ORIGINAL ARTICLE

Determination of Relative Frequency of Eosinophils and Mast Cells in Gastric and Duodenal Mucosal Biopsies in Adults with Non-Ulcer Dyspepsia

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ABSTRACT

Objective: To determine eosinophil and mast cell populations in gastric and duodenal mucosal biopsies of adults with non-ulcer dyspepsia (NUD) as compared to non-dyspeptic adults.

Study Design: A case control study.

Place and Duration of Study: Shahid Sadoughi University of Medical Sciences, Yazd, Iran, from January 2010 to June 2011.

Methodology: A total of 52 (25 non-ulcer dyspeptic patients as case and 27 non-dyspeptic patients as control) patients underwent endoscopy. All patients had a minimum of 2 forceps biopsies obtained from stomach and duodenum. Routine histological evaluation was performed and additionally evaluated to determine eosinophil and mast cell counts. The statistical analysis was performed on SPSS version 17.0, using Mann-Whitney test with significance at $p < 0.05$.

Results: The mean age in the case and control groups was 31.72 ± 12.17 and 35.74 ± 12.42 years respectively. The median eosinophil density in gastric mucosa in case group was 5.0 (ranging from 1 to 20) and 4.0 in control group (ranging from 0 to 16; $p = 0.140$). The median eosinophil density in duodenal mucosa in case group was 16.0 (ranging from 2 to 24) and 13 in control group (ranging from 2 to 45; $p = 0.147$). The median mast cell density in gastric mucosa in case group was 4.0 (ranging from 0 to 33) and 4.0 in control group (ranging from 0 to 26; $p = 0.827$). The median mast cell density in duodenal mucosa in case group was 4.0 (ranging from 0 to 31) and 3.0 in control group (ranging from 1 to 23; $p = 0.704$). The frequency of *Helicobacter pylori* infection in both the groups was similar.

Conclusion: Although there were not statistically significant differences in eosinophil and mast cell densities between case and control groups, there was a trend toward mild eosinophilia in gastric and duodenal mucosa. The specific role of eosinophils and mast cells in NUD is yet to be completely defined.

Key words: *Eosinophil. Mast cell. Non-ulcer dyspepsia.*

INTRODUCTION

Dyspepsia is seen in approximately 40% of adults each year and is often diagnosed as functional (non-ulcer) dyspepsia.[1] Functional bowel disorders (FBDs) are defined by symptoms of gastrointestinal (GI) dysfunction, discomfort and pain in the absence of a demonstrable organic cause. These symptoms may co-exist with anxiety and depression.[1] Owing to un-known pathogenesis of this disorder, appropriate treatment remains challenging. In 2006, the Rome III working group defined functional dyspepsia as presence of one or more of following symptoms: bothersome post-prandial fullness, early satiation, epigastric pain, epigastric burning with no endoscopic evidence of structural disease which is likely to explain the symptoms.[2] Criteria should be fulfilled for at least 3 months with symptom onset at least 6 months previously. Recent studies show controversy about the nature of functional dyspepsia, and there remains a divide between the symptom experience and abnormal pathophysiology which may not be addressed by the new classification.[3]

A wide variety of pathophysiological mechanisms have been postulated to contribute to the development of symptoms in patients with FBD. Of the abnormalities proposed, alterations in gastroduodenal motor and reflex function have been most extensively studied. A role for inflammation in the pathogenesis of FBD has been postulated but the specific role of inflammation has yet to be completely defined.[4] There is evidence to implicate both mast cell and eosinophil in functional dyspepsia. The purpose of this case control study was to determine the frequency of gastric and duodenal eosinophils and mast cells in adult patients with NUD as compared to non-dyspeptic patients.

METHODOLOGY

This study was approved by the Shahid Sadoughi University Ethics Committee. This study was a case

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control study performed on 25 adult patients with functional dyspepsia and normal endoscopy (all were without gross pathology including nodularity, erosion, or ulceration) and 27 controls who had no dyspepsia and no abnormality on their endoscopy.

