ORIGINAL ARTICLE

Effectiveness of nonsurgical periodontal therapy on the levels of *Helicobacter pylori* in dental plaque and saliva of patients with chronic periodontitis

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ABSTRACT

Objectives: Presence of *Helicobacter pylori* in the oral cavity may enhance the risk for gastric re-infection; hence, the present study was carried out to detect and compare the levels of *H. pylori* in subgingival plaque and saliva of patients with periodontal health and chronic periodontitis at baseline and 3 months following scaling and root planing (SRP). **Methods:** A total of 45 patients with 30 patients having chronic periodontitis (test group) and 15 periodontally healthy patients (control group) were considered. *H. pylori* was detected in subgingival plaque and saliva samples, collected at baseline and at 3 months using polymerase chain reaction. Clinical parameters were recorded at baseline, 1 month, 2 months, and 3 months. **Results:** At the baseline, in test group 60% and 40% of the samples were positive for *H. pylori* and in control group 26.7% and 13.3% of the samples were positive for *H. pylori* in subgingival plaque and saliva, respectively. At 3 months, 26.7% and 20% samples were positive for *H. pylori* in the test group and in control group 13.3% and 6.7% samples were positive for *H. pylori* in subgingival plaque and saliva, respectively, demonstrating higher levels of *H. pylori* in dental plaque than in saliva. There was a significant reduction in the percentage of *H. pylori* in subgingival plaque following SRP. **Conclusion:** Periodontal treatment has shown to be effective in reducing the levels of *H. pylori* from the oral cavity.

Key words
Chronic periodontitis, dental plaque, scaling and root planing

INTRODUCTION

An oral cavity is a permanent reservoir of several bacterial pathogens of medical importance.[1] *Helicobacter pylori* is a Gram-negative, spiral-shaped, microaerophilic, flagellated bacterium implicated in the etiology of numerous gastrointestinal diseases such as peptic, duodenal ulcers, gastric carcinoma, and mucosa-associated lymphoid tissue lymphoma.[2-4] There is evidence that the primary extragastric reservoir of *H. pylori* is an oral cavity.[5-7]

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H. pylori infection appears to involve in the oral route as an important mode of transmission. *H. pylori* detected in the oral cavity has been observed in saliva,[8-11] supragingival plaque,[12-16] subgingival dental plaque,[12,16-22] microbiota of the dorsum of the tongue,[23] on the surface of oral ulcerations,[24] and on the surface of oral neoplasias.[25] An association between the presence of *H. pylori* in dental plaque and gastritis has been demonstrated. Higher prevalence of *H. pylori* has been

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observed in dental plaque than in stomach in patients with chronic gastritis.[26]

Although *H. pylori* has been detected in different sites, periodontal pocket can be regarded as a natural reservoir for *H. pylori* as it provides microaerophilic conditions suitable for its growth.[27] Poor oral health and periodontitis have shown to be associated with high prevalence of gastritis caused by *H. pylori*.[28-30] Oral hygiene parameters such as the presence of plaque and gingival bleeding have positively correlated with *H. pylori* in the oral cavity.[15,31]

Oral *H. pylori* is seldom eliminated by systemic eradication therapy,[7] therefore the importance of oral hygiene and periodontal procedures such as scaling and root planing (SRP) needs to be emphasized as an adjunct to eliminate *H. pylori* from the oral cavity.[32] There is a need to elucidate whether the oral cavity is a permanent or transient reservoir of *H. pylori* and the effect of nonsurgical periodontal therapy in eliminating *H. pylori* from the oral cavity. Hence, the aim of the present study was to evaluate the effect of plaque control and SRP in terms of eliminating *H. pylori* from oral cavity in dental plaque and saliva of patients with periodontal health and chronic periodontitis.

MATERIALS AND METHODS

Forty-five patients (23 males and 22 females) in the age group of 18–60 years (with a mean of 39 years) were selected. Patients for the study were recruited from the outpatient section, Department of Periodontology, Krishnadevaraya College of Dental Sciences and Hospital, affiliated to the Rajiv Gandhi University of Health Sciences, Bengaluru, and ethical guidelines outlined in the Helsinki Declaration of 1975 as revised in 2000 were followed. All the eligible cases, who volunteered, were informed about the nature, potential risks, and benefits of their participation in the study and written informed consent was obtained from all the cases. The study protocol was approved by the Institutional Ethical Committee (approval KCDS/302/PG/2011-12).

