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EXPRESSION OF WT1 IN COLORECTAL ADENOCARCINOMA AND ITS ASSOCIATION WITH HISTOLOGICAL GRADE AND STAGE

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ABSTRACT

Background: Colorectal carcinoma (CRC) is one of the most prevalent malignancies globally. Based on GLOBOCAN 2018 data, more than 2 million new cases of colorectal carcinoma (CRC) were recorded and caused around 1million deaths that year. **Objectives:** The aim of the present study was to determine the expression of WT1 in histomorphologically diagnosed colorectal adenocarcinoma and its association with histopathological grade and stage. **Methodology:** This cross-sectional observational study was conducted in the Department of Pathology, Dhaka Medical College (DMC), Dhaka, from March 2021 to February 2023. Large bowel resection specimens of 50 colorectal adenocarcinomas (CAC) were included in this study. Sections were stained with hematoxylin and eosin. Immuno staining with WT1 antibody was also done in Bangabandhu Sheikh Mujib Medical University (BSMMU). Relevant information was collected and recorded in a predesigned data sheet. The association of expressions of selected markers were evaluated with Chi-square test. P value significance was defined as $p<0.05$. **Results:** Fortyone cases (82.0%) showed WT1 positive expression. Out of 13 grade 3 cases 12(92.3%) were WT1 positive. The difference was not statistically significant ($p>0.05$) between two groups. Out of six T4a tumor cases all 100% were WT1 positive. All N_2 nodal status cases 9(100%) and 23 (95.8%) stage III cases were WT1 positive. Tumor extension, Lymph node status and stage were statistically significant ($p<0.05$) between two groups. Higher WT1 expression is significantly ($p<0.05$) associated with higher grade and stage. **Conclusion:** About 32% cases of colorectal adenocarcinoma showed high expression of WT1 and there was significant association of high expression of WT1 with histological gradeand stage of colorectal adenocarcinoma.

KEYWORDS: WT1 immunomarker (WT1), immunohistochemistry (IHC), colorectal adenocarcinoma (CAC).

INTRODUCTION

Colorectal cancer (CRC) is one of the most commonly diagnosed cancers worldwide and accounts for an estimated 10% of all malignancies.[1] Incidence rates are approximately 4-fold higher in more developed regions.[1] In Bangladesh CRC is the 7th most prevalent cancer and 10th commonest cause of death from cancer.[2] In Asian countries,

the incidence of it has increased approximately 2–4-fold over the past few decades.[3] Colorectal cancer is a heterogeneous group of diseases with distinctive genetic and epigenetic background.[4] In cancer biology, WT1 gene plays a major role in normal development, most notably in the urogenital system and is also expressed in the developing cancer.[4]It is located at chromosome locus 11p13, encodes a zinc finger transcription factor, binds to GC-rich sequences.[5] It also works through interaction with Wnt signalling/ beta catenin pathway.[6]

RATIONALE

The most effective treatment for it is surgery but approximately 60% of patients experience local recurrence or distant metastasis, around 20% of patients die from recurrence.[3] CD8 cytotoxic T lymphocytes (CTLs) recognize epitope peptides derived from tumor- associated antigens (TAA) that is WT1. So, new immunotherapies that target TAA have been used to treat colorectal adenocarcinoma (CAC), in addition to conventional one.[7] Clinical trials of immunotherapy (PhaseI)on colorectal adenocarcinoma is ongoing on targeting WT1 protein, which have shown promising results and enhanced disease free survival for more than 2 to 2.5 years.[8] So, anti-WT1 therapy may improve patient's survival. In cancers, immunohistochemical (IHC) study is much less costly than molecular study but give almost similar result and also guide about tumor biology and aggressiveness.[9] The present study evaluated the expression of WT1 in colorectal adenocarcinoma in accordance with its histopathological grade and stage among Bangladeshi patients.

