RESEARCH ARTICLE

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Diffusion-Weighted MRI as Non-Invasive Diagnostic Tool for Rectal Cancer Aggressiveness and Correlation with KI-67 Expression in Tumor Tissue

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Abstract

Background and aim: Apparent diffusion coefficient (ADC) was suggested as a prognostic marker in rectal carcinoma (RC). However, reported data are inconsistent. The present study aimed to assess the relation between ADC value and Ki-67 expression index and other pathological parameters in Egyptian RC patients. **Materials and Methods:** The study included 39 patients with newly diagnosed RC (non-mucinous adenocarcinoma). All patients underwent magnetic resonance imaging (MRI) scan by 1.5T magnet. Mean ADC value was calculated. Pathological features were assessed and Ki- 67 immunohistochemical expression was applied as a proliferative index (PI) biomarker. **Results:** It was shown that patients with T4 tumors had significantly lower ADC values when compared with patients with T2 and T3 (0.903 ± 0.24 versus 1.157 ± 0.31 and 0.971 ± 0.26 respectively, p<0.001). Also, patients with circumferential resection margin (CRM) involvement had significantly lower ADC values when compared with patients without (0.905 ± 0.24 versus 1.109 ± 0.30, p=0.036). Patients with T4 tumors expressed significantly higher ki-67 PI when compared with patients with T2 and T3 tumors (75.71 ± 5.14 versus 46.25 ± 5.18 and 75.71 ± 5.14 respectively, p<0.001). Pearson's correlation coefficient identified a significant inverse correlation between ADC values and ki-67 PI (r=-367, p=0.027). **Conclusion:** ADC values of RC may reflect tumor staging and Ki-67 is closely related to the ADC value confirm this result.

Keywords: Colorectal carcinoma- Diffusion-weighted imaging MRI- apparent diffusion coefficient

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Introduction

Rectal cancer (RC) is the second most common cancer in females and the third most common cancer in males worldwide. Common prognostic factors for RC include older age, higher carcinoembryonic antigen levels and advanced histological grade (Choi et al., 2021).

In recent years, mortality rates have decreased due to significant changes in therapeutic management, in particular the standardization of the operative procedure and more important accurate pre-operative evaluation depending on imaging (Islami et al., 2021).

Preoperative imaging for rectal cancer (RC) staging is useful for choice of the appropriate surgical technique and proper tumor staging (Nougaret et al., 2012). Magnetic resonance imaging (MRI) is currently one of the most accurate noninvasive modalities for staging RC (Horvat et al., 2019). Diffusion weighted imaging (DWI) is increasingly incorporated into standard magnetic resonance imaging (MRI) protocols for tumor imaging due to its ability to detect and characterize tumors. Moreover, when the DWI is co-registered with conventional MRI, tumor staging as well as the circumferential resection margin (CRM) status can be assessed with high accuracy (Schurink et al., 2019).

However, the conventual DWI sequence has important limitations and artifacts which leads to decreased resolution and subsequently accuracy of tumor stage assessment (Padhani et al., 2009). Apparent diffusion coefficient (ADC) was suggested as a prognostic marker in RC. However, reported data are inconsistent with most studies identifying a relation between high ADC values and better tumor behavior (Gurses et al., 2019).

Ki-67 is a nuclear antigen expressed in proliferating cells from G1 to M-phase of the cell cycle (Luo et al., 2019). Many studies have shown a predictive role of Ki67 in a wide range of human malignancies, including gastrointestinal, prostate and breast cancers (Melling et

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al., 2016). The present study aimed to assess the relation between ADC values and Ki-67 expression index and other pathological parameters in Egyptian RC patients.

Materials and Methods

The present study was conducted at Al-Zahraa Hospital, Al Azhar University, Egypt in the period from July 2017 and June 2021. All patients gave informed consent before participation in the study.

The study included 39 patients with newly diagnosed RC (non-mucinous adenocarcinoma). Diagnosis of RC was established on the basis of colonoscopic findings and pathologic features of the resected surgical specimens. Patients were excluded if they had neoadjuvant therapy before MRI examination or recurrent RC or if the tumor did not have a sufficiently large parenchymal area for selecting round/oval-shaped regions of interest (ROIs). All patients were submitted to careful history taking, thorough clinical examination and standard laboratory work-up.

Magnetic resonance imaging

All patients underwent MRI scan by 1.5T magnet (Philips Achieva) using pelvic phased array coil 1 week prior to operation. Mean ADC values was calculated from a sample of three ROIs that were manually placed within solid tumor parts. The size and position of the ROIs was chosen to include as much of the solid tumor area as possible (Figure 1).