Selection criteria

The inclusion criteria of cases were as follows:

- Systemically healthy patients
- Fifteen patients having clinically healthy gingiva with probing depth (PD) <3 mm and ≤10% sites with bleeding on probing as Group I
- Thirty chronic periodontitis patients having PD more than or equal to 5 mm at more than 30% of sites with relative attachment level (RAL) more than or equal to 3 mm and more than 10% sites with bleeding on probing positive as Group II.

Exclusion criteria were as follows:

- Chronic gastritis patients were excluded in the present study based on rapid urease test (RUT)
- Patients who were under medications such as antibiotics, prolonged use of anti-inflammatory drugs, or the use of antacids in past 6 months
- Patients who had undergone professional tooth cleaning or any other periodontal treatment within the past 6 months
- Patients with less than twenty teeth
- Smokers
- Aggressive periodontitis patients.

Clinical examination

Full-mouth periodontal examination was carried out in all cases. Patients were divided into two groups as described in inclusion criteria. The clinical parameters assessed were plaque index (PI),[33] modified sulcular bleeding index (mSBI),[34] probing pocket depth,[35] and RAL.[36] Clinical parameters were recorded at baseline, 1st, 2nd, and 3rd month follow-up visits.

Sample collection

Before collecting the subgingival plaque, tooth was dried and isolated with cotton rolls. Supragingival plaque was removed from the sampling site by hand scaling. In the test group, plaque samples were collected from the deepest periodontal pocket in each quadrant and pooled.[37] In the control group, samples were collected from randomly selected sites. Subgingival plaque was collected by an upward scrape against the tooth surface with a sterile periodontal curette.[37] The unstimulated whole saliva was allowed to pool in the floor of the mouth and subsequently aspirated using 5 ml disposable syringe.[38] Dental plaque and saliva samples were collected at baseline and at the end of 3rd month follow-up from both the groups. The collected samples were stored at − 20°C temperature and transferred to TE buffer solution (Tris-HCl 10 mm and ethylenediaminetetraacetic acid (EDTA) 1 mm, pH 7.6) to the laboratory for polymerase chain reaction (PCR) analysis.

Periodontal treatment

All the cases received SRP and oral hygiene instructions. SRP was performed in two to four appointments of approximately 60 min each under local anesthesia (2% lignocaine hydrochloride with 1:200,000 adrenaline) using area-specific periodontal curettes and an ultrasonic device. The treatment was concluded without the use of systemic antibiotics or local antimicrobials. After baseline recording of clinical parameters, collection of dental plaque and saliva samples were done. All the patients were instructed to perform plaque control using toothbrush, dental floss, and interdental toothbrushes as necessary. Samples were again collected at the end of 3 months following SRP.

Microbiological analysis

Detection frequency of *H. pylori* was analyzed by means of "hot start PCR," which is a variation in conventional PCR. Subgingival plaque and saliva samples suspended in phosphate buffered saline were washed thrice in TE

buffer (Tris-HCl 10 mm and EDTA 1 mm, pH 7.6) and once in lysis buffer I and II and then incubated with 100 µg/ml of proteinase K for 2 h at 65°C. Following this, samples were boiled for 10 min at 90°C, and supernatant is stored at −20°C till processed. Primers used were JW21, the upstream primer (5' GCGACCTGCTGGAACATTAC 3' nucleotides 691-710) and JW22, the downstream primer (5' CGTTAGCTGCATTACTGGAGA 3', nucleotides 829-809). The reagents were added to the sample in laminar airflow. DNA polymerase enzyme was added in the last and mixed by pipetting up and down. DNA amplification was carried out in a thermocycler that heats and cools the reaction tubes within it to the precise temperature required for each step of the reaction. Taq polymerase has allowed the automatization of the reaction using specific appliances such as thermocycler. The final PCR amplification product obtained was subjected to gel electrophoresis and further analyzed under ultraviolet transilluminator. Finally, PCR amplification products were compared with standard molecular markers. The result obtained was subjected to statistical analysis.

