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Dissertação de Mestrado

Eosinófilos Duodenais: Potencial Associação com a Infecção pelo *Helicobacter pylori* e com os Sintomas da Dispepsia Funcional

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Sumário

1. Resumo

Introdução e objetivos: Eosinofilia duodenal está associada com parasitoses intestinais e com alergias alimentares, e tem sido sugerida como possível fator etiológico da dispepsia funcional, pela capacidade de causar alterações na motilidade e na sensibilidade do aparelho digestivo. Sua relação com o *Helicobacter pylori* é pouco conhecida, tendo sido avaliada apenas como achado secundário em alguns estudos, com resultados controversos. Esse estudo tem como objetivos avaliar o papel da infecção gástrica pelo *H. pylori* no número de eosinófilos duodenais e avaliar a relação dos eosinófilos duodenais com os sintomas da dispepsia funcional.

Métodos: foram avaliados 100 pacientes dispépticos funcionais, de acordo com os critérios de Roma III, dos quais 50 foram *H. pylori* positivos e 50 negativos. Os pacientes foram submetidos à endoscopia digestiva alta com biópsias gástricas e duodenais. A positividade do *H. pylori* foi avaliada pelo teste de urease e pelo exame histológico (Hematoxilina-eosina e Giemsa). As biópsias duodenais foram avaliadas com hematoxilina-eosina e a número de eosinófilos duodenais foi quantificada pela média de eosinófilos por 5 campos de grande aumento (CGA) aleatórios e não sobrepostos. Eosinofilia duodenal foi definida pela presença de >22 eosinófilos/CGA. As medianas das médias aritméticas dos eosinófilos duodenais por cinco CGA foram comparadas entre os pacientes *H. pylori* positivos e negativos. Também foi avaliada a relação do número de eosinófilos duodenais com a intensidade e tipo de sintomas dispépticos, determinados por questionário validado (PADYQ). Os eosinófilos duodenais foram avaliados para variáveis demográficas e endoscópicas.

Resultados: Pacientes do sexo feminino representaram 88% da amostra e a idade média foi de 41,7 anos. As características basais dos pacientes *H. pylori* positivos e *H. pylori* negativos foram semelhantes. Apenas um paciente, no grupo *H. pylori* positivo, apresentou eosinofilia duodenal. As medianas dos eosinófilos duodenais/CGA foram 4,6 [P25-75: 2,8-7,2] nos pacientes *H. pylori* negativos e 4,7 [P25-75: 3,4-8,4] nos *H. pylori* positivos (p= 0,403). O número de eosinófilos

duodenais foi significativamente maior em pacientes com sintomas mais intensos: pacientes com escore do PADYQ >22 (>50% da pontuação máxima) apresentaram mediana de eosinófilos duodenais/CGA de 5,4 [P25-75: 3,4–7,6] e pacientes com escore ≤22 de 3,4 [P25-75: 2,2–6,0] (p= 0,018). Os pacientes foram divididos em tercis, de acordo com a intensidade dos sintomas: grupo 1 com 31 pacientes (sintomas leves); grupo 2 com 30 pacientes (sintomas moderados); e grupo 3 com 31 pacientes (sintomas acentuados). A mediana dos eosinófilos duodenais/CGA no grupo 1 foi de 3,4 [P25-75: 2,2 -6,0]; no grupo 2 de 4,7 [P25-75: 3,2-6,4]; e o grupo 3 de 5,8 [P25-75: 3,6-8,2] (P=0,033). Houve diferença estatisticamente significativa no número de eosinófilos duodenais entre fumantes e não fumantes (*p*= 0,030) e entre pacientes com índice de massa corporal (IMC) <25 kg/m2 e IMC ≥ 25 kg/m2 (*p*= 0,035). Na análise multivariada por regressão linear, os fatores que tiveram influência sobre o número de eosinófilos duodenais foram o tabagismo (*p*= 0,026) e a intensidade dos sintomas dispépticos (p= 0,039).

Conclusões: Esse estudo não mostrou associação entre a infecção pelo *H. pylori* e a contagem de eosinófilos duodenais, nessa população de pacientes dispépticos funcionais. Entretanto, foi demonstrada uma relação diretamente proporcional e estatisticamente significativa entre o número de eosinófilos duodenais e a intensidade dos sintomas dispépticos.

Palavras chave: Eosinófilos; *H. pylori*; dispepsia funcional; eosinófilos duodenais; duodeno.

2. Abstract

Background and Aims: Duodenal eosinophilia is associated with intestinal parasitosis and food allergies. It has also been implicated as a potential factor on the etiology of functional dyspepsia, probably by causing changes in digestive tract motility and sensitivity. The association with *Helicobacter pylori* is poorly understood, and has been only evaluated as a secondary finding in

previous studies, with conflicting results. This study aims to evaluate the potential role of gastric *H. pylori* infection in the duodenal eosinophil count, and the influence of duodenal eosinophils on symptoms in functional dyspeptic subjects.

Methods: One hundred functional dyspeptic subjects, according to Rome III criteria, were evaluated, and 50 were *H. pylori* positive and 50 *H. pylori* negative. Patients were submitted to upper gastrointestinal endoscopy with gastric and duodenal biopsies. *H. pylori* positivity was evaluated by urease test and gastric histology (Hematoxylin-eosin and Giemsa). Duodenal biopsies were evaluated with Hematoxylin-Eosin staining, and the duodenal eosinophil count was determined by the mean of eosinophil by 5 random nonoverlapping high power fields (HPF). Duodenal eosinophilia was defined as >22 eosinophils/HPF. The median of the arithmetic means of the duodenal eosinophils counts per high power field were compared between *H. pylori* positive and *H. pylori* negative subjects. The relationship between the number of duodenal eosinophils and the intensity and type of dyspeptic symptoms was determined by validated questionnaire (PADYQ). Duodenal eosinophils counts were also evaluated by demographic variables and endoscopic findings.

Results: 88% of the subjects were female and the mean age was 41.7 years. Baseline characteristics were similar between *H. pylori* positive and *H. pylori* negative subjects. Only one patient, in the *H. pylori* positive group, had duodenal eosinophilia. The median duodenal eosinophils/HPF were 4.6 [Percentiles 25-75(P25-75): 2.8-7.2] in *H. pylori* negative and 4.7 [P25-75: 3.4-8.4] in *H. pylori* positive subjects (p= 0.403). The duodenal eosinophil count was greater in subjects with higher symptoms severity: patients with PADYQ score more than 22 (>50% of the maximum score) had median duodenal eosinophil/HPF of 5.4 [P25-75: 3,4–7,6] and subjects with PADYQ score ≤22 of 3.4 [P25-75: 2.2–6.0] (p= 0.018). The patients were divided into terciles, according to symptoms severity: group 1 with 31 subjects (mild symptoms); group 2 with 30 subjects (moderate symptoms); and group 3 with 31 subjects (severe symptoms).

The median duodenal eosinophils/HPF was 3.4 [P25-75: 2.2-6.0] in group 1; 4.7 [P25-75: 3.2-6.4] in group 2; and 5.8 [P25-75: 3.6-8.2] in group 3 (*p*=0.033). There was a higher duodenal eosinophils count in smokers (current or former) (p=0.030), and subjects with BMI ≥ 25 kg/m^2 (p=0.035). In the multivariate analysis by linear regression, the duodenal eosinophil count were influenced by smoking (p = 0.026) and dyspeptic symptoms severity (p= 0.039).

Conclusion: This study did not show an association between *H. pylori* infection and the number of duodenal eosinophils, in this population of functional dyspeptic patients. However, a directly proportional and statistically significant relationship between the number of duodenal eosinophils and the intensity of dyspeptic symptoms has been demonstrated.

Keywords: Eosinophils; *H. pylori*; functional dyspepsia; duodenal eosinophils; duodenum.

3. Apresentação

A presente dissertação é composta de uma introdução, que aborda os principais tópicos do estudo que são: os eosinófilos, a infecção gástrica pelo *H. pylori*, a dispepsia funcional e as associações entre essas variáveis, através da revisão bibliográfica. Após, será apresentada a justificativa, a hipótese e os objetivos do estudo.

O artigo em inglês irá detalhar os materiais e métodos, os resultados, a discussão sobre os resultados e as conclusões, seguido das referências bibliográficas.

