H. pylori is an important factor in the pathogenesis of numerous diseases including gastro-intestinal, metabolic and vascular disorders. Therefore, identification of individuals at risk of this infection remains of critical importance. Dentists and dental professionals may be at increased risk due to the contact with oral cavity of patients with the presence of H. pylori in the oral cavity where it may serve as reservoir for gastric infections and participate in the pathogenesis oral mucosal lesions and ulceration. However, evidence regarding the occurrence of H. pylori infections and colonization in dentists is conflicting, but has been based mainly on serological studies, which carry significant limitations. Therefore, we attempted to characterize H. pylori infection in practising dentists in relation to the duration of their work as dental professionals. Moreover, apart from seropositivity, which was used by majority of previous studies, we have performed urea-breath test (UBT), which has been shown to represent active H. pylori infection in stomach as well as the H. pylori culture from the oral cavity. We found that while the occurrence of either gastric or oral H. pylori in dentists is not greater than in general population, it seems that in male dentists there is a greater risk of gastric H. pylori infection. Moreover, we found a relationship between the length of dentist occupation with the presence of H. pylori in gingival sulcus. In conclusion, while overall occurrence of H. pylori in dentists did not differ from that reported for stomach or oral cavity in general population, there was an increased occurrence of H. pylori in male dentists and the presence of this germ in the oral cavity appears to be related to the length of professional exposure.

Key words: oral cavity, gingival sulcus, Helicobacter pylori infection, urea breath test, culture, urea-breath test

INTRODUCTION

There are some studies indicating an increased risk of H. pylori infection in dental professionals. This would have critical importance for the risk of serious gastro-intestinal diseases including duodenal and gastric ulcers as well as gastric cancer (1).

The prevalence of H. pylori gastric infection is high all over the world and reaches 80% and 40% in developing and developed countries, respectively. In spite of numerous investigations, the modes of its transmission in the population remain still unclear. It has been shown that both oral-oral and fecal-oral transmission occur in humans, and they appear to be the main reservoir of H. pylori in nature. Therefore, the transfer of H. pylori between individuals via infected saliva or contaminated food and eating devices appear to be the major routes of spreading of the bacteria (2). Taking all these into account it could be reasonable to assume that the oral cavity should play a critical role in the process of H. pylori transmission from patients to dentists. This germ transmission to dentists could occur particularly in the case of restorative dentistry and oral surgery routine or any other dental procedures, associated with creation of aerosol spray which is known to reach the distance of up to 1.5 m away from the patient (2, 3). This spray may be a source of chemical contamination and professional risk for the dentist, but may also provide a very important microbial transmission route and another work related hazard in dentists.

H. pylori is a spiral-shaped gram-negative bacterium, which has been discovered by Warren and Marshall in 1982 and soon afterwards was associated with pathogenesis of serious disorders such as chronic antral gastritis of type B, peptic ulcers, mucosa-associated lymphoid tissue (MALT) lymphoma (4, 5). In 1994, an International Agency for Research on Cancer Working Group decided on the basis of long retrospective evidence to classify H. pylori as a human carcinogen for gastric cancers (6). Acute gastric infection results in accompanied by dyspepsia symptoms but after few weeks it change into chronic gastric infection with chronic gastritis that is generally asymptomatic. However, after many years of chronic gastric inflammation, patients suffer from dyspepsia, gastric ulcers and cancers (7). As described, the consequences of H. pylori infections can be severe, therefore, the identification of the professional hazard, as possibility of H. pylori infection, is very important for taking an action to prevent this risk.

Japanese investigators have shown that the occurrence of seropositivity for H. pylori in young Japanese dentists was higher than in controls (9). Moreover, the most compelling evidence regarding the relationship between dental profession and H.
pylori infection comes from a prospective study showing increased risk of new infection of H. pylori in dental professionals (8). However, there is also a large number of contrasting evidence, thus the problem remains highly controversial. Lin et al (11), using ELISA assay for detection of H. pylori IgG antibodies, showed that there is no significant difference in the H. pylori prevalence between dentists, dental nurses and fifth year dental students as compared to age- and sex-matched healthy controls, indicating that H. pylori infection is not more common in dental professionals working in the oral cavity. Therefore, in current study we have undertaken to characterize H. pylori infection in practising dentists in relation to the duration of their work as dental professionals. Moreover, apart from seropositivity, which was used by majority of previous studies we have employed the UBT, which has been shown to reliably detect active H. pylori infection in stomach as well as the H. pylori cultures from the oral cavity. We find that, while the occurrence of either gastric or oral H. pylori in dentists is not greater than in general population, it appears that in male dentists there is a greater risk of gastric H. pylori infection. Moreover, we find a relationship between length of work as dental professionals with the presence of H. pylori in gingival sulcus.

