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Association of duodenal eosinophilic infiltrate with *Helicobacter pylori* infection, but not with functional dyspepsia

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ABSTRACT – **Background** – The role of *Helicobacter pylori* infection on eosinophilic infiltration in duodenal mucosa is poorly studied. An increase in the number of eosinophils in duodenum has been associated with functional dyspepsia. **Objective** – To evaluate the influence of *H. pylori* infection on duodenal eosinophil count and the role of eosinophilic infiltrate of duodenum in functional dyspepsia. **Methods** – Positive and negative *H. pylori* individuals were included. Both functional dyspeptic patients according to Rome III criteria (cases) and individuals without gastrointestinal symptoms (controls) were enrolled. They were submitted to upper endoscopy and *H. pylori* infection was verified by gastric histopathology and urease test. Eosinophils in the duodenal mucosa were counted in five high-power fields, randomly selected on slides of endoscopic biopsies. **Results** – Thirty-nine *H. pylori* positive (mean age 40.5 and 69.2% women) and 24 negative patients (mean age 37.3 and 75% women) were included. The influence of the infection was observed in the duodenal eosinophil count, which was higher in infected individuals: median 13.2 vs 8.1 in non-infected individuals (P=0.005). When we analyzed patients according to symptoms, cases – mean age 39.6; 71.4% women – and controls – mean age 38.7; 71.4% women – had similar duodenal eosinophil count: median 11.9 and 12.6 respectively (P=0.19). **Conclusions** – We did not demonstrate association of duodenal eosinophil count with functional dyspepsia but found association with *H. pylori* infection.

HEADINGS – Dyspepsia. Biopsy. *Helicobacter pylori*. Duodenum. Eosinophils.

INTRODUCTION

Dyspeptic symptoms affect around a third[1] of the world's population. Most of these individuals suffer from functional dyspepsia (FD), defined by symptoms arising from the gastroduodenal region in the absence of any explanatory organic disease[2].

The pathophysiology of FD is still poorly understood. Hypothesis of its etiology include gastroduodenal motility disorders[3], gastric hypersensitivity to distension and acids[4,5], microbiological triggers such as gastroenteritis, *H. pylori* infection[6,7] and psychosocial distress[8,9].

Eosinophils participate in the interaction between innate and acquired immunity and are important in the inflammatory response of type 2 hypersensitivity reactions. Infections cause their recruitment and degranulation, including *H. pylori*[10]. They may cause symptoms due to release of cytokines, which lead to neural excitation, muscle spasms and pain[11]. Recent studies have reported a greater number of eosinophils in the duodenal mucosa of patients with functional dyspepsia than in asymptomatic controls, but the issue is controversial[12-14].

H. pylori infection is very common in south Brazil, affecting more than 2 thirds of functional dyspeptic patients[6]. The association of this bacteria and duodenitis has been reported[15]. However, there is uncertainty about the role of *H. pylori* in number of duodenal eosinophils. It was not reported by Zhao in a study that included patients with FD[16]. The infection was not related to eosinophilic infiltration in previous studies[12,14,17], with infection rates as low as 16.3%.

So, the findings of previous studies might not be extrapolated to Brazilian patients. This study aims to evaluate further the influence of *H. pylori* infection on eosinophil count and the role of duodenal eosinophilic infiltrate in FD in our population.

METHODS

The functional dyspeptic patients (cases) in this investigation participated in the randomized double-blind study Heroes Trial (Helicobacter Eradication Relief of Dyspeptic Symptoms) – ClinicalTrials.gov number NCT00404534. In this paper[6], it was evaluated the effect of *H. pylori* eradication on the symptoms of functional dyspepsia. The study has been conducted in our institution, *Hospital de Clínicas de Porto Alegre* (HCPA). Individuals aged 18 or older who fulfilled the Rome III criteria for functional dyspepsia were included. Exclusion criteria are better described elsewhere[6].

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The control group consisted of individuals with no symptoms in the gastrointestinal tract selected among donors for the blood bank at the same hospital. They were subjected to the same exclusion criteria as the cases.

