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Original Article

Oral Helicobacter pylori (Hp) is correlated with the occurrence of periodontitis

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Abstract: Objective: To investigate the effect of Helicobacter pylori (Hp) on periodontitis dominant bacteria and define the importance in clearing oral Hp for removal of gastric Hp and good periodontal health. Methods: In total, 320 patients (40±20 years old) received ^{13}C breath test to treat digestive tract symptoms. The correlation between Hp infection and periodontitis by rapid oral urease test were investigated. They were divided into Hp Positive and Negative groups. Meanwhile, 42 periodontitis patients who were oral Hp negative were selected as the control subjects. The correlation between Pg (Porphyromonas gingivalis), Pi (Periodontitis pathogen), Bf (Bacteroides forsythus) amount and oral Hp infection was determined. There were 30 patients experiencing crown lengthening surgery without alveolar ridge crest absorption that were screened with periapical film. After root canal therapy, gingival tissue was removed for TUNEL test. The epithelial apoptosis indexes were compared. Results: Oral Hp positive rate was 68.75%. The oral Hp positive rates in periodontitis groups (PG) were higher than that in NPG. However, it did not increase with the severity of periodontal disease. Gastric Hp infection was an important factor of oral Hp infection. Oral Hp was positively correlated with the expression of Pg and Pi. The oral Hp positive rate in the NPG was 40%. Apoptosis staining was observed in gingival epithelial cells of patients. These patients needed to receive crown lengthening due to crown fracture. However, the index in the Hp infection group was higher ($P < 0.05$). Conclusions: Oral Hp infection is closely related to gastric Hp infection. The expression rate of oral Hp in patients with periodontitis increases. The deep periodontal pocket environment of severe periodontitis propotes Hp proliferation. Oral Hp can copolymerize with the periodontal Pg and Pi. The occurrence and development of periodontitis is indirectly promoted. Hp promotes apoptosis in periodontal tissue. It may have direct effects on the development of periodontitis.

Keywords: Helicobacter pylori, periodontitis, genotype, CBCT

Introduction

Periodontitis is a chronic inflammatory disease [1]. In the disease, the periodontal tissues (gingiva, alveolar bone, periodontium, cementum) are invaded [2]. Periodontitis has a high morbidity worldwide. An epidemiological investigation in 2005 found that the portion of the population with periodontal disease accounts for more than 90% of total population in China [3]. The morbidity has already surpassed decayed teeth. Periodontal disease has become the most prominent major disease endangering the oral health of Chinese people. The morbidity of chronic periodontitis accounts for approximately 95% of periodontitis [4]. Dental plaque is the initial factor of the disease. Bacterial plaque and its metabolites can induce the destruction of periodontal supporting tissue [5]. Moreover, a favorable environment is produced for periodontal pathogen growth [6]. Meanwhile, its pathogenesis is also related to host and environment [7]. The clinical manifestations of periodontitis are gingival bleeding, alveolar bone absorption and periodontal pocket formation [8]. As a result, tooth movement and tooth loss are caused. The resulting complications include periodontal abscess, bad breath, etc. Great pain is brought to people's life. Scholars believe that periodontitis has a cause and effect relationship to the distribution of subgingival plaque [9]. The key of periodontitis treatment is to comprehensively analyze and evaluate the subgingival bacteria [10]. Therefore, the pathogenic bacteria related to the occurrence and development of periodontitis should be studied [11].

It is of great significance for early diagnosis and best treatment of patients with periodontitis [12].

