ANALYSING SUBGINGIVAL PLAQUE WITH REGARD TO *H. PYLORI* AT CHRONIC AND AGGRESSIVE PERIODONTITIS PATIENTS

Kronik ve Agresif Periodontitisli Hastalarda H.pylori Açısından Subgingival Plak Analizi

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ABSTRACT

Background: *Helicobacter pylori (H. Pylori)* is a Gram (-), microaerophilic bacteria and the etiological factor of chronic active gastritis and peptic ulcer. Some studies indicated that this bacterium found at oral cavity which is a potential reservoir for stomach. Several studies showed that *H. pylori* may found in saliva and subgingival plaque of chronic periodontitis patients. However, there is no data related to aggressive periodontitis patients. In this study, we aimed to determine the prevalence of *H. pylori* in subgingival plaque samples of chronic, aggressive periodontitis and gingivitis patients and to increase the awareness of the patients for gastric problems.

Materials and Methods: This study included 155 patients (61 with gingivitis, 60 with chronic periodontitis, and 34 with aggressive periodontitis) who did not have gastric disease symptom and did not use antibiotics in the last 3 months. The subgingival plaque samples were taken using sterile paper points. The existence of *H. pylori*, *A. actinomycetemcomitans*, and *P. gingivalis* was detected by RT-PCR.

Results: *H. pylori* was not detected in any groups at the end of microbiological analysis. However, a high occurrence of *A. actinomycetemcomitans* (97.1%) and *P. gingivalis* (100%) was observed in the aggressive periodontitis group. However, *A. actinomycetemcomitans and P. gingivalis* were found in 30% and 21.7% of patients, respectively, with chronic periodontitis. *A. actinomycetemcomitans* and *P. gingivalis* were found in 24.6% of patients in the gingivitis group.

Conclusions: *H. pylori* were not detected in samples, indicating that subgingival plaque may not be a primary reservoir for this bacterium.

Key words: Aggressive periodontitis, Chronic periodontitis, *Helicobacter pylori*, *Polymerase Chain Reaction*

ÖZ

Amaç: Helicobacter pylori (H. Pylori), bir gram (-), mikroaerofilik bakteri olup, kronik aktif gastrit ve peptik ülserin etyolojik faktörüdür. Bazı çalışmalar, bu bakterinin, oral kavitede bulunduğunda, mide için potansiyel rezervuar olabileceğini göstermiştir. Çeşitli çalışmalar, H. pylori'nin kronik periodontitisli hastaların tükrük ve subgingival plaklarında görülebileceğini göstermiştir. Bununla birlikte agresif periodontitis hastaları ile ilgili herhangi bir veri yoktur. Bu çalışmada, kronik, agresif periodontitis ve gingivitis hastalarının subgingival plak örneklerinde H. pylori prevalansını saptamayı ve hastaların gastrik problemler konusunda bilinçlenmesini arttırmayı amaçladık.

Gereç ve Yöntem: Bu çalışma, gastrik hastalık semptomu olmayan ve son 3 ayda antibiyotik kullanmayan 155 hasta (61 adet gingivitis, 60'ı kronik periodontitisli ve 34 agresif periodontitisli) içermekteydi. Subgingival plak örnekleri steril paper point kullanılarak alındı. H. pylori, A. actinomycetemcomitans ve P. gingivalis'in varlığı RT-PCR ile tespit edildi.

Bulgular: Mikrobiyolojik analizin sonunda herhangi bir grupta H. pylori tespit edilmedi.

Bununla birlikte, agresif periodontit grubunda yüksek oranda A. actinomycetemcomitans (%97.1) ve P. gingivalis (%100) görülmüştür. Bununla birlikte, A. actinomycetemcomitans ve P. gingivalis, kronik periodontitisli hastaların sırasıyla %30 ve %21.7'sinde bulunmuştur. A. actinomycetemcomitans ve P. gingivalis gingivitisli hastaların %24.6'sında bulundu.

Sonuç: H. pylori, örneklerde saptanmamış olması, subgingival plağın bu bakteri için birincil rezervuar olmayabileceğini gösterdi.

