

*Significance of (IMP3) In Dysplasia- Adenocarcinoma Sequence in Barrett's Esophagus
(Immunohistochemical Study).*

Sara A. Madkour (M.B,BCh), Rasha Mahmoud (MD), Mohebat H. Gouda (MD), Samia A. Youssef (MD).

Abstract:

Background: Barrett's esophagus (BE) is the replacement of the squamous epithelium of the distal esophagus by a metaplastic columnar epithelium. It has been considered the most important precursor lesion for esophageal adenocarcinoma (EAC) through a series of genetic and epigenetic mutations. The oncofetal protein IMP3 is a member of a family of RNA-binding proteins that promotes tumor cell proliferation, adhesion, and invasion.

Aim of the Work: The aim of this study was to evaluate IMP3 expression in BE and its associated pathological changes through the malignant progression.

Materials and Methods: This retrospective study was done upon 51 different esophageal lesions designated as; 20 cases of EAC and 31 cases of BE. IMP3 immunostaining was done and assessed for each case.

Results: IMP3 positivity was seen in 3/11 cases (27.5%) of BE-ND, 6/10 cases (60%) of BE-LGD, 9/10 cases (90%) of BE-HGD, and in all (100%) of EAC cases. IMP3 IHC expression was positively correlated with the different grades of BE (BE-ND, BE-LGD, and BE-HGD) and EAC cases ($P<0.01$). A positive significant statistical correlation was found between IMP3 expression and the BE segment length ($P<0.05$), the grading of EAC ($P<0.01$), tumor necrosis ($P<0.05$), and tumor infiltrating neutrophils ($P<0.01$) and a negative statistically significant correlation with lymphoplasmacytic infiltration in EAC ($P<0.01$).

Conclusions: IMP3 can be a promising marker for risk stratification of BE cases, and may be a predictor of the poor prognosis in EAC cases specially when combined with other histopathological findings.

Keyword: Barrett's Esophagus, Esophageal Adenocarcinoma, IMP3.

Abbreviations: Barrett's esophagus (BE), Negative for dysplasia (ND), Low grade dysplasia (LGD), High grade dysplasia (HGD), Esophageal adenocarcinoma (EAC).

Introduction:

Barrett's esophagus (BE) is a common pre-neoplastic condition which is capable of progression to esophageal adenocarcinoma. The most accepted definition for BE is an endoscopic visualization of a change in the lining of the distal esophagus and histologic confirmation with columnar metaplasia.(1) BE results from chronic long-standing gastroesophageal reflux disease (GERD). Approximately, 10-15% of patients who undergo endoscopy for evaluation of GERD symptoms are found to have Barrett's epithelium (2).

Barrett's esophagus (BE) has been identified as the single most important precursor lesion and risk factor for adenocarcinoma in the distal esophagus; through a series of genetic and epigenetic alterations. It undergoes the sequential progression to low-grade dysplasia (LGD), and high-grade dysplasia (HGD), up to esophageal adenocarcinoma (EAC)(3). The incidence rate of progression of BE patients with no dysplasia to HGD or invasive cancer equals 0.5-0.6%. In contrast, the ratio of progression is increased in BE patients with LGD, being 9.1–13.4% per year (4,5).

Esophageal adenocarcinoma constitutes about 30% to 40% of primary esophageal cancers worldwide. There is an increased incidence of EAC in the United states of America (USA) over the past four decades(6).

Insulin-like growth factor II mRNA-binding protein 3 (IMP3) is a member of the IMP family, consisting of IMP1-3, that plays an important role in RNA trafficking, stabilization and translation during embryogenesis. Although it is absent in human adult tissues, IMP3 is detected in high levels in various types of cancers including lung cancer, germ cell cancer, pancreatic cancer and gastric cancer (7,8,9,10). IMP3 promotes tumor cell proliferation, migration, invasion, and aggressiveness(11).

The aim of this study is to evaluate the IHC expression of IMP3 in BE and its associated pathological changes to clarify the role of IMP3 in the sequencing of BE to EAC.

Material and Methods:

This study was conducted retrospectively on 51 selected esophageal biopsies designated as 20 cases of EAC and 31 cases of BE (11 cases BE-ND, 10 cases of BE-LGD and 10 cases of BE-HGD) Six cases of chronic non-specific esophagitis were used as a control group. Cases were obtained through collection of archived formalin fixed, paraffin embedded blocks from Pathology department, Faculty of Medicine, Benha University, during

the period from January 2015 to December 2018. Cases were selected on basis of availability of demographic data and clinicopathological data approved by ethical committee.

Hematoxylin and eosin-stained slides on all cases were reviewed by two observers simultaneously to confirm the diagnosis and to classify the lesions into one of the study categories. At this review, blocks were selected for immunohistochemistry (IHC).

