Detection of *Helicobacter pylori* in dental plaque and posterior dorsal surface of tongue in dyspeptic patients

연세대학교 대학원

치 의 학 과

이 상 섭

감사의 글

본 논문이 완성되기까지 시종일관 아낌없는 배려와 세심한 지도를 해주시며 항상 아버지와 같은 큰 사랑을 베풀어주신 김종열 교수님께 진심으로 감사드리며, 그동안 논문 작성과 심사의 지도편달과 많은 격려와 조언을 아끼지 않으셨던 너무나 자상하시고 제자의 고충을 들어주시던 최종훈 교수님과 어려운 부탁을 마다하지 않으시고 흔쾌히 들어주신 소화기내과 이용찬 교수님께도 진심으로 감사드립니다. 아울러 분자생물학이라는 어려운 학문을 공부하는데에 항상 몸소 가르쳐주시고 길을 보여주신 신경진 교수님과 멀리 광주에서도 아낌없는 사랑으로 지도하여 주신 윤창륙 교수님께도 감사드립니다. 또한 학문에 대한 뜨거운 열정을 보여주신 김성택 교수님과 지금은 미국에서 유학중이신 김재홍 선생님, 분자생물학 연구에 매진할 수 있게 많은 도움을 준 동기 심우현 선생 및 같이 수고한 의국원들에게도 깊은 감사의 뜻을 전합니다.

저에게 배움의 기회를 허락하시고 끝없는 후원을 아끼지 않으신 아버지, 어머니와 기도로 길러주시고 항상 걱정해주시는 할머니, 같은 공부를 하며 항상 모든 일에 열심인 든든한 윤섭이와 어릴적부터 끈끈하고 우정어린 격려와 조언으로 도와준 민수, 상준이와도 작은 기쁨을 나누고 싶습니다. 논문의 시작부터 항상 옆에서 도와주신 소화기내과 박사과정에 계신 주이신 선생님께도 더불어 감사의 뜻을 전합니다.

마지막으로 이 모든 분들을 만나게 해주시고 이 모든 환경과 믿음을 허락하여 주신 주님앞에 겸손한 마음으로 이 논문을 바칩니다.

2003년 12월

저자 씀

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Detection of *Helicobacter pylori* in dental plaque and posterior dorsal surface of tongue in dyspeptic patients

Helicobacter pylori is known as one of the most common infecting bacteria and is infecting half the world's population. However, the exact mechanism of transmission is still unclear. The dental plaque was suggested as a possible resorvoir in transmission route. In this report, dental plaques and posterior dorsal surface of tongue samples were collected from 62 randomly selected dyspeptic patients referred to the division of gastroenterology, Yonsei University, Severance Hospital. To determine the prevalence of *H. pylori* in oral cavity and to assess the role of dental plaque as a permanent resorvoir in transmission route, samples were analyzed for the presence of *H. pylori* with nested PCR. The results are summerized as follows.

1. In Study group (group of *H. pylori* infected patients), eight of 36 (22.2%) dental plaque samples and twelve of 36 (33.3%) posterior dorsal surface of tongue samples turned out to be positive. In Control group (group of *H. pylori* non-infected patients), two of 26 (7.7%) samples of each subgroup were positive.

2. The detection rate of *H. pylori* DNA in samples from Study group was significantly higher than in that from Control group ($P < 0.05$). Comparison of T subgroups (group of posterior dorsal surface of toungue) showed significant difference in detection of *H. pylori* ($P < 0.05$), whereas no difference was found between subgroup P (group of dental plaque) of Study group and that of Control group.

3. Statistical significance was observed in subgroup P from Study group and in Control group ($P < 0.01$) when positivity of *H. pylori* in each groups was compared statistically with the presence of regurgitation symptoms.

Based on the data stated above, this study supports the hypothesis that dental plaques may play an important role as a permanent oral resorvoir of *H. pylori* in transmission route. In addition, dental plaque may be a potential source for reinfection following eradication therapy. However, more profound and comprehensive study using larger number of subjects are required and typing of *H. pylori* isolates from both the oral cavity and stomach will play a major role in studing the modes of transmission. Furthermore, the ability to detect *H. pylori* in dental samples may offer the potential role for a non-invasive test for infection.

Key Words : dental plaque, toungue, *Helicobacter pylori*, reservoir, PCR

Detection of *Helicobacter pylori* in dental plaque and posterior dorsal surface of tongue in dyspeptic patients

(Directed by Prof. Chong-Youl Kim, D.D.S., M.S.D., Ph.D.)

Department of Dental Science,

The Graduate School, Yonsei University

Sang-Seob Lee, D.D.S.

Ⅰ. Introduction

Helicobacter pylori (*H. pylori*) is a microaerophilic, gram-negative, rod-shaped and motile bacterium which colonizes the human stomach. It was first isolated from a human gastric biopsy specimen by Warren and Marshall in 1983. It resides beneath the gastric mucus, adjacent to the gastric epitherial cells and is well adapted to life in the hostile acidic environment of the stomach. Although it is not invasive, it causes inflammation of the gastric mucosa. It is now recognized as an important cause of active chronic gastritis in humans and plays a important pathogenic role in development and recurrence of gastric ulcers

(Graham, 1989, Taylor and Blaser, 1991) and perhaps gastric cancer (Forman et al., 1991, Nomura et al., 1991, Parsonnet et al., 1991). Moreover, *H. pylori* infection significantly increases the risk of gastric MALT (Mucosa-Associated Lymphoid Tissue) lymphoma (Parsonnet et al., 1994, Wotherspoon, 1998).

H. pylori is one of the most common bacterial infections of mankind. It has been estimated that half of the population in developed countries (Graham et al., 1991) and over 80% of the population in developing countries is infected by the *H. pylori* (Holcombe et al., 1992). Risk of infection is increased in lower socioeconomic groups, at younger ages, in many ethnic groups, and in certain geographical populations. Although *H. pylori* infection is widespread throughout the world (Feldman et al., 1997, Goodwin et al., 1997, Stone, 1999), the mode of transmission, the natural history and other aspects of the epidemiology of *H. pylori* infection are still unclear (Go, 2002). Reported observations support a person-to-person mode of transmission via fecal-oral (Leung et al., 1999), oral-oral (Li et al., 1996) , or gastro-oral routes (Goosen et al., 2002).

