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Article

Evaluation of the effects of Curcumin gel against Periodontopathic Bacteria (Porphyromonas gingivalis) using real-time time-polymerase chain reaction technology

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ABSTRACT: Periodontal disease is typically treated with mechanical debridement of the tooth surface. It may, however, be insufficient to eradicate pathogenic microorganisms on its own. Because of the microbial etiology of periodontitis, systemic or local antibiotic therapy is used as an adjunct treatment. The present study aimed to determine the effects of curcumin gel on Porphyromonas gingivalis. Eleven patients with stage II and III periodontitis were registered in the study. A double-blinded split-mouth design followed. Periodontal pockets were distributed into 2 groups; the test group received scaling and root planing along with curcumin gel, while the control group received scaling and root planing along with a placebo gel. Plaque index, probing pocket depth and relative attachment level were recorded with the collection of subgingival plaque samples at different time intervals for bacterial analysis using real-time time-polymerase chain reaction. Results showed a significant reduction in the bacterial outcomes in the test group. There was a significant improvement in the Plaque index, probing pocket depth and relative attachment level in the test group compared to the control group. On intra-group comparison, both groups showed a significant reduction of Plaque index and probing pocket depth with a more significant reduction in the test group, and only the test group showed a significant reduction of relative attachment level. A strong positive correlation of P.gingivalis with probing pocket depth and relative attachment level in the test group was estimated. Curcumin gel has an antibacterial effect against Porphyromonas gingivalis and showed a potent improvement in the outcomes of the periodontal parameters.

Keywords: Curcumin gel, periodontal pocket, Porphyromonas gingivalis

Introduction

Periodontitis is a chronic inflammatory disease defined by the teeth' supporting tissue breakdown. Dental plaque and the microorganisms that exist in it are considered vital aetiologic factors in periodontitis. Biofilm flora of dental plaque offers a particular condition for the organisms, confirming its vitality and pathogenicity. The goal of periodontal treatment is the elimination of bacterial plaque. Scaling and root planing are the most common periodontal treatments that involve the elimination of supragingival and subgingival plaque and calculus, restoring tissue health [1].

Porphyromonas gingivalis, Treponema denticola, and Tannerella forsythia are the most common pathogens in the periodontal disease. These bacteria are frequently detected together in the periodontal pockets, demonstrating that they may act together to destroy periodontal tissue. [2] Porphyromonas gingivalis has been related to the development of periodontitis as a primary etiologic agent. This anaerobe can also persist within the host epithelium without causing any symptoms. [3]. P. gingivalis at high levels triggers an immunological response that stimulates the production of cytokines and proinflammatory mediators, resulting in tissue damage [4]. The equilibrium of the aggressiveness of the subgingival biofilm and the human immune response determines the disease's progression and severity [5].

Oral bacterial analysis is essential for the diagnosis of periodontitis and for the evaluation of disease risk. Microbiological culture, enzyme assays, DNA-DNA hybridization, immunoassays, and polymerase chain reaction (PCR) tests are just a few methods for identifying and quantifying periodontal pathogens developed due to technological advancements. However, most of these procedures are time-consuming and inaccurate when determining periodontal infections. Real-time PCR (RT-PCR) was created to address these limits, permitting more precise quantification with increased sensitivity, specificity, simplicity, and speed [6].

Antibiotics and nonsteroidal anti-inflammatory drugs have been investigated as periodontal therapy adjuvant. These drugs have previously been linked to antimicrobial resistance, systemic changes, and gastrointestinal distress [7,8,9]. As a result, phytochemicals can be used as a substitute for medications that have the necessary qualities and have fewer adverse effects [10].

Researchers have discovered that phytotherapeutic compounds can inhibit microorganisms that cause periodontal diseases. As a result, there is much interest in improving phytotherapeutic substances as a periodontal treatment adjunct [11].

Polyphenols are a group of phytochemicals that are a significant source of antioxidants. Turmeric, an ancient spice produced from the rhizome of Curcuma longa, an everlasting plant belonging to the Zingiberacae (ginger) family, is the most widely used polyphenol. Turmeric contains curcumin, demethoxycurcumin, and bisdemethoxycurcumin, which belong to the curcuminoids class of chemicals. Curcumin is the most essential curcuminoid, making up about 2-5 percent of turmeric. It is responsible for the spice's yellow color and most of turmeric's therapeutic properties [12]. Curcumin possesses antioxidant, anti-inflammatory, anti-carcinogenic, and antimicrobial properties [13].

This study used microbiologic analysis (RT-PCR) to determine the antibacterial efficiency of commercially available curcumin preparations (Curenext Oral Gel®, Abbott Health Care, Mumbai, MH, India, each gm gel contains Curcuma longa extract (Rhizome) 10.00 mg) and its effect on periodontal parameters (PLI, PPD and RAL).

Material and methods

Study population

This study was conducted at the Department of Periodontics/College of Dentistry /University of Baghdad. All samples were selected from April 2021 till July 2021. The study approved by the ethical committee/College of Dentistry/ University of Baghdad at reference no.322 on 24/3/2021 subordinates the guidelines of Helsinki and Tokyo for humans.

