Role of WT1 Immunoexpression in Colorectal Adenocarcinoma: A Study in a Tertiary Care Hospital

*Nupur FP,[1] Jeba R,[2] Khan ZB,[3] Karim SS,[4] Zabin SG,[5] Khan RR,[6] Dewan RK,[7] Jinnah MS[8]

Abstract

Background: Wilms Tumor 1 (WT1) is a transcription factor involved in cellular proliferation and differentiation, with emerging relevance in various malignancies including colorectal adenocarcinoma (CRC). The aim of this study was to assess the immunohistochemical expression of WT1 in colorectal adenocarcinoma and determine its association with age, gender, histological type, grade, stage, lymphovascular invasion (LVI), and perineural invasion (PNI).

Methods: This cross-sectional observational study included 97 histopathologically diagnosed cases of colorectal adenocarcinoma, selected using purposive sampling. The study was conducted at the Department of Pathology, Dhaka Medical College Hospital, while WT1 immunohistochemistry was performed at the Immunohistochemistry Laboratory of Bangladesh Medical University, Dhaka. The study duration was from March 2021 to February 2025. Qualitative data were expressed as frequency and percentage. The Chi- square and Fisher's exact test were used to assess the relationbetween qualitative variables. A p-value less than 0.05 were considered as significant.

Results: Among 97 cases, 55% showed low and 45% showed high WT1 expression. High WT1 expression was significantly associated with mucinous type (p=0.0214), poorly differentiated tumors (p=0.0012), and advanced pathological T (p=0.0007) and N stages (p=0.0003). No significant association was observed with age, gender, LVI, or PNI.

Conclusion: WT1 immunoexpression associates with aggressive histopathological features in colorectal adenocarcinoma, suggesting its potential role as a prognostic marker.

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1. **Dr. Farjana Pervin Nupur, MBBS, MD (Pathology), Pathologist, Dhaka Medical College, Dhaka, pervin_farjana@yahoo.com*
2. *Dr. Ruksana Jeba, MBBS, MD (Pathology), Professor and head, Department of pathology, Dhaka Medical College, Dhaka,ruksanajeba67@gmail.com*
3. *Dr. Zubaida Bahroon Khan, MBBS, MD (Pathology), Associate professor, Department of pathology, Dhaka Medical College, Dhaka, zubaida@gmail.com*
4. *Dr. Saiyeda Sinthia Karim, MBBS, MD (Pathology), Associate professor, National Institute of Laboratory Medicine and Referral centre, Dhaka, sinthiakarim330@gmail.com*
5. *Dr. Shirin Gull Zabin, MBBS, MD (Pathology), Assistant professor, Department of pathology, Dhaka Medical College, Dhaka, gullzabin23@gmail.com*
6. *Dr. Rizwana Rahman Khan, MBBS, MD (Pathology), Assistant professor, Department of pathology, Dhaka Medical College, Dhaka, drizwanasbmc@gmail.com*
7. *Dr. MD. Rezaul Karim Dewan, MBBS, MD (Pathology), Professor and head, Department of Pathology, Greenlife Medical College Hospital, Dhaka, rezadewan22@gmail.com*
8. *Dr. Mohammed Shahed Ali Jinnah, MBBS, MD (Pathology), Professor and ex-director, National Institute of Laboratory Medicine & Referral Centre, Dhaka, shahed.jinnah63@gmail.com*

** For correspondence*

Introduction

Colorectal adenocarcinoma (CRC) remains a global health challenge, ranking as the third most commonly diagnosed cancer and the second leading cause of cancer-related deaths worldwide.[1] Its incidence continues to escalate, particularly in developing nations, a trend attributed to the adoption of Westernized diets, sedentary lifestyles, and an aging demographic.[2] Despite significant strides in diagnostic modalities and therapeutic strategies over recent decades, the prognosis for advanced CRC remains suboptimal, underscoring the urgent need for novel biomarkers that can facilitate early detection, predict disease progression, and guide personalized treatment approaches. The molecular pathogenesis of CRC is characterized by genetic and epigenetic alterations that drive uncontrolled cellular proliferation, invasion, and metastasis.[3]

