Evaluating the expression of immunohistochemical panel of p53, CDX2, IMP3 and AMCAR in grading dysplasia of Barrett's esophagus and predicting the progression to esophageal adenocarcinoma

Omneya Y. Bassyoni[a], Rana M. Abdalla[a], Hiam A. Eleleimy[b]

[a]Department of Pathology, Faculty of Medicine, Benha University, [b]Department of Internal Medicine, Hemato-Oncology Unit, Faculty of Medicine, Benha University, Qalyubiyah, Egypt

Correspondence to Rana M. Abdalla, MD, Pathology Department, Faculty of Medicine, Benha University, Qalioubia 11936, Egypt
Mobile: +0115 570 7733;
Tel: 002011557077;
e-mail: rana.elsaied@fmed.bu.edu.eg

Received: 09 July 2023
Revised: 14 July 2023
Accepted: 28 July 2023
Published: 13 December 2023

Egyptian Journal of Pathology 2023, 43:94–104

Objective
Barrett's esophagus (BE) is an established precursor to esophageal adenocarcinoma, which has a poor prognosis unless detected at an early stage. The progression of BE to adenocarcinoma is slow and unpredictable. Currently, the best predictor of adenocarcinoma is histological detection of dysplasia.
Accurate grading of dysplasia and especially discriminating low-grade dysplasia LGD from high-grade dysplasia HGD is important for management. Marked variability exists when diagnosing dysplasia in BE. This highlights the need for a diagnostic adjunct, especially in histologically challenging cases.
This study aims at evaluating the role of immunohistochemical expression of (p53, IMP3, AMCAR and CDx2) in Barrett's oesophagus spectrum, to increase the diagnostic accuracy of grading dysplasia and predicting progression risk.
Methods
This is a retrospective immunohistochemical study, performed on selected 52 cases of esophageal biopsies.
Results
The p53 was negative in nondysplastic Barrett's esophagus (ND-BE) and LGD and strong positivity towards HGD/esophageal adenocarcinoma (EAC). CDX2 showed highest expression among ND-BE and decreased towards EAD. IMP3 and AMCAR were negative in all cases of ND-BE with gradual increase among HGD/esophageal adenocarcinoma cases.
IMP3, AMCAR, and CDX2 were found to be more sensitive in detecting HGD (80%, 70% & 70%). Meanwhile, p53 is more specific (100%), IMP3 and AMCAR are more sensitive discriminating LGD from HGD (80%) than p53 and CDX2 (60%, 70%).
Conclusion
This combined panel of p53, CDX2, IMP3, and AMACR could be used in conjunction with histology as a promising tool to accurately predict progression form BE to HGD/adenocarcinoma with a great value for early detecting high grade dysplasia, discriminating it from LGD, improving risk stratification in BE, and optimizing patient care.

Keywords:
AMCAR, Barrett's oesophagus, CDX2, IMP3, oesophageal adenocarcinoma, p53

Egypt J Pathol 2023, 43:94–104

1687-4277

Introduction

Esophageal cancer is a global health problem with more than 572 000 newly diagnosed cases worldwide in 2018 (International Agency for Research on Cancer, 2018). There are two main histological subtypes of esophageal cancer, esophageal adenocarcinoma (EAC) and esophageal squamous cell carcinoma, and there has been a dramatic shift in its epidemiology. In Egypt, esophageal carcinoma represents 1.2% of cancers among the newly diagnosed cases according to Global cancer statistics 2020 (Malik and colleagues 2021).

Barrett's esophagus (BE) is the established precursor to esophageal adenocarcinoma (Frei and colleagues 2021). The progression of BE through a metaplasia-dysplasia-adenocarcinoma sequence is slow and unpredictable. Some studies have suggested rate of progression about (2–28%/year) for low-grade dysplasia (LGD) and about (6–60%/year) for high-grade dysplasia (HGD) (Kambhampati and colleagues 2020; Song and colleagues 2020). Currently, in many institutions, detection of BE with LGD necessitates periodic endoscopic surveillance, whereas HGD and EAC warrants more aggressive interventions like

 DOI: 10.4103/egjp.egjp_18_23

endoscopic resection, ablation and/or esophagectomy (Badgery and colleagues 2020).

Grading of dysplasia in BE can represent a real histologic challenge. A recent multi-institution international study has documented over-diagnosing of HGD in BE of about 40% of the cases, which can lead to possible unnecessary therapy and mismanagement (Davison and colleagues 2020). Although, histological examination of BE biopsies is subjected to interobserver variability (Srivastava and colleagues 2017). It remains the gold standard for risk assessment (Sali and colleagues 2020).

Thus, there is a need for adjunct markers that can be used to support histologic decision for more accurate diagnosis, especially in diagnostically challenging cases, and most probably for prediction of cases of nondysplastic Barrett's esophagus (ND-BE) and LGD that have the potential to progress to HGD and EAC, given the treatment implications. Identifying ancillary stains was targeted by numerous studies aiming to help pathologists confirm the presence of BE and dysplasia, and to better predict the progression to adenocarcinoma (Werling and colleagues 2003).

We selected proteins involved in signaling pathways that are independently and differentially regulated in the transition from ND-BE to EAC (Denlinger and Thompson, 2012). The p53 is an important tumor suppressor gene. Inactivation of p53 seems to be a fundamental event occurring early and often during Barrett's carcinogenesis (Bian and colleagues 2001).

CDX2 is the key regulator of intestinal differentiation especially normal esophageal squamous epithelium that transformed into columnar epithelium. (Burdelski and colleagues 2018) Its expression is high in ND-BE and progressively decreases in dysplasia and EAC. (Hayes and colleagues 2011).

Insulin-like growth factor II m-RNA-binding protein 3 (IMP3) is an oncofetal protein that is essential for cellular proliferation, adhesion, and invasion of malignant neoplasms. Its high and strong expression was discovered in a large variety of human types of cancers but not in adjacent benign tissues (Neal and colleagues 2020) It is considered a new biomarker being evaluated in the diagnosis of dysplasia and neoplasia in BE. (Gadara and colleagues 2017).

AMACR is an enzyme that catalyzes the racemization of α-methyl branched-chain carboxylic acid coenzyme (Hasan and colleagues 2020). Its role in cancer biology explains the frequent overexpression in prostate cancer (Elmubrak, 2021). Currently, the probable utility of AMACR expression in diagnosing dysplasia is widely studied, especially in gastrointestinal tract pathology (Snyder and colleagues 2019).

In this study, we analyze the expression of p53, CDX2, IMP3, and AMACR proteins in the four studied categories to evaluate their value in accurately grading dysplasia in BE biopsy and detect their role in progression of BE into oesphgeal adenocarcinoma.

Materials and methods

Study groups

This is a retrospective study, performed on formalin fixed paraffin embedded biopsy specimens of selected 52 cases of esophageal lesions subdivided as 11 specimen of ND-BE, 10 specimens of Barrett's esophagus with LGD, 11 specimens of Barrett's esophagus with HGD, 20 specimens of EAC.

