The effect of warming specimens of rapid urease test on its diagnostic accuracy

El efecto del calentamiento de las muestras de la prueba de ureasa rápida en su precisión diagnóstica

Jafar Nasiri[1], https://orcid.org/0000-0003-3624-6849, Arshya Allahdin[2], https://orcid.org/0000-0002-7370-9227, Reza Imani[3], https://orcid.org/0000-0001-8149-9349, Soleiman Kheiri4, https://orcid.org/0000-0001-8285-8681, Abolfazl Khoshdel 5https://orcid.org/0000-0002-7023-5951*

[1]Gastroenterologist, Assistant Professor, Department of Internal Medicine, Faculty of Medicine, Shaharekord University of Medical Sciences, Shahrekord, Iran

[2]Assistant of Pathology, Department of Pathology, Faculty of Medicine, Jundishapour Medical Sciences University, Ahwaz, Iran

[3]Associate Professor, Department of Infectious Diseases, Shahrekord University of Medical Sciences, Shahrekord, Iran

[4]Associate Professor, Department of Epidemiology and Biostatistics, Faculty of Health, Shahrekord University of Medical Sciences, Shahrekord, Iran

5Associate Professor, Department of Pediatrics, Faculty of Medicine, Shahrekord University of Medical Sciences, Shahrekord, Iran

**corresponding author: Jafar Nasiri, Gastroenterologist, Assistant Professor, Department of Internal Medicine, Faculty of Medicine, Shaharekord University of Medical Sciences, hahrekord, Iran, Postal address: Department of Internal Medicine, Shaharekord University of Medical Sciences, Shahrekord, Iran, Postal code: 88157-13471, Tel: +9838-32223350. Fax: +9838-32243715. Email: jhnasiri@yahoo.com*

Abstract

Introduction: Helicobacter pylori (H. pylori) is a prevalent cause of dyspepsia, peptic ulcer and gastric cancer in developing countries. Among various diagnostic methods, rapid urease test (RUT) is the ideal test for its diagnosis in patients undergoing endoscopy. Studies have identified the factors causing false negative and positive results. One of these factors is proposed to be the temperature of keeping specimens, but the results have been controversial. We aimed to compare the diagnostic accuracy of RUT specimens warmed at 37°C or kept at room temperature (22-25°C) in different time intervals until 24 hours.

Methods: 100 patients with dyspepsia who were indicated for endoscopic examination were selected based on convenience sampling method from patients referring to Endoscopy center of Hajar Hospital during August-September 2006. After recording the demographic and medical history of patients, three biopsy specimens were taken from one portion of the antrum; the first two samples were placed in home-made RUT solution and the third biopsy was placed in formalin solution; one tube was placed in incubator with 37°C and the other at room temperature (22-25°C). Positivity of RUT test at different times was compared to histopathological examination.

Results: Infection with Helicobacter pylori was confirmed by histological examination in 66% of patients. After 24 hours, sensitivity of incubated RUT was 71% at 37°C, and 68% at room temperature (P=0.85). Specificity at 37°C was 59% and at room temperature was 76% (P=0.19). Median time to positive tests was 2 hours at 37°C and 3 hours at room temperature, while RUT became positive faster at 37°C (P=0.553).

Conclusion: The non-significant difference between groups revealed that warming could not improve the diagnostic accuracy of RUT test, thus, the standard RUT method is recommended.

Keywords: Helicobacter pylori; Diagnostic Tests, Routine; Endoscopy, Gastrointestinal; Urease.

Resumen

Introducción: Helicobacter pylori (H. Pylori) es una causa frecuente de dispepsia, úlcera péptica y cáncer gástrico en países en desarrollo. Entre los diversos métodos de diagnóstico, la prueba rápida de ureasa (RUT) es la prueba ideal para su diagnóstico en pacientes sometidos a endoscopia. Los estudios han identificado los factores que causan resultados falsos negativos y positivos. Se propone que uno de estos factores es la temperatura para conservar las muestras, pero los resultados han sido controvertidos. El objetivo fue comparar la precisión diagnóstica de las muestras RUT calentadas a 37°C o mantenidas a temperatura ambiente (22-25°C) en diferentes intervalos de tiempo hasta 24 horas.

