

THE ORAL CAVITY - RESERVOIR OF INFECTION WITH *HELICOBACTER PYLORI*

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ABSTRACT

The oral cavity has been established as an entrance door and second ecological niche of *Helicobacter pylori* (HP). This question remains open – is the oral cavity a source of stomach infection or a cause of re-infection, recurrence or persistence of the infection? The aim of this study is to evaluate the frequency of HP infection into dental plaque and saliva on the base of performed screening, parallel and post-eradication investigation. In the context of this research the method of PCR has been implemented. In the course of the screening examination HP infection has been proved in 66% of all the cases. By the means of parallel investigation a combined infection has been confirmed in 62% of all the cases – presence of HP in the stomach and into the dental plaque. Concerning the HP infection into the dental plaque, it is in co-relation with both of the symptoms – periodontitis and halitosis. Taking into consideration these facts, we can conclude that oral cavity serves as a reservoir of infection, as well as a source for its transfer into the stomach. Poor oral health status, including clinical findings of periodontitis and halitosis, have to be accepted as alarming markers for HP infection into the oral cavity and stomach. It is of great necessity for the patient to organize regular visits to the dentist for dental plaque control and tartar removal and maintenance of proper oral-dental condition as a measure of prevention of HP infection.

Keywords: *Helicobacter pylori* infection, oral cavity, prevalence, PCR, dental plaque, combined HP infection

INTRODUCTION

Infection with *Helicobacter Pylori* is a very old, mass and widespread disease all over the world, affecting over 50% of the world population. It is large-scaled in the developing countries, at the same time characterized with lower distribution concerning the developed countries /its frequency rates at 25-40% in Western Europe, in our country it amounts to 80%/. The process of contamination is being performed during the first years after birth, transferred directly from the infected mother to the newly-born. This bacterium infects the macroorganism, provoking clinical manifestation only in 15-20% of the carriers.

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Most of the infected are characterized by no symptoms. On the base of our previously performed investigation (1) there is a tendency of reduction of the distribution of HP infection among dyspeptic patients, totaling up to 15% today. It has been established that the infection with *Helicobacter pylori* plays the role of an etiological and pathogenic factor for initiation and progression of chronic gastritis, atrophic gastritis, ulcer disease (gastric and duodenal ulcer), gastric cancer and MALT-lymphoma. In the year 1992 the infection was defined by the WHO as a cancerogenic agent of first class. For this reason it has been determined as a disease with social impact, associated with the progression of a significant socio-pathology with great public response. So, the strategy of **testing for *Helicobacter pylori* and successful treatment is established as a step ahead concerning the prevention of gastric cancer.** The oral cavity is an entrance door for the infection and the main reservoir of *Helicobacter pylori* out of the gastric (2), being a second ecological medium. It has been confirmed that by patients with dyspeptic complaints *H. pylori* is recorded in 80% to 97% of all cases into the dental plaque and in 55% of all cases into saliva (3, 4). Gebara et al. (5) have reported that the concentration of *H. pylori* into the sub-gingival dental plaque is almost twice higher compared with that into supra-gingival one (amounting to 22%). According to Songet et al. (3) these bacteria predominantly inhabit periodontal pockets with deepness over 4 mm (57%), in comparison to pockets under 4mm (34%). There are investigations about various rates of its distribution into the plaque on different teeth surfaces (3). The isolation of *H. pylori* in dental plaque has been recorded to be intermittent (6).

Taking into consideration the importance of oral cavity as a source of *H. pylori* infection and its role for re-infection of the stomach, we have determined these **TASKS:**

- ❖ To perform an investigation concerning ascertainment of the bacterium of *H. pylori* in the oral cavity (dental plaque and saliva) into various groups: screening, parallel to stomach examination and after HP eradication treatment;
- ❖ To investigate the co-relation between the presence of the *H. pylori* bacterium in oral cavity and symptoms of clinical manifestation, as well

as the combined infection in the oral cavity and stomach.

- ❖ To investigate the influence of the status of *H. pylori* on the effect of eradication treatment.