Specimens are collected from archives of Pathology Department, EARLY CANCER DETECTION UNIT (ECDU), Faculty of Medicine, Benha University, during the period from November 2018 to May 2022. The study was approved by the Ethical Committee of Benha Faculty of Medicine, Benha University (M.S. 13.5.2023).

10 cases of nonspecific esophagitis considered as control were included in this study. The specimens were 45 endoscopic biopsies and 7 were esophagectomy.

Exclusion criteria: Cases diagnosed as BE with epithelial alterations indefinite for dysplasia and cases with history of chemotherapy or cases with no available paraffin blocks-were excluded from this study.

All collected blocks were cut at 4 μm and stained with routine hematoxylin and eosin and re-reviewed independently by two pathologists to confirm the diagnosis.

Immunohistochemistry

Immunohistochemical analysis was applied using the standard streptavidin–biotin technique following the manufacturer's instructions. The details of used primary antibodies are shown in Table 1. For the secondary developing reagents, a labeled streptavidin–biotin kit (Neomarker; Labvision, Waltham, USA) was used. The sections were stained with 0.02% diaminobenzidine solution used as chromogen, and negative controls for all markers were obtained by omitting the primary antibody.

Table 1 Data for using p53, CDX2, IMP3, and AMCAR antibodies

Markers	Vendor	Clone	Host/isotope	State	Dilution	Incubation	Positive control	Antigen retrieval
CDX2	Thermo Fisher scientific	monoclonal	Rabbit IgG	Concentrated	1:100	20 min at RT	Colon carcinomas	Citrate buffer (pH 6.0)
IMP3	Thermo Fisher scientific	polyclonal	Rabbit/ IgG	Concentrated	1:50	60 min at RT	Lung squamous carcinoma	Citrate buffer (pH 6.0)
AMACR	Thermo Fisher scientific	monoclonal	Rabbit/ IgG	Concentrated	1:100	30 min at RT	Prostate cancer	Citrate buffer (pH 6.0)
p53	Thermo Fisher scientific	Monoclonal	Mouse/ IgG2a	Concentrated	1:20	1 hour at RT	colon carcinoma	Citrate buffer (pH 6.0)

Evaluation of immunohistochemical staining

Positive nuclear staining was examined for p53 and CDX2. The number of positive cells were interpreted in adherence to the methodology utilized by Çetinaslan and colleagues (2012) and Elshafey and colleagues (2016). The statistical analysis of CDX2 immunohistochemical results were initially made according to semi quantitative positivity scores of staining intensity, as 0 - +3. Since the number of cases were small in each group, final statistical correlation was searched from both negative and the positive groups, regardless of their staining score.

IMP3 and AMACR staining were cytoplasmic, scored as the methodology followed by Jankowski and Odze (2009); Sonwalkar and colleagues (2010).

We used SPSS v20 (Statistical Package for Social Sciences, IBM Corp., NY, and USA for all statistical analyses. The IHC data were analyzed using Fischer's exact test, and *P* values less than or equal to 0.05 were considered statistically significant. ROC curves were constructed to assess the performance of p53, CDX2, IMP3 and AMCAR in detection of patients with different grades of dysplasia, in addition to those without dysplasia.

Results

Clinicopathological data

The age of studied cases ranged from 20 to 84 (mean 50.76 ± 17.5) with 31 case were male and 20 cases of female. The included cases in this study were classified into four groups: 11 (21.6%) specimens of ND-BE, 10 (19.9%) specimens of LGD, 10 (19.6%) specimens of HGD, 20 (39.2%) specimens of EAC, and 10 specimens of nonspecific esophagitis used as control.

Immunohistochemical results

Expression of p53, CDX2, IMP3, and AMCAR among the studied groups (ND-BE, LGD, HGD and EAC)

All cases of ND-BE were negative Fig. 1a and 70% of LGD were negative Fig. 1b for p53 while 60% and 45% of HGD Fig. 1c and EAC Fig. 1d were positively expressing p53 with statistically significant positive correlation among the four studied groups ($P = 0.012$) as shown in Table 2.

CDX2 was negatively expressed in control cases while positively expressed in ND-BE Fig. 2a and LGD Fig. 2b (90.9 & 80%, respectively) while it was expressed only in 30% of HGD Fig. 2c and 45% of EAC cases Fig. 2d with statistically significant correlations ($P < 0.001$) illustrated in Table 2.

Up regulation of IMP3 Cytoplasmic staining was detected among the four groups of the studied cases, all cases of ND-BE (100%) Fig. 3a and 60% of LGD cases Fig. 3b showing +1 score of IMP3.While 80% of HGD Fig. 3c and 70% of EAC Fig. 3d cases were highly expressing IMP3 respectively (score +3) with highly significant positive correlations ($P < 0.001$) as shown in Table 2.

Similarly, cytoplasmic expression of AMCAR was overexpressed in 50% HGD Fig. 4c and 55% EAC Fig. 4d respectively. However, 100% of ND-BE Fig. 4a and 90% of LGD Fig. 4b were lower expressing AMCAR (score +1 & +2) with highly significant positive correlations ($P < 0.001$) as shown in Table 2.

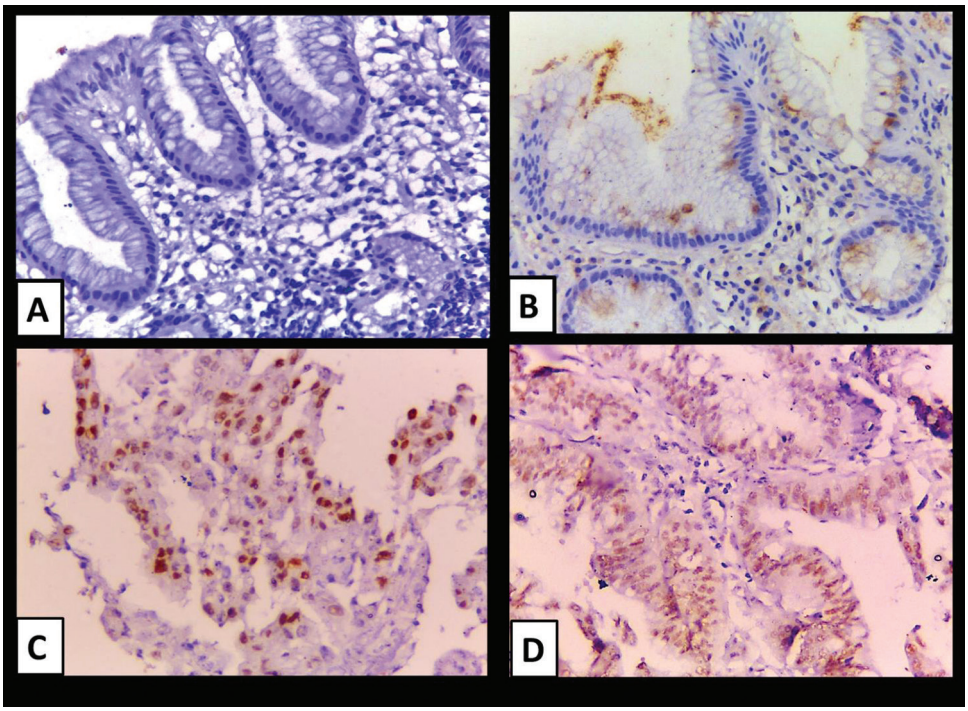
ROC curves analysis

p53, CDX2, IMP3, and AMCAR and have high specificity but low sensitivity in detection of LGD as shown in Fig. 5a. IMP3, AMCAR, and CDX2 were found to be more sensitive for detection of Barrett's with hHGD (80, 70 & 70%, respectively). While p53 is more specific, less sensitive (100% specificity and 60% sensitivity) as shown in Fig. 5b. IMP3 and AMCAR are more sensitive in discrimination of LGD from HGD (80% sensitivity and 90 and 70% specificity, respectively) while p53 and CDX2 were found to be less sensitive (60, 70%, respectively) as shown in Fig. 5c.

