Turk J Gastroenterol *2014; 25: 253-6*

Usefulness of insulin-like growth factor II mRNA-binding protein 3 (IMP3) as a new marker for the diagnosis of esophageal adenocarcinoma in challenging cases

ESOPHAGUS

Behrang Kazeminezhad, Seyed Abbas Mirafsharieh, Kamran Dinyari, Davood Azizi, Abdolali Ebrahimi

Department of Pathology, Shahidbeheshti University of Medical Sciences, Tehran, Islamic Republic of Iran

Original Article

ABSTRACT

Background/Aims: Insulin-like growth factor II mRNA-binding protein 3 (IMP3) is an oncofetal protein with vital function during human embryogenesis in terms of cellular growth and migration. Although it has minimum and undetectable expression in human adult tissues, it is highly expressed in various types of cancer. Few studies have recommend application of IMP3 expression to diagnose challenging cases of esophageal adenocarcinoma. This survey was aimed to evaluate the benefit of IMP3 expression detection in the diagnosis of esophageal adenocarcinoma and high-grade dysplasia by using immunohistochemistry (IHC).

Materials and Methods: An immunohistochemistry study of IMP3 oncofetal protein was performed on paraffin-embedded blocks of 76 cases, including Barrett's esophagus, esophageal squamous epithelium, Barrett's esophagus with low-grade dysplasia, Barrett's esophagus with high-grade dysplasia, moderately differentiated esophageal adenocarcinoma, and poorly differentiated esophageal adenocarcinoma. Two pathologists reevaluated the diagnosis and evaluated the positivity and intensity of the IHC staining as well.

Results: Insulin-like growth factor II mRNA-binding protein 3 expression was intensely positive in all cases of esophageal adenocarcinoma and Barrett's esophagus with HGD. Only mild positivity in 30% of Barrett's esophagus with LGD was seen. However, Barrett's esophagus and esophageal squamous epithelium had, in fact, no IMP3 expression.

Conclusion: The percentage and intensity of IP3 IHC staining showed a significant difference between high-grade dysplasia and adenocarcinoma versus Barrett's esophagus with low-grade dysplasia, Barrett's esophagus, and esophageal squamous epithelium. Therefore, IMP3 oncofetal protein could be a very useful marker for the diagnosis of high-grade dysplasia and adenocarcinoma. However, to test the validation, a larger number samples is required.

Keywords: IMP3, esophageal adenocarcinoma, Barrett's esophagus

INTRODUCTION

Barrett's esophagus is associated with low-grade dysplasia, high-grade dysplasia, and adenocarcinoma of the esophagus (1-3). Though Barrett's esophagus and low-grade dysplasia only need regular endoscopic evaluation, adenocarcinoma and high-grade dysplasia require surgical intervention (1-4). Hence, to choose the best therapeutic option, accurate histopathologic diagnosis of high-grade esophageal glandular dysplasia and adenocarcinoma are mandatory. Diagnosis of dysplasia and adenocarcinoma, however, could be a challenge for the pathologist, particularly in small esophageal biopsies (5). Finding a marker with high sensitivity and specificity to differentiate low-grade dysplasia from high-grade/malignant is a requirement to make a definitive histopathologic diagnosis in challenging cases and to choose the best therapeutic option for patients, as well.

Insulin-like growth factor II mRNA-binding protein 3 is an oncofetal protein that has a crucial role in cellular proliferation, adhesion, and invasion of malignant tumors (6,7). It also has a vital role in the early phases of embryogenesis (8).

