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THE RELATIONSHIP BETWEEN THE PRESENCE OF *HELICOBACTER PYLORI* IN THE ORAL CAVITY AND GASTRIC IN THE STOMACH

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There are numerous studies suggesting that inflammation of the oral cavity caused by bacteria or fungi is accompanied by gastric inflammation. This is particularly relevant in patients using complete dentures. Since the presence of *H. pylori* in the oral cavity can be easily discovered by bacteria culture and that in the stomach by ^{13}C urea breath test (UBT) and histology of gastric endoscopic biopsy samples it is reasonably to state that the majority of the patients show the presence of *bacterium* in oral cavity and active gastric *H. pylori* infection. When comparing, however, the bacteria culture originating from the oral mucosa to those from the gastric mucosa, employing molecular biology examination, such as polymerase chain reaction (PCR), we found that the oral bacteria and those originating from stomach are completely different, suggesting that *H. pylori* may be present only transiently in oral cavity and does not play major role in gastric *H. pylori* infection. Thus, oral cavity does not serve as bacterial reservoir to infect gastric mucosa. Most important finding of our study is that patients with recognized inflammation in the oral cavity in the form of *stomatitis prothetica hyperplasica* both *fibrosa* as well as *papillaris* showed in nearly 100% gastric *H. pylori* infection, usually without the presence of the same *bacterium* in the oral cavity, suggesting that gastric *H. pylori* infection affects oral mucosa at distance by some, as yet, unknown mechanism.

Key words: *Oral cavity, bacterial reservoir, Helicobacter pylori, stomatitis prothetica*

INTRODUCTION

Oral cavity is an initial portion or the gate of the gastro-intestinal tract (GIT), that is of crucial importance for the whole organism. Numerous diseases of oral cavity may affect the integrity of mucous cover of oral activity and the remaining

portions of the GIT. Disorders of oral cavity may be associated with the changes in oral biology, including its microbial colonization and infection in the oral cavity. Indeed, oral cavity may be divided into several different micro-environmental compartments that may provide the niche for the various microorganisms, particularly bacteria and fungi. Oral cavity may serve either as a reservoir of those micro-organisms and the source of the infection of the stomach and the gut, or, alternatively, it may serve as the transmission gate of external germs for further colonization of GIT (including its first part). There are data indicating the connections between the stomach and further portions of the GIT or between the stomach and the pathological states of oral cavity, particularly in patients using dentures. The aim of the present report was the critical analysis of the literature and our own data concerning the relationship between the oral cavity and the remaining parts of GIT, especially the stomach.

Dentistry as a part of medicine

Dentistry is tightly integrated with the general medicine as the health of oral cavity may contribute to health conditions of various organs of the body. This fact stresses the important role of oral cavity as the first part of GIT in numerous functions including;
a) participation in articulation of sounds and generation of speech as well as participation in the perception of the taste and b) serving as passage-way of air to lungs, especially during strong physical exercises or habitually as well as in some pathological conditions that involves nose and mouth. The major role of oral cavity is, however, the food intake and its preparation for swallowing and the transport of ingested food into further segments of GIT. This includes biting and mixing of food particles with saliva to form food "bolus" ready for swallowing and passing through the pharynx and the esophagus to the stomach. Numerous organs are involved in this process such as masticate muscles, temporo-mandibular joint, teeth as well as auxiliary tissues of tongue, lips and chicks. Additionally, saliva which is a product of three pairs of salivary glands (parotid, sublingual and submandibular) as well as numerous small salivary glands dispersed within the mucosa of the oral cavity, enables the binding of the food particles into a single easy to swallow "bolus". Saliva enzymes start the digestion of carbohydrates with alpha-amylase and triglycerides with lingual lipase secreted by salivary glands (1).

During recent decades it has become apparent that oral cavity is inhabited by million of various microorganisms forming an oral bacterial biofilm. This biofilm does not appear to cause any damage to the host, but oral bacteria remain in dynamic physiological symbiosis with the immunological system of the host. This system prevents in healthy individuals the excessive growth of microbes in oral cavity and the invasion of pathological bacteria. Additionally, the bacteria themselves competing for food and place to survival and vegetation, may restrict

the growth of pathogenic microorganisms which otherwise could harm the organism. Oral cavity as a very heterogeneous environment consisting of the numerous organs, provide different conditions for different types of bacteria. Some germs permanently inhabit oral cavity, while others may be only transiently contaminating this cavity, building a very complex environment and under physiological conditions living in symbiosis with the host. Nevertheless, this balance can be disturbed by numerous internal and external factors (1, 2).

