INCIDENCE OF HELICOBACTER PYLORI IN DENTAL PLAQUE OF SAUDI GASTRITIS PATIENTS

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خلفية: لقد تم التعرف على بكتيريا البوات اللولبية فى اللويحات الجرثومية مما أدى الى احتمالية أن تكون مستودع لتلك البكتيريا عند مرضى التهاب وقرح المعدة.
هدف الدراسة: دراسة العلاقة بين وجود بكتيريا البوات اللولبية فى اللويحات الجرثومية والمعدة عند مرضى التهاب المعدة وأيضا لمعرفة تأثير العناية بصحة الفم وحالة اللثة على وجود بكتيريا البوات اللولبية فى اللويحات الجرثومية والمعدة.
طريقة الدراسة: تم أخذ عينتين من كل مشارك فى البحث من اللويحات الجرثومية من الميزاب اللثوى أو من أعمق جيوب لثوية حيث تم حفظ العينة الأولى فى أنبوبة أجار يوريا كريستنسن بها 2% يوريا وتم نقلها بسرعة الى معمل البكتيريا حيث تم تحضينها فى 37درجة مئوية وذلك لبحث امكانية وجود بكتيريا البوات اللولبية فيها بواسطة اختبار البولة السريع حيث تنتج بكتيريا البوات اللولبية كمية كبيرة من انزيم اليوريا الذى يقوم بتكسير اليوريا الى ثانى كربونات الصوديوم ونشادر وحيث أن هذه المواد قاعدية فانها تغير لون محتوى الأنبوبة من اللون الأصفر الى اللون الأحمر خلال مدة نصف ساعة الى ساعة على أكثر تقدير وسرعة التغير فى اللون يعتمد على كمية انزيم اليوريا أو بكتيريا البوات اللولبية. أما العينة الثانية فتم حفظها فى أنبوبة تحتوى على 5% أجار دم الغنم وأجار شيكولاتة ووسط انتقائى لزرع بكتيريا البوات اللولبية.
نتائج الدراسة: أظهر فحص انزيم البولة السريع فى اللويحات الجرثومية أن 89% من المرضى كانت نتائجهم ايجابية. وكان معدل تراكم اللويحات الجرثومية معتدلا في 24% من الحالات ومتوسطا في41% بينما كان شديدا فى 35% من المرضى. أما معدل التهاب اللثة: كان معتدلا فى 17% ومتوسطا فى 48% وشديدا فى 35% من المرضى على التوالى. ومعدل المجتمع لمرضى النسيج الحول سنى: كان معتدلا فى 50% ومتوسطا فى 23% وشديدا فى 27% من المرضى على التوالى. وأظهرت نتائج فحص انزيم البولة السريع فى المعدة ايجابية فى 87% من الحالات. كما كانت كل نتائج مزرعة بكتيريا البوات اللولبية سلبية.
ألخلاصة: ان الكشف عن وجود بكتيريا البوات اللولبية فى اللويحات الجرثومية بالفم يتيح طريقة سهلة لاختبار وجودها بالمعدة ويعطينا تصورعن احتمال اصابة المعدة مرة ثانية عن طريق الفم بعد علاجها. وكذلك يمكن استخدامها للكشف عن مدى تأثير العلاج.

الكلمات المرجعية: بكتيريا البوات اللولبية، اللويحات الجرثومية، التهاب المعدة.

Background: *Helicobacter pylori (H. pylori) was identified in dental plaque, raising the possibility of future gastritis and peptic ulceration.*
Objective: *This trial was to study the association between presence of H. pylori in dental plaque and in the stomachs of patients with gastritis; the effect of oral hygiene and periodontal condition on the stomach.*

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Patients and Methods: *Seventy-five Saudi adult dyspeptic patients, together with 60 healthy persons as control. Two samples of dental plaque were taken from gingival crevice of deepest pocket. One sample was kept in Christensen's urea agar and incubated for H. pylori detection by rapid urease test. The second sample was kept in 5% sheep blood agar , chocolate agar and a selective medium to culture the H. pylori.Gastric urease test was done for the same patients.*

Results: *(1) Plaque urease test results showed 89% positive patients. (2) Dental plaque Index:- Mild dental plaque accumulation in 24%, moderate in 41%, while severe accumulation was in 35% of the patients. (3) Gingival Index: Showed mild, moderate and severe gingivitis in 17%, 48% and 35% of patients, respectively.*

(4) Community periodontal index of treatment needs (CPITN) : Showed gingivitis, mild periodontitis and moderate periodontitis in 50%, 23% and 27% of patients, respectively. (5)Gastric urease results: 87% of patients were positive. (6)All cultured samples results were negative.

