

Original Article:**Ki-67 proliferation index and clinicopathological patterns in colorectal carcinomas****A. Bhagyalakshmi,¹ A. Sreelekha,¹ A. Kasi Babu,² S.V. Kumar,³ P. Muralikrishna⁴***Departments of ¹Pathology, ²Biochemistry, ³Surgery, ⁴Gastroenterology,
Andhra Medical College, Visakhapatnam***ABSTRACT**

Background : Tumour, node, metastasis (TNM) staging system provides useful prognostic information in patients with colorectal carcinoma (CRC). An improved prognostication and patient survival may be achieved by employing immunohistochemistry studies with proliferation markers like Ki-67.

Material and methods: We prospectively studied 51 patients with CRC and evaluated the clinicopathological patterns of CRC and the relationship of with the clinicopathological variables

Results: Their mean age was 48 (range 17-75) years; majority (64.7%) were males. Rectum was the most common subsite affected (45.1%). Histopathologically most of the tumours (86.3%) were usual type adenocarcinomas and were of grade 1 morphology (51%). The Ki-67 proliferation index (PI) ranged from 8.4% to 84.4%. The mean PI was greater in patients aged less than or equal to 50 years than in those aged above 50 years, in males than females, in rectal cancers than colonic cancers. It was greater in mucinous carcinomas than usual type adenocarcinomas, in grade 3 tumours than lower grade tumours (grades 1 and 2) and in T4 than T3 and T2 tumours. There was a significant positive correlation between the PI values and grade of the tumour.

Conclusion: We concluded that Ki-67 proliferation marker may be useful as an additional tool to assess the tumour aggressiveness with respect to certain clinicopathological parameters in colorectal carcinomas.

Key words: *Colorectal carcinoma, Adenocarcinoma, Ki-67 proliferation index*

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INTRODUCTION

Colorectal carcinoma (CRC) is the fourth most common cancer worldwide, with high incidence rates in developed countries and lower incidence in developing countries.^{1,2} Environmental factors, chiefly dietary are thought to influence these differences. Diet rich in calories, red meat, poor in vegetable fibre is thought to contribute to cancer occurrence while diet rich in vegetables, fruits and fibre is protective. Lifestyle factors like obesity, smoking and alcoholism are also considered risk factors. With westernization, an increasing incidence of CRC is being noted in the

previously low incidence countries, including India.³ Age is the most powerful risk factor for CRC.² Incidence increases with age, predominantly affecting late middle-aged and elderly individuals.² Studies from else-where as well as from India report that higher age standardized incidence rates are noted among the young in low incidence countries.⁴⁻⁸ The mean age at onset is 50 years, at diagnosis, in developing countries.² CRC is more common in males. These cancers develop insidiously, thus, often present at an advanced stage. Colonoscopic biopsy and pathological examination is the standard diagnostic

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approach. Pathologic tumour stage is the strongest prognostic factor. Currently Tumour, node, metastasis (TNM) staging system provides useful prognostic information in colorectal carcinomas. However, there is great heterogeneity in the clinical features and survival rates among individual patients with CRC, with disease at equivalent TNM stage. In this context, an improved prognostication and patient survival may be achieved by employing immunohistochemistry studies with proliferation markers like Ki-67, a cell nuclear proliferating antigen. The utility of Ki-67 has been well established in determining the prognosis of lymphomas, gliomas and breast cancer; however, its utility in gastro-intestinal malignancies is debated.⁹ The present study was done to evaluate the clinicopathological patterns of colorectal carcinomas and the relationship of tumour proliferative activity with the clinicopathological variables.

MATERIAL AND METHODS

We prospectively studied 51 patients diagnosed to have histopathologically confirmed CRC at the surgery and Gastroenterology Departments of Andhra Medical College, Vishakapatnam during the period August 2011-July 2013. This study was approved by Institutional Ethics Committee. Informed consent was obtained from all the patients. Cases wherein specimen was insufficient for immunohistochemistry (IHC) studies were excluded from the study.

