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Research Article

Histological correlates of gastro esophageal reflux disease in South Indian population

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ABSTRACT

Background: Diagnosing gastroeosopageal reflux disease (GERD) accurately is a complex problem. This study was conducted to examine the histological findings of GERD in Indian subjects. Esophageal biopsy can be combined with pH monitoring and endoscopy to define the histological damage that occurs due to acid regurgitation. The sensitivity and specificity of the individual findings needs clarity in this clouded area in order to be of use to the pathologist.
Methods: A total of 102 patients with dyspepsia were included in this study. Those with heartburn and /or regurgitation were identified as patients with GERD and those without these symptoms were treated as cases of non GERD dyspepsia. Biopsies were taken 2cm above 'Z' line in all cases. The biopsies were read by a single pathologist. Basal cell hyperplasia (BCH), neutrophilic exocytosis (NE), dilated intercellular spaces (DIS), papillary elongation (PE) and lymphoid aggregates (LA), necrosis (NEC) and eosinophilic infiltration were studied.
Results: 68 patients had GERD dyspepsia and 34 had non GERD dyspepsia. The histological findings of BCH, NE, PE, DIS, LA were found to be found much more often in patients with GERD symptoms (p values ranged from 0.0001 to 0.0008). We found BCH and papillary elongation together were the most sensitive histological findings. Specificity was highest when DIS combined with NE.
Conclusions: In this study we found basal cell hyperplasia is the most common histological finding, and when combined with DIS or papillary elongation enhances its sensitivity. However to exclude other causes of dyspepsia, a combination of DIS, PE and NE can be used effectively.

Keywords: Dyspepsia, Dilated intercellular spaces, Basal cell hyperplasia, Endoscopy

INTRODUCTION

Diagnosing gastroeosopageal reflux disease (GERD) accurately is a complex problem. The diagnostic tests that are used examine three different issues. The 24 hour pH testing tests for reflux but this is not equivalent to having disease as patients with reflux are often asymptomatic and the refluxate does not cause tissue damage.[1] The response to proton pump inhibitors which is the simplified form of the acid perfusion test is complicated by the presence of false positives due to a placebo response and may unnecessarily lead to lifelong use of the drug.[2]

The presence of histological damage indicates disease but is both lower than expected in sensitivity and specificity, because of an inadequate gold standard and possibly poor sites of sampling.[3] It would seem that the best diagnostic motif would be a combination of two tests.[4] For this good histological definition is required. We conducted the following study as the nature of histological changes has not been charted in the Indian population.

In this study we looked at three questions regarding the mucosal histology of GERD. The first was to ask what the dominant mucosal histological changes were in patients who had symptoms of GERD. For the purposes of the study we used a simple eight segment questionnaire Table 1 and the specific symptoms for GERD were heartburn and acid regurgitation; as these were assumed to be the cardinal and distinctive symptoms of GERD.

Table 1: Questionnaire appended.

Symptoms	No	Yes	Duration in months
Upper abdominal pain			
Heart burn			
Regurgitation			
Dysphagia			
Anorexia			
Any bleeding disorder			
Drug intake Anticoagulants NSAIDS PPI			
Vomiting			

We had a population of patients without these symptoms and they were classified as having non GERD dyspepsia. The second question raised was whether there were histological differences between those who suffered from heart burn alone and those who had acid regurgitation alone. The third issue was whether a set of histological changes would emerge as strong sensitive and specific criteria for GERD in our study population.

Initially we examined biopsies for the classical histological features namely basal cell hyperplasia (BCH), neutrophilic exocytosis (NE) and congested Papillae in upper 1/3rd of mucosa. However as recent studies had reported that dilated intercellular spaces (DIS), lymphocyte aggregates (LA), necrosis (NEC) and eosinophils were also histological correlates of GERD, we conducted a post hoc examination of the histology *vis-a-vis* the new histological characteristics.[5-7] We also examined the reliability of endoscopic assessment for evidence of gastroeosopageal reflux disease (GERD).

METHODS

The present study was a descriptive cross sectional study in which a total of 106 consecutive cases of dyspepsia were solicited for this study in our gastroenterology department, Pondicherry Institute of Medical Sciences, India.

Out of which 102 agreed to undergo this study. Institutional ethical clearance was obtained prior to the study. The definition of dyspepsia for this study included any patient whose symptoms appeared to be upper gastro intestinal origin, i.e. either related to feeding or associated with epigastric pain. Patients with associated lower bowel symptoms such as frequent bowel stools and flatulence were not excluded. However patients with bloody stools fever, systemic symptoms were excluded. For the purposes of defining GERD only two symptoms were used: acid regurgitation and heart burn.

