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Original Article

Diagnostic Utility of Beta-Catenin in Poorly Differentiated Colorectal Carcinoma

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ABSTRACT

Objective: To determine the diagnostic ability of Beta-catenin in poorly differentiated colorectal carcinomas.
Study Design: Cross-sectional study
Place and Duration of Study: Department of Histopathology PNS Shifa Hospital, Karachi Pakistan from Jun 2019 to Jun 2020
Methodology: After ethical approval from the Institutional Review Board, 60 patients of both gender and all age group, diagnosed with a case of colorectal carcinoma on biopsy and resection specimens analyzed on histopathology on H & E staining at PNS SHIFA were included in the study. The resection specimens of CRC included those obtained from hemicolectomy, abdominoperineal resection and biopsies. Beta-catenin was interpreted using immunohistochemistry as cytoplasmic and nuclear staining with varying intensity. The scoring method was 0 as negative, 1+ as weakly positive, 2+ as moderate positive, and 3+ as strongly positive.
Results: Among the 60 patients with resection specimens included from hemicolectomy, abdominoperineal resection and colorectal biopsies, strong nuclear positive results were observed in 30(50 %) patients, moderate nuclear positivity in 12 (20 %), weak nuclear positivity in 6(10 %) and positive cytoplasmic staining in 12(20 %) patients.
Conclusion: Beta-catenin can be used successfully as a diagnostic utility in poorly differentiated colorectal carcinoma patients.

Keywords: Beta-catenin, Colorectal carcinoma, Immunohistochemistry.

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INTRODUCTION

The majority of colorectal carcinomas (CRCs) are assumed to arise from pre-malignant polyps, which are treated through endoscopic resection; even then, CRC remains the second most common reason for cancer-related death and the third most commonly diagnosed cancer among both males and females.[1,2]

CRC pathogenesis is thought to be a multi-stage process, beginning as a benign polyp and then progressing into adenoma and carcinoma.[3] In the process, multiple tumour suppressor genes and oncogenes are mutated or deleted in CRC.[4] Amongst them, the antigen-presenting cell (APC) gene implications in genetic predisposition to familial adenomatous polyposis (FAP) is regarded that the most vital "genome safeguard" for normal tissues of the colon; however, the precision of APC in tumorigenicity has been determined until recently when Beta-catenin was recognized as a key mediator of Wnt signalling.[5,6]

Beta-catenin, known as a multifunctional protein, is now known to play a dual role in cells. Initially reported as a protein linked to E-cadherin, it maintained cell-to-cell interactions.[7] Beta-catenin is an independent transcription factor in the Wnt signalling transduction pathway. Beta-catenin remains under strict control of Wnt-signaling cascade upstream regulators in normal conditions. At the surface of cells, Wnt interaction and mutated receptors trigger the activation of pathways that inactivate glycogen synthase kinase-3 beta. This inactivation leads to the failure of NH2 phosphorylation at the terminus end of Beta-catenin.[8] Unphosphorylated Beta-catenin cannot make a complex with APC for forming a ubiquitin-mediated protein complex. Beta-catenin accumulation in the cytoplasm, presumably leading to protein translocation into the nucleus, where it interacts with the DNA-binding T-cell factor complex, acting as a transcriptional activator.[9] This, in turn, causes activation of target genes, which include c-myc and cyclin D1, as in the case of colorectal carcinomas. In addition to the down-regulation of Beta-catenin in CRC, it is found to occur in other cancers as well.[10]

The present study seeks to establish the diagnostic efficacy of Beta-catenin in poorly differentiated colorectal carcinomas through a cross-sectional study conducted at the Department of Histopathology PNS SHIFA from June 2019 to June 2020, aiming to contribute to improved diagnostic approaches for colorectal carcinoma patients.

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METHODOLOGY

The cross-sectional study was conducted, from June 2017 to June 2018, at the Histopathology Department of PNS Shifa Hospital, Karachi Pakistan after ethical approval from the Institutional Review Board. Sample size calculation was done using the formula for prevalence, keeping the prevalence rate of colorectal carcinoma in a Pakistani study to be 4.2 %.[11]

Inclusion Criteria: Patients of both gender and all age group, diagnosed with a case of colorectal carcinoma on biopsy and resection specimens analyzed on histopathology on H & E staining at AFIP were included in the study.

Exclusion Criteria: Poorly fixed specimens of colorectal carcinomas were excluded from the study.

The resection specimens of CRC included those obtained from hemicolectomy, abdominoperineal resection and biopsies. The streptavidin-biotin and Bio SB antibody methods were utilized for immunohistochemistry staining and evaluation. For staining, brief tissue sections of four-micrometer thickness were placed upon silane-coated glass slides, air-dried overnight, and then, using xylene, rehydrated and graded with alcohol. Antigen retrieval was done using boiled tissue sections in EDTA (Ethylene Diamine Tetra Acetate) buffer at a pH of 8.0 in a pressure cooker for about two and a half minutes. Then, the section was cooled off for 30 minutes under running water. Then, immunohistochemical staining was done by Ventana-ES automated immune-stainer at 37 degrees (Ventana, Tucson, AZ). After this, the preparatory steps included an inhibitor for quenching endogenous peroxidase complex, a Beta-catenin antibody at a dilution of 1:200 for about 32 minutes, a labelled secondary antibody, a complex of Streptavidin-Biotin Peroxidase, Copper Sulfate for colour enhancement and Diaminobenzidine Tetrahydrochloride with Hydrogen Peroxide. Then, the sections were counter-stained using Harris hematoxylin and mounted using Permount after dehydration by graded alcohol.