Conclusion*: The ability to detect H. pylori in dental plaque samples offers a potential for a noninvasive test for gastric infection and would lend support for oral spread of H. pylori as the principal mode of transmission. However, the presence of H. pylori in dental plaque and in the stomach (in gastritis patients) could permit not only a target for therapeutic procedures but also a monitoring tool for the efficacy of therapy.*

Key Words: *Helicobacter pylori, Dental Plaque, Gastritis, Saudi patients*

INTRODUCTION

Helicobacter pylori (H. pylori), a microaerophilic gram negative spiral bacteria, first isolated from a human gastric biopsy specimen in 1983, is well adapted to life in the hostile acidic environment of the stomach.[1]

The association between H. pylori and the increased risk of duodenal ulceration and antral gastritis has been well established. Hence the importance of preventing reinfection by identifying the potential natural reservoirs of H. pylori.[2] The reservoir of H. pylori and its mode of transmission are unclear, a fecal-oral, oral-oral, and in developing countries a water borne route of infection have been suggested.[3,4] Studies on gastritis reinfection by H. pylori from an oral reservoir has produced conflicting reports as both supragingival and subgingival dental plaque provide an optimal microaerophilic environment required for the survival of H. pylori.[2,5]

H. pylori was identified in dental plaque in 1989. Some researchers have hypothesized that dental plaque might be the reservoir for H. pylori in those patients with associated gastritis and ulceration. As techniques have improved, this bacterium has been frequently isolated in dental plaque, with some reports showing 100% correspondence between H. pylori–containing dental plaque and patients with H. pylori–associated gastritis and oral ulceration.[6]

Various methods have been used to detect H. pylori in dental plaque, suggesting that dental plaque may be responsible for the transmission of the bacteria and possibly serve as a source of reinfection after eradication treatment. H. pylori has also

been isolated from saliva and denture fitting surfaces.[3]

The aims of this study

(1) To study the association between presence of H. pylori in dental plaque, and its presence in the stomach of the patient group. (2) To know the effect of oral hygiene on the presence of H. pylori in dental plaque and stomach. (3) To determine the correlation between presence of H. pylori in dental plaque and periodontal condition.

PATIENTS AND METHODS

PATIENTS

1. Patient group

Seventy-five Saudi adult patients of both gender attended the gastrointestinal (GI) clinic complaining of dyspepsia. They were referred to Dental clinics in Dammam Central Hospital where a complete medical and dental history on oral hygiene was taken including the number of times the teeth are brushed, flossed, the use of miswak (chewing sticks derived from Salvadora Persica). No antibiotics were taken nor was or mouth wash used for at least one month before the study. Clinical oral examination including periodontal charting, dental plaque index, gingival index and CPITN index were determined.

2. Control cases

Sixty apparently healthy adult persons were taken as controls. They followed the same methodology as the patients.