In this study we aim to evaluate the eosinophil infiltrate in duodenal mucosa of functional dyspeptic and asymptomatic individuals. This research was approved by the HCPA Internal Review Board Committee and informed consent was obtained from all cases and controls prior to inclusion. The study is in accordance with Resolution 466/2012 of the National Health Council of the Ministry of Health (Brazil).

Study procedures

Cases and controls were submitted to a medical consultation, during which the demographic data collection and the physical examination, including anthropometric measurements, were performed.

Symptoms were better assessed by the PADYQ (Porto Alegre Dyspeptic Symptoms Questionnaire)[18]. It comprises 11 questions about the frequency, duration and intensity of dyspeptic symptoms over the last 30 days, with scores ranging from 0 (no symptom) to 44 (severe symptoms).

Cases patients were classified into two subgroups according to the predominant symptom established by the Rome III criteria: epigastric pain syndrome (EPS) and postprandial distress syndrome (PDS). Both cases and controls included were submitted to laboratory tests and upper endoscopy with gastric (body, *antrum* and *incisura*) and duodenal mucosal biopsies (second portion). Endoscopies were performed by two trained endoscopists. In case of disagreement, a third endoscopist was consulted. Those individuals whose findings were not only gastritis, duodenitis or hiatal hernia were excluded.

Histopathology

Fragments from gastric endoscopic biopsies of the *incisura*, *antrum* and body were analyzed for generic histological findings (H&E) and *H. pylori* presence (Giemsa staining). Rapid urease test to verify this bacterium was performed immediately after endoscopy, using one fragment from each portion. Patients were considered *H. pylori* carriers when it was confirmed by both methods. In case of disagreement a third pathologist was consulted.

Biopsies of duodenum were stained with H&E and evaluated by a pathologist DMU blind to the patient's group (case or control). Samples were analyzed for villus architecture, intraepithelial lymphocyte count, chronic inflammation signs, metaplasia, presence of pathogens and erosions. Five high-power fields (40X) were randomly selected on each fragment, eosinophils were counted in each field, then sum and mean was obtained. A second and independent pathologist JCA evaluated all these biopsies by the same method, in order to verify the histopathological reliability.

Sample size

Twenty-three patients in each group were needed to detect a difference of one standard-deviation of eosinophil count between *H. pylori* positive and negative patients, with a power of 90% and *alpha* 0.05%[12].

Statistical analysis

SPSS 21.0 software for Windows (IBM co, NY) was used, applying chi-square tests with and without Yates correction for categorical variables and Student's *t*-test for quantitative variables with normal distribution. The distribution curve of the mean eosinophil count/high-power field of individuals was found to be asymmetrical, and the median and interquartile ranges were obtained.

Mann-Whitney test was used to assess the primary outcome, namely the association of duodenal intramucosal eosinophil count with dyspepsia and *H. pylori*. The intraclass correlation coefficient (ICC) was calculated for comparing values obtained by the independent pathologists. The level of significance was set at 5%.

RESULTS

Thirty-nine *H. pylori* positive and 24 negative patients were included, with 66% of cases in each group, comprising 42 cases and 21 controls. Basal characteristics were similar between groups (TABLE 1). The median of score PADYQ was 19.5 for cases and 0 for controls (P<0.001).

TABLE 1. Sample characterization.

Variables	*H. pylori* positive group N=39	*H. pylori* negative group N=24	*P* value
Age (years) – mean ± SD	40.5±10.5	37.3±10.5	0.25
Sex – n (%)			
Male	12 (30.8)	6 (25)	0.84
Female	27 (69.2)	18 (75)	
Cases – n (%)			
Positive	26 (66.6)	16 (66.6)	1
Race – n (%)			
White	34 (87.2)	21 (87.5)	0.98
Black	3 (7.7)	2 (8.3)	
Mixed	2 (5.1)	1 (4.2)	
BMI (kg/m²) – mean ± SD	25.9±4.1	24.6±5.3	0.54

SD: standard deviation; *H. pylori*: *Helicobacter pylori*; BMI: body mass index.

Upper endoscopy was normal in 31 (79.5%) of *H. pylori* positive individuals. Abnormal findings were duodenitis (three enanthematous and five erosive). Among negative, endoscopy was normal in 21 (87.5%). Alterations were three with enanthematous duodenitis. Values were statistically similar (P=0.16).