Anahtar kelimeler: Agresif Periodontitis, Kronik periodontitis, Helicobacter pylori, Polimeraz zincirleme reaksiyonu

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INTRODUCTION

Helicobacter pylori is a spiral-shaped, motile, and microaerophilic gram-negative microorganism shown to cause gastritis, repetitive peptic ulcer, duodenal ulcer, and gastric cancer. Also, 50%–90% of the world population is estimated to be infected with this pathogenic microorganism and this organism is taken into the body during childhood.[1, 2] *H. pylori* can be isolated from the oral cavity, dental plaque, dorsum of the tongue, or salivary secretions. Its colonization is thought to increase in the presence of periodontal diseases.[3, 4]

Periodontal diseases are extremely prevalent worldwide, affecting roughly half of the adult population. Gingivitis, the mildest form of periodontal disease, is a rapidly inducible and reversible inflammation of gingiva mainly caused by the accumulation of bacterial biofilms. The combination of bacterial infection and persistent inflammatory response can eventually induce the progressive destruction of the deeper periodontal tissues, a worse form of periodontal disease called periodontitis. Aggressive periodontitis is a disease that affects mostly young individuals and can result in fast bone or teeth losses.[5] Several studies have shown the presence of *H. pylori* in the subgingival plaque and saliva of individuals with chronic periodontitis.[3] However, no study has explored the status of *H. pylori* in the oral cavity of individuals with aggressive periodontitis.

H. pylori infections are systemically treated with antibiotics and proton pump inhibitors in a short time.[6] However, complete eradication of *H. pylori* is possible only if its potential reservoirs detected. Bacteria in the oral reservoirs may cause recurrence of the disease after some time.

The numbers of periodontopathogenic bacteria in periodontal lesions increase with the development of periodontitis. Strains of *Porphyromonas gingivalis* and *Fusobacterium nucleatum* were found to be strongly co-aggregated with *H. pylori* strains.[7] Urease test, culture, immunofluorescence, and polymerase chain reaction (PCR) are the most commonly used techniques for detecting *H. pylori* in dental plaques.[8] Real-time (RT)-PCR is a widely used technique in the clinical practice for diagnosing several bacterial and viral infections because it is able to amplify small amounts of genetic sequences with a very high sensitivity.[9]

The aim of this study was to determine the *H. pylori* strains in the subgingival plaques of patients who had no dyspeptic complaints with chronic periodontitis, aggressive periodontitis and gingivitis.

MATERIALS AND METHODS

Study population

This study included 155 individuals aged between 18 and 65 years who did not have any systemic problems affecting periodontal tissues, did not take antibiotics in the last 3 months, did not have dyspeptic symptoms, were not treated for specific reasons, and were not pregnant or lactating in the case of women. The patients applied to Periodontology Department for their gingival disease. All participants signed an informed consent before undergoing research procedures and voluntarily participated in the clinical protocol. Approval was obtained from the Karadeniz Technical University Faculty of Medicine Ethics Council (number 17522305/547; date 01/10/2013) at the beginning of this single-center, cross-sectional study.

Sample collection

After the clinical periodontal examination, subgingival plaque samples were taken from four teeth with the deepest probing depth in each quadrant. For the subgingival plaque sampling, the supragingival plaque was removed using sterile curettes, and the sterile paper points were placed at the bottom of the pocket for 20 s. These samples were placed in an Eppendorf tube containing 0.1 mL of 1M Tris-EDTA (TE) (10 mM Tris-HCl, 1 mM EDTA, pH 7.6) and stored at –80°C until analysis.

Microbiological procedures

The microbiological analyses were carried out at Karadeniz Technical University Medical Faculty Microbiology Department Laboratory. The presence of *Aggregatibacter actinomycetemcomitans*, *P. gingivalis*, and *Helicobacter pylori* species in the subgingival plaque samples was detected by using the PCR method. The suspensions of control strains such as *P. gingivalis* (clinical isolate), *A. actinomycetemcomitans* (clinical isolate), and *H. pylori* (SS1) were prepared at 0.5 McFarland turbidity standard and stored as earlier.

The paper points in the samples, which were kept in 1.5-mL centrifuge tubes, were removed after vortexing, and the tubes were centrifuged for 5 min at 10,000 rpm. The pellet at the bottom was suspended in 0.4 ml distilled water and 200 µL of the sample was used for DNA isolation. The DNA isolation was carried out using ExiPrep 16 Plus instrument and ExiPrep Bacteria Genomic DNA Kit (Bioneer, South Korea).

Detection of agents with RT-PCR

The accuracy of the DNA isolation process was confirmed by showing the presence of bacterial DNA with RT-PCR. The RT-PCR was also used for determining the presence of pathogens. The sequences of primers and probes used in the experiments are given in Table 1. Test compounds were prepared in AccuPower Plus DualStar qPCR Master Mix (Bioneer) with 0.2µM primers and 0.1µM probes. The amplification was done using LightCycler 480 (Roche, Salt Lake City, UT, USA). The temperatures used in this instrument were 95°C for 10 min for the initial denaturation, followed by 45 amplification cycles at 95°C for 15 s, 60°C for 20 s, and 72°C for 20 s. The amplification curves were generated by the instrument that measured the intensity of the fluorescence-labeled primers.

