#### DOI: 10.5455/msm.2025.37.48-53

Received: Feb 03 2025; Accepted: Mar 06, 2025

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**ORIGINAL PAPER** 

Mater Sociomed. 2025; 37(1): 48-53

# Clinical Evaluation and Microbiological Identification of Helicobacter Pylori in Patients with Oral Ulcerations Using the Polymerase Chain Reaction (PCR) Method

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#### **ABSTRACT**

Background: The etiology of oral ulceration is multicausal with numerous predisposing factors. Studies by various authors cite Helicobacter pylori infection as a possible cause of certain oral ulcerations. Objective: The aim of the study is to prove the presence of Helicobacter pylori in the oral cavity of patients with oral ulcerations, as well as to examine the relationship between the presence of Helicobacter pylori in the oral cavity and the development of oral ulcerations. Methods: The study included regular patients at the Department and Clinic of Oral Medicine and Periodontology, Faculty of Dental Medicine, University of Sarajevo, suffering from oral ulcerations, as well as healthy patients without oral diseases. The diagnosis of oral ulceration is based on a thorough history, clinical examination, and exclusion of other oral diseases. All patients were taken anamnestic data on the existence of digestive system diseases, and by reviewing medical documentation, a previously diagnosed digestive system disease by a gastroenterologist was recorded. A cytological smear was taken in all 80 cases, in patients with ulcerative lesions a smear was taken from the lesion and in healthy subjects from the mucous membrane of the palate, cheek and tongue. Highly sensitive and specific polymerase chain reaction (PCR) was used to determine the presence of Helicobacter pylori in the oral cavity and oral lesions. Results: The

results of our study showed that there is no statistically significant difference between the presence of Helicobacter pylori in the oral cavity of patients with oral ulcerations and subjects with healthy mucosa. **Conclusion:** Helicobacter pylori is not a risk factor for the development of oral ulcerations and can be found on the oral mucosa as a transient pathogen.

Keywords: oral mucosa, ulcerations, Helicobacter pylori, Real-time PCR.

#### 1. BACKGROUND

Oral ulceration (ulcer, canker sore) is a deep defect of the mucosa that results from pathological processes occurring in the dermis. The ulceration is described by its shape, size, depth, base, edge, consistency, surroundings, and sensitivity. The base of the ulceration is located in connective tissue and muscle and heals with a scar.

Ulcerative diseases of the oral mucosa have a very similar clinical picture and therefore a detailed history and clinical examination are of great importance for their diagnosis. The etiology of oral ulceration is multicausal with numerous predisposing factors (Figure 1).

The classification of ulcerations, with regard to the etiological factor that led to their occurrence, includes: oral mucosal ulcerations caused by injury, oral mucosal ulcer-



Figure 1. Recurrent aphthous ulcerations localized on the alveolar mucosa

ations in hereditary diseases, oral mucosal ulcerations caused by malignant change, oral mucosal ulcerations caused by immune and autoimmune diseases, and oral mucosal ulcerations caused by infection. In the last 15-20 years, much attention has been paid to the so-called extragastric or extraintestinal manifestations of H. pylori bacterial infection, which vary from iron-deficiency anemia and chronic urticaria to severe neurological, immune, and rheumatic conditions (1).

Studies conducted by various authors indicates infection with Helicobacter pylori as a possible cause of certain oral ulcerations. Although there is a large number of studies on this topic, the results are mixed. Some studies confirm the link between the presence of Helicobacter pylori and the development of oral ulcers, while others deny it. Helicobacter pylori has been detected in dental plaque, saliva, (2, 3) the subgingival region (4) and oral ulcerations (5).

The exact mode of transmission of H. pylori is unknown, but it is thought to be most common through the oral route. The oral cavity is also thought to be a source of reinfection and eradication of H. pylori in the oral cavity is much more difficult than in the gastro-intestinal tract (6). As a consequence, microorganisms may persist in the oral cavity after successful eradication from the stomach (7). It is of great importance to determine the presence of bacteria in oral lesions, which can further explain its pathogenicity in the oral cavity.

