Evaluation of
Ki67 in Detecting
Early Malignant
Changes in
Colorectal
Adenomatous
Polyps

Original Article

The Importance of Immunohistochemical Evaluation of Ki67 in Detecting Early Malignant Changes in Colorectal Adenomatous Polyps

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ABSTRACT

Objective: To assess the prevalent clinicopathogical parameters of Adenomatous polyps and association of ki67 with them in order to elucidate its significance as potent marker in determining early malignant changes

Study Design: Retrospective study

Place and Duration of Study: This study was conducted at the Histopathology department and Molecular diagnostic Research laboratory (MDRL), Dr. Ziauddin University and Hospital, Karachi from November 2021 to November 2023.

Methods: A total of 55 colorectal adenomatous polyps and clinical data was retrieved. Fresh frozen plasma sections were stained under Hematoxylin and eosin stain and further analyzed with KI67 IIHC stain. A P-value of less than 0.05 was deemed statistically significant.

Results: Among all 55 cases, 12 (21.8%) cases had age <50 years and 43 (78.2%) cases had age >50 years. Males predilection was observed. Most common clinical symptom was bleeding per rectum in 35 (63.64%) cases, followed by weight loss and chronic diarrhea, anemia, chronic abdominal pain, constipation while one case was accidentally reported on routine endoscopy (1.81%). 41 polyps (74.54%) were <20mm and 14 polyps (25.45%) were >20mm. Most common site was rectum (41.8%), followed by ascending and sigmoid colon, descending colon and transverse colon. Strong association was observed between size of colorectal polyps and grade of dysplasia with ki67 score.

Conclusion: When identifying the individuals with colorectal adenomatous polyps who require close surveillance in follow-up, size of polyp, high grade dysplasia and significant positive immunohistochemistry markers of Ki-67 may be useful criteria. This shall set a target population for screening of pre malignant changes.

Key Words: Dysplasia, Adenomas, ki-67 score

INTRODUCTION

Intraepithelial pedunculated pre-neoplastic lesions are known as colorectal adenomas. By 50, they are found in almost half of adults and serve as the primary precursors of most cancers in this organ.[1] Adenomas are becoming more common in Asian population due to increased adaptation of Western diets and lifestyles. The epithelial dysplasia that is present in colorectal adenomas is a characteristic.

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The frequency of colorectal adenomas and colorectal carcinoma are correlated which can link the risk of recurrence and the emergence of colorectal malignancies to adenomas.15% of all adenomas larger than 1 cm are predicted to develop into carcinomas within ten years of initial detection.[2]

Colorectal polyps are intraepithelial neoplasms growing through from the wall of the colon and rectum to its origin can be found in different sizes ranging from small polyps to large ones. Generally, colon polyps are more founded pathology and commonly seen over 50 aged patients and are not only the malignant pathology but more the precursor of malignant neoplasms, where the polyps have a high likelihood of cancer. Cancer normally develops in around 5% of adenomatous polyps.[1,2] Colorectal polyps are intraepithelial neoplasms growing through from the wall of the colon and rectum to its origin can be found in different sizes ranging from small polyps to large ones. Generally, colon polyps are more founded pathology and commonly seen over 50 aged patients and are not only the malignant pathology but

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Ki-67 protein is an additional immunohistochemical marker used to identify proliferating cells. With the exception of the G0 phase, it manifests in every stage of the cellular cycle. Ki-67 is a nuclear protein as well as a nucleolus protein[8] Ki67 protein is continually lacking in dormant cells and is undetectable during DNA repair processes.[9] Because of the nucleus's significant function in the upkeep or control of the cell cycle, the presence of the Ki-67 antigen is thus exclusively associated with the cell cycle. [10] Ki67 has been used by numerous researchers as a backup indicator to track the proliferation activity of tumor cells in different systems [11].

