DETECTION OF HELICOBACTER PYLORI INFECTION BY RAPID UREASE TEST IN ORAL LESIONS OF PATIENTS WITH RECURRENT APHTHOUS STOMATITIS

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ABSTRACT

Recurrent aphthous stomatitis (RAS) is characterized by ulcers restricted to the oral mucosa. The possibility of bacterial involvement in the progression of aphthae has been suggested due to the histological similarities between peptic ulcers and RAS and due to the identified role of Helicobacter pylori (HP) in peptic ulcer. Although there are many studies showing the relationship between HP and RAS, rapid urease test (RUT) has not been studied in oral ulcers yet. The purpose of this study is to investigate the presence of HP by using RUT in the lesions of RAS. 32 patients with RAS and 21 control subjects were included in the study. HP colonization was investigated by RUT under local anesthesia in oral lesion of RAS patients and oral mucosa in healthy control group. Color change was respectively checked at the 30th, 60th minute and at the 4th hour. If the color of the test is yellow or yellow-green the result was negative but red or pink color was considered as positive. RUT showed HP colonization in the 22 out 32 patients with RAS (68.75%) and in the 5 of the control group (23.80%). The positivity of RUT in patients with RAS were statistically and significantly higher than the control (p<0.05). According to results of our study; HP may play a role in the etiology of RAS, also it is likely that RUT may be rapid and reliable for investigation of HP in RAS lesions.

Key words: Mouth Mucosa, oral ulcer, Helicobacter

INTRODUCTION

Recurrent aphthous stomatitis (RAS) is known that the most common disorder of oral mucosa characterized by minor (< 1 cm in size and several in number), major (> 1 cm
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and usually single), and herpetiform (tiny and numerous) aphthae. The causative agents considered as genetical, immunological, physical, psychological, and microbiological factors\(^1-4\). Nutritional, (especially iron, vitamin B 12, and folic acid deficiencies), emotional, hormonal (menstruation, pregnancy, menarche, and menopause), immunological, and environmental factors and various infections (especially some viruses, trauma), have all been accused in the etiology of RAS\(^5-7\). In 5% of the cases this disease is shown with gastrointestinal disorders\(^8-9\). There is a family history in 1/3 of patients with RAS\(^8\).

The important etiological agents in the pathogenesis of RAS suggested as *Helicobacter pylori* (HP) in recent years. The HP infection is diagnosed by various methods. The invasive methods are based on collection of endoscopic gastric biopsy specimens that are subject to rapid urease test (RUT), staining, culture, histology and molecular diagnostic techniques. The non-invasive methods are urea breath test and serology. RUT is a quick method that has advantages such as easy application, and no need for any special equipment\(^1-4\). Although there are many studies showing the relationship between HP and RAS, RUT test has not been studied in oral ulcers yet. The aim of this study was to determine probable HP infection in oral aphthous samples by RUT in patients with RAS.

**MATERIALS AND METHODS**

This study was approved by the ethics committee of Kahramanmaras Sutcuimam University, School of Medicine and conducted according to the ethical standards of the Helsinki Declaration of 2000. All subjects signed the written informed consent. The data collection has been done from April to September 2011. A total of 40 patients with minor RAS and 21 healthy control group were included in the study. 32 patients and 21 healthy control were completed this study. They had no chronic illness in both patients and control group. A detailed history (family history of the RAS, time of onset, annual recurrence rate, predisposing factors, number of aphthous lesions in last three months, localizations, diameters and improvement time of the lesions) and complete dermatological examination were performed. Pathergy test, ophthalmologic examination, complete blood counting (CBC), routine biochemical tests, serum iron, folic acid, and vitamin B 12 levels were assessed. Hepatitis markers and anti-HIV antibodies were studied. The patient with normal labatory examination were included in this study. Behcet’s Disease was excluded. 8 patient ( 1 Behcet’s disease and 7 laboratory abnormal ) were excluded from this study. The patients were given a questionnaire, which includes questions on the presence of gastrointestinal disorders or a positive history of peptic ulcer. Of the patients referred to RUT, only those who had not consumed any antibiotics in the last month and did not use any steroidal drugs were tested. The active aphthae of these patients were sampled with RUT. The Kits were obtained from the Orbak Firm. %10 lidocain sprey was applied to oral ulcer of the patient and buccal mucosa of the control. The samples were obtained by scraping the lesion of RAS in patients and buccal mucosa of the controls with a scalpel blade, by the dermatologist. Then, oral scraping samples were inserted into the RUT. RUT was storage at +4 °C until usage. The scraping samples from ulcer and mucosa were stored at the room temperature then placed into kits. Color change were checked respectively at the 30\(^{th}\), 60\(^{th}\) minute and at the 4\(^{th}\) hour (as
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recommended by the company). If the color of the test is yellow or yellow-green the result is negative but red or pink color is considered as a positive test result.