People were registered volunteers for the study after signing the informed consent sheet to share in the research and submitting a questionnaire comprising

their name, age, gender, medical history, dental history, and smoking, followed by a complete examination of periodontal parameters. Exclusion criteria included subjects taking antibiotics or other medications in the last 3 months, Smokers and Pregnant or lactating women.

Preparation of the placebo gel

Carbopol 934 (1 gm) was dissolved in the distilled water (25 ml) to create the placebo gel. Triethanolamine was added to alter the pH above 7, loading in the syringes.

Trial design

Eleven patients were selected to participate in this study. A randomized split-mouth, double-blinded design was followed to evaluate the antibacterial effect of curcumin against the periodontal pathogen (P. gingivalis). Two sites have been selected from each patient; the involved sites must be equal to or more than 5 mm. Measurement of periodontal parameters was done at the first and third visits with bacterial analysis of subgingival plaque samples for P.gingivalis using RT-PCR.

At the first visit, measurement of the clinical Parameters (PLI&PPD), full mouth supragingival scaling, oral hygiene instructions, collection of subgingival plaque sample from the pocket and impression taken for constructing the acrylic stent for RAL recording.

At the second visit, the pockets were treated conventionally with root planing and random application of the gel in the pockets by coin flip:

Group I (control): Scaling and root planing followed by placebo gel application at the second visit.

Group II (test): Scaling and root planing followed by curcumin gel application at the second visit.

The third visit included measuring the periodontal parameters and collecting subgingival plaque samples from the same previous pockets.

Statistical analysis

Statistical analysis was done using a statistical package for social sciences (SPSS). In testing normality among groups for both parameters, the Shapiro-Wilk test was used. The data were not normally distributed and non-parametric. The Wilcoxon sum rank test was used to demonstrate the difference in rank for two independent groups with equal sample sizes, and the Wilcoxon sign rank test was used to demonstrate the difference in rank for two related points. Spearman correlation test was used to assess the correlation between bacterial outcomes and clinical periodontal parameters—Cohen D (effect size) test for analysis of the behavioral sciences.

Results

Findings in Table 1 presented that before gel application, there was no significant difference in P.gingivalis between groups (p=0.385). After gel application, there was a substantial difference (P=0.001) with a medium effect size (0.680). Regarding the reduction of P.gingivalis in each study group, in the placebo group, there was no significant reduction (P=0.790). In contrast, in the curcumin group, the reduction was significant (P=0.003) with a medium effect size (0.626).

Groups		Before	After
Placebo gel	Median	80	87
	Mean rank[1]	12.82	15.91
	Mean rank[2]	7.2	5
Wilcoxon sign rank test		0.267	
P value		0.790 ^	
Curcumin gel	Median	28	4
	Mean rank[1]	10.18	7.09
	Mean rank[2]	6	0
Wilcoxon sign rank test		2.937	
P value		0.003*(ES=0.626)	
Wilcoxon sum rank test		0.952	3.187
P value		0.385^	0.001*(ES=0.680)

*=significant at p<0.05, ^=not significant at p>0.05.,1=between groups, 2=within group(change), ES=effect size

Table 1. Descriptive and Analytic Statistics of Mean rank Value of P. gingivalis between study groups.

Findings in Table 2 showed that before gel application, there was no significant difference in PLI, PPD and RAL between groups (P>0.05). After gel application, the difference in all clinical parameters was statistically significant between groups (P<0.05). Regarding the change of PLI in each study group, both groups have significantly reduced the PLI with slightly more reduction in the curcumin group (p=0.001) than in the placebo group (p =0.003) and more effect size for curcumin gel (ES=0.707) than placebo gel (ES=0.643). The change of PPD in each study group, both groups had a significant reduction of PPD with slightly more in the curcumin group (p=0.001) than the placebo group (p =0.003) and more effect size for curcumin gel (ES=0.707) than placebo gel (ES=0.638). Intra-group change of RAL showed that both groups had reduced RAL. However, it was not significant in the placebo group (P=0.083) and significant in the curcumin group (P=0.003) with a medium effect size (0.633).

Groups		PLI		PPD		RAL	
		Before	After	Before	After	Before	After
Placebo gel	Median	2.0	1.0	6	5	9	8
	Mean rank[1]	11	14	12	17	11.32	9.18
	Mean rank[2]	7	1	8	2.6	6.7	2.1
Wilcoxon sign rank test		3.017		2.994		1.732	
P value		0.003*(ES=0.643)		0.003*(ES=0.638)		0.083^	
Curcumin gel	Median	2.0	1.0	6	3	8	6
	Mean rank[1]	12	9	11.5	6	11.68	6.32
	Mean rank[2]	5	0	7	0	6	0
Wilcoxon sign rank test		3.317		3.317		2.971	
P value		0.001*(ES=0.707)		0.001*(ES=0.707)		0.003*(ES=0.633)	
Wilcoxon sum rank test		1	2.485	0	4.301	0.140	3.881
P value		0.748^	0.013*	1^	0.000003*	0.898^	0.000020*

		(ES=0.530)		(ES=0.917)		(ES=0.827)

*=significant at p<0.05, ^=not significant at p>0.05.,1=between groups, 2=within group(change), ES=effect size

Table 2. Descriptive and statistical test of Mean rank Value of PLI, PPD and RAL between study groups.