Among positive *H. pylori* individuals, the duodenum was histologically normal in 31 of 39 (79.5%) and among negative was normal in 17 of 24 (70.8%). These values were also statistically similar (P=0.3). Regarding the alterations found among positive, seven had duodenitis, one had duodenal lymphangiectasia. Among negative five had duodenitis and two gastric metaplasia. Gastric biopsies from antrum, body and *incisura* were also obtained and set in different jars. All specimens were normal (no atrophy or inflammatory activity) in 20.8% of noninfected patients, and in none of infected patients (P<0.001).

Cases and controls had similar duodenal endoscopic findings, with 81% exhibiting normal duodenum in both groups. Among functional dyspeptic patients, the duodenum was histologically

normal in 31 of 42 (73.8%) cases and normal in 18 of 21 (85.7%) controls, *P*=0.64. As for the alterations found among cases, eight had duodenitis, one had duodenal lymphangiectasia, and two showed gastric metaplasia, whereas three controls exhibited duodenitis, *P*=0.63.

Duodenal eosinophil count

Biopsies were analyzed by a second pathologist. The eosinophil counts obtained from the independent observers were compared using the intraclass correlation coefficient. There was good agreement: the calculated ICC was 0.79 (95% IC 0.62–0.88, *P*<0.001).

The influence of *H. pylori* was observed on the median of eosinophil counts. This value was 13.2 for the 39 infected individuals, whereas it was 8.1 among the 24 non-infected. The difference was statistically significant (*P*=0.005), FIGURE 1.

The best cut-off for eosinophil count that correlated to *H. pylori* infection was 11.1 (FIGURE 2). Among individuals infected with *H. pylori*, 71.8% had values above this limit, and among noninfected, only 25% (*P*<0.001); refer to FIGURE 3.

The median of eosinophil count per high-power field was similar between functional dyspeptic patients and the asymptomatic controls. The median was 11.9 and 12.6 respectively (*P*=0.194). They were also similar in the analysis of the FD subgroups containing 26 EPS patients and 16 PDS patients (TABLE 2). There was no relationship between serum eosinophil value and duodenal count (*P*=0.08).

DISCUSSION

Our study demonstrated association of duodenal eosinophil count with *Helicobacter pylori* infection, but not with FD. The strength of our findings is that the included cases were carefully selected for participation in Heroes, a randomized clinical trial. Our

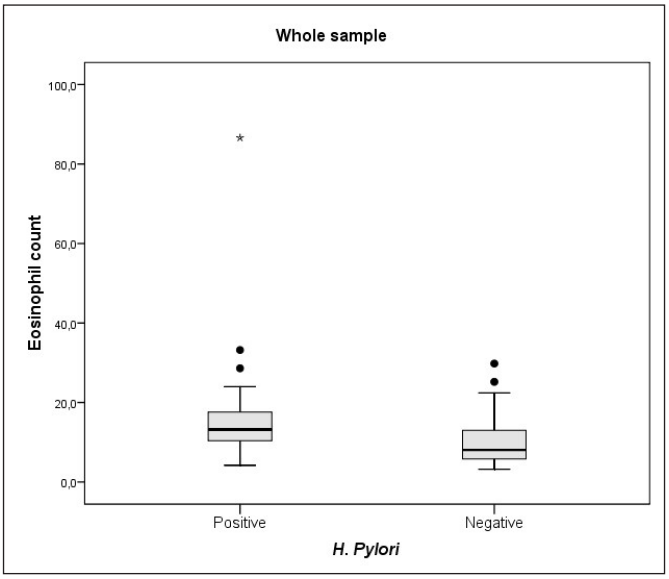

FIGURE 1. Eosinophil count in HP infected X non-infected patients. The band inside the box represents the median. The bottom and top of the box represent 25th and 75th percentiles of the sample [interquartile range or IQR]. The whiskers are the minimum and maximum values in a range of 1.5[IQR]. Dot and asterisks represent the outliers that are above this range.