Pathological assessment

Resected tumors were assessed for histological type, differentiation grade, CRM and staging using TNM staging system according to The Union Of International Cancer Control (Amin et al., 2017). CRM is the distance between the outer margin of the tumor and the mesorectal fascia. It is critical for surgical planning, and for determining potential recurrence after total mesorectal excision. An involved CRM was considered if the shortest distance from either the extramural tumor extension, a suspected lymph node, or a tumor deposit in the mesorectum, to the mesorectal fascia was ≤ 1 mm.

Immunohistochemical staining

Tumor samples were collected after surgical resection then fixed in 10% neutral buffered formalin solution for 24h then subjected to paraffin embedding. We prepared one paraffin section from each specimen and 4-µm sections were cut and subjected to immunohistochemical staining for assessment of Ki- 67 expression using Ki-67 rabbit polyclonal antibody (Thermo Scientific-Lab Vision Corporation, Fermont, USA, Catalog No # PA5-19462). Dilution incubation with secondary antibody was performed. For visualization, product was incubated with diaminobenzidine substrate (Dako-Cytomation, Denmark) for 5-10 minutes. Sections were counterstained with Mayer's hematoxylinfor (2-5) minutes (BioGenex Laboratories, USA) and washed with distilled water.

Sections of breast carcinoma were used as positive control samples for Ki-67. ki-67 gives distinct brown nuclear staining. We recorded the number of tumor cells with distinct nuclear staining by manual counting of at least 1000 tumor cells in 10 different consecutive high-power fields (≤400) in the most reactive areas of the slides. Then, we calculated the percentages of the positive tumor cells as ki-67 proliferating index (ki-67 PI). The tumor is considered positive with significant proliferating activity when at least 10% of tumor cells showed nuclear staining for Ki-67 (Figure 2) (Ahmed et al., 2012).

Statistical analysis

Data were statistically described in terms of mean ± standard deviation (SD), median and range, or frequencies and percentages when appropriate. Categorical data were compared using chi-square test while numerical variables were compared using t test or one-way ANOVA as appropriate. Correlation analysis was achieved using Pearson's correlation coefficient. P value less than 0.05 was considered statistically significant.

Results

The present study included 39 RC patients. They comprised 16 (41.0 %) males and 19 females (59.0 %) with an age of 52.6 ± 18.4 years. The most common tumor location was in the lower third (30.8 %). Lymph node involvement was reported in 89.7 % of patients. Other pathological criteria are shown in Table 1.

It was shown that patients with T4 tumors had significantly lower ADC values when compared with patients with T2 and T3 (0.903 ± 0.24 versus 1.157 ± 0.31 and 0.971 ± 0.26 respectively, p<0.001). Also, patients with CRM involvement had significantly lower ADC values when compared with patients without (0.905 ± 0.24 versus 1.109 ± 0.30, p=0.036) (Table 2).

Table 1. Clinical Findings in the Studied Patients (n=39)

Age (years) mean ± SD	52.6 ± 18.4
Male/female n	16/23
Tumor location n (%)	
Upper third	2 (5.1)
Middle third	2 (5.1)
Lower third	12 (30.8)
Upper two thirds	7 (18.0)
Lower two thirds	6 (15.4)
Whole rectal length	10 (25.6)
Lymph node involvement n (%)	35 (89.7)
Tumor stage n (%)	
T2	4 (10.3)
T3	20 (51.3)
T4	15 (38.4)
Circumferential resection margin n (%)	
+ve	29 (74.4)
-ve	10 (25.6)
ADC mean ± SD	0.957 ± 0.268
Ki-67 proliferation index mean ± SD	69.97 ± 13.68

