Journal of Gastroenterology and Hepatology

JGHF

doi:10.1111/jgh.16051

ORIGINAL ARTICLE - GASTROENTEROLOGY (CLINICAL)

Low-grade duodenal eosinophilia is associated with *cagA* in *Helicobacter pylori*-related dyspepsia

Fernando Javier Barreyro,[*,†] Nicolas Sanchez,[*] Maria Virginia Caronia,[*] Karina Elizondo,[§] Graciela Jordá,[‡] Adolfo Schneider[§] and Pedro Dario Zapata[*,†]

[*]Laboratorio de Biotecnología Molecular (BIOTECMOL), Universidad Nacional de Misiones, Facultad de Ciencias Exactas Químicas y Naturales, Instituto de Biotecnología de Misiones "Dra. Maria Ebbe Reca" (InBioMis), [‡]Departamento Microbiología, Universidad Nacional de Misiones, Facultad de Ciencias Exactas Químicas y Naturales, Misiones, [†]CONICET, Buenos Aires, [§]Fundación HA Barceló, Instituto Universitario en Ciencias de la Salud, Santo Tomé, Argentina

Key words
cagA, duodenal eosinophilia, duodenal inflammation, functional dyspepsia, *H. pylori*.

Accepted for publication 29 October 2022.

Correspondence
Dr Barreyro, Fernando Javier, Biotechnology Institute of Misiones (InBioMis), National University of Misiones, National Scientific and Technical Research Council (CONICET), 7 ½ KM Route 12, National University of Misiones Campus, Posadas, Misiones, Argentina.
Email: ferbarreyro@hotmail.com

Author contributions: FJB: study concept and design, acquisition of data, analysis and interpretation of data, statistical analysis, drafting of the manuscript, obtained funding. NS, GJ, and AS: acquisition of data, analysis and interpretation of data, statistical analysis. VC and KE: acquisition of histological data, analysis and interpretation of data, critical revision of the manuscript. PDZ: analysis and interpretation of data, critical revision of the manuscript. All authors have approved the final version of the manuscript.
Financial support: The study is supported by a grant of the clinical research fund of Fundación HA Barceló.
Guarantor of the article: Fernando Javier Barreyro

Abstract

Background and Aim: Functional dyspepsia (FD) is a multifactorial disorder. *Helicobacter pylori* (*H. pylori*)-related dyspepsia (HpD) may be considered a separate entity. Duodenal eosinophilia is a potential pathogenic mechanism in FD. However, the impact of duodenal eosinophilia and *H. pylori* virulence genes in HpD was not explored. We aim to evaluate the association of *H. pylori* virulence genes and low-grade duodenal eosinophilia in HpD.
Methods: A multi-center cross-sectional study was conducted. A total of 301 patients who meet Rome-III criteria were selected before upper endoscopy, and 95 patients were included after normal endoscopy and positive *H. pylori* in gastric biopsies were assessed. Clinical parameters, *H. pylori* virulence genes (*cagA*, *oipA*, and *vacA*) and duodenal histology were evaluated.
Results: Sixty-nine (72%) patients had epigastric pain syndrome (EPS), 17 (18%) post-prandial distress syndrome (PDS) and 9 (10%) EPS/PDS overlap. FD syndromes were not associated with *cagA* or *oipA* strains. A significantly trend of *vacA s1/m1* (78%) and s1/m2 (80%) positive strains in EPS was observed. Histological duodenal grading of chronic inflammation, low-grade duodenal eosinophilia and intra-epithelial lymphocytes showed no difference in *oipA* and *vacA* strains. Low-grade duodenal eosinophilia was significant in *cagA* positive strain, and the OR for low-grade duodenal eosinophilia with *H. pylori cagA* positive strain was 4.2 (95% CI, 1.78–9.93). Adjusting for age, gender, smoking, diabetes, alcohol, PPI, and NSAID, the OR was 5.44 (1.989–14.902).
Conclusion: Our findings suggest that low-grade duodenal eosinophilia is significantly associated with *cagA* strain in HpD.

Introduction

Functional dyspepsia (FD) is a highly prevalent disease affecting approximately 10% of the general adult population,[1] with wide variation related to country and criteria used to define its presence.[2] FD symptoms include epigastric pain, epigastric burning, postprandial fullness, or early satiety, present for at least 6 months with no structural disease on routine investigation.[3] According to Rome-III criteria FD is classified as syndromes,[3,4] including postprandial distress syndrome (PDS) and epigastric pain syndrome (EPS). Indeed, FD is a chronic functional gastrointestinal disorder associated with increased health care cost, impaired work productivity,[5] and substantially reduced health-related quality of life.[6]

The etiopathogenesis of FD is considered multifactorial including associations with the gut-brain axis such as psychological distress, altered microbiota, post-infectious gastroenteritis, and *H. pylori*, although dyspeptic symptoms associated with *H. pylori* by Kyoto consensus may also be considered as a separate entity.[7] *H. pylori* eradication therapy with antibiotics

© 2022 Journal of Gastroenterology and Hepatology Foundation and John Wiley & Sons Australia, Ltd.

improves symptoms in *H. pylori*-related dyspepsia (HpD) with a number needed to treat (NNT) of 9.[8] This effect was greater with metronidazole-containing regimen,[9] in patients with microscopic duodenitis[10] and with successful eradication of *H. pylori*.[8]

Several neuro-gastroenterological mechanisms have been proposed to explain dyspeptic symptoms; however, the pathophysiology is not fully clarified.[2] In recent studies, no correlations between gastric motor disorders with either PDS and EPS were found,[11] raising the possibility that primary gastric dysfunction is not the main trigger of FD symptoms.[12] In the last decade, the theoretical basis of the concept of FD has begun to change, in light of new data focus on the duodenum.[13] It was observed that in FD patients the duodenal mucosa was characterized by a low grade-duodenal eosinophilia illustrated by an increase in eosinophils count[14] and degranulation,[15] mast cells degranulation,[16] and impaired permeability.[16] This low-grade duodenal eosinophilia was associated with submucosal neural dysfunction.[17] Noteworthy, *H. pylori* infection alone does not have a clinical impact on the symptoms of FD patients; however, the co-existence of duodenal inflammation and *H. pylori* infection significantly enhanced the severity of dyspeptic symptoms.[18] These findings suggest that duodenal inflammation may be an important pathogenic feature in HpD. Furthermore, *H. pylori cagA* positive strain was associated with epigastric pain rather than distention/bloating symptoms score in a German study of patients with non-ulcer dyspepsia.[19] However, another study form China observed that postprandial distress syndrome was associated with *cagA* positive strain.[20] Notably, histological evaluation of duodenal inflammation by *H. pylori* virulence genes in HpD was not assessed. The aim of this study was to evaluate the association of *H. pylori* virulence genes and low-grade duodenal eosinophilia in HpD.