Table 1: Primers and probes used in the study

Primer	Sequence	Source
UNIVF	TGGAGCATGTGGTTTAATTCGA	1
UNIVR	TGCGGGACTTAACCCAACA	1
UNIVP	FAM-CACGAGCTGACGACARCCATGCA-TAMRA	1
AAF	GAACCTTACCTACTCTTGACATCCGAA	2
AAR	TGCAGCACCTGTCTCAAAGC	2
AAP	FAM-AGAACTCAGAGATGGGTTTGTGCCTTAGGG-BHQ1	2
PGF	GCGCTCAACGTTCAGCC	3
PGR	CACGAATTCCGCCTGC	3
PGP	HEX-CACTGAACTCAAGCCCGGCAGTTTCAA-BHQ1	3

Statistical analysis

SPSS for Windows version 17.0 (SPSS, IL, USA) was used for statistical analyses. For age, the Kruskal–Wallis test was used with Bonferroni correction besides the Mann–Whitney *U* test. The pearson chi-square test was used to compare the prevalence rate and sex distribution of bacteria. Calculations at the 5% significance level showed that 33 patients in each group were sufficient to detect a difference between groups with 90% statistical power.

RESULTS

Participants were classified according to their disease status after clinical and radiographic examinations conducted at the Periodontology Department. Of these participants, 60 were diagnosed with chronic periodontitis, 61 with gingivitis, and 34 with aggressive periodontitis. The age and gender status of the patients are shown in Table 2.

Table 2: Age and gender distributions in groups

Group	Female/Male	Age
G (n=61)	30/31	33,97±5.54
CP (n=60)	29/31	45.17±8.6*
AP (n=34)	15/19	34.03±6.82
p	NS	**p* <0.001

The average ages of the participants in the chronic periodontitis, gingivitis, and aggressive periodontitis groups were 45.17± 8.6, 33.97±5.54, and 34.03±6.82, respectively.

When these three groups were compared, the chronic periodontitis group was found to be significantly different from the other groups ($p < 0.001$). No statistically significant difference was found between groups in terms of gender distribution ($p > 0.05$).

The prevalence of bacteria in the subgingival plaque samples is shown in Table 3. H. *pylori* was not determined in any of the study groups. The prevalence of A. *actinomycetemcomitans* was 24.6%, 30%, and 97.1% in the gingivitis, chronic periodontitis, and aggressive periodontitis groups, respectively. When these three groups were compared, a significantly higher prevalence was determined in the aggressive periodontitis group ($p < 0.001$). Furthermore, the prevalence of P. *gingivalis* was 24.6%, 21.7%, and 100% P. *gingivalis* in the gingivitis, chronic periodontitis, and aggressive periodontitis groups, respectively. Therefore, significantly higher bacteria were seen in the aggressive periodontitis group compared with the other groups ($p < 0.001$).

Table 3: Real Time PCR Results

Group	*AA(%)*	*PG(%)*	*HP(%)*
G (n=61)	24.6	24.6	0
CP (n=60)	30	21.7	0
AP (n=34)	97.1*	100*	0
p	*p <0.001	*p <0.001	NS

AA, Agregatibacter actinomycetemcomitans; *PG, Porphyromonas gingivalis*; *HP, Helicobacter pylori*

DISCUSSION

Both periodontal disease and *H. pylori* infection were reported in more than 50% of the population and share some common risk factors. The multivariate analysis showed that age, poor oral hygiene, smoking behavior, and diabetic status of the individuals were some of the risk factors for periodontal disease.[10, 11] Poor oral hygiene and tooth loss can potentially influence gastrointestinal flora and nutritional situation and consequently be implicated in the development of chronic gastrointestinal diseases.[12, 13] So some authors have concluded that elimination of bacterium from the oral cavity should be regarded as an important role of the treatment of *H. pylori* associated diseases, since the oral cavity may serve as a temporary reservoir.[14, 15]

In our study *H. pylori* was analyzed in subgingival plaque samples in periodontal diseases to answer the crucial questions that are whether the oral cavity is a reservoir and whether it plays a role in *H. pylori* transmission.

There are controversial results in the literature. Several studies suggested a positive association between oral and gastric *H. pylori* detection[16-19], although, some studies failed to demonstrate such an association.[20-23] Poor periodontal health, characterized by deep periodontal pockets, was introduced in relation to *H. pylori* infection in some studies.[24, 25]

Silva Rossi-Aguiar[22] analyzed saliva, dorsum of the tongue, and supragingival dental plaque samples of 43 patients with gastric disease and did not detect *H. pylori* in these oral samples by PCR. Their results were consistent with the results of Olivier *et al*[23], who also failed to detect *H. pylori* in dental samples by PCR. Savoldi *et al*. reported results similar to the present study results using different techniques such as urease test, culture, and PCR to demonstrate that *H. pylori* is not generally present in dental plaques. They also observed, in accordance with other studies, that oral hygiene did not significantly influence the prevalence of *H. pylori*.[20]