Fecal-oral spread of *H. pylori* infection could occur directly from the infected person or with crowded or suboptimal sanitary conditions, as in institutionalized subjects, or indirectly from contaminated water or foods. Supporting evidence includes detection of *H. pylori* from human feces by culture and polymerase chain reaction (PCR) (Thomas et al., 1992). Moreover, the report about high concordance rates of *H. pylori* infection and hepatitis A (Rudi et al., 1997) further supports a fecal-oral transmission. However, poor correlation between these two infections was also reported (Fujisawa et al., 1999) and at present, this issue remains controversial.

Oral-oral passage of *H. pylori* infection has been based largely on PCR

detection of homologous DNA in dental plaque, saliva and the subgingival region (Krajden et al., 1989, Vallentine et al., 1991, Hammar et al., 1992, Bernander et al., 1993, Wahlfors et al., 1995, Chong et al., 1996, Oshowo et al., 1998). Cultures are difficult because of many other bacteria inhibiting growth of *H. pylori* (Nguyen at al., 1993) and poor sensitivity of the culture methods to date. CLO™ (Deltawest, Bentley, Australia) test, which is for the detection of urease, can not be applicable in oral cavity because the oral cavity contains many urease-containing bacteria other than *H. pylori*. PCR offers the advantages of sensitivity, specificity, and rapidity for the detection of microbial pathogens. At present, there are many reports of *H. pylori* residue in dental plaque, detected by PCR (Hammar et al., 1992, Banatvala et al., 1993, Mapstone et al., 1993a, Olsson et al., 1993, Shimada et al., 1994).

The detection of *H. pylori* in dental plaque suggests that the oral cavity, especially dental plaque, may be an important reservoir for this bacterium. The reports that *H. pylori* strains in mouth and stomach are identical (Shames et al., 1989, Khandaker et al., 1993, Oshowo et al., 1998) further support this theory. Many studies have been published recently that support and contradicts this theory. Most of these studies have used PCR assays on dental plaque and saliva specimens and reported a 0 to 97% incidence of positive samples. As these results are not in agreement with the prevalence of *H. pylori* infection in the stomach, it is doubtful as to whether they represent the real prevalence of this microorganism in the human oral cavity or are artifacts of the methods applied. Moreover, transmission by gastric regurgitation has been suggested by investigators. Thus, the hypothesis that the oral cavity may be a permanent resorvoir of viable *H. pylori* still remains as a controversial issue.

There are some reports about incidence of *H. pylori* in dental plaque and saliva in Korea (Kim et al., 2000, Kim et al., 2002). Kim et al. (2000) reported the prevalence of *H. pylori* in dental plaque and saliva, 6.9% and 28.6% respectively. In addition, Kim et al. (2002) reported 32.5% positive rate in dental plaque in recurrent apthous ulcer patients. However, *H. pylori* infection status was not considered in those reports. This study was done to investigate the presence of *H. pylori* in dental plaque and posterior dorsal surface of tongue in relation to the infection state of gastric mucosa by PCR. In doing so, we may be able to assess the role of dental plaque as a possible resovoir in *H. pylori* transmission.

Ⅱ. Materials and Methods

1. Materials

Dental plaque and posterior dorsal surface of tongue samples used in this study were collected by a dentist from 62 randomly selected dyspeptic patients referred to the division of gastroenterology, Yonsei University, Severance Hospital. Those who had been treated for *H. pylori* infection or had recently received antibiotics or acid suppressing agents in the last three months before investigation were excluded to avoid false negative results due to interference with *H. pylori* detection methods. Informed consents of the patients were obtained. Multiple samples of dental plaques from different sites were taken from each patient and trasnsfered to a 1.5㎖ microcentrifuge tube containing phosphate buffered saline (PBS). The samples from posterior dorsal surface of tongue were taken by swabbing the surface with sterile cotton tips and transfered to 1.5㎖ microcentrifuge tubes. DNA extraction were done within 2 hours after sampling.

CLO™ tests, histological examinations and microbiological cultures were used to determine their *H. pylori* status. *H. pylori* infection was diagnosed when at least one of the above tests was positive. The presence of upper gastrointestinal reflux symptoms including such as regurgitation and heartburn were assessed. The Study group consisted of patients with *H. pylori* infection (n=36) while those of Control group were not infected with *H. pylori* (n=26). Each group was subdivided according to the sample location, dental plaque (Subgroup P) and

poesteior dorsal surface of tongue (Subgroup T). The statistical difference in age, sex distribution and presence of regurgitation symptoms between Study and Control group were not observed. ($P>0.05$, chi-square test)

2. Methods

A. DNA Extraction

The DNA from the collected samples were extracted with QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany). The sample was placed in 1.5㎖ microcentrifuge tube containing 400㎕ of phosphate buffered saline (PBS). 20㎕ of QIAGEN Protease and 400㎕ of Buffer AL were added into the tube and mixed immediately by vortexing for 15 seconds. After incubating at 56℃ water bath for 10 minutes, the tube was centrifuged brifely, then 400㎕ of 100% ethanol was added to the sample and mixed again by vortexing. From the mixture, 700㎕ was taken and applied carefully to the QIAamp spin column, then the column was centrifuged at 6000×g for 1 minute. The tube containg filtrate was discarded. After that, the column was centrifuged at 6000×g for 1 minute with 500㎕ of Buffer AW1 followed by centrifuge at 20000×g for 3 minutes with 500㎕ of Buffer AW2. Finally, the column was placed in a clean 1.5㎖ microcentrifuge tube and centrifuged at 6000×g for 1 minute with 150㎕ Buffer AE. The extracted DNA was stored at -20℃ for future PCR (QIAGEN, Hilden, Germany).

B. PCR primers

The primer set EHC-U/EHC-L was directed to the 860-bp fragment of *H. pylori* genomic DNA, and the expected product size was 417 bp (Li et al., 1995, 1997). The region targeted by the primers EHC-U/EHC-L is located in 80,076 - 80,492 bp of the genome of *H. pylori* 26695 (GeneBank Accession No. AE000511). Based on the sequence of the 860-bp fragment of *H. pylori* genome of 26695, additional primers, ET-5U/ET-5L, internal to the fragment amplified by EHC-U/EHC-L were used, and the expected product size was 230 bp (Song et al., 1999, 2000a, 2000b). The oligonucleotides were synthesized by Bioneer corporation (Daejeon, Korea). The Table 1 shows the nucleotide sequences of primers and Figure 1 illustrates the location of EHC-U/EHC-L and ET-5U/ET-5L primers in *H. pylori* genomic DNA.

