 DOI: 10.5958/2394-6792.2015.00008.3

# Expression of CDX2 protein in gastric mucosa with intestinal metaplasia and gastric carcinoma

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## ABSTRACT

**Background**: *CDX2 is a caudal homeobox gene essential for intestinal proliferation and differentiation. Expression of its protein product CDX2 in gastric mucosa is strongly associated with intestinal metaplasia and gastric carcinoma.*

**Objectives:** ***1.*** *To study the incidence of intestinal metaplasia in gastric cancer* ***2****.To study the expression of CDX2 protein in intestinal metaplasia and gastric carcinoma.*

**Materials and Methods**: *The study was done for a period of 11/2 years on 50 gastrectomies. The gastric cancer was typed according to Lauren's classification. Adjacent mucosa was assessed and those with intestinal metaplasia were typed into type I, II and III by histochemistry. Immunohistochemistry for CDX2 was done in all cases of intestinal metaplasia and corresponding gastric carcinomas.The staining was graded semiquantitatively and statistical analysis was done.*

**Results**: *There were 34(68%), 9(18%) and 6(12%) cases of intestinal, diffuse and mixed type of gastric adenocarcinoma respectively. The most frequent change in adjacent mucosa was chronic gastritis with intestinal metaplasia. CDX2 immunostaining was positive in 24 out of the total 27 cases (88.8%) of intestinal metaplasia and 21 out of 27 cases (77.7%) of gastric cancer. All controls (100%) turned out to be negative, making the above observations statistically significant.*

**Conclusions**: *Chronic gastritis with intestinal metaplasia is the commonest precursor lesion of gastric adenocarcinoma. CDX2 homeodomain protein which is not expressed in normal gastric mucosa is a sensitive marker for intestinal metaplasia and is closely associated with intestinal type of adenocarcinoma.*

**Key words**: *Gastric adenocarcinoma, chronic gastritis, intestinal metaplasia, CDX2*

## INTRODUCTION

Gastric cancer is a leading cancer type in Kerala. It has various histological classifications. According to Lauren's classification it is typed into intestinal, diffuse and mixed types. Intestinal type of gastric cancer is associated with intestinal metaplasia of the stomach.

Intestinal metaplasia is a precancerous lesion. It is typed into complete and incomplete types based on histochemistry and more recently by mucin IHC. Currently complete intestinal metaplasia is referred to as type I and incomplete intestinal metaplasia is further divided into type II and type III intestinal metaplasias. CDX2 is a member of caudal related homeobox family. It is an intestine specific transcription factor that regulates both proliferation and differentiation. Antibody to the above protein is available which stains the nuclei of epithelial cells in the presence of the protein.The present study is intended to examine the relationship between intestinal metaplasia, neoplasia of stomach and CDX2 expression.

## MATERIALS AND METHODS

It was a prospective observational study done on 50 gastrectomy specimens received in our department during the period April 2011 to October 2012. Detailed history regarding clinical presentation, peroperative findings and gross appearance was recorded. Sections from tumor and adjacent gastric mucosa were assessed by H&E. Gastric carcinomas were classified into intestinal, diffuse and mixed types according to Lauren's classification. Adjacent mucosa was analysed for evidence of chronic/ atrophic gastritis and intestinal metaplasia. Alcian blue – PAS staining was used as an adjunct to H&E to detect even focal areas of metaplasia. Those cases which showed intestinal metaplasia were subdivided into type I, II, and III metaplasias using Alcian blue – PAS and High iron diamine – Alcian blue techniques.

CDX2 immunostaining was done on all cases of intestinal metaplasia and corresponding cases of gastric carcinoma. Ten histologically normal gastric biopsies were used as negative controls and tissue sections from colon were used as positive controls. 4 µm sections of formalin fixed paraffin embedded tissues were stained by standard immunohistochemical methods using peroxidase linked antibody. Primary antibody used was anti CDX2 antibody CDX2 – 88 (Biogenex).

CDX2 immunostaining was graded semiquantitatively combining the score of percentage of positive cells (nuclear staining) and the score of the staining intensity. It was as follows. Score 0 (0-

5% positive cells), 1 (6-25%), 2 (26-50%), 3 (51-75%), 4 (76 – 100%). The intensity of staining was graded on a scale from 1 to 3 as 1(mild), 2(moderate) and 3(strong). Total score came upto 7. Total score of 1 to 2 were taken as negative and scores 3-7 were taken as positive for CDX2 expression. Data was analysed using Epi info software version 3.5.4. Qualitative data were assessed using Chi- square test. A p value <.05 was considered to be statistically significant. The protocol was approved by institutional review board and ethics committee.

