The Journal of Teachers Association

ISSN 1019-8555 (Print) & ISSN 2408-8854 (Online)
Frequency: Bi-Annual
DOI: https://doi.org/10.62469/taj.v037i01.024

OPEN ACCESS
Website: www.tajrmc.com
Email: editor@tajrmc.com
Volume-37 | Issue-1 | Jan-Jun 2024

Expression of WT1 (Wilms' Tumor 1) Marker in Breast Cancer and Its Relationship with Histological Grading

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Abstract: ***Background:*** Breast cancer is the most common cancer among women all over the world. Recently many scientists have been trying to evaluate the usefulness of the Wilms' tumor protein (WT1) as a promising therapeutic target in breast cancer. ***Methods:*** This cross-sectional study was conducted in the Department of Pathology, Rajshahi Medical College, Rajshahi over a period of 12 months from March 2022 to February 2023. A total of 65 biopsy samples from the patients with a histopathological diagnosis of breast carcinoma were included in the study. Expression of WT1 was evaluated in formalin fixed and paraffin embedded specimens by immunohistochemistry. WT1 expression was categorized as negative, mild, moderate and strong. ***Results:*** The mean (±SD) age of the patients was 48.4 (±6.6) years and range 36-65 years. WT1 expression was present in 28/65 (43.1%) patients and WT1 staining expression was found to be mild, moderate and strong in 16.9%, 13.9% and 12.3% of the samples, respectively. Positive WT1 expression was significantly higher in grade I tumor than in the grade II and III tumor (78.6% versus 33.3%, p=0.01). ***Conclusions:*** WT1 expression in breast carcinoma is found to be a common occurrence and its expression has been negatively related with tumor grade.

Keywords: Breast cancer and Wilms' tumor protein expression.

Original Researcher Article

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How to cite this article:
Ferdous, T. N., Ferdousi, S., Maua, J., Awal, R., Chowdhury, G; Expression of WT1 (Wilms' Tumor 1) Marker in Breast Cancer and Its Relationship with Histological Grading. Taj 2024;37 (1): 161-167.

Article history:
Received: December 15, 2023
Revised: January 21, 2024
Accepted: February 26, 2024
Published: April 26, 2024

Article at a glance:

Study Purpose: *The purpose of this study was to evaluate the expression of WT1 in breast tumors and their relation with histological grading.*
Key findings: *Positive WT1 expression was significantly higher in grade I tumors than in grade II and III tumors (p=0.01).*
Newer findings: *WT1 expression decreased significantly with increasing grade of tumor.*
Abbreviations: *ER: Estrogen receptor, HPF: High power field, IHC: Immunohistochemistry, IGF-IR: Insulin-like growth factor 1 receptor, MIB-1: Mind bomb E3 ubiquitin protein ligase 1, SD: Standard deviation, TCF: T-cell factor and WT1: Wilms' tumor gene.*

INTRODUCTION

Worldwide breast cancer is the most commonly diagnosed cancer and its burden has been increasing over the past few decades. Breast cancer continues to impact the global number of cancer deaths significantly. Globally over 2.3 million new cases and 685,000 deaths from breast cancer occurred only in 2020 which is alarming for us. The burden of breast cancer is predicted to increase over 3 million new cases and 1 million deaths yearly because of population growth and aging alone by 2040.[1] According to data on 2021, breast cancer is a fatal disease among women in Bangladesh and has become a hidden burden that accounts for 69% of death among women.[2] High morbidity rate is common in breast cancer patients

as like as other aggressive cancers. The 5 years survival rate is higher than 10 years survival rate and it is approximately 91% and 84% respectively, according to the American Cancer Society's biennial update on female breast cancer statistics.[3] Due to advancing stage of carcinoma of breast, the survival rates are decreased.[4,5] So, early detection by applying different diagnostic test application such as immune markers and target-based therapy of breast cancer can improve survival rate.

