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Duodenal eosinophilia and the link to anxiety: A population-based endoscopic study

Short title: Duodenal eosinophilia and anxiety

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Abstract

Introduction

The concept of gut-to-brain communication via microbial or inflammatory pathways is gaining increased attention but genuine pathology directly linking gut perturbation to anxiety are lacking. We hypothesized that duodenal eosinophilia, as known to occur in functional dyspepsia (FD), may be an underlying cause of anxiety and may help explain the striking association between FD and anxiety.

Methods

Randomly selected subjects from the national population register of Sweden completed the validated Abdominal Symptom Questionnaire; 1000 completed esophagogastroduodenoscopy and the Hospital Anxiety and Depression Scale questionnaire. Duodenal biopsies were obtained from 1st (D1) and 2nd portion (D2). Eligible subjects who underwent endoscopy (n = 887) were invited to participate in a 10-year follow-up study with the same questionnaires. Among endoscopy normal subjects, FD was identified by Rome criteria and controls were symptom free. Duodenal eosinophilia was based on pre-defined cut-offs. Finding are reported as odds ratios (ORs) with 95% confidence interval and p-value.

Results

The study population comprised 89 cases with FD and 124 healthy controls (mean age 62 years, SD 12, 34% male). Clinical anxiety at follow-up was elevated in those with D1 eosinophilia at baseline considering either new onset anxiety (OR=4.5, 95% CI 0.8, 23.8; p=0.08) or follow-up anxiety adjusting for baseline anxiety (OR=4.51 (95% CI 1.03, 19.81; p=0.046).

Conclusion

Duodenal eosinophilia may potentially be a mechanism linked to anxiety independent of FD.

Key words: Anxiety; functional dyspepsia; eosinophils; duodenum; duodenal eosinophilia

Introduction

Functional dyspepsia (FD) is a chronic distressing unexplained gastroduodenal disorder (1). FD affects about 10% to 15% of the population globally, is more prevalent in women than men, overlaps with the irritable bowel syndrome (IBS) more than expected by chance, and may arise sporadically or after an episode of acute gastroenteritis (2-4). FD is subdivided into the more common postprandial distress syndrome (PDS), characterized by postprandial fullness or early satiety, and the epigastric pain syndrome (EPS) although overlap occurs (5, 6).

Not only is quality of life impaired, but FD is commonly associated with distressing comorbid anxiety or depression (1, 7, 8). A previous population-based prospective endoscopic study from Sweden identified anxiety was strongly associated with new-onset FD (9). In a prospective Australian population-based study, over 1000 subjects from a random population completed a 12 year follow-up; a functional gastrointestinal (GI) disorder diagnosis at baseline was associated with significantly higher levels of anxiety and depression at follow-up among subjects who did not have elevated levels of psychological distress at baseline (10), work that has since been replicated in other studies (11, 12). While these epidemiological data suggest functional gut disorders may precede the development of anxiety in about 50% of cases, the underlying mechanisms are unknown (11).

Whilst FD has not previously been attributed to detectable mucosal pathology, an important observation has been the finding of increased duodenal eosinophils and/or eosinophil degranulation in FD with evidence of systemic immune activation, including peripheral small intestinal homing T-cells expressing $\alpha4^+$, integrin $\beta7^+$, and chemokine receptor 9 ($CCR9^+$) (13-15). The presence of duodenal eosinophilia is associated with postprandial distress syndrome, and not epigastric pain, and has been reported in studies from Western and Eastern countries (16, 17). Further, duodenal

inflammatory changes are now known to be associated with increased upper small intestinal permeability and neural damage in the duodenum (18, 19), and alterations of the duodenal microbiome have been observed in FD (20, 21).

The concept of gut-to-brain communication via microbial or inflammatory pathways is gaining increased attention (22). We have utilized a unique population-based endoscopy study to start to address the question could low-grade upper intestinal inflammation potentially account for anxiety or depression? We hypothesized that duodenal eosinophilia may be an underlying cause of anxiety and may help explain the striking association between FD and anxiety (9).