CLINICAL MATERIAL AND METHODS

This study included 146 patients with a mean age of 49. In 111 of them the dental plaque, and in the other 35 – saliva has been examined for the presence of *H. pylori*. The screening test of the dental plaque has been performed in 18 patients, chosen at random during dentists' visits. In 58 patients a parallel investigation has been provided with confirmed HP infection in the stomach. In 31 patients the dental plaque has been examined after eradication treatment.

The oral-dental status of patients with diagnosed HP infection into the dental plaque has been estimated as well during screening tests. Examination of the teeth and other structures in the oral cavity was conducted by dentist. The aim to obtain additional information about clinical symptoms, anamnesis data of gastro-duodenal pathology and tests concerning the diagnosis of HP infection, is performed by the application form and clinical card. We used a question form containing 10 "yes" or "no" questions. The clinical card has been provided to the dental specialist to register alterations of the oral status. After accomplishment of the study the results have been summarized and analyzed.

Among the greater portion of patients a PCR test was conducted, a quality method for proof of HP – DNA into the dental plaque and saliva. A specific procedure for collection of biological material was applied. A small particle of purified dental plaque was taken (the procedure is conducted by dental specialist), put into 0.1 ml of sterile physiological solution, then transferred into the laboratory. Saliva – several drops were taken, equal to 1 volume, and added to 1 volume β -mercaptoethanol – 0.1 ml M.Vortex for incubation at room temperature for 30 minutes during stirring. 1.0 ml of the clinical material is put into a sterile propylene micro-tube, afterwards being centrifuge processed at the rate of 10 000 revolutions per minute with duration of 10 minutes. A portion of the supernatant was taken with a pipette and an amount of 100 μ l provided for DNA extraction. For the purpose of DNA separation out of the clinical

material were applied 5 types of solution: 1- a lysis solution, 2- a solution for first irrigation, 3- a solution for second irrigation, 4- sorbent universal solution, 5- buffer solution. During the process of centrifuging separation of the fluid containing DNA was provided. Four main processes were performed – extraction, amplification, detection by electrophoresis and photo-analysis. Positive and negative results were recorded. The method of One Step *H. pylori* Test Disk-anti-gene test for proof of HP urease in saliva was applied. This is an immune-chromatographic examination for qualitative detection of urease with HP infection. The buffer solution is mixed with several drops of saliva. A portion of the mixture (saliva/buffer) is put upon the test. The results are registered in the time interval between the 5th and 15th minute. The analytic sensitivity of the test amounts to 5 ng/ml. The appearance of a pink-red strip serves as a proof of a positive result. An essential requirement concerning the roper performance of the test is that the patient is not allowed to consume food and drinks for 1 hour before it. This method was applied in 35 patients, predominantly for infection monitoring, after unsuccessful eradication.

Usually prescribed as a first choice of eradication therapy is the standard triple one: Proton pump inhibitor + Claritromycine + Amoxicilline/Tinidazole. Another option is a stage therapy pattern including 2 consecutive stages with a duration of 5 days each:

1. Proton pump inhibitor + Amoxicilline for the first 5 days.

2. Proton pump inhibitor + Claritromycine + Tinidazole for the next 5 days.

A probiotic has also been applied – *Lactobacillus reuteri* (Bio Gaia). The concentration of lactic bacteria in 1 tablet Bio Gaia equals to 100 million alive *Lactobacillus reuteri* bacteria in a dose; intake of 1 tablet twice daily. *Lactobacillus reuteri* (Bio Gaia-prodentis) with a local effect on the oral cavity has been applied, too.

RESULTS

Examination of HP by means of PCR into dental plaque.

The results of study for proving HP into dental plaque in the determined 3 groups are summarized and represented in table 1.

Table 1. Distribution according to HP status in dental plaque

HP status	HP in dental plaque		
	Screening	Parallel examination	After HP eradication
	n	n	n
Positive	12	36	13
Negative	6	22	18
Total	18	58	31

18 patients participated in the screening test. In 12 of them (66.7%) the result was positive, i.e. HP bacteria was present in the dental plaque.