Methods

Patients with FD symptoms based on Rome-III criteria who underwent upper endoscopy at IOT Medical Center, Simes Medical Institute (Posadas City, Province of Misiones) and University Hospital San Juan Bautista (Santo Tomé City, Province of Corrientes), Argentina, were evaluated in this prospective observational study. We consecutively recruited patients newly diagnosed with FD who were scheduled to undergo upper gastrointestinal endoscopy from January 2019 to November 2020. The criteria for inclusion were (i) age between 18 and 70 years; (ii) symptoms meeting Rome-III criteria; (iii) positive Giemsa stain for *H. pylori* in gastric biopsies; and (iv) unremarkable endoscopic findings. The criteria for exclusion before upper endoscopy were (i) progressive, severe diseases requiring active medical management (e.g. advanced congestive heart failure, uncontrolled diabetes, decompensated cirrhosis, end-stage renal failure, neurological disease, advanced cancer, or psychiatric disorder); (ii) those with a bleeding tendency or taking warfarin, aspirin, or antiplatelet drugs; (iii) medical conditions known to increase peripheral and tissue eosinophilia (inflammatory bowel disease, celiac disease, vasculitis, connective tissue disease, hypereosinophilia syndrome, active infection, and transplantation); (iv) atopic disease such as food allergies (milk, eggs, peanuts, tree nuts, fish, shellfish, fruit, and vegetables), asthma, allergic rhinitis or drug reaction, and eczema; (v) patients who had taken an antibiotic and corticosteroids drugs within the past 3 weeks; and (vi) history of previous significant gastrointestinal pathology (gastro-esophageal reflux disease and peptic ulcer disease) and history of gastrointestinal surgery (except appendectomy, cholecystectomy, and hernia repair). Patients receiving proton pump inhibitors (PPI) or non-steroidal anti-inflammatory drugs (NSAID) were advised to suspend 14 days before endoscopy. The criteria for exclusion after upper endoscopy were (i) evidence of active peptic ulcer disease or gastro-esophageal erosive esophagitis; (ii) evidence of gastric malignant disease; (iii) signs of celiac disease; and (iv) not available gastric or duodenal biopsies. The study was approved by the local ethical committee. Written informed consent was obtained from all participants.

Abdominal symptom evaluation. All participants completed the simplified abdominal symptom questionnaire at entry and after 6 months of eradication therapy, which contains the frequency (times per week) and severity of abdominal symptoms (encompass how the symptoms affect daily activities by numeric analog scale from 0 to 5). FD patients satisfied the Rome-III criteria for the past 3 months with symptom onset at least 6 months prior to diagnosis.[3] The diagnostic criteria for FD included one or more of the following: early satiety, postprandial fullness, epigastric pain, or epigastric soreness. In this case, early satiety and postprandial fullness were defined as experiencing symptoms at least 3 times/week, and epigastric pain or epigastric soreness were defined as experiencing symptoms at least 1 time/week.[3] FD was divided into two subtypes depending on the symptoms: Epigastric pain syndrome (EPS) is associated with epigastric pain or epigastric soreness, and postprandial distress syndrome (PDS) is associated with early satiety or postprandial fullness. Those who meet both criteria were classified as EPS/PDS overlap syndrome.

Endoscopy. All recruited participants underwent upper gastrointestinal endoscopy performed by experienced endoscopists (F. J. B. and A. S.). Biopsy specimens were collected from lesser curvature of gastric body (two biopsies), lesser curvature of gastric antrum (two biopsies), and second portion of duodenum (D2) (three biopsies) using a Radial Jaw 3 forceps (Boston Scientific, MA, USA). Only patients with normal endoscopy findings were included.

Histopathologic analysis. Biopsies were fixed in 10% formalin and processed to paraffin embedding for hematoxylin and eosin (HE) staining by routine methods. The presence of *H. pylori* was assessed on gastric biopsies using Giemsa staining in all patients. Duodenal inflammation was assessed semi-quantitatively on HE slides for presence and severity of microscopic duodenitis according to the Updated Sidney Criteria.[18,21] For the purpose of this study, to determine duodenal eosinophils (D-EO) at D2 lamina propria, five non-overlapping fields on the slides (40× field, HPF) were selected with the highest eosinophils density and quantified, data is expressed as mean x HPF, grading as follow: mild: <10, moderate: ≥ 10 to < 20, and marked: ≥ 20. Although there are no universally accepted criteria for defining abnormal eosinophil counts, we graded semi-quantitatively each by 10/HPF based on previous data of Whittington,[22] Kokkonen,[23] Lowichik,[24] Kalach,[25] Lee,[26] Leite[27] and Genta[28] where a threshold of >10 or >20/HPF was used to discriminate higher duodenal eosinophil

© 2022 Journal of Gastroenterology and Hepatology Foundation and John Wiley & Sons Australia, Ltd.

densities. Duodenal architecture and villi/crypt ratio were assessed to exclude celiac disease,[29] and intra-epithelial lymphocytes (IEL) were quantified per 100 enterocytes in 3 to 5 villi on 40× field and grading: very mild: 0–9, mild: ≥ 10 to <20, moderate: ≥20 to <40, and marked: ≥40. For the purpose of this study, low-grade duodenal inflammation was defined as the presence of both ≥ moderate chronic inflammation and eosinophils densities ≥10 HPF.

DNA extraction and polymerase chain reaction (PCR). DNA extraction from gastric biopsies was performed according to the manufacturer's instructions (ADN PuriPrep-T kit, InbioHighway, Argentina). Samples were stored at −20°C until used. PCR was performed by using specific primers. Target gene, amplicon size, primer names, and sequences are shown in Table 1. For PCR amplification, 50 ng of DNA samples was added to a PCR mixture containing 20 µmol forward and reverse primers, 15 µL of MINT Master Mix 2× (InbioHighway, Argentina) to the total volume of 25 µL. PCR amplification was performed under the following conditions: initial denaturation at 94°C for 4 min followed by 35 cycles of denaturation at 95°C for 30 s, annealing for 30 s (Table 1), extension at 72°C for 30 s, and final polymerization at 72°C for 5 min (Labnet MultiGene MiniThermocycler). The PCR reaction products were electrophoresed on 2% agarose gel (Invitrogen, USA), and the bands were visualized by ethidium bromide staining. The *cagA*,[30] *oipA*,[31] and *vacA*[32] statuses were determined from *H. pylori* positive samples by PCR using their respective primers as described in Table 1.