In patients presenting with symptoms suggestive of CRC, a detailed history was obtained and a thorough physical examination was

carried out. Details regarding age, history of bleeding per rectum, altered bowel habits, abdominal swelling, loss of weight or appetite were recorded. Clinical examination was done to look for signs of intestinal obstruction, anaemia, mass per rectum. Patients clinically suspected to have CRC were posted for colonoscopy or proctoscopy/sigmoidoscopy respectively for colonic and rectal/rectosigmoid cancers. The site was, thus, confirmed accurately and biopsy was taken for histopathological confirmation. Radiological examination (computed tomography (CT) and magnetic resonance imaging (MRI) for colonic/rectal tumours respectively) was done in histopathologically confirmed cases of CRC to look for extent of tumour and contiguous spread and for distant spread like liver metastases. Other laboratory investigations that were conducted included complete blood picture, liver function tests, chest radiograph. Patients with resectable tumours were operated. Unresectable tumours were managed by chemotherapy and radiation for colonic cancers and neoadjuvant chemotherapy and radiation followed by surgery for rectal cancers. Type of surgery was decided based on the tumour site, as shown in Table 1. Both colonoscopic biopsies (n=24) and excision specimens (n=27) were studied. Age at presentation was categorized into 'age less than or equal to 50 years' and 'more than 50 years.' The clinicopathological features were studied and compared among different age groups, genders, tumour sites, histopathologic types, grades and in tumours at varying depth of invasion. The antigen identified by monoclonal antibody Ki-

Table 1 : Type of surgery based on tumour type

Site of tumour	Type of surgery
<i>Right colon</i>	<i>Right hemicolectomy</i>
caecum and ascending colon	radical right hemicolectomy
hepatic flexure	extended radical right hemicolectomy
transverse colon	extended radical right hemicolectomy
<i>Left colon</i>	<i>Left hemicolectomy</i>
splenic flexure	extended radical left hemicolectomy
descending colon	radical left hemicolectomy
sigmoid colon	sigmoid colectomy

67 proliferation index (Ki-67 PI) was assessed and correlated with clinicopathological parameters.

The formalin fixed paraffin embedded sections were stained with haematoxylin and eosin. Micropolymer method was employed for immunohistochemical studies with Ki-67; antigen retrieval was done by microwave technique. The primary antibody used was concentrated and prediluted rabbit monoclonal antibody, clone SP6 (Biocare Medical; Concord, CA, U.S.A.). Cells showing diffuse or granular brown nuclear stain were considered Ki-67 positive. A total of 1000 tumour cells were counted (200 cells in each of 5 fields) under high power magnification ($\times 400$) and Ki-67 labelling index (LI)/proliferation index (PI) was ascertained by the percentage of positive labelled nuclei.¹⁰ Intense nuclear positivity of the lymphocytes in the stroma served as internal positive control. The normal mucosa also showed intense nuclear staining confined to the base of crypts.

The data were tabulated and frequencies and percentages were calculated for qualitative variables. Quantitative variables were reported as mean and standard deviation. Categorical variables were compared using chi-square test and quantitative variables were compared using unpaired t-test or one-way analysis of variance (ANOVA) as appropriate. Bivariate correlation studies were done for age, grade and Ki-67 PI values; correlation coefficient (r) and p value were calculated. A p-value less than 0.05 was considered statistically significant.

RESULTS

The mean age was 48 (range 17-75) years; male:female = 1.8:1. Most of the patients were in the 31-60 years age group (72.5%).

Patients with rectal cancer commonly presented with bleeding per rectum, patients with left colonic carcinoma presented with pain abdomen and altered bowel habits. Out of the

17 patients with right colon cancer, eight complained of fatigue and were anaemic (hemoglobin ≤ 10.5 g/dL).

Rectum was the most commonly affected subsite (45.1%) followed by right and left colon in that order (Table 2). However, right and left colon cancers put together (n=28), accounted for more number of cases than rectal cancers. Histopathologically most were adenocarcinomas (usual type) (86.3%), others being mucinous adenocarcinomas and signet ring carcinomas. Majority showed grade 1 tumour differentiation (51%) (Table 2).

When we compared the clinicopathological features of patients aged less than or equal to 50 years (younger age group) with those of patients aged more than 50 years (older age group) (Table 3), majority (56.9%) were in the younger age group. In the younger age group, rectal cancers were more common and in the older age group colon cancers were more common.

Among the young, male: female ratio was relatively higher and higher grade tumours (grade 2 and grade 3) were more common. However, there were no statistically significant differences in both the age groups in terms of sex, tumour site, histopathological type and grade.