Segue-se com a conclusão a respeito dos resultados obtidos para cada objetivo do estudo, seguido das perspectivas e considerações finais. Ao final, seguem as referências bibliográficas utilizadas para a elaboração dessa dissertação e o anexo, detalhando o questionário utilizado para a realização do estudo (PADYQ).

4. Lista de abreviaturas (Português)

CEP	Comitê de Ética em Pesquisa
CGA	Campos de Grande Aumento
DF	Dispepsia Funcional
DPM	Desvio Padrão da Média
ED	Eosinofilia Duodenal
FAP	Fator de Ativação Plaquetária
FCT	Fator de Crescimento Tumoral
GPPG	Grupo de Pesquisa e Pós-Graduação
H2	Receptor Histamínico tipo 2
HCPA	Hospital de Clínicas de Porto Alegre
H&E	Hematoxilina e Eosina
H. pylori	*Helicobacter pylori*
IC	Intervalo de Confiança
IL	Interleucina
IMC	Índice de Massa Corporal
LT	Leucotrienos
M2	Receptor Muscarínico tipo2
Md	Mediana
P25–75	Percentis 25 e 75

PBP	Proteína Básica Principal
SDE	Síndrome da Dor Epigástrica
SDPP	Síndrome do Desconforto Pós-Prandial
TCLE	Termo de Consentimento Livre e Esclarecido
Th	T-helper (linfócito)

5. Lista de abreviaturas (inglês)

BMI	Body mass index
EPS	Epigastric pain syndrome
Heroes	*Helicobacter* Eradication Relief Of dyspeptic Symptoms
HPF	High power fields
MPB	Major Basic Protein
PADYQ	Porto Alegre Dyspeptic Symptoms Questionnaire
PPDS	Postprandial distress syndrome
SPSS	*Statistical Package for Social Sciences*

6. Lista de Tabelas e Figuras (Revisão Bibliográfica)

7. Lista de Tabelas e Figuras (Artigo em inglês)

8. Introdução

Infiltrados inflamatórios na mucosa duodenal, como eosinofilia duodenal, linfocitose intraepitelial e mastocitose duodenal, têm sido cada vez mais estudadas.[1] Esses processos geralmente não são acompanhados por alterações endoscópicas significativas, nem por alterações microscópicas das vilosidades/criptas. A etiologia e as repercussões clínicas dessas alterações ainda são pouco conhecidas. Alguns estudos têm avaliado a possível associação desses infiltrados duodenais com doenças funcionais do trato gastrointestinal, como dispepsia funcional e síndrome do intestino irritável.[2] Entretanto, poucos estudos avaliaram o papel do *H. pylori* na etiologia dessas alterações histológicas duodenais, apesar dessa bactéria ser o principal agente etiológico das úlceras duodenais.[3]

Eosinofilia duodenal está associada a parasitoses intestinais e alergias alimentares,[4] e vem sendo implicada como potencial fator na etiologia da dispepsia funcional,[1,5,6] possivelmente por causar alterações na motilidade e sensibilidade do aparelho digestivo, como observado em estudos com modelos animais.[7] Sua relação com o *H. pylori* é pouco conhecida, tendo sido

avaliada apenas como achado secundário em alguns estudos, os quais apresentaram resultados controversos.[1,8,9]

9. Revisão bibliográfica

9.1. Eosinófilos

Eosinófilos são leucócitos da linhagem dos granulócitos, envolvidos na resposta imune a infecções, remodelamento tecidual, vigilância tumoral e manutenção de outras células imunológicas.[10] Os eosinófilos representam 1-3% dos leucócitos circulantes e apresentam ciclo vital de 13 dias. Durante a metade deste período permanecem nos tecidos, em especial timo, glândulas mamárias e lâmina própria do trato gastrointestinal, exceto no esôfago. Deslocam-se a estes locais em processos inflamatórios, mas já estão alojados nos tecidos em pequeno número desde o período pré-natal, independente de estímulo antigênico. Após este período, citocinas presentes nas células epiteliais intestinais, denominadas eotaxinas, são os principais reguladores da carga eosinofílica local.

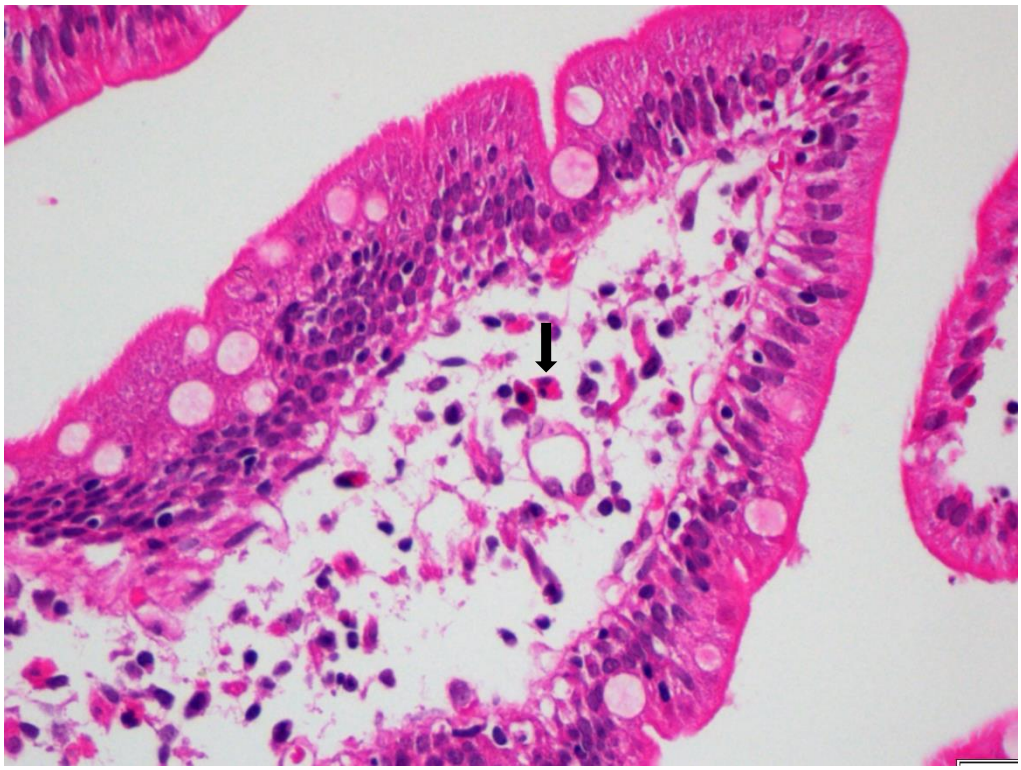
Após a exposição do intestino a algum alergeno ou parasita, o antígeno é fagocitado, processado e exposto pelas células apresentadoras de antígeno aos linfócitos T helper 2 (TH2). Tais células produzem IL-5, que estimula a diferenciação e, em especial, a liberação de eosinófilos pela medula óssea. Estes polimorfonucleares são atraídos ao foco inflamatório pelas eotaxinas, principalmente do tipo 1 e 3.[11]

Os eosinófilos influenciam a motilidade e sensibilidade do aparelho digestivo. A proteína básica principal, liberada pelos eosinófilos, gera disfunção em receptores muscarínicos do tipo M2, levando à hipereatividade da musculatura lisa.[12] Também estimula a síntese de leucotrienos (via do ácido araquidônico), os quais geram contração muscular visceral, vasoconstrição, edema e recrutamento de mais eosinófilos. Tais células também foram relacionadas à necrose axonal,

que gera distúrbio motor.[13] A contração de musculatura lisa, isoladamente gera cólica por dor visceral. No caso de contrações lentificadas ou desordenadas provoca a dismotilidade, que pode estar relacionada com sintomas dispépticos.