The Wilms Tumor 1 (WT1) gene, initially identified as a tumor suppressor gene in Wilms tumor, a pediatric renal neoplasm, has earned considerable attention in the context of various adult malignancies.[4] WT1 encodes a zinc finger transcription factor that plays a critical role in cellular differentiation, proliferation, and apoptosis.[5] While its classical role is intimately associated with kidney development and tumorigenesis, aberrant expression of WT1 has been increasingly implicated in the oncogenesis of numerous other cancers, including leukemia, breast cancer, lung cancer, and ovarian cancer.[6,7,8] Interestingly, in many of these adult cancers, WT1 appears to function as an oncogene in contrast to its established tumor suppressor role in Wilms tumor.[9]

In the gastrointestinal tract, the precise role of WT1 in carcinogenesis is still under active investigation. Some studies have suggested its involvement in gastric and pancreatic cancers, demonstrating varying expression patterns and prognostic implications.[10,11] However, data specifically pertaining to the expression and clinical significance of WT1 in colorectal adenocarcinoma are relatively sparse and often contradictory.[12,13,14]This study aimed to observe the immunoexpression of WT1 in colorectal adenocarcinoma tissue samples obtained from patients managed at Dhaka medical college hospital and its association with various clinicopathological parameters, including age and sex of the patients, tumor grade, stage, location, type, lymph node metastasis, lymph vascular invasion and perineural invasion. These data could provide valuable insights into its potential role in disease progression. Furthermore, identifying WT1 as a reliable biomarker could pave the way for its integration into diagnostic algorithms, aid in risk stratification, and potentially identify a subset of patients who might benefit from WT1-targeted therapies, if such interventions become available in the future and ultimately improve patient management and outcomes in this challenging malignancy.

Methods

This cross-sectional observational study was observed to see the WT1 immunoexpression in resected colorectal adenocarcinoma specimens. The study was conducted at the Department of Pathology, Dhaka Medical College Hospital (DMCH), Dhaka from March 2021 to February 2023. Purposive sampling technique was followed. A total of 97 formalin-fixed, paraffin-embedded (FFPE) tissue blocks from patients who underwent surgical resection for colorectal adenocarcinoma at DMCH during this timeframe of all age and both sexes were included. Cases with inadequate tissue blocks, autolysis, or those pre-treated with neoadjuvant chemoradiotherapy were

excluded to ensure accurate immunohistochemical evaluation. Clinicopathological data for each patient, including age, sex and relevant others were retrieved from hospital medical records. Cases were reevaluated for tumor location, macroscopic type, histological type, tumor grade, pathological T, N, and M stages, lymphovascular and perineural invasion. Variables of this study were including age and sex of the patients, tumor grade, pathological T-stage, location, histologic type, lymph node metastasis (N-stage), lymph vascular invasion (LVI) and perineural invasion (PNI). Ethical approval for the study were obtained from the Institutional Review Board (IRB) of Dhaka Medical College. WT1 immunohistochemistry was performed at the Immunohistochemistry(IHC) Laboratory of Bangladesh Medical University, Dhaka. IHC was performed on 4-µm thick sections from selected FFPE blocks. Sections were deparaffinized, rehydrated, and subjected to antigen retrieval. Endogenous peroxidase activity was blocked, followed by incubation with a primary antibody against WT1 (clone and dilution to be specified, e.g., clone 6F-H2, Dako, 1:100 dilution). Visualization was achieved using a diaminobenzidine (DAB) chromogen system. Negative and positive controls were run concurrently with each batch of staining.WT1 immunoexpression was assessed semi-quantitatively by two independent histopathologists. Both nuclear and cytoplasmic staining were recorded.

***WT1 Immunoexpression interpretation*:**

- Presence/Absence: Whether WT1 protein is detected in tumor cells (both nuclear and cytoplasmic).
- Proportion Score: Percentage of nuclear-positive tumor cells (e.g., categorized as 0, <10%, 10-50%, 50-80%, >80%).
- Intensity Score: Average intensity of nuclear staining (e.g., graded as 0: no staining, 1: weak, 2: moderate, 3: strong).
- Final Composite Score: Calculated by multiplying the proportion and intensity scores (e.g., range 0-12), categorized as (range 0-6 as Low expression and (range 7-12 as High expression).[8,18]

Statistical analysis was performed using SPSS software (version 25.0). Chi-square test or Fisher's exact test were used to analyze associations between WT1 expression and clinicopathological variables. A p-value of <0.05 were considered statistically significant.

