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IS THE IMMUNOCROMATOGRAPHIC FECAL ANTIGEN TEST EFFECTIVE FOR PRIMARY DIAGNOSIS OF *HELICOBACTER PYLORI* INFECTION IN DYSPEPTIC PATIENTS?

Magali **DALLA NORA**[1], Rosmari **HÖRNER**[2], Diego Michelon **De CARLI**[3], Marta Pires da **ROCHA**[4], Amanda Faria de **ARAUJO**[5] and Renato Borges **FAGUNDES**[1,5]

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ABSTRACT - ***Background*** - The diagnosis of *H. pylori* infection can be performed by non-invasive and invasive methods. The identification through a fecal antigen test is a non-invasive, simple, and relatively inexpensive test. ***Objective*** - To determine the diagnostic performance of fecal antigen test in the identification of *H. pylori* infection. ***Methods*** - *H. pylori* antigens were identified in the stools of dyspeptic patients undergoing upper gastrointestinal endoscopy. For the identification of *H. pylori* antigen, we use ImmunoCard STAT! HpSA with immunochromatography technique. Histopathology plus urease test were the gold standard. ***Results*** - We studied 163 patients, 51% male, mean age of 56.7± 8.5years. *H. pylori* infection was present in 49%. Fecal test presented: sensitivity 67.5% (CI95% 60.6-72.9); specificity 85.5% (CI95% 78.9-90.7); positive predictive value 81.8% (CI95% 73.4-88.4) and negative predictive value 73,2% (CI95% 67.5-77.6); Positive likelihood ratio was 4.7 (CI95% 2.9-7.9) and Negative Likelihood Ratio 0.4 (CI95% 0.3-0.5). The prevalence odds ratio for a positive test was 12.3 (CI95% 5.7-26.3). The index kappa between FAT and histology/urease test was 0.53 (CI95% 0.39-0.64). ***Conclusion*** - Immunochromatographic FAT is less expensive than the other methods and readily accepted by the patients but its diagnostic performance does not recommend its use in the primary diagnosis, when the patient may have an active infection.

HEADINGS - *Helicobacter* infections, diagnosis. Immunochromatography. Antibodies. Endoscopy. Digestive system diagnostic techniques.

INTRODUCTION

Invasive and non-invasive methods can be used for the diagnosis of *Helicobacter pylori* (*H. pylori*) infection. The invasive methods include histopathology, urease test, and culture. All of them require gastric biopsy through upper GI endoscopy. Non-invasive methods include urea breath test, serologic tests, and fecal antigen test. These tests do not need endoscopy and biopsy. Due to the character invasive of endoscopy and biopsy, there is a growing interest in the use of non-invasive methods[1]. Among the non-invasive methods, urea breath test is the most reliable due to its high sensitivity and specificity. However, this test has limitations, such as costly laboratory equipment and the possibility of false-negative results. On the other hand, serologic tests detect markers of exposure to *H. pylori* but do not indicate the existence of active infection, because the antibodies against *H. pylori* may remain present long after its eradication[8].

Fecal antigen test (FAT) identifies antigens of *H. pylori* through monoclonal and polyclonal antibodies in a fecal sample. In 1997 appeared the first FAT with polyclonal antibodies. Currently, most FAT uses monoclonal antibodies. The test can be performed by immuno-enzymatic assay or by immunochromatography assay[8]. FAT conducted by immunochromatography is fast and cost effective, because it does not require additional equipment making possible its use in primary care settings[20]. Due to these characteristics, the use of FAT by immunochromatography in developing countries is attractive. To the best of our knowledge, only nine studies[2,3,11,13,14,16-18,21] in the past ten years evaluated FAT to diagnosis *H. pylori* infection in Brazil, most of them used immuno-enzymatic assay and only one used immunochromatography[18].