Os eosinófilos participam da resposta inflamatória contra a infecção pelo *H. pylori*, com o papel de modular a capacidade da mucosa gástrica de manter ou recuperar sua integridade.[14]

Figura 1. Eosinófilos duodenais. Coloração Hematoxilina-Eosina (amostra de paciente desse estudo)



9.2. *Helicobacter pylori* (*H. pylori*)

O *Helicobacter pylori* é uma bactéria Gram negativa, com formato em espiral e é uma das mais prevalentes infecções da humanidade. Estima-se que 4,4 bilhões de pessoas estejam infectadas em todo o mundo.[15]

A reação inflamatória causada pelo *H. pylori* (gastrite por *H. pylori*) pode apresentar alterações de inflamação gástrica aguda e/ou crônica, com infiltração de neutrófilos, linfócitos, mastócitos e células plasmáticas.[16] Os neutrófilos, mastócitos, eosinófilos e células dendríticas podem infiltrar diretamente o epitélio faveolar, enquanto a lâmina própria é permeada por células mononucleares, tais como linfócitos, macrófagos e células plasmáticas.[17]

Eosinófilos são encontrados em grande densidade no estômago, nas gastrites crônicas causadas pelo *H. pylori*. Particularmente, podem ser encontrados em uma densidade aumentada na camada superficial da lâmina própria. Infiltrado eosinofílico intraepitelial também pode ocorrer, e esse achado deve sempre ser interpretado como não usual.

9.3. Dispepsia Funcional

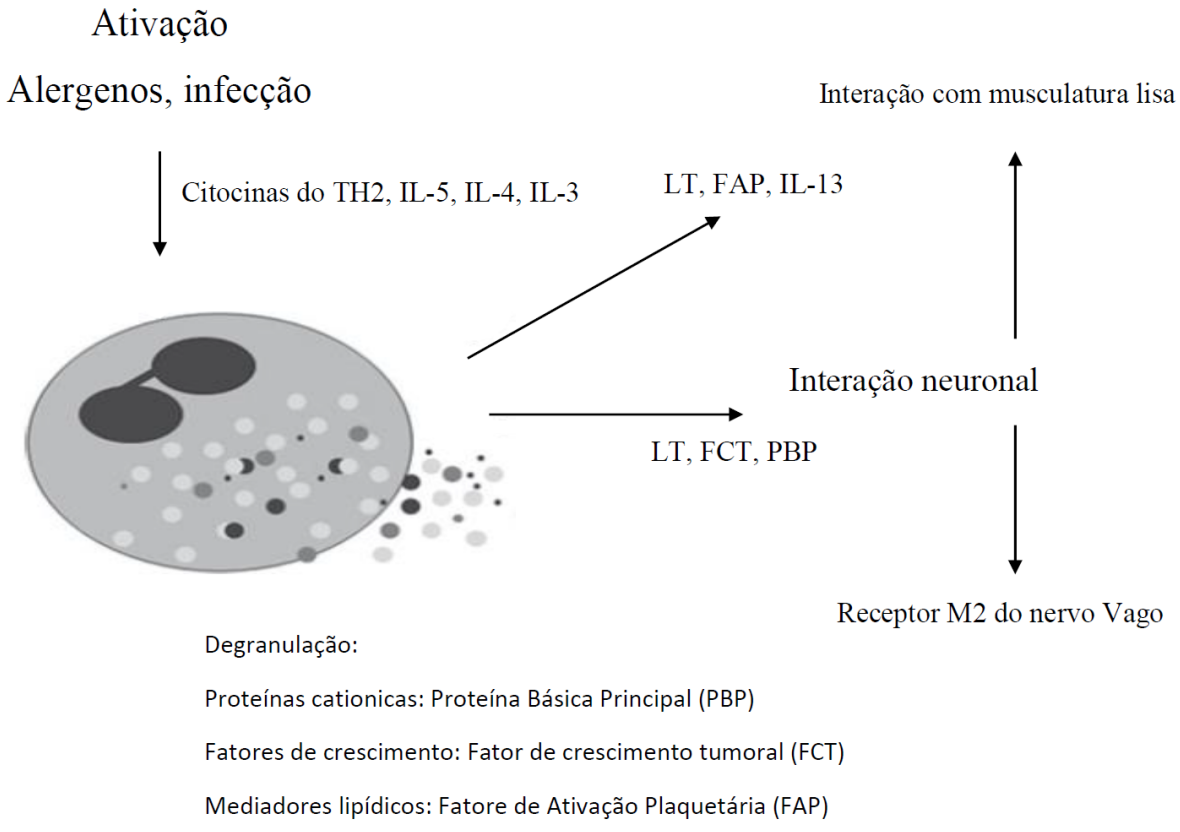
O Consenso Roma III para desordens funcionais gastrointestinais, define dispepsia funcional (DF) como a presença de sintomas que se presumem serem originados da região gastroduodenal na ausência de doença orgânica, sistêmica ou metabólica que os justifiquem. Para o diagnóstico dessa entidade, pelo menos um dos seguintes sintomas deverá estar presente: dor ou queimação epigástrica, plenitude pós-prandial e saciedade precoce. Os sintomas devem estar ativos nos últimos 3 meses, com início dos sintomas (primeira manifestação) pelo menos 6 meses antes do diagnóstico. Recentemente, o Consenso Roma IV reavaliou os critérios para definição de dispepsia funcional, e manteve praticamente inalterados os critérios estabelecidos pelo Consenso Roma III.[18]

A etiologia da dispepsia funcional ainda é pouco compreendida. Alguns potenciais mecanismos fisiopatogênicos são: alterações na motilidade gástrica e duodenal (capacidade reduzida de acomodação gástrica, hipomotilidade antral), hipersensibilidade gástrica e duodenal, alterações no funcionamento do eixo “cérebro-intestino” e infecção pelo *H. pylori.*[14] A presença de eosinofilia duodenal também tem sido relacionada como um possível fator etiológico da

dispepsia funcional,[5,6,19] através da potencial atuação dos eosinófilos na motilidade e sensibilidade do aparelho digestivo.

Os eosinófilos são ativados por alergenos, infecções e citocinas dos linfócitos T helper (TH2), Interleucinas (IL)-5, IL-4 e IL-13. Os produtos da degranulação dos eosinófilos, como o fator de crescimento nervoso, tem ação direta na inervação sensorial e a proteína básica principal pode induzir disfunção dos receptores muscarínicos M2 do nervo Vago. Fator de agregação plaquetária, leucotrienos e IL-13 podem agir diretamente na musculatura lisa, aumentando a reatividade e contratilidade. Portanto, os eosinófilos podem causar disfunção neurológica e provocar sintomas viscerais- Figura 2.[4]

9.4. **Figura 2.** Eosinófilos duodenais e dispepsia funcional.[4]



Legenda: TH2- linfócitos T helper 2, IL- Interleucinas, LT- leucotrienos, FAT- Fator de Ativação Plaquetária, PBP- Proteína Básica Principal, FCT- Fator de Crescimento Tumoral, M2- muscarínico tipo 2.

9.5. Relação entre a infecção gástrica pelo *H. pylori* e o número de eosinófilos duodenais

Um estudo do nosso grupo de pesquisa mostrou que a mediana da média dos eosinófilos duodenais por cinco campos de grande aumento (CGA) em indivíduos *H. pylori* positivos (13,2 eosinófilos/CGA) foi maior que em indivíduos *H. pylori* negativos (8,1 eosinófilos/CGA), com diferença estatisticamente significativa (*p*: 0,005), embora tenha sido um achado secundário do estudo.[9] O objetivo principal foi avaliar a relação entre infiltrado de eosinófilos duodenais com dispepsia funcional.

No estudo de Chaudhari e colaboradores, foram avaliados 50 pacientes dispépticos funcionais, com uma media de eosinófilos/CGA de 40,7 ± 26,9. Desses 50 pacientes, 24 eram *H. pylori* positivos e 26 *H. pylori* negativos. Não houve diferença na prevalência de eosinofilia duodenal entre pacientes *H. pylori* positivos e *H. pylori* negativos, mas foi observado correlação positiva entre a contagem de eosinófilos duodenais com a infecção gástrica pelo *H. pylori* (p= 0,012). O objetivo principal também foi avaliar a relação entre eosinofilia duodenal com dispepsia funcional.[1]

Outro estudo de Talley e colaboradores não encontrou diferença na prevalência de eosinofilia duodenal entre pacientes *H. pylori* positivos e *H. pylori* negativos, mas essa relação foi avaliada como achado secundário.[8] O estudo de Lijun Du e colaboradores mostrou um aumento na densidade de eosinófilos da mucosa gástrica, mas não na mucosa duodenal.[20]

Pelos nossos conhecimentos, não existem estudos na literatura que tenham avaliado, como objetivo primário, a relação da infecção pelo *H. pylori* com o número de eosinófilos duodenais. Kwang Jae Lee e colaboradores, sugerem que o *H. pylori* esteja envolvido na etiologias das inflamações duodenais, e reforça a necessidade de estudos para avaliar essa possível associação.[13]

9.6. **Tabela 1.** Eosinófilos duodenais por status *H. pylori* (estudos prévios).

