Agreement Rate of Rapid Urease Test, Conventional PCR, and Scorpion Real-Time PCR in Detecting *Helicobacter Pylori* from Tonsillar Samples of Patients with Chronic Tonsillitis

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ABSTRACT

Background: *Helicobacter pylori* is capable of inducing systemic inflammatory reactions through immunological processes. There are several methods to identify the presence of *H. pylori* in clinical samples including rapid urease test (RUT), conventional polymerase chain reaction (PCR), and the Scorpion real-time PCR. **Aim:** The aim of the present study is to compare the agreement rate of these tests in identifying *H. pylori* in tonsillar biopsy specimens collected from patients with chronic tonsillitis. **Materials and Methods:** A total of 103 tonsil biopsy samples from patients with clinical signs of chronic tonsillitis were examined with RUT, PCR, and Scorpion real-time PCR. The degree of agreement between the three tests was later calculated. **Results:** There was a poor degree of agreement between RUT and PCR and also RUT and Scorpion real-time PCR (Kappa=0.269 and 0.249, respectively). In contrast with RUT, there was a strong degree of agreement between PCR and Scorpion real-time PCR (Kappa=0.970). **Conclusion:** The presence of a strong agreement between the Scorpion real-time PCR and PCR as well as its technical advantage over the conventional PCR assay, made the Scorpion real-time PCR an appropriate laboratory test to investigate the presence of *H. pylori* in tonsillar biopsy specimens in patients suffering from chronic tonsillitis.

Key words: Degree of agreement, *H. pylori*, PCR, Tonsillitis

INTRODUCTION

Pharyngitis and tonsillitis are among the most common infections of the upper respiratory tract. There are many studies confirming the presence of *Helicobacter pylori* in the saliva,[1,2] oral cavity,[3] nose, and mucus of the sinuses,[4-6] middle ear,[7] dental plaques,[8,9] tonsils, and adenoid glands.[9,10] *H. pylori* has the potential to induce systemic inflammatory reactions through the immunological processes leading to the development of pharyngitis.[11] Moreover, Zhang *et al.*,[2] in their study have clearly shown the absence of *H. pylori* in the pharyngeal region of healthy people and concluded that chronic pharyngitis might be associated with *H. pylori* infection. Bacterial infections of the tonsils and adenoids are treated with various antibiotics and surgical removal is considered in situations resistant to medical therapy or in frequently recurrent infections. As *H. pylori* does not respond to routine antibacterial drugs that are used for chronic tonsillitis, diagnosis of *H. pylori* as a causative agent of chronic tonsillitis may play a role in the decision-making for the treatment of patients.

The *Helicobacter* is a small coma-shaped, gram-negative, and microaerophilic organism, with a number of species differentiated based on the genetic analysis of 16S rRNA, cellular fatty acids, and the presence of polar flagella.[12] This bacterium produces great amounts of urease and has a widespread distribution in the world. Previous studies have shown that *H. pylori* has the potential to invade and colonize the stomach, gastric juice, oral mucus, and saliva of patients.[1,3-5,7,13] The transmission route of this organism is not well known, but the presence of this microorganism in drinking water is reported through epidemiological studies.[14,15] There are several methods to identify the presence of *H. pylori* in clinical samples and the most important ones are the rapid urease test (RUT), conventional polymerase chain reaction (PCR), and real-time PCR.

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The studies carried out concerning the involvement of *H. pylori* in tonsillitis are highly controversial, as in some studies the reports are indicative of the successful isolation of *H. pylori* from tonsillar specimens in the culture media,[16,17] while in other investigations, attempts to isolate this organism from similar samples were unsuccessful.[18] In several studies, it has been mentioned that the application of the RUT is an appropriate method to detect the presence of *H. pylori*. However, the sensitivity and specificity of this test in identifying this organism, in samples obtained from the tonsil and throat, is reported to be insufficient.[19,20] Likewise, the PCR technique is described by some authors to be a sensitive test in detecting *H. pylori*,[21,22] whereas, in other reports PCR is demonstrated to fail identifying this microorganism in similar clinical specimens.[23]

Considering the above-mentioned controversies and with none of these methods being regarded as the gold standard in detecting *H. pylori* in tonsillar samples, and also taking into account the bulk of publications regarding the high sensitivity and specificity of both real-time PCR and Scorpion real-time PCR in the detection of various infectious agents,[24-26] the current study has attempted to investigate the degree of agreement between these three tests in identifying *H. pylori* in the tonsillar biopsy of patients with chronic tonsillitis.