Table 1. Nucleotide sequences of primers used in nested PCR

Name	Sequence
EHC primers*	
EHC-U	5′-CCCTCACGCCATCAGTCCCAAAAA-3′
EHC-L	5′-AAGAAGTCAAAAACGCCCCAAAAC-3′
ET primers*	
ET-5U	5′-GCCAAATCATAAGTCCGCAGAA-3′
ET-5L	5′-TGAGACTTTCCTAGAAGCGGTGTT-3′

* Identical to EHC and ET primers reported by Song *et al.,* 1999

```
80041 CACAAACATG GGGGTGAGTT TCACCCCTGC TTTACCCCTC ACGCCATCAG TCCCAAAAAT
80101 TTTCATCGTT ATAAAATACC TTTTAAACTA TTTTTAATCA ATTTTTAGAT AGAATTATGC
80161 CAAATTTTAC ATTACAAAGG GATTAAAACA AGGCTATGGC AAATCATAAG TCCGCAGAAA
80221 AGCGAATCAG ACAGACCATT AAGAGAACCG AACGCAACAG GTTCTATAAA ACTAAAATTA
80281 AAAATATCAT TAAAGCCGTG CGTGAAGCCG TTGCTGTCAA TGATGTAGCA AAAGCTCAAG
80341 AGCGTTTGAA AATCGCTAAT AAAGAGTTGC ATAAATTTGT CAGCAAGGGG ATTTTAAAGA
80401 AAAACACCGC TTCTAGGAAA GTCTCAAGGC TTAACGCTTC AGTGAAAAAA ATCGCTCTCG
80461 CTTAGTTTTG TGGCGTTTTC AACTTCTTTA AGCTCAGTAA TGGGTTTTTA TTATTGGGCT
```

Fig 1. Location of EHC and ET primers in genomic DNA of *H. pylori*

The diagram contains partial sequences of *H. pylori* strain 26695 that is registered in the GeneBank (GeneBank Accession No. AE000511). The sequences of designed forward and reverse primers of EHC are lightly shadowed. And the sequences of those of ET are darkly shadowed.

C. Nested PCR amplification

The first round of PCR amplification consisted of 40 cycles comprising a 45 sec denaturation step at 94℃, a 30 sec primer annealing step at 59℃, and a 45 sec primer extension step at 72℃. For the initial cycle, denaturation was for 5 min at 95℃ and for the final cycle, primer extension was extended to 10 min at 72℃. All reaction were carried out in a final volume of 20 ㎕ with the following composition: AccuPower PCR Premix (including 10 mM Tris-HCl (pH 9.0), 40 mM KCl, 1.5 mM $MgCl_2$, 250 μM dNTP, and 1U of *Taq* DNA polymerase; Bioneer, Daejeon, Korea), 0.5 μM of each primer, 1 ㎕ of template DNA.

The profile of the second round was similar to the first round, except that 1 ㎕ of the first round product served as template for the second round PCR and only 25 amplification cycles were used.

Confirmation of successful extraction of PCR-amplified DNA from the respective sample was obtained by carrying out PCR on each DNA sample with primers specific for the beta hemoglobin gene, as previously described (Riggio et al., 2000). Positive and negative controls were performed for each batch of amplifications. The genomic DNA of *H. pylori* (type strain 26695) served as a positive control. As negative controls, the autoclaved tertiary distilled water was used. To prevent false positive results due to contamination, very extensive care was taken such as preparing template DNA and pre- and post-PCR materials in separating places. Amplification was performed with a thermocycler, DNA Engine PTC-0200D (MJ Research, U.S.A.).

D. Detection of PCR product

5 ㎕ of each PCR reaction product was electrophoresed on a 1% agarose gel containing ethidium bromide (0.5 ㎍/㎖) and visuallized under ultraviolet (UV) illumination. A 100-bp DNA ladder (Bioneer, Daejeon, Korea) was used as a size marker. All electrophoresis was performed with RunOne Electrophoresis System EP2000 (Embitec, San Diego, U.S.A.). The result of electrophoresis was taken and to be recorded with Polaroid GelCam (Polaroid corporation, Cambridge, U.S.A.).

E. Statistical analysis

1) The positivity of *H. pylori* infection of both Study and Control group were examined. And the positivity of Subgroup P and T of both groups was also observed.
2) The differences between the frequency of *H. pylori* infection in Study group and Control group was evaluated with chi-squre test.
3) The differences between the frequency of *H. pylori* in Study subgroup P, T and that in Control subgroup P, T were evaluated with Fisher's exact probability test.
4) The relation between the existence of regurgitation symptoms and prevalence of *H. pylori* in each group was evaluated with Fisher's exact probability test.

These statistical analysis were calculated with SAS program 8.1 version (SAS institute, U.S.A.).

Ⅲ. Results

1. Detection of *H. pylori* DNA in specimens

The PCR assay was applied to genomic DNA extracted from dental plaque and posterior dorsal surface of tongue samples. On each occasion, when PCR was carried out, negative controls were always negative and positive controls were always positive, therby excluding the possibility of contamination and validating the PCR results obtained. The expected product of amplification was 230 base pairs in length.

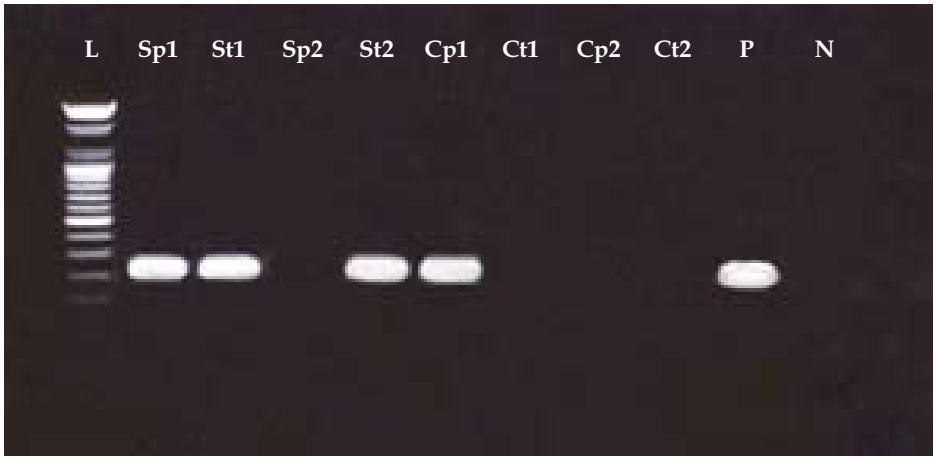

Fig 2. Electrophoretic patterns of PCR amplification products of *H. pylori* genomic DNA

The amplified products were separated in 1% agarose gel and directly visualized with ethidium bromide under ultraviollet light.