Results

In the current study among the 97 colorectal adenocarcinoma cases studied, 55% (n=53) showed low WT1 immunoexpression, while 45% (n=44) exhibited high expression (Table I).

Table I: Distribution of WT1 Immunoexpression among study cases (n=97)

WT1 Immunoexpression	Frequency(n)	Percentage (%)
Low	53	55%
High	44	45%
Total	97	100%

WT1 expression was not significantly associated with age (p=0.197) or gender (p=0.3986). Both low and high WT1 expressions were distributed across all age groups and genders without a statistically significant pattern (Table II).

Table II: Association of WT1 immunoexpression by patient demographics (n = 97)

Variables	WT1 Low Expression (n, %)	WT1 High Expression (n, %)	Total	P-value (Test)
Age (years)				0.197 (Chi-square)
< 50	15 (15.5%)	19 (19.6%)	34	
50–64	30 (30.9%)	17 (17.5%)	47	
≥ 65	8 (8.2%)	8 (8.2%)	16	
Gender				0.3986 (Chi-square)
Male	37 (38.14%)	27 (27.84%)	64	
Female	16 (16.49%)	17 (17.53%)	33	

A significant association was found between WT1 expression and both histological type (p=0.0214) and histological grade (p=0.0012). High WT1 expression was more common in mucinous and poorly differentiated adenocarcinomas, while low expression was predominant in conventional and well-differentiated types (Table III).

Table III: Association of WT1 immunoexpression by histological type and grade (n = 97)

Variables	WT1 Low Expression (n, %)	WT1 High Expression (n, %)	Total	P-value (Test)
Histological Type				0.0214 (Fisher's exact test)
Conventional Adenocarcinoma	52 (53.61%)	37 (38.14%)	89	
Mucinous Adenocarcinoma	1 (1.03%)	7 (7.22%)	8	
Histological Grade				0.0012 (Chi-square)
Well-differentiated	22 (22.68%)	8 (8.25%)	30	
Moderately differentiated	30 (30.93%)	26 (26.80%)	56	
Poorly differentiated	1 (1.03%)	10 (10.31%)	11	

WT1 expression was significantly associated with both pathological T-stage (p=0.0007) and N-stage (p=0.0003), with high WT1 expression more frequently observed in advanced stages (pT3, pT4, pN2). However, there was no significant association between WT1 expression and lymphovascular invasion (p=0.3353) or perineural invasion (p=1.0000) (Table IV).

Table IV: Association of WT1 immunoexpression by pathological T- stage, N-stage, lymph vascular invasion and perineural invasion (n=97)

Variables	WT1 Low Expression (n)	WT1 High Expression (n)	Total (N=97)	P-value
Pathological T-stage				
pT2	29 (29.90%)	8 (8.25%)	37	
pT3	22 (22.68%)	30(30.9%)	52	0.0007[s](Chi-square)
pT4	2 (2.06%)	6 (6.19%)	8	
Pathological N-stage				
pN0	14 (14.43%)	4 (4.12%)	14	0.0003[s]
pN1	36 (37.11%)	24 (24.74%)	64	(Chi-square)
pN2	3 (3.09%)	16 (16.49%)	19	
Lymph vascular Invasion (LVI)				
Present	8 (8.25%)	3 (3.09%)	11	0.3353[ns] (Fisher's exact test)
Absent	45 (46.39%)	41 (42.27%)	86	
Perineural Invasion (PNI)				
Present	1 (1.03%)	1 (1.03%)	2	1.0000 [ns] (Fisher's exact test)
Absent	52 (53.61%)	43 (44.33%)	96	

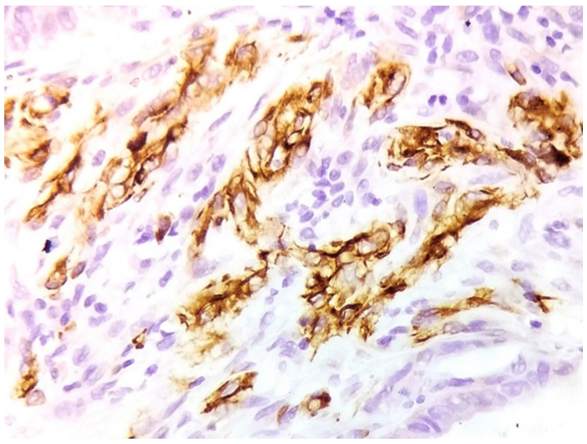

Figure 1. Photomicrograph of poorly differentiated adenocarcinoma showing high staining of WT1 (IHC X400)