Consecutive patients were administered the questionnaire prior to endoscopy. The investigator administering the questionnaire was not involved in making the qualifying pre-endoscopy clinical diagnosis.

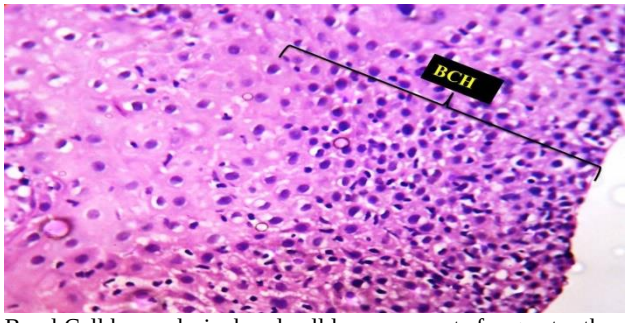
Endoscopy was conducted by a single experienced endoscopist. Protocol biopsies were taken from the lower end of the esophagus 2 cm above the Z line, irrespective of whether endoscopic damage was seen or not.[8]

The endosocopist was blinded to the response of the questionnaire, although the out-patient records were available for him with the pre-test clinical diagnosis.

Histology and interpretation

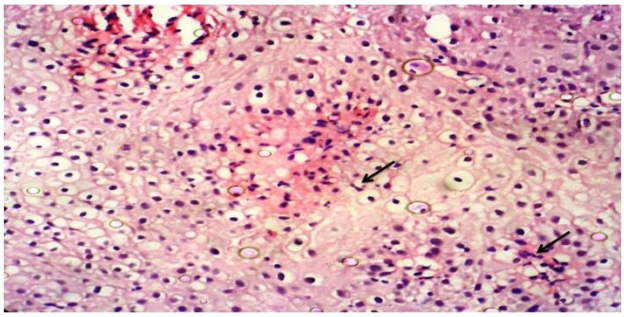
The biopsies were fixed in 10% buffered formalin; routinely processed and embedded in paraffin. The histologic sections were stained with H and E. The slides were first studied by one of us without reference to the questionnaire and the findings reported as present or absent.

However the final decision on each slide was made by an expert histopathologist who was blinded to the nature of the symptoms and endoscopic findings. The following characteristics were noted in the first survey: Basal cell hyperplasia (BCH), neutrophilic exocytosis (NE) and congested papillae in upper 1/3rd of mucosa. Post hoc the following findings were also examined: Dilated intercellular spaces, lymphocyte aggregates, necrosis and eosinophils. The following were the definitions used for these histological criteria. Figures illustrate each criterion.



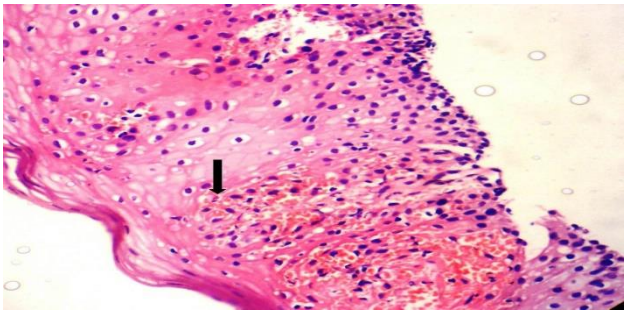
Basal Cell hyperplasia: basal cell layer accounts for greater than 15% of mucosal thickness.

Figure 1: Basal cell hyperplasia of esophageal mucosae.



Neutrophilic exocytosis is the presence of neutrophils in superficial layers of epithelium.

Figure 2: Neutrophilic exocytosis in superficial layers of epithelium.



Congested papillae in upper one third of mucosa: the subepithelial papillae reach greater than two thirds of the mucosal thickness.

Figure 3: Congested papillae in upper 1/3rd of esophageal mucosa.

Analysis

For this study we used symptoms as the gold standard for diagnosis and assessed the histological correlates of that. In the first instance we examined it for the two groups – i.e. those with GERD symptoms and those with Non GERD dyspepsia.

In the second instance we separated out those with only acid regurgitation and those with only heart burn and examined each subgroup separately for histological correlates. Finally we picked out histological changes that separated out GERD from Non-GERD and based on frequency formed a set of criteria that could be used as the histological basis of GERD related mucosal disease.