## RESULTS

Out of the 50 cases of gastric cancers, 34(68%) were intestinal type adenocarcinomas. There were 9(18%) cases of diffuse cancers and 6(12%) cases of mixed cancers. One case belonged to special type – Anaplastic gastric carcinoma with neutrophilic infiltrate. The age group ranged from 25 – 85 yrs (mean 58.1 yrs). The male: female ratio was 2.6:1. The most frequent change in adjacent mucosa was chronic gastritis with intestinal metaplasia (50%). 2 cases (4%) showed atrophic gastritis with intestinal metaplasia. [Fig 1,2,3]

Out of the 27 cases with intestinal metaplasia, 13 belonged to the type III subtype (48.1%). There were 11 cases of type II and 3 cases of type I metaplasia.[Table1,2][Fig.5] Metaplastic change was seen to be more frequently associated with mixed type adenocarcinoma (71%). However 20 cases (59%) of intestinal type adenocarcinoma also had intestinal metaplasia in adjacent mucosa. Distribution of the subtypes of intestinal metaplasia varied among the three types of gastric cancer. But the results were not statistically significant. 24 out of 27cases with intestinal metaplasia showed positive immunostaining with anti CDX2 antibody.[Fig. 6] All the 10 normal gastric biopsies were negative, making the above observation statistically significant. [Table 4] Colonic biopsies showed positive immune-staining consistently.

CDX2 was positive in all cases of complete intestinal metaplasia and 21 cases of incomplete metaplasia. The difference in CDX2 expression between different subtypes of intestinal metaplasia was not statistically significant. However when compared with the control samples the individual observations were statistically significant ($p<0.05$). [Table 5] Out of the 27 cases of gastric cancer for which CDX2 immunostaining was done, 21 turned out to be positive.[Fig 7] This included 16 cases of intestinal, 2 cases of diffuse and 3 cases of mixed type adenocarcinoma.[Table 6]

**Table 1: Frequency of intestinal metaplasia**

| Intestinal Metaplasia | Number | Percent |
|---|---|---|
| PRESENT | 27 | 54.0% |
| ABSENT | 23 | 46.0% |
| **Total** | **50** | **100.0%** |

**Table 2: Distribution of subtypes of intestinal metaplasia**

| Type of Intestinal metaplasia | Number | Percent |
|---|---|---|
| TYPE 1 | 3 | 11.2% |
| TYPE 2 | 11 | 40.7% |
| TYPE 3 | 13 | 48.1% |
| **Total** | **27** | **100.0%** |

**Table 3: Distribution of intestinal metaplasia among gastric cancers**

| Type of carcinoma | Total | IM -present | IM - Absent |
|---|---|---|---|
| Intestinal | 34 | 20 (58.8%) | 14 (41.2%) |
| Diffuse | 9 | 2(22.2%) | 7 (77.8%) |
| Mixed | 7 | 5 (71.4%) | 2 (28.6%) |
| **Total** | **50** | **27 (54%)** | **23(46%)** |

IM = Intestinal metaplasia

**Table 4: Frequency of CDX2 expression**

| CDX2 | IM Positive (cases | IM Negative (controls) |
|---|---|---|
| Positive | 24 (88.8%) | 0 (0%) |
| Negative | 3 (11.2%) | 10 (100%) |
| TOTAL | 27 (100%) | 10 (100%) |

**Table 5: CDX2 positivity in intestinal metaplasia subtypes**

| Type | Cases | CDX2+ve cases | Percentage | χ 2 | P value |
|---|---|---|---|---|---|
| **Total no: of intestinal metaplasia** | 27 | 24 | 88.8% | 21.55 | 0.000 |
| **Type I** | 3 | 3 | 100% | 7.98 | 0.005 |
| **Type II** | 11 | 10 | 90.9% | 13.9 | 0.000 |
| **Type III** | 13 | 11 | 84.6% | 13.00 | 0.000 |

**Table 6: Distribution of CDX2 in tumor**

| Type of Carcinoma | CDX2 positive | CDX2 negative |
|---|---|---|
| Intestinal | 16 (80%) | 4 (20% ) |
| Diffuse | 2 (100%) | 0 (0%) |
| Mixed | 3 (60%) | 2 (40%) |
| **Total** | **21(77.7%)** | **6 (22.3%)** |