ADC, Apparent diffusion coefficient; CRM, Circumferential resection margin

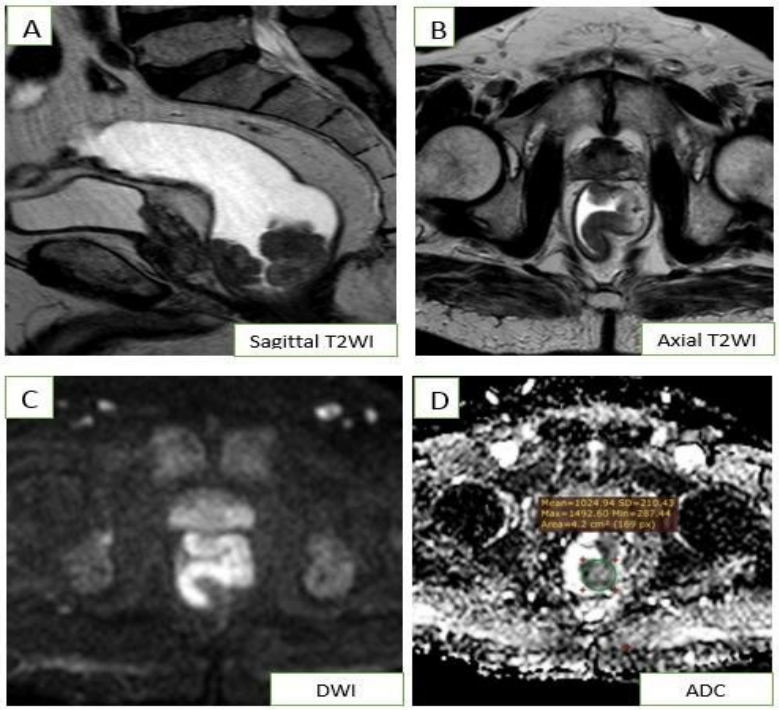

Figure 1. (A) Sagittal T2 shows circumferential irregular mural thickening involving lower rectum and anal canal narrowing its lumen. The involved segment measures 8.5 cm from the anal verge with maximal thickness of lesion measuring 2.2 cm. (B) Axial T2 shows near circumferential mural thickening of the lower rectum with intact serosal line and clear mesorectal fat. (C) This anal and lower rectal mucosal thickening shows diffusion restriction. (D) The ADC value is 1.024.

Table 2. Relation between Pathological Findings and ADC Values

	ADC Mean ± SD	p value
Lymph node involvement		
+ve	0.968 ± 0.26	0.449
-ve	1.074 ± 0.29	
Tumor stage n (%)		
T2	1.157 ± 0.31	<0.001
T3	0.971 ± 0.26	
T4	0.903 ± 0.24	
Circumferential resection margin n (%)		
+ve	0.905 ± 0.24	0.036
-ve	1.109 ± 0.30	

As regard KI-67 PI, expression was higher in poorly differentiated (grade III) tumors rather than well differentiated (grade I) tumors and this relation was statistically significant ($p < 0.01$) (Figure 2). In addition, we found that patients with T4 tumors expressed significantly higher ki-67 PI when compared with patients with T2 and T3 tumors (75.71 ± 5.14 versus 46.25 ± 5.18 and 75.71 ± 5.14 respectively, $p<0.001$) (Table 3). Pearson's correlation coefficient identified a significant inverse correlation between ADC values and ki-67 PI (r=-367, p=0.027) (Figure 3).

Discussion

The present study identified a significant association between ADC values and prognostic pathological markers

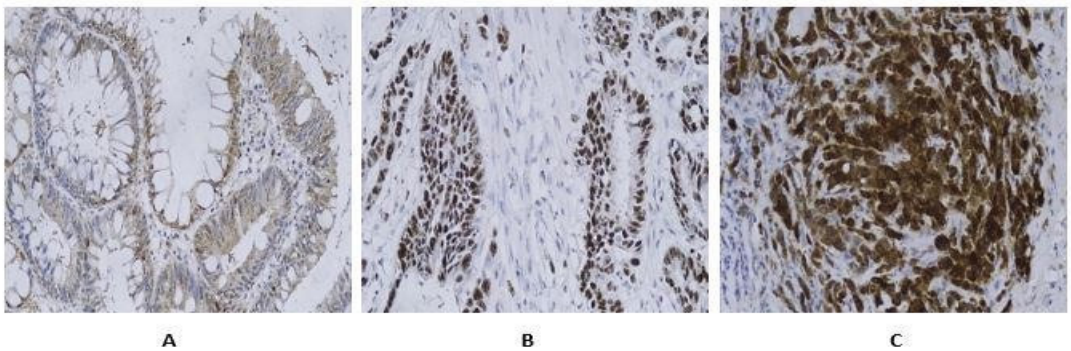

Figure 2. Ki-67 Immunostaining Reveals: (A) low Ki67 proliferation index in well differentiated rectal adenocarcinoma, (B) moderate proliferation index in moderately differentiated rectal adenocarcinoma (C) high proliferation index in poorly differentiated rectal adenocarcinoma.