Methods

Subject selection

The Kalixanda study has previously been described in detail (15, 23). The study was performed in two adjacent communities in the northern part of Sweden (Kalix and Haparanda), with a total population of 28 988 inhabitants (as of December 1998). A randomly selected sample of 3000 adults from the two communities was sent the validated Abdominal Symptom Questionnaire (ASQ) and the 2122 responders who completed the ASQ were phoned to find participants willing and able to undergo an esophago-gastro-duodenoscopy (EGD) (15). A total of 1001 participants attended the visit, of whom 1000 had a successful EGD and completed the Hospital Anxiety and Depression Scale (HADS)(24). The response rate for those eligible for investigation was 73% (15). The age and sex distribution in the 1001 subjects who responded to the questionnaire at both assessments (488 males [48.8%]; mean age, 54 y) closely reflected the Swedish population (15). The study subjects who refused endoscopy were very similar demographically to the 1001 subjects evaluated (15, 23).

The Kalixanda study was approved by the Umeå University Ethics Committee (dnr 98-99, §156/98) and the follow-up study by the Ethical Approval Committee of the Karolinska Institutet (2010/576-31/1).

Follow-up

All eligible from the Kalixanda cohort (n=887, response rate 79%) were invited to a follow-up in 2010 with the ASQ and HADS (9, 24). Endoscopy was not performed at follow-up. All available cases of FD with histological evaluation of the duodenum at baseline (n=89) and healthy controls with histological evaluation of the duodenum at baseline (n=124) comprised the study cohort to assess

if duodenal eosinophilia (regardless of FD status) is a risk factor for anxiety and depression (Figure 1).

Functional dyspepsia and controls

A nested case-control design was applied[15]. Functional dyspepsia was defined by the Rome III criteria (9, 25). Controls were symptom free. Both groups had a normal esophagogastroduodenoscopy.

Anxiety and depression

Anxiety and depression cases were defined by a score of 11 or higher on the validated HADS (24).

Duodenal histology

Histology was dual read blinded to clinical status. Duodenal histology was evaluated at baseline. Eosinophils were quantified by counting the number per high-power field (magnification x40); five high-power fields were selected in each section starting with any areas where eosinophils were greatest. The sum, mean and median over the five-field counts then were calculated in every subject. Eosinophil counts were considered as discrete predictors (using the corresponding median over all subjects as the breakpoints) the pre-specified cut-off being the mean, 23 eosinophils in the bulb (D1) and 24 eosinophils in the second part of duodenum (D2) defining abnormal as previously identified (26).

Statistical analysis

Data were analyzed by unconditional and exact logistic regression. Three sets of models examining the association between eosinophilia and elevated anxiety were considered:

1. The cross-sectional association at the baseline time point

2. A longitudinal analysis in which baseline eosinophilia predicts follow-up new onset anxiety ten years later. This analysis is restricted to subjects free of anxiety at baseline.
3. As a sensitivity analysis, longitudinal modelling in which baseline eosinophilia predicts follow-up anxiety 10 years later in all subjects, controlling for baseline anxiety state and other potentially confounding variables. This model was included because model 2 necessarily limits the sample used and hence lowers statistical power.

Due to missing data on anxiety scores at both baseline (n=14) and follow-up (n=11), models in step 3 were estimated using multiple imputation (27) with five imputation samples, except where indicated in Table 2a, to enable all subjects to be retained in all analyses. For the same reason the percentage of elevated anxiety reported in Tables 2a and 2b were also calculated from logit models estimated using multiple imputation with five imputation samples except where indicated. We also ran the models with and without multiple imputation which provided very similar results (data not shown).

In terms of study power, the results presented here are proposed as indicative due to the relatively small number of cases of elevated anxiety at follow-up and we argue that effect size should be considered as important as statistical significance. From a prevalence of 4% when a risk factor is absent, an odds ratio needs to be 4.5 or greater to have statistical power 0.8 at the 0.05 (two-tailed) level of statistical significance.

Results

In total there were 89 cases with FD and 124 healthy controls. The mean age was 62 years (34% male). The demographic and clinical characteristics of the study subjects is presented in Table 1. No subject had celiac disease.

At baseline duodenal eosinophilia was observed in 78 subjects in D1 and in 84 subjects in D2 (in 46 subjects, both in D1 and in D2, P<0.001 for both). Anxiety at baseline was found in 9 subjects (4%) and at follow-up in 12 subjects (6%) (Figure 2). Depression was found in 2 subjects (1%) both at baseline and at follow-up, respectively. No further modelling was undertaken with depression.