Discussion

Barrett's esophagus represents a significant complication of gastroesophageal reflux disease that primarily remains asymptomatic in most cases. It is recognized as

Figure 1



p53 IHC expression in the studied group Negative staining in ND-BE and LGD (A &B). Positive nuclear staining of p53 in HGD and EAC (C &D), (IHC X400).

Table 2 Expression of p53, CDX2, IMP3, and AMCAR among the studied groups

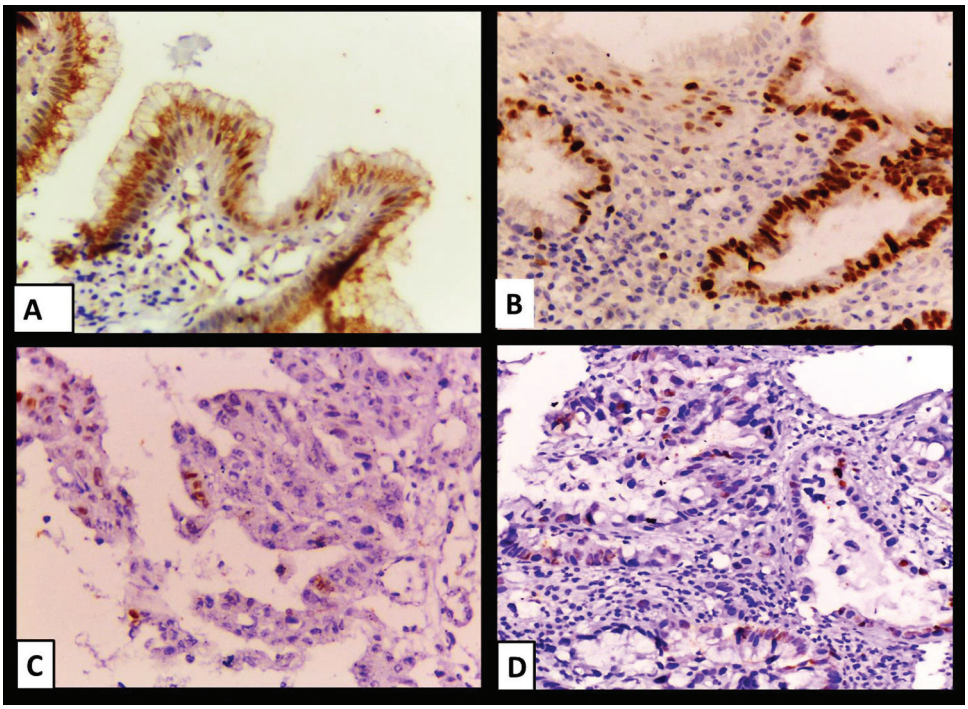
Clinicopathological data	Non dysplastic Barrett (11) No (%)	Low grade dysplasia (10) No (%)	High grade dysplasia (10) No (%)	Adenocarcinoma (20) No (%)	*P* value
Sex					
Male	7 (63.6)	7 (70.0)	5 (50.0)	12 (60.0)	0.85
Female	4 (36.4)	3 (30.0)	5 (50.0)	8 (40.0)	
P53					
+ve	0	3 (30.0)	6 (60.0)	11 (55.0)	0.012*
-ve	11 (100)	7 (70.0)	4 (40.0)	9 (45.0)	
CDX2					
+ve	10 (90.9)	8 (80.0)	3 (30.0)	9 (45.0)	<0.001**
-ve	1 (9.1)	2 (20.0)	7 (70.0)	11 (55.0)	
IMP3					
<10% (+1)	11 (100)	6 (60.0)	1 (10.0)	0	<0.001**
10–50(+2)	0	3 (30.0)	1 (10.0)	6 (30.0)	
>50%(+3)	0	1 (10.0)	8 (80.0)	14 (70.0)	
AMCAR					
<10% (+1)	10 (90.9)	3 (30.0)	2 (20.0)	3 (15.0)	<0.001**
10–50(+2)	1 (9.1)	6 (60.0)	3 (30.0)	6 (30.0)	
>50%(+3)	0	1 (10.0)	5 (50.0)	11 (55.0)	

*significant.
**highly significant.

a risk factor for esophageal adenocarcinoma possessing an unfavorable prognosis in the absence of timely detection at an early stage (Jankowski and Odze, 2009).

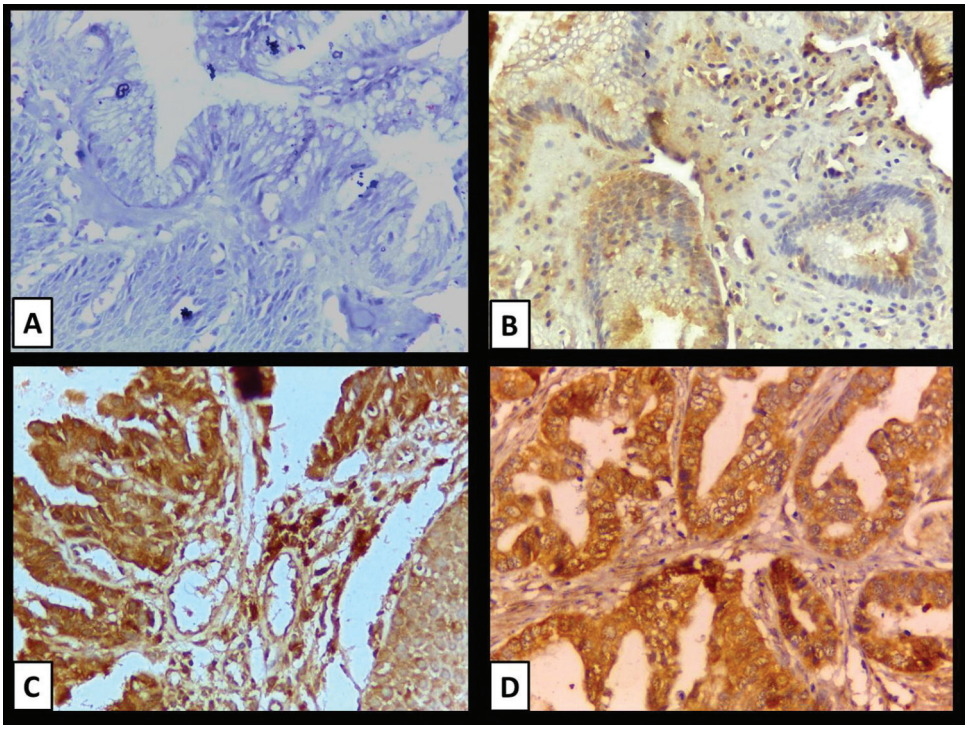
Histological identification of dysplasia remains the best predictor of future carcinoma development in individuals with Barrett's esophagus, with high grade intraepithelial neoplasia carrying the highest relative risk of malignant progression. (Jankowski and Odze, 2009; Kaye and colleagues 2010). Various studies have been conducted based on the hope that, in future, specific molecular changes can be used as reliable markers to identify individuals at risk of developing cancer.