Hp is a highly infectious conditional pathogen [4]. Recent literature has confirmed that Hp is closely related to gastritis, gastric ulcer, even gastric cancer and other gastrointestinal diseases [13]. Humans are the primary host. It was classified as a class I carcinogen by World Health Organization (WHO) in 1994 [14]. Clearance of gastric Hp can reduce the risk of gastric cancer by 40%. Currently, more than half of the world's population is infected with Hp. As a developing country, China's infection rate of gastric Hp is up to 70%. Epidemiological investigation has shown that Hp is easy to spread in the people sharing meals. Therefore, the prevalence rate is high in family groups. In addition, unclean eating habits outside is also an important cause of Hp infection [15]. Hp cannot be cleared by autoimmunity after infection due to its strong colonization ability. Thus, chronic inflammation is caused. However, only approximately 20% of Hp positive patients have obvious clinical symptoms [16]. With the increasing awareness of prevention, more and more people spontaneously go to the hospital for Hp test. Thus, transmission in the family is avoided. At present, ^{13}C-urease breath test (^{13}C-UBT) is often used as the diagnostic basis of Hp infection in domestic hospitals.

There is no uniform standard for the diagnosis of oral Hp infection. Therefore, there are great differences in the positive rates obtained by different test methods. How to diagnose Hp quickly and accurately is a hot topic. With further study on the pathogenicity of Hp, it has been found that all virulence factors expressed and secreted by Hp have their own pathogenic mechanism. After oral Hp infection, the effect of virulence factors on periodontal tissue has not been confirmed. As a "storage pool" for large microbiota, the pathogenic bacteria are not isolated. Bacteria interact with each other. They can copolymerize or be competitive, synergetic or antagonistic. Thus, microbiological balance is achieved. Once the environment is out of balance, it is easy to cause immune disturbances and disease.

Therefore, the study on the toxicity of Hp alone may not be sufficient to demonstrate its pathogenicity. It is necessary to study the interaction between Hp and different periodontal pathogens. In this study, the association of Hp and its associated toxins with chronic periodontitis was investigated. The symbiotic relationship between Hp and suspected periodontal pathogens was evaluated. The pathogenic role of Hp in the occurrence and development of periodontitis was speculated.

Inclusions criteria

There were 320 samples collected in this study. They received a Hp ^{13}C-UBT test from May 2016 to April 2017 in our hospital. The inclusion criteria were as follows: ① Before the test, all the subjects had not taken anti-Hp drugs within two weeks prior. The patients had no special medication history, including receptor antagonists, bismuth agents, chemotherapeutic drugs, etc., within four weeks. ② No periodontal treatment was performed within the previous half of a year. No obvious occlusal abnormality was observed. ③ No serious systemic disease was diagnosed in patients. ④ The patients were aged 40±20. This study was approved by the Ethics Committee of our hospital. The research subjects and their families were informed and they signed an informed consent form.

Exclusion criteria

Patients with congenital heart, kidney, liver and lung dysfunction; patients with immunodeficiency disorders, severe infection and trauma before treatment, or with other malignant tumors; patients with incomplete clinical data; and patients who couldn't cooperate with treatment.

Materials and methods

(1) ^{13}C-Urea capsule breath test kit (HTA Co., Ltd.), executive standard (YBH06682005). Main ingredients: urea (^{13}C). Molecular formula: $(NH2)_2{}^{13}CO$. Indications: diagnosis of Hp infection. (2) HCBT-01 breath test tester (Shenzhen Headway). (3) Williams periodontal probe (Shanghai Dentistry Device Factory). (4) Gracy periodontal scaler (Shanghai Dentistry Device Factory). (5) Oral Hp rapid test paper (Guangzhou Beisiqi Reagent Co., Ltd.). (6) KODAK9000C 3D (KODAK, U.S.).

Table 1. Periodontitis grading diagnosis criteria

	Imaging features	Periodontal pocket depth	Mobility degree
Mild	Alveolar resorption < 1/3 of root length	Periodontal pocket depth ≤ 4 mm	None
Moderate	1/3 of root length < Alveolar resorption ≤ 1/2 of root length	4 mm < Periodontal pocket depth ≤ 6 mm	I
Severe	Alveolar resorption > 1/2 of root length	Periodontal pocket depth > 6 mm	II-III

^{13}C-UBT test

The patients were fasted for more than 2 h in the morning before testing. The general information was filled out on two gas collection bags with labels. Normal breath was maintained. The expiration at 0 min was collected. The latter half of the gas was blown into the collection bag. The cap was immediately tightened after filling. One ^{13}C urea capsule was orally taken with 100 ml of water. Then, the timing was started. Breath was collected again into the bag after 30 min. Cap was tightened.

