Original Article

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Pathology Section

Role of Immunohistochemical Expression of p53 in Intestinal Epithelial Cells to Detect Dysplasia in Patients with Inflammatory Bowel Disease

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ABSTRACT

Introduction: Inflammatory Bowel Disease (IBD) has a genetic predisposition, in which patients are more prone to develop colorectal carcinoma. Dysplasia, a precursor of malignancy, can be very difficult to detect based on histopathological features alone. Tumour suppressor gene, p53, is overexpressed in IBD even when there is no histological evidence of dysplasia. Therefore, p53 can be used as a tissue biomarker for routine surveillance to initiate treatment for prevention of carcinoma.

Aim: To identify the diagnostic utility of p53 immunohistochemistry in the detection of dysplasia in patients with IBD.

Materials and Methods: This cross-sectional diagnostic study was conducted in the Department of Pathology at Pushpagiri Institute of Medical Sciences and Research Centre, Tiruvalla, Kerala, India, from June 2016 to June 2018. Total 31 cases of intestinal biopsies in patients with chronic bloody diarrhoea were selected for the study. Immunohistochemical (IHC) staining using anti p53 antibody was done in all the cases to detect the expressed protein. Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) version 20.0. Significance of p53 immunostains was done using Fischer's-exact test.

Results: The age group of patients with IBD ranged from 15-73 years with a bimodal age peak between 20-29 years and 60-69 years. Male predominance was seen among 20 cases while 11 cases were among females. Negative staining for p53 was seen in 28 cases which were negative for dysplasia histopathologically. Only one case which was histopathologically positive for dysplasia and one of the negative cases of dysplasia showed p53 positivity. Another case which was histopathologically positive for dysplasia showed negative staining for p53. Sensitivity of p53 in this study was 50% while the specificity was 96.55%.

Conclusion: The finding of positive p53 expression in dysplasia positive case follows the fact that p53 mutations found in dysplastic mucosa precedes progression to neoplasia. In contrast, positive p53 expression in a case that was negative for dysplasia histopathologically, should be handled cautiously and warrants regular follow-up in order to prevent neoplastic progression. However, diagnosis of dysplasia should not be made in areas of active inflammation as inflammatory changes can lead to regenerative atypia which could result in over interpretation of histological features attributing it to dysplasia.

Keywords: Crohn's disease, Colorectal cancer, Tumour suppressor gene, Ulcerative colitis

INTRODUCTION

Inflammatory Bowel Disease (IBD) is chronic condition due to inappropriate immune activation of the mucosa which is predisposed by environmental, immunoregulatory and genetic factors [1]. The two constituents of IBD are Ulcerative Colitis (UC) and Crohn's Disease (CD). Ulcerative colitis is limited to the colon. CD can affect any segment of the gastrointestinal tract and is transmural with skip lesions. Both of these diseases present in adolescents and young adults with UC being more frequently seen in females. The incidence of IBD is rising especially in Africa, South America and Asia due to improvement in food storage conditions, decreased contamination of food and change in composition of gut microbiome [2]. This phenomenon has been referred to as the "hygiene hypothesis". It is now believed that altered host interactions with intestinal microbiota, intestinal epithelial dysfunction, aberrant mucosal immune responses, and altered composition of the gut microbiome results in IBD [1]. Long standing cases will lead to an increased risk of colorectal cancer which is preceded by dysplasia. Current modality for detection of colorectal dysplasia is extensive biopsy with colonoscopic surveillance to reduce mortality in IBD patients due to colon cancer [2]. However, diagnostic difficulties and uncertainties arise leading to a diagnosis of indefinite for dysplasia in some cases [2].

The p53 is known as the "guardian of genome" as it is the key regulator of cell cycle progression, DNA repair, cellular senescence, and apoptosis [1]. It is a tumour suppressor gene that is most frequently mutated in most of the human cancers with loss of function mutations being the commonest [1]. These mutations are also seen in IBD which predisposes to colorectal cancer. Many studies have shown that overexpression of p53 can help in detecting dysplasia and to predict the progression of colorectal cancer [2-4]. p53 expression can be detected by immunohistochemistry as strong nuclear positivity is associated with neoplasia progression [2]. Other methods for detecting p53 mutation is Deoxyribonucleic Acid (DNA), Complementary DNA (cDNA) sequencing at messenger Ribonucleic Acid (mRNA) level [5], high performance liquid chromatography, Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) and Western Blot method [6].