Figure 2



CDX2 expression in the studied groups. Diffuse strong nuclear expression in ND-BE and LGD (A&B). Lower nuclear staining of CDX2 in both HGD and EAC (C&D) (IHC, x400).

Figure 3

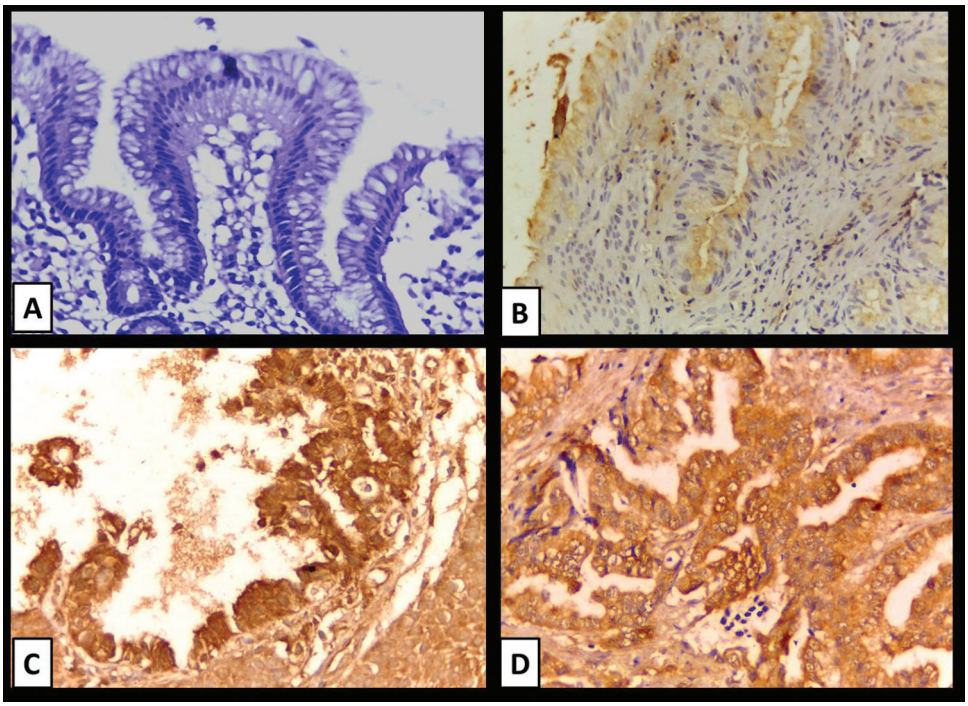


IMP3 expression in the studied categories. ND-BE showed negative staining of IMP3 (A). Low cytoplasmic expression in LGD (B). Diffuse strong cytoplasmic expression of IMP3 in HGD and EAC (C&D), (IHC x400).

In this study, we analysed the expression of four proteins from different signalling pathways (p53, CDX2, IMP3, and AMCAR) in assessing the progression from ND-BE to esophageal adenocarcinoma, with the objective to increase the diagnostic accuracy of grading dysplasia in BE.

The p53 immunoreactivity has emerged as the most extensively studied and highly encouraging biomarker. This is concluded among several supplementary immunohistochemical stains that have been assessed as markers for the increased risk of disease progression in Barrett's esophagus (Srivastava and colleagues 2017).

Figure 4



AMCAR expression in the studied groups. ND-BE showed negative staining (A). Low cytoplasmic expression in LGD (B). Diffuse strong cytoplasmic expression of AMCAR in HGD and EAC (C&D), (IHC x400).

In this study, nuclear expression of p53 shows statistically significant positive correlation during progression from ND-BE, LGD, HGD to oesophageal adenocarcinoma (P = 0.012). All cases with ND-BE were negative. Only 30% of cases with LGD expressed p53 while 60 and 55% of HGD and EAC, respectively were positive for p53.

This in agreement with Hardwick and colleagues 1994 who concluded that only 53% oesophageal adenocarcinoma (ADC) cases overexpressed p53. In those studied cases, p53 up regulation was identified in adjacent dysplastic mucosa, mainly in areas of HGD compared with LGD. In contrast, none of the dysplastic mucosa adjacent to tumours lacking p53 overexpression showed detectable values of p53 in some patients with Barrett's oesophagus. Other studies demonstrated a significant correlation between the expression of aberrant p53 and the progression of ND-BE to LGD and HGD (Younes and colleagues 2017; Chen and colleagues 2023).