Recent research suggests that Helicobacter pylori is a possible cause of oral ulcerations. Given their high incidence and multifactorial etiology, as well as the fact that treatment of oral ulcerations is still symptomatic, testing for the presence of this bacterium on the oral mucosa is warranted.

Oral ulcerations in patients represent a major problem for their daily lives because they make speech and chewing functions difficult, they are painful, and there is an increasing number of patients at the Clinic and Department of Oral Medicine and Periodontology of the Faculty of Dental Medicine with Clinics in Sarajevo, who are seeking answers regarding effective therapeutic modalities.

Highly sensitive and specific polymerase chain reaction (PCR) was used to determine the presence of Helicobacter pylori in the oral cavity and oral lesions. PCR is a method based on the detection and multiplication (amplification) of one or more nucleic acid sequences that are specific for a family, genus, species, strain or type of bacteria, virus, or parasite (8).

# 2. OBJECTIVE

The aim of the study was to prove the presence of Helicobacter pylori in the oral cavity in patients with oral ulcerations, as well as to examine the correlation between the presence of Helicobacter pylori in the oral cavity and the development of oral ulcerations.

## 3. MATERIAL AND METHODS

The study included patients at the Department and Clinic of Oral Medicine and Periodontology of the University of Sarajevo - Faculty of Dentistry with Clinics, suffering from oral ulcerations, as well as healthy subjects without oral diseases.

The sample consisted of 80 subjects of both gender, divided into two groups: Experimental group: patients suffering from oral ulcerations, and Control group: healthy subjects, who do not suffer from oral ulcerations.

The inclusion criteria of subjects in the study were: patients aged 18 to 65 years; oral ulcerations present during the first clinical examination in the experimental group; patients who have a disease from the group of gastrointestinal diseases, but with a verified finding from a gastroenterologist specialist The exclusion criteria from the study were: presence of a systemic disease (infectious and chronic); presence of other oral diseases; use of medication or drug abuse; hormonal imbalance of the body.

The diagnosis of oral ulceration is based on a thorough history, clinical examination, and exclusion of other oral diseases. For the research, a working card (attached) was created, which contains anamnestic data and data from the clinical examination. All subjects were taken anamnestic data on the existence of digestive system diseases, and by reviewing medical documentation, a previously diagnosed digestive system disease by a gastroenterologist was recorded.

Cytological smears were taken from all 80 subjects. In subjects with ulcerative lesions, a smear was taken from the lesion, and in healthy subjects, from the mucous membrane of the palate, cheek, and tongue. Swabs were taken with a cytological brush (Shanghai Kinmed Import, China), which immediately after taking the cytological swab was immersed in an Eppendorf tube filled with 1.5 ml of physiological solution (sodium chloride HZTM 9 mg/ml (0.9%). Until the time of transport to the Institute for Biomedical Diagnostics and Testing "Nalaz", the samples were stored in a freezer at a temperature of -22 °C.

The molecular genetic analysis was performed by

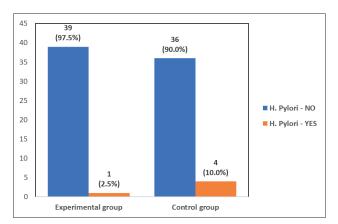


Figure 2. Frequency of H. pylori infection presence in the studied groups

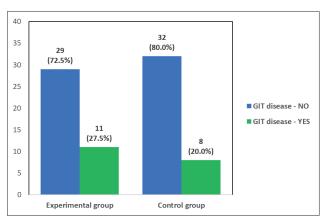


Figure 3. Frequency of presence of gastrointestinal tract diseases in the studied groups

experts and responsible persons at the Institute of Biomedical Diagnostics and Testing "Nalaz".

The process of detecting and identifying H. pylori involved the following steps:

- a) DNA isolation;
- b) Amplification and identification of H. pylori.