$^{13}CO_2$ from the gas collection bags at 0 min and 30 min were tested. $^{13}CO_2$ abundance δ‰ was obtained. The subtractive result was the delta over baseline (DOB). Namely, DOB = δ‰ (30 min) - δ‰ (0 min). DOB ≥ 4.0 implied a positive result. DOB < 4 indicated a negative result.

Collection of clinical data

After the breath test was completed, communication was made with the patient beside the dental unit. The objective and content of survey were explained. Thus, the patients fully understood the items and contents. Meanwhile, the informed consent was obtained. The name, age, gender, residence, general condition, family disease, personal food preference, gastrointestinal symptoms, family Hp infection and other general information were collected and recorded in a tabular form (**Table 1**).

The stress level and the habits of staying up late were recorded in this study. If the patient felt no stress, it was recorded as (-). If the patient felt they are under stress, it was recorded after a stress index scale (attached in **Table 2**) was filled in. If the score was more than 10 points, it was recorded as (+).

If the sleep time was later than midnight, it was recorded as staying up late (+).

Periodontal test

Outcome measures. The gingivival index (GI), probing pocket depth (PD) and attachment loss (AL) were tested and recorded.

The oral panoramic radiography of patients with attachment loss was collected. According to the diagnostic criteria (Version 4) formulated by People's Medical Publishing House, periodontitis was classified into three levels, including mild, moderate and severe [17] (**Table 1**).

Hp rapid urease test

(1) Sample collection. The tartar was collected from the root cervix of the first 4 molars with disinfected gracey periodontal scaler in the morning. The patients were under fasted conditions. To avoid a false positive result, the sample was not contaminated by periodontal bleeding. Therefore, care was taken to not to touch the gingiva forcefully.

(2) Test with Hp test paper. The Hp test paper was placed on the dental unit. The yellow test paper contained urease and phenol red. The sample was directly placed in the center. The test paper was covered to tightly combine with the backing paper. The change in color was observed.

If edge of the sample was changed from yellow to red within 1 min, it was deemed as strongly positive result for Hp infection (++). If it changed from yellow to red within 1~3 min, it was judged as weakly positive result (+). If the color was not changed, it was determined as negative result (-). Strongly positive result and weakly positive result were identified as oral Hp infection.

Statistical analysis

There were 320 patients included in the study. The test results and clinical investigation data were statistically analyzed with SPSS 23.0. The measurement data were expressed with mean

Table 2. Effect of gender, staying up late, stress, dietary habit, age, periodontitis and gastric pH on positive rate

Group	Oral Hp Test		Total	Positive Rate	χ^2	*P*
	Positive	Negative				
Gender					0.256	0.613
Male	88	43	131	67.18%		
Female	132	57	189	69.84%		
Staying up late Group					8.979	0.003
Staying up late (After 12)	132	42	174	75.86%		
Normal sleep	88	58	146	60.27%		
Stress Group					6.705	0.010
Great stress	131	44	175	74.86%		
No obvious stress	89	56	145	61.38%		
Dietary Habit					0.024	0.876
Light diet	102	50	152	67.11%		
Salty, spicy diet	118	60	178	66.29%		
Age					0.832	
< 35	72	31	103	69.9%		
35~50	80	33	113	70.8%		
> 50	68	36	104	65.38%		
Periodontitis					35.107	0.000
Normal periodontal group	53	58	111	47.75%		
Mild periodontitis group	69	16	85	81.18%		
Moderate periodontitis group	45	13	58	77.59%		
Severe periodontitis group	53	13	66	80.30%		
Gastric Hp Group					82.165	0.000
Gastric Hp (+)	177	28	205	86.34%		
Gastric Hp (-)	43	72	115	37.39%		