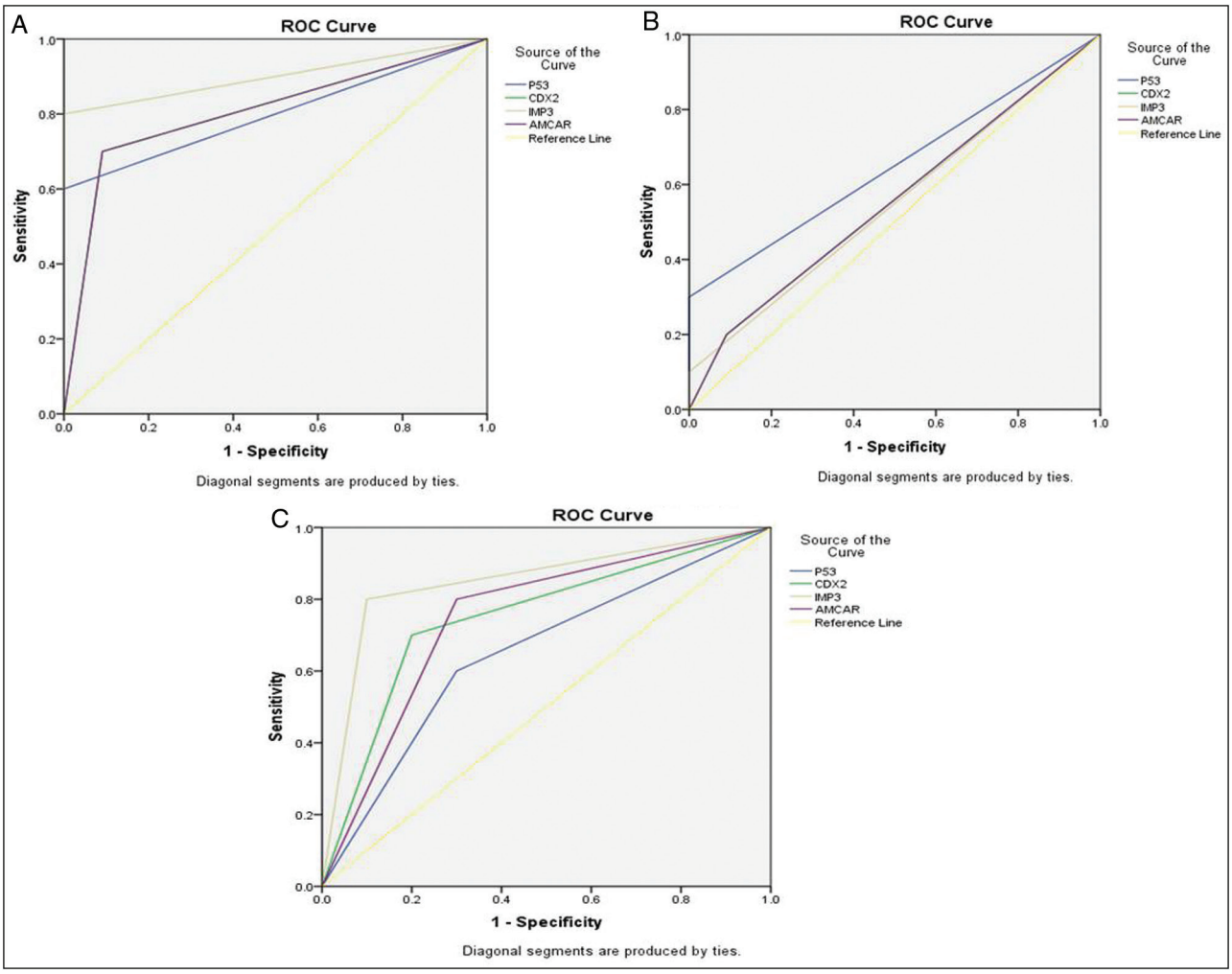
These finding propose the potential involvement of p53 expression in the advancement of dysplasia to carcinoma among individuals diagnosed with Barret's oesophagus. This can possibly be clarified by the main function of tp53, known for its role as a tumour suppressor gene that prevents genomic mutations. The level of p53 protein in normal cells is low. In the event of DNA damage or exposure to stressful stimuli, p53 is up regulated, thereby eliciting growth arrest, DNA repair, and programmed cell death. It has been reported that tp53 gene mutations are highly prevalent in oesophageal adenocarcinoma and HGD but are infrequent in instances of ND-BE (Kaye and colleagues 2010).

Moreover, an increase in the turnover rates of p53 oncoprotein is possibly responsible for decreasing its half-life. This phenomenon consequently poses a challenge for the successful detection of this oncoprotein through immunohistochemical methods. Such complexities could potentially account for the low levels of p53 positivity observed in cases of HGD and EAC.

In the present investigation, CDX2 exhibited a negative expression pattern in the control group. Conversely, a predominantly nuclear positive staining was observed in the majority of nondysplastic Barrett's (90.9%), LGD (80% of cases) and extent of expression lowered within the sequential steps to high-grade dysplasia and invasive adenocarcinoma (60 and 85%, respectively were +1) with statistically significant correlations (P < 0.001)

Previous studies have examined the expression of CDX2 across the metaplastic to dysplastic and adenocarcinoma progression in the esophagus. These studies revealed a lack of CDX2 expression within the

Figure 5



Log Rank curves regarding. A: Performance of P53, CDX2, IMP3 and AMCAR in detecting low grade dysplasia. B: Performance of P53, CDX2, IMP3 and AMCAR in detecting High grade dysplasia. C: Performance of p53, CDX2, IMP3 and AMCAR in discrimination of low grade from High grade dysplasia.

esophageal squamous epithelium but noted a significant up regulation in intestinal metaplasia. Nevertheless, they produced conflicting results concerning expression patterns observed in dysplasia and adenocarcinoma (Ko and colleagues 2005; Hayes and colleagues 2011).

Supporting our results were the studies of Ko and colleagues (2005) and Hayes and colleagues (2011) reached that a reduction in the expression of CDX2 was observed from Barrett's esophagus to LGD (35.3%) and HGD (17.14%). Additionally, evidence of up regulation of CDX2 in ND-BE, followed by marked decrease in its expression through HGD to the adenocarcinoma. Also, gradual decline of CDX2 expression was observed during progression in adenomatous dysplasia, particularly along the intestinal pathway of the Barrett's esophageal cancers.

Reduction in CDX2 immunoreactivity in high-grade dysplastic lesions and adenocarcinomas of the stomach were reported compared with nondysplastic mucosal tissue. A possible explanation for this occurrence could be due to the possibility of neoplastic cells to exhibit less differentiation throughout their progression, resulting in the reduction of CDX2 expression. Moreover, in the context of neoplastic growths, oncogenes are activated, and it has been observed that the CDX2 gene undergoes down regulation because of the oncogenic rat sarcoma in the human colonic cell lines (Ko and colleagues 2005).

The CDX2 gene has been previously considered to be a tumor suppressor gene in both the stomach and colon (Bonhomme and colleagues 2003). According to these previous findings, it can be suggested that CDX2 has a tumor suppressor role in the progression from metaplasia and dysplasia to carcinoma in Barrett's esophagus.

The current study has proposed CDX2 to be strongly expressed by esophageal intestinal metaplasia.

Furthermore, our study has demonstrated with clear statistical significance up regulation followed by linear down regulation of CDX2 expression through the esophageal metaplasia dysplasia adenocarcinoma sequence raising the possibility of its role in carcinogenesis and progression.

A divergence exists between the findings of the current study and those observed by Weimann and colleagues (2010) that nuclear positivity for CDX2 in ~70% of the epithelial cells of the Barrett mucosa, with staining intensity and expression distribution increasing progressively from low-grade to high-grade dysplasia. This discrepancy can be explained by different scoring systems, divergent antibodies used and Different techniques.

The present study analyzed the expression of IMP3 in the cytoplasm of the four groups of the studied cases as follow: All cases of ND-BE and 60% of LGD cases showing lowered demonstration of IMP3. Conversely, 80% of HGD and 70% of ADC cases displayed high expression levels of IMP3 with highly significant positive correlations (P < 0.001). The expression of IMP3 is found to be diminished in ND-BE owing to its role as an oncofetal protein. This protein is physiologically expressed only during the period of embryogenesis and is either absent or present in low level in normal adult tissue (Plum and colleagues 2018).

Lu and colleagues (2009) and Madkour and colleagues (2022) obtained similar outcomes in their studies on different premalignant lesions of the esophagus. In each of these studies, IMP3 was strongly and diffusely expressed in high-grade precursor lesions and malignancies.

According to this study, the significant correlation between IMP3 expression and grade of dysplasia to adenocarcinoma (P < 0.01) could predict a possible role in development and progression of dysplastic changes in BE. The phenomenon could be elucidated based on the findings of numerous *in vitro* studies which have suggested that IMP3 acts as an oncogene and promote the development of malignancy through modulating multiple aspects of cellular functions including morphology, polarization, proliferation, migration, and differentiation (Mancarella and colleagues 2018).