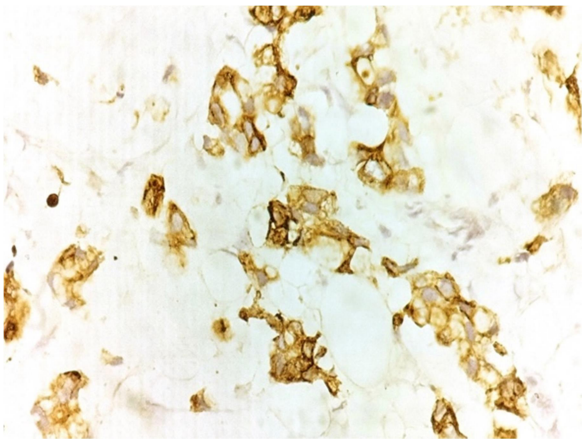

Figure 2. Photomicrograph of mucinous (poorly differentiated) adenocarcinoma showing high staining of WT1 (IHC X400)

Discussion

WT1 expression was detected in a substantial number of cases, with 45% exhibiting high expression and 55% low expression. This distribution aligns with findings from previous studies reporting WT1 expression across various solid tumors, including colorectal carcinoma, supporting its possible involvement in tumor development and progression.[8,9,18]

A significant association was found between high WT1 expression and adverse histopathological parameters, particularly histological type ($p = 0.0214$), histological grade ($p = 0.0012$), pathological T-stage ($p = 0.0007$), and N-stage ($p = 0.0003$). Notably, high WT1 expression was more prevalent in poorly differentiated tumors. These findings are consistent with studies by Xu et al. and Gao et al., who reported similar correlations between WT1 expression and higher histological grades and advanced TNM stages in CRC, implicating WT1 in aggressive tumor behavior and dedifferentiation.[12,13]WT1 in these contexts may affect the regulatory effects in CRC pathogenesis on differentiation pathways and proliferative signals, promoting an undifferentiated and invasive tumor phenotype.[15]

Moreover, the positive association between WT1 expression and advanced T and N stages suggests its role in local invasion and lymphatic spread. Similar patterns were noted in a meta-analysis by Qi et al., which found that WT1 overexpression correlated with advanced tumor stages and poor prognosis in multiple cancers.[15] WT1 may exert its pro-invasive effects through regulation of genes involved in adhesion, migration, and extracellular matrix remodeling, facilitating tumor progression.[5]

A notable finding was the significant association of WT1 overexpression with mucinous adenocarcinoma. This histological subtype of CRC is known for distinct molecular characteristics and a poorer prognosis compared to conventional adenocarcinomas.[16,17] Our observation suggests that WT1 may contribute to the mucinous differentiation pathway or reflects the aggressive nature of this subtype. Although few studies have specifically explored WT1's role in mucinous CRC, this association underscores the need for further research into its molecular function in this context.

In our study, WT1 expression did not show a statistically significant association with patient demographics (age, gender), tumor location, macroscopic growth pattern, lymphovascular invasion (LVI), or perineural invasion (PNI). Our findings contrast with those of Sangkhathat **et al.**[9] and Bejrananda **et al.**[18] who similarly reported no significant association between WT1 expression and tumor location and differentiation in colorectal cancer. In our study, however, no such correlation was observed with patient demographics or tumor location, suggesting possible population-based or methodological differences. In contrast to Al-Sukhni et al.[17]who emphasized the strong prognostic importance of lymphovascular invasion (LVI) and perineural invasion (PNI) in colorectal cancer, our study did not find a significant association between WT1 expression and either LVI or PNI. This difference may be due to the limited number of LVI and PNI-positive cases in our sample, reducing the ability to detect a true relationship. It may also suggest that WT1 influences tumor progression through different biological mechanisms than those directly causing LVI or PNI. Differences in findings across studies could be attributed to methodological variations, including differences in antibody clones, immunohistochemistry protocols, scoring systems, or sample heterogeneity.[14]

This study adds valuable regional data on WT1 immunoexpression in CRC from Bangladesh and highlights WT1's potential role as a prognostic biomarker.