Address for Correspondence: Abdolali Ebrahimi, Department of Pathology, Shahidbeheshti University of Medical Sciences, Tehran, Islamic Republic of Iran
E-mail: ebrahimibeheshti@gmail.com

Received: 29.4.2013 **Accepted:** 6.2.2014

 • Available online at www.turkjgastroenterol.org • DOI: 10.5152/tjg.2014.5454

Insulin-like growth factor II mRNA-binding protein 3 is a type of insulin-like growth factor II mRNA-binding protein (IMP), which has three subtypes, including IMP1, IMP2, and IMP3 (9). IMP3 has a significant role in cell proliferation, cell adhesion, and tumor invasion (6,7). Although IMP3 expression is normal in embryonic epithelium, muscle, and placenta of the human fetus, its expression is either minimal or absent in adult human tissues (8,10). This oncofetal protein is highly expressed in various types of human malignant neoplasm, including lung, colon, pancreas, and stomach cancers; soft tissue sarcomas; endometrial serous carcinoma; and endocervical adenocarcinoma; there is, however, no expression in the surrounding benign tissues (9,11-15). Few studies have shown that IMP3 is a very useful marker to diagnose low-grade from high-grade Esophageal dysplasia/adenocarcinoma (5,16). In this study, we analyzed the expression of IMP3 in Barrett's esophagus, esophageal low-grade and high-grade glandular dysplasia, and adenocarcinoma to determine whether IMP3 has diagnostic application in esophageal biopsies with doubtful adenocarcinoma or high-grade glandular dysplasia.

MATERIALS AND METHODS

In this study, the pathology slides of 76 patients with a diagnosis of Barrett's esophagus or esophageal adenocarcinoma that were admitted and diagnosed in Modarres Hospital, Tehran, Iran from 2008 to 2011 were reevaluated by two pathologists. Approval from the hospital's ethics committee was obtained. In doing so, approval from the hospital's ethics committee was obtained. In all cases, the previous pathology reports were confirmed. The cases included Barrett's esophagus with no dysplasia, with low-grade dysplasia, those with high-grade dysplasia, and esophageal adenocarcinoma.

Immunohistochemistry (IHC) was performed on all 76 cases using mouse monoclonal anti-IMP3 antibody with code M3626 (DAKO manufactured, Denmark). Positive control was prepared using colorectal adenocarcinoma specimens (+4 positivity with cytoplasmic staining) according to the manufacturer's instruction. The slides with no application of primary antibodies were used as negative control.

Two pathologists analyzed the IHC slides and scored the results from 0 to 4+. The data were analyzed by chi-square test and Spearman correlation test using SPSS 10 software (IBM Company, USA). P values less than 0.05 were considered significant.

RESULTS

In this study, the 76 cases were as follows: 12 cases of poorly differentiated adenocarcinoma (PDAC), 15 of moderately differentiated adenocarcinoma (MDAC), 11 of dysplasia (one high-grade dysplasia (HGD) and 10 low-grade dysplasia (LGD)), 26 of Barrett esophageal mucosa (BEM) without dysplasia or carcinoma, and 12 of nonneoplastic esophageal mucosa without dysplasia or BEM. For each case, there were two IHC reports, which were identical.

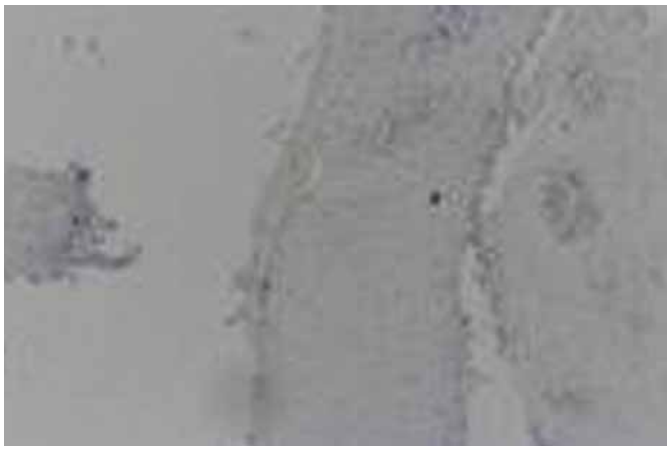

Figure 1. IHC staining: esophageal squamous epithelium shows no staining for IMP3.

