DOI: 10.7860/JCDR/2022/55446.16286

Original Article

Pathology Section

Immunohistochemical Expression of CDX2 in Gastroesophageal Junction Biopsies: An Emerging Marker for Early Intestinal Differentiation of Barrett's Metaplasia

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ABSTRACT

Introduction: Histological diagnosis of Barrett's oesophagus (BE) in mucosal biopsies is challenging and affected by multiple factors. Goblet Cells (GCs) are not distributed uniformly in BE and is dependent on sampling probabilities. Furthermore, GC Mimickers (GCM) are potential pitfalls in the diagnosis of Intestinal Metaplasia (IM). Alcian Blue (AB) stain has been extensively used in detection of GC's although it has the limitation of low specificity with positive staining for GCM. Recently, CDX2 Immunohistochemistry (IHC) is reported to be highly sensitive and specific marker which has shown to identify early intestinal phenotype even in absence of diagnostic GCs and especially pertaining to conditions where characteristic morphological changes are not apparent.

Aim: To study the histomorphology of non neoplastic and neoplastic lesions of the Gastroesophageal Junction (GEJ) in reflux patients and evaluate the diagnostic role of CDX2 IHC versus AB stain in detecting IM.

Materials and Methods: This retrospective study was conducted in Department of Pathology at Kasturba Medical College, Manipal, Karnataka, India, on 55 patients with clinical features of reflux and adequate records of GEJ biopsies, diagnosed over 6 years from January 2012 to August 2018. Clinical presentation, endoscopic findings and histomorphology (18 parameters) were recorded. AB stain and CDX2 (IHC) were performed and evaluated in all cases. A detailed histological evaluation was done for all cases and subsequently, sensitivity, specificity, positive predictive value and negative predictive value of CDX2 IHC to identify early intestinal differentiation was calculated.

Results: Of 55 cases, 28 were BE, 19-Reflux oesophagitis (RE) and 8-adenocarcinoma. Heart burn and chest pain were the most common clinical presentations of BE. Endoscopy of BE predominantly showed hiatus hernia with tongue like projections of the gastric mucosa. Histologically, intraepithelial eosinophils and spongiosis were more common features in RE. Barrett's oesophagus showed columnar epithelium with multilayering, presence of IM with GC (1-20/crypt) along with sub-squamous buried epithelium and splitting of muscularis mucosa. By IHC, as compared to AB; CDX2 IHC was more sensitive (100% vs 78.2%) and specific (96.5% vs 82.6%) for detecting an intestinal phenotype. The five cases (22%) of BE contained only GCM in the biopsy, were CDX2 negative but showed a false positivity for AB. In BE, CDX2 additionally highlighted positivity in non GC columnar cells which were AB negative. The CDX2 showed diffuse positivity in dysplasia with focal strong to absent expression in adenocarcinoma.

Conclusion: The CDX2 efficiently differentiated between GC and pseudo GC. Its presence in the absence of AB in non GC columnar cells suggests that it effectively detects intestinal phenotypic features even before morphological features are evident.

Keywords: Alcian blue, Barretts oesophagous, Goblet cells, Intestinal metaplasia, Reflux

INTRODUCTION

Reflux of the acidic gastric contents into the oesophagus with resultant irritation of the oesophageal mucosa, causes Gastro-Oesophageal Reflux Disease (GERD). Difficulty in swallowing, burning sensation of throat, belching are features of GERD. Patients with GERD could progress to BE. BE predisposes to cancer development , the incidence of BE to cancer progression has been increasing over the years and was seen to be 0.1-0.4% per year in the recent studies [1]. The reflux symptoms might not be present in every patient and they may also have normal endoscopic findings. So, an accurate assessment might be difficult in those cases [2-4]. Hence, a good histopathological analysis is the key for management. Needless to say, histological diagnosis of Barrett's oesophagus (BE) can be quite challenging. Goblet Cells (GCs) are not distributed uniformly in BE, their proportion varies amongst patients and specimens and biopsy may fail to pick up GCs. The columnar cells which are present in between GCs may look a lot like gastric foveolar cells or intestinal absorptive cells. Goblet Cells Mimickers (GCM) are potential pitfalls in the diagnosis of IM [5-12]. The GCMs can look like GCs with their ample accumulation of mucinous cytoplasm and are called as the pseudo-goblets. The columnar epithelial cells may also contain AB positive acid mucins, even though the intensity of staining is less than that of GCs. In addition, these cells have a tendency to be distributed more diffusely than the true GC, which has a more dispersed distribution [5,6].