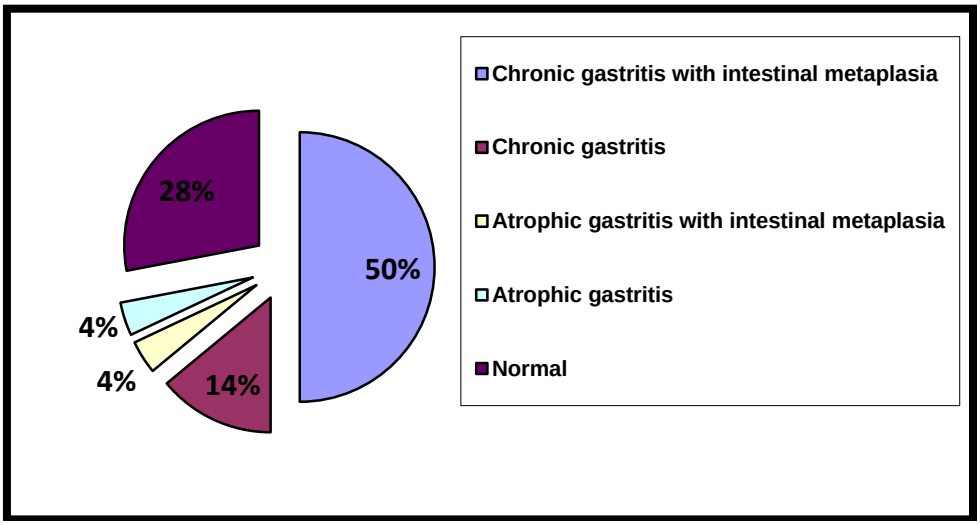


**Fig. 1 Changes in adjacent gastric mucosa**

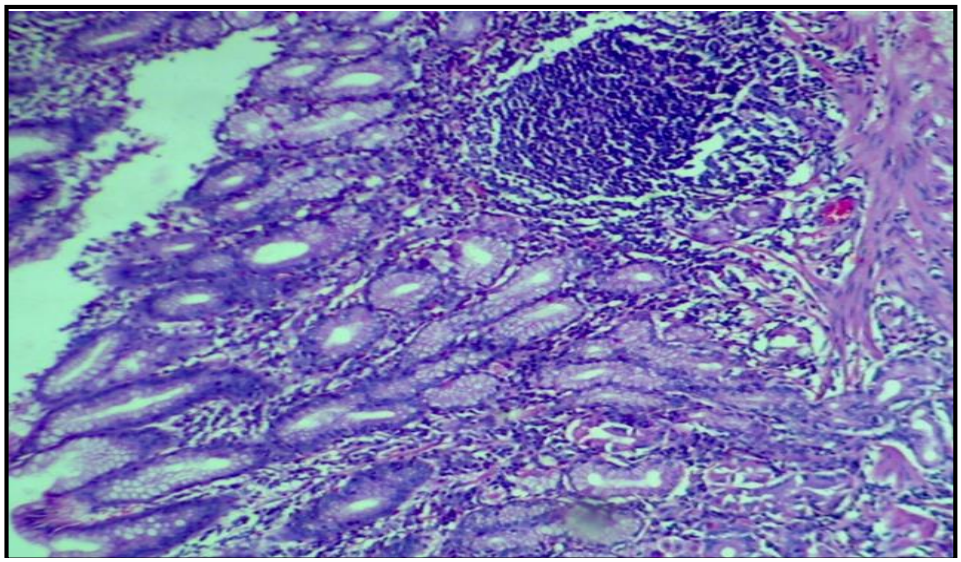

**Fig. 2 (a): Chronic gastritis (H&E 100x)**

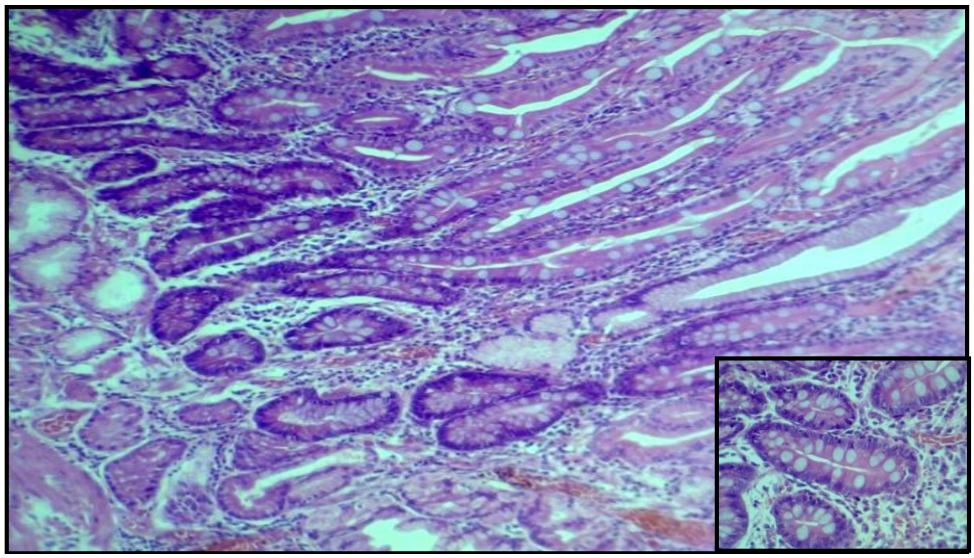

**Fig. 2(b): Chronic gastritis with intestinal metaplasia (H&E 100x), Inset 400x**

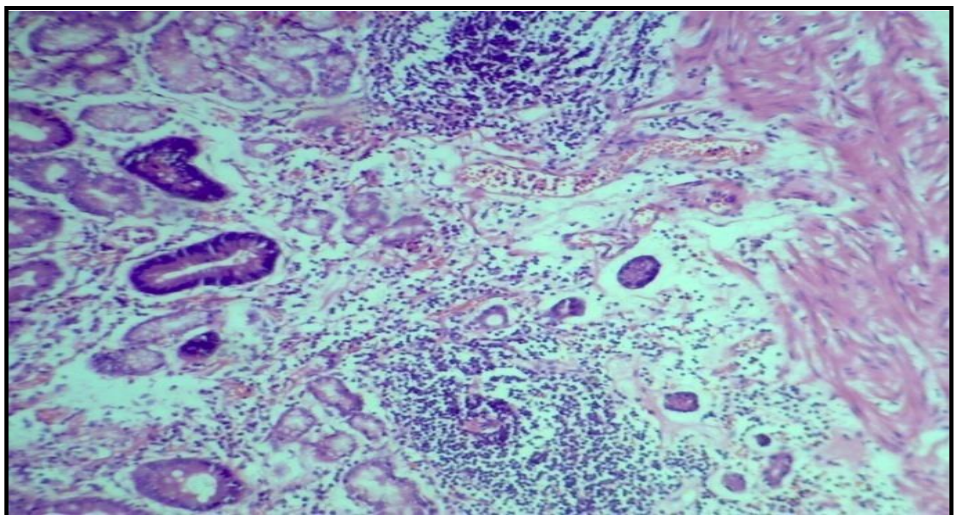