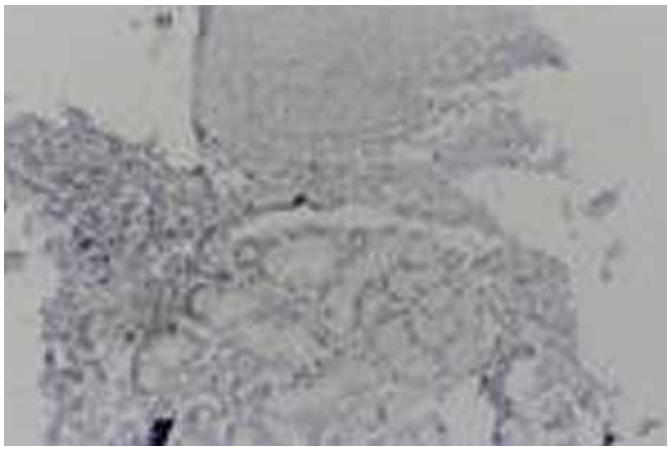

Figure 2. IHC staining: very weak IMP3 positivity in just 11.5% of Barrett's esophagus.

Our results showed that IMP3 was positive in all (100%) cases of esophageal adenocarcinoma (MD and PD) and Barrett's esophagus with high-grade dysplasia.

Just 30% of Barrett's esophagus (BEM) with low-grade dysplasia showed positivity. BEM without dysplasia was positive in only 11.5% of cases. However, 100% of the nonneoplastic esophageal mucosas were IMP3-negative.

Moreover, 91% of PDAC (11 of 12) and 86% of MDAC (13 of 15) and high-grade dysplasia cases showed higher IMP3 staining intensity (3+ to 4+) compared with low-grade dysplasia (1+ positivity). These data showed a significant relationship ($p<.001$) between staining intensity and the presence of adenocarcinoma (Figure 1-6).

DISCUSSION

The results showed that all esophageal adenocarcinomas and HGDs as well as 30% of LGDs were positive for IMP3, whereas all cases of normal esophageal mucosa were negative; All adenocarcinomas have statistically significant higher intensity (3+ and 4+) for IMP3 staining compared with mild dysplasia and normal esophageal mucosa ($p<.001$). A study by Feng et al. (16)

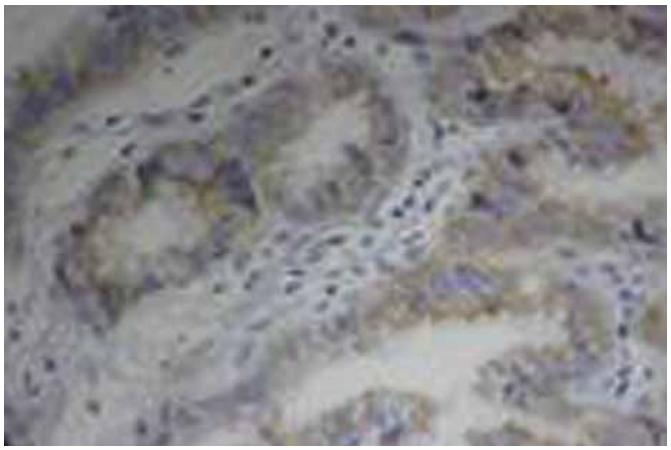

Figure 3. IHC staining: mild IMP3 positivity in Barrett's esophagus with low-grade dysplasia.

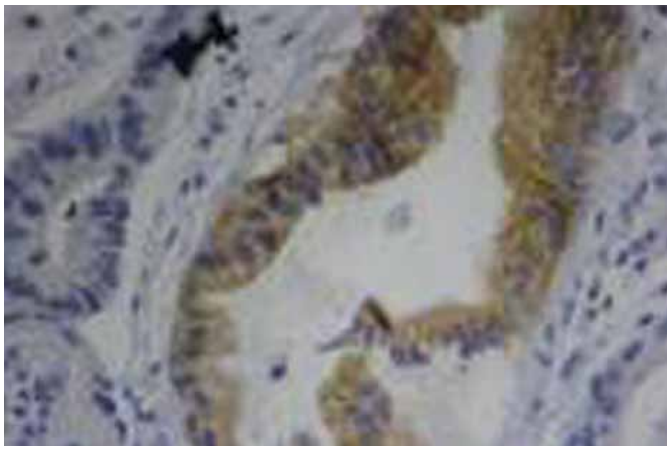

Figure 4. IHC staining: intense IMP3 positivity in Barrett's esophagus with high-grade dysplasia.