DNA extraction was performed using the QIAGEN DNA Mini Kit according to the manufacturer's instructions.

The process of amplification and identification of H. pylori was performed by real-time PCR method using Sacace Helicobacter pylori REAL-TM on 7500 Applied biosystem RT-PCR machine.

#### 4. RESULTS

The results were processed using standard statistical methods, using the SPSS computer program for statistical analyzes (SPSS - Statistical Package for Social Sciences) version 21.0. Results are expressed as absolute numbers N and percentage values %, as mean ( $\chi$ ) and standard error of the arithmetic mean (SEM), and as median and interquartile range (25-75 percentile). The Shapiro-Wilk test was used to test the significance of the difference in deviation from the normal distribution. The results were analyzed with a t-test for variables that met the conditions for application, or with corresponding non-parametric tests (Mann-Whitney U test) for variables with irregular distribution. In the analysis of dependence between

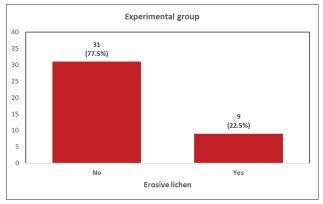


Figure 4. Frequency of occurrence of erosive lichen in the experimental group of subjects

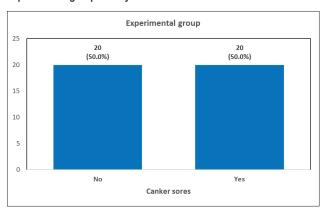


Figure 5. Frequency of canker sores in the experimental group of subjects

categorical variables, the Chi-square test and Fisher's exact test were used.

### 5. DISCUSSION

Ulcerative diseases of the oral mucosa represent a challenge for the dentist for several different reasons. Ulceration is a clinical sign of a wide spectrum of diseases, and the overlap of clinical characteristics between ulcerations of different etiologies represents a difficulty in establishing a diagnosis. In the treatment of ulcerative, as well as all other diseases of the oral cavity, it is important to identify and therapeutically eliminate local and systemic etiological factors, to remove unpleasant subjective complaints, but also to prevent the spread of oral diseases to other organs and organ systems.

Although the classification of oral ulcerations in our work includes a division according to the etiological factors that led to their occurrence, the knowledge that Helicobacter pylori is one of the causes of stomach and small intestine diseases, as well as the fact that these diseases are often associated with oral cavity diseases, justifies the examination of the presence of this bacterium on the oral mucosa.

We confirmed the presence of Helicobacter pylori in the oral cavity through our research. Out of a total of 80 subjects, 12.5% of subjects had bacteria present on the mucous membrane of the oral cavity. To determine the presence of Helicobacter pylori in the oral cavity and oral lesions, we used a highly sensitive and specific polymerase chain reaction (real-time PCR). With the

development of technology and the increasing need for new tests, new "versions" of the polymerase chain reaction are introduced. Real-time PCR is a method that is increasingly used for the detection and typing of the bacterium Helicobacter pylori (9,10).

Different PCR techniques ("Novel" and "Nested") can even be used to detect HP in samples from the oral cavity, such as saliva, dental plaque or inflamed dental pulp, and this is a great advance (11). Saliva as a medium and its composition are an increasingly common cause of research by many scientists because it is accessible by non-invasive methods and represents a mirror of systemic events in the body (12).

Based on the aforementioned research, we believe that the PCR method for detecting Helicobacter pylori in oral cavity samples is justified and that its use has ensured almost complete sensitivity and specificity of the results. The possible role and connection between the presence of Helicobacter pylori in the oral cavity and the development of gastrointestinal tract diseases, as well as the possible role and connection between the presence of Helicobacter pylori in the oral cavity and the development of ulcerative mucosal diseases, has been discussed for years. The main question that arises is whether the bacteria, if present in the oral cavity, is pathogenic.