Estudo	N Dispépticos Funcionais	Eosinófilos duodenais/CGA	p valor
Leite, C.[9]		Mediana:	
H. pylori positivo	26	13,6	<0,001
H. pylori negativo	16	6,1	
Chaudhari, AA.[1]			
H. pylori positivo	24	- *	0,012
H. pylori negativo	26		
Talley, NJ.[8 #]			
H. pylori positivo	27	- *	0,4 [β]
H. pylori negativo	72		
Du, L.[20]		Media:	
H. pylori positivo	25	49,04	0,502
H. pylori negativo	71	61,15	

Legenda: OR- odds ratio. *media/mediana não informada. [#] Incluídos pacientes dispépticos funcionais e controles assintomáticos. [β]Comparado apenas quanto a prevalência de eosinofilia duodenal.

10. Justificativa

Não existe definição na literatura, de qual é o papel da infecção gástrica pelo *H. pylori* sobre o número de eosinófilos duodenais. Poucos estudos avaliaram essa relação e os dados são insuficientes e contraditórios para uma conclusão mais definitiva.

Metanálises e estudos recentes têm demonstrado que a infecção pelo *H. pylori* pode ser uma das causas da dispepsia funcional.[21,22] E, embora a associação entre DF e eosinofilia duodenal (ED) seja controversa, alguns estudos demostraram associação positiva entre essas duas condições.[5,6]

Portanto, é importante saber se a infecção pelo *H. pylori* causa eosinofilia duodenal, que poderia explicar a origem dos sintomas em alguns pacientes dispépticos funcionais.

11. Questão da pesquisa

Existe diferença no número de eosinófilos duodenais entre pacientes dispépticos funcionais *H. pylori* positivos em relação aos *H. pylori* negativos?

12. Hipótese

O número de eosinófilos duodenais é significativamente maior nos pacientes dispépticos funcionais *H. pylori* positivos do que nos pacientes *H. pylori* negativos.

13. Objetivos

13.1. Principal

Avaliar o potencial papel da infecção pelo *H. pylori* no número de eosinófilos da mucosa duodenal, em pacientes dispépticos funcionais.

13.2. Secundários

Avaliar se o número de eosinófilos duodenais tem influência nos sintomas dispépticos.

Avaliar se variáveis demográficas ou ambientais podem influenciar no número de eosinófilos da mucosa duodenal.

Avaliar o papel dos eosinófilos duodenais, nos sintomas específicos da dispepsia funcional e nos sub grupos de dispépticos funcionais (dispepsia tipo dor epigástrica, dispepsia tipo desconforto pós-prandial ou sobreposição das síndromes).

14. Artigo em inglês

Duodenal Eosinophils: Potential Association to *Helicobacter pylori* Infection and Symptoms of Functional Dyspepsia

Authors

Felipe Mazzoleni; Luiz Edmundo Mazzoleni; Carlos Fernando de Magalhães Francesconi; Tobias Cancian Milbradt; Diego de Mendonça Uchoa; Sacha Allebrandt da Silva Ries; Pedro Guilherme Schaefer; Alexandra Cauduro Ponso Fernandes; Liane Golbspan; Daniel Simon

Introduction

Inflammatory infiltrates in the duodenal mucosa, such as duodenal eosinophilia, intraepithelial lymphocytosis and duodenal mastocytosis, have been increasingly studied.[1] These infiltrates usually do not cause significant endoscopic changes, nor microscopic changes in the villi and crypts. The etiology and clinical repercussions of these changes are still poorly understood. Some studies have evaluated the possible association of duodenal infiltrates with functional diseases of the gastrointestinal tract, such as functional dyspepsia and irritable bowel syndrome.[2] And, few studies have evaluated the role of *H. pylori* in the etiology of these duodenal histological changes, despite of this bacterium is the main etiologic agent of duodenal ulcers.[3]

Duodenal eosinophilia is associated with intestinal parasitosis and food allergies,[4] and has been implicated as a potential factor in the etiology of functional dyspepsia,[1,5,6] possibly by causing changes in digestive tract motility and sensitivity, as observed in animal models.[7] The association with *H. pylori* is poorly understood and was only evaluated as a secondary finding in previous studies, with controversial results.[1,8,9]

This study aims to evaluate the potential role of gastric *H. pylori* infection in the duodenal eosinophil count. We also evaluated the influence of duodenal eosinophils on the types of

dyspeptic symptoms and their intensity. The association between duodenal eosinophils and demographic variables was also evaluated.

Methods

Study Design

A cross-sectional study, carried out at the Department of Gastroenterology and Department of Pathology, from Hospital das Clínicas de Porto Alegre (HCPA), from the Universidade Federal do Rio Grande do Sul.

Population

We included 100 subjects (50 *H. pylori* positive and 50 *H. pylori* negative) from the HEROES (Helicobacter Eradication Relief Of Dyspeptic Symptoms) Trial.[10] Community-based and primary care subjects, of both sexes, were enrolled if aged 18 years or more, and having a diagnosis of functional dyspepsia according to the Rome III criteria. Exclusion criteria were: predominant symptoms of heartburn or irritable bowel syndrome, previous history of peptic ulcer disease; clinical manifestations of organic diseases: anorexia, anemia, dysphagia, digestive bleeding, weight loss greater than 10% of body weight and abnormal physical exam suggestive of organic diseases; a clinical picture suggestive of symptomatic biliary colic; previous upper endoscopic investigation with organic alterations; gastrointestinal surgery; previous treatment for *H. pylori*; presence of significant comorbidities (hepatopathies, heart diseases, nephropathies and neuropathies); prior diagnosis of systemic diseases or use of medications that are known to interfere with the motility or sensitivity of the upper gastrointestinal tract; gestation or women of childbearing potential; subjects with endoscopic findings other than gastritis, duodenitis or hiatal hernia: low intellectual level that impairs adequate understanding of the study procedures; not acceptance in participating in the study and/or signing the written informed consent.

Study Procedures

Subjects were recruited in the Helicobacter Eradication Relief Of Dyspeptic Symptoms (HEROES) Trial, between November 2006 and June 2008. At the screening visit were performed medical history, demographic data evaluation and physical exam. Subjects who met the inclusion criteria and did not present exclusion criteria were referred for laboratory tests and upper gastrointestinal endoscopy. The severity of the dyspeptic symptoms was evaluated by the PADYQ questionnaire (Porto Alegre Dyspeptic Symptoms Questionnaire).[11] This 11-item validated instrument evaluates the three most important symptoms of functional dyspepsia (upper abdominal pain, abdominal distension and early satiety) and also nausea and vomiting in the previous 30 days. This procedure allows assessing the severity of each symptom through its frequency, intensity and duration. In this questionnaire, the severity of symptoms varies from 0 (absence of symptoms) to 44 (severe symptoms) points, and subjects with more than 7 points are considered dyspeptic.

Endoscopies were performed at HCPA. Two experienced endoscopists performed the endoscopic procedures. In cases of disagreement between endoscopic diagnoses between the first two examiners, a third endoscopist was consulted. Endoscopic gastritis were classified according to the Endoscopic and Histological Classifications of Sydney.[12]

Two pathologists performed histologic exams. The severity of histological gastritis was graded according to the updated Sydney System.[12] The positivity of *H. pylori* was evaluated by urease test and gastric histology (Hematoxylin-eosin and Giemsa), and it should be positive for both methods. In case of disagreement between results, another pathologist defined the diagnosis.

In the present study, we used biopsies of the second duodenal portion of a sample from HEROES trial subjects, for hematoxylin-eosin staining. The eosinophil count in the duodenal mucosa was performed in five random high power fields (HPF), of the villi. All slides were

evaluated by four experienced pathologists, blinded for the gastric histology findings, *H. pylori* status and patients symptoms. In the five-nonoverlapping high power fields, eosinophils count was obtained and a simple arithmetic mean of those counts were made. The duodenal eosinophil count was evaluated between *H. pylori* positive and *H. pylori* negative subjects.