The biological events such as occurrence, invasion and metastasis of malignant tumors are regulated at multiple levels by various factors. Imbalance between oncogenes and tumor suppressor genes is one of the key factors. It is necessary to explore new functions of existing oncogenes or tumor suppressor genes to clarify the mechanism of malignant tumor occurrence and progression which will be helpful to determine the effective diagnostic and therapeutic targets.[6,7] Currently a constant search for molecular markers to aid in the diagnosis of cancer is going on which ultimately will be helpful in prognosis of patients. To date, several genes and their products have been introduced to predict the prognosis of breast cancer patients, including WT1 gene.[8] WT1 gene is originally isolated as a tumor suppressor gene which spans about 50kb and is located at chromosome 11p13.[9] WT1 is over expressed in several solid tumors such as breast and other non-solid tumors, suggesting its role as an oncogene.[10] WT1 is an immunohistochemistry (IHC) marker. IHC is an important auxiliary method for pathologists as it specifically visualizes distribution and amount of a certain molecule in the tissue using specific Ag-Ab reaction. A positive test means that a marker or receptor is found on the cell during the biopsy or indicates a certain change in the protein of the tumor. WT1 may play a role to maintain the biological characteristics of stem cells in breast cancer. WT1 is usually positive in some smooth muscle cells of the arterial wall and epithelial cells of the terminal duct lobular unit.11 WT1 staining is also more evident in ER-positive premenopausal tumor compared with ER-negative tumors and negatively associated with histopathological grade. Usually, low ER expression is found with increasing rate, so WT1 expression is also dropped as grade increased.[11]

In most solid tumors, WT1 is over-expressed and acts as a potential target antigen for cancer immune therapy due to its specificity, immunogenicity, oncogenicity and therapeutic functions.[12] Combination of neoadjuvant chemotherapy or a transtuzumab-chemotherapy impair or blunt the humoral response. Impairment occurs due to either co-administration of corticosteroids and/or the chemotherapies themselves. Concurrent administration of WT1 immunotherapeutic and standard chemotherapy is well tolerated. That causes induction of WT1 specific antibody response in breast cancer patient. WT1 protein is highly immunogenic and could serve as a therapeutic target. Currently, efficacy and adverse reactions of WT1 vaccines are under evaluation but phase I or II clinical trials are undergoing against leukemia, breast cancer, lung cancer and pancreatic cancer.[8] Though WT1 acts as tumor suppressor gene in breast cancer but it is controversial as others suggesting that WT1 may act as an oncogene. So, it is important to determine whether WT1 plays a significant role in breast cancer and explores its molecular mechanism or not. This study was conducted to evaluate the expression of WT1 in breast cancer and find out its relationship with histological grading.

METHODS

This was a cross-sectional type of descriptive study in the Department of Pathology, Rajshahi Medical College, Rajshahi from March 2021 to February 2023to evaluate the expression of WT1 in breast tumors and their relationship with histological grading. Paraffin blocks of histopathologically diagnosed cases of invasive breast carcinoma at the Department of Pathology, Rajshahi Medical College and BSMMU during specified time duration were the study population. Approval from the Ethical Review Committee (ERC) was obtained prior to the commencement of the study and a purposive sampling technique was used to collect data from 65 samples. Cases of breast cancer receiving chemotherapy or irradiation and poorly preserved specimens were excluded from the study.

Consulting with the guide and reviewing the previous published literature, the questionnaire was developed for the study. In this study, 65 cases of paraffin blocks were graded according to

Nottingham modification of Bloom-Richardson system (assigns a score of 1 to 3 for each parameter; degree of tubular formation, nuclear pleomorphism and mitosis. The final histological grade is based on a sum of the individual scores of the three parameters: 3-5 = Grade 1; 6 or 7 = Grade 2 and 8 or 9 = Grade 3). IHC with WT1 was done for immunohistochemical evaluation. For immunohistochemistry, 3-4 micrometer thick sections of formalin fixed, paraffin-embedded tissues were used. The sections were incubated with a MIB-1 monoclonal antibody in appropriate dilutions. Standard immunohistochemical method was applied for subsequent staining.

WT (F-6) mouse monoclonal antibody kit (Santa Cruz Biotechnology Cat #sc -7385; 1:200 dilution) for WT1 was used to determine the WT1 protein expression. Serous papillary carcinoma of ovary was taken as positive control for WT1. The fraction of positively stained tumor cells was scored semi-quantitatively after examining under 10 HPF (X 400) for each case. Both nuclear and cytoplasmic WT1 staining was scored. Immunohistochemical results for WT1 was scored as:

Negative: Fraction of positive stained tumor cells: < 10%.

Weak (1+): Fraction of positive stained tumor cells: 10-25%.

Moderate (2+): Fraction of positive stained tumor cells: 26-50%.

Strong (3+): Fraction of positive stained tumor cells: > 50%.[16]

Data were entered into excel worksheet to generate a master sheet. Data processing and analysis were done via Statistical Package for the Social Sciences (SPSS) software, version 28.0. Categorical variables were summarized by using number and percentage while continuous variables by mean ± standard deviation (SD). Chi square (χ^2) test was used to see the relationship between tumor grade and WT1 expression. The statistical significance was evaluated as appropriate probability level $p < 0.05$ for all tests.