For the purpose of early diagnosing and predicting the prognosis of cancer, various biomarkers linked to growth factors, angiogenesis, tumour suppressor genes, oncogenes, and proliferating cells factors are employed. Because of its excellent sensitivity, Ki67 is widely used to evaluate the rate at which cancer cells proliferate. [12]

Aim of current study was to examine the Immunohistochemical activity of Ki67 alongwith its correlation to size of polyp and grades of dysplasia in colorectal Adenomatous polyps in order to elucidate its significance as marker of early pre malignant changes.

METHODS

This retrospective study was conducted at Histopathology department and Molecular diagnostic Research laboratory (MDRL), Dr. Ziauddin University and Hospital, Karachi.55 polypectomy specimens from patients in the form of Fresh Frozen Plasma Embedded tissues diagnosed as colorectal Adenomatous polyps were included.

Preparation of H&E sections: Hematoxylin and Eosin was used to stain paraffin-embedded sections from each colorectal polyp according to proper H&E staining protocols[13]

Preparation of IHC slides (immunohistochemical analysis):In order to establish a clear connection between the presence of protein ki67, and dysplasia in neoplastic tissues, IHC ki67 interventions have been performed on all adenomatous polyps exhibiting dysplasia following the IHC staining protocols [14]

Ki67 scoring technique: Without introducing the primary antibody (Ki-67) to the specimen, a negative control was employed.

Ki-67 expression counted in three high power fields (40X). Fraction of positive cells was calculated from atleast 500 tumor cells from the region where positivity was obvious and highlighted. 15 % or more Tumors cell staining positive was considered high ki67 score. Less than 15% tumors cells when stained positive were considered as low ki67 score [15]

Data analysis was carried out by SPSS 24.0. Chi-square test was performed for association between two variables. P value <0.05 was deemed statistically significant.

RESULTS

Clinicopathological parameters of patients included in study are expressed in table 1. 10 out of 21 polyps with mild dysplasia showed low ki67 score and 11 showed high ki67 score, all 3 polyps with moderate dysplasia showed high ki67 staining meanwhile 13 of 31 severely dysplastic polyps had low ki67 score and remaining 18 had ki67 score.

Table No. 1: Baseline demographics of the colorectal adenomatous cases

Variables	Frequency (n=55)	%
Gender		
Male	36	65.5 %
Female	19	34.5 %
Mean age (years)	53.27±10.84	
Clinical symptom		
Bleeding per rectum	35	63.64%
Chornic diarrhoea and weight loss	8	14.55%
Anemia	6	10.91%
Chronic abdominal pain	3	5.45%
constipation	2	3.64%
Accidental finding	1	1.81%
Site of Colorectal Adenomatous		
rectum	23	41.81%
ascending colon	10	18.2%
Sigmoid colon	10	18.2%
Descending colon	7	12.7%
transverse colon	5	9.09%
Size of Colorectal Adenomatous Polyps		
>20mm	14	25.45%
<20mm	41	74.54%
Dysplasia in colorectal adenomatous polyps		
Mild	21	38.19%
moderate	3	5.45%
Severe	31	56.36%

Table No. 2: showing Association of size of colorectal Adenomatous polyps with ki67 score

Ki-67 Score

		High	Low	Total
size	<20mm	19	22	41
	>20mm	13	1	14
Total		32	23	55

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	9.281[a]	1	.002
Continuity correction[b]	7.468	1	.006
Likelihood Ratio	10.944	1	.001
N of Valid Cases	55		

Chi square test was performed and P value of less than 0.05 was obtained showing strong association between size of polyps and ki67 score.