L : 100bp DNA ladder

Sp1, Sp2 : Samples from the dental plaque of *H. pylori* infected patients.

St1, St2 : Samples from the posterior dorsal surface of tongue of *H. pylori* infected patients.

Cp1, Cp2 : Samples from the dental plaque of *H. pylori* non-infected patients.

Ct1, Ct2 : Samples from the posterior dorsal surface of tongue of *H. pylori* non-infected patients.

P : Positive control (the genomic DNA of *H. pylori* 26695 was used as template)

N : Negative control (the autoclaved tertiary distilled water was used as template)

2. The positivity of *H. pylori* in Study group and Control group

In Study group, eight of 36 (22.2%) dental plaque samples and twelve of 36 (33.3%) posterior dorsal surface of tongue samples analysed were found to contain *H. pylori* DNA. In contrast, two of 26 (7.7%) samples of each subgroup were positive in control group. (Table 2)

Table 2. The positivity of *H. pylori* PCR in Study and Control group

Group*	Frequency	Positivity (%)
Study (n=72)	20	27.8
Study P (n=36)	8	22.2
Study T (n=36)	12	33.3
Control (n=52)	4	7.7
Control P (n=26)	2	7.7
Control T (n=26)	2	7.7

* Study : Samples from *H. pylori* infected patients

Study P : Samples from dental plaque of *H. pylori* infected patients

Study T : Samples from posterior dorsal surface of tongue of *H. pylori* infected patients

Control : Samples from *H. pylori* non-infected patients

Control P : Samples from dental plaque of *H. pylori* non-infected patients

Control T : Samples from posterior dorsal surface of tongue of *H. pylori* non-infected patients

3. Comparison of positivity of *H. pylori* PCR between Study and Control group

The detection rate of *H. pylori* DNA in samples from Study group was significantly higher than in that from Control group ($P < 0.05$). Comparison of T subgroups showed significant difference in detection of *H. pylori* ($P < 0.05$). In case of P subgroups there was no significant difference. (Table 2)

Table 3. Statistical significances of positivities of *H. pylori* PCR between Study and Control group

Group	P value
Study P - Control P	0.1705
Study T - Control T	0.0291*
Study - Control	0.0491*

* : significant difference ($P < 0.05$)

4. Comparison of positivity of *H. pylori* PCR in both groups with presence of regurgitation symptoms

Prevalence of *H. pylori* in each groups was compared with the existence of GERD. Statistical significance was observed in subgroup P from Study group and in control group ($P < 0.01$). Dental plaque from patient with *H. pylori* infection showed significantly higher rates of *H. pylori* PCR rate compared to control group.

Table 4. Statistical significance of prevalence of *H. pylori* in Study and Control groups with presence of regurgitation symptoms

Group	P value
Study	0.0895
Study P	0.0013*
Study T	0.7199
Control	0.0023*
Control P	0.0646
Control T	0.0646

* : significant difference ($P < 0.01$)

5. Comparison of positive ratio of *H. pylori* PCR in Control group according to presence of regurgitation symptoms

The positive ratio of *H. pylori* PCR in control group according to presence of regurgitation symptoms was 28.6% in both Subgroup P and Subgroup T. In contrast, *H. pylori* was not detected among patients with no regurgitation symptoms (Figure 3).

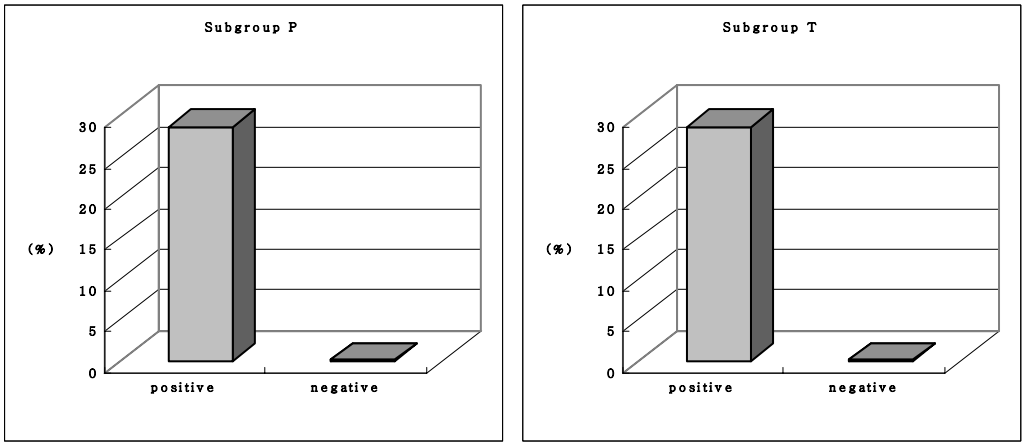

Fig 3. The positive ratio of *H. pylori* PCR in Control group according to presence of regurgitation symptoms.

Ⅳ. Discussion

Helicobacter pylori is a omnipresent micro-organism infecting half the world's population. Although *H. pylori* has been found in cats (Handt et al.,1994, Fox et al., 1996), the worldwide incidence indicates that human is the major resorvior of infection. Transmission occurs via person to person passage, and unclean water sources have been implicated in infection transmission. Risk factors for infection are low socioeconomic status, including overcrowding during childhood and no fixed hot water supply and in adults the risk rises with the number of children living in home. (Goodwin et al., 1997)

The exact mechanism of *H. pylori* transmission is still unclear, but fecal-oral and oral-oral routes are the most widely accepted modes. Although *H. pylori* has been isolated from feces by culture (Thomas et al., 1992), detected in fecal samples (Mapstone et al., 1993a) and in drinking water by PCR assay (Hulten et al., 1996), to date any environmental sources of *H. pylori* have not been revealed for sure. Mapstone et al. (1993a) reported 90% detection rate in stool while Li et al. (1996) reported 25% and in case of study from van Zwet et al. (1994), no positive result was obtained about presence of *H. pylori* in feces. Nevertheless, a lot of studies have provided the proof that there is a significant association of *H. pylori* in the stomach and mouth, it has been suggested that the *H. pylori* in the mouth plays a important role in transmission and recurrence after eradication therapy especially in developed countries (Lee at al., 1993).