The disruption of this balance may lead to pathological states in the oral cavity, which can further influence the health of the whole organism by carrying infection to other portions of the GIT and other body organs through airways as well as blood stream. Oral bacteria can also become a source of systemic inflammatory response, thus, affecting the prognosis and outcomes of therapies for general medical diseases such as cardiovascular, especially coronary and brain artery diseases.

H. pylori is present in both the oral cavity and the stomach

H. pylori is a microaerophilic, Gram negative, spiral and mobile *bacterium* which is believed to be one of the major factors responsible for gastritis, gastro-duodenal ulcers as well as gastric cancer (3 - 5). There are different opinions concerning the presence of *H. pylori* in the oral cavity. The major question remains whether bacteria are only transiently contaminating oral environment during oral processing of food (6, 7) or whether they constitute an integral portion of residual flora of the oral cavity that remains in symbiotic relationship with its host (8). Nevertheless, it is now quite certain that *H. pylori* may be present in oral cavity either temporarily or permanently. The first person who isolated *H. pylori* from dental plaques, soon after its discovery by R. Warren and B. Marshall was Krajden in 1989 (9).

It seems to be logical and most likely, that in the case of the gastric infection with *H. pylori*, oral cavity serves as the gate of this germ transmission to the GIT ("person to person transmission"). However, it is not clear, what is the role of this germ in oral cavity in the transmission process. Is it not clear whether the *bacterium* is only transiently stored in the mouth when passing to the stomach or whether oral cavity is a real bacterial reservoir, where *H. pylori* can multiply, achieving high enough number for entering the stomach and its infecting.

It is well-established that the principal ecological niche for *H. pylori* is the gastric mucosa. The *bacterium*, when reaching the gastric lumen quickly passes through the thick mucus-HCO_3^- layer adhering to the surface of the gastric mucosa due to its mobility by using its flagellas and attaches *via* its adhesins to the glycol-lipid receptors on the apical membrane of surface epithelial cells. Once mucosal infection is established it can last many years or even whole life time. The question arises if in the oral cavity similar ecological niche exists, where *H. pylori* could by attached and grow. Such *locum* for the germ in oral cavity could

be e.g. dental pockets where from the bacteria could be spread to the esophagus and the stomach, where its natural ecological niche exists.

One of the first investigations on the influence of oral *H. pylori* on the stomach condition was carried out by Miyabayashi *et al.* (10). This study was performed on 47 patients and confirmed the existence of the relationship between the gastritis caused by *H. pylori* infection and with oral colonization with this germ. Moreover, these authors also attempted to elucidate the resistance of oral *H. pylori* to typical (triple) anti- *H. pylori* therapy used to eradicate the germ from the stomach. They reported that patients with oral *H. pylori* were at significantly greater risk of gastric reinfection following successful therapy. Therefore, this study emphasized a clear link between the presence of *H. pylori* in oral cavity and its infection of gastric mucosa. Other authors also showed direct correlation between poor oral cavity health and *H. pylori* reinfection of stomach (11). *H. pylori* was found in the oral cavity of virtually each patient who presented with poor hygienic status. Song *et al.* (12) investigated further the particular characteristics of oral cavity microenvironment. They found that distal parts of the oral cavity, which are less oxygenated, contain higher numbers of bacteria. Our previous studies (13, 14) carried out in patients using dentures, indicated that all patients with the *H. pylori* present in the oral cavity showed also the gastric infection with this germ. According to our finding, if the *H. pylori* was present in the oral cavity of patients with dentures, the infection with this germ also occurred in the stomach. On the contrary, gastric *H. pylori* infection does not necessarily indicates the presence of bacteria in the oral cavity. It does not exclude the possibility that *H. pylori* for stomach infection passes through the oral cavity. Oral cavity seems to be the only gate of the *H. pylori* infection of the stomach, which occurs in 70% among Polish population, most probably by "person-to-person" transmission.