The aim of this study was to identify *H. pylori* infection in dyspeptic patients and determine the diagnostic performance (accuracy, sensitivity, specificity,

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[1] Programa de Pós-Graduação em Ciências da Saúde, Universidade Federal de Santa Maria, RS, Brasil; [2] Laboratório de Microbiologia, Departamento de Análises Clínicas e Toxicológicas, Faculdade de Farmácia, Universidade Federal de Santa Maria, RS, Brasil; [3] Serviço de Gastroenterologia do Hospital Universitário, Universidade Federal de Santa Maria, RS, Brasil; [4] Serviço de Patologia do Hospital Universitário, Universidade Federal de Santa Maria, RS, Brasil; [5] Departamento de Clínica Médica, Centro de Ciências da Saúde, Universidade Federal de Santa Maria, Santa Maria, RS, Brasil.
Correspondence: Renato Borges Fagundes. Av. Grécia 1000, ap. 1002, Torre B. CEP: 91350-070 - Porto Alegre, RS, Brasil. E-mail: fagundesrb@gmail.com

positive and negative predictive values and likelihood ratios) of the FAT by immunochromatography using as the gold standard histological examination plus urease test.

METHODS

Dyspeptic adult patients, undergoing to upper GI endoscopy at the Gastroenterology Unit of University Hospital of Santa Maria, from March 2013-June 2015, were invited to participate in the study. Institutional Internal Review Board approved the protocol study under the number CAAE 11979613.7.0000.5346. We excluded patients treated with antibiotics in the last 30 days or proton pump inhibitors (PPIs) in the last two weeks[21], as well as patients with gastric cancer, gastrectomy, bleeding disorder, acquired immunodeficiency syndrome, congestive heart failure, renal failure, and chronic liver disease.

After upper GI endoscopy examination, we collect six gastric biopsies from each patient. Two biopsies from antrum, one biopsy from incisura angularis and two from the body for histological examination, and one biopsy from the antrum to the urease test. The gastric biopsies were stored in vials containing 10% formalin and sent to the pathology lab. The histological diagnosis was according to the Sydney system. We followed the manufacturer's guidelines to perform the urease test.

Patients brought fecal samples stored in a box with ice. We froze the samples at -20ºC until the completion of the test with the ImmunoCard STAT immuno TAF! Hpsa (Meridian Bioscience, Ohio/USA). This test is based on immunochromatography technique. After homogenization, we collected and transferred 5-6 mL of sample to a diluent vial. We put four drops of the suspension in the appropriate area on the device test. We incubated the test at room temperature (20-26ºC) for 5 minutes. We executed the reading for 1 minute based upon the appearance of colored lines in the central window. We interpreted as a positive test when it showed a blue control line (C) and a test line (T) in pink-reddish. Appearance only the line C indicated a negative test. No line C, with or without the line T, pointed out to invalid test. An observer (MDN) unaware of the results of histopathology or urease test read TAF results and classified them as positive, negative or invalid. We used histopathology findings and urease test results, as the gold standard, to assess the accuracy of FAT in the diagnosis of *H. pylori* infection.

Statistical analysis

We based our sample calculation on a previous study[12], which showed 76% prevalence of *H. pylori* infection among dyspeptic patients attending the Gastroenterology Unity at Hospital Universitario de Santa Maria. Previous studies demonstrated the sensitivity of the fecal antigen test (ImmunoCard Stat HpSA) to be around 70%[4]. Assuming 70% of accuracy for fecal antigen test compared to histology and urease test with a difference of ±10% (95%CI), we estimate our sample size in 160 patients.

We used Statistical Package for the Social Sciences v. 17 for data analysis. We organized the data in 2x2 contingency tables for calculation of sensitivity, specificity, positive and negative predictive values, and the likelihood ratios (positive and negative) of FAT in the identification of *H. pylori* infection. We used Fisher exact test to appraise the statistical significance and Cohen's kappa coefficient to assess the agreement between FAT and the combination of histopathology examination and urease test at the significance level of $\alpha<0.05$.