Métodos: se seleccionaron 100 pacientes con dispepsia que estaban indicados para un examen endoscópico según el método de muestreo de conveniencia de los pacientes que se referían al centro de Endoscopia del Hospital Hajar entre agosto y septiembre de 2006. Después de registrar la historia demográfica y médica de los pacientes, se tomaron tres muestras de biopsia. Una porción del antro; las dos primeras muestras se colocaron en una solución de RUT hecha en casa y la tercera biopsia se colocó en una solución de formalina; un tubo se colocó en una incubadora con 37°C y el otro a temperatura ambiente (22-25°C). La positividad de la prueba de RUT en diferentes momentos se comparó con el examen histopatológico.

Resultados: la infección con Helicobacter pylori se confirmó mediante examen histológico en el 66% de los pacientes. Después de 24 horas, la sensibilidad del RUT incubado fue del 71% a 37°C y del 68% a temperatura ambiente (P=0,85). La especificidad a 37°C fue del 59% y la temperatura ambiente fue del 76% (P=0.19). El tiempo medio para las pruebas positivas fue de 2 horas a 37°C y 3 horas a temperatura ambiente, mientras que el RUT se convirtió en positivo más rápido a 37°C (P=0.553).

Conclusión: la diferencia no significativa entre los grupos reveló que el calentamiento no podría mejorar la precisión diagnóstica de la prueba RUT, por lo que se recomienda el método RUT estándar.

Palabras clave: Helicobacter pylori; Pruebas de diagnóstico, de rutina; Endoscopia gastrointestinal; Urease.

Introduction

Helicobacter pylori (H. pylori) is a gram-negative bacterium that affects a large number of patients worldwide, with a significantly high prevalence in developing countries and low socio-economic communities, although its prevalence differs by age, sex, race, and other factors[1]. H. pylori infection is reported to have a high prevalence in Iran with a prevalence of more than 80% reported in many cities[2].

H. pylori infection is of great importance, as it leads to chronic gastritis, peptic ulcer disease, gastric cancer and mucosa-associated lymphoid-tissue (MALT) lymphoma[3]. The age-standardized risk of gastric cancer varies from 10.2 to 50 per 100,000 person-year in different cities[2]. Therefore, H. pylori infection requires early and accurate diagnosis[4].

Although international guidelines suggest endoscopy after empirical acid suppression, it is suggested that this approach, named as "test and treat", is not beneficial in countries with high prevalence of H. pylori infection[5], like Iran, and endoscopic-based methods are recommended for eradication of H. pylori infection[4].

Although none of the various methods suggested have been proven as the gold standard, studies have suggested various invasive and noninvasive methods for diagnosis of H. pylori infection: non-invasive methods include serology, immunoblot, stool antigen test, and urea breath test (UBT), and invasive tests include, histologic examination polymerase chain reaction (PCR) for antibiotic-resistant types of H. pylori, and rapid urease test (RUT)[6].

RUT and histopathologic tests are suggested as excellent accurate diagnostic tests[7]. RUT is a rapid, cheap, and simple test that can indirectly assess the presence of bacteria through urease existence[8]. It requires about 10^5 H. pylori bacteria to change color in an agar-based like CLO test (campylobacter–like organism) and has high sensitivity varying from 80-100% and specificity of 97-100%, based on the methods and techniques used[8]. Obtaining two samples from antrum can result in a sensitivity of 95% and specificity of 100%[9]. In addition, although most specimens turn positive in the first 120-180 minutes, investigation of specimens for 24 hours and no longer results in most accurate detection of H. Pylori bacteria[8].

Studies have investigated other factors that play a role in diagnostic accuracy of RUT; some have suggested that using proton pump inhibitors (PPI) by the patient reduces its sensitivity and increases false negative results[10]. Moreover, other bacterial species may rarely cause false positive[11]. Some studies have also advised that the temperature of reaction might influence the diagnostic value of RUT; some researchers suggested that using a warmer with 38°C had a 20% higher diagnostic ability in the first 30 minutes, while overall results did not differ significantly[12], while other researchers proposed that warming the specimens at 37°C increased the sensitivity of RUT until 2 hours and had an earlier mean time to positive test[13].