**Fig. 3 Atrophic gastritis (H&E 100x)**

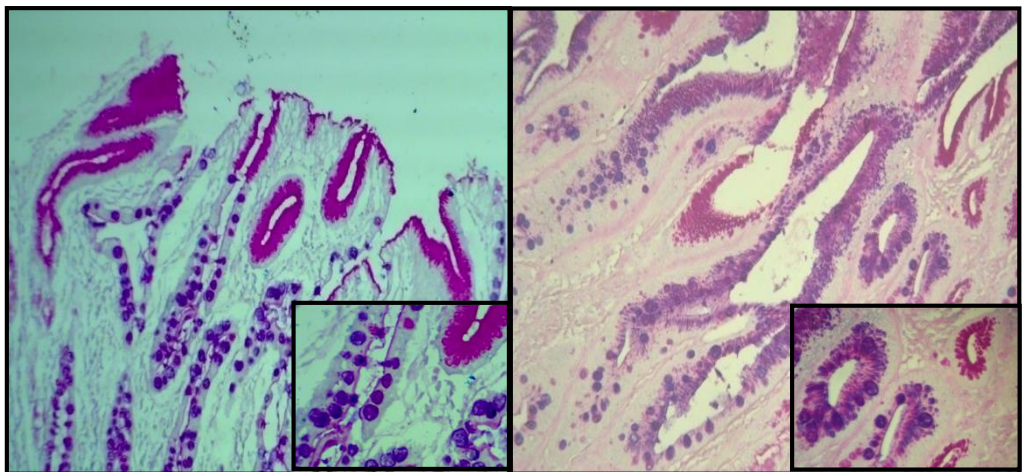

**Fig. 4 - Alcian blue-PAS: a)** Complete intestinal metaplasia (100x). Only goblet cells take up purple blue colour. Normal gastric glands (neutral mucin) take up magenta colour. Inset 400x; **b)** Iincomplete intestinal metaplasia (100x). Both goblet cells and columnar