UREA BREATH TEST AND SPECIFIC IgA IN HELICOBACTER PYLORI INFECTION

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ABSTRACT

• ***Objective:*** *H. pylori is a bacterium which causes infection in the stomach and duodenum and infects more than half the world's population. Various methods are used in the diagnosis of infections. Among these methods is the urea breath test (UBT) which is a noninvasive and reliable test, and has a sensitivity and specificity value of over 95%. In addition, specific serum, IgG, IgM and IgA antibodies are investigated in different studies. The purpose of our study is to analyze specific IgA in dyspeptic patients who have been diagnosed with H. pylori using the urea breath test, and to evaluate the diagnostic value of the specific IgA in different gastric disorders.*

• ***Material and Method:*** *Hundred and fifty four patients, who were admitted to Gaziantep University Faculty of Medicine Gastroenterology polyclinic were included in the study. These patients had dyspeptic complaints and were pre-diagnosed with non ulcerative dyspepsia, erosive gastritis, gastritis, gastro esophageal reflux, gastric ulcers, duodenal ulcers, gastric carcinoma after an upper endoscopy. A urea-breath test was carried out for H. pylori diagnosis. Blood samples were taken simultaneously. H. pylori specific IgA antibodies were analyzed with a commercial ELISA kit.*

• ***Results:*** *Hp specific IgA was positive in 83 of the 96 UBT positive patients (86%); and in 33 of 58 UBT negative patients (57%). In the study, a correlation was detected in the patient groups between UBT and Hp specific IgA results in H. pylori positive cases.*

• ***Conclusion:*** *Although searching for IgA antibodies in serum is a simple, easy and repeatable method among serologic methods, it was thought that the use of these tests would be more appropriate in seroprevalence studies rather than for diagnostic purposes.*

• ***Key Words:*** *Helicobacter pylori, urea breath test, IgA ELISA* ***Nobel Med 2010; 6(3): 46-50***

HELİCOBACTER PYLORİ İNFEKSİYONUNDA ÜRE NEFES TESTİ VE SPESİFİK IgA

ÖZET

• **Amaç:** *H. pylori*, mide ve duedonumun birçok hastalığından sorumlu ve dünyadaki insanların yarısından fazlasını infekte eden bir bakteridir. İnfeksiyonun tanısında çeşitli metodlar kullanılmaktadır. Bunlardan üre nefes testi (ÜNT), duyarlılığı ve özgüllüğü %95'in üzerinde bulunan noninvaziv ve güvenilir bir testtir. Bunun yanında, çeşitli çalışmalarda spesifik serum IgG, IgM ve IgA antikorları araştırılmaktadır.

Çalışmanın amacı, üre nefes testi ile *H. pylori* tanısı alan dispeptik hastalarda, spesifik IgA araştırmak ve farklı hasta gruplarında iki testi karşılaştırmaktır.

• **Materyal ve Metod:**: Gaziantep Üniversitesi Tıp Fakültesi Gastroenteroloji polikliniğine dispeptik şikayetle başvuran ve üst endoskopiyle; non ülser dispepsi, eroziv gastrit, gastrit, gastro özefajial reflü, gastrik ülser, duodenal ülser, gastrik karsinoma ön tanısı konulan 154 hasta çalışmaya alındı. *H. pylori* tanısı için üre-nefes testi yapıldı. Aynı zamanda hastaların kan örnekleri alınarak ticari ELISA kiti ile Hp spesifik IgA antikorları araştırıldı.

• **Bulgular:** ÜNT pozitif 96 hastanın 83'ünde (%86), UNT negatif 58 hastanın ise 33'ünde (%57) Hp spesifik IgA pozitif saptandı. Çalışmada hasta gruplarında *H. pylori* pozitif olgularda UNT ve Hp spesifik IgA sonuçları arasında korelasyon saptandı.

• **Sonuç:** Serolojik yöntemlerden IgA antikorlarının serumda aranması basit, kolay ve tekrarlanabilir bir yöntem olmasına rağmen bu testlerin, tanı amacıyla değilde seroprevalans çalışmalarında kullanılmasının daha uygun olacağı düşünüldü.