In 58 patients with a parallel investigation for the presence of HP diagnosed infection in the stom-

Table 2. Distribution of symptoms related to HP status in dental plaque

Symptom	HP positive	HP negative	p
	n (%)	n (%)	
periodontitis	38 (82.6)	3 (37.5)	0.015*
bleeding	23 (51.1)	3 (33.3)	0.47
gum bleeding	16 (34.8)	1(12.5)	0.411
presence of dental plaque	45 (97.8)	6 (75.0)	0.054
decayed teeth	30 (65.2)	4 (50.0)	0.45
erosion on teeth cervices	15 (32.6)	4 (50.0)	0.431
tongue coating	6 (13.0)	2 (25.0)	0.588
aphthae	7 (15.6)	2 (25.0)	0.611
halitosis	41 (91.1)	4 (50.0)	0.013*

ach – DNA in dental plaque was performed. HP infection was established in 36 (62%) of them. A combined infection was diagnosed in all of these patients. Simultaneously, HP infection in the stomach and oral cavity has been proven. 10 of them were tested after HP eradication treatment. In 8 of these 10 patients the result of the HP bacteria test was negative.

The test for HP in dental plaque after eradication therapy was performed in 35 patients. The results have been registered in 31 of these patients. In 18 of them (58%) the result was negative, so in more than half of them HP infection was not confirmed. Therefore, eradication therapy was fully successful.

There has been accentuated on an eventual relation between HP in dental plaque in the context of parallel examination with dental and oral status.

From Table 2 it is obvious that the presence of HP in dental plaque is in statistically significant relation with two symptoms – periodontitis and halitosis. Regarding the other symptoms was not established such a co-relation, which can be explained with the few patients in the group showing negative HP result.

The most common symptom was the presence of bacterial plaque in 97% of all the cases. The next were halitosis – in 91.1% and periodontitis – in 82.6% of the examined.

In the group of the screening test we investigated the relation between HP in dental plaque and diagnosed upper-dyspeptic syndrome or other symptoms.

A statistically significant relation between the presence of HP in dental plaque and gastro-duodenal syndrome has not been established. At the same time no relation was confirmed between HP in den-

tal plaque and anamnestic data for gastro-duodenal pathology. The efficiency of eradication therapy was estimated on the base of HP status in dental plaque. No difference has been established of the effect of therapy according to HP presence in dental plaque by parallel investigation ($p=0.673$) (Fig. 1).

Investigation of HP into saliva

35 people were included in the investigation of HP-antigen in saliva. HP infection has been diagnosed in 18 of them (51.4%). In 11 of the participants – 31.4%, examination was performed in patients with recurrent HP-infection, proven more than twice. Detected HP-infection in 4/11 – 36.3%. HP-infection parallel to the syndrome of “burning mouth” was established in 9/35 patients – 25.7%. Parallel investigation in patients with diagnosed HP-infection in the stomach, by means of fecal HP antigen test was conducted in 15/35 – 42.8%. A combined infection affecting stomach and saliva was proven in 5/15 – 33.3%.

DISCUSSION

On the base of the screening test and parallel investigation of HP in dental plaque, applying the PCR technique, it has been clarified that the frequency of HP infection in oral cavity is high, corresponding with the rate of this infection in the stomach, relatively 66.7% and 62%. Similar to other authors (7), we also established higher frequency in dental plaque, but the difference was not significant. These results coincide with results by other researchers (4,5,8) and we accept the thesis that oral cavity plays the role of a primary natural reservoir of infection, facilitating its transmission to the stomach. According to Siddiq et al. (7) the oral cavity is the initial location of HP colonization, from where these bacteria inhabit the stomach. Yee et al. have also performed studies concerning HP in dental plaque and established a high frequency of the infection, similar to our results. Applying a PCR method, Panov (9,10) has proved the presence of HP bacteria in oral cavity, amounting to 3% into saliva. The detection of HP in the oral cavity, precisely into dental plaque, is often associated with poor periodontal health, deep periodontal pockets and halitosis. Krasteva et al. (11) concluded in a study that 90% of investigated patients with halitosis complaints suffered from disturbances into the oral cavity. The rest 10% of the participants were with diag-

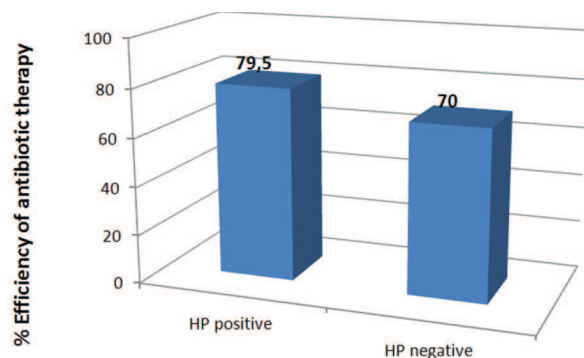


Figure 1. Relative portion of HP eradication depending on the presence of HP in dental plaque

nosed systemic disorders. There were confirmed IgG antibodies to *Helicobacter pylori* in 35% of the investigated. In the group of 56 controls with recorded gastric symptoms the rate of H. Pylori infection amounted to 27%.