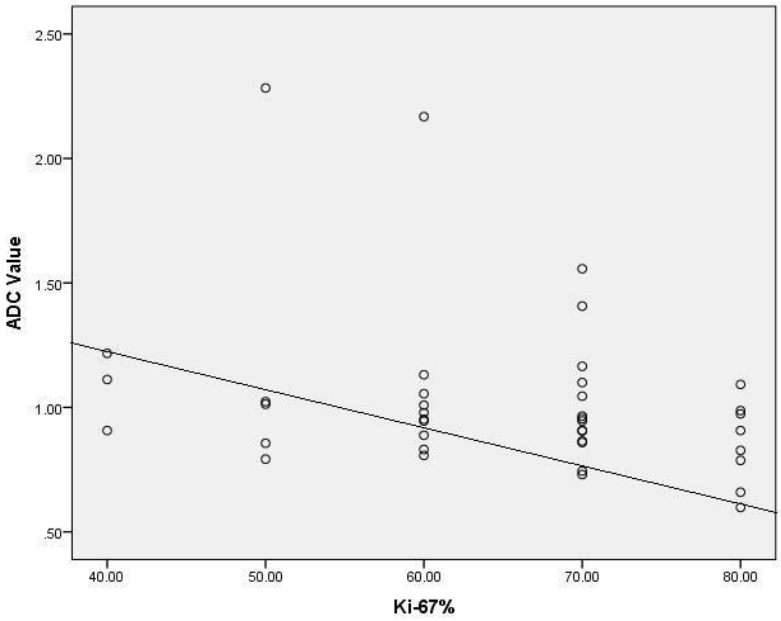

Figure 3. Correlation between Ki-67 Proliferation Index and ADC Values

Table 3. Relation between Pathological Findings and Ki-67 Proliferation Index

	Ki-67 proliferation index Mean ± SD	p value
Lymph node involvement		
+ve	70.62 ± 13.86	0.39
-ve	64.32 ± 12.14	
Tumor stage n (%)		
T2	46.25 ± 5.18	<0.001
T3	64.44 ± 5.11	
T4	75.71 ± 5.14	

in Egyptian RC patients. ADC values were significantly lower as T stage advances and in cases with CRM involvement. Moreover, significant inverse correlation was found between ADC values and Ki-67 PI. Our conclusions shows some agreements and disagreements with previous works.

In Curvo-Semedo et al., (2012) 50-patient study, there was a statistically significant correlation between ADC value and clinical MRF status (p value =0.013) and nodal status (p value = 0.011) on MR imaging and tumor differentiation grade upon histological examination (p value = 0.025) while there was no significant correlation between ADC and the T stage at primary MRI (p value = 0.064). The study demonstrated that the lack of significance in correlation between ADC values and T stage could be that the assumption of the subgroups T1–2 and T3–4 having different prognosis (good versus bad) may not be correct. There is a huge variability in prognosis within the group of T3 tumors: whereas large, bulky T3 tumors are associated with a poorer prognostic outcome and would behave more closely like T4 tumors, the smaller (borderline) T3 tumors are known to have a better prognosis, behaving more closely like T2 tumors.

In a study done by Sun et al. (2014) on 49 patients, ADC values were significantly lower for higher T stages (p value = 0.003), the N stage also increased as the ADC value decreased, although this trend was not statistically significant (p value = 0.055) and the patients with positive CRM had lower ADC values but this result was also not statistically significant (p value =0.312). Also, Akashi et al. (Akashi et al., 2014) study on 40 patients showed a significant correlation between ADC values and tumor differentiation grade (p = 0.019) with no significant correlation between ADC and T stage (p= 0.59) and the presence of MRF invasion (p =0.71) and N stage (p=0.41).

Interestingly. our results showed a highly significant association between the Ki-67 PI and the pathological T stage of rectal cancer (P < .001). This finding is matched with that of Aladherai et al., (Aladhraei et al., 2019) and Li et al., (Li et al., 2016), Tong et al (Tong et al., 2020) who found that Ki-67 expression showed a significant association with tumor size (P < 0.05). Our work showed a significant association between the Ki-67 PI and the tumor grade of rectal cancer (P < 0.05). This finding is matched with that of Li et al., (Li et al., 2016) and Tong et al (Tong et al., 2020) who found that Ki-67 expression showed a significant association with tumor grade (P < 0.05).

Moreover, our results observed a significant inverse correlation between preoperative ADC value and Ki-67 proliferation index in accordance with Ao et al., (Ao et al., 2020) and Meng et al., (Meng et al., 2016). Also, our findings are in concordance with other previous results done on bladder cancer (Kobayashi et al., 2014; Sevcenco et al., 2014).

In conclusion, ADC values of RC may reflect tumor staging; Ki-67 is closely related to the ADC value, and this is valuable for determining the preoperative T stage and biological behavior of low rectal cancer. Therefore, ADC values have potential as biological imaging indicators of tumor aggressiveness and may provide patients with more options in terms of treatment plans.

Author Contribution Statement

All authors equally shared in formulating the idea, conception, and data collection statistics, writing and drafting the manuscript.

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Ethical approval
The present study approved by the ethical committee of Al-Azhar Faculty of Medicine.

Conflict of Interests
None

Data Availability Statement
Data of this research will be available upon reasonable request.

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