MATERIALS AND METHODS

The study was a cross-sectional descriptive study and was carried out at the Department of Pathology, Dhaka medical college (DMC), Dhaka and BSMMU during the period from March 2021 to February 2023. Total 50 cases of large bowel resection specimens histologically diagnosed as adenocarcinoma were collected from all age groups and sexes. Consecutive purposive sampling technique was followed to collect samples in this study and enrolled in data collection sheet. Patients exposed to neo adjuvant or adjuvant chemotherapy and radiological evidence of distant metastasis were excluded from the study. Cases were reevaluated for gross feature, histological type, tumor grading, local extension, nodal metastasis and staging. The cases were histologically graded (WHO) and staged according to TNM staging system. Representative sections from each case were selected for immunohistochemical stain with WT1.

Immunohistochemical Analysis

Immunohistochemistry was done in immunohistochemistry laboratory, BSMMU. Formalin fixed paraffin-embedded tissues sections of 3-4 micrometer thickness were used. FLEX Monoclonal mouse anti-Human WT1 protein clone 6F-H2 Ready-to- Use(Link) which react to N terminus of WT1 protein was used as primary antibody. EnVision HRP (ready to use DAKO) was used for WT1 as secondary antibody. Serous papillary carcinoma of ovary was taken as positive control. Brownish cytoplasmic staining of tumor cells was considered positive. The expression of WT1 were analyzed in ten microscopic fields. The mean expression of ten microscopic fields has been evaluated.

The WT1 index score and expression was calculated.[10,12] The area of staining positivity (ASP) staining was scored as follows: 0=0%, 1= 1-50% stained cells; 2= > 50% stained cells; The intensity was scored as follows: 0= Negative; 1= Mild; 2=Moderate; 3=Marked. WT1 index was determined by the multiplication of frequency and intensity scores. Six indices (0, 1, 2, 3, 4 and 6) were found and it was further divided as follows: 0 index was considered negative and (1 -6 indices) were considered positive. Among positive indices: (1 -3 indices) were considered low expression and (4 or more indices) were considered high expression.

The statistical analysis was carried out using the Statistical Package for Social Sciences version 22.0 for Windows. The association of expressions of selected markers were evaluated with Chi-square test. P value significance was defined as $p<0.05$.

OBSERVATIONS AND RESULTS

Table 1: Distribution of the cases according to demographic variable (n=50).

Age (year)	Frequency	Percent
o ≤40	6	12.0
o 41-50	11	22.0
o 51-60	12	24.0
o 61-70	14	28.0
o >70	7	14.0
o Male	32	64.0
o Female	18	36.0
Family history		
o Yes	1	2.0
o No	49	98.0
Smoking status		
o Smoker	24	48.0
o Non smoker	26	52.0
CEA level (ng/ml)		
o ≤5 ng/ml	28	56.0
o >5 ng/ml	22	44.0

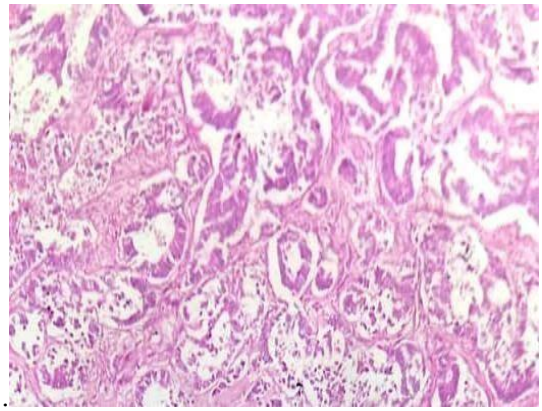
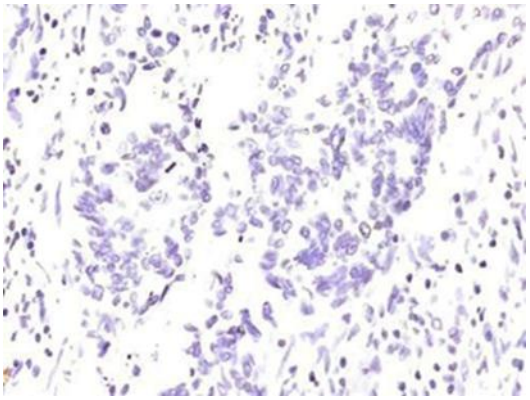
WT1 expression		
o WT1 Positive	41	82.0
o WT1 Negative	09	18.0