Statistical analysis was carried out using SPSS 17.0 for Windows statistical software. The conformability of the quantitative data to the normal distribution was examined using Kolmogorov–Smirnov test. As the age and gender were distributed normally, the descriptive statistics were presented as mean±standard deviation (SD). Mann–Whitney U test was used in terms of groups. Furthermore, the descriptive statistics were also shown as median, minimum and maximum. The statistical difference was taken as probability value < 0.05.

**RESULTS**

A total of 32 patients with minor RAS 18 female (56.25%) and 14 male (43.75%), with a mean age of 37.30±13.57 years (range; 17-73, median; 34 years) were enrolled in the study. The control group (n=21) included 9 males (42.85%) and 12 females (57.14%), with a mean age of 34.09±10.05 years (range; 19-54, median; 34 years) The mean duration of RAS was 70.78±2.79 months (range; 12-240 months). There were no significant differences in age and male/female ratio between the both groups (p>0.05). 20 of patient group and 10 of the control group had at least one kind of gastric complaints. RUT was positive in 22 patients (68.75%) and in 5 of control group (23.80%). RUT positivity in RAS patients was significantly higher than control groups (p<0.05). (Table 1). Laboratory finding were normal in the RAS and the control group.

Table 1. Socio-demographic features and RUT results of the patient and control groups.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age years mean±SD(median)</th>
<th>RUT (positive)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patient (n:32)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female 18</td>
<td>37.30±13.57 (34)</td>
<td>22(68.75%)*</td>
</tr>
<tr>
<td>Male 14</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Control(n:21)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female 12</td>
<td>34.09±10.05 (34)</td>
<td>5(23.80%)*</td>
</tr>
<tr>
<td>Male 9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*RUT positivity in RAS patients was significantly higher than control group.

**DISCUSSION**

We have found that RUT is positive in 68.25% of the patients and 23.81% of the control group. We think that HP may play a role in the etiology of RAS, also it is likely that RUT may be rapid and reliable for investigation of HP in RAS lesions.

HP is Gram (−) spiral bacterium that has been established to play an important role in the development of the ulcerative diseases of the digestive systems. This microorganism is identified in dental plaque and saliva also as well as in oral ulcers. Elsheikh et al. and Gebara et al. isolated, HP from saliva, gingiva, gingival plaques, and lymphoid tissues of the patients with oral pathologies. The results of studies with the relation of HP and RAS are controversial. In some studies, it has been said that HP had no role in the pathogenesis of RAS using PCR and serology by Mansour-Ghanaei et al., Iamaroon.
et al., Shimoyama et al. and Fritscher et al. 1,2,18-19. Whereas a positive correlation has been observed using PCR and serology in others 4,8,20-21. But there has been no clear conclusion in some studies 22. This controversy is probably due to methodological and technical differences. Many invasive and noninvasive technique are used to diagnose HP infections. Bacteriologic culture and histological staining of tissue are the conventional methods used to detect HP. Although HP culture can be carried out in most laboratories, it has some restrictions, including the long delay (4 days) in obtaining results, the low sensitivity of the culture isolation, and the need for strict transport conditions because of the fastidious nature of the bacterium 23. On the other hand, histological analysis is time consuming and requires an expert pathologist. The modified Giemsa and Silverstains, have good specificity and sensitivity, but false-positive readings can occur in the case of abundant mucus and contaminating organisms resembling HP are present 23-24. Serologic tests identify circulating IgG or IgA antibodies. However, despite the cost effectiveness, the diagnostic significance of ELISA test is restricted because it cannot differentiate between current and past infections 24. Likewise, in the urea breath test, a patient drinks an oral solutions containing urea labeled with carbon 13 or 14. HP bacteria metabolizes the urea to produce carbon that was absorbed into the bloodstream. The carbon travels through the bloodstream into the lungs. When the lungs exhale the carbon, measurement of carbon 13 or 14 determines the presence or absence of HP infection. However false-positive results due to the presence of other enteric bacteria remain as the main disadvantage of the urea breath test 13.