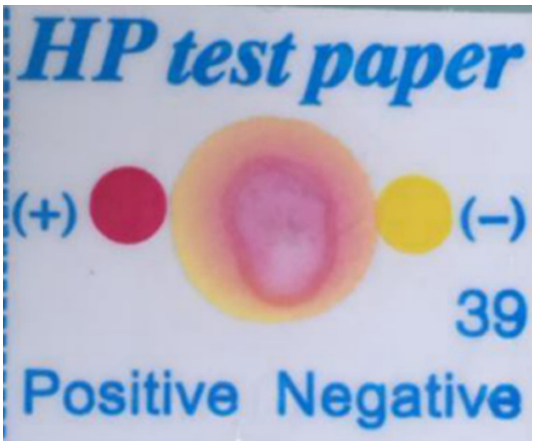

Figure 1. Positive urease test result.

± standard deviation (x±s). t test was used for comparison among groups. x^2 test was adopted for comparison of rate among enumeration data. We used the Peason test analysis to verify the association of Helicobacter pylori with chronic periodontitis. Stratified analysis was introduced for comparison among multi-factor groups. P < 0.05 implied a significant difference.

Results

Gastric Hp infection

The results of the breath test showed that 320 patients had digestive symptoms. There were 205 patients with DOB > 4.0. The positive rate was 86.34%.

Oral Hp infection

The results of a positive Hp urease reaction showed that the edge of the sample was red (**Figure 1**). There were 220 patients with positive Hp urease reaction. The positive rate was 68.75%.

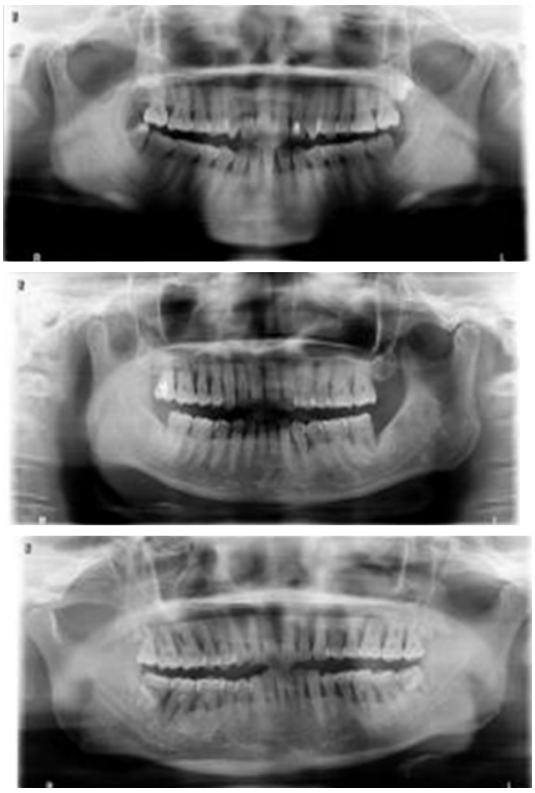

Figure 2. Periodontitis panoramic radiograph. (1) Mild periodontitis panoramic radiograph; (2) Moderate periodontitis panoramic radiograph; (3) Severe periodontitis panoramic radiograph.

Periodontitis infection

The clinical periodontal probing and dental panoramic radiograph results showed that 209 patients were diagnosed with periodontitis (positive rate 65.31%). The imaging tests were shown in **Figure 2** below.

Correlation between oral Hp infection positive rate and test factors

The effect of gender, staying up late, stress, dietary habit, age, periodontitis and gastric pH on oral Hp infection positive rate was tested (**Tables 2**, **3**).