Subjects were evaluated for the association of duodenal eosinophils and the severity of dyspeptic symptoms, measured by the PADYQ questionnaire as a continuous variable and as categorical variables. PADYQ score were divided into two groups: up to 22 (up to 50% of maximum score) and higher than 22. PADYQ scores were also analyzed into terciles. Duodenal eosinophil count was further evaluated according to specific functional dyspeptic symptoms. Duodenal eosinophils counts were also evaluated by demographic variables and endoscopic findings.

Statistical analysis

The variables were analyzed through the SPSS program (Statistical Package for Social Sciences) version 21.0; values of $p < 0.05$ (two-tailed) were considered statistically significant. The median and the percentile 25-75 were used to evaluate the differences in eosinophil counts per high power field according to variables. The median of the arithmetic means of the duodenal eosinophils counts per five high power field were compared between *H. pylori* positive and *H. pylori* negative subjects.

Categorical variables were assessed using the chi-square test and quantitative variables were evaluated using Student's t-test or Mann-Whitney test, according to the results of the Shapiro-Wilk normality test. For comparisons between categorical variables with more than two groups, with non-parametric continuous variables, the Kruskal-Wallis test was used. The Spearman test was used to assess the correlation between variables. An age- and sex-adjusted

multivariate analysis was performed to evaluate the variables significantly associated to duodenal eosinophil count, including all variables with p-value ≤ 0.20 on univariable analysis.

Ethical Considerations

The study was developed according to Resolution 466/2012 of the National Health Council and approved by the Research Ethics Committee of the Hospital de Clínicas de Porto Alegre (HCPA). Written informed consent was obtained from all patients prior to enrollment. None of the authors of this study presented conflicts of interest.

Results

Women accounted for 88% of the sample of subjects, 82% were white and the average age was 41.7 years. There was no statistically significant difference in the baseline characteristics of *H. pylori* positive and *H. pylori* negative subjects (Table 1).

The biopsies were evaluated by four pathologists (25 slides per pathologist), and there was no significant difference in duodenal eosinophil counts among the pathologists, as measured by the Kruskal-Wallis test (p = 0.454). There was a loss, on the *H. pylori* negative group, because of an insufficient sample of duodenal biopsy.

Table 1. Baseline characteristics of subjects according to *H. pylori* status

Variable	**Total n= 100**	***H. pylori* positive n= 50**	***H. pylori* negative n= 50**	***p***
Age (years) – Mean ± SD	41.8 ± 13.8	39.7 ± 13.4	43.8 ± 13.9	0.137[a]
Sex – n (n%)				
Male	12 (12.0)	5 (10.0)	7 (14.0)	0.758[b]
Female	88 (88.0)	45 (90.0)	43 (86.0)	
Color – n (n%)				
White	82 (82.0)	39 (78.0)	43 (86.0)	0.435[b]
Non white	18 (18.0)	11 (22.0)	7 (14.0)	

BMI (n= 97)*– md [P25–75]	24,96 [22,72–28,89]	24,77 [22,72– 28,89]	25,59 [22,64– 28,96]	0.608[e]
Education (n= 98)*– n (n%)				
< 10 years	46 (47.0)	24 (49.0)	22 (45.0)	0.840[c]
≥ 10 years	52 (53.0)	25 (51.0)	27 (55.0)	
Marital status				
Single or without partner	56 (56.0)	27 (54.0)	29 (58.0)	0.840[c]
Married or with partner	44 (44.0)	23 (46.0)	21 (42.0)	
Income (R$) – md [P25–75]	1.200 [700– 2.000]	1.200 [800– 2.000]	1.150 [600– 2.000]	0.380[e]
Smoking status – n (n%)				
Never smoked	65 (65.0)	34 (68.0)	31 (62.0)	0.675[c]
Current/former	35 (35.0)	16 (32.0)	19 (38.0)	
Alcohol consumption– n (n%)				
No consumption	83 (83.0)	43 (86.0)	40 (80.0)	0.594[b]
Current/ former	17 (17.0)	7 (14.0)	10 (20.0)	
Local tea drinking (Mate) – n (n%)				
No	62 (62.0)	30 (60.0)	32 (64.0)	0.837[c]
Yes	38 (38.0)	20 (40.0)	18 (36.0)	
Coffee drinking– n (n%)				
No	31 (31.0)	16 (32.0)	15 (30.0)	>0,999[c]
Yes	69 (69.0)	34 (68.0)	35 (70.0)	
PADYQ (n= 92)*– md [P25–75]	26,00 [20,00–30,5]	26,00 [18,00–31,00]	25,50 [20,50 – 30,00]	0.888[e]
Functional dyspepsia subtype – n (n%)				
EPS	55 (55.0)	24 (48.0)	31 (62.0)	0.228[c]
PPDS	45 (45.0)	26 (52.0)	19 (38.0)	
Gastric endoscopic fingdings (n=96)*– n (n%)				
Normal	28 (29.2)	17 (35.4)	11 (22.9)	0.224[d]
Enanthematous	28 (29.2)	15 (31.3)	13 (27.1)	
Erosive	38 (39.6)	16 (33.3)	22 (45.8)	
Atrophic	2 (2.0)	-	2 (4.2)	

SD – standard deviation. md – median. P25–75 – Percentiles 25 e 75. BMI – body mass index. EPS – Epigastric pain syndrome. PPDS – Postprandial distress syndrome. PADYQ – Porto Alegre Dyspeptic Symptoms Questionnaire. *reduced n by missing data. [a]Student t test. [b]qui-square by correction for continuity [c] Fisher Exact test. [d]Pearson qui-square test. [e]Mann-Whitney U test

Outcomes

There were no statistically significant differences between the medians of duodenal eosinophils/HPF between *H. pylori* negative (4.6 eosinophils/HPF [P25–75: 2.8 to 7.2/CGA] and

H. pylori-positive subjects (4.7 eosinophils/HPF [P25-75: 3.4–8.4/CGA] (p= 0.403) Table 2 and Figure 1.

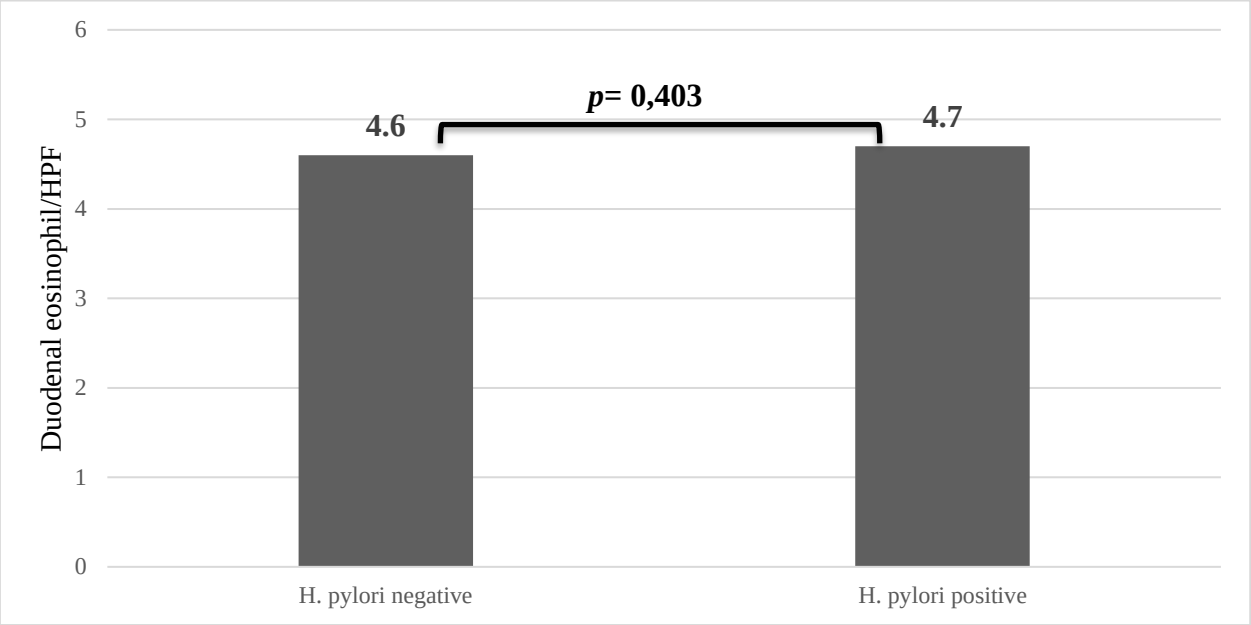
Table 2. Duodenal eosinophils/HPF according to *H. pylori* status and symptoms severity

Variable	**n**	**Duodenal Eosinophils/HPF md [P25–75]**	***p* value[#]**
H. pylori status (N = 99*)			
Negative	49	4.6 [2.8–7.2]	0,403
Positive	50	4.7 [3.4–8.4]	
PADYQ score (N= 92*)			
≤ 22	31	3.4 [2.2–6.0]	0.018
> 22	61	5.4 [3.4–7.6]	
PADYQ score (Terciles) (N= 92*)			
Mild	31	3.4 [2.2–6.0]	0,033**
Moderate	30	4.7 [3.2–6.4]	
Severe	31	5.8 [3.6–8.2]	

Abbreviations: HPF – high power fields, md – median, P25–75 – percentiles 27 e 75.

