RESEARCH ARTICLE

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Polo-Like Kinase 1(PLK1) Immunohistochemical Expression in Triple Negative Breast Carcinoma: A Probable Therapeutic Target

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Abstract

Background: Breast cancer is the most commonly diagnosed female cancer and is a major cause of cancer-related deaths in women. Triple-negative breast cancer (TNBC) is defined as ER, PR and HER2 negative, which are characterized by rapid progression with low survival rates with limited therapeutic choices. Polo-like kinase 1 protein acts as a cell division regulator which is highly expressed in many tumors making it a potentially valuable target for antiproliferative therapies. In this study we tried to evaluate the value of this marker as a possible therapeutic target in TNBC. **Methods:** This research studied the immunohistochemical expression of PLK1 done on 49 paraffin blocks of TNBC female patients and then correlated with the different clinicopathological parameters. **Results:** Our results showed high PLK1 expression in 91.9% of cases. Most of the high grade tumors showed high PLK1 high score (76.9%). All cases showing lymph node metastasis showed high PLK1 expression, implying a statistically significant correlation between PLK1 expression and tumor grade as well as N stage. **Conclusion:** PLK1, although a negative prognostic factor, but is a promising therapeutic target for treating TNBC patients.

Keywords: PLK1- triple negative- breast- carcinoma

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Introduction

Breast cancer (BC) is the most commonly diagnosed female cancer and is a major cause of cancer-related deaths in women (Burstein et al., 2015). In Egypt, BC represents 38% of all female cancers according to the National Cancer Institute, Cairo university (Donizy et al., 2016).

Prognosis and treatment of BC depend on its histological grade, stage, and 3 major immunohistochemical markers: Estrogen receptor (ER), Progesterone receptor (PR) and HER2. Triple-negative breast cancer (TNBC) is defined as ER, PR and HER2 negative. It represents about 15% to 20% of all breast cancer patients (Elston, and Ellis, 2002) who often present with visceral involvement, early recurrence and rapid progression with low survival rates providing limited window of treatment opportunity, moreover exclusion of both hormonal therapy and trastuzumab (herciptin) treatment (Ferlay et al., 2015). TNBC is responsible for significant number of breast cancer-associated deaths due to lack of molecular-targeted therapy. Up till now, a targeted therapeutic approach for the treatment of TNBC patients does not exist, and these patients receive standard chemotherapy only (Giordano et al., 2019). Therefore focusing on the presence of cancer-associated proteins, would help identify relevant new candidate markers for therapeutic targets. In an attempt to offer the best medical opportunity for every patient, modern medicine is targeted towards personalized therapies, which offers a balance between the best approach and avoiding undesired side effects resulting from aggressive treatment, which requires new ideas about drug development and the choice of patients for these drugs; therefore, novel prognostic factors are necessary (Ibrahim et al., 2014). Polo-like kinase 1 (PLK1), protein of the PLK family, is a serine-threonine kinase that plays a crucial role in cell division regulation, regulation of mitosis, induction of cytokinesis and a modulator of the DNA damage so it acts as a novel factor in the maintenance of genome stability during DNA replication. High PLK1 expression is observed within intensively proliferating normal tissues such as placenta and colonic epithelium and in many cancer, including gastric, colorectal, hepatocellular, prostate etc. Moreover, due to its being a controller of mitosis, PLK1 has become a potentially valuable target for antiproliferative therapies (King et al., 2012). Emerging experimental results are promising, and many anti-PLK1 agents are currently being investigated in clinical trials. However, no predictive factor has been specified so far that could be used as a reliable qualifier to the possible use of PLK1 inhibitor therapy in BC treatment

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(Koboldt et al., 2012). Immunohistochemical analysis of PLK1 expression in BC cells and the subsequent decision of initiating therapy or excluding the patient from therapy is of utmost importance, which of course requires verification in extensive, multicentre research studies (Liu et al., 2019).

Therefore, the aim of the present work was to evaluate the expression of PLK1 in TNBC in order to explore its role as a potential therapeutic target for these patients who are deprived of hormonal and Herceptin treatment.

Materials and Methods

This is a retrospective study which includes 49 paraffin embedded tumor tissue sections collected from modified radical mastectomy specimens of female patients with triple negative breast cancer confirmed by reports of immunohistochemical markers. Cases who received neo-adjuvant therapy; either hormonal or chemotherapy were excluded from the study. The data collected from the studied patients' reports included age of patient, size of tumor, LN status and metastatic disease status. The IHC reports include ER, PR, HER2 and Ki67 results for each case.

Histopathologic examination

The paraffin blocks of tumor sections were serially sectioned at 4 μm thickness and stained for pathological examination with routine Hematoxylin and Eosin stains. Tumors histological grading was performed according to the Nottingham Grading System (Nofech-Mozes et al., 2009). For further statistical evaluation, Grades 1 and 2 cases were considered as low grade, while grade 3 cases were considered as high grade.