Statistical analysis and sample size. Sample size calculation was performed assuming a prevalence of duodenal eosinophilia in FD of 47%[33] and 70% in HpD.[27] We calculated, with 90% of power and alpha level of 0.05, that at least 92 patients with *H. pylori*-related dyspepsia would be needed for the study.

Data were presented as mean ± SD or number of subjects (% of total) as appropriate. Differences between groups were analyzed by Student's *t*-test or ANOVA for normal distribution and Wilcoxon rank sum test or Kruskal–Wallis test for non-normal distribution. Categorical values were compared using chi-square tests. The relationship between duodenal eosinophilia and *H. pylori* virulence genes were examined by logistic

Table 1 Primer sets used for genotyping *H. pylori* virulence genes by PCR

Target site	Amplicon size (bp)	Primer names and sequences	Annealing temperature	References
cagA	189	cagA-F (5-TTGACCAACAACCACAAACCGAAG-3) cagA-R (5-CTTCCCTTAATTGCGAGATTCC-3)	62°C	28
oipA	516	oipA-F (5-CCATGAAAAAAGCTCTCTTACT-3) oipA-R (5-GTTGAACGAAGGGTAAAAGGGC-3)	55°C	30
vacAs1	259	VA1-F (5-ATggAAATACAACAAACACAC-3) VA1-R (5-CTgCTTGAATgCgCCAAAC-3)	56°C	31
vacAs2	286	VA1-F (5-ATggAAATACAACAAACACAC-3) VA1-R (5-CTgCTTGAATgCgCCAAAC-3)	56°C	31
vacAm1	290	VA3-F (5-GgTCAAAATgCggTCATgg-3) VA3-R (5-CCATTggTACCTgTAgAAAC-3)	56°C	31
vacAm2	352	VA4-F (5-GgAgCCCCAggAAACATTg-3) VA4-R (5-ATAACTAgCgCCTTgCAC-3)	56°C	31

PCR primer sets, annealing temperature and size of the PCR products used for genotyping *H. pylori*. F – forward; R – reverse.

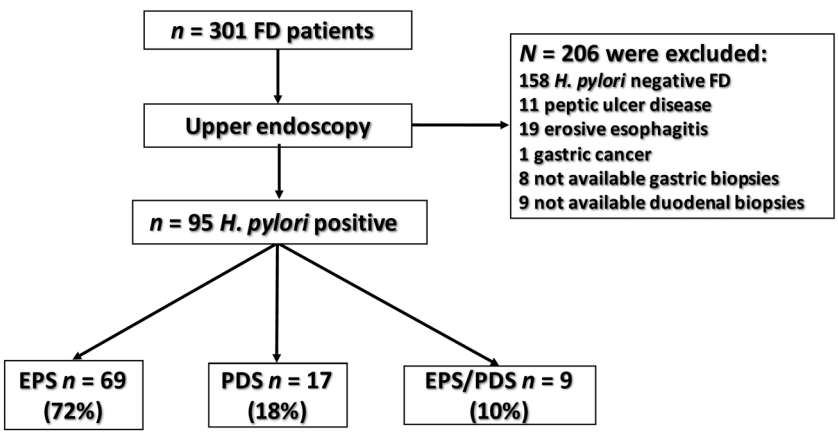

FIGURE 1 Study flow-chart. Functional dyspepsia (FD), epigastric pain syndrome (EPS), postprandial distress syndrome (PDS).

© 2022 Journal of Gastroenterology and Hepatology Foundation and John Wiley & Sons Australia, Ltd.

regression model (binary response variable, presence or absence of low-grade duodenal eosinophilia). Univariate models (only *H. pylori* virulence genes as a predictor of variable) and multiple predictor variable models including age,[28] gender, diabetes/impaired fasting glucose, smoking,[34] alcohol consumption,[35] NSAID,[36] and PPI[37] as covariates were assessed. The covariates of the regression model were selected based on investigator criteria and previous published data associated with a trend of increase or reduced eosinophils at upper gastrointestinal tract. Data were analyzed using SPSS 22.0, Jamovi 2.2.5 and Med-Calc, and a two-tailed $P < 0.05$ was considered statistically significant.

Results

Study population. Three hundred and one patients who meet Rome-III criteria were evaluated before upper endoscopy, 125 patients were *H. pylori* positive after upper endoscopy and 95 were included for analysis. A flowchart of the study and baseline characteristics of patients appear in Figure 1 and Table 2. The cohort of patients was comprised of 52 (55%) women and 43 (45%) men with a median age of 48.6 ± 12.5 years. The FD syndromes were as follows: 69 (72%) patients had EPS, 17 (18%) PDS and 9 (10%) were EPS/PDS overlap. In relation to clinical comorbidities: 27 (29%) patients had type-II diabetes mellitus/impaired

Table 2 *H. pylori*-related dyspepsia subtypes and clinical variables

Variable	Total (*n* = 95)	EPS (*n* = 69)	PDS (*n* = 17)	EPS/PDS (*n* = 9)	*P*
Age (SD)	48.6 (12.5)	47.7 (12.4)	48.9 (13.4)	54.6 (9.7)	ns
Gender (Fem %)	52 (55%)	37 (54%)	12 (71%)	3 (33%)	ns
BMI (SD)	27.2 (4.7)	27.3 (4.7)	26.4 (4.7)	27.6 (4.6)	ns
Obesity (%)	20 (22%)	13 (20%)	4 (25%)	3 (33%)	ns
PPI (%)	34 (36%)	31 (45%)*	2 (12%)	1 (11%)	< 0.05
NSAID (%)	37 (39%)	31 (45%)	1 (6%)	5 (56%)	< 0.05
Diabetes/IFG (%)	27 (28%)	21 (30%)	3 (18%)	3 (33%)	ns
Hypertension (%)	27 (28%)	18 (26%)	4 (24%)	5 (56%)	ns
Smoking (%)	33 (35%)	24 (35%)	6 (35%)	3 (35%)	ns
Alcohol (%)	60 (63%)	44 (64%)	10 (59%)	6 (63%)	ns
Appendectomy (%)	26 (27%)	22 (32%)	2 (12%)	2 (22%)	ns
Cholecystectomy (%)	14 (15%)	11 (16%)	2 (12%)	1 (11%)	ns

Data are expressed as mean (± SD, standard deviation), or percentage (%) of total. For continuous variables, ANOVA was used. For categorical variables, chi-square test was used.
*$P < 0.05$.