Due to the significance of RUT in diagnosis of H. pylori, beside the controversial results regarding the effect of temperature on diagnostic accuracy of RUT, the present study aimed to evaluate the diagnostic accuracy of RUT, in specimens kept at 37°C, compare with specimens kept at room temperature in different intervals until 24 hours.

Materials and methods

Study design: In this prospective study, 100 patients (including 50 males and 50 females) with dyspepsia who were indicated for endoscopic evaluation were selected based on convenience sampling method among patients referring to Endoscopy center of Hajar Hospital from July to September 2006. Sample size was calculated to estimate the sensitivity and specificity of RUT with the 10% precision and 95% confidence, using the overall sensitivity/specificity equal to 50%.

Patients with dyspepsia aged over 14 years who were referred to Endoscopy center of Hajar Hospital from July to September 2006 were included into the study. Any patient with recent gastrointestinal bleeding, and urgent cases undergoing endoscopy was excluded from the study.

Demographic characteristics, past medical and therapeutic history of patients were recorded. Then, three biopsy specimens were taken from the one section of antrum. To prevent formalin contamination, the first two samples were placed in similar RUT and the third biopsy was placed in formalin solution.

Home-made RUT solution was prepared each day by mixing 0.02 g red phenol, 1 g potassium dihydrogen orthophosphate, and 10 g urea (Merk Company, Germany) with de-ionized distilled water; the solution was kept out of fridge 15-20 minutes before each test.

The exact time of sampling, and patients' name were documented on each tube and the tubes were immediately sent to laboratory of Hajar Hospital; one tube was kept in incubator with 37°C and the other at room temperature (22-25°C). The results of the RUT test were evaluated by one laboratory expert each 15 minutes for the first hour, then each hour for the next four hours, and then at 8th, 12th, and 24th hour after sampling; the temperature conditions were maintained during the evaluation period. The results were reported positive, when the color changed to purple and was considered negative when no color change occurred; the time when the test became positive was recorded by the researcher.

Samples were sent to laboratory for pathologic examination at the end of each day. Specimens were stained by Giemsa, and hematoxylin-eosin and were evaluated by a pathologist. The specimens were fixed in formalin 10%; after processing two slides (stained with hematoxylin-eosin and Giemsa) were prepared and the presence of bacteria in any slide was considered positive pathology result and lack of bacteria in both stains were considered as negative pathology result.

Ethical considerations:The protocol of the study was approved by the Ethic Committee of Shahrekord University of Medical Sciences. The design and objectives of the study were explained to all participants and written informed consent was obtained from those who were willing to participate in the study and they were ensured that their data will be kept confidential and analyzed anonymously.

Statistical analysis; Results were presented as mean ± standard deviation (SD) for quantitative variables and were summarized by frequency (percentage) for categorical variables. Continuous variables were compared using T test or Mann-Whitney U test, whenever the data did not appear to have normal distribution or when the assumption of equal variances was violated across the study groups. Categorical variables were, on the other hand, compared using chi-square test and Fisher's exact test. For the statistical analysis, the statistical software SPSS version 21.0 for windows (SPSS Inc., Chicago, IL) was used. P values of 0.05 or less were considered statistically significant.

Results

Mean and SD of age of participants was 47.6±18.9 (range: 14-83) years. Half of patients were male and half were female; 82% of males and 78% of females were married, but patients' sex and marital status had no association with the pathologic results (P=0.832, and 0.774, respectively). Mean years of history of gastrointestinal disorder was 3.8±5.3 (range 1-30) years with 80% giving a history of using medication for their gastrointestinal disorder. The most used medication was omeprazole (48 patients), H_2-blocker (40 patients); 8 patients used both medications, and 8 used bismuth with omeprazole or ranitidine. There was no association between anti-acid therapy and pathologic result (P=0.102).

Infection with Helicobacter pylori was confirmed by histologic examination in 66% patients, among whom 18 patients had negative RUT results (false negative RUT) and 34 patients had positive RUT at both temperatures tested, while 13 cases had positive results only at 37oC, and one case at room temperature (P<0.001) (Tables 1 and 2). Negative histologic and RUT results were reported in 34 patients, among whom 20 had negative RUT results at both temperatures, and 4 patients had positive RUT (false negative RUT). In 10 patients, RUT was only positive at 37oC (P=0.022) (Tables 1 and 2).