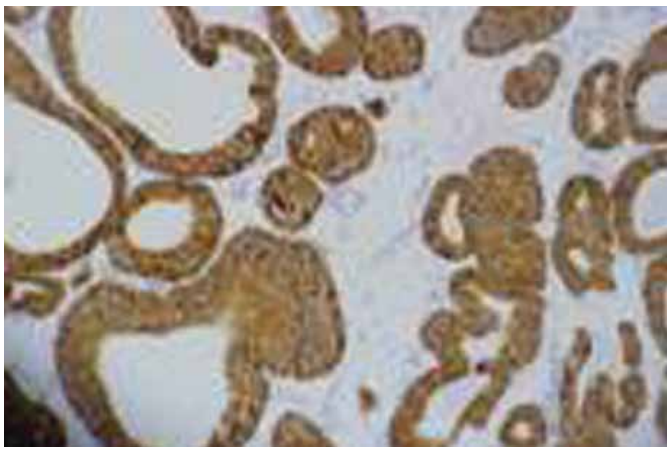

Figure 5. IHC staining: intense IMP3 positivity in moderately differentiated esophageal adenocarcinoma.

in 2010 showed 70% IMP3 intense positivity for adenocarcinoma of the esophagus and 25% IMP3 weak positivity for mild dysplasia. The findings of the study of Lu et al. (5) in 2009 identified 94%, 94%, 14%, and 7% IMP3 positivity in esophageal adenocarcinoma, HGD, LGD, and Barrett's esophagus, respectively. In the studies mentioned above, there was no IMP3 positivity in benign squamous epithelium. Our findings support the suggestion of two recent studies (5,16) regarding the application of IMP3 marker as a very useful test to differentiate between adenocarcinoma and high-grade dysplasia versus low-grade dysplasia or normal esophageal tissue.

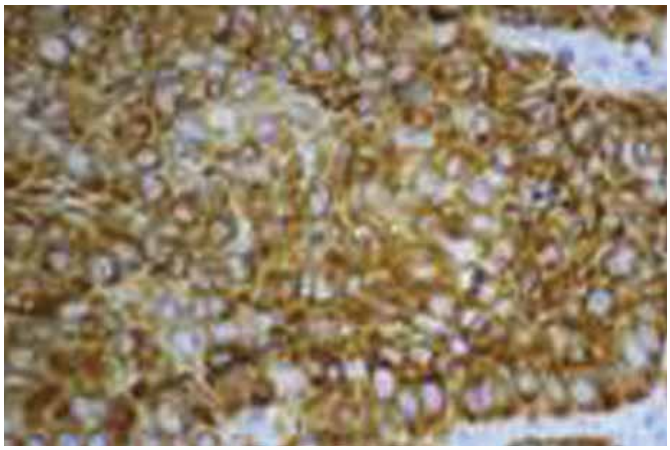

Figure 6. IHC staining: intense IMP3 positivity in poorly differentiated esophageal adenocarcinoma.

Our findings were in line with many other studies; The intense and significantly higher IMP3 positivity in esophageal adenocarcinoma and low rate and weak IMP3 positivity in low-grade dysplasia and Barrett's esophagus could, definitely, suggest IMP3 oncofetal protein as a useful marker for the diagnosis of adenocarcinoma in challenging cases of Barrett's esophagus with dysplasia to choose the best therapeutic option for patients. Test validation with a larger sample, however, is required.

Ethics Committee Approval: Ethics committee approval was received for this study.

Informed Consent: N/A.

Peer-review: Externally peer-reviewed.