Table 3 *H. pylori* virulence genotypes and clinical variables

	cagA		*oipA*		*vacA*			
	Positive (*n* = 43)	Negative (*n* = 52)	Positive (*n* = 65)	Negative (*n* = 30)	s1 m1 (*n* = 41)	s1 m2 (*n* = 25)	s2 m1 (*n* = 4)	s2 m2 (*n* = 17)
Age (SD)	49.8 (12.1)	47.5 (12.8)	47.9 (12.4)	50 (12.5)	47.9 (12.2)	51.2 (11.4)	32.5 (12.1)*	49.7 (12.5)
Gender (Fem %)	24 (56%)	28 (54%)	35 (54%)	17 (57%)	20 (49%)	14 (56%)	3 (75%)	10 (55%)
BMI (SD)	25.8 (4.3)*	28.5 (4.7)	27.3 (4.7)	26.7 (3.9)	26.4 (4.3)	28.2 (4.6)	27.6 (10.6)	27 (4.8)
Obesity (%)	6 (14%)	14 (27%)	14 (21%)	6 (15%)	7 (17%)	5 (20%)	1 (25%)	5 (29%)
EPS (%)	29 (67%)	40 (77%)	50 (77%)	19 (63%)	32 (78%)*	20 (80%)*	4 (100%)	7 (41%)
PDS (%)	9 (21%)	8 (15%)	11 (17%)	6 (20%)	8 (19%)	2 (8%)	0	6 (35%)
EPS/PDS overlap (%)	5 (12%)	4 (8%)	4 (6%)	5 (17%)	1 (2%)	3 (12%)	0	4 (23%)
PPI consumption (%)	14 (33%)	20 (38%)	21 (32%)	13 (43%)	17 (41%)	5 (20%)	2 (50%)	7 (41%)
NSAID consumption (%)	19 (44%)	18 (35%)	23 (35%)	14 (47%)	13 (327)	13 (52%)	1 (25%)	5 (29%)
Diabetes/IFG (%)	10 (23%)	17 (33%)	19 (29%)	8 (27%)	11 (27%)	9 (36%)	1 (25%)	3 (18%)
Hypertension (%)	11 (26%)	16 (31%)	16 (25%)	11 (37%)	9 (22%)	8 (32%)	1 (25%)	5 (29%)
Smoking (%)	18 (42%)	15 (29%)	24 (37%)	9 (30%)	18 (44%)	7 (28%)	1 (25%)	6 (35%)
Alcohol consumption (%)	27 (63%)	33 (63%)	38 (58%)	22 (73%)	26 (63%)	15 (60%)	2 (50%)	10 (59%)
Appendectomy (%)	12 (28%)	14 (27%)	16 (25%)	10 (33%)	11 (27%)	5 (20%)	2 (50%)	6 (35%)
Cholecystectomy (%)	8 (19%)	6 (11%)	12 (18%)	2 (7%)	9 (22%)	2 (8%)	0	1 (6%)

Data are expressed as mean (± SD, standard deviation), or percentage (%) of total. For continuous variables, unpaired *t*-test or ANOVA was used. For categorical variables, chi-square test was used.
*$P < 0.05$.

© 2022 Journal of Gastroenterology and Hepatology Foundation and John Wiley & Sons Australia, Ltd.

fasting glucose (IFG), 27 (29%) patients had hypertension, mean BMI was 27.2 ± 4.7 kg/m^2 and 20 (21%) patients had obesity (BMI ≥ 30 kg/m^2), regular smoking was present in 33 (35%) patients, alcohol consumption daily or occasional was referred by 60 (63%) subjects. NSAID and PPI consumption was detected and discontinued before upper endoscopy in 37 (39%) and 34 (36%) patients respectively (Table 2).

Then, we evaluated the association of FD syndromes and clinical variables in our cohort. There were no differences in FD syndromes at age, gender, BMI, obesity, diabetes/IFG, hypertension,

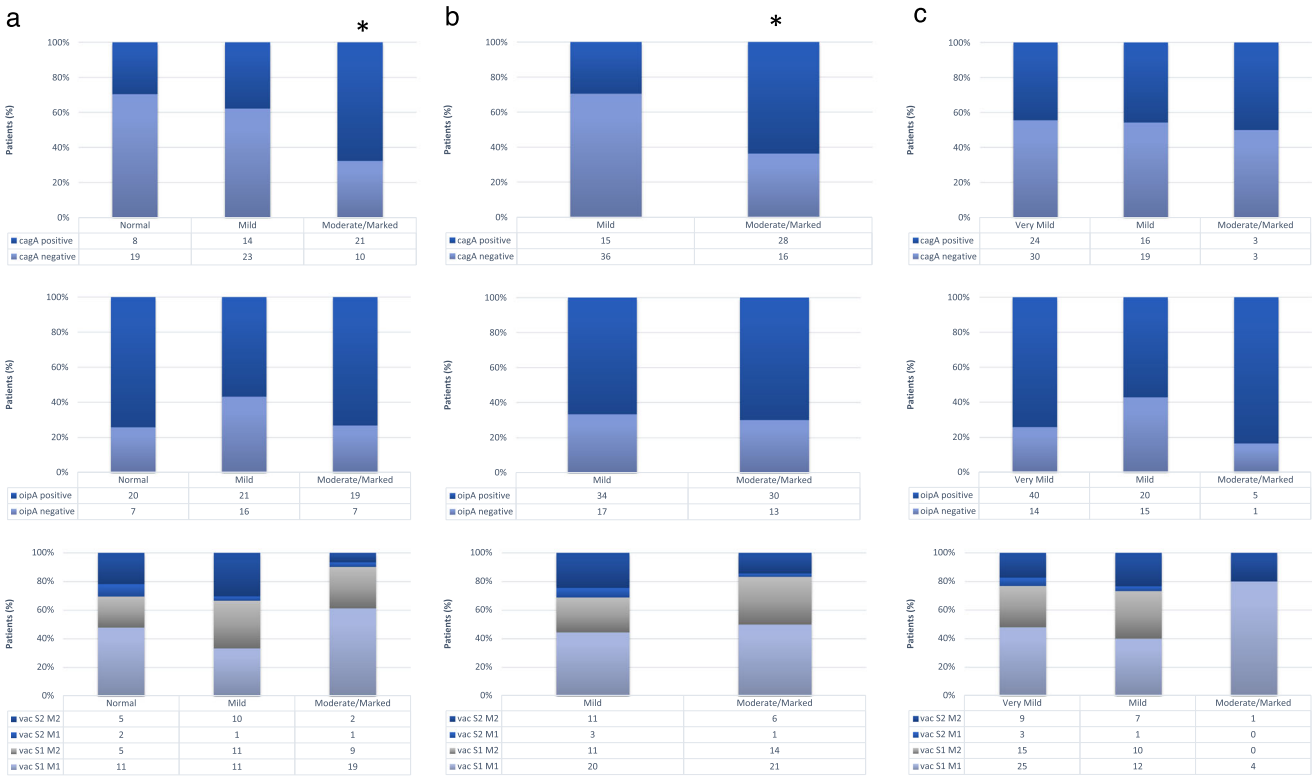

FIGURE 2 *H. pylori* virulence genotypes and histological grading of duodenal inflammation at D2. (a) Chronic duodenal inflammation. (b) Duodenal eosinophils count in lamina propria. (c) Duodenal intra-epithelial lymphocyte (IEL). Data are expressed as (*n*) subjects and percentage %. **P* < 0.05.