All patients had symptoms consistent with non-ulcer dyspepsia.[1] There were 27 caces serving as controls who were referred to endoscopy ward for reasons other than dyspepsia such as check-up, foreign body swallowing and dysphagia. Controls were in the same age and gender ranges. All patients and controls underwent upper endoscopy. All patients and controls had normal gross endoscopic examinations, without ulceration, erosions, and nodularities or masses. Endoscopy was performed in the endoscopy ward under sedation, using Olympus GIF N30 and XP20 endoscopes.

All patients and controls had two grasp mucosal biopsies obtained from gastric mucosa and first part of duodenum. Biopsies were fixed in 10% formalin for standard processing and hematoxylin and eosin (H and E) and Giemsa staining. The specimens were evaluated for abnormal histologic findings and *H. pylori (HP)* microorganism. In addition, a minimum of 5 hpf were evaluated for eosinophil (Figure 1) and mast cell (Figure 2) counts on gastric and duodenal mucosal biopsies and the sum was calculated for each case. All eosinophil and mast cell counts and histological evaluation were performed by a single observer (F. Benish) in a blinded fashion.

Statistical analyses were done using Statistical Package for Social Sciences (SPSS) software version 17.0 (SPSS Inc. Chicago, IL, USA). Continues variables are shown as median and categorical variables are shown as percentage. Since the data was not normally distributed, Mann-Whitney U-test was used to compare the median values of cell counts between study groups. P-value less than 0.05 was considered as statistically significant.

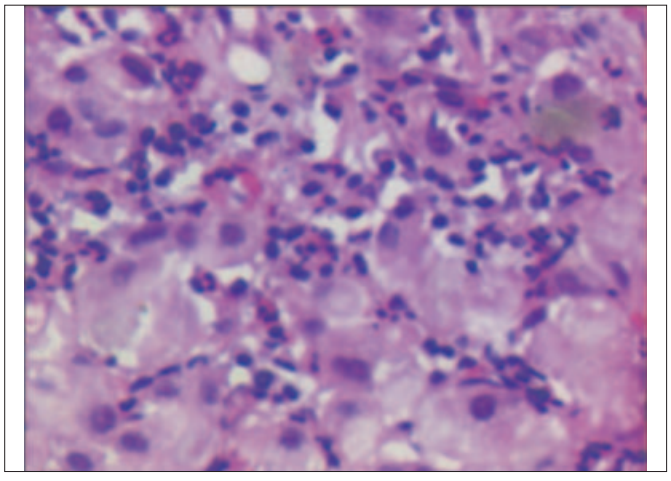

Figure 1: Shows eosinophils in gastric mucosa (H & EX 40).

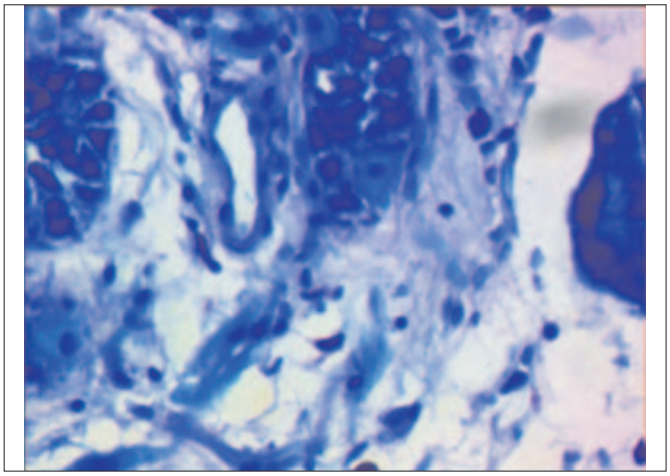

Figure 2: Shows mast cell in gastric mucosa (Giemsa's stain X40).

Table I: The median cell counts in the gastric and duodenal mucosa among study groups.