The PCR is one of the mostly used molecular techniques for detecting specific pathogens. Compared with various methods as histological and cultural, PCR offers most sensitivity in detection of HP. It was used for the detection of HP in gastric tissue samples, where it provides a rapid, sensitive, and specific test result and is particularly useful for a gastroenterologist who does not have access to local routine laboratory facilities 13. RUT is one of the invasive tests. The principle is based on abundant urease enzyme produced by HP hydrolyses urea to ammonia. The phenol red indicator is used in detection of consequent rise in the pH of the medium. Several modifications of Christensen’s original urea medium have been developed obtaining quick results and improving sensitivity and specificity. Various RUTs are available commercially like CLO test, HP test and Pylori-Tek test providing comparable results with high sensitivity and specificity. However, simpler and cheaper in-house urease test medium giving similar results can be made in most of the dermatology departments as done by us. The sensitivity of test depends on pH of the medium, concentration of urea, indicator used and temperature of incubation. Microscopy may be falsely negative in the case quite low number of organisms. RUT yields positive result as HP gets sufficient time to multiply in the urea broth 13. Berry et al and Goh et al suggest that RUT is the gold standard test for HP infection in gastric biopsy material 25,26. It can be used as a rapid diagnostic technique, also results are obtained within 90 minutes. Thus RUT, a simple and cheap test, is quite beneficial in tracing HP infection and thereby helps us in treating the patients on time 25-26. Unver et al. 27 reported 58% positive HP in pharyngeal tissue. Yilmaz et al. 28 reported that they did not find any positivity with RUT in materials of tonsillectomy but they reported positivity in 50% with HP antigen in the stool and 56% HP IgG in the blood in the same patients. Similar to Skinner et al. 29 reported positivity 28% with HP IgG in blood but did not find...
any positivity with RUT in tonsillectomy samples in 50 patients. Bitar et al.\textsuperscript{30} reported 78% positive in adenoidectomy samples in 25 patients, but did not find any positivity with PCR. Dagtekin et al.\textsuperscript{31} reported RUT is not reliable for investigation of HP in their study other than the stomach. We have found that RUT is positive in 68.25% of the patients and 23.81% of the control group. To the best of our knowledge, RUT was not studied in the oral lesions of RAS patients. Therefore, comparisons could not be made. As a result, HP determination at gastric biopsy specimens with RUT in RAS patients may not always be possible. In this case, RUT may be reliable and rapid for investigation of HP in oral lesion with RAS patients. Also, RUT positivity may lead the way for use in antibiotic for RAS patients. Of course, this is just our hypothesis and our study here is the first. Broader patient sized studies are needed on this issue.

When interpreting our results, it is important to note some of the limitations of our study. Firstly we had limited number of patients. Secondly, another diagnostic method showing the existence of HP as histopathology, PCR, antibody was not used. Therefore, false negative and the positivity of the test had not been possible to evaluate exactly. The major and herpetiform RAS was not included in the study.

REFERENCES

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