Original Article

CDX2 EXPRESSION: DEVELOPMENT OF LOWER ESOPHAGEAL ADENOCARCINOMA

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ABSTRACT

Objectives: To look into the expression of CDX2 in normal and various abnormal esophageal epithelial conditions in human subjects.

Material and Methods: This study was conducted at Department of Pathology, Dow University of Health Sciences and Al-Tibri Medical College, Karachi. After ERC (ethical review committee) approval 88 human subjects were selected through non-probability sampling using inclusion and exclusion criteria 22 subjects as control (group-I) and 22 individuals with Barrett esophagus (Group-II), 22 patients with dysplasia (Group-III) and 22 known patients of esophageal adenocarcinoma (Group-IV). Demographic data were collected on proforma while histopathological changes and biomarkers were evaluated by the department of pathology, Chi-square was the statistical techniques used.

Results: There were 70 (79.55%) males and 18 (21.45%) females. The mean age was 43.09±6.52 years in group-I while 56.63±2.64 years in group-II and 61.68±4.23 years in group-III whereas it was 63.90±3.80 years in group IV. CDX-2 was found expressed in 36(40.91%) patients where as it was negative in 52 (59.09%) patients.

Conclusion: There is significant association in CDX2 expression in various epithelial abnormalities of the esophagus from Barrett esophagus to esophageal adenocarcinoma.

Key Words: CDX2, Dysplasia, Adenocarcinoma.

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INTRODUCTION

In the field of diagnosis of esophageal malignancies, a number of methods are available including esophagoscopy, endoscopic optical coherence tomography, positron emission tomography, spectroscopy, biopsy and immunohisto-chemistry. No specific tumor marker exists for the diagnosis of esophageal malignancies therefore rigorous work is going on for introducing combinations of biomarkers [1]. Recently conventional histopathology accompanied by imm-unohistochemistry has played a tremendous role in identification of unclassified tumors and also helped in possible diagnosis of metastatic tumors of unknown origin. In undifferentiated tumors where light microscopy is not providing sufficient features, immunohistochemistry has been of remarkable assistance [2]. These days identification of new biomarker in patient with malignancy can open a new door of diagnosis and facilitate the management in early stage of malignancy [3]. Researchers are working on formulation of biomarkers which will help in early detection of the progression of Barrett to dysplasia and then to adenocarcinoma. Among all available biomarkers CDX2 has a distinct role. CDX2 is a homeobox protein that is expressed in the nuclei of intestinal epithelial cells. It is encoded by the CDX2 gene. This gene is a member of the caudal-related homeobox transcription factor family [4]. CDX2 is significant marker in the development and differentiation of intestinal epithelium. As Barrett esophagus is characterized by development of specialized intestinal epithelium, CDX2 may play role during morphological changes which take place during of development of Barrett esophagus [5]. CDX2 has critical use in diagnosis of Barrett esophagus. Early diagnosis of progression of metaplasia in Barrett esophagus can be helpful in preventing the dysplasia, thus helping to lower the burden of adenocarcinoma in the community. This study is an attempt at diagnosing Barrett esophagus at an early stage with the help of biomarker CDX2, in turn raising opportunity to prevent progression to dysplasia that leads to adenocarcinoma. So, our objectives were set to observe histopathological features of intestinal metaplasia, dysplasia and adenocarcinoma of esophagus in comparison with normal squamous epithelium under routine hematoxylin and eosin and PAS stains and to evaluate the immune-reactivity for CDX2 in intestinal metaplasia (Barrett esophagus), dysplasia and

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adenocarcinoma along with normal squamous epithelium. At early stages better diagnosis of this condition is the key point. Some biomarkers like cyclin-D1, CDK4/6, TP53 and Ki-67 in these patients can be of significant importance. Therefore, this study was done regarding the early detection and comparison of biomarker CDX2 in detection of intestinal metaplasia, dysplasia and adenocarcinoma for better prognosis.