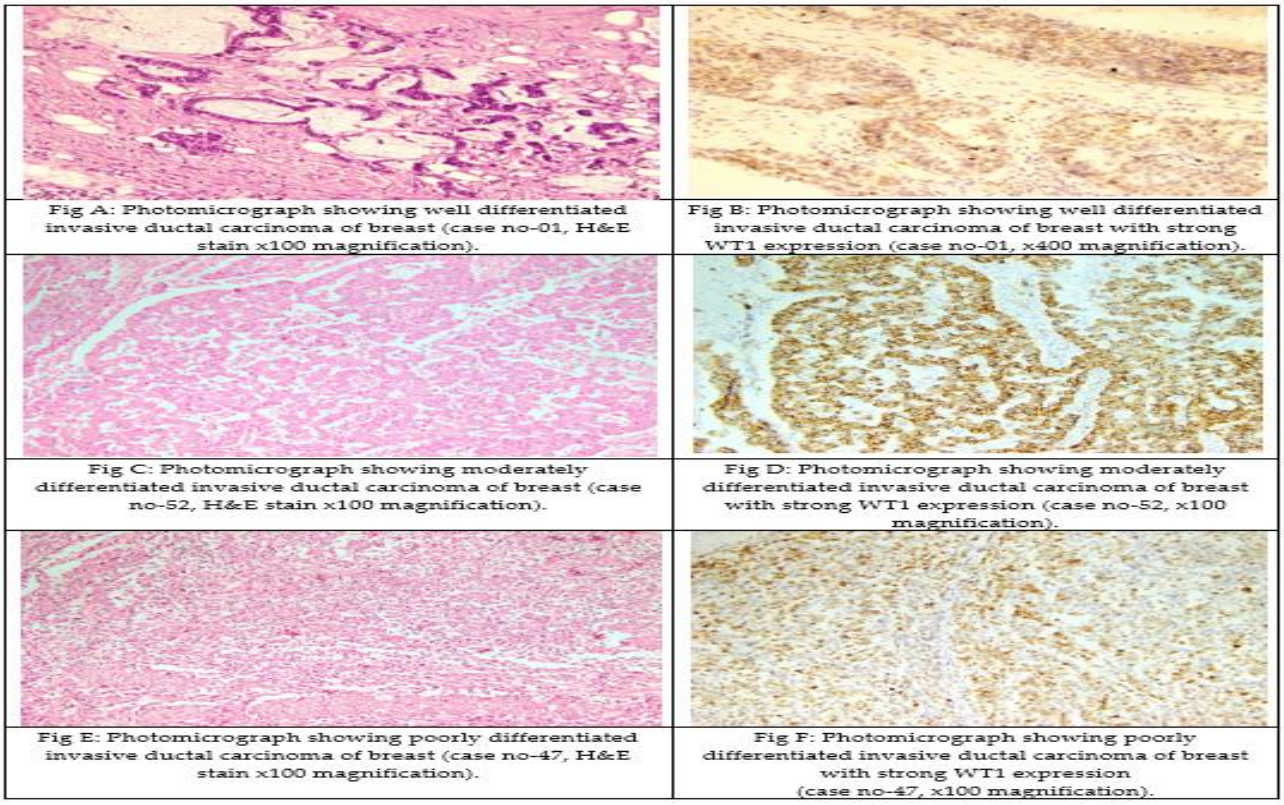

Fig A: Photomicrograph showing well differentiated invasive ductal carcinoma of breast (case no-01, H&E stain x100 magnification).

Fig B: Photomicrograph showing well differentiated invasive ductal carcinoma of breast with strong WT1 expression (case no-01, x400 magnification).

Fig C: Photomicrograph showing moderately differentiated invasive ductal carcinoma of breast (case no-52, H&E stain x100 magnification).

Fig D: Photomicrograph showing moderately differentiated invasive ductal carcinoma of breast with strong WT1 expression (case no-52, x100 magnification).

Fig E: Photomicrograph showing poorly differentiated invasive ductal carcinoma of breast (case no-47, H&E stain x100 magnification).

Fig F: Photomicrograph showing poorly differentiated invasive ductal carcinoma of breast with strong WT1 expression (case no-47, x100 magnification).

RESULTS

The maximum 38 (58.5%) patient's age were between 41-50 years, followed by 16 (24.6%) patients aged 51-60 years. The mean age of the patients was 48.4±6.6 with minimum and maximum age of the patients in this study were 36 years and 65 years, respectively (Table-01).