In this respect, CDX2 IHC is a reported highly sensitive and specific marker which has been shown to identify early intestinal phenotype even in absence of diagnostic GCs and especially pertaining to conditions where characteristic morphological changes are not apparent [7,8].

Study objectives:

- Detailed histomorphological analysis of non neoplastic and neoplastic lesions of GEJ in patients with reflux and to correlate with endoscopic findings;
- Evaluate the diagnostic role of CDX2 IHC in detecting early IM in comparison with AB (pH 2.5); and
- Study CDX2 staining patterns in dysplasia and adenocarcinoma of oesophagus.

MATERIALS AND METHODS

This retrospective study was conducted in Department of Pathology at Kasturba Medical College, Manipal, Karnataka, India, from January 2012 to August 2018. All procedures performed were approved by Institutional Review Board and National Research Ethics Committee (IEC number 595/2016, dated 20/09/2016) in accordance with the 1964 Helsinki declaration and its later amendments. A total of 55 cases with symptoms of reflux and who had adequate GEJ mucosal biopsies were included. The clinical details and endoscopic findings were retrieved from the medical records department. Haematoxylin and Eosin (H&E) sections of mucosal biopsies of all the 55 cases were studied for histomorphological features. AB and CDX2 IHC were subsequently done in all the cases and were studied. For CDX2 IHC, deparaffinised tissue sections were used for DAK-CDX2 (Dako Monoclonal mouse Antihuman CDX2, Ref M3646) and manual staining for AB was done in all the cases.

Inclusion and Exclusion criteria: All cases of reflux with GEJ biopsies were included in the study. Other cases with causes of oesophagitis, squamous cell carcinomas and adenocarcinoma of stomach were excluded from the study.

All cases were studied for the histomorphological features as given in [Supplementary Table-1]. The detailed definitions of the histological variable are provided in [Supplementary Table-2].

Reflux oesophagitis (RE) was diagnosed in the presence of epithelial injury and absence of GCs; and BE was diagnosed in the presence of GCs on H&E stain. The pattern of staining of CDX2 IHC and AB were studied subsequently. CDX2 IHC staining was considered positive when any intensity of nuclear staining was seen. The AB was considered as positive when the cells had any intensity of bluish cytoplasmic staining. CDX2 staining was studied in terms of nuclear expression in GCs and non GCs, along with extent of staining (diffuse>50% of cells/focal <50% of cells). Adenocarcinomas were diagnosed in presence of invasion. Sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV) was derived for AB and CDX2 staining in case of BE, following which the data was compared with other published literature.

STATISTICAL ANALYSIS

Categorical data was expressed as frequency along with percentage n (%). Sensitivity, specificity, PPV and NPV was calculated for CDX2 on comparison with AB. Data processing and statistics were done using Microsoft Excel 2010 version.

RESULTS

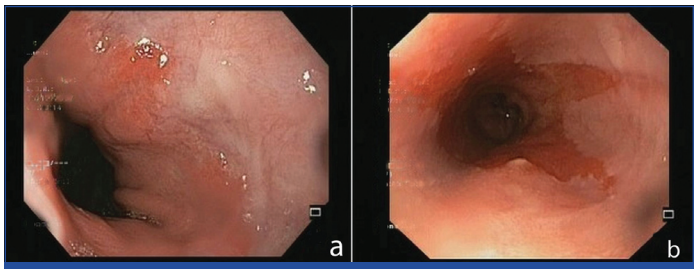
There were a total of 55 cases, 19 had reflux oesophagitis, 28 had BE and 8 had adenocarcinomas. The clinical details with endoscopic findings are given in [Table/Fig-1]. On endoscopy 62.5% cases showed tongue like projection of salmon colored mucosa above the GEJ which ranged from <5-10 cm length above the GEJ. Two (12.5%) presented as Short Segment Barrett's oesophagus (SSBE) with tongue like projection of 2 cm above GEJ [Table/Fig-2a] and ultra-SSBE was seen in 1 case (0.06%) with a length of <5 mm above GEJ. An 87.5% cases (14 out of 16) were long segment BE [Table/Fig-2b]. All the above cases with tongue like projections were clinically given the diagnosis of BE.

[Table/Fig-3] summarises the various histomorphologic features along with [Table/Fig-4-7] showing the various histomorphologic findings in RE and BE including pseudo-GCs. Detailed histopathological changes in both the squamous and columnar component of individual oesophageal biopsies were evaluated in cases of RE and BE.