Among male patients, younger patients (≤ 50 years) were more common, whereas among females, both age groups (≤ 50 and > 50 years) were equally distributed. In both males and females, rectal tumours were most common; however in males right colonic tumours accounted for higher proportions and in females left colonic tumours were proportionately more common. Mucinous carcinomas and grade 3 carcinomas were relatively more common in males; the two patients with signet ring tumours were males. In females grade 2 tumours were relatively more common. However, these differences were not significant statistically (Table 4).

Table 2: Clinicopathological features

Variable	No.	%
Age (years)		
≤50	29	56.9
>50	22	43.1
Gender		
Males	33	64.7
Females	18	35.3
Site		
Right colon	17	33.3
Left colon	11	21.6
Rectum	23	45.1
Histopathologic type		
Adenocarcinoma (usual type)	44	86.3
Mucinous carcinoma	5	9.8
Signet ring carcinoma	2	3.9
Histologic grade		
Grade 1	26	51
Grade 2	13	25.5
Grade 3	12	23.5

Table 3: Comparison of clinicopathological features in patients aged ≤50 and > 50 years

Parameter	Age ≤50 years (n=29) No. (%)	Age >50 years (n=22) No. (%)	p-value
Gender			
Males	20 (69)	13 (59.1)	0.559
Females	9 (31)	9 (40.9)	
Site			
Right colon	7 (24.1)	10 (45.5)	0.277
Left colon	7 (24.1)	4 (18.2)	
Rectum	15 (51.7)	8 (36.4)	
Histologic type			
Adenocarcinoma (usual type)	25 (86.2)	19 (86.4)	0.971
Mucinous carcinoma	3 (10.3)	2 (9.1)	
Signet ring carcinoma	1 (3.4)	1 (4.5)	
Histologic grade			
Grade 1	14 (48.3)	12 (54.6)	0.895
Grade 2	8 (27.6)	5 (22.7)	
Grade 3	7 (24.1)	5 (22.7)	

There were no statistically significant differences between carcinomas at various subsites and of different histologic types and grades. Usual type adenocarcinoma was most common at all three subsites i.e., right colon, left colon and rectum. The proportion of mucinous tumours and grade 3 tumours was greater in right colon than at other.

In the resected tumours majority showed T3 level of invasion followed by T2 and T4 tumours in decreasing order. There were no tumours which were confined to T1 level of invasion. When compared with both T2 and T3 tumours, in T4 tumours, younger aged patients accounted for greater proportions. Rectum was the most common site affected, and both grade

Table 4: Comparison of clinicopathological variables between males and females

Parameter	Males (n=33) No. (%)	Females (n=18) No. (%)	p-value
Age (in years)			
≤50	20 (60.6)	9 (50)	0.559
>50	13 (39.4)	9 (50)	
Site			
Right colon	13 (39.4)	4 (22.2)	0.433
Left colon	6 (18.2)	5 (27.8)	
Rectum	14 (42.4)	9 (50)	
Histologic type			
Adenocarcinoma (usual)	27 (81.8)	17 (94.4)	0.403
Mucinous carcinoma	4 (12.1)	1 (5.6)	
Signet ring carcinoma	2 (6.1)	0	
Histopathologic grade			
Grade 1	17 (51.5)	9 (50)	0.54
Grade 2	7 (21.2)	6 (33.3)	
Grade 3	9 (27.3)	3 (16.7)	

Table 5: Relationship between Ki-67 proliferation index and clinicopathological variables

Variable	No	Ki-67 PI (%)*	p-value
Age (years):			
≤50	29	44.1 (16.7)	0.073
>50	22	35.2 (17.5)	
Gender			
Males	33	41.2 (16.7)	0.621
Females	18	38.5 (19.2)	
Site			
Right colon	17	40.4 (18.1)	0.254
Left colon	11	32.9 (13.9)	
Rectum	23	43.6 (18.1)	
Histologic type			
Adenocarcinoma (usual type)	44	38.4 (17.8)	0.163
Mucinous carcinoma	5	53.6 (12.6)	
Signet ring carcinoma	2	46.7 (9.19)	
Histologic grade			
Grade 1	26	31.2 (14.0)	<0.0001
Grade 2	13	44.7 (13.5)	
Grade 3	12	55 (17)	
Depth of invasion			
T1	—	—	0.054
T2	10	28.7 (17.4)	
T3	12	34.8 (12.6)	
T4	5	52.2 (24)	

*data are expressed as mean ± standard deviation

PI=proliferation index

2 and grade 3 tumours were equal in number in T4 tumours. In T2 and T3 tumours right colon was most common site affected and grade 1 tumours were most common. But none of these differences were statistically significant.