Statistical analysis

Analysis of variance test was applied to determine the intergroup differences in clinical parameters between the test and control groups. Pearson's Chi-square test was applied to compare the percentage of samples positive for *H. pylori* between the control and test group at baseline and 3 months. Spearman's correlation coefficient (*r* value) was used to determine the association between PD and percentage of *H. pylori* detected. If *r* value is positive, it indicates that the association is present and if *r* value is negative, it indicates that the association is not present. The $P = 0.05$ or less was considered to be statistically significant. The data were analyzed using the SPSS Inc. Statistical Package for the Social Sciences (SPSS) is a software package used in statistical analysis of data. It was developed by SPSS Inc.

RESULTS

Clinical results

Table 1 shows the descriptive statistics of clinical parameters including PI, mSBI, and PD of both the groups. There was a reduction in all the clinical parameters from baseline to 3-month follow-up. Although the scores of PI and mSBI improved over time, between group analyses revealed no significant differences (PI: $P = 0.086$, mSBI: $P = 0.090$). Statistically, significant differences were observed in the PD and RAL [Table 1a] when the comparison was made between the control and test groups (PD: $P = 0.031$, RAL: $P = 0.025$) [Graph 1].

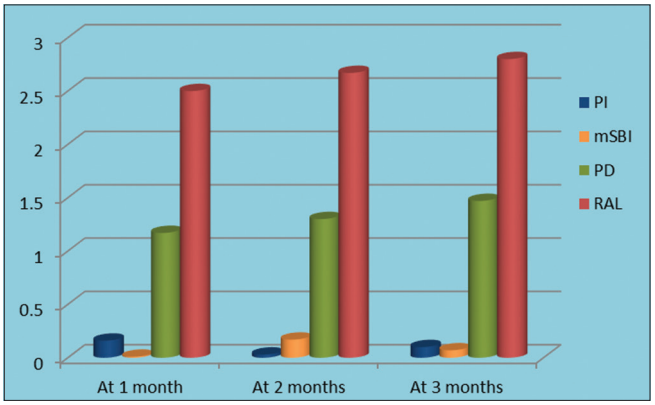
Microbial results

Table 2 represents the intergroup comparison of the percentage of *H. pylori* between control and test group. In the control group, at baseline 26.7% subgingival plaque samples and 13.3% saliva samples were found to be positive for *H. pylori*. At 3 month follow-up, the percentage of detection of *H. pylori* reduced to 13.3% and 6.7%, respectively, in subgingival plaque and saliva. In the test group, at baseline 60% subgingival plaque samples and 40% saliva samples were found to be positive for *H. pylori*. At 3 month follow-up, the percentage of detection of *H. pylori* reduced to 26.7% and 20%, respectively, in subgingival plaque and saliva. There was a significant difference in the reduction in the percentage of *H. pylori* in subgingival plaque when compared from baseline to 3 months ($P = 0.031$). However, in saliva, the reduction is not statistically significant ($P = 0.245$) [Graphs 2 and 3].

Table 1: Descriptive statistics of clinical parameters in control and test groups

Group	Mean±SD		
	PI	mSBI	PD
Control group			
Baseline	2.20±0.56	2.33±0.48	2.73±0.45
1 month	1.47±0.45	1.27±0.45	2.40±0.50
2 months	1.30±0.50	1.13±0.35	2.13±0.35
3 months	1.17±0.46	0.80±0.41	2.07±0.25
Test group			
Baseline	2.40±0.56	2.50±0.50	7.03±1.29
1 month	1.83±0.46	1.43±0.43	5.53±1.10
2 months	1.47±0.45	1.23±0.43	5.13±0.86
3 months	1.27±0.50	0.90±0.40	4.90±0.71

PI – Plaque index, mSBI – Modified sulcular bleeding index, PD – Probing depth, SD – Standard deviation

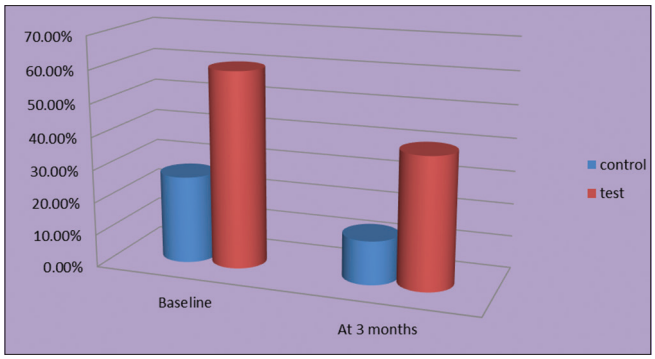


Graph 1: Intergroup comparison of clinical parameters

Spearman's correlation coefficient was applied to determine the association between PD and percentage of *H. pylori*. The correlation coefficient was positive indicating a positive correlation between the PD and percentage of *H. pylori* [Table 3].