In the squamous components, RE had more basal zone hyperplasia (100% versus 88%), intraepithelial lymphocytes (95% versus 82.1%),

Parameters	Reflux oesophagitis (RE) (n=19)	Barrett's oesophagus (n=28)*	Adenocarcinoma (n=8)
Age	24-80 years (mean age-54.1)	41-75 years (mean age-62.3)	49-70 years (mean age- 58.2)
Sex	Male:12 (63.1%)	Male: 15 (53.5%)	Male: 8 (100%)
	Female:7 (36.8%)	Female: 8 (28.5%)	Female: 0 (0%)
Presentation	Symptoms of reflux	Symptoms of reflux-heart burn and chest pain	Difficulty in swallowing solids
Clinical diagnosis	Reflux oesophagitis (95%)	Gastrooesophageal reflux disease (65%), Barret's oesophagus (18%), Hiatus hernia (17%)	Carcinoma (100%)
Endoscopy	-Ulceration and nodularity in oesophageal mucosa (60%) -Hiatus hernia (20%) -Tongue like projection (20%)	Tongue like projection (62.5%) Hiatus hernia (13%)	Growth in Gastroesophgeal junction (100%)

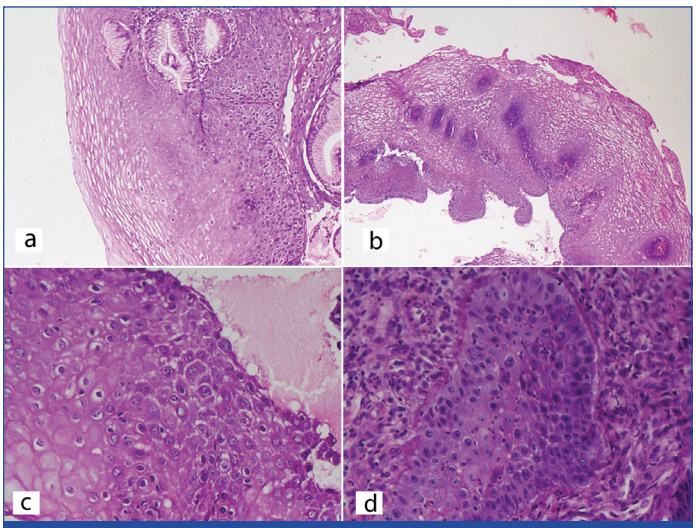
[Table/Fig-1]: Clinical details and endoscopic findings.
*Some samples didn't take up the stain



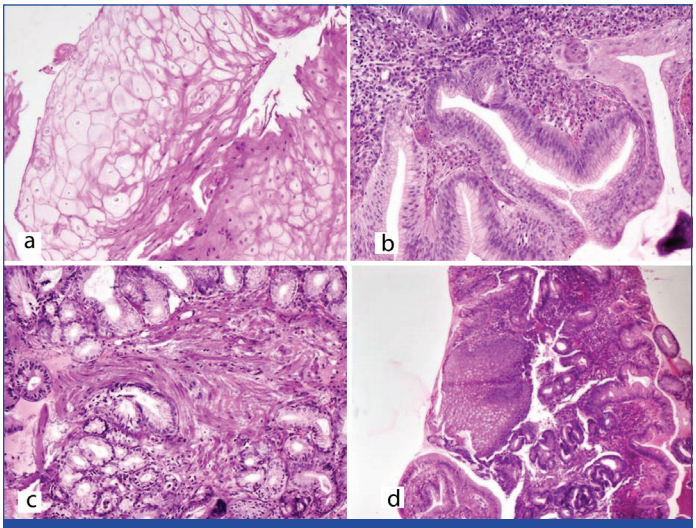
[Table/Fig-2]: Gastric endoscopy images. a): Short segment Barrett's Oesophagus (BE) with tongue like projection, 2 cm above Gastroesophageal Junction (GEJ); b): Long segment Barrett's Oesophagus (BE) with tongue like projection, 3 cm above GEJ.

Features		Reflux oesophagitis	Barrett's oesophagus
Squamous component			
Basal zone hyperplasia		100%	88%
Intraepithelial lymphocytes		95%	82.1%
Intraepithelial eosinophils		37%	24%
Papillary hyperplasia		79%	72%
Spongiosis		68%	72%
Inflammation in lamina		78%	100%
Mucosal ulceration		37%	28%
Columnar component			
Glands beneath crypt epithelium	Only Mucous glands	83%	62.9%
	Only Oxyntic glands	0	0
	Mucous+ Oxyntic glands	17%	37%
Multilayered epithelium		50%	51.8%
Inflammation in lamina propria (100%)		Lymphocytes+Plasma cells+eosinophils: 16.6%) Lymphocytes+Plasma cells: (66.6%) Lymphocytes+Plasma cells+Neutrophils: 16.6% Oedema: 0	Lymphocytes+Plasma cells: 100% Only Eosinophils: 25.9% Only Neutrophils: 29.6% Oedema: 33.3%
Goblet cell number per crypt		0	100% (ranging from 0>20)
Dysplasia		18%	18%
Low grade		15%	15%
High grade		3%	3%
Squamous islands		11%	28.5%
Subsquamous buried epithelium		0	50%
Splitting of muscularis mucosae		53%	35.7%
Changes in gastric cardia	Inflammation	100%	100%
	Goblet cells	0	33.3%
	H.pylori	43%	0