METHOD AND RESULTS

The study involved 40 men (25-70 yrs), who gave their informed consent to participate in the study that has been approved by Ethics Committee of Medical College of Jagiellonian University. The presence of urease active bacteria was demonstrated in the oral cavity by the use of ^{13}C-urea breath testing (UBT) similarly to the technique described in details before (15). Briefly, after collecting two baseline breath samples, each patient was asked to confine in oral cavity 10 ml of water solution containing 40 mg of ^{13}C-urea and phenol red as a volume recovery marker. While the solution was kept in the oral cavity for 5 min, patient was asked to inflate at one min interval plastic bags through his nose (nasal collection). That was followed by spitting of entrapped ^{13}C-solution, washing out the mouth and finally proceeding ^{13}C-UBT by collecting the breath samples at 6, 10 and 20 min time points from the start of testing. The spitted fluid was used to

estimate the recovery factor. Gastric *H. pylori* status was determined using capsulated ^{13}C-UBT as described previously (16).

Fig. 1 demonstrates the UBT in *H. pylori* positive (N=20) and negative patients (N=20). The presence of bacteria originating from the mouth cavity (saliva or supragingival plaques and determined by culture on agar-horse was independent of the presence or absence of gastric *H. pylori* infection determined by UBT. In some cases the bacteria was detected in the oral cavity even when in the same patients that were eradicated from the gastric *H. pylori* by typical triple therapy.

RAPD – PCR

Most important question remained whether the bacteria detected by culture of mouth contents and that cultured from the gastric biopsy samples were identical or not. To answer this question, we employed Random Amplification of Polymorphic DNA (RAPD). Bacterial cultures were prepared from smears collected from oral cavities and from the gastric biopsies of selected patients. Total bacterial DNA was isolated from the samples of *H. pylori* cultures using DNAzol according to the manufacturer's instruction. Isolated DNA was subject to RAPD reaction using specifically designed primers:

1) CCg CAg CCA A;
2) AAg AgC CCg T;
3) AAC gCg CAA C.

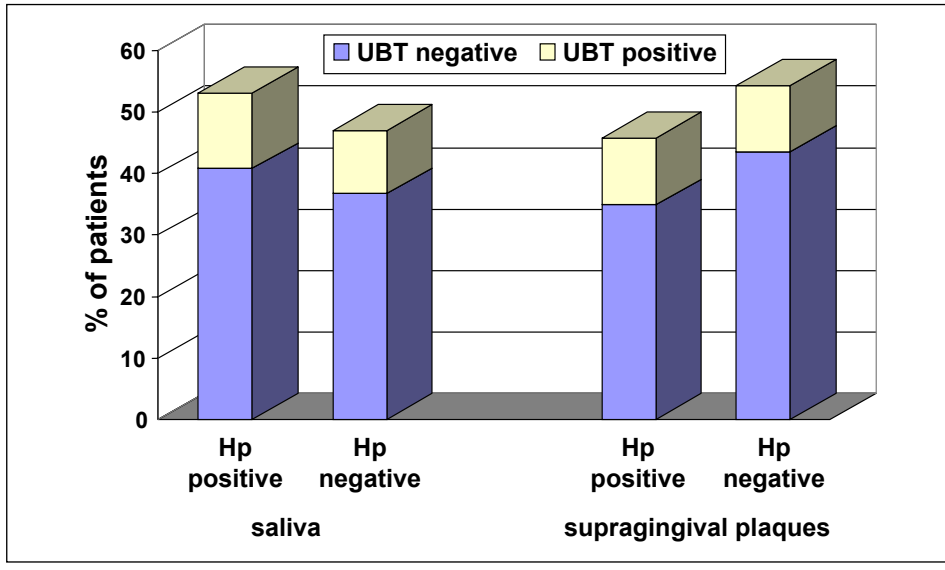

Fig. 1. The positive and negative microbiology tests in saliva (on left) and supragingival plaques (on right). Gray color reflects percent of patients with negative UBT and white with positive UBT.