	Control Median (range)	Case Median (range)	p-value
Eosinophil			
Gastric mucosa	4.0 (0 to 16)	5.0 (1 to 20)	0.140
Duodenal mucosa	13 (2 to 45)	16.0 (2 to 24)	0.147
Mast cell			
Gastric mucosa	4.0 (0 to 26)	4.0 (0 to 33)	0.827
Duodenal mucosa	3.0 (1 to 23)	4.0 (0 to 31)	0.704

RESULTS

The patients aged ranged from 15 – 76 years (mean, 31.72 $\pm$ 12.17 years). There were 35 males and 17 females. The median eosinophil density in gastric mucosa in case group was 5.0 (ranging from 1 to 20) and in control group was 4.0 (ranging from 0 to 16; p = 0.140). The median eosinophil density in duodenal mucosa in case group was 16.0 (ranging from 2 to 24) and in control group was 13 (ranging from 2 to 45), (Table I; p = 0.147). The median mast cell density in gastric mucosa in case group was 4.0 (ranging from 0 to 33) and in control group was 4.0 (ranging from 0 to 26; p = 0.827). The median mast cell density in duodenal mucosa in the case group was 4.0 (ranging from 0 to 31) and in control group was 3.0 (ranging from 1 to 23; p = 0.704). With regard to *HP* infection, the prevalence of *HP* infection in the case and control groups was similar. The median density of eosinophil in the gastric mucosa of the HP+NUD was slightly more than HP-NUD (6.0 vs. 4.0). The median density of eosinophil and mast cell in the duodenal mucosa of the HP+NUD and HP-NUD was 15.0 vs. 16.0 and 3.0 vs. 4.0 respectively.

DISCUSSION

Functional dyspepsia has been defined by Rome III as the presence of one or more chronic dyspepsia symptoms in the absence of any organic, systemic, or

metabolic disease that is likely to explain the symptoms.[9] The etiology and pathophysiology of FBDs are not completely clear, and no single physiologic abnormality can be implicated as the cause of symptoms in every patient. Inflammation of the upper gastrointestinal tract has been implicated in the development of functional gastrointestinal disorders.[5] Among the inflammatory cells, mast cells and eosinophils are especially important and some recent studies suggested that the duodenum has a critical role in symptom generation in FBD. This is attributed to immune activation.[6]

Overall there is very limited data on the cellular pathology of FBD and existing data have rather conflicting results. Toukan *et al*. evaluated 31 cases with non-ulcer dyspepsia and 32 healthy controls; it was found that there was a slight but significant increase in the eosinophil count of the duodenal bulb mucosa but the relationship between duodenal abnormalities and pathophysiologic mechanisms of FBD were not clear.[7] In 2007, Talley *et al*. showed a significant association between the number of eosinophils in the duodenum and non-ulcer dyspepsia,[8] but mast cell density was not considered.

The purpose of this case control study was to determine the frequency of gastric and duodenal eosinophil and mast cell densities in adult patients with FBD. The current study evaluated both mast cell and eosinophil densities in the gastric and duodenal mucosa in adults with NUD and we were unable to find any similar study in the literature. In this study, there was a slight trend toward increased eosinophil in the gastric mucosa of the patients with NUD although this finding just failed to reach a significance. In Talley's study, the gastric eosinophil counts were not altered in FBD. The median eosinophil density in duodenal mucosa in both groups again showed a slight trend toward increased eosinophil density in the duodenal mucosa in patient with NUD that failed to reach a significance. While no generally accepted standard exists for defining increased eosinophil density, the eosinophil count in dyspeptic patients in the current study were lower than Talley's study. To explain this difference, it can be said that the pathophysiology of FBD is multifactorial and many factors such as genetic predisposition, previous episodes of infectious enteritis, undiagnosed food allergies, atopy and even psychological factors may contribute either individually or in combination. For example in one study, the role of eosinophils in the duodenum in functional dyspepsia was studied in 155 randomly selected duodenal biopsies which were designed normal after routine microscopic examination.[9] Significantly increased eosinophil densities were detected in dyspepsic patients and this was associated with allergy (odds ratio 5.04). Also in another research, the prevalence of dyspepsia in asthmatic patients was significantly greater than in control.[10] Duodenal eosinophilia was found in allergic patients in a population attending for endoscopy in one study.[9] These observations show that confounding factors, especially allergy might be responsible for this difference in eosinophilic densities in different studies.