The positive rates of oral Hp test were respectively 67.18% in males and 69.84% in females (x^2=0.256, P=0.613). The positive rate was 75.86% in the staying up late group. It was higher than 60.27% in the normal sleep group (x^2=8.979, P=0.003). The positive rate was 61.38% in the no stress group. It was lower than 74.86% in high stress group (x^2=6.705, P=0.010). The positive rate was 67.11% in light diet group and 66.29% in salty and spicy group (x^2=0.024, P=0.876). We found that there was no difference in the positive rate between light diet group and salty-spicy diet group. The positive rates were respectively 69.9% in the under 35-year old group, 70.8% in 35-50-year group and 65.38% in the above 50-year old group (x^2=0.832, P=0.660). We found that the positive rates were not different among different age groups. The positive rates were accordingly 47.75% in NPG, 81.18% in MIPG, 77.59% in MOPG and 80.30% in SPG. The differences among the four groups were significant (x^2= 35.107, P=0.000). The pairwise comparison results were as follows: the positive rate in the NPG group was lower than those in the 3 periodontitis groups (PG) (x^2=22.890, P=0.000; x^2=13.922, P=0.000; x^2=18.261, P=0.000). The differences among the 3 PGs were not remarkable. The positive rate was 86.34% in gastric Hp positive group. It was higher than 37.39% in gastric Hp negative group (x^2= 82.165, P=0.000).

Stratified analysis on oral Hp test positive rates of patients with different degrees of periodontitis and gastric Hp.

In the gastric Hp-positive populations, the positive rates of oral Hp test in NPG, MIPG, MOPG and SPG were respectively 79.25%, 81.16%, 80.0% and 81.13% (x^2=0.092, P=0.993). The correlation between the severity of periodontitis and the oral Hp positive rate cannot be determined. In the gastric Hp-negative populations, the positive rates were correspondingly 25.18%, 25.0%, 30.77% and 38.46% (x^2= 0.958, P=0.811). The correlation between the severity of periodontitis and the oral Hp positive rate cannot be concluded.

Correlation between Pg, Pi and Bf and oral Hp expression

The difference of Pg, Pi and Bf expression in RT-PCR tests between the two groups of samples was shown in **Table 4**.

The t-test results showed that there was no significant difference in Bf expression between the two groups. Pg and Pi were highly expressed in oral Hp (+) group (**Figure 3**).

Table 3. Stratified analysis on gastric Hp-periodontitis-oral Hp

	Gastric Hp (+)		χ^2	P	Gastric Hp (-)		χ^2	P
	Oral Hp (+)	Oral Hp (-)			Oral Hp (+)	Oral Hp (-)		
Normal periodontal group	42 (79.2%)	11 (20.75%)	0.092	0.993	15 (25.18%)	43 (74.14%)	0.958	0.811
Mild periodontitis	56 (81.2%)	13 (18.84%)			4 (25.0%)	12 (75.0%)		
Moderate periodontitis	36 (80.0%)	9 (20.0%)			4 (30.77%)	9 (69.23%)		
Severe periodontitis	43 (81.2%)	10 (18.87%)			5 (38.46%)	8 (61.54%)		
Total	177	43			28	72		

Table 4. Relationship between the concentrations of the three pathogens and Hp infection

	Pg	Pi	Bf
Hp (+) 47 samples	5.375+0.06231	1.410+0.01133	1.304+0.01099
Hp (-) 42 samples	1.056+0.01132	1.201+0.02648	1.240+0.0854
t	64.63	7.542	5.372
P	< 0.0001	< 0.0001	0.2461

Apoptosis index in gingival tissues of Hp infection group (HIG) and normal control group (NCG)

The samples were divided into two groups by Hp infection. The t-test (overall homogeneity of variance was the same between the groups) showed that the apoptosis index in HIG was higher than that in the NCG ($P < 0.05$) (**Table 5**). Apoptosis positive nuclei were brown. The outline was clear. Many apoptotic cells were observed in HIG (**Figure 4A**). There were fewer apoptotic cells observed in NCG (**Figure 4B**).