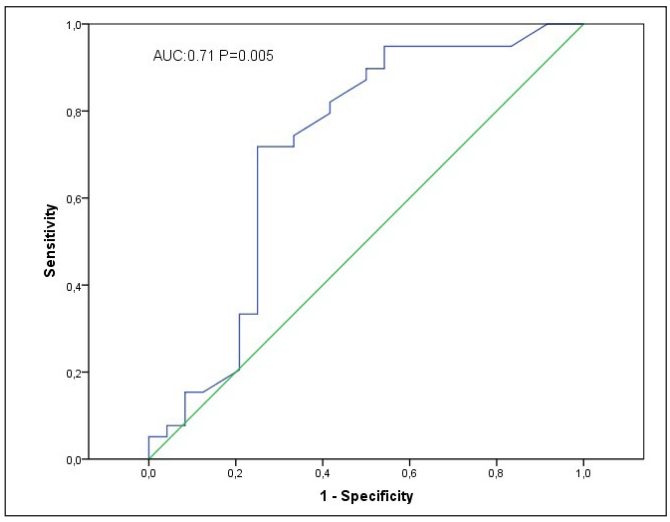

FIGURE 2. ROC curve representing accuracy of eosinophil count to detect *H. pylori* infection.
The AUC was 0.71 (CI 0.56–0.86), *P*=0.005.

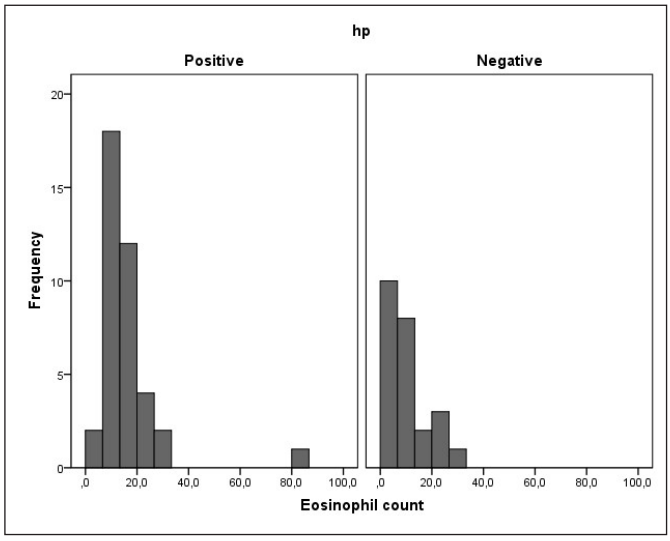

FIGURE 3. Frequency of patients according to eosinophil count fashion.
The histogram shows that the majority of *H. pylori* positive and the minority of *H. pylori* negative patients had eosinophil count higher than the cut-off 11.1.

TABLE 2. Eosinophil count/HPF*- dyspeptic patients x controls.

Group	Eosinophil count/HPF, median (interquartile range)	*P* value vs controls**
Cases	11.9 (6.7–14.7)	0.194
EPS	11.5 (6.7–14.6)	0.181
PDS	12.2 (6.5–16.8)	0.421
Controls	12.6 (8.8–22.6)	–

* HPF high-power field. ** Mann-Whitney test.

controls were asymptomatic regarding digestive tract. None of the individuals (dyspeptic or asymptomatic) showed any gastroduodenal alteration in the endoscopy other than gastritis or duodenitis. In addition, the baseline characteristics of groups were similar.

The association of *H. pylori* and duodenal eosinophilic infiltrate both in dyspeptic and controls has not been previously described. *Helicobacter* infection has been shown to cause inflammatory infiltrates in the stomach, leading to recruitment and degranulation of eosinophils[10,19]. Consequently, there is release of toxic molecules. They can generate gastrointestinal symptoms, as we mentioned before. This recruitment and degranulation cascade might also occur in duodenum.

So, our finding is biologically supported. Most studies neglected this hypothesis, probably because of prevalence of *H. pylori* positive individuals being as low as 10%[12,14,17,20]. A larger sample investigation found no relationship between infection and duodenal eosinophils, but did not include asymptomatic individuals as we did[16].