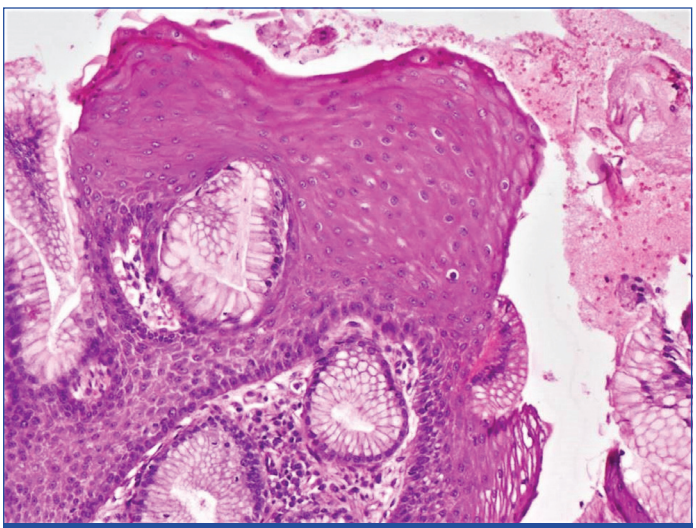
[Table/Fig-3]: Histomorphologic features of Reflux Oesophagitis (RE) and Barrett's Oesophagus (BE).



[Table/Fig-4]: a): Basal zone hyperplasia (100X); b): papillary hyperplasia (40X); c): Spongiosis with intraepithelial lymphocytes (400x); d): Intraepithelial eosinophils (400x).



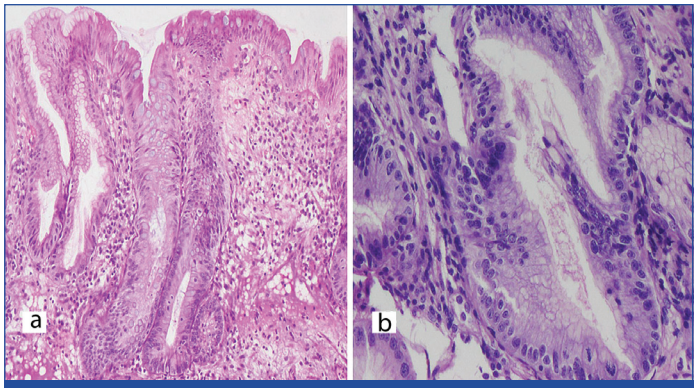
[Table/Fig-5]: a): Ballooning of cells (400X); b): Multilayered columnar pithelium (100X); c): Splitting of muscularis mucosa (100X); d): Squamous islands (40X).



[Table/Fig-6]: Sub-squamous buried epithelium (400X).

intrapepithelial eosinophils (37% versus 24%) and mucosal ulcerations (37% versus 28%) as compared to BE. In the columnar component, BE had more combined mucous and oxyntic glands beneath crypt epithelium (37% versus 17%).

The inflammation in lamina propria was higher in BE than RE (100% vs 78%). The inflammation in BE consisted primarily of lymphocytes and plasma cells (100% vs 67%). In BE, 25.9% and 29.6% of cases showed isolated neutrophil and eosinophil rich inflammation respectively, which was not observed in any case of

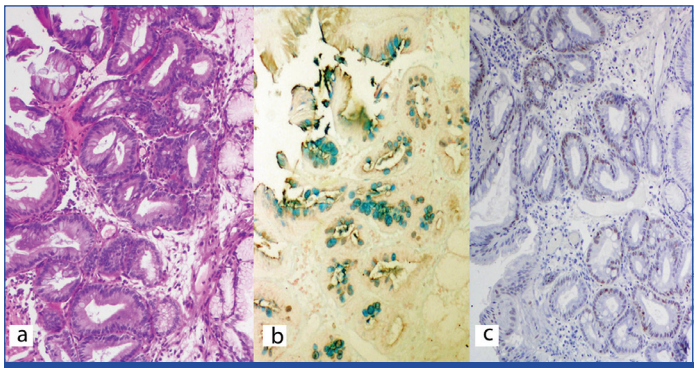


[Table/Fig-7]: a): True Goblet Cells (GC) in Barrett's Oesophagus (BE) (100X); b): Pseudo-goblet cells (400X).