BE cases were graded according to the dysplasia present into negative for dysplasia , BE-LGD, and BE-HGD.(12) EAC cases were classified and graded as stated in the 2019 WHO classification. (13) The remarkable histopathological features was noted such as the degree of lymphoplasmacytic and neutrophilic infiltration in BE and EAC cases by the application of the Updated Sydney system (USCS) into: absent, mild, moderate and severe,(14) the grade of tumor , necrosis; by subjective scoring of necrosis in H&E stained sections into (score 1: absent confluent necrosis), (score 2; mild confluent area of tumor necrosis)(score 3; extensive confluent areas of necrosis), and grouped into Low grade: (scores 0 and 1) or High grade: (scores 2 and 3),(15) and presence of tumor ulceration (16).

The patients' demographic and endoscopic data were obtained from their original files, including patient's age, sex, smoking status and BE segment length which is grouped into (short segment: <3 cm in length) or (long segment: > 3 cm in length) (17).

Immunohistochemical study: Three-micron tissue sections were obtained from formalin-fixed, paraffin-embedded tissue blocks on coated slides. After xylene deparaffinization, the sections were rehydrated in descending grades of alcohol then in distilled water. Antigen retrieval was performed by using 10 mmol/L citrate monohydrate buffer (PH 6.0) and heated for 15 minutes in the microwave. The sections were then incubated in a blocking medium (3% H₂O₂) for 15 minutes followed by washing with distilled water. The slides then were immunostained for IMP3 Rabbit polyclonal antibody (*0.1mg/ml concentration, Chongqing, 400039, China*) at a dilution of 1:100, at room temperature overnight. Immunodetection was executed using a standard labeled streptavidin-biotin system (*DakoCytomation, Denmark, A/S*). Immune staining was performed based on manufacturer's instructions. Immunoreaction was visualized by adding DAB as a chromagen. Counterstaining of slides was performed with the Mayer hematoxylin.

Negative & positive controls:

Aborted fetal liver (16 weeks) was used as a positive control for IMP3. For negative controls, Omitting the primary antibody and replacing it with solution of BSA in phosphate-buffered saline (PBS).

Immunostaining evaluation:

The slides were evaluated for the presence or absence of IHC staining in studied esophageal biopsies; IMP3 expression was detected as cytoplasmic or membranous brownish coloration. Immunoreactivity was assessed by evaluating extent and intensity of the stained cells. In examined tissues, cytoplasmic staining was evaluated by staining intensity (0, 1+, 2+, and 3+), and the fraction of positive cells was scored for each tissue spot. A final score was done from these two parameters according to the following parameters: negative scores had a staining intensity of 0 and 1+ in $\leq 10\%$ of epithelial cells; weak scores had a staining intensity of 1+ in $>10\%$ and $\leq 70\%$ of epithelial cells or a staining intensity of 2+ in $\leq 30\%$ of epithelial cells; moderate scores had a staining intensity of 1+ in $>70\%$ of epithelial cells, a staining intensity of 2+ in $>30\%$ and $\leq 70\%$ of epithelial cells or a staining intensity of 3+ in $\leq 30\%$ of epithelial cells; and strong scores had a staining intensity of 2+ in $>70\%$ of epithelial cells or a staining intensity of 3+ in $>30\%$ of epithelial cells. At least, weak IHC expression is required for the definition of IMP3-positivity of the cells. Expression of IMP3 was then correlated with histopathological data in studied BE and EAC cases.

Statistical analysis: Results were analyzed using SPSS (version 23) statistical package for Microsoft windows. The Pearson correlation coefficient was used for statistical analysis. P value <0.05 was considered statistically significant, and highly statistically significant when it was <0.01 .

Results:

Clinico-pathological features of studied BE cases:

Mean age of the studied BE cases was (44.16). Twenty-three cases (74%) were male and 8 cases (26%) were females. Fifty five percent were smokers and 45% were non-smokers. Forty eight percent were short segment BE and fifty two percent were long segment BE. Non dysplastic BE represented 36%, LGD represented 32% and HGD represented 32% of BE cases.

Table1: Correlation between the grade of dysplasia and histopathological data of BE:

Grade of dysplasia	Segment Length			P value
	Short		Long	
BE-ND	63.5%		36.5%	<0.5*
BE-LGD	60%		40%	
BE-HGD	20%		80%	
	Neutrophilic infiltrate			
	Mild	Moderate	Severe	
BE-ND	27.2%	36.4%	36.4%	>0.5
BE-LGD	40%	20%	40%	
BE-HGD	40%	20%	40%	
	Lymphocytic infiltration			
	Mild	Moderate	Severe	
BE-ND	27%	46%	27%	>0.5
BE-LGD	30%	40%	30%	
BE-HGD	30%	40%	30%	

Table (2): Clinico-pathological features of studied EAC cases:

Variable	No=20 (100%)	
Mean age	63.16	
Gender	Male	12 (60%)
	Female	8 (40%)
Smoking	Smoker	14 (70%)
	Non-smoker	6 (30%)
Histological subtypes	Tubular adenocarcinoma	8 (40%)
	Papillary adenocarcinoma	2 (10%)
	Mucinous adenocarcinoma	4 (20%)
	Signet adenocarcinoma ring	6 (30%)
Necrosis	High grade	13 (65%)
	Low grade	7 (35%)
Ulceration	Present	9 (45%)
	Absent	11 (55%)
Tumor grade	Well differentiated	6 (30%)
	Moderately differentiated	6 (30%)
	Poorly differentiated	8 (40%)

A highly significant statistical correlation was found between tumor grade and the histological subtypes of EAC ($P<0.01$). Also, a significant statistical correlation was found between tumor grade and Tumor infiltrating neutrophils (TINs) ($P<0.05$), and the lymphoplasmacytic infiltrate ($P<0.05$). No significant statistical correlation was found between histological subtypes of EAC and necrosis, Tumor infiltrating neutrophils (TINs) or the lymphoplasmacytic infiltrate ($P>0.05$). There was insignificant statistical correlation between tumor grade and necrosis ($P>0.05$).