RESULTS

We included 165 patients with a mean age of 56.7 (SD±8.5) years. Fifty-one percent were male. Eighty patients (49%) presented *H. pylori* infection assessed by histopathology/urease test (Table 1). We excluded two patients with FAT results classified as invalid. The contingency table 2x2 (Table 2) shows FAT results compared to histopathology/urease test, for 163 patients.

FAT performance for the diagnosis of *H. pylori* infection is detailed in Table 3. Cohen's kappa index for the agreement between FAT and histopathology/urease test was 0.53 (95%CI: 0.39-0.64).

TABLE 1. Sample demographics plus urease test and histopathology results

Variable	Category	N	%
Age (years)	20-40	42	25.7
	41-60	89	54.6
	61-80	32	19.6
Genre	Female	80	49.1
	Male	83	50,9
Urease test*	Positive	75	46.1
	Negative	88	53.9
Histopathology*	Positive	80	49.0
	Negative	83	51.0

* Cohen's kappa index= 0.94 (CI95% 0.79-1.0)

TABLE 2. Comparison between FAT and histopathology/urease test for diagnosis of *H. pylori* infection

		Histophatology/ Urease test		
		Positive	Negative	Total
FAT	Positive	54	12	66
	Negative	26	71	97
	Total	80	83	163

P<0.001

TABLE 3. FAT performance for the diagnosis of *H. pylori* infection

	%	95% CI
Acuracy	76.7	70-82
Sensitivity	67.5	60.6-72.9
Specificity	85.5	78.9-90.7
Positive predictive value	81.8	73.4-88.4
Negative predictive value	73.2	67.5-77.6
Positive likelihood ratio	4.7	2.9-7.9
Negative likelihood ratio	0.4	0.3-0.5

DISCUSSION

In this study, we found that FAT with immunochromatography technique in the diagnosis of *H. pylori* infection presented a moderate agreement with histopathology and urease test. FAT showed high specificity, low sensitivity, high predictive positive value (PPV) and moderate negative predictive value (NPV). Its high specificity pointed out to a low rate of false-positive values, and the low sensitivity raised the probability of high rate of false-negative results. The positive likelihood ratio suggested a small increase in the probability of active *H. pylori* infection in a patient when compared with one who tested negative.

The high specificity of FAT indicates that the exam can provide a significant percentage of correct results when individuals do not have *H. pylori* infection. This high specificity determines the probability that the test does not select erroneously uninfected people. On the other hand, the low sensitivity demonstrates the inability of the test in making the diagnosis when the patient has *H. pylori* infection. The predictive values of a test are not exclusive properties of the test because their values depend on the prevalence of the disease among the population. The high PPV indicates that a patient with a positive test has a high probability of presenting the infection. The moderate NPV means that the exam has an average chance to predict the non-existence of the infection when it does not exist.

The results of this imunocromatographic test are in disagreement with the immunoassay tests executed in Brazil[2,3,11,13,14,16-18,21]. Our results are also in disagreement with some results obtained even in different areas of the world, by immunocromatographic assay[4,9,18]. On the other hand, our results are similar to results achieved in Egypt[1,5]. Egypt has a high prevalence of *H. pylori* infection. It is noteworthy that the performance of immunochromatographic tests depends on the patient's characteristics. The sensitivity and specificity are higher in patients with ulcer. Otherwise, the sensitivity decreases in patients who are more than 60 years[4]. The age could be an explanation for the lower sensitivity achieved in our results, once the mean age of our sample was around 60 years/old. Due to its high specificity and high PPV the test could be useful in younger patients making them candidates for the test and treat approach.

Many others factors exert influence in the FATs results. Watery fecal samples reduce FATs accuracy because of the *H. pylori* antigens dilution, but none of the patients included in this study presented watery samples. Other factors are PPIs. Antibiotics and bismuth salts use. To avoid bias we did not included patients in use of these drugs. Therefore, the cause of false negative results is not related to the temporary inhibition of *H. pylori*. However, false negative results could be due to a low colonization in the gastric mucosa. Decreased colonization of gastric mucosa might lead to low concentration of *H. pylori* antigens in stool, not enough to react in FAT. False-positive results may also have occurred due to the presence of a coccoid form of *H. pylori*. This form despite to be a morphological manifestation of bacterial death, induces antigen detected by the test[21].