Anderson et al. (1998) reported the selective adhesion of *H. pylori* to *Fusobacterium* spp. not to other species. *Fusobacterium* species are important bridging organisms between non-coaggregating bacteria in dental plaque, and so

may allow *H. pylori* to become estabilished in the dental plaque matrix (Kolenbrander, 1993). In addition, Young et al. (2001) reported the presence of *H. pylori* in dental plaque by SEM, and there is no morphological difference in the *H. pylori* cells between gastric biopsy and dental plaque samples. These findings suggests there is a potential for oral-oral transmission of *H. pylori* to occur and possible role of dental plaque in transmission.

Culture of *H. pylori* is recognized as the "gold standard" for the diagnosis of the infection. But the low detection rates have been reported in mouth. The inability to culture the organism from dental plaque suggests that this microenvironment may not be capable of the supporting the growth of *H. pylori*. Some investigators (von Recklinghausen et al., 1994) have suggested that non-culturable coccoid forms of the organism may survive in mouth. *H. pylori* converts from rod to coccoid form when the organism is stressed through nutrient depletion, prolonged incubation or antibiotic therapy (Bode et al., 1993). Coccoid forms are unculturable by conventinal methods, but may still be viable and contain a risk of infection (Krajden et al., 1989, Moshkowitz et al., 1994). The rod shaped *H. pylori* is believed to be responsible for chronic infection of the stomach, while the coccoid form has been considered a important factor in transmission (Lee at al., 1993).

The trait of *H. pylori* to produce abundant amounts of urease has been used to provide a fast diagnostic test for the infection. With the urease test, Desai et al. (1991) found *H. pylori* in dental plaque at rates of 98%, and Majmudar et al. (1990) reported *H. pylori* in dental plaque of all of their 40 healthy volunteers. However, a positive urease test on a specimen taken from the oral cavity should be interpreted with caution. There are many urease-producing bacteria in the oral

cavity, such as *Actinomyces viscosus* and *Streptococcus vestibularis,* which may cause false-positive results, therefore it may be a leap in argument to conclude that high urease activity in dental plaque is positive sign of the presence of *H. pylori*.

The development of the PCR has given researchers a powerful tool for the detection of microbial pathogens. PCR has been used successfully to detect *H. pylori* in biopsy specimens and gastric juice. To date, many PCR assays have been developed for detecting *H. pylori* in dental plaque. Different sets of primers have been proposed for the detection of *H. pylori* including those based on the urease A gene (Banatvala et al., 1993, 1994), urease C gene (Bickley et al., 1993), 16S ribosomal RNA (Mapstone et al., 1993b), a gene coding for a specific 26-K protein (Hammar et al., 1992), and 860-bp DNA (Li et al., 1995). The reported detection rates of *H. pylori* in many previous studies ranged from 0% to 100% with PCR, therefore the results still remains controversial. Song et al. (1999) reported the study designed to detect *H. pylori* in the same series of dental plaque samples with three different sets of primers. The detection rates were 26.5% for HPU1/HPU2 (urease A gene), 78.9% for HP1/HP2 (16S rRNA) and 100% for EHC-U/EHC-L and ET-5U/ET-5L (860bp DNA), respectively. As the nested PCR with primers directed to the 860-bp DNA had relative strong detection ability in case of *H. pylori* detection in dental plaque, we conducted the study with this set of primers.

In this study, the detection rate in test group was 27.8%, and in control group was 7.7%. Similar studies show various results from non-detection (Bickley et al., 1993) to detection rate of 72.2% (Banatvala et al., 1994). Technical difficulty is considered to yield the various results depending on the location of DNA assigned as target of primer for PCR process. Song et al. (1999) reported

the possibility of false positive result from HPU primer set or HP primer for the presence of similar binding sites in DNA of another bacterial species. Unlike gastric mucosa, various species of bacteria coexist in oral mucosa, therefore, competitive inhibition of primer would result in dramatic decrease in PCR detection of *H. pylori* from oral mucosa. In order to decrease the error, EHC primer set and ET primer set, which were confirmed to be very specific and sensitive in detection of *H. pylori* from previous studies, were used.

Different results could be obtained depending on the methods of PCR. In this study, nested PCR, which is effective in case of minimal template concentration through the increase in number of PCR amplification cycle using two primer sets, was used. Since relatively low number of *H. pylori* exist in oral mucosa, nested PCR could be used to decrease the chance of false negative result. Kim et al. (2000) used similar study and control group as in this study, and reported the positive detection rate as 6.9.% and 0%, respectively. The difference between Kim et al.'s (2000) result and result of this report can be thought to be due to the different PCR methods used. In 2002, Kim et al. used the same primer set and PCR method, although exact comparison is not possible, and reported 20% of *H. pylori* overall positivity within dental plaque which was similar to that of our study. This shows that the different PCR method and primer design could result in different results.

Additionally, the area difference in sample collection should be considered. Nguyen et al. (1993) reported that since *H. pylori* is not uniformly distributed in oral environment, *H. pylori* was detected in one site among the various sites from the same person. Furthermore, Song et al. (2000a) reported uneven distribution of *H. pylori* in oral environment through higher frequency of *H.*

pylori positivity at molar area than anterior teeth area. In order to minimize such errors, plaques from anterior teeth as well as molars were collected in this study.

The rate of positivity was significantly higher in Study group than that of Control group ($P<0.05$), especially at posterior dorsal surface of tongue ($P=0.0291$), however, in the case of dental plaque, there was no significant difference between the two groups. For there was no report about presence of *H. pylori* in gastrointestinal organs except stomach, the presence of *H. pylori* in mouth has a significance in transmission route of *H. pylori*. Because the presence of *H. pylori* in dental plaque had no connection with *H. pylori* infection status of stomach, dental plaque may act as reservoir in transmission route of *H. pylori*. *H. plyori* detection from the dental plaque of the control group and non-significant difference in the rate of *H. pylori* positivity in dental plaque between the Study and the Control groups support the above hypothesis. In addition, the above results show the possibility of *H. pylori* within dental plaque as a source of reinfection in patients who had received *H. pylori* elimination therapy. Butt et al. (2001) recently reported that effective elimination of *H. pylori* from dental plaque can be achieved by local dental measures and conluded that it would be prudent to evaluate periodontal status, give treatment to remove dental plaque and improve periodontal health, in addition to administering elimination therapy for patients with *H. pylori* gastritis. Results of this report show similarities to that of Butt's study, and the importance of oral hygiene in *H. pylori* transmission can be suggested.