cells take up purple blue colour. Normal gastric gland (neutral mucin) takes up magenta colour. Inset 400x.

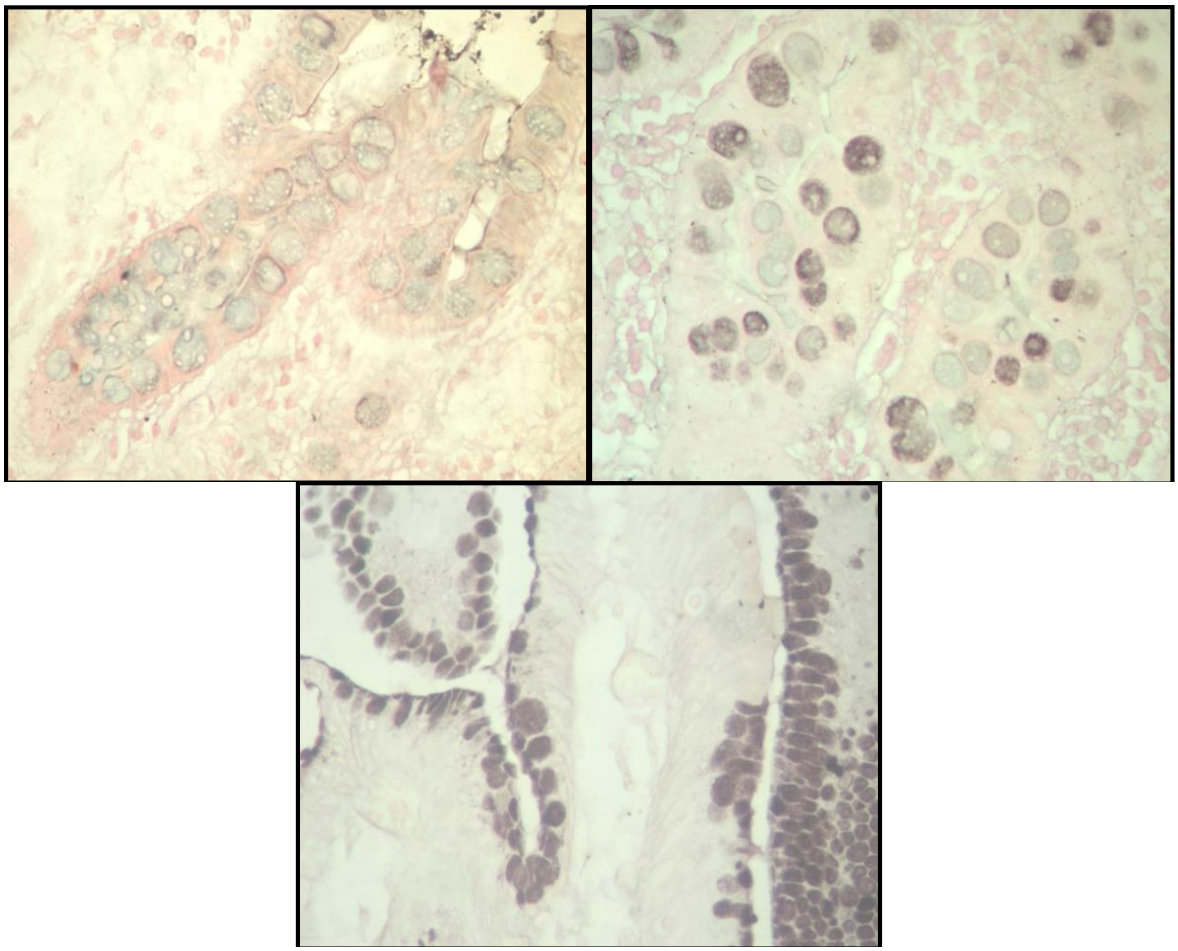

**Fig. 5 High iron diamine - Alcianblue 400x**

**(a):** Type I intestinal metaplasia -Goblet cells stain blue (sialomucin) whereas the columnar cells do not take up any mucin stain. (absorptive cells)