Table 1: Comparison of RUT results with pathologic results in both groups

	Positive pathology	Negative athology	Total	p-value
Positive at 37°C	47	14	61	0.004
Negative at 37°C	19	20	39	
Total	66	34	100	
Positive at room temperature	45	8	53	<0.001
Negative at room temperature	21	26	47	
Total	66	34	100	

Table 2: Comparison of RUT results between the two temperatures tested

	Sensitivity (%)			Specificity (%)			Positivity (%)	
	At 37°C	At room temperature	P-value	At 37°C	At room temperature	P-value	At 37°C	At room temperature
After 15 minutes	23	20	0.83	100	100	1.00	22.7	19.69
After 30 minutes	26	21	0.68	100	100	1.00	3.03	1.5
After 45 minutes	32	21	0.85	100	100	1.00	7.5	0
1st hour	42	30	0.205	100	100	1.00	9.09	9.09
2nd hour	50	38	0.22	94	100	0.49	7.5	7.5
3rd hour	52	41	0.29	94	97	1.00	1.5	3.03
4th hour	53	45	0.49	91	97	0.61	1.5	4.5
8th hour	59	52	0.48	91	94	1.00	6.06	6.06
12th hour	62	53	0.38	79	88	0.51	3.03	1.5
24th hour	71	68	0.85	59	76	0.19	9.09	15.05

Among all positive RUT results, 26 cases were positive at both temperatures and 23 cases got positive faster at room temperature; the difference was more than one hour in 19 cases and less than one hour in 4 cases; also, 3 cases became positive later at 37°C.

Mean response time at 37°C was 7.69±9.41 hours (median: 2 hours), which was 8.55±9.95 (median: 3 hours) at room temperature (P=0.553).

The endoscopic results revealed gastric ulcer in 8 patients (7 positive patients), duodenal ulcer in 7 patients (5 positive patients), antral nodularity in 50 cases (30 positive patients), and other pathologies in 22 patients (14 positive patients); 12 patients had antral nodularity with duodenal ulcer (10 positive patients), and one patient had simultaneous gastric and duodenal ulcer. The endoscopic presentation was not associated with pathologic results (P=0.352).

The sensitivity of RUT increased gradually and was higher at all intervals at 37°C than room temperature, but was not statistically significant; the sensitivity at 24th hour was 71% at 37°C and at room temperature was 68% (P=0.85) (Table 3). The RUT specificity was greater than 90% until 8th hour in both temperatures, but then decreased and was higher at room temperature after the first hour (Table 3). At the 24th hour, specificity of the test was 59% at 37°C and 76% at room temperature, but was not statistically different (P=0.19).

Table 3: Comparison of sensitivity and specificity between two temperatures

	Positive at room temperature	Negative at room temperature	Total	P-value
Positive at 37°C	52	1	53	<0.001
Negative at 37°C	9	38	47	
Total	61	39	100	

Conclusion: The results of the present study revealed a prevalence of 66% for H. Pylori infection in patients with dyspepsia. But the higher sensitivity of RUT at 37°C was not statistically different from room temperature and the specificity did not differ statistically, as well[25,26].

Although some endoscopists keep the RUT specimens in their pocket to keep them warm, few studies have evaluated the effect of temperature on diagnostic accuracy of RUT[8]. Yousfi and colleagues suggested that using a warmer with 38°C for RUT specimens resulted in a 20% higher diagnostic accuracy in the first 30 minutes, while overall results did not differ significantly[12]. Also, in the present study, the diagnostic accuracy of warmed specimens were higher in first hours, although the difference was not statistically significant, which could be a result of sampling error or other confounding factors, such as high prevalence of using PPIs by patients in the present study, as studies have indicated that anti-acid medications reduce the sensitivity of RUT[10,14]. Laine and coworkers have obtained four antral specimens from 200 patients; two for histologic examination, and two for CLO test, one of which were incubated at 37°C and the other at room temperature[13]. The prevalence of H. pylori infection in their study was 61%, which is close to the prevalence reported in the present study. Laine and colleagues reported that the specimens kept at 37°C had a significantly earlier median time to a positive test and greater sensitivity until 2 hours, while specifities were similar[13]. The differences between the results of the studies might be due to the different RUT techniques, as we used home-made solution, while Laine and colleagues have used CLO solution for RUT. Moreover, there are many factors affecting sensitivity of the RUT, including using anti-acid medications[10,14], presence of blood in samples[15], the duration of the gastric ulcer, and the underlying gastric disease that may reduce the bacterial load[8,16], which might have resulted in negative results in the present study.