Our results are in agreement with other studies[7,8,15,19]. It is reported an irregular performance of FAT in comparison to other methods, a wide variation of accuracy and antigenicity differences among strains of *H. pylori* in distinct populations[9,10,20]. Thus, the tests' sensitivity must be investigated in each population prior its use in practice. It is also possible to occur misinterpretation of FAT results with immunochromatography technique. A weaker band in the test, as well as problems of affinity in antigen-antibody binding and insufficient analytical sensitivity are responsible for the diagnostic misinterpretation[22]. Some authors observed considerable differences in FAT evaluations of dyspeptic patients and suggested that FAT with immunochromatography technique presents unpredictable results as a tool for the primary diagnosis and good results for the assessment of *H. pylori* eradication[6,20].

The considerable genetic heterogeneity and the wide geographic variation in *H. pylori* strains may be compromised our results. The differences between antigens and antibodies may have influenced the results[9,20]. The older age of our sample and the predominance of women could be another possible bias. Intestinal constipation is more frequent among older people and female, and slow movement of intestines can determine a greater chance of *H. pylori* antigen degradation[5].

CONCLUSION

Although immunochromatographic FAT is less expensive than the other methods and readily accepted by the patients its diagnostic performance does not recommend its use in the primary diagnosis, when the patient may have an active infection.

Authors' contributions

Fagundes RB e Dalla Nora M designed the research protocol. Dalla Nora M conducted the process for IRB permission and coordinated the samples collection. Dalla Nora M and Hörner R performed the immunochromatography technique for fecal antigen test. De Carli DM and Araujo AF collected biopsies and performed the urease tests. Rocha MP performed the anatomopathological diagnosis. Fagundes RB analyzed the data and wrote the manuscript. All authors read and approved the manuscript final version for submission.

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RESUMO - ***Contexto*** - O diagnóstico da infecção por *Helicobacter pylori* (*H. pylori*) pode ser realizado por métodos invasivos e não invasivos. A identificação através do teste do antígeno fecal é um método não invasivo, simples, fácil e relativamente barato. ***Objetivo*** - Determinar o desempenho diagnóstico do teste fecal imunocromatográfico na identificação da infecção pelo *H. pylori*. ***Métodos -*** A pesquisa de antígenos fecais do *H. pylori* foi realizada através do ImmunoCard STAT! HpSA em pacientes dispépticos submetidos à endoscopia digestiva alta com coleta de biópsias para histopatologia e teste da urease, utilizados como padrão ouro. ***Resultados -*** Foram estudados 163 pacientes, 51% do sexo masculino, com idade média de 56,7± 8,5 anos. A infecção por *H. pylori* esteve presente em 49%. O teste fecal apresentou o seguinte desempenho diagnóstico: sensibilidade 67,5% (IC95% 60,6-72,9), especificidade 85,5% (IC95% 78,9-90,7), valor preditivo positivo 81,8% (IC95% 73,4-88,4) e valor preditivo negativo 73,2% (IC95% 67,5-77,6). A razão de probabilidade positiva foi 4,7 (IC95% 2,9-7,9) e a razão de probabilidade negativa foi 0,4 (IC95% 0,3-0,5). A razão de chances de prevalência para teste fecal positivo foi 12,3 (IC95% 5,7-26,3). O índice kappa para a concordância do teste fecal com histologia/teste da urease foi 0,53 (IC95% 0,39-0,64) ***Conclusão -*** O teste fecal imunocromatográfico apresenta baixo custo e é facilmente aceito pelos pacientes, no entanto seu desempenho diagnóstico não o recomenda para diagnóstico primário.

DESCRITORES - Infecções por *Helicobacter*, diagnóstico. Imunocromatografia. Anticorpos. Endoscopia. Técnicas de diagnóstico do sistema digestório.

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