METHODS

1. Dental plaque samples

Two samples of dental plaque were taken from gingival crevice at deepest pocket reading and removed from the clinical site using a sterile universal curette. The tip of the curette was inserted into the depths of crevice/pocket, moved coronally while in contact with the tooth surface, to remove both sub and supragingival plaque . (a) The first dental plaque sample from each patient was immediately placed in a vial of Christensen's urea agar containing 2% urea, kept at room temperature and transported to a microbiology laboratory. The tubes were incubated at 37°C for H. pylori detection by the commercially available rapid urease test, CP-Test (Brocades pharma spa Milano-Italia). H. pylori produces large amounts of urease enzyme, which breaks urea into bicarbonate and ammonia. These substances are strong bases. This reaction is important for the diagnosis of H. pylori . When the urease enzyme of H. pylori is present in an inserted tissue or bacteria culture sample, the degradation of urea causes the pH rise, and the color of reagent vial turn from yellow to red or red-violet. The speed of color change depends on the amount of enzyme or density of germs in the specimen. In most cases the color change occurring between 30 to a maximum of 60 minutes (as recommended by the manufacturer), was considered positive. This test has a sensitivity of 94% and specificity of 100%.[10] To confirm this, when pure proteus (urease producing organism) culture was injected, there was no change in color for up to 24 hours (no further observation was made). (b) The second dental plaque sample from each patient was cultured, by being directly introduced into a sterile tube containing 5% sheep blood agar , chocolate agar and a selective medium, and transported to the microbiology laboratory to be incubated microaerophically at temperature of 37°C for seven days. The plates were examined for characteristic growth, identified as H. pylori by colony morphology, Gram stain and motility.

2. Gastric biopsy

All the patients underwent upper gastro-

intestinal endoscopy and were found to have gastritis. At least two biopsies were taken from antrum and the body of the stomach. Each patient's biopsies were inoculated into a vial of Christensen's urea agar containing 2% urea , kept at room temperature and transported to the microbiology laboratory for rapid urease test as was done with the dental plaque samples.

INDICES USED IN THIS STUDY

1. Dental plaque index (PI)

Dental PI was scored according to Silness and Loe[7] where: 0=no plaque in the gingival area; 1=a film of plaque adhering to the free gingival margin and adjacent area of the tooth (plaque may only be recognized by running a probe across the tooth surface); 2=moderate accumulation of soft deposits within the gingival pocket, on the gingival margin and/or adjacent tooth surface, which can be seen with the naked eye; 3=abundance of soft matter within the gingival pocket and/or on the gingival margin and adjacent tooth surface.

To make statistical analysis applicable, we considered the following: Plaque score 0.1-1.0=mild, 1.1-2.0=moderate and 2.1-3.0=severe.

2. The gingival index

The gingival index was scored according to Löe and Silness[8] where: 0: normal gingival; 1= mild inflammation, slight change in color, slight edema and glazing, and bleeding on probing; 2=moderate inflammation, redness, edema and glazing, and bleeding on probing; 3=severe inflammation, marked redness and edema, ulceration, and tendency for spontaneous bleeding.

Gingivial score from 0.1-1.0 was considered mild, 1.1-2.0 as moderate and 2.1-3.0 as severe.

3. Community periodontal index of treatment needs (CPITN)[9]

Community periodontal index of treatment need were scored as follows: 0=healthy periodontium; 1=bleeding observed, directly or by using mouth mirror, after sensing; 2=calculus felt during probing, but the entire black area of the probe is visible; 3=pocket 4 or 5 mm (gingival margin is situated on black area of probe); 4=pocket ≥ 6 mm (black area of probe not visible).

These data of CPITN were used to compute a single periodontal status index value for each subject and was scored: I=gingivitis, for subjects with at least one CPITN sextant recording of (score 1 or 2); II=mild periodontitis for subjects with at least one CPITN sextant recording of (score 3); III=moderate periodontitis for subjects with at least one CPITN sextant recording of (score 4).

STATISTICAL ANALYSIS

The data were analyzed using SPSS for Windows (Release 8.0) statistical software. Chi-squared test was used, a significance level was set at a p-value of 0.05 to find statistical association: between plaque urease and gastric urease, and between each of dental plaque urease and gastric urease and each of oral hygiene parameters, dental plaque index, gingival and periodontal indices.

RESULTS

Patient group consisted of 75 Saudi gastritis patients, 33(44%) of whom were males and 42(56%) females, aged between 21-76 years old, with a mean age of 37.23 years. Control group consisted of 60 normal adult Saudis, 30 (50%) of whom were males, and 30 (50%) females, aged 21-65 years old, with mean age of 36.37 years old. Results of all cultured samples were negative.