Regarding AMCAR expression, overexpressed cytoplasmic staining was observed in 50% of HGD and 55% of ADC, respectively in our study. While all cases of ND-BE and 90% of LGD showed lower expression of AMCAR (score +1 & +2) with highly significant positive correlations (P < 0.001).

This is concur with the study conducted by Dorer and Odze (2006) which found a significant elevation in AMACR expression in the ND-BE progressed to LGD, HGD and eventually to EAC. This coincides with the findings presented by Huang and colleagues (2008) whose results suggested that AMACR may play an important role in the intermediate stage of gastric carcinogenesis with notice of negative AMACR expression in the groups without dysplasia and indefinite for dysplasia despite, in the groups of high-grade dysplasia and invasive intestinal-type adenocarcinoma (76%) and (52.9%) were positive for AMACR respectively. This could be explained by the fact that AMCAR is being involved in lipid metabolism. Its overexpression may lead to alterations in the balance of cellular oxidants, which in turn may contribute to the development of neoplasia. The prevalence of this phenomenon is particularly pronounced in tumors that have been linked with high fat diet such as prostatic and colonic cancer (Jiang and colleagues 2003).

In disagreement with these findings, a meta-analysis reporting infrequent or negative expression of AMCAR in various cancer types, including those of the breast, pancreas, bile duct, adrenal, salivary gland, ovary, thyroid, and endometrium. Additionally, the authors observed complete absence of AMCAR expression in melanomas, squamous cell carcinomas, basal cell carcinomas, thymus tumors, and germ cell tumors (Jiang and colleagues 2003). This may be explained by studying different tissue cancers with different pathogenesis involved in each.

The proper grading of dysplasia and, more importantly, discrimination of low-grade from high-grade dysplasia is of great value because of the aggressive therapeutic interventions still used for HGD in various institutions. There is considerable variability exists when diagnosing dysplasia in Barrett's esophagus, even amongst specialized gastrointestinal pathologists. Therefore, there is a necessity for diagnostic adjunct, particularly in cases with diagnostic challenges (Karamchandani and colleagues 2016).

In this study, p53 demonstrated high specificity in identifying cases of HGD and LGD (100%) from nondysplastic Barrett's esophagus. However, its sensitivity was comparatively lower at 60 and 30% for identifying HGD and LGD cases, respectively. The aforementioned findings demonstrate a degree of similarity with the conclusions reached by a study of Kastelein and colleagues (2013) reported 86% specificity of p53 in detecting malignancy, and a relatively low sensitivity rate of 47%. Another study of Krothapalli and colleagues (2018) reached that p53

exhibited a sensitivity of 90% and a specificity of 89.3% when distinguishing between nondysplastic Barrett's and dysplastic Barrett's. The difference in reported sensitivity results may be due to different clone of antibody used and different staining protocols.

Preston and colleagues (2006) reported that p53 had lower sensitivity towards malignant progression. Consequently, positive p53 expression is suggested to be only reported as strong nuclear staining instead of scattered nuclei.

The p53 protein demonstrates reduced sensitivity and specificity in differentiating LGD and HGD (60 and 70%, respectively. This is supported by the results reached by Kaye and colleagues (2016) in their study. They reported a significant interobserver reproducibility for the interpretation of p53. They have proposed that including p53 can be a useful benefit in dysplasia diagnosis. However, the effectiveness of p53 in distinguishing between LGD and HGD was poor.

Disaccording with these results was a study done by Giménez and colleagues (1998) who approved higher sensitivity and specificity (96.7 and 97.5%, respectively) of p53. This may be attributed to using alternative p53 evaluation methods including flow cytometer or microsatellite primers.

This discrepancy in results can be attributed to different methods in interpretation of p53 expression. Positive p53 staining pattern in this study was evaluated as the strong nuclear staining in more than 10% of glandular epithelium (Sonwalkar and colleagues 2010). In another study, clonal expansion of cells with abnormal p53 expression was represented by aggregates of p53 positive cells. Dispersed p53 positive cells are considered negative because it may only represent physiological accumulation of p53. The study further reported that in dysplastic conditions, p53 expression in aggregates of cells and in multiple biopsies within the oesophagus may serve as an indicator for the rising risk of malignant transformation (Younes and colleagues 2017).

It is frequently observed that mutations in tp53 gene are associated with increased nuclear accumulation of a defective protein, but in the case of truncating mutations, p53 can be entirely lost. Thus, weak, and dispersed p53 immunoreactivity observed within the nuclei is predominantly associated with a wild-type gene status, while either intense nuclear positivity or complete absence of staining is more indicative of tp53 mutations (Kaye and colleagues 2010).

This work proposed that the use of p53 immunohistochemistry may aid in improving the efficacy of diagnostic reproducibility in the detection of dysplasia as well as enhance risk stratification in Barrett's esophagus.

The current study revealed high specificity of IMP3 in detecting dysplasia (100%) with high sensitivity in discriminating HGD and ADC from ND-BE more than LGD (80, 85 and 10%, respectively). It has also showed (80%) sensitivity in discriminating LGD and HGD with high specificity (90%) being suitable for clinical use in the latter condition. Agreeing with our results were shown in Gadara and colleagues (2017) study demonstrated that IMP3 has higher sensitivity in detecting HGD/adenocarcinoma compared with LGD (62.5 and 93.7% vs. 41.7%) with 100% specificity for identifying HGD.

The variation in sensitivity reported between the two studies could be explained by different samples size and the use of distinct antibody clones. However, both studies yield good results regarding the expression of IMP3 in dysplasia and highlight its significance as a reliable predictor of cases with increased susceptibility to malignant transformation.

The current study revealed high specificity of AMCAR in detecting dysplasia (90.9%) in cases of BE with high sensitivity in detecting HGD and carcinoma from ND-BE more than LGD (70, 80, and 20%, respectively). Also, in discriminating LGD from HGD (90 and 80%, respectively). The remarkable specificity exhibited by AMACR towards dysplasia in Barrett's Esophagus qualifies it for clinical use in these conditions.

Agreeing with these findings is a study conducted by Rasha and colleagues (2021) which found significant up regulation of AMACR in high-grade dysplasia and carcinoma indicates its potential as a valuable biomarker for accurately distinguishing high-grade dysplasia and carcinoma from low-grade dysplasia (Jiang and colleagues 2003). In another study, comparable results were reached. They stated 100% specificity detecting dysplasia in Barret's oesophagus.

In the present study, CDX2 was more sensitive in determining HGD from ND-BE rather than LGD (70 and 20%) and more specific in detecting Barret's with dysplasia. Also, CDX2 can discriminate LGD from HGD (80% specificity and 90% sensitivity). This in partial agreement with results of Krothapalli and colleagues (2018) that reports that CDX2 acts as a highly sensitive and specific diagnostic marker for

distinguishing between subsets of ND-BE/LGD and HGD/EAC. However, CDX2 does not possess the capability to differentiate between ND-BE and LGD. In contrast to our study, Plum and colleagues (2018) which reported.a discrepancy in the findings regarding CDX2 expression as a dependable marker for various degrees of dysplasia were reported.