DISCUSSION

Dental plaque consists of a variety of microorganisms which are responsible for initiation and progression



Graph 2: Percentage of *Helicobacter pylori* detected in dental plaque

Table 1a: Comparison of relative attachment level within Group II

Parameter	1 month	2 months	3 months	*P*
RAL	1.03±0.10	1.57±20	1.97±0.41	0.038

RAL – Relative attachment level

Table 2: Intergroup comparison of the percentage of *Helicobacter pylori*

Timeline	Sample	*P*
Baseline	Dental plaque	0.035*
	Saliva	0.069
At 3 months	Dental plaque	0.031*
	Saliva	0.245

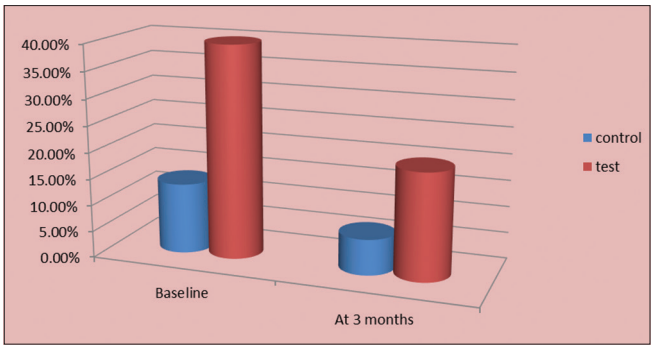
*Significance

Table 3: Spearman's correlation coefficient to determine the association between probing depth and percentage of *Helicobacter pylori* detection

Group	Correlation coefficient (*r*)	*P*
Group I (control)	+0.158	0.663
Group II (test)	+0.448	0.019*

*Significance

of periodontal diseases. It has been shown that there is a significant increase in the number of these microorganisms once the gingival sulcus is converted into the periodontal pocket.[39] Periodontal pockets can serve as an ideal habitat for microaerophilic and anaerobic microorganisms due to its favorable architecture and microcosm.[22] Dental plaque and periodontal pockets can act as reservoirs of *H. pylori* since it is a microaerophilic organism. Recent studies[7,32] have reported that the presence of *H. pylori* in dental plaque can cause recurrence of gastric *H. pylori* following its eradication, thereby stressing the significance of plaque control. Since the literature is lacking in the arena of the efficacy of periodontal treatment to prevent gastric re-infection, the present study emphasizes the need for periodontal treatment to eliminate or reduce the oral *H. pylori* levels.



Graph 3: Percentage of *Helicobacter pylori* detected in saliva

Helicobacter pylori in dental plaque

Numerous studies in literature have shown oral cavity as a reservoir of *H. pylori*. According to some studies,[40,41] *H. pylori* has only a transient presence in the oral cavity and if present may be the result of occasional gastroesophageal reflux. On the other hand, some authors viewed *H. pylori* as a permanent resident in the oral cavity. These variations are attributed to the differences in the techniques used to detect *H. pylori*. Studies carried out on Indian population have shown a high prevalence of *H. pylori* in the oral cavity.[22,28] A recent study on the same population by Agarwal and Jithendra[22] demonstrated *H. pylori* in 60% of subgingival plaque samples in chronic periodontitis patients whose gastric biopsies were *H. pylori* positive, whereas control group (chronic periodontitis, who never had gastritis) demonstrated 15% samples positive for *H. pylori*. Similar results were reported by Anand *et al.* in 2006.[28]

Distinguishingly, decreasing pattern of distribution of *H. pylori* has been observed in the supragingival plaque on molars, showing their high prevalence when compared to the anterior teeth.[42] Reasons for this high prevalence in posterior teeth are less access for oral hygiene and also posterior teeth provide a more microaerophilic environment for the survival of *H. pylori*. Dental biofilms provide urea and further facilitate the growth of urease producing bacteria such as *H. pylori*.[5] In correlation to these findings, the present study showed a higher prevalence of *H. pylori* in subgingival plaque from chronic periodontitis patients. Similarly, Al Asqah *et al.*[20] demonstrated *H. pylori* in 78% of subgingival plaque samples obtained from the periodontitis patients.