• **Anahtar Kelimeler:** Helicobacter pylori, üre nefes testi, IgA ELISA **Nobel Med 2010; 6(3): 46-50**

INTRODUCTION

Helicobacter pylori is one of the most common infectious agents in the world; as a result half of the world's population is infected by this organism.[1] Starting from birth, this bacterium colonizes in the stomach by oral transmission, and causes various diseases such as dyspepsia, erosive gastritis, peptic ulcer, duodenal ulcer and stomach carcinoma.[2] This infection is present in 95% of patients who have duodenal ulcers, 70-80% of patients with stomach ulcers, and in approximately 50% of non ulcer dyspeptic patients. In prospective and retrospective studies, *H. pylori* was found in 90% of gastric cancer and gastric lymphoma cases.[3,4] From studies conducted in Turkey, *H. pylori* prevalence varies between 80-90% in patients with duodenal ulcers; 60-75% with gastric ulcers; 40-100% with gastritis; 30-90% with stomach cancer and 40-80% with non-ulcer dyspepsia.[5]

H. pylori is a gram-negative, unipolar, spiral or curved, active, microaerofilic bacterium. It generally survives in the stomach and needs urease enzymes in order to colonize in a mucus layer.[6,7] In the diagnosis of *H. pylori*, invasive methods (culture, histopathologic analysis, rapid urease test, molecular diagnostic methods) and non invasive methods (Urea breath test, serologic tests, stool antigen tests, stool polymerase chain reaction) are used.[1] The most important component of a systemic antibody response is IgG1. IgA is part of a local response in the stomach mucosa and it remains in the blood for long periods. While IgA antibodies prevent *H. pylori* from sticking on to the stomach epithelia, IgG usually serves to complement fixation and activation.[8]

In this study, *H. pylori* was analyzed in patients who were admitted to the hospital with upper gastrointestinal complaints using UBT and ELISA noninvasive methods. According to UBT results, specific IgA in serum was evaluated by observing the infected groups.

MATERIAL and METHOD

A total of 154 patients (81 females, 73 males), who were admitted to Gaziantep University Faculty of Medicine, Şahinbey Research Hospital, Gastroenterology department between the dates of 10-05-2006 to 29-12-2006 with upper gastrointestinal complaints were included in the study. The patients were required to complete the pre-determined questionnaire form which contained questions about gender, age, cigarette smoking, stomach symptoms, existence of the same disease, drug use (antibiotic, PPI) etc. After the patients were examined and, their upper endoscopy was carried out and their pre-diagnosis was made. During the pre-diagnosis of 154 patients, 14 of them were found to have non ulcer dyspepsia; 103 of them had gastritis; 11 of them had gastroesophageal reflux; 8 of them had stomach ulcers; 13 of them had duodenum ulcer and 5 of them had gastric carcinoma. Immediately after a GIS endoscopy, the patients were administered a urea breath test. In addition, 7 cc blood was injected from the peripheric venous vein of each patient. The collected serum was frozen and kept at -80 °C until it was to be used.→

Table 1: UBT and IgA status

Urea breath test (UBT)	Helicobacter pylori IgA					
	Positivity		Negativity		Total	
	n	%	n	%	n	%
Positivity	83	86	13	14	96	100
Negativity	33	57	25	43	58	100
Total	116	75	48	25	154	100

Table 2: UBT and IgA status in diseas groups

Upper GIT diseases	UBT positivity				UBT negativity				Total	
	Hp IgA positivity		Hp IgA negativity		Hp IgA positivity		Hp IgA negativity			
	n	%	n	%	n	%	n	%	n	%
Gastroesophageal reflux diseases	3	27	0	0	6	55	2	18	11	100
Nonulcus dyspepsi	7	50	1	7	0	0	6	43	14	100
Gastritis	58	56	9	9	21	20	15	15	103	100
Peptic ulcer (Stomach)	5	63	2	25	1	12	0	0	8	100
Duodenal ulcer	8	62	1	8	1	8	3	22	13	100
Adeno Ca	2	67	0	0	0	0	1	33	3	100
MALT	0	0	0	0	1	50	1	50	2	100
Total	83	54	13	8	30	20	28	18	154	100