Anxiety at baseline or at follow-up was not associated with smoking, use of alcohol, allergy, *Helicobacter pylori* infection or medications at baseline (Fischer's exact test). Only use of NSAIDs (OR=2.29, 95% CI 1.12, 7.41, p=0.03) was associated with eosinophilia in D1 but not in D2 at baseline. All other medications, use of alcohol and smoking were not associated with eosinophilia in D1 or D2.

Univariately, anxiety at baseline was not statistically significantly associated with eosinophilia in D1 at baseline (Table 2a) but was associated with eosinophilia in D2 at baseline (Table 2b). These associations were not substantially altered by controlling for age and gender.

Of 202 individuals who could be evaluated for anxiety at baseline, 9 (4.5%) were elevated and of 199 evaluted at follow-up 12 (6.0%) had elevated anxiety. New onset anxiety at follow-up was higher among individuals meeting criteria for D1 eosinophilia at baseline (n=5, 7.5%) than those not meeting criteria (n=2, 1.8%) but just failed to reach statistical significance (OR 4.5, 95% CI 0.8, 23.8; p=0.08), and this association was not substantially altered by controlling for age and gender. In this analysis

using only individuals free of anxiety at baseline, the statistical power is slightly low as indicated in the earlier power calculation. These models were run without multiple imputation due to numerical estimation limitations.

Since the new onset analysis can only use a subset of the entire sample, a sensitivity analysis that included all subjects was conducted using logistic regression in which baseline anxiety status was controlled for and estimation based on multiple imputation to retain all individuals. The odds ratio measuring the association between baseline D1 eosinophilia and follow-up elevated anxiety was 4.51 (95% CI 1.03, 19.81, p=0.046), and this association was not substantially altered by further controlling for age, gender and NSAID use (OR 4.58 95% CI 1.01, 20.88; p=0.049). We conducted a further sensitivity analysis also including the potentially confounding variables of baseline functional dyspepsia status, to allow for potential overlap with eosinophilia, and baseline anxiety status, to estimate pure predictive versus cross-sectional associations between eosinophilia and anxiety state given the potential for autocorrelation between baseline and follow-up anxiety. While further controlling for baseline FD status results in a p-value slightly greater than 0.05, the effect size is not noticeably diminished (OR 4.43 95% CI 0.88, 22.34; p=0.07) and the borderline statistical significance is likely due to a complex model fitted to modest sample size.

Discussion

In a novel prospective population-based 10-year follow-up study, anxiety at baseline was independently associated with a nearly 12-fold increase in odds of eosinophilia in D2 at the same timepoint. However, these cross-sectional data do not identify directionality, and in particular whether duodenal low-grade inflammation increases the risk of psychological distress. We further observed, as hypothesized, that anxiety at follow-up was associated with a nearly 5-fold increased risk with duodenal eosinophilia in D1 10 years earlier. Presumably because duodenal eosinophilia can be patchy (15, 26) the associations varied by duodenal site. The study lacked power to show an association of new-onset anxiety with duodenal eosinophilia although there was a nearly five-fold increase in odds. This is the first study to our knowledge to demonstrate a possible link between duodenal inflammation and the later onset of psychological distress.

In clinical practice it has been recognized for nearly 100 years that unexplained chronic GI symptoms are associated with high levels of psychological distress in many cases, leading to the hypothesis that IBS and related gut conditions are primarily stress and anxiety driven (28). Based on the current Rome criteria, anxiety and depression are considered to be comorbid conditions, and have not been included as part of the diagnostic criteria for FD or any functional GI syndrome (5). This is in part because past studies had suggested the association of anxiety and depression with IBS in patients was accounted for by selection bias, as those who were anxious or depressed appeared more likely to consult, although other population-based studies came to the opposite conclusion (29-31). Further, more recent work has directly challenged the concept psychological distress is accounted for by selection bias, including the present study, and suggest anxiety and depression may be integral to the very nature of many suffering with functional GI disorders (9-12, 32).

Population-based studies (10, 11) and studies in general practice (12) have shown about 50% of cases with a functional GI disorder have their GI symptoms preceed the later onset of psychological distress (the other 50% had prior anxiety or depression then developed chronic GI symptoms). In the Kalixanda study, we have previously reported (9) that anxiety was associated with postprandial distress syndrome at baseline (OR, 4.83; 99% CI, 1.24-18.76) and 10 years later (OR, 8.12; 99% CI, 2.13-30.85), and anxiety at baseline was associated with a 7-fold increased risk of new-onset FD 10 year later (OR, 7.61; 99% CI, 1.21-47.73) but we did not examine low grade duodenal inflammation and its potential role in precipitating anxiety. These previous studies and the current study together suggest a central nervous system (CNS) process can result in gut dysfunction and symptoms, but similarly a primary intestinal process may result in psychological distress presumably secondary to CNS dysfunction. While GI symptoms have been shown to precede psychological distress, the current study is the first to provide evidence low grade intestinal inflammation may play a role in provoking anxiety.