Table 1: Oral hygiene in patients and controls

Frequency	Brushing No. (%)	Flossing No. (%)	Using Miswak No. (%)
No oral hygiene:			
Patients	25 (33.3)	40 (53.3)	41 (54.7)
Controls	7 (11.7)	28 (46.7)	27 (45.0)
Once daily:			
Patients	16 (21.3)	28 (37.3)	10 (13.3)
Controls	16 (26.7)	12 (20.0)	9 (15.0)
Twice daily:			
Patients	23 (30.7)	7 (9.3)	8 (10.7)
Controls	18 (30.0)	16 (26.7)	13 (21.7)
Thrice daily:			
Patients	11 (14.7)	0	16 (21.3)
Controls	19 (31.7)	4 (6.6)	8 (13.3)
Five times daily:			
Patients	0	0	0
Controls	0	0	3 (5.0)

Table 2: *Periodontal indices in patients and controls*

Indices	Mild No. (%)	Moderate No. (%)	Severe No. (%)	Mean	Standard Error
Plaque index:					
Patients	18 (24.0)	31 (41.3)	26 (34.7)	2.11	8.8
Controls	20 (33.3)	25 (41.7)	15 (25.0)	1.92	9.8
Gingival index:					
Patients	13 (17.3)	36 (48.0)	26 (34.7)	2.17	8.1
Controls	17 (28.3)	28 (46.7)	15 (25.0)	1.97	9.5
CPITN index:					
Patients	38 (50.6)	17 (22.7)	20 (26.7)	1.76	9.8
Controls	22 (36.6)	22 (36.7)	16 (26.7)	1.90	0.1

1. Oral hygiene findings (Table 1):

a. Brushing of teeth: Twenty-five patients (33.3%) of the patient group did not use the tooth brush, while 50 patients (66.7%) did. Seven of the controls (11.7%) did not brush their teeth, while 53 (88.3%) did brush their teeth.

b. Flossing of teeth: While 40 patients (53.3%) did not floss their teeth, 35 (46.7%) flossed their teeth regularly. Twenty-eight of the controls (46.7%) did not floss their teeth, but 32(53.3%) flossed their teeth regularly.

c. Use of miswak: In the patient group, 41 (54.7%) did not use Miswak, while 34 (45.3%) did. In the control group, 27 (45.0%) persons did not use Miswak, while 33 (55.0%) did.

2. Findings of periodontal indices
(Table 2)

a. Dental plaque Index: In the patients, mild dental plaque accumulation was detected in 18(24%), moderate in 31

(41.3%), while severe accumulation was in 26(34.7%). On the other hand, in the controls, mild dental plaque accumulation was detected in 20(33.3%), moderate in 25(41.7%), while severe accumulation was in 15(25.0%) persons.

b. Gingival Index: In the patient group, mild gingivitis, in the form of mild inflammation, slight change in color, slight edema and glazing, and bleeding on probing was seen in 13(17.3%) patients. Moderate gingivitis manifested as moderate inflammation, redness, edema and glazing, and bleeding on probing in 36(48.0%) patients, and severe gingivitis seen as severe inflammation, marked redness and edema, ulceration, and tendency for spontaneous bleeding in 26(34.7%) patients. In the controls, mild gingivitis was seen in 17(28.3%), moderate gingivitis in 28(46.7%), and severe gingivitis in 15(25.0%) patients.

c. Community periodontal index of treatment needs (CPITN): When calculus was probed in the patients, gingivitis was seen in 38(50.6%), while mild periodontitis Pocket 4 or 5 mm (gingival margin is situated on black area of probe) was found in 17(22.7%) patients and moderate periodontitis pocket > 6 mm (black area of probe not visible) in 20(26.7%) patients. In the control cases when calculus was probed, gingivitis was seen in 22(36.6%) while mild periodontitis in 22(36.7%) and moderate periodontitis in 16(26.7%) patients.

3. Statistical analysis results (Table 3)
Highly significant association resulted statistically for Plaque Urease / Gastric urease (chi-squared = 42.629), which correspond to (P<0.0001). No statistically significant association was seen between each of plaque urease, and gastric urease, and each of oral hygiene parameters (use of tooth brush, dental floss silk or miswak), or dental plaque, gingival and periodontal indices used in this study.