Some of the strains of Helicobacter pylori found in the oral cavity are identical to those found in the gastric mucosa (13, 14, 15). This may indicate that a gastro-oral route of transmission of infection is possible or that Helicobacter pylori may primarily colonize certain sites in the mouth.

In the research of Cześnikiewicz-Guzik et al., it was concluded that the oral cavity is only transiently contaminated with bacteria and that there is no clear connection with the stomach infection (16). Similar results were obtained by Loster et al., who also concluded that the oral cavity does not serve as a reservoir of bacteria for infecting the gastric mucosa (17). In the study by Silva Rossi-Aguiar, 43 patients with epigastric pain syndrome were studied, in whom Helicobacter pylori infection was diagnosed by rapid urease test and urea breath test. Saliva, dorsum of the tongue and supragingival dental plaque samples were collected from the oral cavity of each subject, and subgingival dental plaque samples were collected from patients with periodontitis.

H. pylori infection was confirmed by polymerase chain reaction. The conclusion of the research was that the oral cavity is not a reservoir of bacteria in patients with epigastric pain syndrome and that it is present exclusively in the stomach (18). Chinese scientists investigated the presence of Helicobacter pylori in the oral cavity of patients with gastritis. One of the diagnostic methods was polymerase chain reaction. The results of the study showed that Helicobacter pylori exists in the oral cavity of patients with gastritis and that oral infection may be an important source of gastric infection (19).

In a study by Turkish scientists, the study group

consisted of children with symptoms of dyspepsia. The oral parameters included in the study were saliva and dental plaque, and the method used to analyze the samples was the polymerase chain reaction method. The conclusion of the study is that Helicobacter pylori can be found in the oral cavity of patients regardless of whether the bacteria are present or not in the stomach of the patients, although the incidence of the bacteria is higher in children with gastric infection (20). In the following research, samples of dental plaque and gastric mucosa were analyzed by PCR, histopathologically and by rapid urease test in patients with dyspepsia. Research results indicate that gastric infection is associated with the presence of Helicobacter pylori in the mouth (21). In our research, we examined the presence of bacteria in the oral cavity of patients suffering from various gastrointestinal diseases: gastritis, GERD, gastric ulcer, and patients with various dyspeptic complaints.

We did not confirm a link between the presence of bacteria in the oral cavity and gastrointestinal diseases. The inconsistency of the above studies, including our study, can be explained by the fact that Helicobacter pylori lives in the environment in a coccoid form that is difficult to cultivate and remains in this form for a short time in the oral cavity, and in the stomach it transforms into a form in which it begins to multiply and thus causes infection. Also, different diagnostic procedures used to detect the bacteria in the oral cavity and stomach could have led to the above results.

On the other hand, data from the literature on the presence of Helicobacter pylori in the oral cavity, as well as its possible role in the development of ulcerative lesions, are quite scarce and contradictory. Our research has confirmed that Helicobacter pylori is not present in the oral cavity of patients with oral ulcerations and does not represent a risk factor for their development. The results of our research are confirmed by a large number of studies that have also attempted to link the presence of Helicobacter pylori in the oral cavity and the development of various ulcerations.

In a study that included subjects diagnosed with recurrent aphthous stomatitis and duodenal ulcer, as well as subjects diagnosed with aphthous stomatitis but without the presence of duodenal ulcer, no link was found between Helicobacter pylori infection and aphthous stomatitis (22).

Shimoyama T. examined the role of Helicobacter pylori in the development of oral ulcerations using culture as the gold standard in diagnosis. The subjects had diagnoses of recurrent aphthous stomatitis, erosive Lichen planus and herpetic gingivostomatitis. All cases of recurrent aphthous stomatitis and erosive Lichen planus had a negative culture for Helicobacter pylori, while two cases of herpes were positive. The conclusion of the research is that Helicobacter pylori has no direct connection with oral ulcerations (23). In his research, using polymerase chain reaction and biopsy samples from patients with recurrent aphthous

stomatitis and oral Lichen planus, Riggio does not find a connection between infection with Helicobacter pylori and their occurrence, but also states that the role of Helicobacter pylori in a small number of cases of recurrent aphthous stomatitis cannot be ruled out (24).