The mean Ki-67 PI was 41% (range 8.4%-84.4%). The tumour cells showed more extensive nuclear immunostaining compared to adjacent normal mucosa. There was marked heterogeneity in staining in different areas

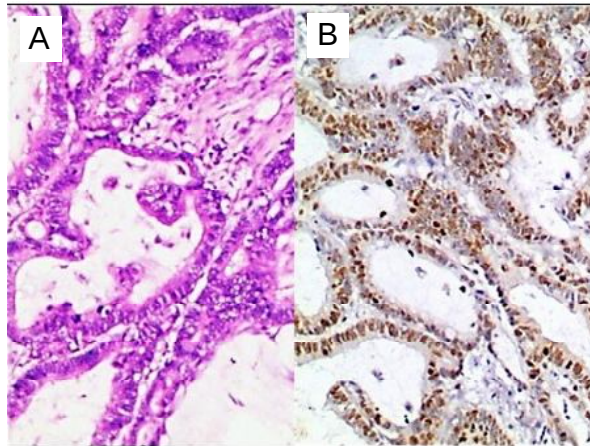


Figure 1: Mucinous adenocarcinoma. Photomicrograph showing malignant glands, lumen filled with mucin and mucin pools (Haematoxylin and eosin, $\times 100$) (A). Immunohistochemistry showing Ki-67 PI 70% (B)

PI = proliferation index

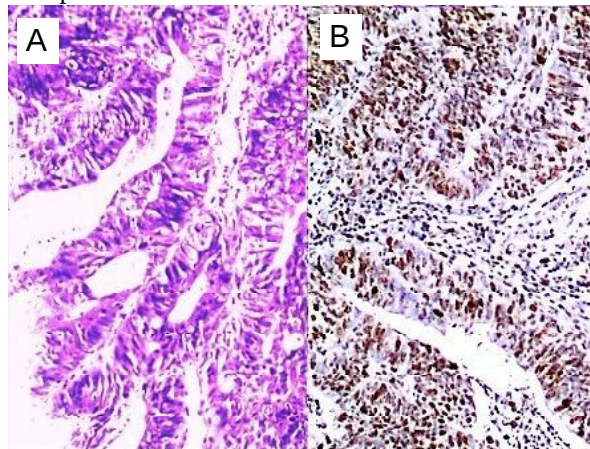


Figure 3: Photomicrograph showing gland formation and arrangement in sheets; suggestive of moderately differentiated adenocarcinoma (Haematoxylin and eosin, $\times 100$) (A). Immunohistochemistry showing Ki-67 PI 55.5% (B)

PI = proliferation index

within a tumour. Relationship between Ki-67 PI and various clinical pathological variables is shown in (Table 4). The mean Ki-67 PI was higher in patients aged 50 years or younger than in patients aged above 50 years. It was greater in males than females, in rectal cancers than colonic cancers, in T4 than T3 and T2 tumours. The mean Ki-67 PI was highest in mucinous carcinomas, lower in signet ring carcinomas and lowest in usual type adenocarcinomas (Table 5, Figure 1). However, these differences were not significant statistically. The mean Ki-67 PI value increased

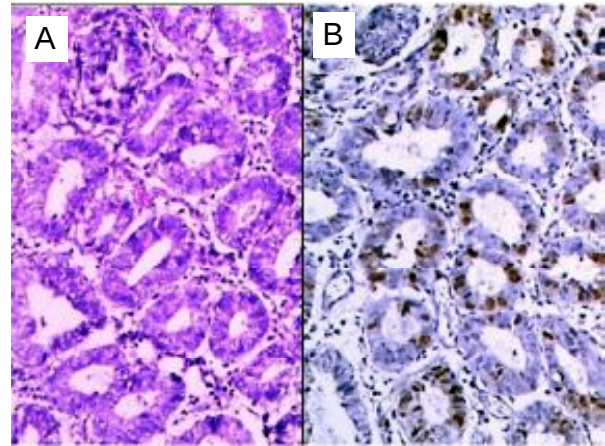


Figure 2: Photomicrograph suggestive of well-differentiated adenocarcinoma (Haematoxylin and eosin, $\times 100$) (A). Immunohistochemistry showing Ki-67 PI 38.3% (B)