Immunohistochemical results:

A highly significant correlation was found between IMP3 IHC expression and the different grades of BE and EAC cases. Regarding BE, a high significant statistical correlation between IMP3 expression and the segment length and grade of dysplasia in the studied cases.

Both neutrophilic and lymphoplasmacytic infiltrate showed no statistically significant correlation with the IHC expression of IMP3. **Table (3) (Figure 1; a,b,c)**

IMP3 expression had a significant positive statistical correlation with tumor necrosis and a high significant statistical correlation with grading, neutrophilic and lymphoplasmacytic infiltration in the studied EAC cases. In contrast, there was no significant statistical correlation between the histological subtypes of EAC and the IHC expression of IMP3. **Table (4) (Figure 1).**

Table (3): Correlation between the Clinico-pathological variants of BE and IMP3 IHC expression

Clinico-pathological variants of BE		IMP3 expression				P value
		Negative	Weak	Moderate	Strong	
Segment length	Short segment BE	66.5%	27%	0%	6.5%	0.001**
	Long segment BE	19%	19%	25%	37%	
Grade of dysplasia	BE-ND	72.5%	27.5%	9%	0%	0.000**
	BE-LGD	40%	40%	20%	0%	
	BE-HGD	10%	0%	20%	70%	
Neutrophilic infiltrate	Mild	55%	18%	18%	9%	>0.05
	Moderate	37.5%	25%	12.5%	25%	
	Severe	33.5%	25%	8%	33.5%	
Lymphoplasmacytic infiltrate	Mild	44.5%	22%	11.5%	22%	>0.05
	Moderate	53.5%	15.5%	8%	23%	
	Severe	22.2%	33.4%	22.2%	22.2%	