Asikainen *et al*. used PCR for detecting *H. pylori* in subgingival plaque of patients with periodontitis. Patients were not evaluated for their any dyspeptic disease or gastric *H. pylori* presence. They concluded that periodontal pockets did not constitute a natural reservoir for *H. pylori* and paper point sampling method also might affect the detection of *H. pylori* if periodontal pockets only harbor low numbers

of *H. pylori* cells, which predominantly might attach to the pocket epithelium.[26] The result of our study is similar with study of Asikainen *et al*. We also did not find *H. pylori* in the RT-PCR evaluation of the subgingival plaque samples in either of the three periodontal disease conditions. One possible reason for not detecting *H. pylori* in subgingival plaque may be paper point sampling method.

On the other hand, there is also a great deal of evidence that *H. pylori* is found in the oral mucosa and gastric mucosa (in tongue, saliva, and sub-supra gingival plaque samples) and also these evidences prove that there is a positive correlation between them.[17, 19, 27-29]

In a study of Gebara *et al*. urease-positive patients divided into 2 groups according to their periodontal disease status: 15 with gingivitis and 15 with chronic periodontitis. The plaque and saliva samples were analyzed for *H. pylori* using PCR. The gingivitis and chronic periodontitis groups were not different in terms of the prevalence of bacteria.[3] In our study the patients who did not have any gastric symptoms were included to the study.

However, we did not make any test related to gastric *H .pylori*. At the end of the study we did not find difference in terms of the prevalence of *H. pylori* among groups. In another study that was conducted on patients who do not have any gastric symptoms, Souto *et al*. investigated saliva and subgingival samples periodontally healthy and chronic periodontitis patients. *H. pylori* was detected significantly more often in the saliva and subgingival samples of subjects with periodontitis (23.5% and 50%, respectively) compared with samples of periodontally healthy subjects.[28]

H. pylori exists in a microaerobic atmosphere and it seems to prefer supragingival plaque or shallow pocket sites than deep periodontal pockets. *H. pylori* grows well in an atmosphere with low levels of oxygen, rather than in a strictly anaerobic atmosphere.[30] Since the plaque samples were taken from the deepest pockets subgingivally, the microaerobic aspect of the bacteria might be the reason for not finding any *H. pylori* in either of the periodontal disease groups. Another reason might be that the patients who had dyspeptic complaints were not included to eliminate the possibility of transition from the stomach to oral cavity such as reflux.

This novel study investigated the presence of *H. pylori* in the patients who had generalized aggressive periodontitis. A high prevalence of *P. gingivalis* and *A. actinomycetemcomitans* were found in the plaque samples of the patients with aggressive periodontitis. This result verifies that the patients diagnosed as aggressive periodontitis clinically, have harbored the bacterias that are specific in this disease. In literature, some microorganisms such as *A. actinomycetemcomitans*, some *Capnocytophaga* species, *Eikenella corrodens*, *P. intermedia*, and *Campylobacter rectus* were reported as frequently detected in the patients with aggressive periodontitis.[31] *P. gingivalis* very frequently appears in the subgingival plaque of patients with chronic periodontitis, ranging from 29.6 to 97.5% prevalence. [32-34]

While *P.gingivalis* is considered one of the bacteria most closely associated with chronic periodontitis, it is also present in the subgingival plaque of periodontally healthy patients, ranging from 1.5 to 57.8% depending on the studies.[35, 36]

In our study prevalence of *P. gingivalis in* gingivitis and chronic periodontitis group was lower rate (24.6%, 21.7% respectively) Similar to our work Krishnan *et al*. found prevalence of *P. gingivalis* fim A type I gene among chronic periodontitis and chronic gingivitis patients were 8.7% and 30.4% respectively.[37]

We conducted this study at north-east site of country, at Black-Sea region. So, procedural variations and geographical differences between the studied populations may have a strong

influence on the reported results. Also, the microbial composition of dental plaque varies from tooth to tooth and from site to site within the same tooth. Differences in the prevalence of *H. pylori* in the oral cavity might also have resulted from differences in the population, oral health condition, *H. pylori* infection, the type and number of clinical specimens and the definition methods used.[17, 38]

This study used RT-PCR to investigate the presence of bacteria in the oral cavity of patients with gingivitis, chronic periodontitis, and aggressive periodontitis without dyspeptic symptoms. As a result, *H. pylori* bacteria was not found in any of the patients. This supported the view that the subgingival environment may not be a reservoir of *H. pylori* bacteria. However, more studies are needed to explore the rate of infection in the mouth and different periodontal disease groups.

Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical approval: "All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards."

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