* reduced N by missing data.[#] Mann-Whitney U test.** Kruskal-Wallis test [pairwise comparison between groups 1 and 3 (p= 0,027)]

Figure 1. Duodenal eosinophil median/HPF according to *H. pylori* status



Subjects with a PADYQ score >22 (>50% maximum score) n= 61, had a significantly higher duodenal eosinophils count compared to subjects with a score of ≤22 (≤50% maximum score), n= 31 (p = 0.018)- Table 2 and Figure 2a.

The patients were divided by terciles, according to symptoms severity: group 1 with 31 subjects (mild symptoms) and PADYQ score up to 22; group 2 with 30 subjects (moderate symptoms) and PADYQ scores ranging from 23 to 29; and group 3 with 31 subjects (severe symptoms) and PADYQ score of 30 or more. The duodenal eosinophils count progressively increased within the groups: median eosinophils /HPF [Percentiles 25–75] in group 1 of 3.4 [2.2-6.0]; in group 2 of 4.7 [3.2-6.4]; and group 3 of 5.8 [3.6-8.2] (*p*= 0.033) – Tab 2 and Figure 2b. There was a statistically significant difference between groups 1 and 3 (p = 0.027), with Kruskal-Wallis test pairwise comparison. A trend of correlation between the continuous PADYQ score and the duodenal eosinophil count, when evaluated by the Spearman test (p = 0.071; Spearman's Rho = +0,189). This trend was even more pronounced, when we excluded one eosinophil count with the larger Cook's distance (1.063), that distorts the result of the test (p= 0,055; Spearman's Rho = +0,202).

Figure 2a. Duodenal eosinophil median/HPF according to symptoms severity

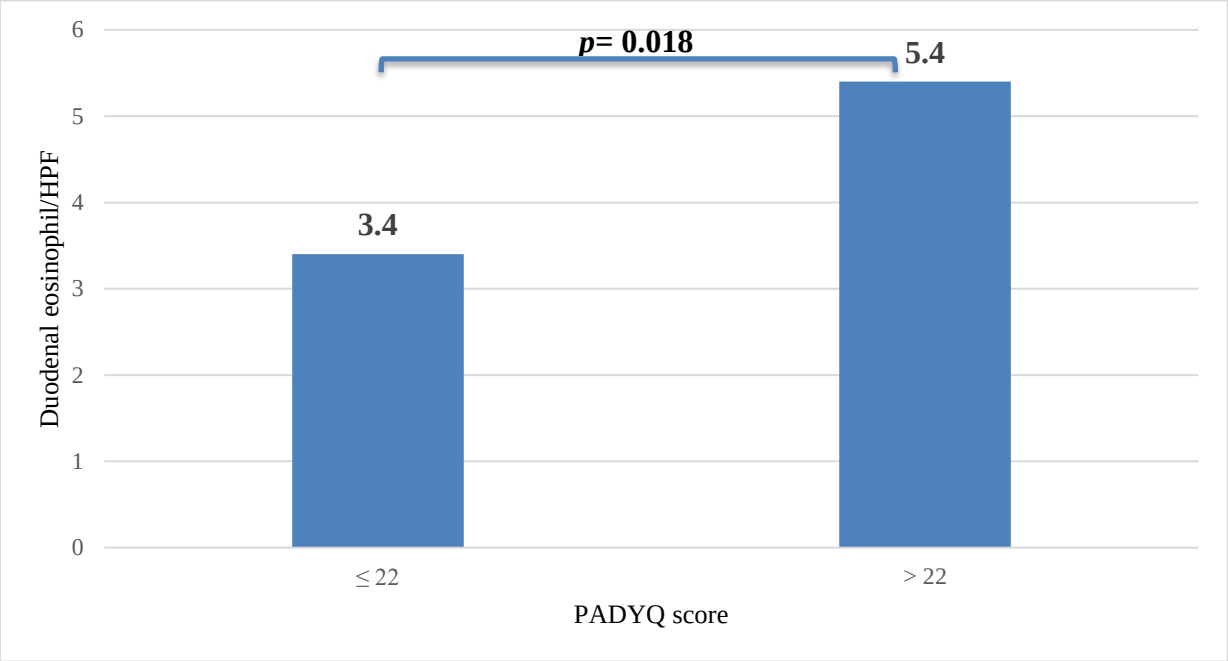
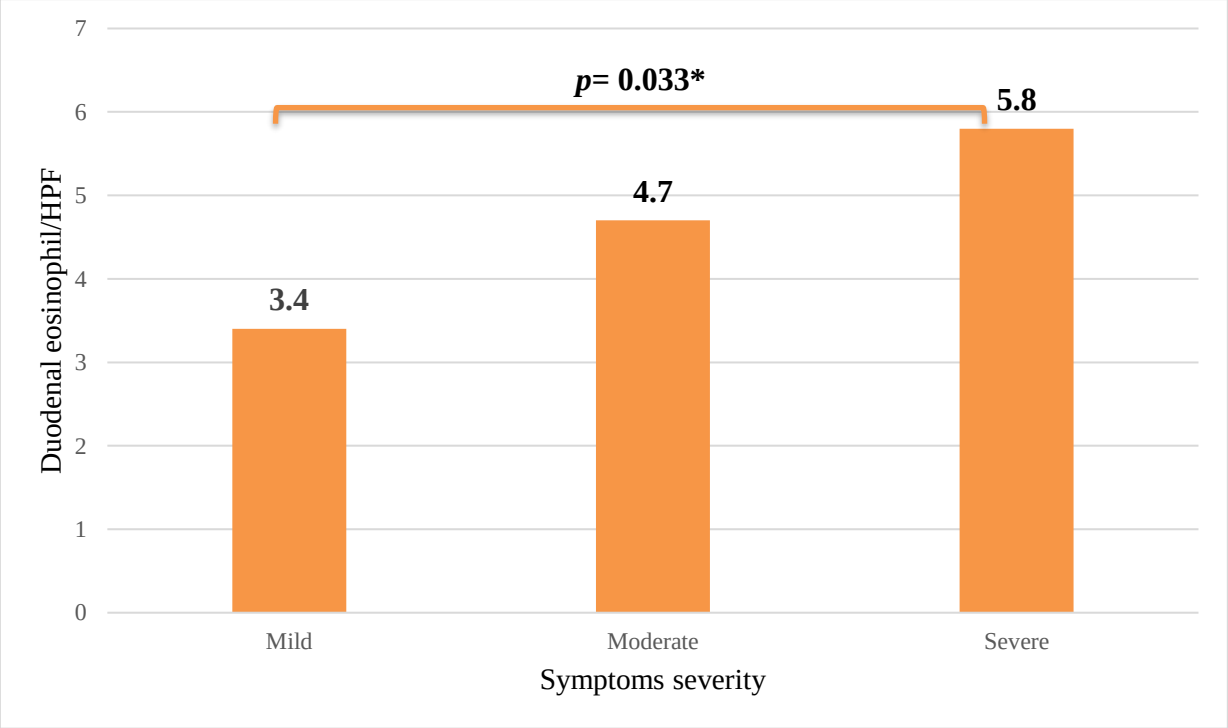


Figure 2b. Duodenal eosinophil median/HPF according to symptoms severity (groups)



*Kruskal-Wallis test pairwise comparison between groups 1 and 3 (p= 0.027)

No difference was observed in the duodenal eosinophil count for PADYQ-specific symptoms severity, but there was a trend to higher duodenal eosinophil count with severe

symptoms, when abdominal bloating was evaluated with early satiation (symptoms of postprandial distress syndrome)- Table 3. When Spearman test was performed between duodenal eosinophils and all the PADYQ questionnaire answers individually, only the duration of distension showed a statistically significant correlation (p = 0.037, Spearman's Rho +0.218). Also, there was no difference in the duodenal eosinophil count for the two dyspeptic syndromes, epigastric pain syndrome and postprandial distress syndrome (p = 0.602) - Table 4. The subtype of dyspepsia syndrome was self-reported by the patient at the first visit, taking into account the main symptom. A overlap of dyspeptic syndromes was observed in 82.6% of the subjects, when analyzed by the PADYQ score.