Author contributions: Concept - B.K.; Design - B.K., D.A.; Supervision - B.K., S.A.M.; Resource - D.A.; Materials - D.A.; Data Collection&/or Processing - D.A., A.E.; Analysis&/or Interpretation - B.K., D.A., A.E.; Literature Search - D.A., A.E.; Writing - A.E.; Critical Reviews - A.E., B.K., S.A.M.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received financial support from Shahidbeheshti University of Medical Sciences, School of Medicine, Tehran, Iran.

REFERENCES

1. Enzinger PC, Mayer RJ. Esophageal cancer. N Engl J Med 2003; 349: 2241-52. **[CrossRef]**
2. Montgomery E, Bronner MP, Goldblum JR, et al. Reproducibility of the diagnosis of dysplasia in Barrett esophagus: a reaffirmation.Hum Pathol 2001; 32: 368-78. **[CrossRef]**
3. Montgomery E, Goldblum JR, Greenson JK, et al. Dysplasia as a predictive marker for invasive carcinoma in Barrett's esophagus: A follow-up study based on 138 cases from a diagnostic variability study. Hum Pathol 2001; 32: 379-88. **[CrossRef]**
4. Rice TW, Falk GW, Achkar E, et al. Surgical management of highgrade dysplasia in Barrett's esophagus. Am J Gastroenterol 1993; 88: 1832-6.

5. Lu, D., Poon am Vohra, Peigou G. Chu, et al. An oncofetal protein IMP3: A new molecular marker for the detection of esophageal adenocarcinoma and high-grade dysplasia. Am J Surg Pathol 2009; 33: 521-5. **[CrossRef]**
6. Liao B, Hu Y, Herrick DJ, et al. The RNA-binding protein IMP-3 is a translational activator of insulin-like growth factor II leader-3 mRNA during proliferation of human K562 leukemia cells. J Biol Chem 2005; 280: 18517-24. **[CrossRef]**
7. Vikesaa J, Hansen TV, Jonson L, et al. RNA-binding IMPs promote cell adhesion and invadopodia formation. EMBO J 2006; 25: 1456-68. **[CrossRef]**
8. Mueller-Pillasch F, Pohl B, Wilda M, et al. Expression of the highly conserved RNA binding protein KOC in embryogenesis. Mech Dev 1999; 88: 95-9. **[CrossRef]**
9. Nielsen J, Christiansen J, Lykke-Andersen J, et al. A family of insulin-like growth factor II mRNA-binding proteins represses translation in late development. Mol Cell Biol 1999; 19: 1262-70.
10. Nielsen FC, Nielsen J, Christiansen J. A family of IGF-II mRNA binding proteins (IMP) involved in RNA trafficking. Scand J Clin Lab Invest 2001; 61: 93-9. **[CrossRef]**
11. Li C, Rock KL, Woda BA, et al. IMP3 is a novel biomarker for adenocarcinoma in situ of the uterine cervix: an immunohistochemical study in comparison with p16(INK4a) expression. Mod Pathol 2007; 20: 242-7. **[CrossRef]**
12. Mueller-Pillasch F, Lacher U, Wallrapp C, et al. Cloning of a gene highly overexpressed in cancer coding for a novel KH-domain containing protein. Oncogene 1997; 14: 2729-33. **[CrossRef]**
13. Wang T, Fan L, Watanabe Y, et al. L523S, an RNA-binding protein as a potential therapeutic target for lung cancer. Br J Cancer 2003; 88: 887-94. **[CrossRef]**
14. Yantiss RK, Woda BA, Fanger GR, et al. KOC (K homology domain containing protein overexpressed in cancer): A novel molecular marker that distinguishes between benign and malignant lesions of the pancreas. Am J Surg Pathol 2005; 29: 188-95. **[CrossRef]**
15. Zheng W, Yi X, Fadare O, et al. The oncofetal protein IMP3: a novel biomarker for endometrial serous carcinoma. Am J Surg Pathol 2008; 32: 304-15. **[CrossRef]**
16. Feng W, Zhou Z, Peters JH, 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; 135: 1024-31. **[CrossRef]**