Conclusion

WT1 immunoexpression was significantly associated with adverse clinicopathological features in colorectal adenocarcinoma, suggesting its role in tumor aggressiveness. These findings highlight WT1 as a potential prognostic biomarker.

Limitation

This study was limited by its single-center design and relatively small sample size, which may affect generalizability.

Recommendation

Further multicenter studies with larger cohorts and integrated molecular analysis are recommended to validate WT1 as a prognostic biomarker in colorectal adenocarcinoma.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram J, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021 May;71(3):209-49.
2. Dekker E, Tanis PJ, Vleugels ME, Kasi PM, Wallace MB. Colorectal cancer. Lancet. 2019 Aug 17;394(10207):1467-80.
3. Dienstmann R, Salazar R, Tabernero J. Personalizing Colon Cancer Treatment: The Clinical Promise of Molecular Biomarkers. J Clin Oncol. 2015 Feb 1;33(15):1701-18.
4. Call KM, Glaser T, Ito CY, Buckler AL, Pelletier J, Haber DA, et al. Isolation and characterization of a zinc finger polypeptide gene at the human chromosome 11p13 Wilms' tumor locus. Cell. 1990 May 4;60(4):509-20.
5. Roberts SG, Brieger A, Piskol R, Zuo X, Xu X, Zhang H, et al. WT1 as a therapeutic target in cancer. Cancer Treat Rev. 2017 Jul;58:91-100.
6. Cilloni D, Saglio G. WT1 as a new prognostic factor and a suitable molecular target in acute leukaemia. Haematologica. 2004 Oct;89(10):1243-7.
7. Lo CH, Ma W, Chen SC, Su JL, Chang WC, Lai MJ, et al. Wilms' tumor 1 expression is correlated with triple-negative breast cancer. Mod Pathol. 2016 Nov;29(11):1327-37.
8. Oji Y, Yamamoto H, Nomura M, Nakano Y, Abeno S, Ikeba A, et al. Overexpression of the Wilms' tumor gene WT1 in colorectal adenocarcinoma. Cancer Sci. 2003;94(8):712–7.
9. Sangkhathat S, Maneechay W, Chaiyapan W, Kanngern S, Boonpipattanapong T. Association of Wilms’ tumor 1 gene single-nucleotide polymorphism rs16754 with colorectal cancer. Mol Clin Oncol. 2015;3(6):1401–5.
10. Fujimura S, Kuwano H, Hirohashi Y, Tsuboi A, Oji Y, Oka Y, et al. Wilms tumor 1 gene expression in pancreatic cancer. Hum Immunol. 2007 Aug;68(8):722-6.
11. Oji Y, Tsuboi A, Oka Y, Kumagai T, Soma T, Nakajima H, et al. Wilms' tumor 1 (WT1) protein induces apoptosis in leukemia cells. Jpn J Cancer Res. 1999 May;90(5):548-55.
12. Xu D, Zhao Y, Zhang Q, Guo Y, Hou M, Zhang X. Clinicopathological significance of WT1 protein in colorectal cancer. Oncol Lett. 2015 Jun;9(6):2613-8.
13. Gao X, Wu T, Han N, Li H, Chen Z, Gu L, et al. Expression of Wilms' tumor 1 (WT1) in colorectal carcinoma and its clinicopathological significance. Oncol Lett. 2017 Jan;13(1):315-20.

14. Ramos-Vara JA. Immunohistochemistry: methods and applications. Vet Pathol. 2005 Jan;42(1):1-16.
15. Qi XW, Zhang F, Wu H, Liu JL. Wilms' tumor 1 (WT1) expression and prognosis in solid cancer: a systematic review and meta-analysis. *Oncotarget*. 2015;6(7):4723–37.
16. Li Y, Li X, Li J. Mucinous adenocarcinoma: A unique clinicopathological subtype in colorectal cancer. *World J Gastrointest Oncol*. 2022;14(1):1–16.
17. Al-Sukhni E, Attwood K, Gabriel EM, LeVea CM, Kanehira K, Nurkin SJ. Lymphovascular and perineural invasion are associated with poor prognostic features and outcomes in colorectal cancer: A retrospective cohort study. *Int J Surg*. 2017;37:42–9.
18. Bejrananda T, Phukaoloun M, Boonpipattanapong T, Wanitsuwana W. WT1 expression as an independent marker of poor prognosis in colorectal cancers. *Cancer Biomark*. 2010;8(1):35–42.