Duodenal eosinophilia is associated with atopic disease, and in functional GI syndromes atopic disease is now a newly recognized risk factor (33, 34), although atopy was not associated with duodenal eosinophilia in the present study. A number of studies indirectly support the concept that atopic diseases may be linked to increased psychological distress (35, 36), similar to our finding of an association between duodenal eosinophilia and anxiety. For example, atopic dermatitis was associated with more psychological distress and more depression in a large representative sample of the US adult population (35). Other data suggest stress, exhaustion, and anxiety are increased in allergic asthma compared with controls (36) although in younger subjects this association may be weak or non-existent (37).

In celiac disease, increased duodenal intraepithelial lymphocytes occur as part of a gluten-sensitive enteropathy. Notably, duodenal eosinophils are also significantly increased in celiac disease (38) and in those with a diagnosis of celiac disease there is a significantly increased prevalence of functional dyspepsia by Rome criteria (39). In a nationwide Swedish study of over 19000 children with biopsy verified celiac disease, there was a 19% increased risk of a new diagnosis of psychiatric disease including anxiety disorders, and the risk persisted into adulthood (40). However, any link of anxiety with celiac disease may be explained by other factors including anxiety induced by the diagnosis itself and need to be on a gluten free diet or fears about long term complications (41, 42). In this study, none of the participants had celiac disease or were on a gluten free diet.

The association between low-grade duodenal inflammation and anxiety is therefore biologically plausible. Microbial or inflammatory pathways may both be involved in gut to brain communication potentially driving disease (22). For example, a fermented milk product with probiotics given for four weeks to healthy women altered brain region activity related to sensation and emotion, suggesting microbial signaling via the intestinal tract may be one mechanism that could drive CNS dysfunction (22). In the present study we have no data on the duodenal microbiome but other studies suggest there is a specific duodenal dysbiosis in FD and research is ongoing to ascertain if microbial alterations explain the upper gut symptoms (20, 21). In FD in addition to duodenal inflammation, immune activation with increased cytokine release has been reported including TNF-alpha (1, 14). Further, TNF-alpha blockade with infliximab was reported to reduce gut visceral sensitivity presumably via changes centrally in the brain (43). Whether cytokine release is a mechanism driving anxiety in duodenal eosinophilia remains to be established. However, the finding of duodenal pathology as a factor directly linked to anxiety may have important therapeutic implications because it is potentially easier to target intestinal pathology than the central nervous system. The intriguing possibility treating intestinal low-grade inflammation may possibly be able to relieve anxiety now needs to be studied.

The strengths of this study include that a random sample of a true general population was prospectively studied, and the response rate was high at all time points with no evidence of major selection or measurement bias. There are also a number of potential limitations. The number of cases with new-onset anxiety at follow-up was not large, and we only had data on duodenal eosinophilia at baseline not at follow-up 10 years later. We were likely underpowered to detect any associations with depression, but conclude if depression is important it is less likely to follow intestinal perturbations and is less relevant to disease burden compared with anxiety. While we applied a validated measure of anxiety and depression, we did not evaluate other psychological risk factors nor could we explore the stress response or alterations in the hypothalamic pituitary adrenal axis which may be highly relevant. We have reported the study population is representative of the Swedish population (23) but the findings may not generalize to other parts of the world.

We conclude duodenal eosinophilia is linked to anxiety, a novel observation. While the current data are exciting, only by testing if healing of the low-grade duodenal inflammation relieves anxiety will it be possible to conclude this is likely a casual pathway.