Beta-catenin was interpreted using immunohistochemistry as cytoplasmic and nuclear staining with varying intensity. The scoring method was 0 as negative, 1+ as weak positive, 2+ as moderate positive, and 3+ as strong positive. Statistical Package for Social Sciences (SPSS) version 24.0 was used for the data analysis. For quantitative data, frequency and percentages were reported.

RESULTS

Among the 60 patients with resection specimens included from hemicolectomy, abdominoperineal resection and colorectal biopsies, strong nuclear positive results were observed in 30(50 %) patients, moderate nuclear positivity in 12(20 %) patients, weak nuclear positivity in 6(10 %) patients and positive cytoplasmic staining in 12 (20 %) patients (Table). Graphical representation of beta-catenin diagnostic utility in poorly differentiated colo-rectal carcinoma.

Table: Interpretation of Beta-catenin diagnostic utility in poorly differentiated colo-rectal carcinoma (n=60)

Immunohistochemistry	Frequency (%)
Strong nuclear positive	30(50 %)
Moderate nuclear positive	12(20 %)
Weak nuclear positive	06(10 %)
Cytoplasmic staining	12(20 %)

DISCUSSION

According to the results of our study, a strong nuclear staining immune-histochemically was reported regarding the diagnostic utility of beta-catenin in CRC. Similar to the findings in our study, another study observed that immune-staining of colorectal cancer specimens showing nuclear beta-catenin expression correlating with the stages of progression of cancer showed that 100 %, i.e. 60 out of 60 colorectal carcinomas and 92 %, i.e. 55 out of 60 colorectal adenomas were positive for nuclear staining of beta-catenin. However, in the study, only 08 %, i.e. 05 out of 60 colorectal polyps, were positive for nuclear staining with beta-catenin.[12] Another research reported that the tumour histologic grade of nuclear staining density with beta-catenin was mild (+).[13] A positive relationship was observed between beta-catenin's expression and differentiation grade, metastasis of lymph node, and stage and size of the tumour but not with the vascular invasion of CRC. The study concluded that beta-catenin, which plays an important role in cell homeostasis and as an antigen-presenting cell gene, has a substantial role in the carcinogenesis of colorectal cancers.[14]

Many researchers have regarded that beta-catenin is implicated in the development of colorectal carcinoma. Abnormal expressions of the APC gene, along with mutation at phosphorylation sites of beta-catenin, are the main factors which are responsible for over-expression as well as subsequent translocation (cytoplasmic/ nuclear) of beta-catenin which are reported in colorectal cancers. Roseweir *et al*. reported that beta-catenin's cytoplasmic expression is not always correlated to the protein's nuclear expression.[15] De Smedt *et al*. reported differing results in terms of

beta-catenin's diagnostic utility in CRCs. These discrepancies in the frequency of positive nuclear signals might be associated with the differences in the retrieval of antigens and the procedure of staining used by each laboratory.[16]

Moreover, our study observed beta-catenin in the nuclei and cytoplasm of tumour cells. Lately, researchers have reported that among patients with advanced CRCs, a greater expression of cellular and nuclear levels of beta-catenin was observed. In stabilizing beta-catenin in the cytoplasm, its transportation towards the nucleus, and activating gene expression, all are increased by a wingless pathway.[17] The ligand Wnt-1 is one such ligand that triggers the signalling cascade. Our study and Serafino et al., in a European study in 2014, found that a higher expression of Wnt-1 ligand was seen in the epithelium of tumour tissues of the colon as compared with the expression in the normal epithelium of the colon. This shows that beta-catenin accumulates in Wnt-1 activation among CRCs.[18]

Likewise, another research by Bourroul *et al*. reported that beta-catenin's expression was substantially higher in colonic carcinomas (p<0.001). The increased expression of beta-catenin showed that the destruction complex of beta-catenin was disrupted.[19] Yet another study by Kazem et al. observed that beta-catenin played a vital role in colonic carcinoma. Its increase in expression can be utilized as a marker of tumour progression and poorer prognosis.[20]

CONCLUSION

According to the results of this study, strong nuclear staining of beta-catenin was observed as compared with the cytoplasmic staining among poorly differentiated colon carcinoma patients. Therefore, in conclusion, beta-catenin can be used successfully as a diagnostic utility in poorly differentiated colorectal carcinoma patients.

Conflict of Interest: None.

Authors Contribution

Following authors have made substantial contributions to the manuscript as under:

FMM & FA: Data acquisition, data analysis, drafting the manuscript, critical review, approval of the final version to be published.

NJ & RA: Study design, data interpretation, drafting the manuscript, , critical review, approval of the final version to be published.

MA & FW: Concept, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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