Table 3 demonstrated the correlation of P.gingivalis with periodontal clinical parameters in the curcumin group; there was a significant solid correlation of P. gingivalis with PPD (p=0.022) and RAL (p=0.026), while it was weak, not significant with PLI (p=0.213).

Variables		Rsp	P value
	PLI	0.408	0.213^
	PPD	0.677	0.022*
	RAL	0.663	0.026*

*=significant at p<0.05, ^=not significant at p>0.05, rsp= spearman correlation coefficient

Table 3. Correlation between P.gingivalis and periodontal parameters in the curcumin group.

Table 4 revealed the correlation of P.gingivalis with periodontal clinical parameters in the placebo group; the correlation was weak and insignificant (p>0.05).

Variables		rs	p-value ^
r	PLI	0.265	0.445
	PPD	0.242	0.474
	RAL	0.238	0.481

^=not significant at p>0.05, rsp= spearman correlation coefficient

Table 4. Correlation between P.gingivalis and periodontal parameters in the placebo group.

Discussion

The current study effectively used the RT-PCR method to detect and count P. gingivalis in subgingival microbial samples. The inter-comparison in groups presented that there was a substantial difference (p=0.001) in microbiological outcomes after 30 days when comparing the test group (SRP and curcumin gel) with the control group (SRP and placebo gel) with medium effect size. The significant bacterial decrease noted in the test group may be related to curcumin's antibacterial, anti-inflammatory and antiplaque activity [20]. Curcumin prevented the formation of P. gingivalis homotypic and P. gingivalis–Streptococcus gordonii biofilms and inhibiting the activities of Arg-gingipain (Rgp) and Lys-gingipain (Kgp) in P. gingivalis [14]. Curcumin also decreased P.gingivalis LPS (Lipopolysaccharide) induced TNFα and IL-1 β production [15,16,17] suggesting that curcumin's suppression of these cytokines may help to minimize the effect of cytokine-mediated tissue breakdown in periodontitis [18].

The results of the comparison group of SRP with curcumin gel application displayed a significant decrease in the microbial count after gel application. In contrast, there was a slight but not statistically significant decrease in the control group. This could be related to the difficulty of performing extensive mechanical

debridement of deep periodontal pockets, the pathogenic microorganisms found within the gingival and dental tissues, and other sites unreachable to periodontal instruments [19]. So, local administration of antimicrobials like curcumin gel is done because of the microbial etiology of periodontitis. These results agreed with studies conducted by Vijayapremakumar et al. [26] and Bhatia et al. [18,] which demonstrated that a better reduction in the number of periopathogens was shown in the test group.

Regarding the PLI, on inter-group comparison, there was a statistically significant difference between groups (P=0.013). The significant reduction of PLI in the test sites (curcumin) in this study can be attributed to its antibiofilm activity, as suggested by Chusri et al. [21] and Savita et al. [22], who pretended that curcumin impedes the production of biofilm and disbanded the biofilm constructed by many microbes. This study was by Pandy et al. [23], who showed a significant difference in the PLI between study groups. In the intra-group comparison of PLI, both groups presented a significant decrease in the PLI, which was more in the test group than in the control group. This is due to the effectiveness of SRP in removing bacterial plaque and retentive factors, as well as the antiplaque properties of curcumin. These results agreed with a study conducted by Anuradha et al. [13], who found a significant reduction in the PLI in both study groups.

Concerning PPD and RAL, the reduction in the test group was more than in the control group, with a significant difference. This finding could be attributed to an anti-inflammatory mechanism of curcumin, which modulates the inflammatory response, inhibits the production of proinflammatory cytokines, suppresses the activation of AP-1 and NF-kβ, inhibits the biosynthesis of inflammatory prostaglandins, enhances neutrophil function during inflammatory response [20]. These results agreed with studies by Bhatia et al. [18] and Vijayapremakumer et al. [26]. They found a significant difference in PPD and CAL between the test and control groups.

The intra-group comparison showed a significant reduction of PPD in the curcumin and placebo groups. These findings agreed with the study done by Behal et al. [25], which presented a significant decrease in the PPD in both study groups. At the same time, RAL has decreased in both groups but with no significant gain for the placebo group (P=0.083) and a significant gain for the curcumin group (P=0.003).

The correlation of P.gingivalis with periodontal clinical parameters showed a robust and significant correlation when comparing this bacteria with PPD and RAL in the curcumin gel group. This finding may be attributed to the antibacterial activity of curcumin that was mentioned previously. In addition to the anti-inflammatory properties of curcumin, it was known to enhance wound healing by regeneration.

Conclusion

RT-PCR analysis revealed that curcumin gel (Curenext®) has antibacterial action against P.gingivalis. Quantitative real-time PCR (qRT-PCR) is a fast and accurate approach for studying specific organisms. Because PPD and RAL were much reduced in the test group, curcumin may have enhanced periodontal wound healing. In the test group, P.gingivalis was substantially correlated with PPD and RAL.

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