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Table 1. Baseline characteristics of the study cohort

Characteristic	Summary
Age: mean (SD), N	62 (12), 213
Male gender: % (n)	34 (72)
Rome III functional dyspepsia: % (n)	42 (89)
Body Mass Index: mean (SD), N	26.1 (4.0) 211
HADS anxiety: mean (SD), N	4.0 (3.4) 202
HADS depression: mean (SD), N	2.6 (2.3) 203

HADS = Hospital Anxiety and Depression Scale

Table 2a. Univariate associations with anxiety at baseline

	Risk factor status %[A]		
Risk factor	**Absent**	**Present**	**[B]OR, 95% confidence interval, p-value**
D1 eosinophils elevated	3.2	6.8	2.14 (0.56, 8.26) 0.3
D2 eosinophils elevated	0.9	10.5	11.71 (1.40, 97.67) 0.02
Smoking	3.7	8.1	2.19 (0.52, 9.16) 0.3
Snuff	4.5	4.5	1.01 (0.12 (8.46) >0.9
H. pylori	6.1	1.4	0.23 (0.03, 1.91) 0.2
Alcohol (>100g)	5.1	0.0	n/a
Rome III FD	2.6	7.2	2.75 (0.66, 11.37) 0.2
NSAIDs	3.7	10.0	2.87 (0.55, 14.88) 0.2
PPI use	[C]5.9	[C]8.3	1.26 (0.15, 10.53) 0.8
Allergy	[C]6.0	[C]6.3	[D]0.91 (0.00, 6.19) 0.9

[A]% without and with the risk factor who have clinical anxiety on HADS

[B]Odds ratio (OR) estimated via multiple imputation

[C]Estimated without multiple imputation due to numerical estimation problems

[D]Estimated via exact logistic regression

D1=duodenal bulb. D2=duodenal 2nd portion

NSAIDs = non-steroidal anti-inflammatory drugs

PPI=proton pump inhibitor

Table 2b. Univariate associations with anxiety at follow-up

	Risk factor status % [A]		
Risk factor	**Absent**	**Present**	**[B]OR, 95% confidence interval, p-value**
D1 eosinophils elevated	3.0	13.7	4.54 (1.21, 17.10) 0.03
D2 eosinophils elevated	6.2	7.7	1.27 (0.41, 3.91) 0.7
Smoking	6.6	6.7	1.00 (0.18, 5.68) >0.9
Snuff	7.5	0.0	n/a
H. pylori	7.7	4.7	0.61 (0.16, 2.31) 0.5
Alcohol (>100g)	6.8	5.3	0.77 (0.09, 6.81) 0.8
Rome III FD	2.2	13.5	6.06 (1.22, 30.11) 0.03
NSAIDs	5.9	10.0	1.77 (0.36, 8.62) 0.5
PPI use	6.2	7.7	1.26 (0.15, 10.53) 0.8
Allergy	6.2	6.8	1.10 (0.12, (9.93) 0.9

[A]% without and with the risk factor who have clinical anxiety on HADS

[B]Odds ratio (OR) estimated via multiple imputation

D1=duodenal bulb. D2=duodenal 2nd portion

FD=functional dyspepsia

NSAIDs = non-steroidal anti-inflammatory drugs

PPI=proton pump inhibitor

Figure 1. Nested case control endoscopic study subject flow chart.
EGD=esophagogastroduodenoscopy.

Figure 2. Proportion with new onset anxiety according to baseline first portion of duodenum (D1) eosinophilia status (normal versus elevated). Low baseline anxiety=HADS score<11. High baseline anxiety=HADS score ≥11.

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	Page No.	Relevant text from manuscript
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1	
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	4	
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	6	
Objectives	3	State specific objectives, including any prespecified hypotheses	7	
Methods				
Study design	4	Present key elements of study design early in the paper	7	
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7	
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	8	
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	8	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	8	
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*8*	
Bias	9	Describe any efforts to address potential sources of bias	8	
Study size	10	Explain how the study size was arrived at	8	

Continued on next page

Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8
		(*b*) Describe any methods used to examine subgroups and interactions	8
		(*c*) Explain how missing data were addressed	8
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	8
		(*e*) Describe any sensitivity analyses	N A
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	9
		(b) Give reasons for non-participation at each stage	9
		(c) Consider use of a flow diagram	Fig 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	12
		(b) Indicate number of participants with missing data for each variable of interest	10
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	9
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	*12*
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence	

		interval). Make clear which confounders were adjusted for and why they were included	12
		(*b*) Report category boundaries when continuous variables were categorized	12-13
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	

Continued on next page

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	18	Summarise key results with reference to study objectives	10
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	10
Generalisability	21	Discuss the generalisability (external validity) of the study results	13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	2

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.