Comparing the performance of p53, IMP3, AMCAR, and CDX2 in predicting dysplasia associated with Barrett's esophagus, this study revealed that IMP3, AMCAR, and CDX2 are more sensitive in detection of cases of Barrett's with HGD (85, 80, and 70%, respectively) rather than p53 which considered more specific (100%). This agrees with Rasha and colleagues (2021) who concluded in their study on Barrett's oesophagus cases that concomitant IMP3 and p53 immuno-staining could effectively detect HGD because of high sensitivity of IMP3 in combination with the significant specificity of p53 towards these cases. This agreed also with Strehl and colleagues (2014) who suggested that the co-expression of P53 and IMP3 may aid in distinguishing between neoplastic and reactive alterations in the gastric mucosa. Thus, combining of p53, CDX2, IMP3, and AMCAR is of great value in early detection of HGD and increased risk of progression to carcinoma.

Conclusion

To that end, a panel of four biomarkers (p53, CDX2, IMP3, and AMCR) assessed in this study could be a vital tool for accurately predicting progression from ND-BE to EAC. Furthermore, this panel is suggested to have high value for early detection of high-grade dysplasia and distinguishing it from LGD. This may contribute to enhancing diagnostic consistency in dysplasia identification and risk stratification in BE cases. Finally, the combined morphologic/IHC method successfully stratified patients into ND-BE/LGD and HGD/EAC groups, optimising patient management.

Limitation

One of the primary limitations of this study belong to the inability of the studied panel of biomarkers to effectively distinguish between ND-BE and LGD. Consequently, this panel may not be considered a reliable diagnostic tool, particularly in challenging cases where discrimination between ND-BE and LGD is required.

Recommendation

To predict a potential role for these markers in the onset and progression of dysplastic alterations in BE, more molecular research should be conducted. Even though p53 IHC is currently used widely, we think that more research is necessary to establish and validate exact criteria and demonstrate how incorporating p53 testing into standard practice could improve patient care.

Authors contributors

Dr O. Y. B. and Dr R. M. A. developed the concept and design and wrote the main manuscript. Dr O. Y. B. and Dr R. M. A. examined and revised cases and methods, interpreted slides and prepared figures. Dr H. A. E. was involved in acquisition of data and statistical analysis. The author(s) read, revised, and approved the final manuscript.

Financial support and sponsorship

Nil.

Conflicts of interest

No conflict of interest

References

Badgery H, Read M, Winter NN, Taylor AC, Hii MW. (2020). The role of esophagectomy in the management of Barrett's esophagus with high-grade dysplasia. Ann N Y Acad Sci 1481:72–89.

Bian YS, Osterheld MC, Bosman FT, Benhattar J, Fontolliet C. (2001). p53 gene mutation and protein accumulation during neoplastic progression in Barrett's esophagus. Mod Pathol 14:397–403.

Bonhomme C, Duluc I, Martin E, Chawengsaksophak K, Chenard MP, Kedinger M, *et al*. (2003). The Cdx2 homeobox gene has a tumour suppressor function in the distal colon in addition to a homeotic role during gut development. Gut 52:1465–1471.

Burdelski C, Jakani-Karimi N, Jacobsen F, Möller-Koop C, Minner S, Simon R, *et al*. (2018). IMP3 overexpression occurs in various important cancer types and is linked to aggressive tumor features: A tissue microarray study on 8,877 human cancers and normal tissues. Oncol Rep 39:3–12.

Çetinaslan İT, Başsüllü N, Uraz S. (2012). CDX2, COX2 and MUC2 expressions in barrett's esophagus: Can they be useful in determination of the dysplasia?

Chen X, Liu BL, Harpaz N, Zhu H, Polydorides AD, Liu Q. (2023). Aberrant p53 expression is associated with neoplastic progression in Barrett oesophagus diagnosed as indefinite for dysplasia. Histopathology 82:454–465.

Davison JM, Goldblum J, Grewal US, McGrath K, Fasanella K, Deitrick C, *et al*. (2020). Independent Blinded Validation of a Tissue Systems Pathology Test to Predict Progression in Barrett's Esophagus Patients. Am J Gastroenterol 115:843.

Denlinger CE, Thompson RK (2012). Molecular basis of esophageal cancer development and progression. Surgical Clinics 92:1089–1103.

Dorer R, Odze RD (2006). AMACR immunostaining is useful in detecting dysplastic epithelium in Barrett's esophagus, ulcerative colitis, and Crohn's disease. Am J Surg Pathol 30:871–877.

Elmubrak AIE (2021). Expression of Alpha-Methyl acyl-Coenzyme A Racemase (AMACR) in Patients with Colorectal Cancer using, TMA, Elsafwa Medical Center, Khartoum State, Sudan (2020) (Doctoral dissertation, University of Gezira)

Elshafey MR, Ahmed RA, Mourad MI, Gaballah ET. (2016). The oncofetal protein IMP3 is an indicator of early recurrence and poor outcome in mucoepidermoid carcinoma of salivary glands. Cancer Biol Med 13:286.

Frei NF, Khoshiwal AM, Konte K, Bossart EA, Stebbins K, Zhang Y, *et al*. (2021). Tissue systems pathology test objectively risk stratifies Barrett's esophagus patients with low-grade dysplasia. Official journal of the American College of Gastroenterology| ACG 116:675–682.

Gadara MR, Gonzalez M, Cartun RW, Ligato S. (2017). IMP3 immunoreactivity is more sensitive than AMACR in detecting dysplastic epithelium and early adenocarcinoma in barrett esophagus. Appl Immunohistochem Mol Morphol 25:386–391.

Giménez A, Minguela A, Parrilla P, Bermejo, Pérez D, Molina J, *et al*. (1998). Flow cytometric DNA analysis and p53 protein expression show a good

correlation with histologic findings in patients with Barrett's esophagus. Cancer: Interdisciplinary Inter J Am Cancer Soc 83:641–651.

Hardwick RH, Shepherd NA, Moorghen M, Newcomb PV, Alderson D, *et al*. (1994). Adenocarcinoma arising in Barrett's oesophagus: evidence for the participation of p53 dysfunction in the dysplasia/carcinoma sequence. Gut 35:764–768.

Hasan IA, Gaidan HA, Al-Kaabi MM (2020). Diagnostic value of cytokeratin 34 beta E12 (Ck34βE12) and α-Methylacyl-CoA racemase (AMACR) immunohistochemical expression in prostatic lesions. Iran J Pathol 15:232.

Hayes S, Ahmed S, Clark P (2011). Immunohistochemical assessment for Cdx2 expression in the Barrett metaplasia–dysplasia–adenocarcinoma sequence. J Clin Pathol 64:110–113.

Huang W, Zhao J, Li L, Huang Y, Yang X, Wang J, Zhang T. (2008). a-Methylacyl coenzyme A racemase is highly expressed in the intestinal-type adenocarcinoma and high-grade dysplasia lesions of the stomach. Histol Histopathol

International Agency for Research on Cancer. (2018). Cancer today: data visualization tools for exploring the global cancer burden in 2018. Available online: Gco. iarc. fr (accessed on 24 September 2020).