Table 1: Distribution of the study population according to age (n=65)

Age (Years)	Frequency	Percentage (%)
31-40	8	12.3
41-50	38	58.5
51-60	16	24.6
>60	3	4.6
Total	65	100.0
Mean ± SD	48.4±6.6	
Range (Min-Max)	36-65	

Out of 65 patients, WT1expression was present in 28 (43.1%) patients. On the other hand, 37 (56.9%) patients had no expression of WT1 (Figure-I).

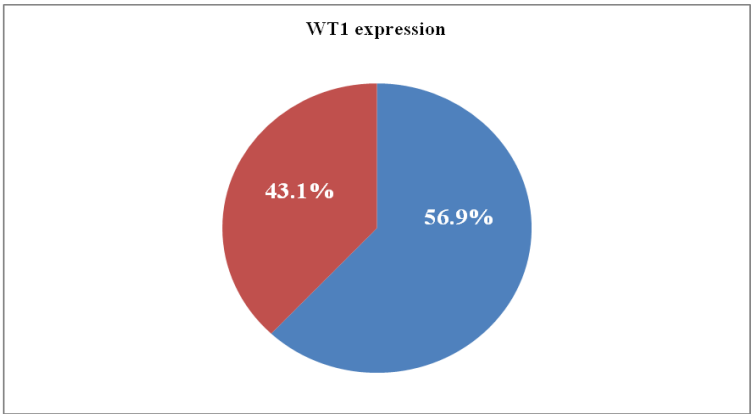

Figure 1: Expression of WT1 in study population (n=65)

WT1 expression was mild in 11 (16.9%) of the samples followed by moderate in 9 (13.9%) and strong in 8 (12.3%) samples (Figure-II).

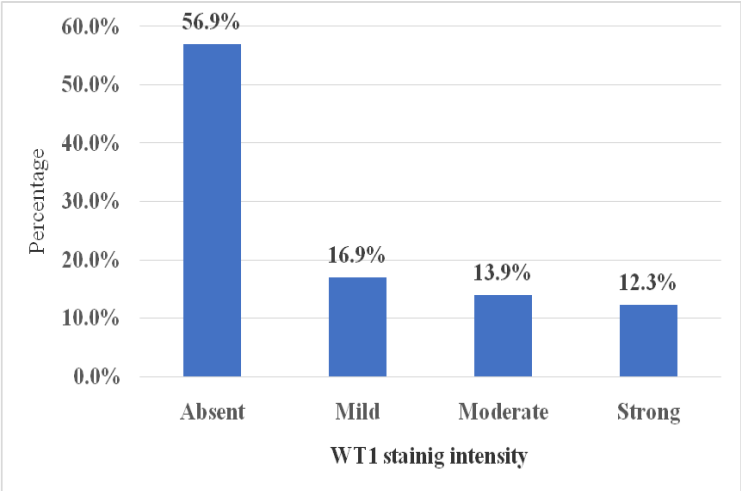

Figure 2: Distribution of the study population according to the WT1 expression (n=65)

Out of 14 Grade I tumor, 11 (78.6%) had positive WT1 expression. In grade II and Grade III tumor, only 33.3% were positive for WT1. The results indicated that WT1 expression was significantly more in grade I than from Grade II and III (Table-02).

Table 2: WT1 expression status in different tumor grades (n=65)

Tumor grade	n	WT1 expression status		
		Absent	Present	p-value
Grade I	14	3 (21.4)	11 (78.6)	
Grade II	30	20 (66.7)	10 (33.3)	0.010
Grade III	21	14 (66.7)	7 (33.3)	

* Chi-square test, significant statistically

Out of 14 Grade I tumor, strong WT1 stain was detected in 5 (35.7%) of the sample, which was respectively, 1 (3.3%) and 2 (9.5%) in Grade II and Grade III tumors (Table-03).

Table 3: Degree of WT1 expression in breast tumor grades (n=65)

Tumor grade	n	WT1 staining expression				
		Absent	Weak	Moderate	Strong	p-value
		0	10-25%	26-50%	> 50%	
Grade I	14	3 (21.4)	4 (28.6)	2 (14.3)	5 (35.7)	0.012
Grade II	30	20 (66.7)	3 (10.0)	6 (20.0)	1 (3.3)	
Grade III	21	14 (66.7)	4 (19.0)	1 (4.8)	2 (9.5)	

* Chi-square test, significant statistically

DISCUSSION

Breast carcinoma is a heterogeneous disease with variable biological and clinical determinants. Over the last decade, despite the plethora of multigene molecular tests are becoming available, immunohistochemistry still remains as the workhouse for most pathologists in the diagnostic work-up of breast cancer. A wide variety of immunohistochemistry-based tumor markers have been studied for better characterization of breast carcinoma.[13] The age of the patients, anatomical characteristics of the tumor, histopathological findings and immunohistochemistry WT1 staining scores were noted in a preformed data sheet. Regarding age distribution, present study demonstrated that more than half of the patients (58.5%) were in 41-50 years age group with a mean age of 48.4 (±6.6) years ranging from 36-65 years.

