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## Original Article

# Clinical-pathological and p53 protein expression study in dysplasia associated with ulcerative colitis<sup>☆,☆☆</sup>



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## ABSTRACT

**Background:** The association between ulcerative colitis and adenocarcinoma determined strategies for patient follow-up and early detection of dysplastic and neoplastic lesions.

**Aims:** To analyze the incidence of dysplasia in patients with ulcerative colitis, comparing clinical data of patients with and without dysplasia and check immunohistochemical expression of p53 protein in dysplasias.

**Materials and methods:** We analyzed biopsy samples and clinical data of 124 patients with ulcerative colitis at Hospital de Clínicas, Federal University of Paraná, Curitiba, Brazil.

**Results:** Dysplasia incidence was low (9.67%) and all cases with low-grade dysplasia. Patients clinical data comparison with and without dysplasia did not show significant statistical differences with regard to the race, age at the start of the disease, age at last biopsy, duration and anatomic extent of ulcerative colitis. Significant difference was found between males and females with predominance of males (58.34%) for dysplasia. Seventeenth biopsy samples of 12 patients with dysplasia, 5 (29.4%) were p53 positive.

**Conclusions:** From these results it is concluded that the incidence of dysplasia was low, higher in males and there was positivity of p53 protein in dysplasia.

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## Estudo clínico-patológico e da expressão da proteína p53 nas displasias associadas à retocolite ulcerativa

### R E S U M O

#### Palavras-chave:

Retocolite ulcerativa

Displasia

Proteína p53

**Racional:** A associação entre retocolite ulcerativa e adenocarcinoma determinou estratégias para seguimento dos pacientes e detecção precoce das lesões displásicas e neoplásicas.

**Objetivos:** Analisar a incidência de displasia nos pacientes com retocolite ulcerativa, comparar dados clínicos dos pacientes com e sem displasia e verificar a expressão imunoistoquímica da proteína p53 nas displasias.

**Material e Métodos:** Foram estudados os exames anatomopatológicos e dados clínicos de 124 pacientes com e sem displasia, portadores de retocolite ulcerativa no Hospital de Clínicas da Universidade Federal do Paraná.

**Resultados:** A incidência de displasia foi de 9,67% e todos os casos foram de displasia de baixo grau. Na comparação dos dados clínicos dos pacientes com e sem displasia não houve diferença estatisticamente significativa com relação à cor, idade no início da doença, idade na última biópsia, extensão da doença e tempo de evolução da doença. Houve diferença estatística com predomínio de pacientes do sexo masculino (58,34%) em relação ao feminino para displasia. Dos 17 exames avaliados de 12 pacientes com displasia, em 5 exames (29,4%) a expressão da proteína p53 foi positiva.

**Conclusões:** Desses resultados conclui-se que a incidência de displasia foi baixa, maior no sexo masculino e houve positividade da proteína p53 nas displasias.

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## Introduction

Ulcerative colitis (UC) falls within the group of chronic inflammatory bowel diseases of unknown cause. With outbreaks of remission and exacerbation, UC has an incidence of 3–20 new cases per year per 100,000 inhabitants.<sup>1</sup> UC with over 8 years of evolution represents an important risk factor for the development of dysplasia and subsequent development of adenocarcinoma.<sup>2,3</sup> The increased replacement (turnover) of intestinal epithelial cells damaged by chronic inflammation is considered a risk factor for the development of dysplasia and adenocarcinoma in patients with long progression.<sup>4</sup> The risk of developing colorectal cancer varies from 5.5% to 13.5%, and risk factors include the extent of disease and the progression time.<sup>5</sup> Monitoring and follow-up of patients are performed by means of colonoscopies and serial biopsies, but these cover less than 0.05% of the colic surface.<sup>6</sup> Genetic alterations have been studied to characterize dysplastic lesions and for an early detection of carcinogenesis.<sup>5</sup> Of all the markers studied, the expression of p53 protein has demonstrated significant correlation with duration of disease, as a risk factor of developing colorectal cancer associated with UC.<sup>7</sup>

Although the expression of p53 is a late event in colorectal carcinogenesis, it is considered an early event in the onset of dysplasia associated with UC.<sup>8</sup> The onset of expression of p53, by an abnormal protein, in the nucleus of epithelial cells of the intestinal mucosa occurs even without the presence of dysplasia and precedes the development of dysplasia and colorectal cancer.<sup>9</sup>

## Material

A retrospective study was performed since 2004 in 124 patients with clinical and endoscopic diagnosis of UC followed at the inflammatory bowel diseases outpatient service, Hospital de Clínicas, Universidade Federal do Paraná, and who underwent pathological examination of the colonic mucosa. This study was approved by the Research Ethics Committee, Hospital de Clínicas, with CEP/HC 732.151/2003-10 protocol. In the database of the Department of Pathology of the same hospital, all patients and their respective exams were reviewed. A clinical data survey was conducted for age, gender, race, progression time of the disease until the time of examination, and extent of disease. Pathological lab workup included endoscopic biopsies and surgical specimens. The pathological reports in which were mentioned “indefinite for dysplasia (IND)”, “presence of dysplasia” or “adenocarcinoma” were selected.