MATERIALS AND METHODS

This was a comparative study, in which 103 tonsil biopsy samples obtained from chronic tonsillitis patients, with chronic inflammation of the throat, cervical dysphagia, and permanent rough voice, for greater than three months. These patients had undergone the adenotonsillectomy operation at the Rhinolaryngoscopy Ward of Qods Hospital (Qazvin University of Medical Sciences) during 2010.

The RUT was immediately performed in all samples according to the manufacturer's instruction (RUT Chem. Enzyme, Tehran, Iran) and the biopsies kept in paraffin blocks until use.

Following the collection of all clinical specimens, Deoxyribonucleic acid (DNA) extraction was carried out using a commercial extraction kit (Roche, Germany). The amount of extracted DNA from 20 mg of each sample was 200±10 ng.

Rapid urease test

The RUT was performed[19] using a commercial kit obtained from RPT Chem. Enzyme (Tehran, Iran). For positive control of the RUT, PCR, and Real-time PCR, a standard strain of *H. pylori*, ATCC 26695, was used, and distilled water was employed as the negative control.

Conventional PCR

DNA amplification was performed using a *H. pylori* detection kit (Cinnagen, Iran) in a final volume of 30 µl, with the cycling program of the PCR instrument (Applied Biosystem, USA), which consisted of a cycle of 72ºC/30 seconds, 50ºC/20 seconds, and 94ºC/45 seconds, followed by 30 cycles of 72ºC/30 seconds, 50ºC/20 seconds, and 94ºC/20 seconds.[19] A volume of 10 µl of the amplified sample was directly electrophoresed on 1.5% agarose gel. The presence of a 492-bp DNA fragment was indicative of a positive reaction targeting the Ure C gene.

Scorpion real-time PCR

PCR was performed at a final reaction volume of 25 µl, containing 5 µl of the biopsy-extracted DNA or 1 µl of extracted DNA from the culture of a standard strain of *H. pylori* ATCC 26695, as a positive control,[27] with few necessary modifications made to be used in the ABI Prism 7500 Sequence Detection System (Applied Biosystem, USA) as follows: denaturation step (95ºC/15 seconds) followed by 50 cycles of reactions containing denaturation (95ºC/15 seconds), annealing (55ºC/34 seconds), and extension (72ºC/20 seconds), targeting the peptidyltransferase of 23SrRNA.[27] Reading of the fluorescent probe was performed during the annealing step. The accuracy of extraction was confirmed by the appearance of the associated signal in the VIC channel, which amplified a B-globulin gene.[27]

Statistical analysis

The Kappa coefficient (K) was calculated to assess the degree of agreement between the RUT, conventional PCR with UreC target, and the Scorpion real-time PCR with 23S rRNA, by SPSS 14. The Kappa coefficient was defined as a measure of agreement.

RESULTS

Detection of *H. pylori* by the rapid urease test

Out of 103 samples from patients with clinical symptoms of chronic tonsillitis, 50 samples showed positive RUT, whereas, 53 samples demonstrated negative results.

Detection of *H. pylori* by conventional PCR

When conventional PCR was used to detect *H. pylori* in

biopsy specimens of a total of 103 samples, 20 produced positive PCR results, and 83 were negative for *H. pylori*. As the present study was performed on paraffinized biopsies, there was no possibility of testing the samples by culture and recommended routine diagnostic tests.

Detection of *H. pylori* by scorpion real-time PCR

Initially, the Scorpion real-time PCR was performed on *H. pylori* ATCC 26695. Of the total of 103 samples, 21 biopsies were found to be positive for *H. pylori* by the Scorpion real-time PCR, whereas, 82 samples showed negative results. Among samples with a positive Scorpion real-time PCR test, 17 samples were also RUT positive and four samples had RUT negative test results. The results of RUT, conventional PCR, and Scorpion real-time PCR are demonstrated in Tables 1–3.