Jankowski JA, Odze RD (2009). Biomarkers in gastroenterology: between hope and hype comes histopathology. Official journal of the American College of Gastroenterologyl ACG 104:1093–1096.

Jiang Z, Fanger GR, Woda BA, Banner, Algate P, Dresser K, *et al*. (2003). Expression of α-methylacyl-CoA racemase (P504S) in various malignant neoplasms and normal tissues: a study of 761 cases. Hum Pathol 34:792–796.

Kambhampati S, Tieu AH, Luber B. (2020). Risk factors for progression of Barrett's esophagus to high grade dysplasia and esophageal adenocarcinoma. Sci Rep 10:1–10.

Karamchandani DM, Lehman HL, Ohanessian SE, Massé J, Welsh PA, Odze RD, *et al*. (2016). Increasing diagnostic accuracy to grade dysplasia in Barrett's esophagus using an immunohistochemical panel for CDX2, p120ctn, c-Myc and Jagged1. Diagn Pathol 11:1–13.

Kastelein F, Biermann K, Steyerberg EW, Verheij J, Kalisvaart M, Looijenga LH, *et al*. (2013). Aberrant p53 protein expression is associated with an increased risk of neoplastic progression in patients with Barrett's oesophagus. Gut 62:1676–1683.

Kaye PV, Haider SA, James PD, Soomro I, Catton J, Parsons SL, *et al*. (2010). Novel staining pattern of p53 in Barrett's dysplasia–the absent pattern. Histopathology 57:933–935.

Kaye PV, Ilyas M, Soomro I, Haider SA, Atwal G, Menon S, *et al*. (2016). Dysplasia in Barrett's oesophagus: p53 immunostaining is more reproducible than haematoxylin and eosin diagnosis and improves overall reliability, while grading is poorly reproducible. Histopathology 69:431–440.

Ko S, Chu KM, Luk JMC, Wong BWY, Yuen ST, Leung SY, Wong J. (2005). CDX2 co-localizes with liver–intestine cadherin in intestinal metaplasia and adenocarcinoma of the stomach. The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland 205:615–622.

Krothapalli M, Kini JR, Kini H, Sahu KK, Shenoy S, Krishna SG, Tantry BV. (2018). Evaluation of p53 protein expression in Barrett esophagus. Indian Journal of Pathology and Microbiology 61:170.

Lu D, Vohra P, Chu PG, Woda B, Rock KL, Jiang Z. (2009). An oncofetal protein IMP3: a new molecular marker for the detection of esophageal adenocarcinoma and high-grade dysplasia. Am J Surg Pathol 33:521–525.

Madkour SAEF, Youssef SA, Goda MH, Abd Rabh RM. (2022). Significance of (IMP3) In Dysplasia-Adenocarcinoma Sequence in Barrett's Esophagus. (Immunohistochemical Study). Benha Medical Journal, 39(Special issue (Academic)):125–140.

Malik S, Marwah S, Bidhuri S, Mushahary D. (2021). Challenges in Cancer Prevention in HIV. Indian Journal of Gynecologic Oncology 19:43.

Mancarella C, Pasello M, Manara MC, Toracchio L, Sciandra EF, Picci P, Scotlandi K. (2018). Insulin-like growth factor 2 mRNA-binding protein 3 influences sensitivity to anti-IGF system agents through the translational regulation of IGF1R. Front Endocrinol 9:178.

Neal DJ, Amin MB, Smith SC (2020). CK20 versus AMACR and p53 immunostains in evaluation of Urothelial Carcinoma in Situ and Reactive Atypia. Diagn Pathol 15:1–4.

Plum PS, Ulase D, Bollschweiler E, Chon SH, Berlth F, Zander T, *et al*. (2018). Upregulation of insulin-like growth factor II mRNA-binding protein 3 (IMP3) has negative prognostic impact on early invasive (pT1) adenocarcinoma of the esophagus. J Cancer Res Clin Oncol 144:1731–1739.

Preston S, Jankowski J, Murray L, Sedo A, Scott M, Mcmanus D (2006). TP53 and progression from Barrett's metaplasia to oesophageal adenocarcinoma in a UK population cohort. Commentary. Gut 55:10.

Rasha MA, Ghada A, Kandill M (2021). Studying the Role of IMP3 and P53 in Detection of Dysplastic Changes in Barrett's Esophagus. Med J Cairo Univ 89(September):1899–1906.

Sali R, Moradinasab N, Guleria S, Ehsan L, Fernandes P, Shah TU, *et al*. (2020). Deep learning for whole-slide tissue histopathology classification: A comparative study in the identification of dysplastic and non-dysplastic Barrett's esophagus. J Pers Med 10:141.

Snyder P, Dunbar K, Cipher DJ, Souza RF, Spechler SJ, Konda VJ. (2019). Aberrant p53 immunostaining in Barrett's esophagus predicts neoplastic progression: systematic review and meta-analyses. Dig Dis Sci 64:1089–1097.

Song KY, Henn AJ, Gravely AA, Mesa H, Sultan S, Shaheen NJ, *et al*. (2020). Persistent confirmed low-grade dysplasia in Barrett's esophagus is a risk factor for progression to high-grade dysplasia and adenocarcinoma in a US Veterans cohort. Dis Esophagus 33:doz061.

Sonwalkar SA, Rotimi O, Scott N, Verghese E, Dixon M, Axon AT, Everett SM. (2010). A study of indefinite for dysplasia in Barrett's oesophagus: reproducibility of diagnosis, clinical outcomes and predicting progression with AMACR (α-methylacyl-CoA-racemase). Histopathology 56:900–907.

Srivastava A, Appelman H, Goldsmith JD, Davison JM, Hart J, Krasinskas AM. (2017). The Use of Ancillary Stains in the Diagnosis of Barrett Esophagus and Barrett Esophagus–associated Dysplasia. Am J Surg Pathol 41:e8–e21.

Strehl JD, Hoegel J, Hornicek I, Hartmann A, Riener MO. (2014). Immunohistochemical expression of IMP3 and p53 in inflammatory lesions and neoplastic lesions of the gastric mucosa. Int J Clin Exp Pathol 7:2091.

Weimann A, Zimmermann M, Gross M, Slevogt H, Rieger A, Morawietz L. (2010). CDX2 and LI-cadherin expression in esophageal mucosa: use of both markers can facilitate the histologic diagnosis of Barrett's esophagus and carcinoma. Int J Surg Pathol 18:330–337.

Werling RW, Yaziji H, Bacchi CE, Gown AM. (2003). CDX2, a highly sensitive and specific marker of adenocarcinomas of intestinal origin: an immunohistochemical survey of 476 primary and metastatic carcinomas. Am J Surg Pathol 27:303–310.

Younes M, Brown K, Lauwers GY, Ergun G, Meriano F, Schmulen AC, *et al*. (2017). p53 protein accumulation predicts malignant progression in Barrett's metaplasia: a prospective study of 275 patients. Histopathology 71:27–33.