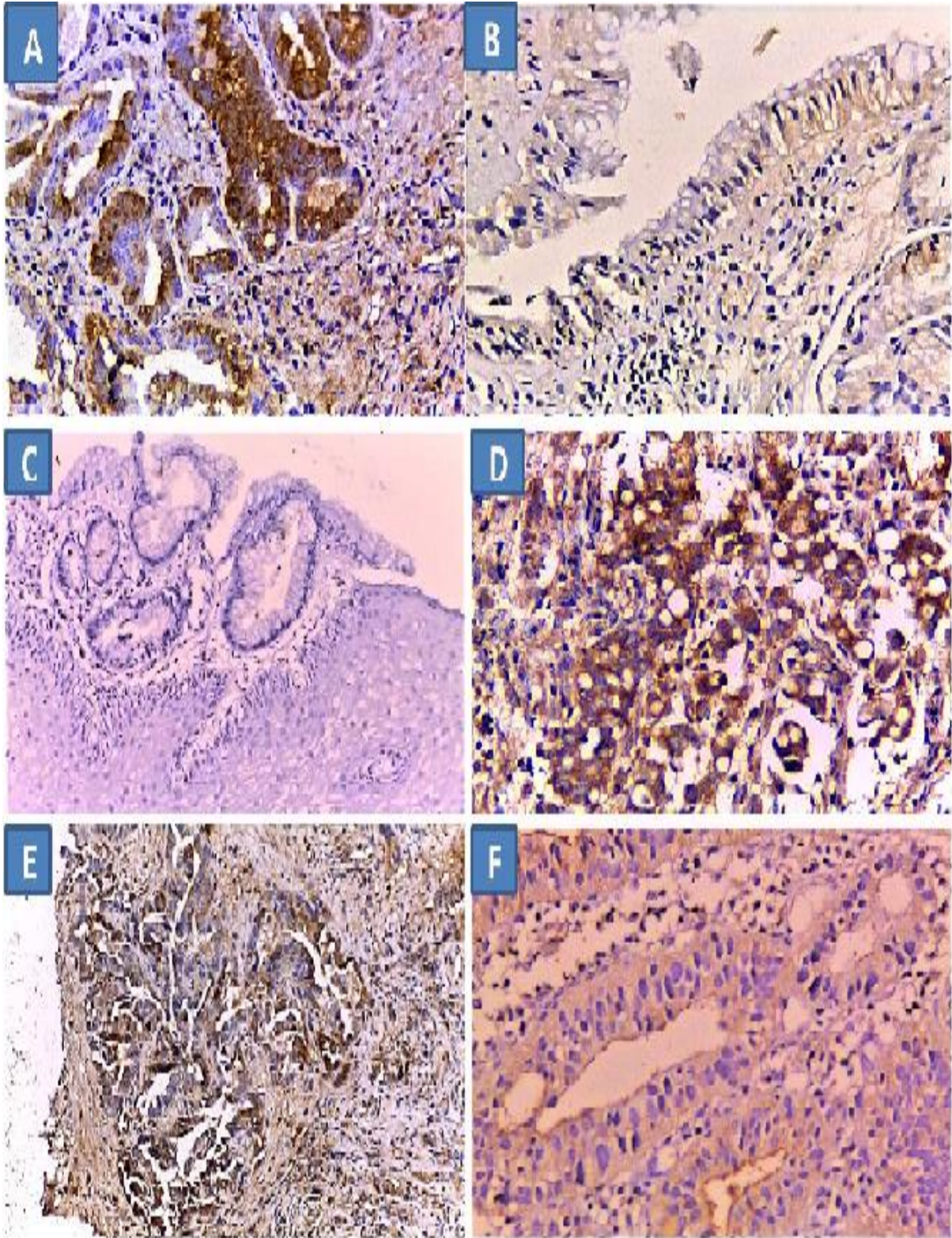


Figure 1: Immunohistochemical staining for IMP3 in different grades of BE. Negative staining for IMP3 in BE-ND, X200 (A), weak cytoplasmic staining in BE-LGD, X400 (B), Strong cytoplasmic staining in BE-HGD, X400 (C) Immunohistochemical staining for IMP3 in different grades of EAC. Weak cytoplasmic staining for IMP3 in well differentiated EAC, X400 (D), moderate cytoplasmic staining for IMP3 in moderately differentiated EAC, X00 (E), Strong cytoplasmic and membranous staining in poorly differentiated signet ring adenocarcinoma, X400 (F).

Table (4): Correlation between IMP3 expression and clinicopathological variables of EAC.

Clinico-pathological variants of EAC		IMP3 IHC expression			P value
		Weak	Moderate	Strong	
Grading	Well differentiated	33.3%	50.0%	16.7%	0.003**
	Moderately differentiated	0%	16.7%	83.3%	
	Poorly differentiated	0%	12.5%	87.5%	
Histological subtypes	Tubular adenocarcinoma	25%	25%	50%	>0.05
	Papillary adenocarcinoma	0%	50%	50%	
	Mucinous adenocarcinoma	0%	25%	75.0%	
	Signet ring carcinoma	0%	16.7%	83.3%	
Necrosis	Low grade	15.5%	38.5%	46%	0.027*
	High grade	0%	0%	100%	
Neutrophilic infiltrate	Mild	33.5%	50%	16.5%	0.001**
	Moderate	0%	28.6%	71.4%	
	Severe	0%	0.0%	100.0%	
Lymphoplasmacytic infiltrate	Mild	0%	20%	80%	0.01**
	Moderate	0%	20%	80%	
	Severe	40%	40%	20.0%	

Discussion:

BE is a major risk factor for esophageal adenocarcinoma (EAC), which has shown a dramatic increase in incidence over the past 50 years with a very poor prognosis.(18)

This retrospective study was done on fifty-one cases of esophageal lesions including BE and EAC. IMP3 was immunohistochemically stained and evaluated for each case. Then its expression was correlated with different clinical and histopathological variables.

An obvious male predominance was found in both BE and EAC studied cases. This was in accordance with the finding of **Nguyen et al., (2019)**,(19) **Chen et al., (2019)**(20) and **Coleman et al., (2018)**(21) in their studies in the esophagus. **Sanikini et al., (2019)**(22) explained this male predominance in BE by the gender related differences in the known risk

factors of BE such as increased prevalence of tobacco use and alcohol consumption, being more common in men. The male predominance in BE, being the strongest predisposing factor of EAC, can explain the emerging male predominance in EAC.

The current work revealed an increased prevalence of smoking in BE and EAC cases. This agreed to the work of **Huang et al., (2018)**(23) who found that current smokers have an increased risk of both BE and EAC, as compared to non-smokers. **Hardikar et al., (2013)**(24) explained the role of smoking in BE and EAC through exposure to chemicals such as N-nitrosoamines, the promotion of GERD, and the continuous inflammatory effects of smoking promoting the cellular proliferation.

The present study showed a significant statistical correlation between the grade of dysplasia and segment length of the studied BE cases (P value<0.5). While no significant statistical correlation was found between the the grade of dysplasia and the neutrophilic infiltrate in studied BE cases. In contrast, a high significant statistical correlation was found between the grading of EAC cases and tumor infiltrating neutrophils (TINs) (P value<0.01). In agreement with this, **Xiao et al., (2014)**(25) concluded that high neutrophil to lymphocyte ratio was associated with a poor tumor grade in hepatocellular carcinoma and **Tang et al., (2017)**(26) proved that neutrophilic count was significantly associated with high-grade bladder carcinoma. This can be explained by the carcinogenic role of neutrophils. It releases reactive oxygen species that causes genetic instability. Chemokines secreted from tumor cells themselves such as interleukin8 (IL8) recruit neutrophils to the site of tumorigenesis. Neutrophils also cause constitutive activation of the inflammatory signaling pathways leading to downstream activation of gene transcription and enzymatic activity that have a key role in tumor growth and survival. The IL-6/STAT3 signaling pathway is upregulated in esophageal cancer and several studies have correlated increased epithelial IL-6/STAT3 activity with cell proliferation and apoptotic resistance in BE and EAC.(27)