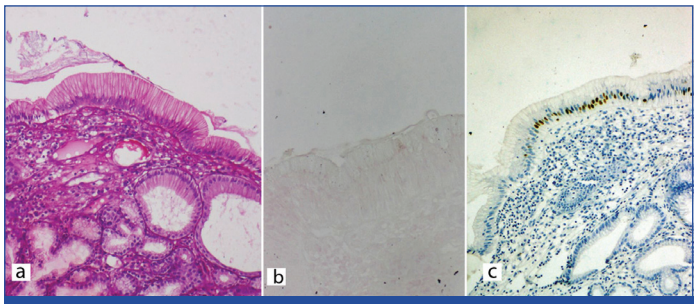
RE. A 33.3% cases of BE also showed diffuse oedema, whereas oedema was not noted in RE. Presence of GCs, dysplasia (18%) and subsquamous columnar cell nests (50%) were only seen in BE whereas presence of H.pylori was only seen in RE (43%). A splitting of muscularis mucosa was more in RE (53% versus 35.7%) as compared to BE. Out of the eight cases of adenocarcinomas, six were well differentiated and two were moderately differentiated. Two cases (33.3%) were associated with BE while RE did not show any association with carcinoma.

CDX2 and Alcian Blue (AB) Staining in Reflux Oesophagitis (RE) and Barrett's Oesophagus (BE):

AB was done in 24 cases of BE and CDX2 was done in 23 due to the unavailability of blocks in remaining cases. All the cases of BE were positive for AB in the GCs (100%) and also in 18 cases (75%) where along with GCs, non GCs were also stained [Table/Fig-8]. All the cases of RE were negative for AB [Table/Fig-3]. The CDX2 was positive in only 18 out of 23 cases (78.2%) of BE (true positive cases), which showed positivity of non GCs more than the GCs [Table/Fig-8]. Only one case (0.05%) of RE showed nuclear CDX2 positivity [Table/Fig-9].



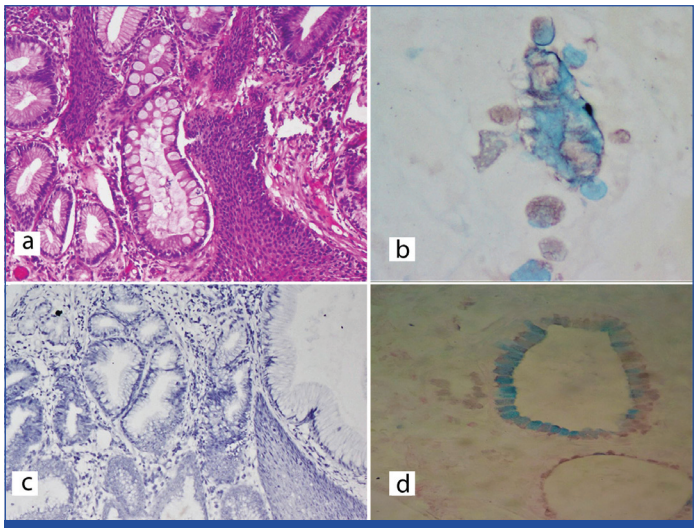
[Table/Fig-8]: a): H&E of true Goblet Cells (GC) (100X); b): Alcian Blue (AB) positive GC (100X); c): Nuclear CDX2 expression in goblet and non GC (100X).



[Table/Fig-9]: Reflux Oesophagitis (RE). a): H&E; b): Alcian Blue (AB) negative in columnar cells; and c): CDX2 positivity in the metaplastic columnar epithelium (40X).

After further detailed review, the remaining (CDX2 negative) five cases (5/23) (37%) by histology had only GCMs , surprisingly all of which were AB positive and were thus interpreted to be histologically incompatible with a diagnosis of BE. CDX2 IHC on these five cases

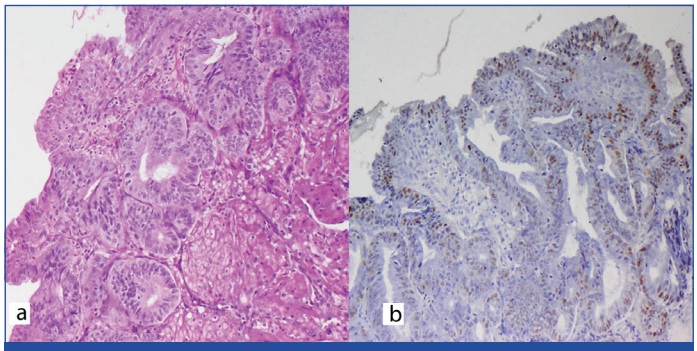
was repeatedly negative [Table/Fig-10a-d]. Out of these five cases, two had pseudo-GCs and three had columnar blue cells which had AB positivity in a stretch of the epithelium.