Anderson *et al.*[43] justifies that dental plaque act as a reservoir for *H. pylori* by demonstrating the ability of *H. pylori* to coaggregate with *Fusobacterium nucleatum* and *Fusobacterium periodonticum*, which are early and late colonizers of the oral cavity, respectively. The present study also demonstrates the increase in the percentage of *H. pylori* in dental plaque of chronic periodontitis patients.[43] On contrary, it has been reported that early colonizers such as *Streptococcus* and *Actinomyces* species

produce bacteriocin-like inhibitory proteins against *H. pylori*. This inhibitory activity on *H. pylori*[44] explains the low prevalence of *H. pylori* in periodontally healthy patients.

In the current study, our objective was to determine whether the oral cavity could act as a reservoir for *H. pylori* regardless of gastric infection. In contrast to most of the studies,[6,12,18,22,28,29] we evaluated cases who reported no history of gastric symptoms. The findings of our study are similar to Souto and Colombo[19] who observed a higher prevalence of *H. pylori* in subgingival biofilm samples (33.3%) compared to saliva samples (20%) in nondyspeptic patients. Berroteran *et al.*[45] detected *H. pylori* in the dental plaque of 15% of nondyspeptic patients which is low when compared with our study, whereas Martinez-Gomis *et al.*[46] did not detect this bacterium in any of the ten selected cases without dyspepsia, which is in contrast to the present study.

Olivier *et al.*[47] failed to detect *H. pylori* in dental plaque from cases without periodontitis who had positive stomach biopsies. Umeda *et al.*[29] reported that eradication of *H. pylori* from the stomach did not eliminate it from the oral cavity. This shows that periodontal pockets can act as a reservoir of *H. pylori* even after its eradication from the stomach. Gebara *et al.*[7] showed that 60% of cases with periodontal disease were still positive for *H. pylori* in their oral cavity, whereas only 10% were positive in the stomach after triple therapy (triple therapy is a combination of omeprazole 20 mg, amoxicillin 1 g, and clarithromycin 500 mg twice daily for 7 days). These findings suggest that the frequency of *H. pylori* in the oral cavity is related more to the presence of periodontal disease than to the existence of gastric infection. In a recent meta-analysis, Zou and Li[48] demonstrated that the prevalence of *H. pylori* infection in oral cavity was significantly increased in gastric *H. pylori*-positive patients compared with gastric *H. pylori*-negative patients with an odds ratio (OR) of 3.61 (range: 1.91–6.82). OR differs with different diagnostic methods[49] with PCR showing the highest (5.11) and RUT showing the lowest (2.00). This study also showed that the eradication efficiency of *H. pylori* in the stomach is significantly higher (85.8%) compared with that in the oral cavity (5.7%) when triple therapy is used. *H. pylori* in the oral cavity are more difficult to be eradicated than in the stomach.

Helicobacter pylori in saliva

H. pylori are commonly transmitted person-to-person by saliva. Saliva is therefore considered as an important vehicle for *H. pylori*. Li *et al.* (1995, 1996)[8,49] reported a high prevalence of *H. pylori* (75%, 84%) in saliva specimens in patients with proven gastric *H. pylori* infection. Similarly, Song *et al.*[42] demonstrated a high prevalence of *H. pylori* (100%) in saliva specimens. These studies reported a higher prevalence rate compared to the present study. In contrary, Ferguson *et al.*[50] were able to isolate *H. pylori* from only one saliva specimen out of the nine samples examined. Bürgers *et al.*[51] showed that *H. pylori* can occur in the oral cavity independent of gastric colonization. Several other studies[14,16,17,52,53] failed to detect *H. pylori* in the saliva and subgingival plaque or found no association between periodontal status and the prevalence of the bacteria in the oral cavity.