In the current work, there was no significant statistical correlation between the lymphoplasmacytic infiltrate and the grade of dysplasia in studied BE cases (P>0.05). In contrast, we found a significant negative statistical correlation between the lymphoplasmacytic infiltrate and the grading of EAC cases (P value <0.05). This agreed to the result of **Fridman et al., (2012)**(28) that high infiltration by CD8+ T lymphocytes is associated with favorable prognosis in esophageal cancer. In contrast to this **Seo et al., (2013)**(29) found that higher tumor infiltrating lymphocytes are associated with higher histologic grades in breast carcinomas. **Giraldo, et al. (2019)**(30) suggested that the more

poorly differentiated tumor cells recruit more tumor infiltrating lymphocytes from the circulation due to either the expression of pro-inflammatory factors or changes in their immunogenicity. This argument in results could be due to different tissues examined and different methods of lymphocytes evaluation.

In our study, tumor necrosis showed no statistically significant correlation with histopathological subtypes of EAC cases. Also, the correlation between necrosis score and grading of EAC cases was statistically insignificant (P value >0.05).

The insulin-like growth factor-II mRNA-binding protein 3 (IMP3) is an oncofetal protein that affects cellular proliferation, adhesion, and invasion of malignant neoplasms through the control of the translation or turnover of various candidate target genes, including IGF2, CD44, HMGA2, and MMP9. The IHC expression of IMP3 was detected in various neoplasms.(31) So, we aimed to evaluate this protein expression in the pathological changes occurring in BE through its progression to adenocarcinoma.

There was complete negative IHC staining of IMP3 in chronic non specific esophagitis cases (control group). A significant statistical correlation was found between IHC expression of IMP3 and different grades of dysplasia in BE and esophageal adenocarcinoma (P value <0.01). IMP3 was expressed in 27.5% of BE-ND, 60% of BE-LGD and in 90% of BE-HGD. All esophageal adenocarcinoma cases (100%) showed IHC positivity of IMP3. Our findings were compatible with the results of other series in literature evaluating the expression of IMP3 in BE (32,33,34,35). Those results propose that IMP3 have a role in the tumorigenesis and progression from BE to EAC.

IMP3 is known to favor IGF2 translation, activating IGF signaling and promoting cell growth, proliferation, and resistance to ionic irradiation in different tumor types (36) It acts by synergizing with heterogeneous nuclear ribonucleoprotein M (HNRNPM) in the nucleus leading to an enhanced expression of cyclins and was also shown to promote the expression of HMGA2 (high-mobility group AT-hook 2) by preventing miRNA binding, leading to proliferation, migration and invasion of malignant cells (37).

The obvious upregulation of IMP3 expression in malignant lesions rather than benign and dysplastic cases in the current work and similar previous studies in other tissues, (38,39) approved the fore mentioned oncogenic role in esophageal adenocarcinoma and other human malignancies and making it a promising biomarker for the differentiation between benign and malignant lesions in some doubtful cases specially when integrated with other clinicopathological parameters. Also, its different expression in high and low dysplastic cases

can help for risk stratification and close monitoring for low risk cases or be a guide for rapid intervention in high risk cases limiting the progression to invasive cancer.

In Barrett's esophagus cases, a significant statistical correlation was found between the IHC expression of IMP3 and the segment length (P value<0.01). The result of **Al Nasser et al., (2019) (40)** is in line with our work. They concluded that the longer Barrett's was associated with increased risk of dysplastic and neoplastic progression. So, we can say that patients with longer Barrett's esophagus segment should undergo a more intense surveillance.

A highly significant statistical correlation was found between IMP3 expression of IMP3 and the grade of dysplasia (P <0.01). This result is compatible with the result of **Daikuhara et al. (2015)(41)** in their study who reported that increased IMP3 IHC expression was associated with a higher degree of dysplasia in small intestinal adenomas.

A significant statistical correlation was found between IMP3 expression and the grading of esophageal adenocarcinoma cases (P <0.01). Against our result was the result of **lu et al., 2009(42)** who reported that no difference in IMP3 IHC expression between the grades of esophageal adenocarcinoma. We also argue with the result of **Yasutake et al., (2018)(43)** who said that there was no statistical difference in IMP3 expression between low and high grade leiomyosarcomas. This difference can be attributed to the different assessment and different tissue examined.

The current study showed a significant statistical correlation between IMP3 expression and the necrosis score in EACs. In agreement to this, **Samanta et al., (2016)(44)** had found that IMP3 expression was correlated with necrosis in triple negative breast carcinomas. **Also Sasaki and Sato (2017)(39)** concluded a stronger IHC expression of IMP3 in cases of papillary biliary tumors associated with necrosis. But yet, not much research are found in literature about this issue.