Table 2: Association of WT1 expression with tumor grade, tumor extension, lymph node status and stage (n=50)

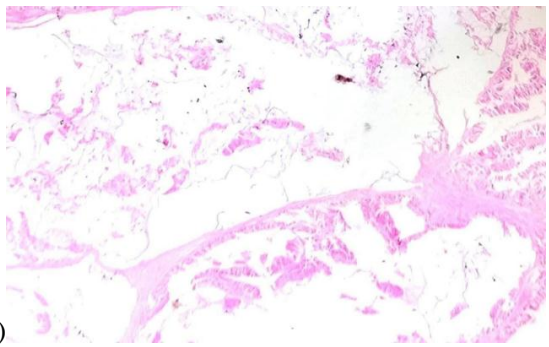
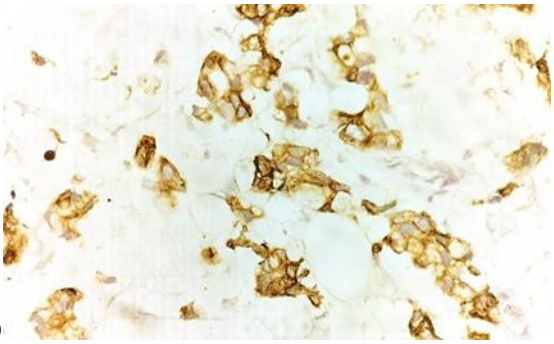
Tumor grade	WT1 positive n=41		WT1 negative n=9		p value
	N	%	n	%	
Grade1	5	55.6	4	44.4	
Grade 2	25	89.3	3	10.7	0.065[ns]
Grade 3	12	92.3	1	7.7	
Grade 4	0	0	0	0	
Tumor extension					
T2	2	25.0	6	75.0	
T3	33	91.7	3	8.3	0.001[s]
T4a	6	100.0	0	0.0	
Lymph node status					
N0	18	69.2	8	30.8	
N1	14	93.3	1	6.7	0.046[s]
N2	9	100.0	0	0.0	
Tumor stage					
Stage 1	4	40.0	6	60.0	
Stage 2	14	87.5	2	12.5	0.001[s]
Stage 3	23	95.8	1	4.2	

Table 3: Association of overall WT1 immunoreactive score (IRS) with tumor grade and tumor stage among WT1 positive cases (n=41)

	Low expression (score 1-3)(n=25)		High expression (score 4-6)(n=16)		p value
	n	%	N	%	
Tumor grade					
Grade1	5	100.0	0	0.0	
Grade2	20	83.3	4	16.7	0.001[s]
Grade3	0	0.0	12	100.0	
Tumor stage					
Stage1	4	100.0	0	0.0	
	12	85.7	2	14.3	0.004[s]
Stage2					
Stage3	9	39.1	14	60.9	

1(A).  1(B) 

Photograph 1(A): Well differentiated (grade 1) colorectal adenocarcinoma (H&E X100)
Photograph 1(B): Well differentiated (grade 1) colorectal adenocarcinoma showing negative staining of WT1 (IHC X400)

2(A)  2(B) 

Photograph 2(A): Mucinous (poorly differentiated) adenocarcinoma (H&E X200)
Photograph 2(B): Mucinous (poorly differentiated) adenocarcinoma showing high expression of WT1 (IHC X400)