In the present study, the rate of sensitivity and specificity of both groups were higher than some previous studies[17,18], while lower than some others[7,19], although some studies have adjusted the cut-off reported by the manufacturer[7]. The differences in diagnostic accuracy rates can be justified by the differences in the methods and techniques used.

The prevalence of positive H. pylori infection was similar to some studies[13], whereas most studies in developed countries have reported a prevalence of less than 40% and developing countries have reported a prevalence of 80-90%[1,20]. Also, Iranian studies have determined various prevalence rates in different cities of Iran[2]. In Ardebil, Shiraz and Babol, a prevalence of 80% has been reported[21,22], while it was reported about 60-70% in Tehran, Nahavand, and Rafsanjan[23,24]. As indicated by studies, the prevalence depend of various factors such as age, gender, race, and other factors[1].

Conclusions

One of the strengths of the present study included reporting sensitivity and specificity in several intervals that can give researchers a wider spectrum through analysis of RUT. In addition, all the results of RUT and histologies were reviewed by one expert, thus increasing the reliability of the results. On the other hand, the present study had also some limitations, including the confounding factors in the results, such as duration of the disease, the underlying gastric disease, high prevalence of using PPIs by patients, and other factors. Thus, it is suggested that future studies evaluate the effect of warming samples in different RUT methods, considering the confounders, especially duration of the disease and anti-acid therapy, in order to be able to evaluate the pure effect of temperature on diagnostic accuracy of RUT. In conclusion, the results of the present study indicated no significant difference in overall

diagnostic accuracy of RUT by warming the specimens.

Conflict of interest: all authors declare that they have no conflict of interest.