Discussion

Using ^{13}C-UBT as the diagnostic method of current infection, the infection rate of Helicobacer pylori (Hp) is more than 50% worldwide [4]. The average Hp infection rate in China is 42%~64%. It is higher than that in developed countries. With the isolation of oral Hp, the relationship between its pathogenicity and gastric Hp is controversial in the medical field [18]. However, there is no uniform test standard for oral Hp infection [19]. Due to the limitation of test methods, there are great differences in the study data on oral Hp [20]. Therefore, a highly sensitive and specific method is urgently needed. The specificity of PCR is relatively stable. However, as an epidemiological test, the sensitivity is not high enough. The cost is higher. In addition, the probability of false negative is higher due to the influence of sampling site and the specificity of primers. Therefore, oral Hp urease test paper or HPS test is more commonly used in large clinical samples (> 100 cases). Its defect is the increase of false positive rate. However, it is convenient, efficient and easy to collect samples. After the samples are screened, PCR and other techniques can be used to test.

The subjects in this study were aged 20-60 years. The statistical results showed that there was no remarkable correlation between dietary habit, gender, age and oral Hp. Both gastric Hp and periodontitis were positively correlated with oral Hp infection in single-factor analysis. In this study, the oral Hp positive rate in healthy periodontal samples without attachment loss was 47.75%. It was much lower than 79.9% in PG. The differences in oral Hp positive rate of periodontitis groups were not significant. The pairwise comparison results showed that the positive rates in PGs were much higher than that in NPG. It indicated that Hp was involved in the generating process of periodontitis. The ecological characteristics of the bacterial plaque were more favorable for parasitism of Hp. Chronic periodontitis can increase the infection rate of oral Hp [21]. Poor periodontal conditions may provide a good living environment for oral Hp. The conclusion is similar to the study result of Sudhakar, et al. [22]. The stratified analysis showed that the oral Hp positive rates were not remarkably different among the PG. Therefore, we cannot conclude that the oral Hp infection is associated with the severity of periodontitis. The reason may be that the sample size was too small. Therefore, it is necessary to increase the sample size in subsequent study. In addition, this study suggested that the periodontal pocket was an influence factor of oral Hp infection.

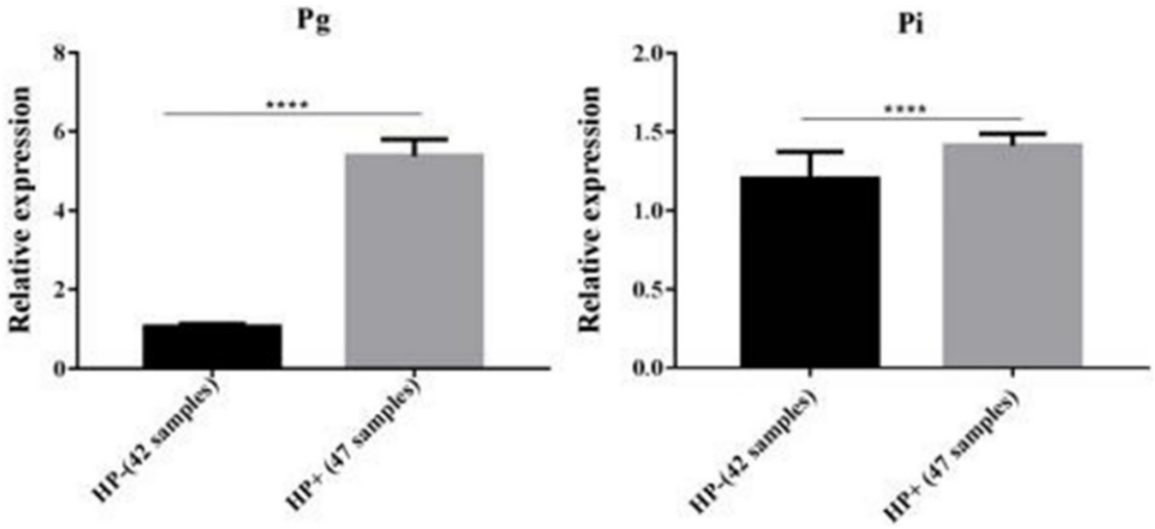

Figure 3. Comparison of Pg, Pi expression between oral Hp (+) and Hp (-) groups.