Immunohistochemical examination

For the detection of PLK1, tissue sections were incubated with a monoclonal mouse antibody against PLK1 (BD Transduction Laboratories™; BD Biosciences, Franklin Lakes, NJ, USA) was diluted 1:500 in the Antibody Diluent with Background Reducing Components (DakoCytomation; Dako, Glostrup, Denmark) for 1 h at room temperature. Subsequent incubations involved biotinylated antibodies (15 min, room temperature) and a streptavidin-biotinylated peroxidase complex (15 min, room temperature) (LSAB 2 System-HRP; DakoCytomation; Dako). As a chromogen, 3,3′-diaminobenzidine (DakoCytomation; Dako) was used (10 min, room temperature). All sections were counterstained with Mayer's hematoxylin. A human colon tissue serves as a positive control.

PLK1 expression was evaluated and scored under (Olympus microscope model BX 53) microscope at low power then high power magnification PLK1 expression was evaluated using the semi-quantitative scale of the immunoreactive score (IRS), which calculates the percentage of reactive cells (no staining=0, <25%=1, 25–50%=2, 51–75%=3 and >75%=4) together with the intensity of staining (no staining=0, weak=1, intermediate=2 and strong=3), with the final result being the product of both variables. Consequently, nine possible scores (0, 1, 2, 3, 4, 6, 8, 9 and 12) were obtained. For subsequent statistical analyses, a two-grade scale system was used, allocating 0 points for expression of PLK1 <8 (low PLK1 immunoreactivity) and 1 for expression of PLK1 ≥8 (high PLK1 immunoreactivity) (Kinget al., 2012).

Statistical analysis

The clinical, histopathological and immunohistochemical data were transferred to the Statistical Package of Social Science (SPSS) Software program, version 25 to be statistically analyzed. Data was summarized using mean, standard deviation, frequency and percentages. Comparison between groups was then performed using Chi square test. A P value of ≤ 0.05 was considered statistically significant and of ≤ 0.001 were considered highly significant.

Results

This study included 49 female cases of invasive BC with triple negative immunohistochemical profile. The age of pateints ranged from 30 to 75 with mean 55±9.9. The most prevalent histological type was invasive BC no special type (IBC-NST) accounting for 65.4% of cases (32 cases), followed by BC with medullary pattern accounting for 16.3% of cases (8 cases). Two cases (4.1%) for each of mixed IBC-NST with invasive lobular carcinoma, carcinoma with osteoclast like stromal giant cells and metaplastic squamous cell carcinoma. Invasive papillary carcinoma, pleomorphic lobular carcinoma and carcinoma with secretory pattern were seen in one case for each (2%). As regards the histological grade, 36 cases (73.5%) were of low grade, while 13 cases (26.5%) were of high grade. Concerning the stage, the most prevalent T stage was T2 (31 cases, 63.3%) followed by T3 (14 cases, 28.5%) then T1 (4 cases, 8.2%). While, the most prevalent N stage was N0 (15 cases, 30.6%) followed by N1 (13 cases, 26.5%), N3 (11 cases, 22.5%) and finally N2 (10 cases, 20.4%). As for M stage, most of the cases were M0 (37 cases, 75.5%) while M1 was seen in only 12 cases (24.5%). Speaking of lymphovascular invasion (LVI) it was seen in only 12 cases (24.5%). Ki67 index was high in most of our studied cases (43 cases, 87.8%).

PLK1 expression in our studied cases

High PLK1 expression (defined as IRS score ≥8) was seen in 45/49 cases accounting for 91.9% of cases (Figures 1 and 2). On the other hand, low PLK1 expression (defined as IRS<8) was seen in only 4/49 cases accounting for 8.1% of cases (Figures 3 and 4). When the relation between the tumor histological type and PLK1 expression was studied, we found that 96.9% (31 cases) of IBC-NST showed high expression, as well as 62.5% (5 cases) of carcinoma with medullary features and 100% of all the other types, but unfortunately these results were statistically insignificant (p value 0.14). LVI didn't seem to be affected by the PLK1 expression as high IRS score was seen in the majority of cases showing present LVI as well as absent LVI (91.6% and 91.9% respectively). Again these results were statistically insignificant (p value 0.10).

Table 1. Relation between PLK1 Expression and Clinicopathological Variables with Significant Results

Clinicopathological parameter	PLK1 expression		Total		P value
	High	Low			
Grade					
High	10 (76.9%)	3 (23.10%)	13	49	0.02
Low	35 (97.20%)	1 (2.80%)	36		
N stage					
N0	11 (73.30%)	4 (26.70%)	15		
N1	13 (100%)	0 (0.0%)	13	49	0.02
N2	10 (100%)	0 (0.0%)	10		
N3	11 (100%)	0(0.0%)	11		