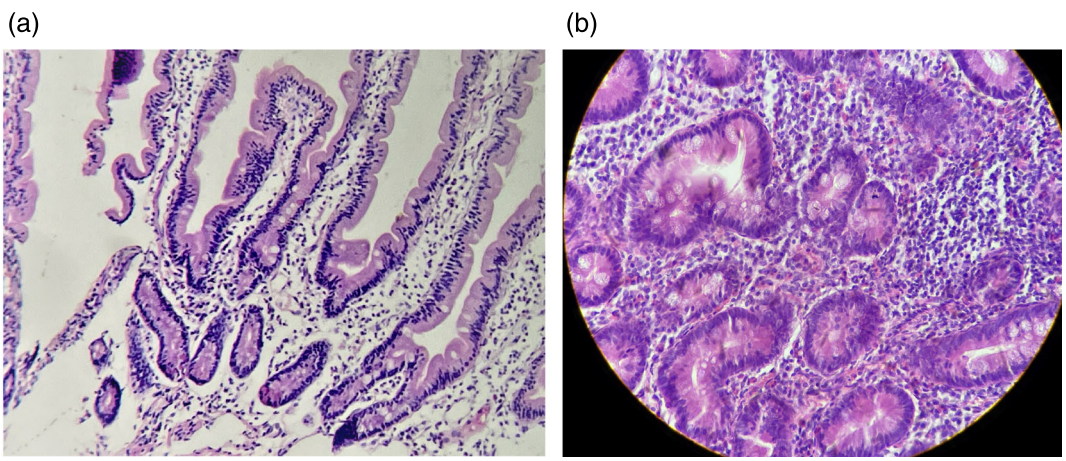

FIGURE 3 Duodenal chronic inflammation and duodenal eosinophilia, representative histological photomicrograph (40×). (a) Duodenal mucosa without chronic inflammation. (b) Duodenal mucosa with chronic inflammation and high duodenal eosinophils density adjacent to glands.

© 2022 Journal of Gastroenterology and Hepatology Foundation and John Wiley & Sons Australia, Ltd.

smoking, alcohol use and prior appendectomy or cholecystectomy (Table 2). EPS subjects had significantly increased PPI consumption (45%) (Table 2). Analysis of NSAID consumption showed that PDS patients had significantly less consumption than EPS and EPS/PDS. There was a trend to more proportion of female patients in PDS (70%) than EPS (53%) and EPS/PDS overlap (33%) subtypes.

***H. pylori* virulence genes and clinical variables.** Patients with *H. pylori cagA* positive strains had significantly lower BMI compared to *cagA* negative strain (Table 3). No difference was observed at clinical variables between *oipA* strains. FD syndromes were not associated with *cagA* or *oipA* strains. We observed a significantly trend of more *vacA s1/m1* and *s1/m2* positive strains in EPS as compared with PDS and overlap syndrome (Table 3).

***H. pylori* virulence genes and histological grading of low-grade duodenal inflammation.** Histological duodenal grading of chronic inflammation, low-grade duodenal eosinophilia and intra-epithelial lymphocytes showed no difference between *oipA* and *vacA* genotypes in HpD subjects (Fig. 2). Furthermore, no difference in *cagA* genotype was observed at intra-epithelial lymphocytes. However, duodenal chronic inflammation (21/31, $P < 0.05$) and low-grade duodenal eosinophilia (> 10 per HPF) (28/44, $P < 0.05$) were significantly increased in *cagA* positive strain subjects (Figs. 2 and 3). Moreover, *cagA* positive strain was significantly associated with the combination of chronic duodenal inflammation and eosinophilia but not *oipA* nor *vacA* virulence genes (Table 4). Then, we evaluated low-grade gastric eosinophilia and virulence genes of *H. pylori* in our cohort, no differences were observed between groups (Table 5).

Finally, we evaluated clinical variables, *H. pylori* virulence genes and low-grade duodenal eosinophilia. Indeed, a higher proportion of patients with *cagA* positive strain (64%) were significantly associated with low-grade duodenal eosinophilia. But no difference in low-grade duodenal eosinophilia was detected in *oipA* positive strain, *vacA s1/m1* positive strain, age, gender, BMI, previous NSAID or PPI consumption, diabetes, hypertension and FD syndromes (Table 6). The OR for low-grade duodenal eosinophilia with *H. pylori cagA* positive strain was 4.2 (95% CI, 1.78–9.93; $P = 0.001$). After adjusting for age, gender, smoking, diabetes/IFG, alcohol, PPI and NSAID the OR was 5.44 (1.989–14.902; $P < 0.001$) (Table 7). These findings showed that low-grade duodenal eosinophilia is independently associated with *cagA* in *H. pylori*-related dyspepsia.

Table 4 *H. pylori* virulence genotypes and low-grade duodenal inflammation

	Low-grade duodenal inflammation	
Virulence genes	Positive (n = 27)	Negative (n = 68)
cagA		
Positive (%)	21 (78%)*	22 (32%)
Negative (%)	6 (22%)	46 (68%)
oipA		
Positive (%)	20 (74%)	45 (66%)
Negative (%)	7 (26%)	23 (34%)
vacA		
s1 m1 (%)	15 (56%)	26 (43%)
s1 m2 (%)	9 (33%)	16 (27%)
s2 m1 (%)	1 (4%)	3 (5%)
s2 m2 (%)	2 (7%)	15 (25%)

Data are expressed as *n* (percentage %). Chi-square test was used.
*$P < 0.05$.