This study revealed a high significant statistical correlation between tumor infiltrating neutrophils and IMP3 IHC expression (p<0.01). Also, IMP3 expression showed a significant inverse correlation with the lymphoplasmacytic infiltration in EAC cases. But yet, no much research in literature explained the association between IMP3 expression and the neutrophilic or lymphoplasmacytic infiltrate. A wider cohort studies are needed to evaluate it.

Conclusion:

IMP3 is involved in the neoplastic progression of BE, and can be a potential diagnostic marker for dysplastic BE. Thus, it is considered a promising marker for the risk

stratification of BE cases allowing a better surveillance and rapid intervention in the high risk groups. IMP3 may be a marker of poor prognosis in EAC. A wider cohort studies are needed to confirm the role of IMP3 in BE progression and esophageal carcinogenesis.

Histopathological findings such as neutrophilic and lymphoplasmacytic infiltration in EAC cases, could predict the prognosis as it correlates significantly with the degree of differentiation. But this still need confirmation in literature by further wider studies.

Conflicts of Interest :

No conflict of interest

References:

1. Clermont M, Falk GW. Clinical Guidelines Update on the Diagnosis and Management of Barrett's Esophagus. Vol. 63, Digestive Diseases and Sciences. Springer New York LLC; 2018. p. 2122–8.
2. Hsu S-C, Huang S-H, Lee C-L, Tzeng C-C, Wu C-H. Correlation between Endoscopically Suspected Esophageal Metaplasia and Barrett's Esophagus: A Single Center Experience. *Br J Med Med Res.* 2016;11(4):1–6.
3. Kambhampati S, Luber B, Wang H, Meltzer SJ. Risk Factors for Progression of Barrett's Esophagus to High Grade Dysplasia and Esophageal Adenocarcinoma: A Large Retrospective Cohort Study. *Gastroenterology.* 2017;152(5):S455.
4. Duits LC, Phoa KN, Curvers WL, Ten Kate FJW, Meijer GA, Seldenrijk CA, et al. Barrett's oesophagus patients with low-grade dysplasia can be accurately risk-stratified after histological review by an expert pathology panel. *Gut.* 2015 Apr 9;64(5):700–6.
5. Kastelein F, Van Olphen SH, Steyerberg EW, Spaander MCW, Bruno MJ, Biermann K, et al. Impact of surveillance for Barrett's oesophagus on tumour stage and survival of patients with neoplastic progression. *Gut.* 2016 Apr 1;65(4):548–54.
6. Njei B, Mccarty TR, Birk JW. Trends in esophageal cancer survival in United States adults from 1973 to 2009: A SEER database analysis. *J Gastroenterol Hepatol.* 2016 Jun 1;31(6):1141–6.
7. Goodman S, Zhang L, Cheng L, Jiang Z. Differential expression of IMP3 between male and female mature teratomas-immunohistochemical evidence of malignant nature. *Histopathology.* 2014 Oct 1;65(4):483–9.
8. Lok T, Chen L, Lin F, Wang HL. Immunohistochemical distinction between intrahepatic cholangiocarcinoma and pancreatic ductal adenocarcinoma. *Hum Pathol.* 2014;45(2):394–400.
9. Damasceno EAM, Carneiro FP, de Magalhães AV, Carneiro M de V, Takano GHS, Vianna LM de S, et al. IMP3 expression in gastric cancer: association with clinicopathological features and HER2 status. *J Cancer Res Clin Oncol.* 2014;140(12):2163–8.