Table 3. Duodenal eosinophil count for dyspeptic symptoms (PADYQ)

Symptoms (N= 92)	**N= 92**	**Duodenal eosinophils/HPF md [P25–75]**	***p* value***
Epigastric pain			
Up to 50%	12	4.6 [2.6–7.6]	0.839
> 50%	80	4.6 [3.0–7.2]	
Abdominal bloating			
Up to 50%	11	4.2 [2.2–5.8]	0.224
> 50%	81	4.8 [3.0–7.4]	
Early satiation			
Up to 50%	43	5.0 [2.9–7.5]	0.526
> 50%	49	4.6 [3.0–7.2]	
Nausea			
Up to 50%	48	4.6 [3.0–7.6]	0.966
> 50%	44	4.9 [2.6–7.2]	
Vomiting			
Up to 50%	87	4.6 [3.0–7.3]	0.475
> 50%	5	3.2 [2.4–5.8]	
Abdominal bloating + Early satiation			
Up to 50%	11	2.8 [2.2–5.3]	0,080
> 50%	81	4.8 [3.2–7.6]	

HPF – High Power Field, md – median, [P25–75] – percentiles 25 e 75, *Mann-Whitney U test. Up to 50% – up to 50% score on specific symptom at PADYQ score. > 50% – more than 50% score on specific symptom at PADYQ score.

The duodenal eosinophils count was significantly higher in smokers than in nonsmokers (p = 0.030), and also in subjects with BMI ≥ 25 kg / m^2 compared to those with BMI <25 kg/m^2 (p = 0.035). There was a trend towards a higher duodenal eosinophils count in white subjects compared to non-white, and also in subjects with current or previous alcohol consumption (> 14 weekly doses for men and > 7 weekly doses for women) compared to subjects with no history of alcohol consumption- Table 4.

In multivariate regression analysis, adjusted by sex and age, the positive association of symptoms severity, evaluated by continuous PADYQ score, and duodenal eosinophil count was confirmed (p= 0.039). The other variable that influenced the duodenal eosinophil count was smoking (p = 0.021). There was a trend of white skin color (p= 0.052) and alcohol consumption (p= 0.077) to influence duodenal eosinophil count.

Table 4. Baseline caracteristic of subjects according to duodenal eosinophil/HPF

Variables	**N = 99***	**Duodenal eosinophil/HPF md [P25-75]**	***p* value**§
Age (years)			
< 50 years	70	5,0 [3,0–7,4]	0,444
≥ 50 years	29	4,2 [3,0–7,6]	
Sex			
Male	12	5,1 [2,5–8,3]	0,962
Female	87	4,6 [3,1–7,3]	
Color			
White	81	5,0 [3,2–8,0]	0,058
Non white	18	3,5 [2,4–6,0]	
BMI - kg/m^2 (N= 96)*			
< 25	48	4,5 [2,5–6,5]	**0,035**
≥ 25	48	5,7 [3,4–8,1]	
Education (N= 97)*– n (n%)			
< 10 years	46	4,8 [2,8–7,6]	0,718
≥ 10 years	51	4,6 [3,4–7,1]	

Marital status			
Single or without partner	55	4,8 [2,8–7,2]	0,430
Married or with partner	44	4,6 [3,3–7,9]	
Monthly income			
< R$ 1.200/month	47	4,2 [2,8–6,8]	0,129
≥ R$ 1.200/month	52	5,5 [3,4–7,8]	
Smoking status			
Never smoked	64	4,3 [2,6–7,1]	**0,030**
Current/ former	35	5,8 [3,4–8,1]	
Alcohol consumption			
No consumption	82	4,6 [3,0–7,2]	0,186
Current/ former	17	7,0 [3,4–10,4]	
Local tea drinking (Mate)			
No	61	4,6 [3,2–7,2]	0,587
Yes	38	5,1 [3,0–9,0]	
Coffee drinking– n (n%)			
No	31	4,6 [2,7–6,6]	0,455
Yes	68	4,9 [3,1–7,8]	
Functional dyspepsia subtype			
EPS	55	4,6 [3,0–7.2]	0,602
PPDS	44	4,8 [3,2–7,6]	
Gastric endoscopic findings (N=96)*			
Normal	28	4,9 [2,8–7,2]	
Enanthematous	27	3,6 [2,8–5,8]	0,227¶
Erosive	38	5,9 [3,2–8,2]	
Atrófic	2	7,4 [7,2–7,6]	

Abbreviations: *H. pylori* – *Helicobacter pylori*, HPF – High Power Fields, md – median, [P25–75] – percentiles 25 e 75, NSAIDs – Non steroidal anti-inflammatory drugs, BMI – body mass index, EPS – Epigastric Pain Syndrome, PPDS – Postprandial Distress Syndrome. §Mann-Whitney U test. ¶Kruskal-Wallis. *reduced N by unavailable data.

Discussion

Our results did not demonstrate an association between *H. pylori* infection and the duodenal eosinophil count, in functional dyspeptic patients. However, an association was found between the severity of dyspeptic symptoms and the duodenal eosinophils count. Subjects with mild dyspeptic symptoms had significantly lower duodenal eosinophil counts than subjects with severe symptoms. Smoking also had an influence on the duodenal eosinophil count.

Histological changes caused by *H. pylori* infection in the gastric mucosa have been extensively studied.[13] Lymphocyte and neutrophilic infiltrate in the gastric mucosa occur in almost 100% of the infected individuals. Eosinophilic infiltrates are also observed in gastric mucosa in *H. pylori* positive. Eosinophils, in addition to participating in the inflammatory response against the bacteria, are likely to play a role in the maintenance and recovery of gastric mucosal integrity.[14] However, little is known about the influence of *H. pylori* infection on histology of the duodenal mucosa. A study carried out prior to the evaluation of *H. pylori* as an important gastroduodenal pathogen demonstrated an increase in the duodenal eosinophils count in subjects with duodenal ulcers,[15] which is strongly associated with gastric *H. pylori* infection.[3] A previous study conducted by our research group found, as a secondary finding, a significantly higher duodenal eosinophils count in *H. pylori* positive than in *H. pylori* negative subjects.[9] However, other studies showed no association between *H. pylori* and the duodenal eosinophil count, but also as a secondary finding.[1,8,16] To the best of our knowledge, our current study was the first to evaluate the role of *H. pylori* in the duodenal eosinophil count as the main outcome, and our results showed no difference in the duodenal eosinophil count among *H. pylori* positive and *H. pylori* negative functional dyspeptic patients.

The definition of normal duodenal eosinophil count is controversial in the literature. Although there is no absolute definition of the normal amount of the duodenal eosinophil count, some authors suggest that values equal to or greater than 22 eosinophils per high power field should be considered abnormal, characterizing duodenal eosinophilia.[17] According to this criterion, only one patient in our study presented duodenal eosinophilia. This patient was *H. pylori* positive, but the study had no power to define this condition as being associated with *H. pylori* infection.