Urea Breath Test is based on the principle of the production of urease enzyme by *H. pylori* and detection of the emerging urea. After the patient orally ingests radioactive marked (^{13}C) urea, an ammoniac and carbon dioxide marked gas is released from the urea in which the bacterium has been decomposed by urease enzyme, and this gas is discharged through the patient's breath.[9,10] For this test, the patients took five millicurie (185 kBq) of urea tablets marked with ^{14}C with 50 mL of water, after a minimum of 4 hours of hunger. After waiting ten minutes to allow for the carbon dioxide gas to enter circulation and to be discharged by respiration, the patients were asked to give breath samples. For this reason, the patients exhaled into a specially prepared breath card. To confirm whether the expiration was carried out accurately, as the criterion for this test, the orange color on the indicator membrade had to turn yellow. When the membrane turned yellow, expiration was successfully completed. The results were calculated automatically by a Heliprobe Type device (Noster System AB, Sweden) and were given in DPM ("Disintegrations per Minute") units. They were categorized in three groups; 0 for uninfected patients; 1 for patients at the limit; and 2 for infected patients.

H. pylori specific IgA was analyzed in the 154 patients who were included in the study, using the ELISA method (Trinity Biotech USA). For this reason, serum obtained from blood samples which were taken from the peripheric vein and stored at -80°C was thawed at room temperature. After the serums were vortexed, according to recommendations made by the manufacturing company, the ELISA method was applied. For these applications, an automatic washing device (LP35 Plate Washer, Pasteur Diagnostics, France) and an optical reading device (LP-400, Pasteur Diagnostics, Austria) were used. According to suggestions made by the manufacturing country, the cut-off calibrator value was calculated, and patient optical density results were assessed.

Statistical Assessment: data was shown as sample number (n) and percentage (%). The statistical research was carried out in Windows-based SPSS 10.0 program, using χ^2 test in dependent groups of McNemar. Values statistically smaller than 0.05 were accepted as significant.

RESULTS

With an upper GIS endoscopy 154 patients who were pre-diagnosed with non ulcer dyspepsia, erosive gastritis, gastritis, gastro-esophageal reflux, gastric ulcer, duodenal ulcer, and gastric carcinoma were administered urea breath test.

Of the 154 patients, who participated in the study, 81 (53%) were females and 73 (47%) were males. Of the 96 patients, 49 (51%) UBT positive were females, and 47 (49%) were males. Of the 116 patients, 56 (48%) of the specific IgA positive were females; 60 (52%) were males. No significant difference was found between *H. pylori* positivity and gender (p>0.05).

In 96 out of the 154 patients who took part in the study, UBT positive was detected and 83 (86%) of them had a positive result for Hp specific IgA. When comparing both tests, a statistical correlation was detected (p: 0.00003) (Table 1).

In the study, gastritis was detected in the majority of patients who came to the clinic with gastrointestinal symptoms (67%). In some of the gastritis patients UBT positivity was the case, IgA negativity was observed. In stomach ulcers, duodenum ulcers and stomach adeno Ca disease groups, UBT and IgA positivity was found to have a higher ratio when compared to other disease groups. In gastro esophageal reflux and gastritis patients, while UBT was negative, high ratios of IgA positivity were detected (Table 2). When IgA optical density (OD) values are compared according to disease groups, no significant difference was detected (p>0.05) (Fig).→

DISCUSSION

Helicobacter pylori is the leading infection amongst widespread infections in the world. Epidemiologic studies have indicated that *H. pylori* prevalence is parallel to the development levels of states and societies.[11] While in developed countries yearly *H. pylori* incidence is 0.5-1%, in developing countries this ratio is around 10%.[12] *H. pylori* prevalence is quite high in Turkey for which about 80% of the population is infected by the age of 20. In various seroprevalence studies which are conducted in different regions, ratios were found to be between 60-80%.[13-15]

There are various methods in the diagnosis of *H. pylori*. Among these is the urea breath test which is a reliable and highly sensitive method and is considered to be a gold standard among non-invasive methods.[16,17] The sensitivity of UBT is around 90-100%, and its accuracy is around 89-100%.[18]