10. Yan J, Wei Q, Jian W, Qiu B, Wen J, Liu J, et al. IMP3 Predicts Invasion and Prognosis in Human Lung Adenocarcinoma. *Lung*. 2016 Feb 1;194(1):137–46.
11. Bell JL, Wächter K, Mühleck B, Pazaitis N, Köhn M, Lederer M, et al. Insulin-like growth factor 2 mRNA-binding proteins (IGF2BPs): Post-transcriptional drivers of cancer progression? Vol. 70, *Cellular and Molecular Life Sciences*. 2013. p. 2657–75.
12. Ten Kate FJC, Nieboer D, Ten Kate FJW, Doukas M, Bruno MJ, Spaander MCW, et al. Improved Progression Prediction in Barrett's Esophagus with Low-grade Dysplasia Using Specific Histologic Criteria. *Am J Surg Pathol*. 2018;42(7):918–26.
13. Nagtegaal ID, Odze RD, Klimstra D, Paradis V, Rugge M, Schirmacher P, et al. The 2019 WHO classification of tumours of the digestive system. *Histopathology*. 2019 Aug 21;
14. Qamar S, Bukhari M, Asrar A, Sarwar S, Niazi S, Qamar S. Evaluation of Antral Gastric Biopsies. A Study of 50 Patients at Mayo Hospital. *Spec Ed Ann JAN -MAR*. 2010;16(1).
15. Dutta S, Going JJ, Crumley ABC, Mohammed Z, Orange C, Edwards J, et al. The relationship between tumour necrosis, tumour proliferation, local and systemic inflammation, microvessel density and survival in patients undergoing potentially curative resection of oesophageal adenocarcinoma. *Br J Cancer*. 2012 Feb 14;106(4):702–10.
16. Solanky D, Krishnamoorthi R, Crews N, Johnson M, Wang K, Wolfsen H, et al. Barrett esophagus length, nodularity, and low-grade dysplasia are predictive of progression to esophageal adenocarcinoma. *J Clin Gastroenterol*. 2019;53(5):361–5.
17. Ross-Innes CS, Debiram-Beecham I, O'Donovan M, Walker E, Varghese S, Lao-Sirieix P, et al. Evaluation of a Minimally Invasive Cell Sampling Device Coupled with Assessment of Trefoil Factor 3 Expression for Diagnosing Barrett's Esophagus: A Multi-Center Case–Control Study. *PLoS Med*. 2015 Jan 1;12(1):1–19.
18. Xian W, Duleba M, Zhang Y, Yamamoto Y, Ho KY, Crum C, et al. The Cellular Origin of Barrett's Esophagus and Its Stem Cells. In: *Advances in Experimental Medicine and Biology*. Springer New York LLC; 2019. p. 55–69.
19. Nguyen AD, Spechler SJ, Dunbar KB. Barrett's Esophagus. In: *Evidence-based Gastroenterology and Hepatology 4e*. Chichester, UK: John Wiley & Sons, Ltd; 2019. p. 21–34.
20. Chen YH, Yu HC, Lin KH, Lin HS, Hsu PI. Prevalence and risk factors for Barrett's esophagus in Taiwan. *World J Gastroenterol*. 2019;25(25):3231–41.
21. Coleman HG, Xie SH, Lagergren J. The Epidemiology of Esophageal Adenocarcinoma. *Gastroenterology*. 2018;154(2):390–405.
22. Sanikini H, Muller DC, Sophiea M, Rinaldi S, Agudo A, Duell EJ, et al. Anthropometric and reproductive factors and risk of esophageal and gastric cancer by subtype and subsite: Results from the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort. *Int J Cancer*. 2019 May 3;
23. Huang QD, Zheng SR, Cai YJ, Chen DL, Shen YY, Lin CQ, et al. IMP3 promotes TNBC stem cell property through miRNA-34a regulation. *Eur Rev Med Pharmacol Sci*. 2018;22(9):2688–96.

24. Hardikar S, Onstad L, Blount PL, Odze RD, Reid BJ, Vaughan TL. The Role of Tobacco, Alcohol, and Obesity in Neoplastic Progression to Esophageal Adenocarcinoma: A Prospective Study of Barrett's Esophagus. *PLoS One*. 2013
25. Xiao W-K, Chen D, Li S-Q, Fu S-J, Peng B-G, Liang L-J. Prognostic significance of neutrophil-lymphocyte ratio in hepatocellular carcinoma: a meta-analysis. *BMC Cancer*. 2014 Feb 21;14:117.
26. Tang X, Wang S, An C, Du P, Yang Y. Preoperative high neutrophil-to-lymphocyte ratio is associated with high-grade bladder cancer. In: *Anticancer Research*. 2017. p. 4659–63.
27. Cho W, Jin X, Pang J, Wang Y, Mivechi NF, Moskophidis D. The Molecular Chaperone Heat Shock Protein 70 Controls Liver Cancer Initiation and Progression by Regulating Adaptive DNA Damage and Mitogen-Activated Protein Kinase/Extracellular Signal-Regulated Kinase Signaling Pathways. *Mol Cell Biol*. 2019;39(9).
28. Fridman WH, Pagès F, Sautès-Fridman C, Galon J. The immune contexture in human tumours: Impact on clinical outcome. Vol. 12, *Nature Reviews Cancer*. 2012. p. 298–306.
29. Seo AN, Lee HJ, Kim EJ, Kim HJ, Jang MH, Lee HE, et al. Tumour-infiltrating CD8+ lymphocytes as an independent predictive factor for pathological complete response to primary systemic therapy in breast cancer. *Br J Cancer*. 2013 Nov 12;109(10):2705–13.
30. Giraldo NA, Peske JD, Sautès-Fridman C, Fridman WH. Integrating histopathology, immune biomarkers, and molecular subgroups in solid cancer: the next step in precision oncology. Vol. 474, *Virchows Archiv*. 2019. p. 463–74.
31. Burdelski C, Jakani-Karimi N, Jacobsen F, Möller-Koop C, Minner S, Simon R, et al. 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*. 2018 Jan 1;39(1):3–12.
32. Lu D, Vohra P, Chu PG, Woda B, Rock KL, Jiang Z. An oncofetal protein IMP3: A new molecular marker for the detection of esophageal adenocarcinoma and high-grade dysplasia. *Am J Surg Pathol*. 2009 Apr;33(4):521–5.
33. Feng W, Zhou Z, Peters JH, Khoury T, Zhai Q, Wei Q, et al. Expression of insulin-like growth factor II mRNA-binding protein 3 in human esophageal adenocarcinoma and its precursor lesions. *Arch Pathol Lab Med*. 2011 Aug;135(8):1024–31.
34. Gonzalez M, Cartun RW, Ligato S. IMP3 Immunoreactivity is More Sensitive Than AMACR in Detecting Dysplastic Epithelium and Early Adenocarcinoma in Barrett Esophagus. *Appl Immunohistochem Mol Morphol*. 2017;25(6):386–91.
35. Srivastava A, Odze RD, Lauwers GY, Redston M, Antonioli DA, Glickman JN. Morphologic features are useful in distinguishing Barrett esophagus from carditis with intestinal metaplasia. *Am J Surg Pathol*. 2007;31(11):1733–41.
36. Panebianco F, Kelly LM, Liu P, Zhong S, Dacic S, Wang X, et al. THADA fusion is a mechanism of IGF2BP3 activation and IGF1R signaling in thyroid cancer. *Proc Natl Acad Sci U S A*. 2017;114(9):2307–12.