In statistical comparison of presence of regurgitation symptoms and *H. pylori*, significant difference was observed in dental plaque group of test group ($P<0.01$). This shows the possibility that *H. pylori*, which was transported into oral

envirionment by regurgitaion, could remain within dental plaque. This again suggest the role of dental plaque as permanent reservoir of *H. pylori*. Although *H. pylori* attachment within dental plaque has been proved experimentally, further studies are required for the clarification of exact mechanism. Although the statistical siginificance between the presence of *H. pylori* in mouth and the presence of regurgitaion symptoms was not observed in this study, the possibility was supposed with the results (Figure 3). Hereafter, further studies on positivity of regurgitaion symptioms and dental plaque would be meaningful.

In conlusion, the dental plaque of oral cavity may be a permanant resorvoir for *H. pylori*, underlyining importance of oral-oral transmission of this organism. In addition, the importance of oral hygiene care in elimination therapy of *H. pylori* can be suggested. Further studies with larger number of samples and more accurate and broad studies are required, and studies on clarification of *H. pylori* transmission route by comparing *H. pylori* strain of oral cavity and that of stomach would be significant.

Ⅴ. Conclusion

Dental plaques and posterior dorsal surface of tongue samples were collected from 62 randomly selected dyspeptic patients referred to the division of gastroenterology, Yonsei University, Severance Hospital. To determine the prevalence of *H. pylori* in oral cavity and to assess the role of dental plaque as a permanent resorvoir in transmission route, samples were analyzed for the presence of *H. pylori* with nested PCR. The results are summerized as follows.

1. In Study group (group of *H. pylori* infected patients), eight of 36 (22.2%) dental plaque samples and twelve of 36 (33.3%) posterior dorsal surface of tongue samples turned out to be positive. In Control group (group of *H. pylori* non-infected patients), two of 26 (7.7%) samples of each subgroup were positive.

2. The detection rate of *H. pylori* DNA in samples from Study group was significantly higher than in that from Control group ($P < 0.05$). Comparison of T subgroups (group of posterior dorsal surface of toungue) showed significant difference in detection of *H. pylori* ($P < 0.05$), whereas no difference was found between subgroup P (group of dental plaque) of Study group and that of Control group.

3. Statistical significance was observed in subgroup P from Study group and in Control group ($P < 0.01$) when positivity of *H. pylori* in each groups was compared statistically with the presence of regurgitation symptoms.

Based on the data stated above, this study supports the hypothesis that dental plaques may play an important role as a permanent oral resorvoir of *H. pylori* in transmission route. In addition, dental plaque may be a potential source for reinfection following eradication therapy. However, more profound and comprehensive study using larger number of subjects are required and typing of *H. pylori* isolates from both the oral cavity and stomach will play a major role in studing the modes of transmission. Furthermore, the ability to detect *H. pylori* in dental samples may offer the potential role for a non-invasive test for infection.

References

Andersen RN, Ganeshkumar N, Kolenbrander PE: *Helicobacter pylori* adheres selectively to *Fusobacterium* spp. *Oral Microbiol Immunol* 13: 51-54, 1998.

Banatvala N, Lopez CR, Owen R, Abdi Y, Davies G, Hardie J, Feldman R: *Helicobacter pylori* in dental plaque. *Lancet* 341: 380, 1993.

Banatvala N, Lopez CR, Owen R, Abdi Y, Davies G, Hardie J, Feldman R: Use of the polymerase chain reaction to detect *Helicobacter pylori* in the dental plaque of healthy and symptomatic individuals. *Microb Ecol Health Dis* 7: 1-8, 1994.

Bernander S, Dalen J, Gastrin B, Hedenborg L, Lamke LO, Ohrn R: Absence of *Helicobacter pylori* in dental plaques in *Helicobacter pylori* positive dyspeptic patients. Eur *J Clin Microbiol Infect Dis* 12: 282-285, 1993.

Bickley J, Owen RJ, Fraser AG, Pounder RE: Evaluation of the polymerase chain reaction for detecting the urease C gene of *Helicobacter pylori* in gastric biopsy samples and dental plaque. *J Med Microbiol* 39: 338-344, 1993.

Butt AK, Khan AA, Suleman BA, Bedi R: Randomized clinical trial of *Helicobacter pylori* from dental plaque. *Br J Surg*. 88: 206, 2001.

Bode G, Mauch F, Malfertheiner P: The coccoid forms of *Helicobacter pylori*. Criteria for their viability. *Epidemiol Infect* 111: 483-490, 1993.

Chong SK, Lou Q, Fitzgerald JF, Lee CH: Evaluation of 16S rRNA gene PCR with primers Hp1 and Hp2 for detection of *Helicobacter pylori*. *J Clin Microbiol* 34: 2728-2730, 1996.

Desai HG, Gill HH, Shankaran K, Mehta PR, Prabhu SR: Dental plaque: a permanent reservoir of *Helicobacter pylori*? Scand J Gastroenterol 26: 1205-1208, 1991.

Feldman RA, Eccersley AJ, Hardie JM: Transmission of *Helicobacter pylori*. *Curr Opin Gastroenterol* 8: 8-12, 1997.

Forman D, Newell DG, Fullerton F, Yarnell JW, Stacey AR, Wald N, Sitas F: Association between infection with *Helicobacter pylori* and risk of gastric cancer: evidence from a prospective investigation. *BMJ* 302: 1302-1305, 1991.

Fox JG, Perkins S, Yan L, Shen Z, Attardo L, Pappo J: Local immune response in *Helicobacter pylori*-infected cats and identification of *H. pylori* in saliva, gastric fluid and feces. *Immunology* 88: 400-406, 1996.

Fujisawa T, Kumagai T, Akamatsu T, Kiyosawa K, Matsunaga Y: Changes in seroepidemiological pattern of *Helicobacter pylori* and hepatitis A virus over the last 20 years in Japan. *Am J Gastroenterol* 94: 2094-2099, 1999.

Go MF: Review article: natural history and epidemiology of *Helicobacter pylori* infection. *Aliment Pharmacol Ther* 16(Suppl 1): 3-15, 2002.

Goodwin CS, Mendall MM, Northfield TC: *Helicobacter pylori* infection. *Lancet* 349: 265-269, 1997.

Goosen C, Theron J, Ntsala M, Maree FF, Olckers A, Botha SJ, Lastovica AJ, van der Merwe SW: Evaluation of a novel heminested PCR assay based on the phosphoglucosamine mutase gene for detection of *Helicobacter pylori* in saliva and dental plaque. *J Clin Microbiol*. 40: 205-209, 2002.

