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Original Article

Immunohistochemistry in the diagnosis of dysplasia in chronic inflammatory bowel disease colorectal polyps

Ehsen B. Brahim [a,*], Ali Mrabet [b], Raja Jouini [a], Wafa Koubaa [a], Rimel B. Sidhom [a], Hela Elloumi [c], Aschraf Chadli [a]

[a] Department of Pathology, Habib Thameur Hospital, Faculty of Medicine, University of Tunis El Manar, Tunis, Tunisia
[b] Department of Epidemiology and Public Health, Faculty of Medicine of Tunis, University of Tunis El Manar, Tunisia
[c] Department of Gastroenterology, Habib Thameur Hospital, Faculty of Medicine of Tunis, University of Tunis El Manar, Tunis, Tunisia

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ABSTRACT

Background and study aims: Development of cancer is the most significant complication in inflammatory bowel disease (IBD). Distinguishing true dysplasia from reactive atypia in polyps is difficult, leading sometimes to the unsatisfactory diagnosis of "indefinite for dysplasia". Therefore, there is a need for the development of markers that can help improve diagnosis.
We evaluated the diagnostic value of the expression of AMACR, Ki67 and p53 by immunohistochemistry in the diagnosis of dysplasia in polyps developed on IBD.
Patients and methods: Forty colorectal polyps in IBD were studied. These had been diagnosed over a period of 11 years. Dysplasia was classified according to the Vienna Classification (version 2000). Immunohistochemistry was performed using anti-AMACR, anti-Ki67 and anti-p53 antibodies.
Results: Polyps were classified as follows: 21 negative for dysplasia (ND), 10 indefinite for dysplasia (IFD), 6 low-grade dysplasia (LGD), 1 high-grade dysplasia (HGD) and 2 adenocarcinomas (ACA).
AMACR positivity was observed in all polyps with HGD and ACA, 5 of the 6 LGD polyps and 3 of the 10 IFD ($p = 0.007$).
p53 immunostaining showed nuclear staining in the basal part of the crypts in 8 of the 10 IFD lesions. In ACA and HGD polyps, p53 positivity was typically observed in all epithelial cell layers ($p = 0.004$).
ACA and HGD showed diffuse and scattered staining of Ki67 along the full length of the crypts. Five lesions with LGD had extension of Ki-67 positive cells up to and into the surface epithelium. Ki67 staining in all IFD lesions was restricted to the basal third of the crypt ($p < 0.001$).
By combining the three markers, a relationship with dysplasia was statistically significant ($p < 0.001$). Sensitivity ranged from 66.7% to 88.9% and specificity from 71.4% to 100%.
The positive predictive value (PPV) for detecting dysplasia using these different antibodies ranged from 66.7% to 100% and the negative predictive value (NPV) for excluding dysplasia ranged from 85.7% to 93.3%.
Conclusions: The high degree of sensitivity and specificity of AMACR, p53 and Ki67 for dysplasia in IBD suggests that these antibodies, when combined, may be useful to detect neoplastic epithelium in this condition.

Introduction

Extensive and long standing inflammatory bowel disease (IBD) is well known to predispose patients to the development of colorectal carcinoma. This cancer is preceded by dysplasia, sometimes with polypoid appearance. On the basis of morphologic criteria, dysplasia is classified into 1 of 5 categories according to the Vienna classification: Negative for dysplasia (ND), indefinite for dysplasia (IFD), low-grade dysplasia (LGD), high-grade dysplasia (HGD) and adenocarcinoma (ACA) [1].

Differentiating between regenerative changes and true dysplasia in the IFD category is often difficult even in absence of active inflammation and even for experienced pathologists [2]. A search for more objective methods to identify early neoplastic changes in the epithelium therefore seems warranted.

* Corresponding author at: Department of Pathology, Habib Thameur Hospital, 8 Ali Ben Ayed Avenue, 1008, Montfleury, Tunis, Tunisia. Fax: +216 71135000.
E-mail address: benbrahim_ehsen@yahoo.fr (E. B. Brahim).

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Immunohistochemistry would solve this problem by revealing an expression of potential marker of neoplastic cells, alpha-methylacyl coenzyme A racemase (AMACR) [3], the proliferation index (Ki-67) [4] and of the tumour suppressor protein, p53 [3].

The aim of our study was to evaluate the diagnostic value of the expression of AMACR, Ki67 and p53 in the diagnosis of dysplasia in IBD.

Patients and methods

Our study included all colorectal polyps from patients with IBD selected by a retrospective search through the files of Department of Pathology of Habib Thameur Hospital in Tunis, throughout a period of 11 years between 2001 and 2011.

It included both biopsy and polyps removed from colectomy specimens. Fragmented and / or crushed polyps, and those which are difficult to analyse histologically were excluded. Clinicopathological parameters including the age and gender of the patients, the type of IBD, the duration of disease progression, the site, the type and the size of polyps were obtained from the available reports.