MATERIAL AND METHODS

Study population

Forty six dentists without known co-morbidities were included in the study (36 female, 10 male). Study was approved by the Institutional Research Ethics Committee of the Jagiellonian University Medical College and informed consent was obtained from all individuals before the study. Patients who had taken anti-secretory drugs or antibiotics, bismuth salts or anti-secretory agents within previous two months, were excluded from the study. The samples of the subgingival plaques were collected with a periodontal curette from two different front teeth, or in the case of tooth loss, from premolars and molars. Subjects were asked about their appetite behaviour and dyspeptic symptoms such as upper abdominal pain and discomfort in the abdomen that are considered as dyspeptic symptoms.

Determination of H. pylori status in stomach

The H. pylori infection of the stomach was estimated using UBT as described before (9, 10). Briefly, after obtaining 2 baseline breath samples for registering baseline urea level, gelatin capsule containing 38 mg of 13C-urea was swallowed with 25 ml of water by each subject. Then, breath samples were collected into testing vials after 10 and 20 minutes following 13C-urea administration. Final results of 13CO2/12CO2 ratios were measured with the use of isotope ratio mass-spectometry (IRMS, Heliview, Medicems Seoul, Korea) and were expressed as δ13CO2 (per mil) values. A change of mean δ13CO2 value over baseline (DOB) after urea capsule administration, of more than 2.5 was considered as positive result.

Detection of oral Helicobacter pylori

The presence of H. pylori in the oral cavity was determined using microbiological culture (9, 10). The samples of saliva and subgingival dental plaque were obtained into sterile vials. Aliquots (100 µl) of the samples were cultured on solid selective, enriched medium (H. pylori agar, Becton Dickinson) containing 5% horse blood and incubated microaerobically at 37°C for 5-7 days. Colonies were confirmed as H. pylori by Gram staining to show the presence of gram-negative spiral bacteria and by using the tests for bacterial urease, catalase and oxidase. The API-Campy test (BioMerieux) was performed to confirm diagnosis.

Serological tests

Five ml of vein blood was obtained from each subject. The blood was centrifuged for 10 min at 2000 g and serum was saved for the further investigation. To measure the level of anti-Hp IgG, we used Captia ™ H. pylori IgG (Trinity Biotech) test based on enzyme linked immunosorbent assay (ELISA) (9, 10).

Statistical analysis

The data were expressed as means±standard error of the mean (SEM). Statistical analysis was performed using Student t or Turkey test after analysis of variance. P value <0.05 was considered statistically significant.

RESULTS

H. pylori infection and colonization in dentists

The occurrence of positive UBT, indicating stomach H. pylori infection was about 65%, while the remaining 35% showed negative UBT (Fig. 1A). Similarly, seropositivity determined by ELISA detecting IgG in serum was present in 70% of dentists (Fig. 1B). There was an overall agreement between the detection of gastric H. pylori infection with UBT and the IgG presence in serum. Only few individuals showed IgG and negative UBT indicating recovered infection. However, it is notable that 30% of dentists did not show anti-H. pylori IgG in their serum (Fig. 1B).

Detection of H. pylori in oral cavity in dentists

As expected in general population the occurrence rate observed for H. pylori isolated from either saliva or gingival sulcus was lower than positivity in serum or in UBT and reached level of 57% (Fig. 1C). H. pylori was identified by means of bacterial culture as described in methods. Interestingly, presence of H. pylori in either gingival sulcus or saliva of dentists was 48% and 43% respectively (Fig. 2), which was in complete agreement with previously published rates for non-dentist populations (p>0.1, population structure test; data not shown) (8).

H. pylori in dentists in relation to sex

We also compared presence of oral or gastric infection with H. pylori in males and females separately. While no significant differences were observed in relation to oral presence of H. pylori, we found that gastric infection measured by UBT was significantly more prevalent in male dentists, suggesting they might be at greater risk (Fig. 3).

Gastric and oral H. pylori in relation to the duration of professional activity in dentists

We next investigated the relationship between length of work in dental profession in relation to H. pylori status in the stomach (UBT) or oral cavity (H. pylori culture). This analysis showed that overall subjects with the presence of H. pylori presence had longer professional exposure (Fig. 4), although it reached statistical significance only in relation to dental/gingival sulcus H. pylori (Fig. 4B). Accordingly we next investigated the occurrence of positivity for H. pylori in cultures from gingival
Fig. 1. The prevalence of *H. pylori* in dentists. Panel A: Gastric *H. pylori* was assessed by urea-breath test (UBT). Panel B: Serum anti-*H. pylori* IgG was assessed by ELISA; Panel C: *H. pylori* in the oral cavity assessed by cultures (from saliva and gingival sulcus).