Table 5. Comparison of apoptosis index between HIG and NCG (x±s)

Group	n	AI
Hp (+)	12	0.498+0.092
Hp (-)	18	0.207+0.053

P < 0.05 vs Negative control group.

Many studies have shown that the micro-ecological environment of periodontitis is suitable for oral Hp colonization [23]. By expressing VacA and CagA genes, oral Hp can also induce the release of reactive oxygen and massive inflammatory mediators, such as PGE2, tumor necrosis factor (TNF-α), interleukin and other cytokines [24]. Under the action of cytokines, neutrophils can produce more superoxide anions [25]. As a result, periodontitis is aggravated. However, the concentration of Hp in subgingival plaque is lower. Before excluding the oral hygiene and other class I periodontal risk factors, the outcome of periodontitis may be determined by more complex factors. Among them, periodontal dominant bacteria may have a key function. Therefore, the correlation between Hp infection and periodontitis cannot be confirmed statistically. The reasons may be as follows: on the one hand, although Hp is involved in the pathogenesis of periodontitis, it is not the main dominant bacteria. The amount of bacteria is relatively small. On the other hand, periodontal pocket depth and periodontal recession degree represent the severity of periodontitis. However, the development of periodontitis is influenced by many factors. Therefore, the results can imply that Hp is not the most significant influence factor. The positive correlation between them cannot be denied. To prove the relationship between oral Hp and the severity of periodontitis, it is best to obtain the quantity and activity of Hp. Thus, a relatively direct relationship can be obtained. However, the quantity of Hp is not easy to be obtained. The colonization ability varies from one sampling site to another.

In this study, the relationship between Hp and periodontal destruction was qualitatively compared. The pathogenicity of Hp in periodontitis was analyzed. According to the results above, it is speculated that oral Hp is statistically correlated with the occurrence of periodontitis. Periodontitis is a risk factor of Hp infection. However, the effect of Hp on development of periodontitis and the role in the deepening periodontal pocket still need to be further quantitatively studied. Relevant conclusions cannot be drawn in this study.

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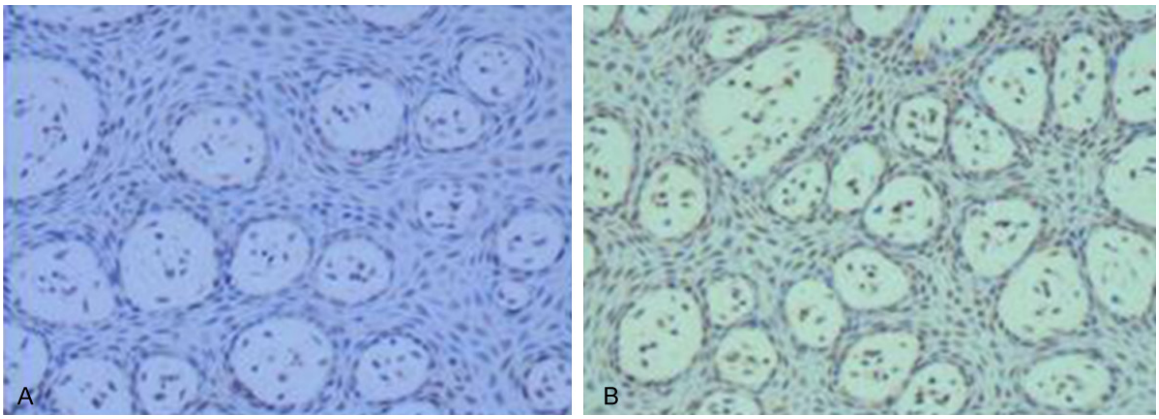

Figure 4. Apoptosis in gingival tissues. A. Apoptosis in Hp (-) gingival tissues; B. Apoptosis in Hp (+) gingival tissues.

Disclosure of conflict of interest

None.

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