Our results showed a weak degree of agreement between the RUT, PCR, and Scorpion real-time PCR with Kappa values of 0.269 and 0.249, respectively [Tables 1 and 2]. A strong degree of agreement between PCR and the Scorpion real-time PCR with a Kappa value of 0.970 was demonstrated [Table 3]. When the degree of agreement between PCR and Scorpion real-time PCR in samples with negative RUT results was calculated, the agreement was complete (Kappa=1), but when the results on the degree of agreement between PCR and Scorpion real-time PCR in samples with positive RUT was compared, the agreement was demonstrated to be strong, but not complete (Kappa=0.955).

Table 1: Agreement rate between rapid urease test and conventional PCR results

Conventional PCR / Rapid urease test	+	–
Positive	16	34
Negative	4	49

Kappa=0.249; Asymp. Std. Error=0.078; Approx. T=3.1369; Approx. Sig.=0.002; PCR: Polymerase chain reaction

Table 2: Agreement rate between rapid urease test and Scorpion real-time PCR results

Scorpion real-time PCR / Rapid urease test	+	–
Positive	17	33
Negative	4	49

Kappa=0.269; Asymp. Std. Error=0.079; Approx. T=3.330; Approx. Sig=0.001; PCR: Polymerase chain reaction

Table 3: Agreement rate between conventional PCR and Scorpion real-time PCR

Scorpion real-time PCR / Conventional PCR results	+	–
Positive	20	0
Negative	1	82

Kappa=0.269; Asymp. Std Error=0.079; Approx T=3.330; Approx. Sig=0.00;1 PCR: Polymerase chain reaction

DISCUSSION

Our findings showed that there was a strong agreement between the Scorpion real-time PCR and PCR, in the detection of *H. pylori* DNA in the tonsillar specimens of chronic tonsillitis patients, but its technical advantage over the conventional PCR assay, made the Scorpion real-time PCR an appropriate laboratory test to investigate the presence of *H. pylori* in the tonsillar biopsy specimens of patients suffering with chronic tonsillitis.

The mucous membrane of the upper digestive-respiratory tract is an extension of the digestive tract. There are several studies confirming the colonization by *H. pylori* of the adjacent sites of the intestinal cavity, with or without stomach bacteria. Among these anatomic sites, are the oral cavity, dental plaque, saliva, and adenotonsillar tissue.[28] As this organism is independent of the stomach, capable of living in the oral cavity, including beyond the digestive tract, it may be assumed that *H. pylori* can colonize in areas within the larynx and pharynx.

Considering the ability of urease production by other bacterial residents of the mouth, throat, and tonsils, the RUT for the detection of *H. pylori* is of low specificity. The results reported by Jabbari Moghaddam *et al.*, confirm the ability of urease production by other bacterial populations in such anatomical areas.[29] Moreover, the ureC gene target in conventional PCR, which identifies the presence of the urease enzyme is also of inadequate specificity.[30] Above all, the negative culture result seen in the *H. pylori*-infected tonsillar samples,[31] is regarded as another hampering factor in the application of RUT and conventional PCR as the standard tests. Hence, it seems that the Scorpion real-time PCR technique, in which the PCR and probing are performed simultaneously, through targeting the peptidyltransferase of the 23SrRNA gene, and thus avoiding repeated manipulations while accelerating the performance of the test, will be an ideal method for diagnosis of *H. pylori,* in the biopsy samples.[24-26] However, the correlation between the Scorpion real-time PCR and the two more convenient-to-perform techniques of conventional PCR and RUT has to be investigated.

The findings of the present study revealed the presence of a poor agreement between the RUT and conventional PCR, and also RUT and the Scorpion real-time PCR. These results are consistent with those reported by Jabbari-Moghaddam *et al.*,[29] in which a low efficacy of

RUT in detecting *H. pylori* in tonsillar biopsy specimens was obtained. In addition, our data regarding the existence of a strong agreement between the conventional PCR and Scorpion real-time PCR are also in harmony with the results described by Burucoa *et al.*,[28] Liu *et al.*,[26] Ben Mansour *et al.*,[25] and Qiu *et al.*,[24] all indicating a high potential of Scorpion real-time PCR in identifying *H. pylori* in the biopsy specimens.

CONCLUSION

Considering the presence of a strong agreement between the results obtained by the Scorpion real-time PCR and conventional PCR, and also the advantage of simultaneous probing and identification, the application of Scorpion real-time PCR as an appropriate method to detect *H. pylori* in the biopsy samples of patients with chronic tonsillitis is suggested.

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