In cases with dysplasia and in two cases of colic adenocarcinoma, immunohistochemical reactions for nuclear p53 protein were performed.

## Method

This study included 124 patients with ulcerative colitis, followed-up by biopsies, and the time elapsed between the onset of the disease (ulcerative colitis) and the time of identification of dysplasia was registered. Patients without dysplasia had recorded their follow-up to the last biopsy. The study variables were: race, gender, age at onset, age at last biopsy and

site of disease. The explanatory variables were dichotomized by testing the null hypothesis of distributions of dysplasia-free time (time elapsed from disease progression to diagnosis of dysplasia, or to the last biopsy) equal in both classifications versus the alternative hypothesis of different distributions of dysplasia-free time. The statistical test used was considered the Cox-Mantel test. The evaluation of the progression time of the groups with and without dysplasia was performed using the non-parametric Mann-Whitney test.  $p$  values  $<0.05$  were considered statistically significant.

The indefinite cases for dysplasia and those with dysplasia were reviewed with the use of a discriminant classification to characterize dysplasia as atypia or repair.<sup>10</sup> After reviewing the pathological examination reports, the cases were classified as "with dysplasia", "without dysplasia" or "indefinite for dysplasia". The paraffin-impregnated material was cut in 4-mm thick-sections. Preparation of immunohistochemistry slides and their deparaffinization and hydration with xylene and decreasing concentrations of ethanol were performed, as well as antigen retrieval with citrate buffer. Mouse monoclonal antibody anti-p53 protein in previously paraffined material was used. This a monoclonal antibody of immunoglobulin G2 class, that binds to both the wild-type and to the mutated protein. The antibody was used at a dilution of 1:100. A reaction using the streptavidin-biotin-peroxidase complex, using colon adenocarcinoma p53-positive as positive control, was performed. A counting of brown-stained nuclei by means of an image analyzer (Image-Pro Plus® – The proven solution™ Version 4.5.1.23 for Windows 98/NT/Me/2000/XP Copyright©1993–2002 Media Cybernetics Inc.) was performed. Positive cells with nuclei strongly brown-stained were counted by the program by color difference versus nuclei in blue (negative cells). Lesions with less than 10% positive cells were negative for p53; between 1% and 25%, positive +, between 26% and 50%, positive ++, greater than 50%, positive +++.<sup>11</sup>

## Results

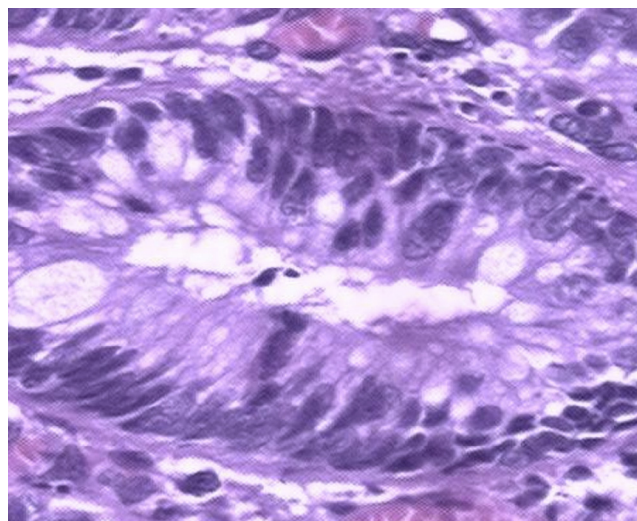
Thirty-eight (30.65%) male and 86 (69.35%) female patients were recruited. Nine (7.26%) were black or mestizo patients and 115 (92.74%) were Caucasians. Clinical data (age, age at onset of disease, disease duration, and number of biopsies) are shown in Table 1.

Regarding the extent of disease, 78 (62.90%) patients had only involvement of left colon and 46 (37.09%) exhibited pancolitis.