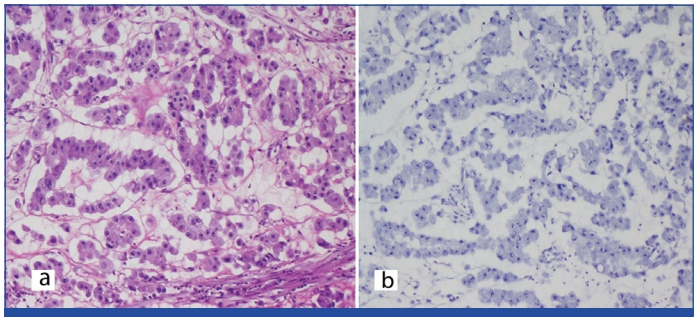
[Table/Fig-10]: Goblet Cell (GC) mimickers, pseudo-Goblet Cells on a): H&E; b): Alcian Blue (AB) positive in pseudo-Goblet cells; c): CDX2 expression negative in pseudo-Goblet cells; d): Columnar blue cells (400X).

CDX2 staining in dysplasia and adenocarcinoma:

All the cases of dysplasia, both low and high grade had diffuse strong positivity for CDX2 IHC [Table/Fig-11a,b]. In case of Adenocarcinomas, CDX2 was available in six out of eight cases. Out of these six cases, 4/6 was well differentiated and 2/6 was moderately differentiated. Both two negative CDX2 cases [Table/Fig-12a,b] were well differentiated adenocarcinomas. In the well differentiated adenocarcinoma category, only one case showed focal (<50% of tumour cells) positive staining of tumour cells. Overall, as compared to AB, overall CDX2 IHC had a higher sensitivity (100%) and specificity (96.5%) with a PPV of 95% to identify early intestinal phenotype in BE [Table/Fig-13].



[Table/Fig-11]: Low grade dysplasia. a): H&E; b): CDX2 expression (40X).



[Table/Fig-12]: Adenocarcinoma well-differentiated. a): H&E; b): Absence of CDX2 expression in the same (100x).

Histopathological diagnosis	Alcian blue positive	CDX2 positive
Barrette's oesophagus	23/23 (100%), mainly in Globet Cells (GC) -(18 true BE+5 with Globet Cells Mimicker incorrectly diagnosed as BE by histology)	18/18 (78.2%), Non GC>GC
Reflux oesophagittis	None (100% negative)	1 (0.05%) in non GC
Sensitivity	78.26%	100%
Specificity	82.76%	96.5%
Positive predictive value	78%	95%
Negative predictive value	83%	100%

[Table/Fig-13]: Alcian Blue (AB) and CDX2 staining in Barrett's Oesophagus (BE) and Reflux Oesophagitis (RE).

DISCUSSION

The BE is a relatively indolent disease and prevalent in 2-7 % of the population [6]. The importance of BE lies in its increased potential risk of oesophageal adenocarcinoma. Till date, there exists a considerable amount of controversy regarding the precise histological diagnostic criteria of BE. The American Gastroenterological Association (AGA) emphasise that IM (with 'GCs') is essential for diagnosing BE [6]. AGA defined BE as "Columnar Metaplasia (CM) of oesophagus that is visible 'endoscopically' and confirmed 'histologically' [6]. It is of the view that IM is the only type of oesophageal columnar epithelium that predisposes to malignancy. In contrast, The British Society of Gastroenterology defines BE as "an oesophagus in which any portion of normal distal squamous epithelial lining , which has been replaced by metaplastic columnar epithelium , clearly visible endoscopically (≥1 cm) above GEJ and confirmed histopathologically by oesophageal biopsy" [6]. Hence, by this definition BE requires presence of CM with or without GCs. However, it is important to note that the risk for malignancy in CM is much less when compared to IM with GCs [6].

On endoscopy, BE has been classically reported to present as circumferential migration with tongues of metaplastic epithelium [12]. The junction with the squamous epithelial lining of the oesophagus may sometimes appear as a symmetric or asymmetric Z line or columnar mucosa seen alternating with squamous mucosa forming islands (island pattern) [13]. An endoscopic diagnosis of BE is given only when salmon colour mucosa extends into tubular oesophagus, extending more than or equal to 1 cm proximal to GEJ, which should also be necessarily confirmed on biopsy [14]. In this study, 64.5% cases of BE and also a large portion (20%) cases of RE presented with the classic endoscopic tongue like presentation. This point to considerable overlap and non specificity of endoscopic findings in suspected cases of BE.