Discussion

In this cross-sectional study, we investigated the potential association of *H. pylori* virulence genes and low-grade duodenal eosinophilia in HpD. Our results suggest that low-grade duodenal eosinophilia is significantly associated with *cagA* positive strain in HpD but not *oipA* and *vacA* genotypes. Increased eosinophils densities in duodenum play an important role in pathogenesis of FD[38]; however, we failed to detect an association with functional dyspepsia syndromes and low-grade duodenal eosinophilia as was demonstrated in *H. pylori* negative FD.[33,37,39]

The etiopathogenesis of FD is considered multifactorial including associations with gut-brain axis such as psychological distress, altered microbiota, post-infectious gastroenteritis and *H. pylori*, although dyspeptic symptoms associated with *H. pylori* by Kyoto consensus may also be considered as a separate entity.[7] Indeed, abnormalities of gastric function including impaired accommodation or hypersensitivity to distention and delayed emptying have been

Table 5 Low-grade gastric eosinophilia and *H. pylori* virulence genotypes

	Low-grade gastric eosinophilia		
Eosinophils	Mild: < 10, (n = 73)	Moderate: ≥ 10 to < 20, (n = 15)	Marked: ≥ 20, (n = 3)
cagA (%)	33 (45%)	6 (40%)	2 (67%)
oipA (%)	48 (66%)	11 (73%)	2 (67%)
vacA s1 m1 (%)	30 (45%)	6 (43%)	2 (67%)
vacA s1 m2 (%)	19 (29%)	5 (36%)	1 (33%)
vacA s2 m1 (%)	4 (6%)	0	0
vacA s2 m2 (%)	13 (20%)	3 (21%)	0

Data are expressed as percentage (%) of total. Biopsies from gastric body and antrum were combined. Chi-square test was used.
*$P < 0.05$.

© 2022 Journal of Gastroenterology and Hepatology Foundation and John Wiley & Sons Australia, Ltd.

reported in FD, but these changes correlate poorly with symptoms.[11] For instance, symptoms severity in HpD correlated with microscopic duodenitis[18] but not to the degree of *H. pylori* gastritis.[40,41] Emerging data increasingly point towards the duodenum as the key integrator in dyspepsia symptom generation, and it has been proposed that gastric motor dysfunction may be attributed to disordered duodeno-gastric feedback and low-grade inflammation.[38] Talley *et al*. proposes a new model of disease that is initiated by internal and external triggers that induce gastrointestinal barrier dysfunction leading to a low-grade duodenal inflammation characterized by an innate immune response with increased activation of eosinophils and mast cells.[13,14,42,43] Emergent studies have reported that duodenal low-grade inflammation and increased small intestinal homing T cells correlates with dyspeptic symptom severity.[16,44] Moreover, recent observation correlates duodenal eosinophils or mast cells activation and calcium transient amplitudes to high K+ or electrical stimulation in FD patients.[17] Duodenal eosinophilia and mast cell degranulation seem to be related to early step in triggering and sustained visceral hypersensitivity and altered motor control, but is not clear what is causing this phenomenon in a group of FD patients but not in others. Recent data suggest that childhood environmental factors (herbivore pet),[45] luminal triggers (e.g. food, microbiota, and bile acids),[33,46] and increased duodenal acid exposure[47] may explain duodenal mucosal hyperpermeability and low grade inflammation mediated inhibitory duodeno-gastric motor reflex.

The influence of *H. pylori* virulence genes on eosinophilic infiltrates in the duodenum has not been well explored. In our study we observed a correlation with low-grade duodenal eosinophilia in patients harboring *cagA*-positive *H. pylori* strain but not in *oipA* or *vacA* genotypes. This association remain significant after adjusting to age, gender, smoking, PPI and *vacA s1/m1*. Our study failed to detect an association with duodenal eosinophilia and FD syndromes. Previous reports on FD showed that duodenal eosinophilia was associated with early satiety and postprandial fullness.[33,34] Indeed, a significant association with *vacA s1/m1-m2* with EPS was observed in this report but not to low grade duodenal inflammation. Perhaps, the higher prevalence of EPS

Table 6 Low-grade duodenal eosinophilia, *H. pylori* virulence genotypes and clinical variables

Variable	Low-grade duodenal eosinophilia	
	Positive (n = 44)	Negative (n = 51)
Age (SD)	44 (11.6)	51 (13)
Gender (Fem %)	22 (50%)	22 (43%)
PDS (%)	9 (20%)	8 (17%)
EPS (%)	32 (73%)	37 (72%)
EPS/PDS overlap (%)	3 (7%)	6 (11%)
BMI (SD)	26.6 (3.9)	27.6 (5.2)
cagA positive (%)	28 (64%)*	15 (29%)
oipA positive (%)	31 (70%)	34 (67%)
vacA s1 m1 positive (%)	21 (48%)	20 (39%)
PPI (%)	14 (32%)	20 (39%)
NSAID (%)	17(39%)	19 (37%)
Diabetes/IFG (%)	9 (20%)	18 (35%)
Hypertension (%)	14 (32%)	13 (25%)
Smoking (%)	13 (29%)	20 (39%)

Data are expressed as mean (± SD, standard deviation), or percentage (%) of total. For continuous variables, unpaired *t*-test was used. For categorical variables, chi-square test was used.
*$P < 0.05$.

Table 7 Association between low-grade duodenal eosinophilia and *H. pylori* virulence genotypes

Low-grade duodenal eosinophilia (LGDEo)	Univariate results			Adjusting for age, gender, smoking, diabetes, alcohol, PPI, and NSAID	
	n (%)	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
cagA +					
LGDEo negative	15 (29%)				
LGDEo positive	28 (64%)	4.2 (1.78–9.93)	< 0.001	5.44 (1.989–14.902)	< 0.001
oipA +					
LGDEo negative	34 (67%)				
LGDEo positive	31 (70%)	1.19 (0.49–2.85)	ns		
vacA s1/m1 +					
LGDEo negative	20 (39%)				
LGDEo positive	21 (48%)	1.25 (0.53–2.91)	ns		
vacA s1/m2 +					
LGDEo negative	11 (21%)				
LGDEo positive	14 (32%)	1.55 (0.61–3.94)	ns		
vacA s2/m1 +					
LGDEo negative	3 (6%)				
LGDEo positive	1 (2%)	0.34 (0.03–3.42)	ns		
vacA s2/m2 +					
LGDEo negative	11 (21%)				
LGDEo positive	6 (14%)	0.51 (0.17–1.55)	ns		

Data are expressed as number of positives genotypes and percentage (%) of total. Low-grade duodenal eosinophilia (LGDEo).

© 2022 Journal of Gastroenterology and Hepatology Foundation and John Wiley & Sons Australia, Ltd.

(72%) in our cohort might have underpowered our PDS patients' number to detect the association with low-grade duodenal eosinophilia.[48] Noteworthy, the bacterial components and toxins of *H. pylori* infection might directly activate mucosal inflammation, peripheral sensory neurons and enhance the visceral hypersensitivity,[40,49] making it possible that HpD could be a different pathophysiological entity from FD.[7] Previous study on HpD patients have shown an upregulation of the chemokine peptide RANTES (Regulated on Activation, Normal T cell-Expressed and -Secreted), which is a chemoattractant to eosinophils.[50] While the correlation between increased duodenal eosinophils densities and *cagA* strain does not prove causality, confirmation from mechanistic studies is needed to clarify the clinical role of low-grade duodenal eosinophilia in HpD. In this regard, the interaction between *H. pylori* virulence genes, host genetic inflammatory background, low-grade duodenal inflammation and FD syndromes could explain HpD phenotypes and will be evaluated in our future research.