**(b):** Type II intestinal metaplasia-Goblet cells stain blue (sialomucin) or brown (sulphomucin). Columnar cells take up pale blue colour. (sialomucin)

**(c)** Type III intestinal metaplasia. Goblet cells and columnar cells stain brown. (sulphomucin)

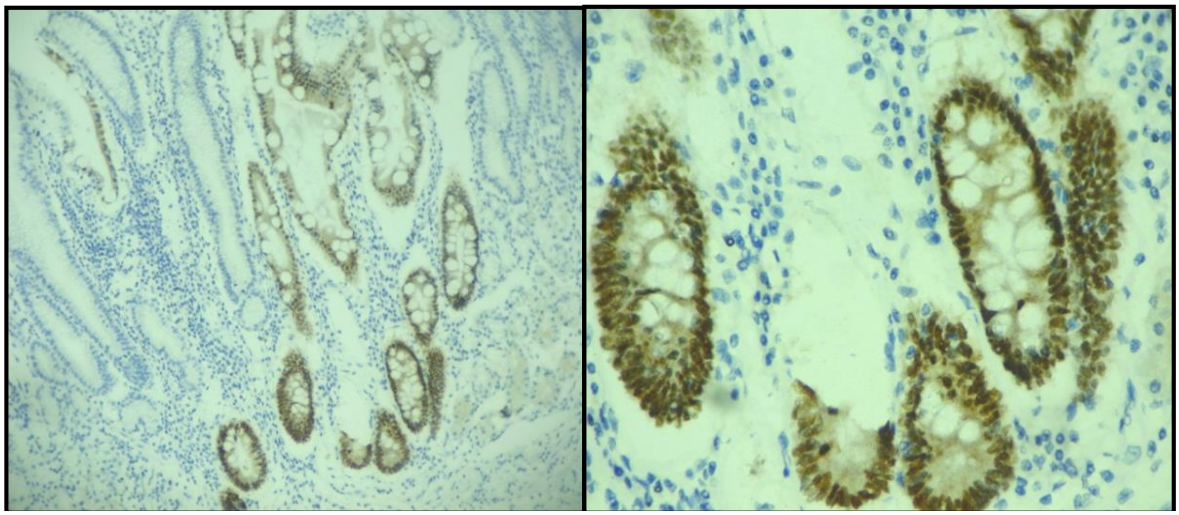

**Fig. 6 Metaplastic glands show nuclear positivity for CDX2 immunostaining. Adjacent gastric glands are negative. a)100x, b) 400x**

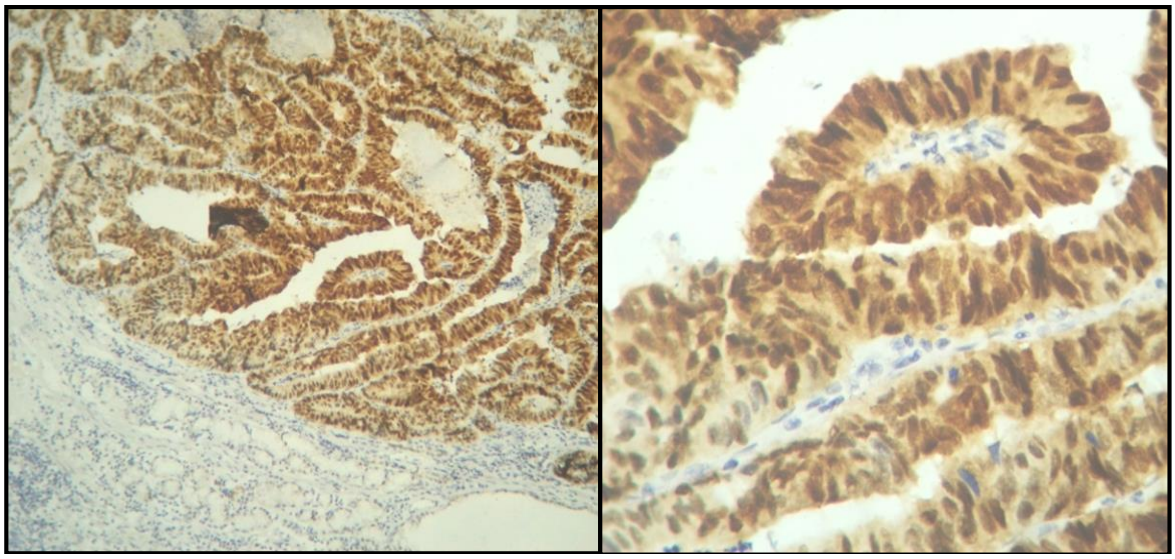

**Fig. 7: CDX2 positivity (nuclear) in gastric adenocarcinoma. Adjacent normal gastric glands are negative. a) 100x b) 400x.**