Antimicrobial therapy fails to resolve *H. pylori* infection from the oral cavity as anti-*H. pylori* doses are difficult to achieve in saliva.[29] *H. pylori* recolonized in gastric mucosa from dental plaque has been found to be inaccessible to systemic antimicrobial therapy.[7,29] Dental plaque can be efficiently removed by professional and daily oral hygiene thus controlling *H. pylori* infection in the oral cavity.[32]

Effect of plaque control on *Helicobacter pylori*

SRP was performed in the present study to examine its effect on *H. pylori* levels. Following, there was a significant reduction in *H. pylori* levels. This finding is similar to a recent case series by Escobar[54] where full-mouth ultrasonic debridement has shown to reduce the percentage of *H. pylori* but not eradicated from all the samples. This result shows that the efficacy of nonsurgical treatment modalities may reduce only the depth of periodontal pockets but cannot treat the deep pockets (>5 mm) completely. Hence, other treatment modalities such as open flap debridement have been advocated.

Results from studies by Zaric *et al.*[55] and Jia *et al.*[5] showed that percentage of *H. pylori* eradication is greater in patients who received combined periodontal and triple therapy when compared to triple therapy alone. A recent meta-analysis concluded that adjunctive use of periodontal treatment to systemic eradication therapy can reduce gastric *H. pylori* recurrence compared with systemic eradication therapy alone.[32] A combination of periodontal treatment with systemic therapy holds a promising approach for eradicating *H. pylori* and thereby decreasing the risk of re-infection.[32,55] Marbaix *et al.*[56] reported an update of scientific data showing the potential localization of *H. pylori* in the oral cavity of periodontitis patients and also proposed a multidisciplinary clinical protocol combining full-mouth disinfection and triple therapy to enhance eradication of oral *H. pylori*.

Multidisciplinary clinical protocol for the eradication of oral *H. pylori*[56] as proposed by Marbaix *et al.*

- Oral hygiene instructions: Tooth brushing, interdental plaque removal, tongue scraping
- Full-mouth disinfection (one or two sessions performed within 24 h): Mechanical scaling/root planning (or ultrasonic debridement), subgingival chlorhexidine irrigation, and tongue disinfection with chlorhexidine gel
- Triple therapy along with chlorhexidine mouthwash for 2 weeks should be started immediately following the last session of full-mouth disinfection.

H. pylori are found in low numbers in the normal oral flora, where their reliable detection is difficult.[13] It has been speculated that dental plaque might harbor *H. pylori* and act as a permanent reservoir thereby causing gastric re-infection. *H. pylori*[40,41] constantly present in dental plaque may be the result of occasional gastroesophageal reflux.

Bernander *et al.*[57] and Krajden *et al.*[58] reported that detection frequency of *H. pylori* in the oral cavity was lower than that in the stomach when culture method was used. This might be due to the lack of culture growth because of an insufficient number of cells or presence of unculturable but viable coccoid forms.[59] These limitations of culture method have been overcome by the use of PCR.

In the present study, at 3 months follow-up, there was 20% and 26.7% samples were positive for *H. pylori* in saliva and subgingival plaque, respectively, in the test group and 6.7% and 13.3% samples were positive for *H. pylori* in the control group. The percentage detected was reduced significantly following SRP. However, some of the limitations of the present study are: First, the sample size of the study was less. Second, repeated sampling of the same site was not done. The actual number of viable *H. pylori* was not detected since PCR permits the detection of nonviable *H. pylori*, which can be noninfectious. It allows the detection of even low numbers of bacteria, which may be too few to influence gastric health.

CONCLUSION

Within the limitations of the present study, dental plaque control is an effective way to reduce the load of *H. pylori* from the oral cavity. Therefore, professional plaque control along with standard daily plaque removal procedures should be recommended to patients with chronic gastritis or peptic ulcer prior to the triple therapy. However, further studies using larger sample size and sensitive and specific methods for detection of *H. pylori* are required to better assess the relationship of dental plaque, oral hygiene status, and periodontal disease with *H. pylori* infection. Once these relationships are better understood, intervention strategies can be designed to better endeavor the burden of *H. pylori* infection in the oral cavity.

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Conflicts of interest
There are no conflicts of interest.

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