The strengths of this study are that is a prospective evaluation from a well-defined *H. pylori*-related FD cohort based on Rome-III criteria with normal upper endoscopy. *H. pylori* virulence genes were genotyped in $>$ 95% of subjects. Furthermore, more than 90% of the duodenal biopsies were evaluated and Pathologists were blinded to medical history before evaluation.

The limitations of the study include (i) population studied: Our cohort comprised of South-American population of *H. pylori*-related functional dyspepsia with high proportion of EPS patients rather than PDS; (ii) histological evaluation of duodenal samples from D2 only: we choose to evaluate D2 based on previous data where duodenal eosinophilia and degranulation was observed in FD subjects at D2 but not D1 when was compared to healthy controls[34,51]; (iii) psychological factors were not evaluated; and (iv) validated questionnaires were not used. Based on this limitations, the results may not apply to other referral centers with different ethnic background and more data are needed to confirm the associations observed here.

In conclusion, the results of this prospective cross-sectional study suggest that low-grade duodenal eosinophilia is associated with *cagA* strain but not with FD syndromes in HpD patients.

Acknowledgments

The authors thank Prof Raul Marucci and Prof Cristina Cirilo for their strong support.

References

1 Aziz I, Palsson OS, Tornblom H *et al*. Epidemiology, clinical characteristics, and associations for symptom-based Rome IV functional dyspepsia in adults in the USA, Canada, and the UK: a cross-sectional population-based study. *Lancet Gastroenterol Hepatol* 2018; **3**: 252–62.
2 Ford AC, Mahadeva S, Carbone MF, Lacy BE, Talley NJ. Functional dyspepsia. *Lancet* 2020; **396**: 1689–702.
3 Drossman DA. The functional gastrointestinal disorders and the Rome III process. *Gastroenterology* 2006; **130**: 1377–90.
4 Stanghellini V, Chan FK, Hasler WL *et al*. Gastroduodenal Disorders. *Gastroenterology* 2016; **150**: 1380–92.
5 Lacy BE, Weiser KT, Kennedy AT, Crowell MD, Talley NJ. Functional dyspepsia: the economic impact to patients. *Aliment Pharmacol Ther* 2013; **38**: 170–7.
6 Aro P, Talley NJ, Agreus L *et al*. Functional dyspepsia impairs quality of life in the adult population. *Aliment Pharmacol Ther* 2011; **33**: 1215–24.
7 Sugano K, Tack J, Kuipers EJ *et al*. Kyoto global consensus report on Helicobacter pylori gastritis. *Gut* 2015; **64**: 1353–67.
8 Ford AC, Tsipotis E, Yuan Y, Leontiadis GI, Moayyedi P. Efficacy of Helicobacter pylori eradication therapy for functional dyspepsia: updated systematic review and meta-analysis. *Gut* 2022.
9 Kim YJ, Chung WC, Kim BW *et al*. Is Helicobacter pylori Associated Functional Dyspepsia Correlated With Dysbiosis? *J Neurogastroenterol Motil* 2017; **23**: 504–16.
10 Mirbagheri SS, Mirbagheri SA, Nabavizadeh B *et al*. Impact of microscopic duodenitis on symptomatic response to Helicobacter pylori eradication in functional dyspepsia. *Dig Dis Sci* 2015; **60**: 163–7.
11 Vanheel H, Carbone F, Valvekens L *et al*. Pathophysiological Abnormalities in Functional Dyspepsia Subgroups According to the Rome III Criteria. *Am J Gastroenterol* 2017; **112**: 132–40.
12 Talley NJ, Ford AC. Functional Dyspepsia. *N Engl J Med* 2015; **373**: 1853–63.
13 Fan K, Talley NJ. Functional dyspepsia and duodenal eosinophilia: A new model. *J Dig Dis* 2017; **18**: 667–77.
14 Talley NJ, Walker MM, Aro P *et al*. Non-ulcer dyspepsia and duodenal eosinophilia: an adult endoscopic population-based case-control study. *Clin Gastroenterol Hepatol* 2007; **5**: 1175–83.
15 Jarbrink-Sehgal ME, Sparkman J, Damron A *et al*. Functional Dyspepsia and Duodenal Eosinophil Count and Degranulation: A Multiethnic US Veteran Cohort Study. *Dig Dis Sci* 2021; **66**: 3482–9.
16 Vanheel H, Vicario M, Vanuytsel T *et al*. Impaired duodenal mucosal integrity and low-grade inflammation in functional dyspepsia. *Gut* 2014; **63**: 262–71.
17 Cirillo C, Bessissow T, Desmet AS, Vanheel H, Tack J, vanden Berghe P. Evidence for neuronal and structural changes in submucous ganglia of patients with functional dyspepsia. *Am J Gastroenterol* 2015; **110**: 1205–15.
18 Mirbagheri SA, Khajavirad N, Rakhshani N, Ostovaneh MR, Hoseini SME, Hoseini V. Impact of Helicobacter pylori infection and microscopic duodenal histopathological changes on clinical symptoms of patients with functional dyspepsia. *Dig Dis Sci* 2012; **57**: 967–72.
19 Treiber G, Schwabe M, Ammon S, Walker S, Klotz U, Malfertheiner P. Dyspeptic symptoms associated with Helicobacter pylori infection are influenced by strain and host specific factors. *Aliment Pharmacol Ther* 2004; **19**: 219–31.
20 Fang YJ, Liou JM, Chen CC *et al*. Distinct aetiopathogenesis in subgroups of functional dyspepsia according to the Rome III criteria. *Gut* 2015; **64**: 1517–28.
21 Dixon MF, Genta RM, Yardley JH, Correa P. Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. *Am J Surg Pathol* 1996; **20**: 1161–81.
22 Whitington PF, Whitington GL. Eosinophilic gastroenteropathy in childhood. *J Pediatr Gastroenterol Nutr* 1988; **7**: 379–85.
23 Kokkonen J, Ruuska T, Karttunen TJ, Niinimäki A. Mucosal pathology of the foregut associated with food allergy and recurrent abdominal pains in children. *Acta Paediatr* 2001; **90**: 16–21.
24 Lowichik A, Weinberg AG. A quantitative evaluation of mucosal eosinophils in the pediatric gastrointestinal tract. *Mod Pathol* 1996; **9**: 110–4.
25 Kalach N, Huvenne H, Gosset P *et al*. Eosinophil counts in upper digestive mucosa of Western European children: variations with age,