In addition, it is suggested that eosinophil counts obtained by routine histology may not give an adequate indication of potential eosinophil involvement in disease processes. Indeed, Erjefalt *et al*. found that the level of degranulation varies significantly between diseases where the eosinophil density on routine histology does not.[11] As a result, the degree of degranulation may be a better indicator of the disease process rather than density. But this study was not designed to evaluate this hypothesis. A link between eosinophils and functional gastrointestinal diseases has therapeutic implications.

There was no statistically significant difference in duodenal mucosal mast cell density between patients with or without dyspepsia. To explain this very mild increase in duodenal mast cells density, it should be noted that eosinophils and mast cells contribute to immune responses or immune activation. Mast cells induce eosinophil migration and eosinophils activate mast cells.[12] In contrast to the current study, Hall *et al*. reported increased gastric mucosal mast cells in patients with and without *H. pylori*-associated FBD. They proposed that these findings might contribute to FBD by altering signalling in the brain-gut axis.[13] Matter *et al*. demonstrated that 13% of patients with NUD had increased antral mast cell density.[14] This study illustrated that gastric mast cells have a possible role in FBD, although the data are not as yet as robust as that for duodenal eosinophilia. Biopsy reports from paediatric populations with functional abdominal pain, compared to those with abdominal pain due to inflammation (Crohn's disease or ulcerative colitis), showed increased mast cells in antral mucosa in one study.[15] As a result, it is suggested that patients with increased mast cells on antral biopsy appear to be subset of patients with non-ulcer dyspepsia amenable specific treatment with H1-antagonists.[14] With regard to *HP* infection, the prevalence of *HP* infection in the case and control groups was similar. A number of trials have been published showing the high prevalence of *H. pylori* in patients with FBD.[16] So an association between *H. pylori* and non-ulcer dyspepsia is more uncertain. Nevertheless *H. pylori* eradication treatment is effective in at least a subset of patients with non-ulcer dyspepsia. The median density of eosinophil in the gastric mucosa of the HP+NUD was slightly more than HP-NUD. But it was not true about the median density of eosinophil and mast cell in the duodenal mucosa of the same groups. It is suggested that *HP* infection may up-regulate eosinophil infiltration in the stomach.[8] Eosinophils, in addition to releasing their preformed cytotoxic proteins, can also

release a variety of cytokines and chemokines.[17] And it is possible that mast cells have a direct cytotoxic function on the *HP* by modifying the gastric mucosa environment.[18] Some studies showed that *H. pylori* is involved in mast cell chemotaxis, and activation and release of pro-inflammatory mediators and cytokines.[19] In contrast, one study showed that there was no significant difference between mast cell numbers in patients with *H. pylori* positive functional dyspepsia compared to inflammatory control subjects and they proposed that increased mast cell density is related to functional dyspepsia, independently of inflammation.[13]

It should be noted that this study had some limitations. Ethically there was no healthy control group. Eosinophil and mast cell activation and degranulation could not be evaluated. The sample size was small.

There is very limited data on the cellular pathology of FBD. Although inflammation has been implicated in functional FBD the specific role of inflammation has yet to be completely defined. Whether gastric and duodenal eosinophilia in humans can influence gastrointestinal sensory and motor function warrants further investigation. Whether *H. pylori* infection could contribute to immune activation observed in the gastric and duodenal mucosa of *H. pylori*, positive FBD patients should be further investigated. Mast cells and eosinophils interact with T-lymphocytes and may alter enteric nerve and smooth muscle function. What role lymphocytes have in functional dyspepsia and indeed which antigens, if any, are responsible for T-cell stimulation remains to be seen. Studies elucidating the role of eosinophils, mast cells and other immune cells, including lymphocytes, in the small and large bowel mucosa of patients with FBD are currently being actively pursued.

CONCLUSION

Although there were not statistically significant differences in eosinophil and mast cell densities between case and control groups, there was a trend toward mild eosinophilia in gastric and duodenal mucosa. The specific role of eosinophils and mast cells in NUD is yet to be completely defined.

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