The peak incidence of carcinoma breast observed by Pervin *et al*., in the 4th decade which is almost similar to that of this study.[14] This indicates carcinoma of breast is more common at early age in our setup. In comparison to developed countries in Asia and the rest of the world, the incidence of breast cancer is lower but mortality is significantly higher in developing Asian countries and patients are about one decade younger in developing countries than in developed nations.[15] Regarding tumor grade out of 65 cases of breast carcinoma, the most frequent tumor grade was Grade II 30 (46.2%), followed by Grade III 21 (32.3%) and Grade I 14 (21.5%). Proportion of patients with grade I tumor were also the lowest in other similar studies.[16,17] WT1 is overexpressed in approximately 90% of breast cancers.[18]

Out of 65 cases, WT1 expression was present in 28 (43.1%) of the patients in the present study. On the other hand, 37 (56.9%) patients had negative expression of WT1. The WT1 staining expression was mild in 11 (16.9%) of the samples, followed by moderate 9 (13.9%) and strong 8 (12.3%). The corresponding positivity rates for WT1 expression were 41.1%, 48.5% and 60% respectively, in the study of Choi *et al*.,[17] Camci *et al*.,[16] and Loeb *et al*.[10] In the present study, a negative association was identified between WT1 expression and histopathological Grade. WT1 expression decreases with histopathological grade of breast tumor. Among 14 cases of grade I tumor, 11 (78.6%) had positive WT1 expression. In grade II and grade III tumors, only 33.3% were positive for WT1. In the study of McGregor *et al*.,[11] WT1 expression decreased with rising histopathological grade. The total numbers of WT1-positive cells were all higher

in Grade I tumors. In addition, the numbers of vessels and WT1-positive vessels were also higher in grade I breast cancers compared to Grade II tumors.

Moreover, the percentage of WT1-positive vessels within the tumors also decreased as tumor grade increased.[11] In the study of Camci *et al*.,[16] the WT1 positivity rate in grade I, II and III were 57%, 29% and 35%, respectively. Similar observation was noted by Choi *et al*. The results of the present study and the mentioned studies indicate that WT1 expression by tumor is more evident in low grade.[17] Zhang *et al*.,[8] reported that WT1 inhibited breast cancer cell growth by regulating the stability of β-catenin. WT1 inhibits the transformed phenotype of breast cancer cells and down-regulates the betacatenin/TCF signaling pathway through destabilization of beta-catenin. The IGF-1 receptor is important for the development and progression of breast cancer and IGF-1 receptor is downregulated by some transcription factors like WT1 protein. Expression of growth factors increases after WT1 inactivation and results in cell proliferation.

Regulation of WT1 transcription is dependent on wild type p53. In the absence of this, WT1 will act as a transcription activator rather than suppressor.19 Reizner *et al*.,[19] showed that WT1 suppresses IGF-IR gene transcription in breast cancer cells and there was an association with estrogen receptor alpha. This finding suggested the potential role of the tumor suppressor activity of WT1 in breast cancer. There were some limitations of the study such as relatively small sample size and at a short period of time. As present study samples were collected from RMCH, it does not reflect the picture of the whole country.

CONCLUSION

WT1 marker expression in low grade breast carcinoma is higher than in higher grade carcinoma. So, immunohistochemical expression of WT1 has the potential to be used as a predictive as well as prognostic marker for invasive breast carcinoma.

Acknowledgements

Contribution of all the stuffs in histopathology laboratory, Department of Pathology, Rajshahi Medical College, Rajshahi and BSMMU in data collection of the study were acknowledged. Authors' contributions: NTF, SF, JM: Concept and design, data acquisition, interpretation, drafting and final approval. NTF, RA and GC: Data acquisition, interpretation, drafting, final approval and agree to be accountable for all aspects of the work.

Declarations

Funding: The authors received no financial support for the research, authorship and/or publication of this article.

Conflict of interest: Authors declared no conflict of interest.

Ethical approval: Ethical approval of the study was obtained from the Ethical Review Committee, Rajshahi Medical College, Rajshahi. All the study methodology was carried out following the relevant ethical guidelines and regulations.

Consent for publication: Taken.

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The Journal of Teachers Association
Abbreviated Key Title: TAJ
Official Journal of Teachers Association Rajshahi Medical College