PI = proliferation index

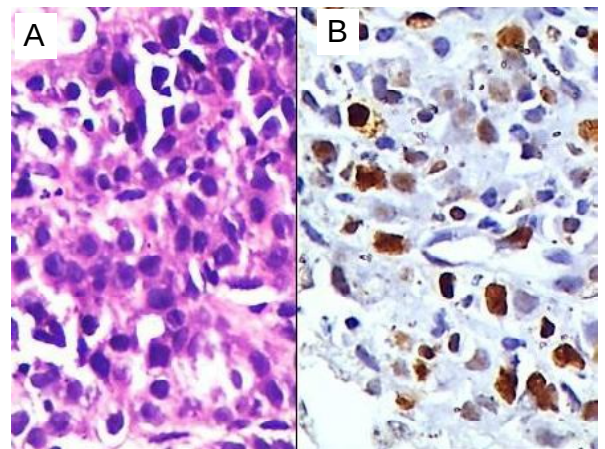


Figure 4: Photomicrograph showing tumour cells in sheets; suggestive of poorly differentiated adenocarcinoma (Haematoxylin and eosin, $\times 100$) (A). Immunohistochemistry showing Ki-67 PI 65% (B)

PI = proliferation index

from grade 1 to grade 3 tumours (Table 5); post-hoc analysis revealed that the difference in mean Ki-67 PI between tumours of grades 1 and 2 ($p=0.028$), and between tumours of grades 1 and 3 ($p=0.000069$) were significant (Table 5, Figures 2, 3 and 4).

There was no correlation between age and tumour grade ($r = -0.1132$; $p=0.4289$). There was a significant positive correlation between the Ki-67 PI values and grade of the tumour ($r = 0.5696$, $p<0.0001$). There was also a significant negative correlation between age

and mean Ki-67 PI values ($r = -0.3301$; $p = 0.018$)

DISCUSSION

Mean age of the patients in the present study (48 years) was similar (mean age 51 years) to another study,¹¹ and less compared to that reported by Rajesh Singh et al⁶ where the most common age group affected was 60-69 years. Compared to the present study, the age of patients reported in another study¹² was a decade younger. Gender distribution observed in the present study was similar to that reported in another study.⁷

In the present study, rectum was the most common subsite affected. However right and left colon cancers put together, accounted for more number of cases than rectal cancer. In one study⁸ colon was more commonly affected site than rectum. In another study⁶ rectum was the most common site affected. In two other studies^{7,12} rectosigmoid was found to be the most common site affected by CRC.

Usual type adenocarcinoma was the most common histologic subtype in the present study and also in other studies.^{7,13,14} In one study⁷ mucinous carcinomas accounted for 11.6% of all cases and signet ring for 4%, almost similar to that in present study.

Majority of CRCs were grade 1 tumours (well differentiated) in the present study as was observed in another study¹² However, in several other studies^{7,8,14-16} CRCs were mostly grade 2 tumours (moderately differentiated).

Majority of the patients in the present study were aged less than or equal to 50 years accounting for 56.9% while 43.1% were aged above 50 years. In one study¹² younger age patients (<50 years) accounted for 65.9%. Aljebreen et al¹⁵ reported that young (<50 years) patients constituted 37% of the patients. The authors concluded that the patients in their geographical region were more likely to present

with colorectal cancer at younger ages compared to Western populations. In one study⁶ 33.3% of the patients were younger than 50 years. It was observed in other studies^{4,5} that age standardized incidence rates among young are higher in the low incidence countries.

In the present study there were no significant differences between young and old patients with respect to sex, tumour site, histopathological type, grade and depth of invasion of tumours. Similar results were seen in other studies.^{15,17} In a study⁶ younger patients (<50 years) presented with poorer grades of tumour differentiation; however, this was not statistically significant. Similar findings were observed in present study. In another study¹³ mucinous and signet ring carcinomas were significantly more common in younger than older age group patients.