Graham DY: *Campylobacter pylori* and peptic ulcer disease. *Gasroenterology* 96: 615-625, 1989.

Graham DY, Malaty HM, Evans DG, Evans DJ Jr, Klein PD, Adam E: Epidemiology of *Helicobacter pylori* in an asymptomatic population in the United States. Effect of age, race, and socioeconomic status. *Gastroenterology* 100: 1495-1501, 1991.

Hammar M, Tyszkiewicz T, Wadstrom T, O'Toole PW: Rapid detection of *Helicobacter pylori* in gastric biopsy material by polymerase chain reaction. *J Clin Microbiol* 30: 54-58, 1992.

Handt LK, Fox JG, Dewhirst FE, Fraser GJ, Paster BJ, Yan LL, Rozmiarek H, Rufo R, Stalis IH: *Helicobacter pylori* isolated from the domestic cat: public health implications. *Infect Immun* 62: 2367-2374, 1994.

Hulten K, Han SW, Enroth H, Klein PD, Opekun AR, Gilman RH, Evans DG, Engstrand L, Graham DY, El-Zaatari FA: *Helicobacter pylori* in the drinking water in Peru. *Gastroenterology* 110: 1031-1035, 1996.

Holcombe C, Omotara BA, Eldridge J, Jones DM: *H. pylori*, the most common bacterial infection in Africa: a random serological study. *Am J Gastroenterol* 87: 28-30, 1992.

Khandaker K, Palmer KR, Eastwood MA, Scott AC, Desai M, Owen RJ: DNA fingerprints of *Helicobacter pylori* from mouth and antrum of patients with chronic ulcer dyspepsia. *Lancet* 342: 751, 1993.

Kim N, Lim SH, Lee KH, You JY, Kim JM, Lee NR, Jung HC, Song IS, Kim CY: *Helicobacter pylori* in dental plaque and saliva. *Korean J Intern Med* 15: 187-194, 2000.

Kim HC, Kim JH, Lee SS, Shin KJ, Choi JH, Kim CY: Incidence of *Helicobacter pylori* detected by PCR and its relation to the potential etiology of recurrent apthous ulcerations. *Korean J Oral Med* 27: 401-413, 2002.

Kolenbrander PE: Coaggregation of human oral bacteria: potential role in the accretion of dental plaque. *J Appl Bacteriol* 74(Suppl): 79-86, 1993.

Krajden S, Fuksa M, Anderson J, Kempston J, Boccia A, Petrea C, Babida C, Karmali M, Penner JL: Examination of human stomach biopsies, saliva, and dental plaque for *Campylobacter pylori*. *J Clin Microbiol* 27: 1397-1398, 1989.

Lee A, Fox J, Hazell S: Pathogenicity of *Helicobacter pylori*: a perspective. *Infect Immun* 61: 1601-1610, 1993.

Leung WK, Sung JJ, Ling TK, Siu KL, Cheng AF: Use of chopsticks for eating and *Helicobacter pylori* infection. *Dig Dis Sci* 44: 1173-1176, 1999.

Li C, Musich PR, Ha T, Ferguson DA Jr, Patel NR, Chi DS, Thomas E: High prevalence of *Helicobacter pylori* in saliva demonstrated by a novel PCR assay. *J Clin Pathol* 48: 662-666, 1995.

Li C, Ha T, Ferguson DA Jr, Chi DS, Zhao R, Patel NR, Krishnaswamy G, Thomas E: A newly developed PCR assay of *H. pylori* in gastric biopsy, saliva, and feces. Evidence of high prevalence of *H. pylori* in saliva supports oral transmission. *Dig Dis Sci* 41: 2142-2149, 1996.

Li C, Ha T, Chi DS, Ferguson DA Jr, Jiang C, Laffan JJ, Thomas E: Differentiation of *Helicobacter pylori* strains directly from gastric biopsy specimens by PCR-based restriction fragment length polymorphism analysis without culture. *J Clin Microbiol* 35: 3021-3025, 1997.

Majmudar P, Shah SM, Dhunjibhoy KR, Desai HG: Isolation of *Helicobacter pylori* from dental plaques in healthy volunteers. *Indian J Gastroenterol* 9: 271-372, 1990.

Mapstone NP, Lynch DA, Lewis FA, Axon AT, Tompkins DS, Dixon MF, Quirke P: PCR identification of *Helicobacter pylori* in feces from gastritis patients. *Lancet* 341: 447, 1993a.

Mapstone NP, Lynch DA, Lewis FA, Axon AT, Tompkins DS, Dixon MF, Quirke P: Identification of *Helicobacter pylori* DNA in the mouths and stomachs of patients with gastritis using PCR. *J Clin Pathol* 46: 540-543, 1993b.

Moshkowitz M, Gorea A, Arber N, Konikoff F, Berger S, Gilat T: Morphological transformation of *Helicobacter pylori* during prolonged incubation: association with

decreased acid resistance. *J Clin Pathol* 47: 172-174, 1994.

Nguyen AM, Engstrand L, Genta RM, Graham DY, el-Zaatari FA: Detection of *Helicobacter pylori* in dental plaque by reverse transcription-polymerase chain reaction. *J Clin Microbiol* 31: 783-787, 1993.

Nomura A, Stemmermann GN, Chyou PH, Kato I, Perez GI, Blaser MJ: *Helicobacter pylori* infection and gastric carcinoma among Japanese Americans in Hawaii. *N Engl J Med* 325: 1132-1136, 1991.

Olsson K, Wadstrom T, Tyszkiewicz T: *H. pylori* in dental plaques. *Lancet* 341: 956-957, 1993.

Oshowo A, Tunio M, Gillam D, Botha AJ, Holton J, Boulos P, Hobsley M: Oral colonization is unlikely to play an important role in *Helicobacter pylori* infection. *Br J Surg* 85: 850-852, 1998.

Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, Sibley RK: *Helicobacter pylori* infection and risk of gastric carcinoma. *N Engl J Med* 325: 1127-1131, 1991.

Parsonnet J, Hansen S, Rodriguez L, Gelb AB, Warnke RA, Jellum E, Orentreich N, Vogelman JH, Friedman GD: *Helicobacter pylori* infection and gastric lymphoma. *N Engl J Med* 330: 1267-1271, 1994.

Riggio MP, Lennon A, Wray D: Detection of *Helicobacter pylori* DNA in recurrent aphthous stomatitis tissue by PCR. *J Oral Pathol Med* 29: 507-513, 2000.