Histologically, classical BE biopsies show relatively similar histopathological features as GERD, except for the additional presence of metaplastic columnar epithelium with or without the presence of GCs. Goblet cells (GCs) should be differentiated from GCMs, which have been sometimes noted to be concomitantly in BE biopsies [15]. In this regard, histochemical stains cannot distinguish between the two as it stains acidic mucin in both. The AB-positive non GCs are generally found in the gastric pit epithelium. If these AB positive columnar cells are present in the surface epithelium then it is an abnormal finding and are known as "Metaplastic AB positive cells" [16]. A distinction between the two lesions is imperative in mucosal biopsies, as one is relatively benign and the other with premalignant potential. In this study, the American system was followed for all the cases of BE which were diagnosed on the basis of presence of GCs.

The presence of IM in a biopsy is also considerably dependent on sampling probability. Harrison R et al., reported the frequency of IM varies from patient to patient, depends on the site and also the number of biopsies taken [15]. Khandwalla HE et al., and Sharma P et al., found presence of IM in 29% and 23% of repeat biopsies, which were initially diagnosed to be negative for IM [17,18]. Takubo K et al., also reported that the mucosa immediately adjacent to adenocarcinomas was more frequently of gastric type (71%) rather than intestinal type (22%) [19].

The CDX2 is a homeobox transcription factor and belongs to the caudal related family of CDX homeobox genes which was first found to be expressed in the mouse intestine [20]. The expression of CDX2 is seen in adult non neoplastic tissues and is limited to normal intestinal epithelium, normal pancreatic epithelial cells, and gastric and IM. In the gastrointestinal tract,CDX2 is seen to be strongly and diffusely positive in small and large intestinal epithelial cells, including absorptive, goblet, endocrine and Paneth cells [21]. It has also been reported that the oesophageal superficial columnar mucosal cells lacking GCs, known as "Columnar blues" can demonstrate positive AB staining, but are consistently CDX2 negative [22]. Normal oesophageal and gastric epithelial cells are CDX2 negative. In the oesophagus, CDX2 is expressed in CM with or without IM.

A study suggests that CDX2 positivity is necessary for embryonic intestinal proliferation [22]. The CDX2 can also be positive in columnar cells in few of the cases without histological features of IM, hence reflects the ability of CDX2 to detect early intestinal phenotypic features even before the histochemical and morphological features are manifested. The expression of CDX2 proximal to Barrett's metaplasia suggests that its expression precedes the phenotypic transformation [20].

Another role of CDX2 staining has been reported to help differentiate low and high grade dysplasia in BE. According to various literature, diffuse staining for CDX2 is seen in non dysplastic BE and BE with low grade dysplasia. The intensity of CDX2 staining and percentage of positive cells decreases in BE with high grade dysplasia and adenocarcinoma. This suggests that with tumour progression, cellular differentiation decreases [23].

The CDX2 was performed in 48 cases in the current study. An 18/23 cases of BE, all of which had true GCs had consistent CDX2 positive staining. The remaining five cases were hence interpreted to be falsely diagnosed as BE and had pseudo-GCs, which were CDX2 negative but had AB false positivity. Only a single case with no evidence of IM and diagnosed as RE showed CDX2 positivity in the non GCs.

Phillips RW et al., observed that 77% cases with IM were CDX2 positive and 20% cases without GCs and only showing columnar epithelium was positive for CDX2 [23]. Streher SA et al., reported CDX2 positivity in 5% cases of oesophagitis and 62.5% cases with IM [24]. Groisman GM et al., observed reported that out of 90 cases with endoscopic diagnosis of BE, 45 showed GCs and 45 did not show [22]. All the cases with GCs showed CDX2 strong reactivity in both GCs and the surrounding non GCs columnar cells and 38% cases showed focal CDX2 expression in the columnar cells without any GCs. In this study, all BE cases showed CDX2 expression both in GCs and the non GCs which ranged from diffuse to focal positive. In 56.5% cases CDX2 was expressed only in the GCs and in 17.3% cases CDX2 was expressed in the non GCs. Therefore the results from the present study validated the findings of Groisman GM et al., and it underlined the capability of CDX2 in detection of early intestinal phenotype even before the morphologic changes are apparent [22]. Another important finding which was uncovered from this study was that CDX2 expression was more present in the non goblet metaplastic columnar epithelial lining than in the GCs which were similar to the findings in literature emphasising CDX2 as a marker of early intestinal differentiation [25].