Statistical analysis was done by using chi square test with SPSS version 19.0 software.

RESULTS

Assessing histological changes against symptoms

Assessing all GERD symptoms together: By the criteria mentioned above 68 had GERD symptoms and 34 had non GERD dyspepsia. Out of 68 GERD patients 48 (70%) had both regurgitation and heart burn, 9 (13%) patients had only heartburn and 11 (16%) had only regurgitation. Out of 68 GERD patients 5 patients had endoscopic GERD. The histological findings are listed in Table 2.

Table 2: Comparison of histological parameters with GERD and Non GERD dyspepsia.

Histological parameters	GERD (68)	Non-GERD dyspepsia (34)	P- value
BCH	55 (81%)	6(18%)	0.008
Neutrophilic exocytosis	46(68%)	3(9%)	0.001
Papillary elongation	47(69%)	6(18%)	0.001
DIS	36(53%)	2(6%)	0.007
Lymphocyte aggregates	39(57%)	5(15%)	0.2
Eosinophils	3(4%)	0	0.5
Necrosis	2(3%)	0	0.9

*The following findings are statistically significant (p value<0.05) Basal cell hyperplasia (0.008), Neutrophilic exocytosis (0.001), papillary elongation (0.001) and Dilated intercellular spaces (0.007). The following were not statistically significant: Lymphocyte aggregates, Necrosis and the presence of eosinophils.

Table 2 shows that the histological findings of basal cell hyperplasia, neutrophilic exocytosis, papillary elongation (PE) and dilated intercellular spaces Figure 4 are common in those biopsies from patients with the GERD symptoms described and further distinguish the GERD group from those with non GERD-dyspepsia. Necrosis and eosinophilia were not significantly present or a distinguishing characteristic of GERD.

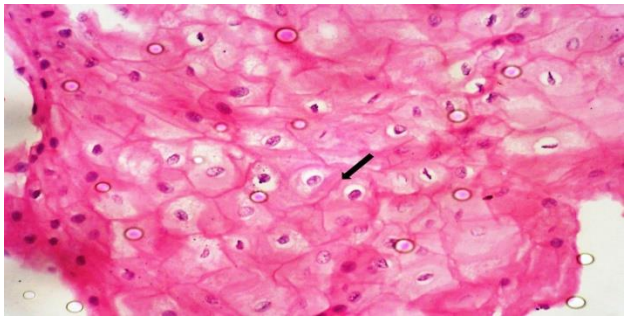


Figure 4: Dilated intercellular spaces in esophageal epitheium.

Assessing individual GERD symptoms separately: When those with regurgitation alone were separately identified from those with heartburn alone and the histological correlates examined, there were distinct differences between these two groups.

Table 3 shows that basal cell hyperplasia, dilated intercellular spaces and lymphoid aggregate was clearly found more in those with heartburn, while there appeared to be a trend towards significance with regard to

neutrophilic exocytosis but no differences as regards papillary elongation and eosinophils, even as papillary elongation was a characteristic of all GERD. In the group with both symptoms, the presence of these histological finding were in between the two extremes.

Table 3: Comparison of histological parameters with GERD symptoms.

Histological parameters	Regurgitation	Percent	Heartburn	Percent	P- value	Comment
BCH+	7	63.6%	9	100%	0.043	Significant
BCH-	4	36.4%	0	0%		
NE +	4	36.4%	7	77.7%	0.06	Trend
NE-	7	63.6%	2	22.3%		
PE+	6	54.5%	8	88.9%	0.398	NS
PE-	5	45.5%	1	11.1%		
DIS +	2	18.2%	7	77.7%	0.008	Significant
DIS-	9	81.8%	2	22.3%		
LA+	3	22.3%	8	88.9%	0.006	Significant
LA-	8	77.7%	1	11.1%		
EO+	0	0%	1	11.1%	0.257	NS
EO-	11	100%	8	88.9%		

*The following findings were statistically significant: Basal cell hyperplasia (0.04), Dilated intercellular spaces (0.008) and Lymphocyte Aggregates (0.006).

Definition of diagnostic criteria: We then looked sensitivity and specificity of the individual histological findings namely basal cell hyperplasia, papillary elongation, neutrophilic exocytosis, dilated intercellular spaces and lymphoid aggregates.