Rudi J, Toppe H, Marx N, Zuna I, Theilmann L, Stremmel W, Raedsch R: Risk of infection with *Helicobacter pylori* and hepatitis A virus in different groups of hospital workers. *Am J Gastroenterol* 92: 258-262, 1997.

Shames B, Krajden S, Fuksa M, Babida C, Penner JL: Evidence for the occurrence of the same strain of *Campylobacter pylori* in the stomach and dental plaque. *J*

Clin Microbiol 27:2849-2850, 1989.

Shimada T, Ogura K, Ota S: Detection of *Helicobacter pylori* in gastric biopsies, gastric juice, salia, and feces by polymerase chain reaction. *Gastroenterol* 106: 178, 1994.

Song Q, Haller B, Schmid RM, Adler G, Bode G: *Helicobacter pylori* in dental plaque: a comparison of different PCR primer sets. *Dig Dis Sci* 44: 479-484, 1999.

Song Q, Lange T, Spahr A, Adler G, Bode G: Characteristic distribution pattern of *Helicobacter pylori* in dental plaque and saliva detected with nested PCR. *J Med Microbiol* 49: 349-353, 2000a.

Song Q, Spahr A, Schmid RM, Adler G, Bode G: *Helicobacter pylori* in the oral cavity: high prevalence and great DNA diversity. *Dig Dis Sci* 45: 2162-2167, 2000b.

Stone MA: Transmission of *Helicobacter pylori*. *Postgrad Med J* 75: 198-200, 1999.

Taylor DN, Blaser MJ: The epidemiology of *Helicobacter pylori* infection. *Epidemiol Rev* 13: 42-59, 1991.

Thomas JE, Gibson GR, Darboe MK, Dale A, Weaver LT: Isolation of *Helicobacter pylori* from human faeces. *Lancet* 340: 1194-1195, 1992.

Valentine JL, Arthur RR, Mobley HL, Dick JD: Detection of *Helicobacter pylori* by using the polymerase chain reaction. *J Clin Microbiol* 29: 689-695, 1991.

van Zwet AA, Thijs JC, Kooistra-Smid AM, Schirm J, Snijder JA: Use of PCR with feces for detection of *Helicobacter pylori* infections in patients. *J Clin Microbiol* 32: 1346-1348, 1994.

Von Recklinghausen G, Weischer T, Ansorg R, Mohr C: No cultural detection of *Helicobacter pylori* in dental plaque. Zentralbl Bakteriol 281: 102-106, 1994.

Wahlfors J, Meurman JH, Toskala J, Korhonen A, Alakuijala P, Janatuinen E, Karkainen UM, Nuutinen P, Janne J: Development of a rapid PCR method for identification of *Helicobacter pylori* in dental plaque and gastric biopsy specimens. *Eur J Clin Microbiol Infect Dis* 14: 780-786, 1995.

Warren JR, Marshall B: Unidentified curved bacilli on gastric epithelium in active chronic gastritis. *Lancet* 1: 1273-1275, 1983.

Wotherspoon AC: *Helicobacter pylori* infection and gastric lymphoma. *Br Med Bull* 54: 79-85, 1998.

Young KA, Allaker RP, Hardie JM: Morphological analysis of *Helicobacter pylori* from gastric biopsies and dental plaque by scanning electron microscopy. *Oral Microbiol Immunol* 16: 178-181, 2001.

국 문 요 약

소화불량증 환자의 치태 및 후방 설배면에서의 *Helicobacter pylori*의 검출

(지도 **김 종 열** 교수)

연세대학교 대학원 치의학과

이 상 섭

Helicobacter pylori 균에 의한 감염은 인류의 질병 중에서 흔히 볼 수 있는 감염증으로 알려져 있으며 약 전 세계 인구의 절반에 걸쳐서 만연되고 있는 것으로 보고되고 있다. 하지만 그 감염 경로가 아직 명확히 밝혀지지 않은 상태이며 그 감염 경로에 있어서 치태가 구강내 저장소로서의 역할을 한다는 가설이 제시되고 있는 실정이다. 이에 *H. pylori*의 구강내에서의 발현 빈도를 조사하고 감염 경로에 있어서 치태의 저장소로서의 역할을 규명하여 보고자 연세대학교 세브란스병원 소화기내과에 내원한 환자 62명을 대상으로 치태 및 후방 설배면에서의 *H. pylori*의 유무 및 검출빈도를 PCR을 이용하여 분석, 평가한 후 다음과 같은 결과를 얻었다.

1. *H. pylori* 감염 환자로부터 얻은 표본의 경우 치태에서는 22.2%의 양성 결과를 보였으며 후방 설배면에서는 33.3%의 양성 결과를 보였다. 대조군으로부터 얻은 표본의 경우 치태와 후방 설배면에서 각각 7.7%의 양성 결과를 보였다.

2. *H. pylori* 감염 환자로부터 얻은 표본과 대조군 표본의 *H. pylori* 유전자 발현 빈도의 비교에서 유의차를 보였다 ($P < 0.05$). 후방 설배면 표본간의 발현빈도 비교

에서는 유의차가 관찰이 되었으나 ($P < 0.05$), 치태 표본간의 발현빈도 비교에서는 유의차를 보이지 않았다.

3. 역류 증상의 존재 여부와 각 표본의 *H. pylori* 유전자 발현빈도와의 비교에서 *H. pylori* 감염 환자로부터 얻은 치태 표본과 ($P < 0.01$) 대조군 전체 표본에서 유의차를 보였다 ($P < 0.01$).

이상의 연구를 종합하여 보았을 때 본 연구를 통하여 구강내 저장소로서 치태의 역할에 대한 가설을 성립시킬 수 있는 중요한 근거가 마련되었으며 *H. pylori*에 대한 치료 후에 나타나는 재감염의 요인으로서의 치태의 역할 또한 제시할 수 있었다. 향후 더 많은 표본을 대상으로한 정밀하고 광범위한 연구의 필요성이 있으며 구강내에서 검출된 *H. pylori* 와 위장내에서 검출된 *H. pylori* 의 유전자형을 비교함으로서 그 감염 경로의 명확한 규명이 이루어져야 할 것으로 사료된다. 게다가 치태의 *H. pylori* 검출을 통하여 위장관의 *H. pylori* 감염 여부에 대한 진단에도 어느 정도 유용하게 적용되리라고 기대되는 바이다.

핵심되는 말 : 치태, 설배면, *Helicobacter pylori*, 저장소, PCR