Fig. 2. Nature of presence of *H. pylori* in the oral cavity of dentists. Distribution between saliva and gingival sulcus.

Fig. 3. Indices of *H. pylori* infection in the stomach and oral cavity in relation to sex.
sulcus isolated from dentists working longer and shorter than 15 years and we observed striking difference in *H. pylori* prevalence, namely, significantly higher *H. pylori* positivity after longer than 15 years of dental profession (Fig. 5).

**DISCUSSION**

*H. pylori* infection and colonization constitute a vital element of pathogenesis of gastro-duodenal ulcer disease and non-cardia gastric as well as gastro-esophageal reflux disease (1). These findings are also important for other pathologies as *H. pylori* ‘s role has been identified in other diseases including some pulmonary as well as cardiovascular disorders (11-17). Therefore, an identification of patients prone for this infection is vital. In particular research has focused on potentially increased prevalence of *H. pylori* in relation to professional activity or living conditions. While most studies emphasized that low living standards are associated with increased prevalence of *H. pylori* infection, mainly due to hygienic conditions, much less is known regarding the relationships between *H. pylori* infections and colonization and professional activities. Dentists are among health professionals that may be particularly prone to *H. pylori* infections. This is related to the fact that *H. pylori* is a commonly present in the oral cavity in patients and can be disseminated during dental procedures (7, 18-20). The latter is associated with generation of so called „aerosol cloud” which reaches up to 1.5 m from its centre in patient’s oral cavity. This aerosol cloud contains majority of microorganisms present in the oral cavity and may be a source of infection in dentists and dental assistants.

However, the relationship between *H. pylori* colonization of the oral cavity and dental professionals has been poorly investigated. In the present study we showed that the prevalence of gastric *H. pylori* infections as measured by urea breath tests (UBT) reaches about 65% in Polish dentists which is relatively similar to reports in general population of Poland (10). The presence of anti-*H. pylori* IgG antibodies in serum of studied subjects is similar to general population and reaches 70% (20), while oral colonization is only modestly increased over other population studies by our group in the past. However, while the general prevalence of *H. pylori* infection is not different between dentists and general population, we observed a significant dependence of presence of *H. pylori* in gingival sulcus in dentists upon the time of their professional activity. Dentists who showed positive presence of *H. pylori* in gingival sulcus were working on average 6 years longer than subjects who did not.
show the presence of \textit{H. pylori} in gingival sulcus. Moreover, our analysis demonstrated that when more than 15 years of professional activity was considered in dentists, there was a dramatic increase in the presence of \textit{H. pylori} in gingival sulcus as compared to those involved for less than 15 years in dental profession. Above differences were not reflected in gastric \textit{H. pylori} as determined by UBT. Interestingly, in male dentists the prevalence of gastric \textit{H. pylori} infection reached about 80%, which is much higher than in females and than that in general population.

Previous studies mainly in American, Japanese and French populations have investigated the problem of \textit{H. pylori} infections in dental professionals, although these studies were usually based on serological markers of infection. Approach presented in our current study seems to be more comprehensive and covers an important gastric \textit{H. pylori} infection, with the use of UBC; serologic marker IgG levels using ELISA as well as direct cultures from oral tissues/saliva.

Most convincing evidence for associations between the dental practice and \textit{H. pylori} infection comes from Japanese population. A study of young Japanese dentists showed that the proportion of dentists with \textit{H. pylori} seropositivity (42% of 60, 70%) was higher than in controls (23 of 60, 38%). The odds ratio for \textit{H. pylori} seropositivity (3.8; 95% CI, 1.76-8.02) was high in the dentists (9).

One of the first studies on seroepidemiologic characterization of \textit{H. pylori} IgG in dental professionals carried out in 1992 in Texas, USA (23), showed the overall prevalence of \textit{H. pylori} to be 24% which consisted of 17% in dentists, 18% in dental hygienists, 34% in dental assistants, and 25% in dental students. The authors showed that logistic regression analysis (dependent variable \textit{H. pylori}) revealed no significant association between \textit{H. pylori} infection and the type, duration, or volume of practice, or the type of cleaning instrument used. It was concluded that dental workers are not at increased risk of \textit{H. pylori} infection. However, the seize of this study might not been sufficient and the rates of infection reported was much lower than observed in Polish or even American populations in major epidemiological studies. Similar low rates of seropositivity for \textit{H. pylori} IgG were reported for Australian population of dentists and dental nurses(21). Similar results were however obtained by a subsequent study of 179 adults performed in Sweden, in whom seropositivity for \textit{H. pylori} IgG was studied. (22). Further studies, performed in populations with greater seropositivity, closer to that observed in our study, have confirmed these observations. The frequency of \textit{H. pylori}-seropositive Italian dentists did not differ between from the control group (56% versus 64%). A positive saliva assay was found in 39% of dentists and in 62% of controls (23) The odds ratio for a dentist being \textit{H. pylori}-positive was 0.7 (95% confidence interval 0.3-1.7) by serology and 0.9 (95% confidence interval 0.4-2.1) by salivary antibody assay (23). All of the studies discussed above do not support the concept that dentists are a high-risk group for \textit{H. pylori} infection based on seropositivity. Our present study is in agreement with above as we have observed seropositivity for \textit{H. pylori} in ca. 70% of dentists while levels between 55-70% have been reported for polish general population from our region.