***Table 3**: Statistical results*

Variables	X^2 test	p-value
Plaque urease/gastric urease	35.747	<0.0001
Plaque urease/plaque index	0.175	NS
Plaque urease/Gingival index	3.232	NS
Plaque urease/CPITN	1.83	NS
Gastric urease/plaque index	0.001	NS
Gastric urease/gingival index	1.736	NS
Gastric urease/CPITN	0.312	NS

NS=Not significant

4. Plaque urease
Plaque urease test results showed that 67 patients (89%) were positive, and 8 (11%) negative, and for the controls, 52 cases (87%) were positive, while 8 (13%) were negative.

5. Gastric urease
Gastric urease results showed that 65 patients (87%) were positive, and 10(13%) were negative.

DISCUSSION

In 1904, Robson and Moynihan suggested that oral sepsis may play a role in the pathogenesis of gastric ulcers.[11] Recently, attention has been focused on the importance of dental plaque in harboring H. pylori, and in having a role in the epidemiology of H. pylori infection. However, published works in this field give conflicting results on the incidence of H. pylori in dental plaque and its significance.[3]

The results of this current limited study have shown that there was a high significant association between dental plaque urease and gastric urease. Thus, it can be suggested that dental plaque may play a role in the development of gastric H. pylori infection.

This is supported by other studies in which rapid urease test for the detection of H. pylori was used. A study done by Gill et al showed that H. pylori was detected in the dental plaque of 82% (18/22) Indian children and 88% (15/17) of their adult family members and concluded that their observations indicate that H. pylori is present in the dental plaque of a majority of children and their family members.[12] Song et al, using rapid urease test for the detection of H. pylori in dental plaque, showed that H. pylori was present in most of the patients' dental plaque.[13]

In other studies using Polymerase chain reaction (PCR) for the detection of H. pylori, Kim et al detected H. pylori in dental plaque in 6.9% of the cases and declared that this rate might have been an underestimation due to the low sensitivity of the PCR method. However, the results that H. pylori was found in dental plaque and saliva, suggest that the oral cavity can be a reservoir of H. pylori.[14] Also, in another study done by Oshowo et al, 7% of the patients tested were positive for H. pylori by PCR in dental plaque. They concluded that the detection of H. pylori in dental plaque could indicate that the oral cavity may act as a reservoir for the organism. Whether H. pylori is a resident or transient oral microorganism is still unclear, although it is more likely to be transient in nature.[15]

Mapstone et al[16] used a nested-PCR test to detect the 16S ribosomal RNA gene of H. pylori in saliva, dental plaque, gastric juice, and gastric biopsy specimens from 13 histologically confirmed, H. pylori gastritis patients. Twelve of these patients had PCR-positive gastric aspirates, and 5(38.5%) had PCR-positive oral specimens. Shimada et a1[17] also reported the presence of H. pylori in the oral cavity of 27 of 100 patients tested. These studies in aggregate suggest that H. pylori is present in dental plaque implicating the oral cavity as an important reservoir for H. pylori.

Khandaker et a1[10] used the ribotyping fingerprinting approach to show that paired strains from the mouth and antrum of their ulcer patients were identical. They hypothesized that dental plaque may be a reservoir for gastric reinfection by H. pylori. However, other studies have showed contradictory results. Von Recklinghausen et al, investigating H. pylori in dental plaque, were unable to cultivate H. pylori from any of the 100 dental plaque specimens from 55 dental surgery patients. Plaque material from 12 patients with moderate and severe gingivitis showed urease activity. These results contradict the hypothesis that dental plaque is a relevant reservoir of viable H. pylori cells, and that non-cultivable forms of H. pylori may survive in dental plaque.[18] Cammarota et al found in a study done by PCR that H. pylori had a low prevalence (3.2%) in dental plaque, with no significant relationship between gastric mucosa and dental plaque colonization.[19]