While analyzing a patient's history for the *H. pylori* infection, searching for an anti *H. pylori* IgG, IgM or IgA antibodies is one of the most beneficial, cheap and rapid tests available. Serologic methods are especially beneficial in epidemiological studies and the follow-up treatment. While in successfully treated cases antibody response decreases, it increases again in recurring cases. Sensitivity of the majority of the ELISA tests on the market, reach to almost 100% and their specificity reach to about 95%.[19] In gastric *H. pylori* infections, specific antibodies can be detected by serologic tests in serum, saliva, urine and gastric secretions of the patient. There are studies which suggest that though the emerging antibodies do not provide spontaneous eradication, they are an important criterion in diagnosis.[20] The detection of serum IgA is especially helpful in a suspected infection.[8,21] In studies that are carried out about the *H. pylori* infection, it is reported that the infection occurrs almost at the same ratio in males and females, and there are no significant differences.[8,22-26] In contrast, there are studies which suggest that the *H. pylori* infection is significantly high among females. (60.6%).[27]

In studies by Lazebnik et al. it was suggested that a high level of antibodies an indication of the *H. pylori* infection, and the fact that these antibodies decrease is an indication that treatment gives a response.[28] In a study by Bhat N et al. on 89 patients who underwent an endoscopy, they found *H. pylori* by using a histological technique in 62 people; and the PCR and culture were positive. In this study they found that patients who have a positive result for IgG , serum IgA and stomach liquid IgA of *H. pylori* were significantly higher than people with *H. pylori* negative.[29] In a study by Dope MP et al. on 86 dyspeptic patients, it was found that

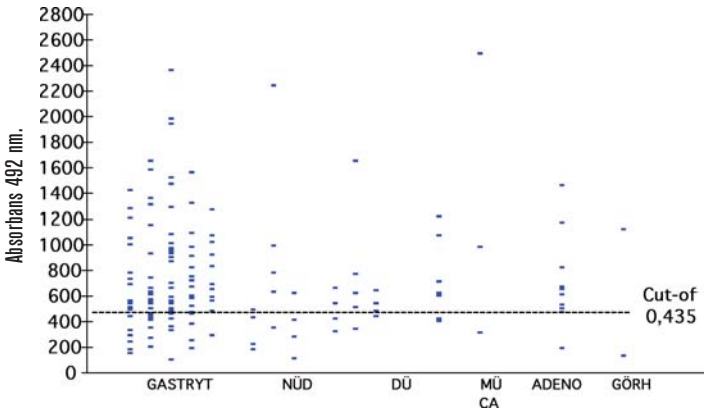

Figure. IgA OD status in diseas groups.

43 (50%) of them were infected with *H. pylori*. In 22 (51%) of *H. pylori* infected patients they found IgG and IgA as positive.[30]

In this study, in 116 (75%) of the 154 patients, Hp lgA was found to be positive. This ratio has correlations with other studies. No statistical relationship was found between disease groups and the optical density values. In our study 86% correlation was detected between UBT and the IgA ELISA test. In addition, 43% of UBT negative cases, IgA negative was found. When UBT was taken as a basis in acute infections, the sensitivity of the IgA ELISA test was detected to be around 86% and specificity was detected to be around 43%. However, in 33 cases (57%), although the UBT result was negative, antibody positivity was observed. In these cases it is thought that there might have been a past or a treated infection. In the study, this was detected especially in gastro esophageal reflux (n:6) and gastritis (n:21). It was found that as the patients in this disease group had more complaints, they received medical treatment. It was observed that the received antibiotic and proton pump inhibitor (PPI) treatment affected UBT results. So we didn't include these patients who used this medicine for the last 2 weeks into the study. Although an antibody response for an H. pylori infection may continue or is eradicated, UBT negativity can still be achieved. In addition, in a UBT application, insufficiency of a patient's adaptation can also affect test results. In the study, despite UBT positive results in 13 (8%) cases, IgA ELISA negativity was detected. In these cases, the infection may have recently started. Furhermore, immune deficiency can occur due to age or malignity. In our stydy, although we didn't investigate the total IgA, there were no circumstances which could have been caused by immune deficiency.

CONCLUSION

In the study, a correlation was found between UBT and IgA results in the disease groups. Where *H.pylori* occurs→

in UBT acute findings, it gives a positive result. Because of a gold standard test, it is influenced by many factors. When a diagnosis is made with this method, it should be determined whether or not the individual has used antibiotics and/or PPI due to an infection. Searching for antibodies with the ELISA, among serologic methods, is a simple and valid method. In conclusion, we suggest that it would be more convenient for this test to be used in seroprevalance studies rather than for diagnostic purposes.

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- *This study was approved by the Ethics Committee of Gaziantep University.*