While these observational studies are interesting the most compelling evidence regarding the relationship between dental profession and \textit{H. pylori} infection has been provided by two Japanese authors Matsuda and Morizane who performed a 6 year long prospective observation of dentists (10). The same authors in a cross-sectional study did not report significant differences between dentists and controls in the seroprevalence of \textit{H. pylori}. In the prospective evaluation, they found in turn that the risk of new infection of \textit{H. pylori} for dental professionals is 1.12%/year and the relative risk is 4.0 and thus, dental professionals are at greater risk of being infected by \textit{H. pylori} than are controls (10). Matsuda and Morizane (10) also determined the risk factors for acquiring \textit{H. pylori} using logistic regression. Adjusted odds ratio being a dental professionals was 2.6, having upper gastrointestinal family history was 4.8, and age over 40 was 8. One of potential explanations for lack of influence of chronic daily exposure for aerosols containing \textit{H. pylori} and other bacteria may be related to protection worn by dentist during procedures. While it is unlikely that such protection can completely prevent colonization of oral cavity and stomach with \textit{H. pylori} it may greatly reduce load of bacteria and therefore affect conditions necessary for the infection. Moreover, it is also possible that dental hygiene is increased in dentists when compared with general population, and in non-dentist population such exposure might have effects on infections. However, some studies, show that there is no correlation of \textit{H pylori} gastritis with either dental hygiene or periodontal disease (26). Finally, a very important explanation which is actually supported by some results presented here as well as our previous studies, in patients without dentures oral \textit{H pylori} may not reflect gastric infection, positive UBT or IgG seropositivity.

One of the very interesting observations of the current study is that the presence of \textit{H. pylori} in the oral cavity in dentists is related to length of work in dental profession. This is in contrast to previous report from Japan, which showed that when dentists were classified in terms of the length of their practice, the odds ratio for seropositivity (10.4; 95% CI, 3.26-32.85) was high in the dentists practising for fewer than 4 years (9). However it needs to be emphasized again that this was a seropositivity study, while we investigated actual presence of bacteria detected by bacterial cultures.

The importance of our finding of relationship between length of time of employment as a dentist with prevalence of \textit{H. pylori} in gingival sulcus for the pathogenesis of gastric or duodenal ulcer disease remains unclear. A study from our own group has shown that in patients with dental prostheses, oral prevalence of \textit{H. pylori} is associated with gastric infection, which could suggest an important role (27). However our studies of subjects without dental prosthesis do not show association, indicating that bacterial load in these patients may not be sufficient as opposed to prosthetic subjects (12, 13). Numerous conflicting studies regarding oral localization of \textit{H. pylori} have been reported. Some authors showed that the occurrence of \textit{H. pylori} is relatively low and equals 38% while others find it in nearly 90% or even in 100% of subjects (18, 19, 24). The major differences in study populations, sample collection and laboratory procedure for bacteria detection between studies described above make it difficult to compare results obtained in these studies. There are two main methods currently accepted for the \textit{H. pylori} detection in oral cavity that is culture of germ and PCR, but each of them has disadvantages. Culture is not that sensitive as PCR and \textit{H. pylori}, a microaerophilic bacteria, may not be able in some cases to survive the sampling process before being transferred into the medium. This could particularly refer to cases in which numbers of colonizing bacteria are low.

The major question, which is related to the presence of \textit{H. pylori} in the oral cavity, is whether this is a residual bacteria composing part of biofilm of the oral cavity, or whether it plays a pathogenic role. Some investigators suggested that \textit{H. pylori} can contribute to the aetiology of recurrent aphthous stomatitis (18, 19, 24).

In conclusion, we showed in present study that the prevalence of gastric \textit{H. pylori} infection in dentists is not increased compared with general population. Moreover, the oral colonization with \textit{H. pylori} is also similar in both dentists and general population. However, we observed the significant increase of frequency \textit{H. pylori} presence in gingival sulcus with
length of time being active in dental profession. Additionally, we revealed that male dentists are more susceptible to *H. pylori* colonization of oral cavity than females.

Conflict of interests: None declared.

REFERENCES