## DISCUSSION

Adenocarcinoma stomach remains the second most common cause of death from malignant disease worldwide.[1] According to western literature, intestinal type adenocarcinoma was more prevalent in males in seventh decade. Diffuse type adenocarcinoma was equally distributed among both sexes with a mean age at diagnosis of 48 yrs. [2,3] In the present study gastric carcinoma was more prevalent in males (72%). Majority fell into the age group of 51-60 yrs (36%), mean age being 58.8 yrs. Intestinal type adenocarcinoma predominated in both males (66.7%) and females (71.4%).

There are various classification systems for gastric carcinoma.The most widely used one is Lauren's classification which lists three histological subtypes – intestinal, diffuse and mixed.[4] Intestinal type adenocarcinoma predominated among the three histological variants of gastric carcinomas in our study (68%). Similar incidence was found on literature review.[5] Practically all gastric carcinomas arise from basal cells of foveolae on a background of chronic atrophic gastritis with intestinal metaplasia and are preceded by various stages of dysplasia, carcinoma in situ and superficial gastric carcinoma.[6] Chronic atrophic gastritis (CAG) is an inflammatory condition characterized by the loss of gastric glandular structures that are replaced by connective tissue (non-metaplastic atrophy) or by glandular structures inappropriate for location (metaplastic atrophy).[7] This definition of atrophic gastritis was proposed by the atrophy club in 2000.[8] Histologically majority of the cases showed chronic gastritis with intestinal metaplasia in the present study (50%).[Fig.1] Atrophic gastritis was seen in 4 cases. The adjacent mucosa was histologically normal in 28% of cases. Total number of cases with intestinal metaplasia numbered 27(54%).[Table 1] Multifocal atrophic gastritis affects both corpus and antrum in a patchy fashion. A diagnosis of MAG can be made only when there is evidence of atrophy within intestinal metaplasia in at least 50% of multiple biopsy specimens (a minimum of two from the antrum and two from the corpus or fundus).[9]

In the study conducted by Arista-Nasr et al multifocal atrophic gastritis with or without intestinal metaplasia was the predominant precursor lesion noted in the adjacent non neoplastic mucosa of intestinal type adenocarcinoma.[10] In the present study, although intestinal metaplasia was detected in a good percentage of cases, atrophic gastritis was not frequent. This is possibly due to the limited sampling of adjacent mucosa in our cases and the relatively small sample size. In the present study, 20/34 cases of intestinal type adenocarcinoma showed intestinal metaplasia in adjacent mucosa (58.8%). Out of the 7 mixed type of adenocarcinomas, 5 showed intestinal metaplasia (71.4%). [Table 3] Dysplasia of non metaplastic gastric mucosa has been proposed as a precursor lesion of diffuse type adenocarcinoma by Ghandhur et al.[11] In the present study, 2 out of 9 cases of diffuse type of cancer showed intestinal metaplasia in adjacent mucosa (22.2%). No dysplasia was found. However a definite conclusion cannot be drawn as the number of diffuse type of gastric carcinoma cases in our study are very few. [Table 3] Intestinal metaplasia can be complete, incomplete or a mixture of two. Complete intestinal metaplasia is characterized by loss of columnar mucus secreting cells and replacement by epithelium containing goblet cells and absorptive cells. In incomplete type of metaplasia, mucin content of columnar cells is reduced and it changes from normal gastric neutral mucin to acidic mucin which includes sulphomucin.