Table No. 3: Shows Association between Grades of dysplasia and ki67 score

		Ki-67 Score		Total
		High	Low	
Grades dysplasia	Mild	7	14	21
	Moderate	2	1	3
	Serve	23	8	31
Total		32	23	55

Chi-Square Tests			
Pearson Chi-Square	8.685[a]	2	.013
Likelihood Ratio	8.811	2	.012
N of Valid Cases	55		

Chi-square test was performed and P-value of < 0.05 was obtained showing stronger association.
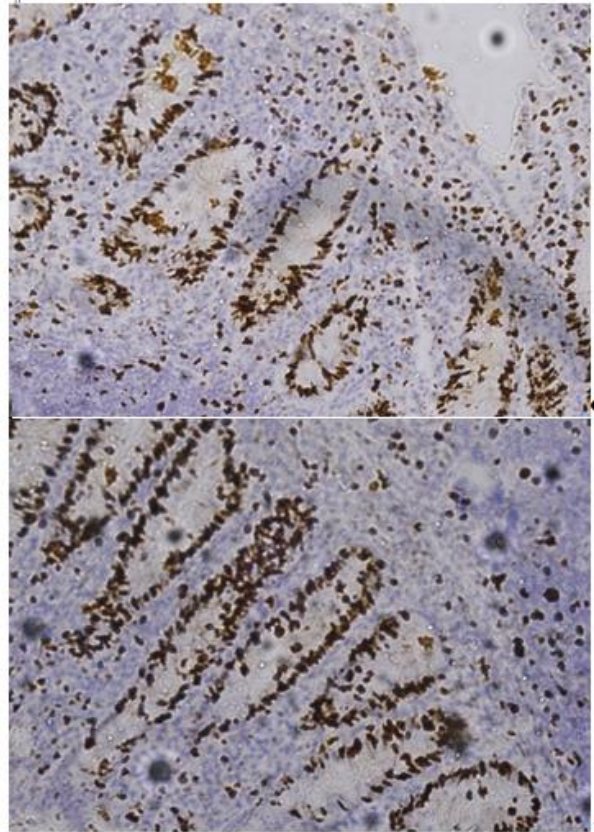

Figure Nos. 1 & 2: showing high Ki67 staining in the glands.

DISCUSSION

A number of tumors in humans have been shown to express Ki-67, which is linked to malignancies.[16] Although some research[17] failed to show the prognostic significance of higher Ki-67 expression, others reported this marker as an independent predictor of human colorectal cancer. [18]

In current study, 55 adenomatous polyps were retrieved. There were 36 (65.5%) males and 19 (34.5%) females in this study. Patients mean age was 53.27±10.84 years. Results were inline with the study done by Nusrat et al[19] Our study reported vast majority of severely dysplastic colorectal Adenomatous polyps, their proportion can be considered quite high when compared to previous research. [17,18]

High expression of Ki67 is also strongly associated with the incidence and progression of colorectal cancer[20] The severity of dysplasia and Ki-67

expression did not significantly correlate, according to research by Vernillo et al[21]. Another study showed significant correlation between size of colorectal Adenomatous polyps and grades of dysplasia with ki67 score, these findings were aligned with the findings of another study.

Ki67 score showed also significant correlation with size, type and high grade dysplasia in a previous study and the role of this marker was highlighted as an ancillary marker for the risk of transformation and as a target for chemo-preventive drugs [22], while our study worked on size and dysplasia correlation with ki67 score and the type of adenoma part should be considered for further research. High levels of ki67 positive cells in colonic samples indicated poor prognosis and comparatively adverse stage of colorectal carcinoma[23] This indicates the significance of ki67 screening in aiding in early diagnosis and prompt treatment of malignantly potential polyps.

Another study revealed significant association between dysplasia and ki67 score while the association remained insignificant in other studies [24].

Overall, the importance of ki67 scoring in dysplastic colorectal Adenomatous polyps with large size is significantly evident in screening and early diagnosis of colorectal Adenomatous polyps with potential of malignant transformation.

CONCLUSION

When identifying the individuals with colorectal adenoma who require close monitoring in follow-up prevention activities, high grade dysplasia and significant positive immunohistochemistry markers of Ki-67 may be useful criteria. Particularly in patients with a large size adenoma and high grade dysplasia.

Author's Contribution:

Concept & Design of Study:	Sabika Batool
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Data Analysis:	Sobia Hassan
Revisiting Critically:	Sabika Batool, Talat Mirza
Final Approval of version:	Sabika Batool

Conflict of Interest: The study has no conflict of interest to declare by any author.

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