Funding: None

References

1. Perez-Perez GI, Rothenbacher D, Brenner H. Epidemiology of Helicobacter pylori infection. Helicobacter. 2004;9:1-6.
2. Malekzadeh R, Derakhshan M, Malekzadeh Z. Gastric cancer in Iran: epidemiology and risk factors. Arch Iran Med. 2009;12:576-83.
3. Malaty HM. Epidemiology of Helicobacter pylori infection. Best Practice & Research Clinical Gastroenterology. 2007;21:205-14.
4. Malfertheiner P, Mégraud F, O'Morain C, Hungin A, Jones R, Axon A, et al. Current concepts in the management of Helicobacter pylori infection—The Maastricht 2-2000 Consensus Report. Alimentary pharmacology & therapeutics. 2002;16:167-80.
5. Ford AC, Moayyedi P. Current guidelines for dyspepsia management. Digestive diseases. 2008;26:225-30.
6. Monteiro L, De Mascarel A, Sarrasqueta AM, Bergey B, Barberis C, Talby P, et al. Diagnosis of Helicobacter pylori infection: noninvasive methods compared to invasive methods and evaluation of two new tests. The American journal of gastroenterology. 2001;96:353-8.
7. Calvet X, Sánchez-Delgado J, Montserrat A, Lario S, Ramírez-Lázaro MJ, Quesada M, et al. Accuracy of diagnostic tests for Helicobacter pylori: a reappraisal. Clinical Infectious Diseases. 2009;48:1385-91.
8. Uotani T, Graham DY. Diagnosis of Helicobacter pylori using the rapid urease test. Annals of translational medicine. 2014;3.
9. Woo JS, El-Zimaity HM, Genta RM, Yousfi MM, Graham DY. The best gastric site for obtaining a positive rapid urease test. Helicobacter. 1996;1:256-9.
10. Yakoob J, Jafri W, Abid S, Jafri N, Abbas Z, Hamid S, et al. Role of rapid urease test and histopathology in the diagnosis of Helicobacter pylori infection in a developing country. BMC gastroenterology. 2005;5:1.
11. Osaki T, Mabe K, Hanawa T, Kamiya S. Urease-positive bacteria in the stomach induce a false-positive reaction in a urea breath test for diagnosis of Helicobacter pylori infection. Journal of medical microbiology. 2008;57:814-9.
12. Yousfi MM, El-Zimaity HM, Cole RA, Genta RM, Graham DY. Does using a warmer influence the results of rapid urease testing for Helicobacter pylori? Gastrointestinal endoscopy. 1996;43:260-1.
13. Laine L, Estrada R, Lewin DN, Cohen H. The influence of warming on rapid urease test results: a prospective evaluation. Gastrointestinal endoscopy. 1996;44:429-32.
14. Chen T, Meng X, Zhang H, Tsang RW, Tsang T-K. Comparing multiplex PCR and rapid urease test in the detection of H. pylori in patients on proton pump inhibitors. Gastroenterology research and practice. 2012;2012.
15. Houghton J, Ramamoorthy R, Pandya H, Dhirmalani R, Kim KH. Human plasma is directly bacteriocidal against Helicobacter pylori in vitro, potentially explaining the decreased detection of Helicobacter pylori during acute upper GI bleeding. Gastrointestinal endoscopy. 2002;55:11-6.
16. Tian X-Y, Zhu H, Zhao J, She Q, Zhang G-X. Diagnostic performance of urea breath test, rapid urea test, and histology for Helicobacter pylori infection in patients with partial gastrectomy: a meta-analysis. Journal of clinical gastroenterology. 2012;46:285-92.
17. Miftahussurur M, Yamaoka Y. Diagnostic Methods of Helicobacter pylori Infection for Epidemiological Studies: Critical Importance of Indirect Test Validation. BioMed research international. 2016;2016.
18. Myint T, Shiota S, Vilaichone RK, Ni N, Aye TT, Matsuda M, et al. Prevalence of Helicobacter pylori infection and atrophic gastritis in patients with dyspeptic symptoms in Myanmar. World J Gastroenterol. 2015;21:620-7.
19. Roy AD, Deuri S, Dutta UC. The diagnostic accuracy of rapid urease biopsy test compared to histopathology in implementing "test and treat" policy for Helicobacter pylori. International Journal of Applied and Basic Medical Research. 2016;6:18.
20. Baydin A, Duran L, Şengüldür E, Katı C, Tomak L. Evaluation of Usefulness of Cardiopulmonary Resuscitation Education on Public Health Physicians. J Clin Exp Invest. 2017;8(4):110-3. https://doi.org/10.5799/jcei.382406
21. Sotoudeh M, Derakhshan MH, Abedi-Ardakani B, Nouraie M, Yazdanbod A, Tavangar SM, et al. Critical role of Helicobacter pylori in the pattern of gastritis and carditis in residents of an area with high prevalence of gastric cardia cancer. Digestive diseases and sciences. 2008;53:27-33.
22. Kalkan, Havva, et al. "Thoracic application of multi-detector CT: A pictorial essay." European Journal of General Medicine14.4 (2017).
23. Nouraie M, Latifi-Navid S, Rezvan H, Radmard AR, Maghsudlu M, Zaer-Rezaii H, et al. Childhood hygienic practice and family education status determine the prevalence of Helicobacter pylori infection in Iran. Helicobacter. 2009;14:40-6.
24. Alborzi A, Soltani J, Pourabbas B, Oboodi B, Haghighat M, Hayati M, et al. Prevalence of Helicobacter pylori infection in children (south of Iran). Diagnostic microbiology and infectious disease. 2006;54:259-61.
25. Bazrafkan L, Shokrpour N, Yousefi A, Yamani N. Management of stress and anxiety among phd students during thesis writing: a qualitative study. The health care manager. 2016 Jul 1;35(3):231-40
26. Sagheb MM, Amini M, Saber M, Moghadami M, Nabiei P, Khalili R, Rezaee R, Bazrafcan L, Hayat AA. Teaching Evidence-Based Medicine (EBM) to Undergraduate Medical Students through Flipped Classroom Approach. Shiraz E-Medical Journal. 2017