In the present study there were no significant differences between male and female genders in terms of age, site affected, histopathological type and grade of tumours, although mucinous carcinoma and grade 3 tumours were relatively more common in males than females. In a study¹⁴ right sided CRCs were more common in females compared to males in whom rectal cancers were more common. In the present study at all three subsites i.e., right colon, left colon and rectum, usual type adenocarcinoma was most common. There were no statistically significant differences in terms of age and gender affected, histopathological type and grade of tumours in these subsites. Mucinous carcinomas were more common in right colon than at other sites in some studies^{7,13} as was observed in the present study. Also, Song Wu et al¹³ reported that signet ring tumours were more common in rectum than at other sites. In another study¹⁷ grade 1 tumours were most common in right colon whereas in the present study grade 1 and grade 3 tumours were equally common in right colon and most of the rectal and left colonic tumours were grade 1 tumours.

The mean Ki-67 PI value observed in the present study was similar to the figures 34.6%,¹⁸ 38%,¹⁹ 48%,²⁰ and lesser than 52.8%,²¹ reported in other studies. In the present study the Ki-67 PI was higher in younger age (<50 years) than in patients aged above 50 years, in males than in females, in rectal than colonic tumours. The mean PI was higher in mucinous tumours than in other histopathological types. However, these findings were not statistically significant.

In the present study the mean Ki-67 PI value increased from grade 1 to grade 3 tumours but the difference in PI between grades 1 and 2, and between grades 1 and 3 were only significant. In another study,²¹ Ki-67 PI positively correlated with grade, showing an increase in mean Ki-67 PI with increasing grade but had no correlation with histopathological type, similar to present study.

In a study the mean Ki-67 PI was significantly higher ($p < 0.05$) in younger patients than in older patients.²² In their study Ki-67 PI did not correlate with tumour grade, histopathological type, tumour site and gender of the patient but it did correlate with tumour stage. The mean Ki-67 PI increased with advanced tumour stage in another study.²³ In a study of 47 CRC cases,²⁴ Ki-67 PI ranged from 26%-94% with a mean of 59% and PI correlated directly with tumor grade. In this study²⁴ there was no correlation between Ki-67 PI and age, gender or depth of tumour invasion. In a study of 50 CRCs²⁵ there was a significant correlation between Ki-67 PI and histopathological type ($p=0.005$) and tumour grade ($p=0.018$) but Ki-67 PI showed no correlation with age, gender or depth of tumour invasion. In one other study²⁶ Ki-67 PI was higher in poorly differentiated, mucinous tumours and T3 tumours than in well/moderately differentiated and T2 tumours

respectively. A high PI was significantly associated with advanced TNM stage but not with age or tumour grade in another study.²⁷ A significant correlation of Ki-67 PI with tumour grade, metastatic disease and local invasiveness was reported in other studies.^{18,21,28,29} A significant inverse relation between Ki-67 PI and tumour grade was observed in several studies^{30,31} in contradiction to findings in above studies and in present study. In one study,³⁰ the mean Ki-67 PI was greater in grade 1 and grade 2 tumours than in grade 3 tumours. Also PI was higher in non-mucinous tumours than in mucinous and signet ring tumours. Similar results were also seen in another study.³¹ In some studies^{27,28,32-34} no correlation was observed between Ki-67 PI and several clinicopathological variables like age, gender, tumour site, histologic type and grade of tumour. These conflicting results from various studies led to the suggestion that the discrepancies could be due to differences in epitope preservation, staining procedures, methods in evaluation and quantification of Ki-67 immunostaining, characteristics of the study population and considerable heterogeneity of colorectal carcinomas.³⁵⁻³⁷ In one study²⁴ intratumour heterogeneity was observed with high and low proliferative activity in different areas within same tumour. In the present study we observed marked heterogeneity in Ki-67 staining pattern among tumours of same histopathological type and grade; also intratumour and intragland variation was noted.

Therefore, we concluded that Ki-67 proliferation marker may be useful as an additional tool to assess the tumour aggressiveness with respect to certain clinicopathological parameters in colorectal carcinomas. However, in view of the small sample size in the present study, additional studies are required for confirmation of these findings.

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