The hypothesis of a relationship between eosinophilic infiltrate and dyspepsia was initially investigated by Toukan et al.[21], who analyzed gastroduodenal endoscopies and biopsies in dyspeptic and control patients. Later, Talley and Walker[12] confirmed this finding in a Swedish community. The same group conducted another case-control study including EPS and PDS patients in London's population, but found only an association of duodenal eosinophilia and symptoms in individuals in the second subgroup[22]. They also conducted a study in Australia, where they observed no association between functional dyspepsia and eosinophilic infiltrate, except for cases of postprandial fullness[17]. A study by Bafutto et al. with 36 dyspeptic patients and nine controls in Brazil showed a similar result[23].

As in the present study, other authors reported negative results for association between eosinophilic infiltrate and dyspepsia, such as the researches carried out by Veerapan et al. (Washington)[24], Binesh et al. among Iranians[14] and Lijun Du in China[25]. A recently published study by Song et al. found a positive association, but the status of the *H. pylori* infection was not evaluated, which, as we reported, may influence the results[13].

Limitations of our study include we did not investigate the presence of parasitic infection or allergic factors in the individuals included. However, we believe this was no longer relevant as negative results were found, and both cases and controls are from the same population and exposed to the same environmental factors. We obtained a lower eosinophil count than the developed countries. This may show less than expected influence of parasitical infection.

The lack of an association between eosinophils and dyspeptic symptoms in some studies does not disagree with previous positive findings. The populations are different, with local genetic and microbiological influences and allergen exposure, factors known to influence the recruitment and accommodation of eosinophils in the mucosa of the gastrointestinal tract[26].

CONCLUSION

We suggest that results from previous studies have little validity to our population. However, functional dyspepsia is a multifactorial disease whose etiology should be tailored to each person. This research contributes with a new finding, influence of *H. pylori* on eosinophil count. How this can contribute to clinical management is an issue that should drive further studies.

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Authors' contribution

Leite C: data collection and analysis. Mazzoleni LE and Sander GB: coordination and design of the study. Uchoa MD and Castanho JA: histopathology. Mazzoleni F: patients' appointments and endoscopies. All the authors have contributed to writing and revision of this manuscript.

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Leite C, Mazzoleni LE, Uchoa DM, Castanho JA, Mazzoleni F, Sander GB. Associação de infiltrado eosinofílico duodenal com infecção por *Helicobacter pylori*, mas não com dispepsia funcional. Arq Gastroenterol. 2020;57(1):74-8.

RESUMO – **Contexto** – O papel de infecção por *Helicobacter pylori* no infiltrado eosinofílico duodenal ainda é pouco compreendido. Um aumento no número de eosinófilos duodenais tem sido associado a dispepsia funcional. **Objetivo** – Avaliar a influência do *H. pylori* na contagem de eosinófilos duodenais e o papel do infiltrado eosinofílico duodenal na dispepsia funcional. **Métodos** – Indivíduos *H. pylori* positivo e negativo foram incluídos. Ambos os grupos, compreendendo dispépticos funcionais pelos critérios de Roma III (casos) e indivíduos sem sintomas gastrointestinais (controles), foram submetidos à endoscopia digestiva alta para pesquisa de *H. pylori,* efetuada por histopatologia e teste de urease. Eosinófilos na mucosa duodenal foram contabilizados em cinco campos de maior aumento, selecionados randomicamente nas lâminas de biópsia endoscópicas. **Resultados** – Trinta e nove indivíduos *H. pylori* positivo (média de idade 40,5 e 69,2% mulheres) e 24 *H. pylori* negativos (média de idade 37,3 e 75% mulheres) foram incluídos. A influência da infecção por *H. pylori* foi observada na contagem de eosinófilos, que foi maior nos positivos: mediana 13,2 vs 8,1 (P=0,005). Quando analisados pacientes de acordo com sintomas, os casos (média de idade 39,6 e 71,4% mulheres) e controles (média de idade 38,7 e 71,4% mulheres), apresentaram semelhante contagem de eosinófilos duodenais: mediana 11,9 e 12,6, respectivamente (P=0,19). **Conclusão** – Não demonstramos associação da contagem de eosinófilos duodenais com dispepsia duodenal, mas encontramos associação com infecção por *H. pylori*.

DESCRITORES – Dispepsia. Biópsia. *Helicobacter pylori*. Duodeno. Eosinófilos.

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