The formalin-fixed paraffin-embedded tissue specimens were selected. Haematoxylin and eosin sections were reassessed and evaluated with regard to inflammation and dysplasia. Dysplasia was classified using the architectural characteristics of polyps, the cytologic and nuclear appearance of mitosis. Inflammation was classified as mild, moderate, or severe. As a result of this study, polyps were classified according to the Vienna Classification (version 2000) which is meant to be applied throughout the entire gastrointestinal tract [1].

Immunohistochemistry

Immunohistochemistry (IHC) was performed by the BOND-MAX System (Leica) on indefinite for dysplasia polyps, dysplastic and a sample from non-dysplastic polyps. The antibodies were the anti-AMACR (rabbit monoclonal anti-human, clone 13H4 Dako), anti-Ki-67 (monoclonal Mouse Anti-Human, clone MIB1 Dako) and anti-p53 (antibody Monoclonal Rabbit Anti-Human, 318-6-11 clone Dako). Positive controls were normal colonic mucosa for the Ki-67 and p53 and prostatic adenocarcinoma for the AMACR.

Considering cytoplasmic staining for AMACR for each tissue sample, the staining intensity (SI) and percentage (P) of positive cells were estimated, and an immunoreactivity score (IRS = P × SI) was used: IRS (0–12 points) = P (0–9%, 1 point; 10–49%, 2 points; 50, 80%, 3 points; >80%, 4 points) × SI (negative, 0; weak, 1 point; moderate, 2 points; strong, 3 points). Only IHC staining with an IRS above 2 was considered positive. Nuclear staining for p53 and Ki67 was evaluated according to the seat of the signal in the crypt: the full height (positive for dysplasia), 1/3 or 2/3 lower (negative for dysplasia) [3,4].

Statistical analysis

Data were entered and analysed by SPSS software (version 18) and Epi Info software package (version 3.5.1), the statistical analysis used the chi-square, and in cases of non validity of this test, Fisher's test is used to compare percentages. Student's test was used for comparison of average. A p value <0.05 was considered statistically significant. In all cases, the diagnostic value of the immunostaining was investigated by calculating the sensitivity, the specificity and the positive and negative predictive values of AMACR, p53 and Ki67 for detecting dysplasia.

Results

Forty polyps were removed in 21 patients with IBD of mean age of 47.5 ± 14.9 years (range: 23–75 years). The male:female ratio was 1.62. Amongst the polyps, 34 were from 16 patients with ulcerative colitis and 6 were obtained from 5 patients with Crohn's disease. The duration of disease progression varied from several days to 31 years. It was less than 10 years in 55% of cases and between 10 and 25 years in 32% of cases. The median size of colorectal polyps was 7 mm (range 3–20 mm). It was less than 10 mm in 67% of cases. Polyps were located in the distal colon in 68% of cases. They were pedunculated in 35% of cases, sessile in 30% of cases and flat in 3% of cases. In the remaining cases, the polyp type was not specified.

Histological features

The overall architecture of the polyps was tubulous in 82% of cases and tubulo-villous in 18% of cases.

The cells showed dedifferentiation in appearance in 47% of cases. Nuclei were slightly increased in size in 80% of polyps. Chromatin was homogeneous and sparse in 80% of cases. Nuclear pseudostratification was absent in 60% of cases. In 32% of polyps, it was present in the lower half. Nucleolus was unique and small in 21 cases (52%), multiple and prominent in the remaining cases. Mitoses were present in the basal crypt in 31 cases (77%). The inflammation was severe in 55% of cases.

According to the Vienna Classification, 77.5% of polyps were of category 1 and 2 (Table 1) (Figs. 1 and 2). Low grade neoplasia was observed in 6 cases (3 developed in ulcerative colitis and the others in Crohn's disease). All high grade and invasive neoplasia (3 cases) were present in ulcerative colitis patients.

There was a significant correlation between increasing degrees of dysplasia and the proportion of specimens with tubulo-villous architecture, nuclear pseudostratification, nuclear hyperchromatism, mitosis, loss of cell polarity and low intensity of the inflammation (p ranged from 0.001 to 0.05) (Table 2).

Immunohistochemistry

AMACR immunostaining was typically cytoplasmic with coarse granularity verified on prostatic carcinoma tissue. In typical cases, AMACR-positive dysplastic areas were sharply demarcated from adjacent non dysplastic AMACR negative epithelium.

AMACR positivity was observed in 12 cases (52%): all polyps with HGD and ACA (score >2), 5 of the 6 polyps with LGD and 3 of the 10 IFD (p = 0.007) (Table 3) (Fig. 3).

Table 1
Classification of polyps according to the Vienna classification.

Vienna classification		Number of cases
1: Negative for neoplasia/dysplasia		21
2: Indefinite for neoplasia/dysplasia		10
3: Non-invasive low grade neoplasia (low grade adenoma/dysplasia)		6
4: Non-invasive high grade neoplasia	4.1: High grade adenoma/dysplasia	1
	4.2: Non-invasive carcinoma (carcinoma in situ)	0
	4.3: Suspicion of invasive carcinoma	–
5: Invasive neoplasia	5.1: Intramucosal carcinoma	2
	5.2: Submucosal carcinoma or beyond	–
Total		40

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