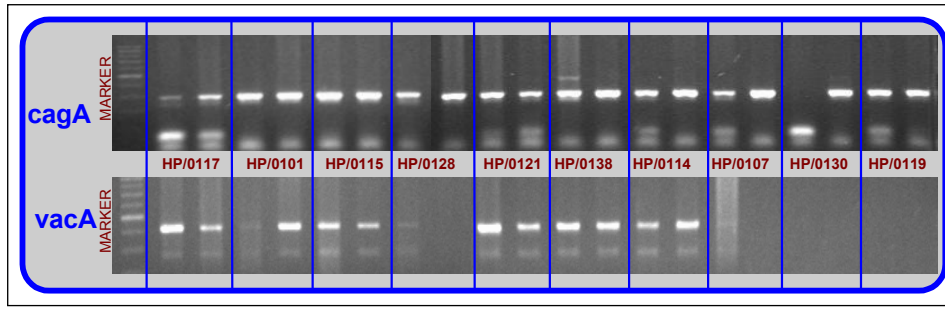

Fig. 2. Cag A and vac A status in *H. pylori* cultures isolated from oral cavity and stomach of 10 patients. Each getween panel represents result of DNA analysis from the bacterial culture prepared from oral cavity (left band) and stomach (right band) of one patient.

Moreover, cagA (298bp), vacA (447bp) (*Fig. 2)* and urease status (not shown) of *H. pylori* isolates was confirmed in PCR reaction with specific primers:

cagA	sense:	ATA ATG CTA AAT TAG ACA ACT TGA GCG A;
	antisense:	TTA GAA TAA TCA ACA AAC ATC ACG CCA T.
vacA	sense:	GCT CAT TAC GGC TTC CAC;
	antisense:	GCC TCG GAC CAG ATA GTT.
urease A	sense:	GCC AAT GGT AAA TTA GTT;
	antisense:	CTC CTT AAT TGT TTT TAC.

RAPD reaction was performed to compare and discriminate strains of *H. pylori* in the collected samples. Analysis of *H. pylori* genomic DNA revealed no resemblance, in any analysed case and these results have been omitted for the sake of clarity. This implies no relation between *H. pylori* strains infecting oral cavity and stomachs.

DISCUSSION

On the basis of our preliminary microbiological studies, we reached the conclusions that *H. pylori* is present in oral cavity of all of the examined patients with the dentures who had *H. pylori* infection in the stomach. In this context the question arises if oral cavity is the reservoir of *H. pylori* for the stomach or if the stomach is the source of the bacteria for the oral cavity. Theoretically, either way is feasible. *H. pylori* might get to the oral cavity in process of gastro-esophageal reflux or regurgitation. Even if there is no direct influence of gastric *H. pylori* on the oral cavity, the gastric *H. pylori* could still exert an indirect effect on the oral cavity through its various virulence and growth promoting factors released in the stomach to general circulation or *via* triggering the gastro-oral neural reflexes that function between various organs of the GIT. Based on the patients with diagnosed prosthetic *stomatitis*, we revealed the connection between gastric *H. pylori*

infection and inflammation of oral mucosa in the area of contact with denture. We think that it is a consequence of the indirect influence of gastric *H. pylori* on the oral cavity mucosa. It is very likely that the *H. pylori* located in the stomach releases cytoxins such as CagA (encoded by *cagA gene*) or lipopolysacharides that reach the mucosa of oral cavity through bloodstream. The combination and overlay of simultaneous stimuli including direct irritation at the basis of the denture together with the toxic effects originating from blood *H. pylori*-related factors might damage the mucosa and result in various clinical manifestations of the symptoms.

On the other hand, Oshowo *et al.* (17) established in their study that although the reinfection of the stomach with the bacteria from oral cavity is possible, this occurs rather rarely and has minor impact on gastric re-infection. The majority of reports, however, indicate that the anti-*H. pylori* triple therapy does not influence the bacteria in the oral cavity, especially those present on dental plaques where they exist in the largest number. The failure of the triple therapy to affect oral *H. pylori* results from the fact that it is not possible to achieve the therapeutic anti-*H. pylori* concentrations of antibiotics in saliva and dental plaques (8, 12, 18-21). If this is true, the *H. pylori* eradication from the stomach in patients with bacteria present in the oral cavity, would be very difficult considering high rate of colonization of *H. pylori* in the saliva and dental plaques, that e.g. in Cracow region reaches the level of 84% and 100%, respectively.

