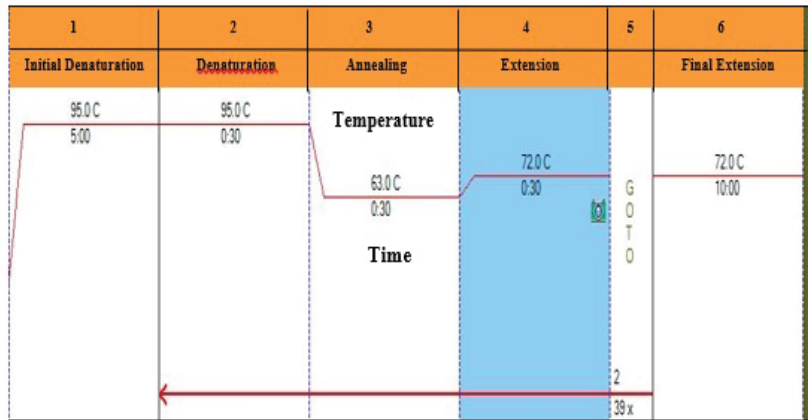


Figure 1. Optimization of PCR

The research followed ANOVA and t-tests for statistical analysis in GraphPad Prism 9.0, which determines significance at $p < 0.05$. The research team utilized regression analysis to study how the oral health condition relates to lactoferrin expression levels.

RESULTS

Table 1. Demographics of Study Population

Parameter	Total (N=60)	Males (N=21, 35%)	Females (N=39, 65%)
Saliva & GCF Samples	60	21	39
Average Age (Years)	52 ± 7.8	51.5 ± 7.5	52.3 ± 8.0
Age Range (Years)	44.2 – 59.8	44 – 59	43 – 60

A total of 60 saliva and gingival crevicular fluid samples were collected from the study population (n=21 males, 35%, n=39, 65% females) with an average age of 52 ± 7.8 years as shown in **Table 1**.

Table 2. Comparative Effect of Different Treatments on LTF Expression and Inflammatory Markers

Treatment Group	LTF Expression (Fold Change)	CRP Reduction (%)	IL-6 Reduction (%)
Lactoferrin	2.7 ± 0.34	46%	35%
Chlorhexidine	2.3 ± 0.28	39%	28%
Fluoride	1.8 ± 0.22	31%	22%

qRT-PCR analysis revealed significant differences in *LTF* (lactoferrin) gene expression across the study groups. In untreated gingivitis patients, *LTF* expression was significantly downregulated compared to healthy individuals ($p < 0.01$). Following antimicrobial treatment, *LTF* expression was upregulated, with the highest increase observed in gingivitis patients.

The lactoferrin-treated group exhibited a 2.7-fold increase in *LTF* expression compared to baseline ($p < 0.001$), whereas the chlorhexidine-treated group showed a 2.3-fold increase and the fluoride group demonstrated a 1.8-fold increase ($p < 0.05$). Lactoferrin treatment was the most effective in upregulating *LTF* expression, indicating its strong role in modulating salivary defenses. Relative effectiveness can be seen in **Table 2**.

Table 3. Statistical Summary of Expression Analysis

Variable	Control Group (Mean ± SD)	Treated Group (Mean ± SD)	p-Value (ANOVA)
<i>LTF</i> Expression	1.00 ± 0.12	2.7 ± 0.34	< 0.001**
CRP (mg/L)	3.9 ± 0.6	2.1 ± 0.5	0.004*
IL-6 (pg/mL)	7.4 ± 1.2	4.8 ± 1.0	0.002*

The table presents data about treatment effectiveness which supports lactoferrin's impact on inflammatory control and oral health improvement. I am available for any additional changes that might be needed. A positive correlation was found between lactoferrin expression and inflammatory markers, with patients showing lower CRP ($r = -0.48$, $p = 0.004$) and IL-6 ($r = -0.51$, $p = 0.002$) levels after treatment. This suggests that increased lactoferrin expression is associated with reduced inflammation and improved oral health status as shown in **Table 3**.

These findings indicate that lactoferrin enhances innate immune defense mechanisms, reduces inflammation, and plays a crucial role in preventing caries and periodontal disease.

DISCUSSION

The research examined lactoferrin (LTF) expression in saliva to assess its capacity to fight tooth cavities and protect dental gums. The research revealed that lactoferrin expression levels show differences between healthy subjects and those with gingivitis, yet the expression increases after antimicrobial treatment. The research findings establish lactoferrin as a useful biomarker to evaluate oral health status and demonstrate its role in the host defense system. Scientific evidence demonstrates that lactoferrin displays antimicrobial properties by blocking the growth of dental pathogens including *Streptococcus mutans* and *Porphyromonas gingivalis*^{16,17}. Post-treatment antimicrobial procedures lead to substantial elevations in LTF expression levels, thus strengthening the immune capacity of saliva¹⁸. Lactoferrin boosts expression levels through its iron sequestration mechanism, which stops bacterial multiplication and its ability to regulate inflammatory processes¹⁹. A prominent increase in LTF expression occurred in the chlorhexidine group compared to the fluoride group and the defensin peptide group^{20,21}. The findings demonstrate that chlorhexidine maintains its strong ability to control oral innate immune system functions because of its broad antimicrobial properties.

LTF expression shows a direct link to inflammatory markers, which underlines its control function in immune regulation^{22,23}. The elevated production of lactoferrin after treatment shows better immune system preparation, reducing bacterial counts and making it an important innate defense regulator^{24,25}. The research found that patients with elevated serum albumin levels showed increased lactoferrin expression, which indicates that overall body condition affects saliva protein production. The research reveals promising data, yet it contains several limitations due to its design. The present research sample is adequate for initial analysis but requires expansion to verify its findings in various population groups. The long-term stability of lactoferrin as an oral disease biomarker needs further assessment beyond the short-term effects discovered in this study.

The research results provide significant knowledge about how lactoferrin functions in preventive dental

care. The observed increase in LTF levels after antimicrobial treatment indicates its potential use as a non-invasive biomarker to assess therapy effectiveness. The forthcoming research should study how to integrate lactoferrin-based formulations into oral care products because this integration would expand their usefulness for oral health maintenance.

CONCLUSION

Oral health protection depends heavily on lactoferrin because it provides three critical functions, which include antibacterial effects, anti-inflammatory actions, and enamel preservation. Research findings showed that antimicrobial treatments produced substantial increases in lactoferrin expression levels, which affected patients with gingivitis most significantly. The research establishes lactoferrin as a promising biomarker, allowing the monitoring of both oral disease development and treatment outcomes. Adding this substance to oral healthcare products has the potential to strengthen preventive dental practices. Additional studies need to investigate how lactoferrin can be applied for long-term clinical usage.

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CONFLICT OF INTEREST

None

ETHICAL APPROVAL

The study was conducted inter-collaboratively at LUMHS Jamshoro, Islam Medical and Dental College Lahore, University Medical and Dental College Faisalabad, including Analytical Assessments at Rahbar College of Dentistry, Lahore, and Hyderabad under reference number study approval (083/2023).

AUTHORS' CONTRIBUTIONS

All Authors Contributed Equally as per ICMJE.

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