In a study done by Sahin et al to detect the presence of H. pylori in the dental plaque of 50 dyspeptic Turkish patients by polymerase chain reaction (PCR) none of the dental plaque samples, even in the 23 patients whose gastric biopsy specimens were positive proved positive. They concluded that there was no correlation between dental presentation of the microorganism and H. pylori gastritis.[20]

The different results in the detection of H. pylori in dental plaque by PCR may be due to the low sensitivity of the PCR method used.[14] The reduced sensitivity of detection in clinical biopsy and dental plaque samples could reflect the low number of organisms in the specimens, problems in isolating DNA, or the presence of inhibitors to the PCR reactions. False positive results can also be due to the contamination of

specimens by PCR product. This can be a real problem since the disinfection of equipment will not render the bacterial DNA refractory to amplification with PCR.[21] Hence, inorder to improve the quality of DNA templates, the use of different DNA extraction and the purification methods may be needed to enhance the sensitivity of the assay without compromising the specificity and vice versa.[2] In a comparison with PCR, rapid urease test has a sensitivity of 94% and specificity of 100%.[10]

In another study by Savoldi et al on 80 patients undergoing gastroscopy, an indirect immunoperoxidase test was done, employing a mixture of two monoclonal antibodies against H. pylori. No immunostained bacteria were shown in any of the examined dental plaque samples. It was concluded that H. pylori was not usually present in dental plaque, indicating that oral-oral transmission of the infection could be due to intermittent esophageal reflux only.[22]

The result of the present study showing that H. pylori was not isolated by culture from any of the dental plaque samples, is in agreement with other studies.[2,3,23]

Kamat et al, evaluated Dental plaque obtained from 156 patients and 92 healthy volunteers for the presence of H. pylori using rapid urease test. He found that H. pylori was not isolated by culture from any dental plaque.[23] Another study done by Cheng et al., showed that dental plaque from all dentate participants was negative for H. pylori culture, and concluded that dental plaque could not be implicated as the major reservoir of H. pylori for gastric reinfection.[2] Also, in a study done by Hardo et al, on sixty two patients for the presence of H. pylori in plaque, all of the cultures of dental plaque were negative.[3]

Although cultures are generally considered the gold standard for the detection of H. pylori in clinical samples, they prove to be efficient when biopsy samples from the stomach are used, the current media may be unsatisfactory when specimens harboring abundant flora, such as from the mouth and stools, or when environmental specimens are tested. Poor sensitivity of the culture methods to date probably reflects the low number of organisms or the loss of viability during processing of dental specimens. Theoretically, interference by commensal microorganisms in the mouth may also play a role either by inhibiting growth in culture media or by inducing transformation of the rod-shaped H. pylori to coccoid forms.[24]

The results of this study are in agreement with Hardo et al[3] and Nguyen et al[25] that there was no association of H. pylori in dental plaque with either dental hygiene or periodontal disease. However, Peach et al concluded that positive H. pylori status was significantly associated with increasing number of tooth surfaces with a high plaque score.[26] Also, Avcu et al in a study done in Australian patients with vitamin B12-deficiency anemia, found that H. pylori positivity in dental plaque was correlated with oral hygiene indices scores.[27] The same result was established in another study done by Von Recklinghausen et al.[18]

Dental plaque has been implicated as a possible source of H. pylori in studies that used culture, biochemical, nucleic acid, and immunologic analyses. Variation in the sensitivities of detection by these different reported assays may reflect the methods used, technical difficulties, microbiota complexes, geographic distribution, and host response.[21]

CONCLUSIONS

(1) There is a correlation between the presence of H. pylori in dental plaque and the stomach as shown by plaque and gastric urease test. Failure to eliminate H. pylori

from the mouth could then lead to recolonization of the stomach. (2) The ability to detect H. pylori in the dental samples offers the potential for a noninvasive test for infection and would lend support for the oral spread as the principal mode of transmission. (3) Presence of H. pylori in dental plaque and in the stomach (in gastritis patients) could permit a target for therapeutic procedures as well as a monitoring tool for efficacy of therapy. (4) No significant association was found between plaque urease and gastric urease tests and oral hygiene parameters or the extent of periodontal destruction.

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