The objectives of this study were to detect proportion of IHC expression of p53 in IBD with no dysplastic changes histopathologically, describe staining pattern of p53 in intestinal biopsies of IBD and thereby assess validity of IHC expression of p53 in detecting dysplasia in histopathologically negative specimens of IBD.

MATERIALS AND METHODS

This was a cross-sectional diagnostic study done in the Department of Pathology of Pushpagiri Institute of Medical Sciences and Research

Centre, Tiruvalla, Kerala, India, from June 2016 to June 2018. This study was started after getting approval from the Scientific Review Committee and Institutional Ethics Committee (IEC no.-PIMSRC/E1/388A/55/2016) of Pushpagiri Institute of Medical Sciences and Research centre, Tiruvalla. The study sample included all intestinal biopsies in patients with chronic bloody diarrhoea.

Inclusion criteria: Histologically proven cases of IBD were included in the study.

Exclusion criteria: Histologically proven cases of colorectal carcinoma and non specific colitis were excluded from the study.

Sample size calculation: Assuming that 97% of intestinal biopsies with histopathological evidence of dysplasia and 15% of histopathologically negative specimens will have p53 overexpression along with a confidence level of 95%, power 80%, α error 5%, β error 10%, sample size was calculated as 30. Consecutive sampling was done until the desired sample size was achieved.

Procedure

Total 31 cases of histopathologically proven IBD from patients with chronic bloody diarrhoea were selected. Formalin fixed paraffin embedded tissue blocks of intestinal biopsies were taken. Sections of 3µ thickness were cut and mounted on poly-L-lysine coated slides. Immunohistochemistry was performed using anti-p53 antibody (Pathnsitu Biotechnologies Pvt., Ltd., mouse monoclonal antibody p53-BP-53-12) [7]. Immunostaining for p53 was interpreted as positive or negative [7]. Positive staining is defined as moderate to strong nuclear staining in >50% of cells. Negative staining is defined as absent or weak nuclear staining in <50% of cells.

STATISTICAL ANALYSIS

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) version 20.0. The significance of p53 immunostain was analysed using Fischer-exact test. The sensitivity, specificity, positive predictive value, negative predictive value, diagnostic accuracy and likelihood ratio for p53 was calculated.

RESULTS

The study sample comprised of 31 histopathologically proven cases of IBD. The age group of patients with IBD ranged from 15-73 years with a bimodal age peak between 20-29 years and 60-69 years. Distribution of cases had a male predominance with 20 cases among males and 11 cases among females. Total 30 cases belonged to the ulcerative colitis group [Table/Fig-1] whereas one case belonged to the CD group [Table/Fig-1]. Among the UC cases, seven cases belonged to active phase, four cases in chronic phase and three cases in early phase. Rest of the UC cases could not be categorised into any of the phases due to overlapping features.

Type of inflammatory bowel disease	p53 staining	
	Positive	Negative
Ulcerative colitis	2	28
Crohn's disease	0	1

[Table/Fig-1]: Relationship between type of Inflammatory Bowel Disease (IBD) and p53 staining.
p-value=0.501, calculated by Fischer's test

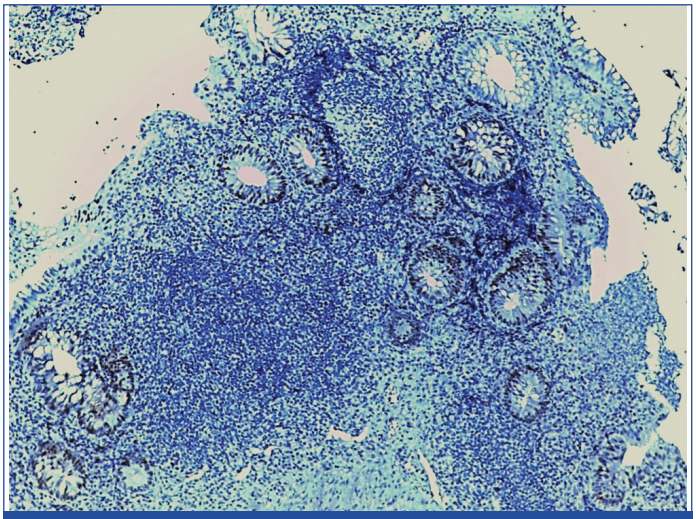
Immunohistochemistry for p53

Out of the 31 cases of IBD, two cases stained positive for p53, one of which was histopathologically negative and the other histopathologically positive for dysplasia, both of which belonged to ulcerative colitis [Table/Fig-2-4]. Another case which was histopathologically positive for dysplasia showed negative staining for p53 [Table/Fig-5].