We used the most common and differentiating findings from this study namely basal cell hyperplasia, papillary elongation, dilated intercellular spaces and lymphoid aggregates. These were the core criteria and looked at their frequency in those with GERD.

Table 4: Sensitivity and specificity of histological findings.

Features	Sensitivity	Specificity
BCH/PE	83.8%	58.8%
NE/PE	76.4%	67.6%
PE/DIS	75%	52.9%
BCH/DIS	80.8%	47.06%
BCH/NE	82.3%	58.8%
NE/DIS	22%	61.7%

*****Basal cell hyperplasia (BCH) and papillary elongation (PE) has the highest sensitivity (83.8%) and specificity being best among neutrophilic exocytosis (NE) and papillary elongation (PE) (67.6%). (DIS- Dilated intercellular spaces).

Table 4 showed that the highest sensitivity of picking up histological correlation of symptomatic GERD are when BCH/PE or BCH/DIS or BCH/NE which means that BCH is the most promising histological finding. BCH alone had a sensitivity of 80.8%. Therefore most of the contribution is because of BCH. Sensitivity was highest with the combination of basal cell hyperplasia and papillary elongation. However the specificity was highest when dilated intercellular spaces was combined with neutrophilic exocytosis or papillary elongation, but not sufficiently high when DIS or any other features taken alone.

DISCUSSION

This study is probably the first of its kind in Indian patients and demonstrates that the histological correlates of symptomatic GERD are BCH, DIS, NE and PE. Necrosis, eosinophils and lymphocyte aggregates (LA) are not seen very often. This is different from a previous report which also used symptoms as the gold standard of diagnosis. Whether it is similar in other study populations in India, we cannot say but this is what is found in South India.

We have not seen similar blinded analysis from elsewhere and therefore this needs further analysis in other populations. Interestingly the value of DIS has varied from not being pathognomic to being highly sensitive and specific.[9]

Is it valid to use a combination of symptoms as a gold standard? The Diamond study demonstrates how single tests or symptom responses to therapy can be misleading. Symptoms alone have been used before this though the questionnaire was more complicated.

The two symptoms that were used here are the only ones used in the reflux disease questionnaire, albeit the

severity and length of symptoms may have given an edge to that diagnosis.[10] It is a moot point whether the two questions used in our questionnaire was less proficient than the complex questionnaire quoted above or that the length and severity added anything to just the presence of regurgitation and heartburn without qualifications.

From the way the evidence has fallen it appears that these two questions are sufficient as the high frequency histological changes such as BCH, PE, NE and DIS seem to separate out GERD from non GERD quite clearly while the low frequency changes such as lymphoid aggregates, necrosis and eosinophils do not help differentiate between the two groups.

From these findings we can also conclude that the latter histological changes are poor indicators of disease and that the former should be taken as the most important correlates of GERD. The findings that a small number of Non GERD subjects have these findings may indicate that there is asymptomatic GERD in the dyspeptic population (9 to 18%).

The second interesting finding of this study is that within the group with symptoms, those with heart burn have a different set of histological correlates than those with symptoms of reflux alone. This was despite a small number in each group. Such findings have not been reported before.

The outstanding finding was that DIS and BCH were more common with heart burn. Further LA which is not often found in patients with gastroeosopageal reflux disease (GERD) still appears to be more common in those with heart burn. This suggests that heartburn indicates a different histological milieu where chronic inflammation and regeneration are part of the process.

The third finding is the specificity and sensitivity of using the core criteria as histological diagnostic tool. We found that the combination of basal cell hyperplasia (BCH) and papillary elongation (PE) has the highest sensitivity (83.8%), closely followed by basal cell hyperplasia and neutrophilic exocytosis (NE) (82.3%). The specificity is much lower in comparison and the best indicators specific to GERD are papillary elongation and neutrophilic exocytosis (67.6%).

CONCLUSION

In this study we identified the most prominent histological findings and used them to identify the most sensitive at picking out those with disease and the ones that are specific for GERD.

Interestingly, basal cell hyperplasia which is the most common histological finding is the one that identifies most cases though the addition of papillary elongation (PE) or dilated intercellular spaces (DIS) enhances its specificity. However to exclude other diseases, a combination of dilated intercellular spaces, papillary elongation and neutrophilic exocytosis can be used effectively.

These criteria we feel should be used whenever there is clinical doubt of disease prior to surgery for gastroeosopageal reflux disease and when the relationship of oesophageal ulcer, stricture or cancer in doubt. The widespread use of pH studies without biopsies should be discouraged.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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