© 2022 Journal of Gastroenterology and Hepatology Foundation and John Wiley & Sons Australia, Ltd.

organs, symptoms, Helicobacter pylori status, and pathological findings. *J Pediatr Gastroenterol Nutr* 2011; **52**: 175–82.
26 Lee CM, Changchien CS, Chen PC *et al*. Eosinophilic gastroenteritis: 10 years experience. *Am J Gastroenterol* 1993; **88**: 70–4.
27 Leite C, Mazzoleni LE, Uchoa DM *et al*. Association of Duodenal Eosinophilic Infiltrate with Helicobacter Pylori Infection, but Not with Functional Dyspepsia. *Arq Gastroenterol* 2020; **57**: 74–8.
28 Genta RM, Sonnenberg A, Turner K. Quantification of the duodenal eosinophil content in adults: a necessary step for an evidence-based diagnosis of duodenal eosinophilia. *Aliment Pharmacol Ther* 2018; **47**: 1143–50.
29 Walker MM, Murray JA. An update in the diagnosis of coeliac disease. *Histopathology* 2011; **59**: 166–79.
30 van Doorn LJ, Figueiredo C, Rossau R *et al*. Typing of Helicobacter pylori vacA gene and detection of cagA gene by PCR and reverse hybridization. *J Clin Microbiol* 1998; **36**: 1271–6.
31 Yamaoka Y, Kikuchi S, el Zimaity HM *et al*. Importance of Helicobacter pylori oipA in clinical presentation, gastric inflammation, and mucosal interleukin 8 production. *Gastroenterology* 2002; **123**: 414–24.
32 Atherton JC, Cao P, Peek RM Jr, Tummuru MKR, Blaser MJ, Cover TL. Mosaicism in vacuolating cytotoxin alleles of Helicobacter pylori. Association of specific vacA types with cytotoxin production and peptic ulceration. *J Biol Chem* 1995; **270**: 17771–7.
33 Walker MM, Salehian SS, Murray CE *et al*. Implications of eosinophilia in the normal duodenal biopsy - an association with allergy and functional dyspepsia. *Aliment Pharmacol Ther* 2010; **31**: 1229–36.
34 Walker MM, Aggarwal KR, Shim LS *et al*. Duodenal eosinophilia and early satiety in functional dyspepsia: confirmation of a positive association in an Australian cohort. *J Gastroenterol Hepatol* 2014; **29**: 474–9.
35 Brauer D, Fellows Z, White A. ALCOHOL INDUCED GASTROINTESTINAL SYMPTOMS IN EOSINOPHILIC ESOPHAGITIS. *Ann Allergy Asthma Immunol* 2018; **121**: S56.
36 Koutlas NT, Eluri S, Rusin S *et al*. Impact of smoking, alcohol consumption, and NSAID use on risk for and phenotypes of eosinophilic esophagitis. *Dis Esophagus* 2018; **31**: 1–7.
37 Wauters L, Ceulemans M, Frings D *et al*. Proton Pump Inhibitors Reduce Duodenal Eosinophilia, Mast Cells, and Permeability in Patients With Functional Dyspepsia. *Gastroenterology* 2021; **160**: 1521–31 e9.
38 Wauters L, Talley NJ, Walker MM, Tack J, Vanuytsel T. Novel concepts in the pathophysiology and treatment of functional dyspepsia. *Gut* 2020; **69**: 591–600.
39 Sarkar MAM, Akhter S, Khan MR, Saha M, Roy PK. Association of duodenal eosinophilia with Helicobacter pylori-negative functional dyspepsia. *Arab J Gastroenterol* 2020; **21**: 19–23.
40 Suzuki H, Moayyedi P. Helicobacter pylori infection in functional dyspepsia. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 168–74.
41 Turkkan E, Uslan I, Acarturk G *et al*. Does Helicobacter pylori-induced inflammation of gastric mucosa determine the severity of symptoms in functional dyspepsia? *J Gastroenterol* 2009; **44**: 66–70.
42 Walker MM, Talley NJ, Prabhakar M *et al*. Duodenal mastocytosis, eosinophilia and intraepithelial lymphocytosis as possible disease markers in the irritable bowel syndrome and functional dyspepsia. *Aliment Pharmacol Ther* 2009; **29**: 765–73.
43 Wang X, Li X, Ge W *et al*. Quantitative evaluation of duodenal eosinophils and mast cells in adult patients with functional dyspepsia. *Ann Diagn Pathol* 2015; **19**: 50–6.
44 Liebregts T, Adam B, Bredack C *et al*. Small bowel homing T cells are associated with symptoms and delayed gastric emptying in functional dyspepsia. *Am J Gastroenterol* 2011; **106**: 1089–98.
45 Koloski NA, Jones M, Weltman M *et al*. Identification of early environmental risk factors for irritable bowel syndrome and dyspepsia. *Neurogastroenterol Motil* 2015; **27**: 1317–25.
46 Beeckmans D, Riethorst D, Augustijns P *et al*. Altered duodenal bile salt concentration and receptor expression in functional dyspepsia. *United Eur Gastroenterol J* 2018; **6**: 1347–55.
47 Vanheel H, Vicario M, Beeckmans D *et al*. Duodenal acidification induces gastric relaxation and alters epithelial barrier function by a mast cell independent mechanism. *Sci Rep* 2020; **10**: 17448.
48 Ronkainen J, Aro P, Walker MM, Agréus L, Johansson SE, Jones M, Talley NJ. Duodenal eosinophilia is associated with functional dyspepsia and new onset gastro-oesophageal reflux disease. *Aliment Pharmacol Ther* 2019; **50**: 24–32.
49 Thumshirn M, Camilleri M, Saslow SB, Williams DE, Burton DD, Hanson RB. Gastric accommodation in non-ulcer dyspepsia and the roles of Helicobacter pylori infection and vagal function. *Gut* 1999; **44**: 55–64.
50 Kikuchi T, Kato K, Ohara S *et al*. The relationship between persistent secretion of RANTES and residual infiltration of eosinophils and memory T lymphocytes after Helicobacter pylori eradication. *J Pathol* 2000; **192**: 243–50.
51 Du L, Shen J, Kim JJ *et al*. Increased Duodenal Eosinophil Degranulation in Patients with Functional Dyspepsia: A Prospective Study. *Sci Rep* 2016; **6**: 34305.

© 2022 Journal of Gastroenterology and Hepatology Foundation and John Wiley & Sons Australia, Ltd.