Of the 124 patients, in 12 (9.67%) we found 20 pathological examinations diagnosed with low-grade dysplasia (LGD) or IND, 17 with LGD (Fig. 1) and 3 with IND. No examination with high-grade dysplasia was found.

Two female patients with adenocarcinoma had concurrent dysplasia. One patient developed adenocarcinoma in hepatic colonic flexure, after 8 years of disease progression, with stage T2 N0 M0; Astler-Coller B1; Dukes A. Another patient presented with rectal adenocarcinoma with signet ring cells areas after 12 years of disease progression, with stage T2 N1 M0; Collier Astler-C1-2; Dukes C.

Of the 17 tests evaluated (from 12 patients with dysplasia), in 5 (29.4%) the result was p53-positive (Fig. 2) and in 12 (70.6%)

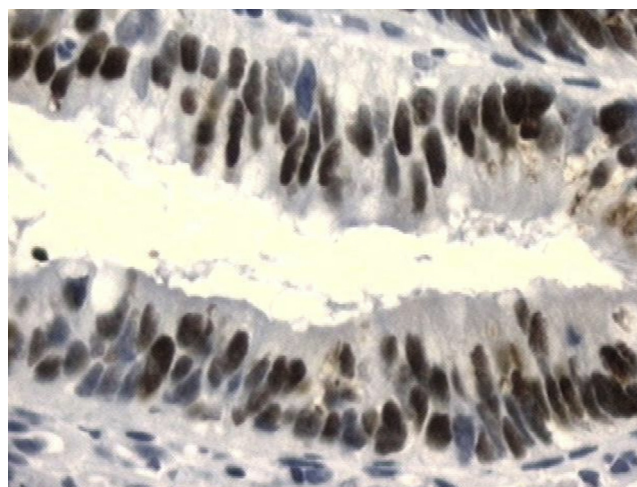


**Fig. 1 – Area of dysplasia. Note: Dysplastic nuclei dark blue-stained. HE 400×.**

**Source: Department of Pathology, UFPR.**

was p53-negative. An examination with positivity + (24% of cells), 1 with positivity ++ (39% of cells) and 3 with positivity +++ (> 50% of cells). Two cases of dysplasia were associated with a mass (DALM), with a positivity +++. The female patient with a rectal tumor was p53-positive +++ and the other patient had her tumor in right colon flexure was p53-negative.

In the statistical analysis with dichotomized variables, dysplasia-free time did not correlate with race ( $p = 0.9467$ ) nor with the extent of the disease ( $p = 0.1551$ ). The age before or after 15 years at disease onset and patient age  $<40$  years or  $\geq 40$  years at the last biopsy did not correlate with dysplasia-free time ( $p = 0.8882$  and  $p = 0.7920$  respectively). In Fig. 3, it appears that males had higher incidence of dysplasia in relation to dysplasia-free time ( $p = 0.0242$ ). The progression of disease in patients with and without dysplasia showed no difference ( $p = 0.8055$ ).



**Fig. 2 – Dysplasia p53-positive. Note: Dysplastic nuclei brown-stained brown before marking for cell count.**

**Immunohistochemistry for p53, 400×.**  
**Source: Department of Pathology, UFPR.**



**Table 1 – Clinical data.**

| Variable                            | n   | Minimum | Maximum | Median | Mean  | Standard deviation |
|-------------------------------------|-----|---------|---------|--------|-------|--------------------|
| Age at last biopsy                  | 124 | 10.00   | 81.00   | 39.00  | 39.71 | 13.48              |
| Age at the beginning of the disease | 124 | 3.00    | 79.00   | 32.50  | 33.90 | 13.56              |
| Disease duration at last biopsy     | 124 | 1.00    | 36.00   | 5.00   | 6.81  | 5.97               |
| Nr. of biopsies                     | 124 | 1.00    | 10.00   | 3.00   | 3.60  | 2.17               |

Source: The Author.