Song (8) found that the presence of *H. pylori* in oral cavity is not associated with the gastric infection with this germ. However, according to out data based on our molecular studies using PCR for detection of *H. pylori* genome, we can conclude that the strain of *H. pylori* from the oral cavity is different from that from the stomach, which indicates that these two locations in GIT are infected independently and separately without any relationship between them. Nevertheless, we cannot exclude the potential influence of *H. pylori* in oral cavity on the stomach. Blaser and Berg (22) proposed that the *H. pylori* is highly diversible *bacterium,* capable of quick exchanging of its genetic (DNA) material from one *bacterium* to another and the strains originating in oral cavity and to those infecting the stomach. Such exchange of the genetic material from one strain to another, depends on the environmental conditions and this could help in best adjustment of germ to the surrounding conditions. The adjusting processing as well as genetic material exchange could take place in numerous different local niches in the oral cavity. The heterogeneous strains created may lead to developing resistance of bacteria to sudden changes in host environment, that is an important element of the symbiosis of *H. pylori* strains with the host. The dynamic balance between the environment and microbiological balance in the stomach may be disturbed when additional pathologic factors are introduced into these systems. These may include disorders of the immune system leading to decreased immune competence such as occurs with advancing age under physiological conditions. It is tempting to speculate if the factors leading to such

disturbance of careful balance could be associated with the introduction into the system of new factor – e.g. oral *H. pylori*. Moreover, it is possible that life of *H. pylori* in the dental plaque, or denture-related inflammatory environment might actually "prepare it" for consecutive gastric infection by e.g. exchanging genetic material discussed above. This could result in the generation of strains, which would be capable of better survival and would push out the other less pathologic strains from the niche and environment. These processes would be very important in altering the balance facilitating oral, gastric and/or systemic infections. However, the responses of mucosa may be equally variable and often unpredictable (22).

We have to admit, however, that the detection of *H. pylori* in the oral cavity does not necessarily provides an evidence that the oral cavity is the reservoir of bacteria for the further parts of GIT. Certain number of viable bacteria is required for successful infection of gastric mucosa and the bacteria may be present in the oral cavity in the number too low to infect gastric mucosa after passing into the stomach with saliva or swallowed food. It was shown for the first time by B. Marshall, who drank the pure culture of *H. pylori*, that caused an immediate acute hemorrhagic and erosive gastritis confirmed by gastroscopy but no ulcer developed. That was the proof that the oral cavity may serve as the gate for the transmission of the *H. pylori* to the stomach (23).

The risk of the stomach infection with *H. pylori* originating from the oral cavity is significantly increased when aphthous ulcerations are present in the mouth. It has been established that the amount of *H. pylori* in the oral cavity is much higher when aphthous ulcerations occur (24 - 26). Therefore, when *H. pylori* is attempted to be eradicated when aphthous ulcerations are present, the risk of the reinfection of the stomach and the possibility of the transmission of the bacteria between humans ("mouth to mouth" transmission) greatly increase.

The group of the patients, which should be considered as a group of high risk of the *H. pylori* gastric reinfection from the oral cavity, includes individuals with extensive dentures. These subjects have been already established as the group with increased detection of fungi in the mouth, which usually manifests as the pathology described as prosthetic stomatopathy (*stomatitis prothetica mycotica*). Fungi can serve as the vector for bacteria *H. pylori* (see below), which allows these bacteria to survive in the oral cavity and after getting to the hostile acidic environment of the stomach, to infect its mucosa. The correlation between *H. pylori* and yeast-like fungi has not been established yet (27).

In our own studies with toothless patients using complete dentures and exhibiting proliferative form of mucosal inflammation type *papillaris* or with fibrosis of the prosthetic area, are in 100% *H. pylori* positive in the stomach. We found that there is no gastric *H. pylori* infection without *H. pylori* presence in the oral cavity in these selected subjects. Moreover, we can further speculate that there is no proliferative prosthetic stomatopathy without gastric *H. pylori* infection, which might indicate that the influence of some *H. pylori* cytotoxins

and noxious substances such as LPS can encourage the proliferative changes even in the distant parts of the organism. We think that such distant changes caused by gastric *H. pylori* infection require the involvement of additional, as yet unrecognized factors required to develop mucosal proliferation. In the case of described patient it may be also the trauma of the oral mucosa caused by the denture. This motion can prove the fact that in the case of patients with gastric *H. pylori* infection who do not use dentures, we didn't observe any proliferative changes within mucous membranes.

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