MATERIAL AND METHODS

This analytical research of cross-sectional nature took place at Pathology Department of Dow University of Health Sciences and Al-Tibri Medical College, Karachi over a period of 6 months after ERC approval. Samples were collected under consent from 66 patients and 22 normal individuals as per sample size calculations using convenient type of sampling [6]. Inclusion criteria were already diagnosed sample of Barrett esophagus, dysplasia or adenocarcinoma while exclusion Criteria was insufficient biopsy samples as well as patients undergoing radio- or chemotherapy. SPSS (Statistical Package of Social Sciences) version 22.0 was used for data analysis using chi-square test at p-value of < 0.05 as significant. 5µ sections were cut from paraffin embedded sections and mounted on microscope glass slide for examination according to published guidelines [7]. Periodic acid-Schiff-aclian blue (PAS-Ab) staining was done for intestinal metaplasia. For immunohistochemistry eighty eight selected sections were processed for CDX2 immunomarker. Slides were stained with Myer hematoxylin and observed using light microscope under 100x and 400x magnification. For assessment of CDX2 staining, intensity and area of each section were evaluated. Immunohistochemical staining was assessed semiquantatively for both extent and intensity. Cells with brown-stained nuclei were considered positive, whereas cells without nuclear staining were considered negative. Staining was categorized1+ (weak) if less than 10% of cells were positive, 2+ (moderate) if staining occurs in 10–50% of cells and 3+(strong) if more than 50% of cells took up CDX2 [8,9].

RESULTS

Mean age in our study subjects was 56.33 years which was further divided into various age-range groups shown in Table-1. Age as mean and SD (Standard deviation) in Group-I (Control) turned out to be 43.09±6.52 years. In Group-II (Barrett Esophagus) it was 56.63±2.64 years, in Group-III (dysplasia) 61.68±4.23 years and in Group-IV (esophageal adenocarcinoma) 63.90 ± 3.80 years and the mean age difference was significant with a p-value of 0.00003. CDX2 expression was observed positive in 2 (2.27%) subjects of group I while it was negative in 20 (22.73%) individuals. In group II 20 (22.73%) patients showed positive expression while it was negative in 2 (2.27%) patients. CDX2 expression was positive and negative in 8 (9.09%) and 14 (15.91%) patients of group III respectively. Group IV only 6 (6.82%) patients were CDX2 positive that were well differentiated esophageal adenocarcinoma while other 16 (18.18%) were negative. Significant statistical difference was observed for CDX2 expression between different groups X2-value 33.85 and p-value 0.00001(Table-2).

Table-1: Distribution of study patients in age range groups.

S. No	Age Groups	Frequency & Percentage
1.	41-50 years	23 (26.17%)
2.	51-60 years	40 (45.54%)
3.	61-70years	25 (28.40%)
	Total Patients	**88 (100%)**

Table-2: Chi-Square of CDX2 expression status in various groups.

Study Groups	CDX2 Expression		Row Total	p-value
	Positive Frequency (%)	Negative Frequency (%)		
Group-I	02 (2.27%)	20 (22.73%)	22 (25%)	0.00001
Group-II	20 (22.73%)	02 (2.27%)	22 (25%)	
Group-III	08 (9.09%)	14 (15.91%)	22 (25%)	
Group-IV	06 (6.82%)	16 (18.18%)	22 (25%)	
Column Total	36 (40.91%)	52 (59.09%)	88 (100%)	

DISCUSSION

In the current study CDX2 expression was found significantly different among various groups, with a p-value of 0.00001 which is parallel to with various previously-published study results [10]. Kang *et al* (2011) found 94.4% positive Barrett esophagus cases on CDX2 Expression [11]. Similarly, Bai *et al* (2002) found 3247 (90%) positive cases on CDX2 expression [12]. Kanwaijit *et al* (2013) reported more esophageal adenocarcinoma cells were positive when stained with CDX2 which was focally positive in esophageal tumors in 92% of cases [13]. Streher *et al* (2014) study results showed that over 70% patients of esophageal adenocarcinoma cases were CDX2-positive on biopsies along with the Barret's esophagus, suggesting that the latter predisposes the

former condition which is also consistent with our findings [14]. Rita *et al* (2016) also reported that CDX2 is more expressed in Barrett esophagus that further predisposes that area to esophageal cancers which is also supported by our findings [15]. Animal models studies where overexpression of CDX2 targeted for esophagus caused Barrett metaplasia or Barrett esophagus which is characterized by intestinal epithelium replacing the normal squamous epithelium [16,17]. Similarly, CDX2 overexpression in stomach results in metaplasia, with a posteriorization of epithelial identity and these tissue alterations model pre-neoplastic metaplasias that are common in humans [18]. This study however have certain limitations like lower number of patients in different groups but it was the first study on this topic in our region so we hope it will ease the path for other researchers in our field and so some diagnostic, preventive and therapeutic role of the marker under study may get developed in future.

CONCLUSION

Present study revealed significant association of CDX2 with metaplasia, low grade dysplasia and well differentiated esophageal adenocarcinoma.

RECOMMENDATIONS

Large scale studies are recommended on patients with types of various cancers

AUTHOR CONTRIBUTION

Humera Jabeen: Study design and concept
Lubna Avesi: Drafting and final approval
Saba Hassan Shamim & Farheen Danish: Data interpretation
Nazia Qamar & Maryam Moula Bakhsh: Literautre review

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