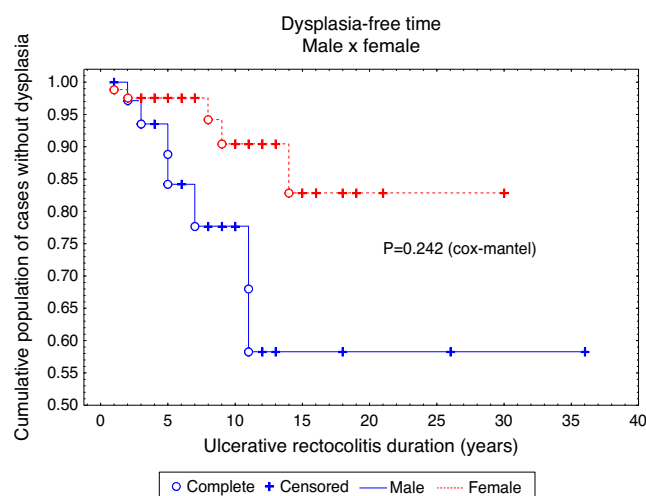
## Discussion

The medical attention is focused on the monitoring of patients with UC, and the dysplasia found in pathological examinations became the most significant predictive factor of cancer.<sup>12</sup> One study found 13.1% of dysplasia in 590 patients undergoing proctocolectomy and determined that the positive predictive value for colonic cancer of a preoperative finding of dysplasia of any grade is 50%.<sup>13</sup>

Low-grade dysplasia can be detected in 17% of patients during follow-up with endoscopic biopsies.<sup>14</sup> In this study, LGD was detected in 12 patients (9.67%). Higher incidence of dysplasia in patients with concomitant adenocarcinoma is related; in our sample, two patients who had adenocarcinoma also had LGD.<sup>13</sup>

Some authors found a five -fold higher incidence of cancer in patients with pancolitis than in patients with left side colitis.<sup>15</sup> Another study found 18 patients with LGD; 17 of them had pancolitis (94%).<sup>16</sup> In this study, disagreeing with the findings of the authors above mentioned, of the 12 patients with dysplasia, seven (58.30%) had pancolitis and five (41.70%) had left colitis. The extent of disease did not influence the occurrence of dysplasia with statistical significance ( $p=0.1551$ ).

The time of disease progression is cited as a risk factor for colorectal cancer.<sup>15,17</sup> In our sample there was no agreement with this observation (Table 2). The comparison of groups with and without dysplasia showed no significant difference with regard to time of disease progression ( $p=0.8055$ ).



**Fig. 3 – Dysplasia-free time and gender.**  
Source: The author.

**Table 2 – Frequency distribution of disease duration at last biopsy.**

| Follow-up (years) | Without dysplasia |            | With dysplasia |            |
|-------------------|-------------------|------------|----------------|------------|
|                   | Frequency         | Percentage | Frequency      | Percentage |
| <5                | 50                | 44.64      | 4              | 33.33      |
| 5–9.9             | 35                | 31.25      | 5              | 41.67      |
| 10–14.9           | 16                | 14.29      | 3              | 25.00      |
| 15–19.9           | 7                 | 6.25       | –              | –          |
| ≥20               | 4                 | 3.57       | –              | –          |
| Total             | 112               | 100.00     | 12             | 100.00     |

Source: The Author.

In this study there was a predominance of white patients, which is also observed in the United States.<sup>18</sup> In this series, the group with dysplasia had 11 white patients and one black patient. However, when analyzing the influence of race and free dysplasia-free time, there was no significant difference ( $p=0.9467$ ).

The expression of the nuclear protein p53 has been studied as a marker for dysplasia.<sup>7</sup> Although the mutation of the p53 gene is considered to be a late event in the carcinogenesis of sporadic colonic cancers, in the cancer associated with colitis, the mutation appears as an earlier event.<sup>7,9</sup> In this sample, five exams with LGD were p53-positive (29.4%). This finding is consistent with the literature, where LGD positivity indexes of 25–50% are found.<sup>9,19</sup>

In this study, the tests with DALM showed dense staining (+++) for p53. The presence of a DALM injury correlates in 43% of cases with a concurrent finding of adenocarcinoma.<sup>2</sup> A strong expression of p53 is found in DALM and not in adenoma.<sup>20</sup>

In the United States, women are more affected by UC than men.<sup>18</sup> Accordingly, we found a female predominance in patients with ulcerative colitis (69.35%). The European epidemiological study was discordant from the USA study and found higher incidence in men in the age groups above 35 years.<sup>21</sup> In our sample, we found more men in the group with dysplasia, and the statistical analysis showed a significant difference ( $p=0.0242$ ). Some studies have found a higher proportion of cases with dysplasia and colorectal cancer in males, but there was no statistical assessment of the significance of this difference in relation to gender.<sup>22–24</sup>

## Conclusions

The incidence of dysplasia was lower than that in the literature. Patients with colorectal carcinoma had concomitant

dysplasia. The incidence of dysplasia was significantly higher in males. The expression of p53 protein in dysplasias was consistent with the literature.

### Conflicts of interest

The authors declare no conflicts of interest.

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