37. Conway AE, Van Nostrand EL, Pratt GA, Aigner S, Wilbert ML, Sundararaman B, et al. Enhanced CLIP Uncovers IMP Protein-RNA Targets in Human Pluripotent Stem Cells Important for Cell Adhesion and Survival. *Cell Rep.* 2016;15(3):666–79.
38. Senoo J, Mikata R, Kishimoto T, Hayashi M, Kusakabe Y, Yasui S, et al. Immunohistochemical analysis of IMP3 and p53 expression in endoscopic ultrasound-guided fine needle aspiration and resected specimens of pancreatic diseases. *Pancreatol.* 2018;18(2):176–83.
39. Sasaki M, Sato Y. Insulin-like growth factor II mRNA-binding protein 3 (IMP3) is a marker that predicts presence of invasion in papillary biliary tumors. *Hum Pathol.* 2017;62:152–9.
40. Alnasser S, Agnihotram R, Martel M, Mayrand S, Franco E, Ferri L. Predictors of dysplastic and neoplastic progression of Barrett's esophagus. *Can J Surg.* 2019;62(2):93–9.
41. Daikuhara S, Uehara T, Higuchi K, Hosaka N, Iwaya M, Maruyama Y, et al. Insulin-like growth factor II mRNA-binding protein 3 (IMP3) as a useful immunohistochemical marker for the diagnosis of adenocarcinoma of small intestine. *Acta Histochem Cytochem.* 2015 Dec 25;48(6):193–204.
42. Lu D, Vohra P, Chu PG, Woda B, Rock KL, Jiang Z. An oncofetal protein IMP3: A new molecular marker for the detection of esophageal adenocarcinoma and high-grade dysplasia. *Am J Surg Pathol.* 2009;33(4):521–5.
43. Yasutake N, Ohishi Y, Taguchi K, Hiraki Y, Oya M, Oshiro Y, et al. Insulin-like growth factor II messenger RNA-binding protein-3 is an independent prognostic factor in uterine leiomyosarcoma. *Histopathology.* 2018 Apr 1;72(5):739–48.
44. Samanta S, Sharma VM, Khan A, Mercurio AM. Regulation of IMP3 by EGFR signaling and repression by ER β : Implications for triple-negative breast cancer. *Oncogene.* 2012;31(44):4689–97.

الملخص العربي

الخلفية: مريء باريت (BE) هو استبدال الظهارة الحرشفية للمريء البعيد بظهارة عمودية ميتابلاستيك. وقد اعتبرت أهم آفة السلانف لسرطان غدي المريء (EAC) من خلال سلسلة من الطفرات الجينية والجينية. بروتين oncofetal IMP3 هو عضو في عائلة من بروتينات ربط الحمض النووي الريبي التي تعزز انتشار خلايا الورم ، الالتصاق ، والغزو.

الهدف: كان الهدف من هذه الدراسة هو تقييم تعبير IMP3 في BE والتغيرات المرضية المرتبطة به من خلال التقدم الخبيث.

طرق البحث: تم إجراء هذه الدراسة بأثر رجعي على ٥١ آفة مريئية مختلفة تم تصنيفها على أنها: ٢٠ حالة EAC و ٣١ حالة BE. تم إجراء IMP3 immunostaining وتقييم لكل حالة.

النتائج: شوهدت إيجابية IMP3 في ١١/٣ حالات (٢٧.٥٪) من BE-ND ، 6/10 حالات (٦٠٪) من BE-LGD ، 9/10 حالات (٩٠٪) من BE-HGD ، وفي جميع (١٠٠٪) من حالات EAC. ارتبط تعبير IMP3 IHC بشكل إيجابي مع الدرجات المختلفة لـ (BE-ND) و BE (BE-LGD و BE-HGD) وحالات (P) EAC (> 0.01). تم العثور على ارتباط إحصائي إيجابي مهم بين تعبير IMP3 وطول مقطع (P) BE (> 0.05) ، وتصنيف (P) EAC (> 0.01) ، ونخر الورم (P) (> 0.05) ، والعدلات المتسللة للورم (P) (> 0.01) و علاقة سلبية ذات دلالة إحصائية مع الارتشاح للمفاوي في (P) EAC (> 0.01).

الخلاصة: IMP3 يمكن أن يكون علامة واعدة لتصنيف المخاطر لحالات BE ، ويمكن أن يكون مؤشرا لسوء التشخيص في حالات EAC خاصة عند دمجها مع النتائج النسيجية الأخرى.