The present study found that AB was positive in all the cases of BE with GCs (100%), which were both diffuse and focal positive and it was present in the non GCs in 75% cases. All the cases of RE were negative for AB. The GCMs showed apical positivity and also positivity in the stretch of epithelium for AB stain. All of these GCM were negative for CDX2. Johnson DR et al., similarly observed that out of 108 biopsies of BE, all the cases (100%) were AB positive, but only 102 (94.4%) cases were CDX2 positive [21]. They had 43 cases with GCMs, all of which were CDX2 negative but AB positive. Similarly, all the cases of BE in this study, were AB positive but only 18/23 cases (78.2%) of them were CDX2 positive and all the GCM were CDX2 negative but AB positive.

So overall, CDX2 appeared to be a specific immunohistochemical marker for also detection of early precursors of IM/ confirmation of IM/BE in oesophageal small mucosal biopsies. As it is a nuclear transcription factor, it showed an "all or none" phenomenon. In this present study, CDX2 had a better PPV (95%) than AB (78%) , similar to that of Johnson DR et al., where the PPV of CDX2 and AB were 95.6% and 71.5%, respectively [21].

CDX2 Expression in Dysplasia and Oesophageal Adenocarcinoma:

Detection of dysplasia is very important, as progression to adenocarcinoma is a very slow and unpredictable process. Although histomorphology is considered as the gold standard when it comes to the diagnosis of dysplasia, IHC markers like CDX2 do provide an essential contributory help. Lord RV et al., reported eight cases of dysplastic BE and five cases of adenocarcinoma, with diffuse strong CDX2 expression and there was no difference in staining intensity between low grade and high grade dysplasia [8]. Hayes S et al., and Phillips RW et al., reported a decrease in CDX2 expression from high grade dysplasia to adenocarcinoma [7,23]. Barros R et al., found that out of four cases of dysplastic BE, three were positive for CDX2 and one was negative, and out of eight cases of adenocarcinoma oesophagus seven were positive and one was negative [25]. In this study, all the cases of dysplasia both high grade and low grade showed diffuse strong positivity for CDX2. In cases of adenocarcinoma, 4/6 was CDX2 positive which ranged from diffuse to focal expression. As denoted above, different studies have reported varying results with regards to intensity of CDX2 expression in different grades of dysplasia to adenocarcinoma and therefore further studies are warranted for precise confirmation.

Finally, the present study contained a morphometric detailed histopathological analysis of the squamous and columnar component of the oesophagus: 1) Basal zone hyperplasia; 2) Papillary hyperplasia; and 3) Increased intraepithelial eosinophils/neutrophils were the most predominating histological parameters noted in the squamous component of RE biopsies. On comparison with published literature by Colleypriest BJ et al., and Soucy G et al., the findings of the present study were in concordance [11,20].

Limitation(s)

Firstly, this was retrospective study which included random 55 patients and could possibly represent a selection bias due to random endoscopic and histopathological evaluation. Secondly, although the study performed a detailed histomorphological and immunohistochemical evaluation, the sample size was limited to 55 cases. This study demonstrates that CDX2 is a marker of intestinal phenotype was seen to be positive in GCs and non GCs, and was more present in the non goblet columnar epithelium, suggesting the fact that it is a marker of early intestinal differentiation which could be missed on H&E and AB stains. With its ability to pick up the early GC precursors/intestinal differentiation may prove to be fruitful in diagnosing more cases of BE than routine H&E. Furthermore, CDX2 expression decreased from dysplasia to adenocarcinoma, suggesting that its expression decreases with decrease in intestinal differentiation and tumour progression.

CONCLUSION(S)

The CDX2 immunohistochemistry is more sensitive and specific compared to AB in picking up intestinal differentiation and is an effective immunohistochemical marker for detection of early BE. Further studies with large cohort of patients, multi-quadrant gastroesophageal junction biopsies, CDX2 IHC staining and staining with other IHC's of intestinal differentiation (SATB2, CDX2) as controls are warranted for validating the diagnostic utility of CDX2 IHC in early diagnosis of BE.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]

- Plagiarism X-checker: Feb 11, 2022
- Manual Googling: Feb 16, 2022
- iThenticate Software: Mar 09, 2022 (5%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:

- Financial or Other Competing Interests: Funded by an Internal Grant (IRB Board) from the Institute
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? NA
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Feb 09, 2022**
Date of Peer Review: **Mar 05, 2022**
Date of Acceptance: **Mar 16, 2022**
Date of Publishing: **Apr 01, 2022**