The presence of Helicobacter pylori on the oral mucosa was also examined by Mravak Stipetić and her associates. In one study, the aim was to examine the colonization of the lingual mucosa with Helicobacter pylori. The results showed that the incidence of presence was increased in atrophic glossitis and burning mouth syndrome. Mucosal changes in these conditions could make the oral environment more receptive to H. pylori colonization compared to physiological mucosa, and this mechanism may play a role in its oro-oral transmission (25). In another study by the same author, the presence of Helicobacter pylori in various oral lesions was examined by polymerase chain reaction. The study included 121 patients and only 21% of the patients were positive. The conclusion of the study is that Helicobacter pylori is not pathogenic in the oral cavity and is not associated with oral pathological processes (26).

In the aforementioned study, the authors concluded that Helicobacter pylori is present in a small number of aphthous lesions. Also, if present, it is almost always associated with chronic gastritis (27). In the study by Birek et al., the results showed the opposite. Helicobacter pylori is present in most aphthous lesions, but the possible pathogenesis of oral ulceration needs to be further investigated and discussed (28).

Study conducrted by Turkish scientists has shown that there is a possibility that infection with Helicobacter pylori, which was confirmed by endoscopic biopsy of the stomach in 30 patients with recurrent aphthous stomatitis, is the cause of its occurrence. This study suggests eradication of Helicobacter pylori in patients with aphthous stomatitis (29).

Our study did not confirm the connection between the occurrence of Lichen planus and the presence of Helicobacter pylori in the oral cavity. 22.5% of our patients were diagnosed with erosive Lichen. Only one patient with the above diagnosis had Helicobacter pylori present in the oral cavity. The patient also had a confirmed diagnosis of erosive gastritis by a gastroenterologist. The association between Lichen planus and infection with Helicobacter pylori was not found by a group of scientists who determined the presence of the bacteria with a urea breath test in two groups of patients: those suffering from Lichen planus as well as healthy ones (30).

In a study conducted by Kazanowska et al. using the polymerase chain reaction method, they found that the presence of Helicobacter pylori in the oral cavity can be associated with the occurrence of leukoplakia and Lichen planus lesions (31). The results of recent studies are mixed. Some studies confirm the link between the presence of Helicobacter pylori and the development of oral ulcers, while others deny it.

Our results confirmed that there is no statistically

significant difference between the experimental and control groups and Helicobacter pylori as a possible etiological factor, which is in agreement with most studies.

The results can be explained, first of all, by the fact that the average age of the subjects of both groups was 30 years, that they were healthy individuals, without systemic diseases except from the gastrointestinal group. It should be remembered that the most common route of transmission of infection is oral-oral or fecaloral in a community with low hygiene standards. The defense factors of the oral cavity that prevent the balance from being disturbed are numerous, and a stable oral flora prevents the occurrence of infection on the oral mucous membranes.

Helicobacter pylori, as previously mentioned, can probably persist in the oral cavity in a coccoid form that cannot be cultivated and reproduces only under special conditions.

#### 6. CONCLUSION

The results of our study showed that there is no statistically significant difference between the presence of Helicobacter pylori in the oral cavity of patients with oral ulcerations and patients with healthy mucosa. Therefore, Helicobacter pylori does not represent a risk factor for their occurrence.

Regardless of the fact that the results of our study showed that Helicobacter pylori does not represent a risk factor for the occurrence of oral ulcerations, additional research in this direction is needed to resolve many dilemmas. Great importance should be given to multidisciplinary cooperation between gastroenterologists and dentists, as well as other medical professionals, in order to discover etiological factors, establish an early diagnosis, therapy and prevention of oral ulcerations.

- Author's conribution: The all authors were involved in all steps of preparation this article. Final proofreading was made by the first author.
- Conflict of interest: None to declare.
- Financial support and sponsorship: Nil.

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