These statements motivate us to draw attention of dental specialists to respect the mandatory testing of dental plaque for HP infection, as well as urgent recovery of proper oral-dental health status. In this context, the topical application of a probiotic containing *Lactobacillus reuteri* (Bio Gaia pro dentis), with confirmed effect upon HP bacteria in vitro, will enhance the process of elimination of the bacterium, resulting in rehabilitation of the oral cavity and its structures. Taking into consideration our previous research (1), the results consequent on application of sequential eradication therapy in combination with probiotic Bio Gaia are quite acceptable. Boyanova et al. (12) investigated and established the effect of inhibition of medicines containing *Lactobacilli*, which suppress the growth and decrease the urease activity of HP bacteria in vitro.

Panov et al. (13) detected an oral *Helicobacter pylori* species characterized by resistance to metronidazole and clarithromycin. This confirmed that antibiotic resistant *Helicobacter pylori* species can be registered in the oral cavity of patients suffering from chronic dental and gastro-duodenal diseases even in child's age.

We have established the distribution of combined HP infection affecting simultaneously the stomach and dental plaque amounting to 62% of all examined. Other authors (4,8) have established a combined infection in almost 50% of all investigated cases. The diagnosed by us combined, simultaneously persisting infection in more than half of the examined patients, serves as a confirmation of the thesis that the oral cavity is a reservoir from which the HP infection is transferred to the stomach, becoming a source of re-infection and recurrence concerning also patients without any symptoms and complaints. We have established a co-relation between the presence of HP in the stomach (by urease test, histological examination, PCR technic) and the oral cavity similar to other authors (14). These results lead to the conclusion that procedures of control upon dental plaque will have as a consequence reduction of the frequency of

re-infection. Krasteva et al. (15) have accentuated on the contribution of dentists for overcoming the risk of *H. pylori* infection and re-infection. The fact that dental practitioners themselves can be in an explicit risk for *Helicobacter pylori* infection cannot be neglected. Related to this, we advise our patients to visit regularly dental specialists for the purpose of thorough and complex prophylactic cares and adequate treatment of the oral cavity. Although it seems logical to expect successful eradication therapy provided for patients with absence of HP bacteria in the oral cavity, our results have stressed on the fact that the presence of HP in the oral cavity does not influence the efficiency of the eradication therapy. The rate of success of eradication concerning the oral cavity has been established to be similar in HP positive and HP negative patients.

Z Ou et al. consider examination of HP infection with PCR in gastric bioplat as a more appropriate method of diagnostics compared to routine histological, rapid urease and urea breath tests. The same test has been applied for proving HP in dental plaque and is as reliable as the respiratory test with C13 urea. Most of the researchers (3, 6, 16) estimate PCR approach as the most sensitive, specific and precise method for the diagnosis of HP bacteria in the oral cavity. Although being labor- and efforts-consuming, we highly value this method because of its certain and reliable results.

On the base of attained results we can conclude:

- ❖ Oral cavity is the prime reservoir of HP bacteria and serves as a source of gastric infecting.
- ❖ A combined, synchronic HP infection affecting oral cavity and gastric is often co-existing.
- ❖ Poor oral health status (manifested by periodontitis and halitosis) is accompanied with HP infection in the oral cavity.

In **CONCLUSION**, examination of the oral cavity for proving HP bacteria can be useful for detection of gastric infection, successful therapeutic influence and prevention of recurrence or re-infection, as well as adequate treatment of oral cavity alterations with proper oral health care. The elimination of the oral cavity reservoir of *Helicobacter pylori* infection results in a significant success concerning *H. pylori* stomach eradication.

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