Currently complete intestinal metaplasia is referred to as type I and incomplete intestinal metaplasia is further divided into type II (few absorptive cells, columnar cells secreting neutral and acid sialomucin, and goblet cells secreting mainly sialomucin but occasionally sulphomucin) and type III (columnar cells secreting predominantly sulphomucin and goblet cells secreting sialomucin or sulphomucin)[12]

Special stains are essential for differentiating the different types of intestinal metaplasias. Neutral mucin is PAS positive and alcian blue negative at pH 2.5 and 0.5. Sialomucin is PAS positive and alcian blue positive at pH 2.5 but alcian blue negative at pH 0.5. Sulfomucin is high iron diamine positive, weakly PAS positive and alcian blue positive at pH 2.5 and 0.5. [13]In PAS –Alcian blue staining neutral mucins stain magenta and acid mucins stain blue. Cells that contain both acid and neutral mucins stain various shades of blue purple to purple. In complete intestinal metaplasia, only goblet cells take up purple - blue colour while in incomplete type both goblet cells and adjacent columnar cells take up the colour. [14][Fig. 4]High iron diamine- Alcian blue staining helps to differentiate type II and type III intestinal metaplasias. Sulfomucins stain black-brown and sialomucins stain blue with this technique. Type II intestinal metaplasia shows sialomucin containing columnar cells and sulphomucin or sialomucin containing goblet cells. In typeIII intestinal metaplasia, both columnar and goblet cells stain brown. [14][Fig. 5]

Type III intestinal metaplasia is said to show a closer association with intestinal type gastric carcinoma[15,16] than other types but the results in literature are conflicting. [17]In this study type III intestinal metaplasia was more prevalent (48.1%) in association with gastric cancer.[Table 2] Similar results were obtained in studies conducted by Filipe et al [18] and Rokkas T et al.[19] CDX2 is a member of caudal related homeobox transcription factor gene family. It regulates proliferation and differentiation of intestinal epithelial cells and is a very useful marker for identification of adenocarcinoma of intestinal origin. [20] Loss of accurate control of CDX2 expression causes serious disruption of mucosal architecture, leading to intestinal diseases and developmental disorders. [21]

In the present study 24 out of 27 cases (88.8%) of intestinal metaplasia showed CDX2 protein expression.[Table 4] None of the histologically normal gastric biopsies showed CDX2 positivity, making the observation statistically significant ($p<0.05$). This was similar to the observation made by Quin et al. [22] Study conducted by Seno et al showed similar results. He studied 40 cases of which 18 had intestinal metaplasia. CDX2 was expressed in 16 cases (89%). [23] In the study conducted by Kim H S et al 89.7% cases of intestinal metaplasia showed CDX2 expression. [24] 14/15 cases (93.3%) of intestinal metaplasia showed CDX2 expression in the study conducted by Satoh K et al. [25] Studies conducted by Almeida et al [26] and Bai et al [27] also gave similar results.

The expression rates of CDX2 were 100%, 90.9%, 84.6% in type I, type II and type III intestinal metaplasias,but the difference was not statistically significant ($p>0.05$). This was similar to the observation made by Quin et al.[22] The individual observations were however statistically significant when compared with the controls.[Table 5] The intensity of CDX2 staining was consistently strong in all cases of type I intestinal metaplasia (100%). This was similar to the observation made by Satoh K et al. [25] The intensity of staining varied from mild to moderate to strong among cases of type II and type III intestinal metaplasia.

CDX2 was positive in 21 out of 27 cases (77.7%) of gastric carcinoma for which the immunostaining was done.[Table 6] It was seen in 16/20 cases of intestinal type, 2/2 cases of diffuse type and 3/5 cases of mixed type of gastric carcinoma. However the number is too small to derive a definite conclusion as to the prevalence of CDX2 expression in gastric cancer associated with intestinal metaplasia. In Quin et al's study the rate of expression of the protein was 48.7%. [22]

## CONCLUSION

Intestinal metaplasia is a frequently observed precursor lesion of gastric adenocarcinoma, especially the intestinal type. Incomplete intestinal metaplasia is more commonly associated with gastric carcinoma compared to the complete type. We could not arrive at a definitive conclusion regarding the incidence of multifocal atrophic gastritis, which is probably due to the small sample size and limited sampling of adjacent mucosa. CDX2 homeodomain protein which is not expressed in normal gastric mucosa is a sensitive marker for intestinal metaplasia. Our study also reveals that CDX2 might be closely related to intestinal type adenocarcinoma.

To conclude, CDX2 expression is frequently associated with intestinal metaplasia and it may be possible that the expression of CDX2 protein in altered gastric mucosa and gastric carcinoma is due to de repression of the *CDX2* gene.

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