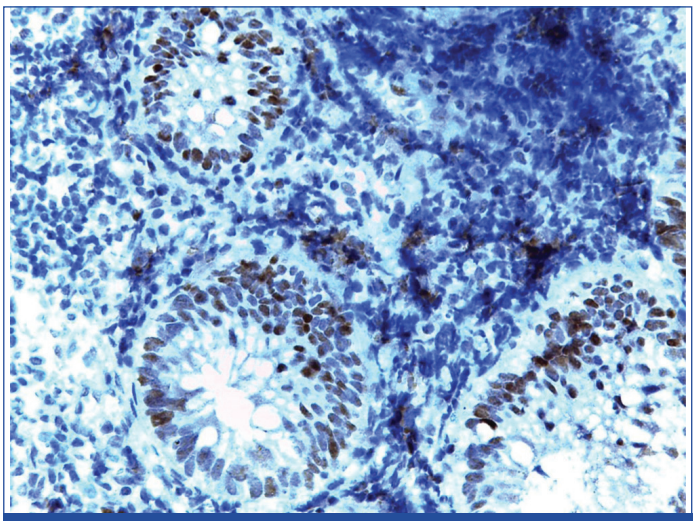
The significance of p53 immunostain was analysed using Fischer-exact test and it was found to be non significant (p-value=0.2694). Sensitivity of p53 in this study was 50% while the specificity was 96.55%. Positive predictive value was 50% and negative predictive value of p53 was 96.55%. Diagnostic accuracy was 93.55%. Positive likelihood ratio of p53 was 14.50. Negative likelihood ratio

p53 staining	Dysplasia positive on histopathology	Dysplasia negative on histopathology	p-value
Positive	1	1	0.2694
Negative	1	28	

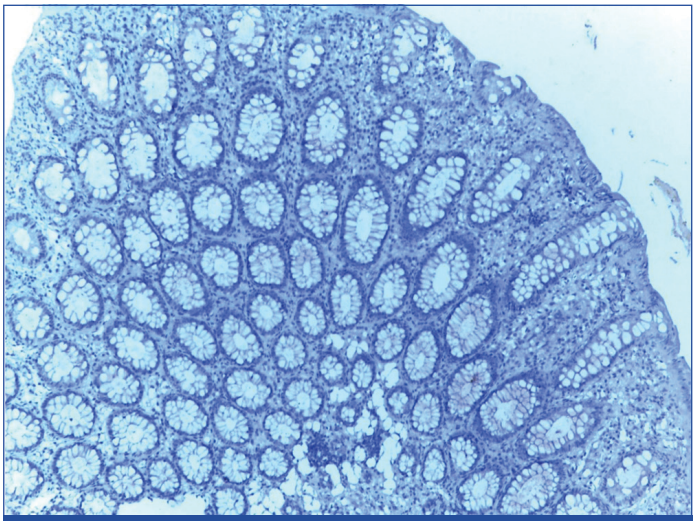
[Table/Fig-2]: Distribution of cases according to p53 staining.
Fisher-exact test used, *p-value <0.05 was considered as statistically significant



[Table/Fig-3]: Positive p53 staining in >50% of cells (10X).
In this photomicrograph, many cells show positivity for p53 immunostaining



[Table/Fig-4]: Positive p53 nuclear staining (40X).
Higher magnification showing the p53 immunostaining in >50% of cells



[Table/Fig-5]: Negative p53 staining (10X).
Fragment of colonic tissue in which p53 immunostain was not taken up by any of the cells making it a negative control

of p53 was 0.52. There was no significant relationship between type of IBD and p53 staining (p-value=0.501) [Table/Fig-1].

DISCUSSION

The IBD carries an increased risk of developing colorectal cancer. Younger age at diagnosis, greater extent and duration of disease, increased severity of inflammation, family history of colorectal cancer and coexisting primary sclerosing cholangitis are the main risk factors for cancer among patients with IBD [3]. The concept of an inflammation-dysplasia-carcinoma sequence provides the basis for current guidelines for the prevention and early detection of cancer in this high risk population. To develop an effective strategy for surveillance and prevention, and to understand the limitations of the current approach to prevention, a meticulous understanding of the definition and natural history of dysplasia in IBD, as well as the challenges associated with detection and interpretation of dysplasia, is highly essential [3].