DISCUSSION

It was observed that 41 (82.0%) patients had WT1 expression positive and 09 (18.0%) had negative WT1 expression. The result was nearly consistent with other studies conducted by Bejrananda et al. 2011 and Oji et al. 2003.[10,11] They found 91%, and 89.0% cases showed positive WT1 expression respectively in their researches. On the contrary, Barresi et al. (2016) showed, only 21% neoplastic cells had positive WT1expression in their study.[12] WT1 immunoreactivity was observed in higher histologic grade in a study performed by Bejrananda et al. (2011).[10] In this study when the cases were segregated according to WHO grading system it was observed that WT1 expression was positive in 12(92.3%) cases of grade -3 tumor, 25(89.3%) cases of grade-2 tumor, 5 (55.5%) cases of grade-1 tumor and WT1 expression were negative in 4cases (44.4%) in grade-1 tumor. The difference was not statistically significant ($p>0.05$) between two groups. So no significant association was found between WT1 expression and histological grade of colorectal adenocarcinoma. This finding is close to the findings described by Salvatorelli et al., (2020) and Barresi, et al., (2016).[13,10] Oji et al. (2003) found that, WT1 expression was positive in all cases of grade-3 tumor and negative or low expression ingrade-1 tumor.[11]In this study, most of the cases 36 cases (72.0%) were belonged to sub serosal invasion (T3).WT1 expression was positive in 33 (91.7%) cases that invaded T3 level and 6 (100.0%) cases that invaded T4a level and 2 (25.0%) cases that invaded upto muscularis propria (T2) showed positive WT1 expression. The difference was statistically significant ($p<0.05$) between two groups. So, a positive association was found between tumor extension and WT1 expression in the present study. Miyata et al. (2015) also found a significant association between depth of invasion and WT1 expression.[3] Miyata et al. (2015) found, WT1 expression was positive in 100% cases with T4 level, 63.6% cases with T3 level.[3] Bejrananda et al. (2011) recorded no significant association between the expression of WT1 and stage of tumour.[10] These dissimilarities of previous study were due to poverty, ignorance and lack of routine screening program for early cancer detection. Patients' treatment becomes delayed which causes progression of disease to an advanced stage. Out of 15 cases of N1, 14(93.3%) were WT1 positive. All the 9(100%) N2 cases and 18 (43.9%) out of 26 N0 cases were WT1 Positive. The difference was statistically significant ($p<0.05$) between two groups. So, a positive association was found between lymph

nodestatus and WT1 expression in this study. Miyata et al.(2015) found statistically significant associatiation (p<.05) with nodal metastasis.[3] Sangkhathat et al. (2015) also demonstrated almost similar findings.[14] Cases were staged according to AJCC cancer staging manual 8th edition in this study. Out of 24 stage III cases 23(95.8%) were WT1 positive and out of 10 stage I cases 4(40%) were WT1 positive. The difference was statistically significant (p<0.05) between two groups. So WT1 expression was significantly associated with histological stage. Miyata et al. (2015) also found 58(42.3%) cases with stage III and 41 (29.9%) stage II cases showed positive WT1 expression and higher frequency of stage I cases with negative WT1 expression.[3] These findings were nearly consistent with the present study. Among all WT1 positive cases 16 (32%) cases showed high expression. In them 12(100%) Grade 3 tumor showed high WT1 expression and all the 5(100%) grade 1 cases showed low WT1 expression. Among all WT1 positive cases 14(60.9%) Stage III tumor showed high WT1 expression and all the 4(100%) stage 1 cases showed low WT1 expression. The difference was statistically significant (p<0.05) between two groups. So higher WT1 expression were significantly associated with higher histological grade and advanced stage. Bejrananda et al. (2011) also found in their study that stronger intensity expression of WT1is associated with poor tumor differentiation and their stronger intensity expression was associated with higher percentage of positive cells.[10] Miyata et al. (2015) also showed in their study that higher WT1 expression was significantly related to advanced clinical stage (p=0.039).[3] So present study corresponds with these previous researches.

CONCLUSION

About 18(32%) cases of colorectal adenocarcinoma showed high expression of WT1 and there was significant association of high expression of WT1 with histological grade and
stage of colorectal adenocarcinoma.

LIMITATIONS

The study population was selected from one place only.

RECOMMENDATIONS

WT1 may be used as a prognostic marker for guiding targeted therapy in colorectal adenocarcinoma.

ETHICAL MEASURES

The study was conducted after ethical clearance from the Ethical Review Committee of Dhaka Medical College.

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