The median duodenal eosinophils by HPF in our sample of functional dyspeptic subjects was lower compared to other studies, from different countries, that showed medians/means

ranging from 16.0 to 58.0 eosinophils/HPF.[6,1,18] Even in asymptomatic controls of these studies, the duodenal eosinophils count was greater when compared to our sample of dyspeptic subjects, with means ranging from 9.5 to 55.1.[1,18] Lijun Du et cols. found the highest duodenal eosinophil count, with mean of 58.0 eosinophil/HPF. They performed evaluation for causes of tissue eosinophilia (stool ova and parasite, serum IgE) in all the subjects, with negative results, and an experienced pathologist reviewed all the slides to verify accuracy of the eosinophil count, confirming the results.[18] With these findings they suggest that different lifestyle and dietary factors may account for the difference in the number of eosinophil counts between populations. Food-induced immune activation in functional gastrointestinal disorders has been previously recognized.[19] There is a possibility that genetic factors also account for this difference.[20] Our results suggest that one environmental factor, smoking, may have influence in the duodenal eosinophil count. This association with smoking was also observed in an Australian cohort.[5]

Although our study did not find a significant association between *H. pylori* and an increase in the duodenal eosinophils count, our data demonstrated a directly proportional correlation between the duodenal eosinophils count and the severity of dyspeptic symptoms as assessed by the PADYQ score. Statistically significant differences were found in the duodenal eosinophils count among subjects with mild dyspeptic symptoms compare to those with severe symptoms. This corroborate with findings from other clinical studies and review articles that suggest an association of increase duodenal eosinophil and functional dyspepsia.[17] The difference found in our study, is that the density of duodenal eosinophils we found was lower when compared to most others in the literature. However, we have shown that variations in the duodenal eosinophil count may influence the severity of dyspeptic symptoms. Our results showed no difference in the duodenal eosinophil count among subtypes of functional dyspeptic syndromes, but most functional dyspeptic patients in our sample had concomitant symptoms of epigastric pain associated with symptoms of early satiety and/or postprandial fullness. There was

also no difference in the duodenal eosinophil count for the different types of dyspeptic symptoms, when analyzed individually.

For this study, we included a sample of the functional dyspeptic subjects from the HEROES Trial,[10] which strictly used the Rome III criteria for inclusion and used two methods for the *H. pylori* diagnosis, ensuring an accurate diagnosis of both functional dyspepsia and *H. pylori* status. Our sample were homogeneous, including only functional dyspeptic patients, avoiding other diseases as confounding factors for the duodenal eosinophil count. Experienced pathologists performed duodenal eosinophil counts. And, to our knowledge, this was the first study to evaluate the association between *H. pylori* and the duodenal eosinophil count as the primary endpoint. Other studies have made this assessment as a secondary finding.[1,8]

This study presents some limitations: it was a cross-sectional study which, therefore, cannot establish a causal association between the greater number of duodenal eosinophils and the more severe symptoms of functional dyspepsia; the sample was predominantly women; was conducted in a single centre, a characteristic that may limit its external validity, though we believe the predominantly white sample of European descent is representative of the Western population; and there were probably low power to determine if there is an association between duodenal eosinophil count and subtypes of dyspeptic syndromes or other demographic variables, such as alcohol consumption and subject color. The association of duodenal eosinophils with the severity of dyspeptic symptoms was a secondary finding in this study, although it was established as one of the secondary endpoints prior to the start of the study, and a validated questionnaire was used to obtain the symptoms data.

Conclusions

Our results do not confirm the finding of our previous study. However, our study corroborate other studies in which *H. pylori* showed no influence on duodenal eosinophil count.[1,8] However, our data showed an association between duodenal eosinophils and dyspeptic symptoms severity, corroborating other studies in literature which suggest a role of duodenal eosinophils in the etiology of functional dyspepsia. More studies, with larger number of subjects, and different designs, are necessary to better evaluate the influence of duodenal eosinophils in symptoms severity and different types of dyspeptic syndromes.

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15. Conclusão

Os resultados do nosso estudo não demonstraram associação da infecção da mucosa gástrica pelo *H. pylori* com o número de eosinófilos duodenais, em pacientes dispépticos funcionais. Entretanto, foi observada associação entre a intensidade dos sintomas dispépticos e a densidade de eosinófilos duodenais. Pacientes que apresentavam sintomas dispépticos leves, tiveram densidade de eosinófilos duodenais significativamente menor do que pacientes com sintomas acentuados. Nossos resultados não mostraram diferença na densidade de eosinófilos duodenais entre subtipos de síndromes dispépticos funcionais, mas a maioria dos pacientes dispépticos funcionais em nossa amostra apresentaram sintomas concomitantes de dor epigástrica associada a sintomas de saciedade precoce e / ou plenitude pós-prandial. Também não houve diferença na contagem de eosinófilos duodenais para os diferentes tipos de sintomas

dispépticos, quando analisados individualmente. Tabagismo também apresentou influência no número de eosinófilos duodenais.

16. Perspectivas / Considerações finais

Diversas hipóteses foram geradas a partir dos dados obtidos e, por isso, como principais perspectivas do presente trabalho podemos:

a) avaliar a evolução da densidade de eosinófilos duodenais em pacientes dispépticos funcionais *H. pylori* positivos antes e após a erradicação do *H. pylori*, através de um ensaio clínico randomizado;
b) avaliar a resposta sintomática de pacientes dispépticos funcionais *H. pylori* positivos, erradicados x não erradicados, de acordo com a densidade de eosinófilos duodenais basal. Essa avaliação pode permitir a identificação de um subgrupo de dispépticos funcionais, em que a presença de eosinófilos duodenais pode apresentar um papel mais relevante do que o *H. pylori* na etiologia da dispepsia funcional;
c) Realizar cálculo amostral para determinar a amostra necessária para avaliar a possível associação dos eosinófilos duodenais com as síndromes dispépticas específicas.

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18. Anexo

ANEXO I - QUESTIONÁRIO DE SINTOMAS DISPÉPTICOS

Com relação aos últimos 30 dias

DOR

Qual a intensidade da dor abdominal (superior) na maioria dos dias neste período? ( )
0. Ausente
1. Muito leve
2. Leve
3. Moderada
4. Forte
5. Muito forte

Qual a duração da dor na maioria dos dias neste período? ( )
0. Não se aplica
1. Alguns minutos (menos que 30 minutos)
2. Menor que 2 horas
3. Maior que 2 horas

Com que frequência os Sr./Sra. apresentou dor abdominal nos últimos 30 dias? ( )
0. Não se aplica
1. Raramente
2. 1 a 2 dias/semana
3. Quase diariamente
4. Diariamente

ESCORE TOTAL DOR _____ (máximo 12 pontos)

NÁUSEAS/VÔMITOS

Qual a intensidade das náuseas na maioria dos dias deste período? ( )
0. Ausente
1. Muito leve
2. Leve
3. Moderada
4. Forte
5. Muito forte

Qual a duração aproximada da maioria dos episódios de náuseas? ( )
0. Não se aplica
1. Alguns minutos (menos que 30 minutos)
2. Menor que 2 horas
3. Maior que 2 horas

Com que frequência o Sr./Sra. apresentou náuseas nos últimos 30 dias? ( )

0. Não se aplica
1. Raramente
2. 1 a 2 dias/semana
3. Quase diariamente
4. Diariamente

Com que frequência o Sr./Sra. apresentou vômitos nos últimos 30 dias? ( )

0. Não se aplica
1. Raramente
2. 1 a 2 dias/semana
3. Quase diariamente
4. Diariamente

ESCORE TOTAL NÁUSEAS/VÔMITOS: _____ (máximo 16 pontos)

<u>DISTENSÃO/SACIEDADE</u>

Qual a intensidade da sensação de distensão ("estufamento"/inchaço) nos últimos 30 dias? ( )

0. Ausente
1. Muito leve
2. Leve
3. Moderada
4. Forte
5. Muito forte

Qual a duração destes episódios nestes períodos? ( )

0. Não se aplica
1. Alguns minutos (menos que 30 minutos)
2. Menor que 2 horas
3. Maior que 2 horas

Com que frequência os Sr./Sra. apresentou esses episódios de distensão/inchaço no abdômen superior nos últimos 30 dias? ( )

0. Não se aplica
1. Raramente
2. 1 a 2 dias/semana
3. Quase diariamente
4. Diariamente

Com que frequência o Sr./Sra. apresentou sensação de estar com o estômago cheio logo após começar a comer, nos últimos 30 dias? ( )

0. Sem saciedade precoce
1. Raramente
2. 1 a 2 dias/semana
3. Quase diariamente
4. Diariamente

ESCORE TOTAL DISTENSÃO/SACIEDADE _____ (máximo 16 pontos)

PONTUAÇÃO TOTAL DOS SINTOMAS DISPÉPTICOS:_____ (máximo 44 pontos)