Fricke H et al., stated that p53 tumour suppressor gene mutations are frequent abnormalities in colorectal cancer especially when associated with UC. p53 mutations can lead to the accumulation of p53 gene product in the cell and trigger an antigen driven humoral response against p53 [8]. p53 mutations have been observed in non dysplastic IBD colonic mucosa adjacent to dysplastic areas [9].

The present study aimed to identify the diagnostic utility of p53 immunostain in the detection of dysplasia in patients with IBD. A total of 31 histopathologically proven cases of IBD were selected for the study. Immunohistochemistry with anti p53 antibodies was done on all cases.

The IBD usually present in teens and early twenties. Most patients present in the second decade of life. However, a smaller, second peak also occurs in the eighth decade of life [10,11]. The age group of the patients in this study ranged from 15-73 years with a bimodal age peak between 20-29 years and 60-69 years. IBD is more common in females with regard to UC. Crohn's disease has a male predominance [10,12]. In this study, majority of the patients were males (64.5%) while the rest were females.

Majority of the cases were UC while only one case belonged to CD though consecutive sampling was done. The paucity of CD cases included in the study could be due to the overlap of histological features between CD and tuberculosis. Subsequently, such cases could not be followed-up for confirmation of diagnosis.

Cases belonging to UC were classified as early, acute and chronic phases of the disease based on histological features. Acute phase is characterised by the presence of neutrophils, cryptitis, and crypt abscesses [10,13,14]. Chronic phase is defined by crypt architectural distortion, mucin depletion, basal plasmacytosis, and paneth cell metaplasia [10,13-15]. Early phase of UC may not show all these microscopic features but focal basal plasmacytosis can be seen [16].

The p53 expression was absent in 29 cases, which were negative for dysplasia histopathologically [Table/Fig-3] while positive staining for p53 was found in two cases [Table/Fig-4,5]. Only one case which was histopathologically positive for dysplasia showed p53 positivity. One of the positive cases of dysplasia showed negative staining for p53.

Sensitivity of p53 in this study was 50% while the specificity was 96.55%. This is in concordance with the study done by Kinra SLP et al., where the sensitivity of p53 was 71.4% and specificity was 90.8% [7]. A study done by Nathanson JW et al., had eight cases out of fourteen showing p53 positivity. Seven of the eight p53 positive cases had dysplasia [11]. One of the cases in the present study which was positive for dysplasia histopathologically showed positive p53 expression.

In a study done by Hamouda HE et al., serum p53 antibodies showed significantly increased levels in UC patients, with significantly higher levels in patients with dysplasia which is associated with overexpression of p53 protein or mutation of p53 gene [17]. Similarly, in present study, both the cases positive for p53 expression were patients with a diagnosis of UC.

The finding of positive p53 expression in dysplasia positive case follows the fact that p53 mutations found in dysplastic mucosa precedes progression to neoplasia [18]. In contrast, positive p53 expression in a case that was negative for dysplasia histopathologically, should be handled cautiously and warrants regular follow-up in order to prevent neoplastic progression. This is in concordance with the study done by Holzmann K et al., who asserted that higher percentage of p53 mutations in histopathologically negative samples is an indication that these genetic alterations is one of the most important and early one in tumour development [19].

One of the cases showed negative staining for p53 which was positive for dysplasia histopathologically. This could be due to over interpretation of histological features and attributing it to dysplasia rather than the inflammatory changes which can lead to regenerative atypia. As a rule, diagnosis of dysplasia should not be made in areas of active inflammation [14].

However, in the present study, comparison of p53 staining with the status of dysplasia on histopathology did not yield a significant result (p-value >0.05). Many studies have concluded that p53 is an important marker to detect dysplasia with few evaluating it at a genetic level [20-22]. In the present study, IHC expression of p53 was evaluated to detect the expressed protein. This could be a reason for discordance in various studies [5,6].

Limitation(s)

Limitations of the study include small sample size and short duration of study.

CONCLUSION(S)

The present study did not show a statistically significant association between p53 staining and various parameters like status of dysplasia on histopathology, gender and type of IBD but it showed low sensitivity and high specificity. Positive p53 staining was observed in a case that was histopathologically positive for dysplasia. This finding states that p53 mutations found in dysplastic mucosa has a risk of progressing to neoplasia. One of the cases which were negative for dysplasia showed positive staining for p53. This indicates that such cases should be handled cautiously and regular follow-up is very essential. New insights can be provided by directing resources to understand the biology of disease and combine them with novel molecular biomarkers to enhance IBD surveillance thus leading to improved clinical outcomes for both clinicians and patients.

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