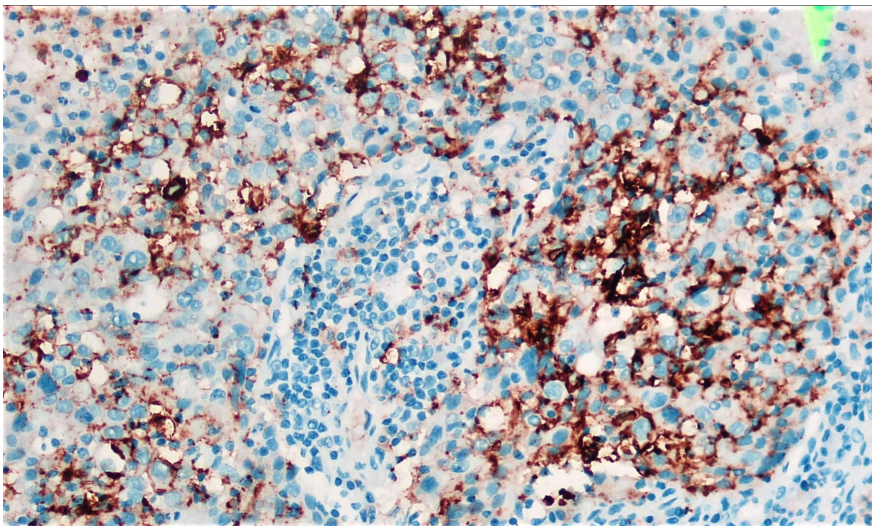

Figure 1. A Case of IBC with Medullary Features Showing Positive PLK1 High Score Expression (x200 Original Power).

Other insignificant relations were observed between PLK1 expression and T stage as well as M stage. (p values 0.09 and 0.2 respectively). Despite our observation that high PLK1 expression was seen in all low Ki67 cases and 90% of high ki67 cases but the results were insignificant (p value 0.4).

The significant relations found in our study are summarized in Table 1.

Discussion

BC is the most prevalent type of cancer in women worldwide (Burstein et al., 2015). In Egypt, BC accounts for (38%) of all female cancers according to the National

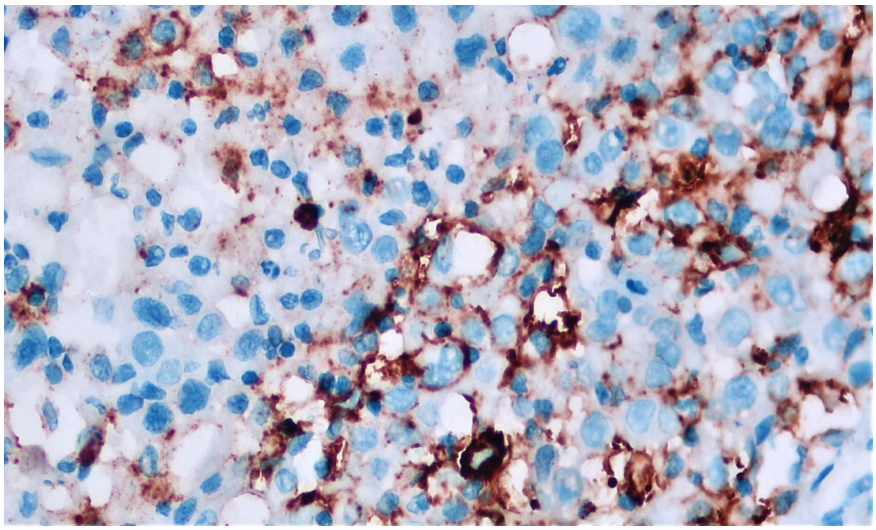

Figure 2. A Case of IBC with Medullary Features Showing Positive PLK1 High Score Expression (x400 Original Power).

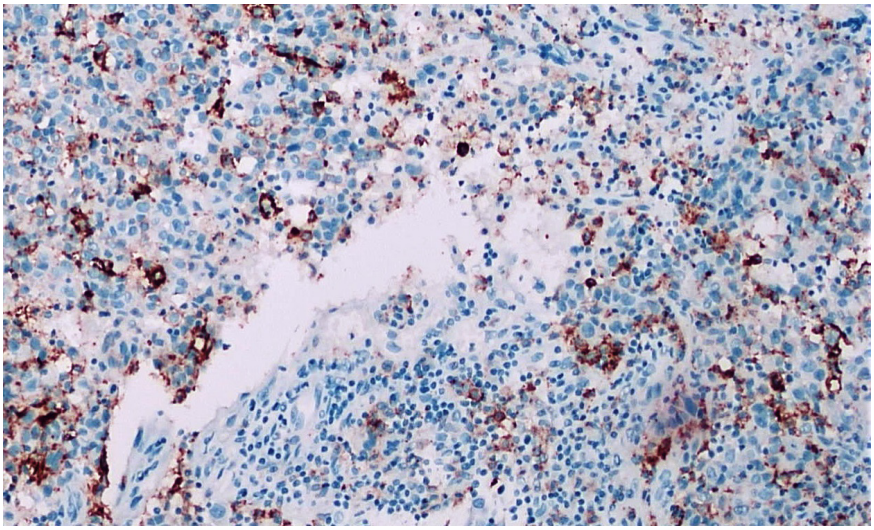

Figure 3. A Case of IBC-NST Showing Positive PLK1 Low Score Expression (x200 Original Power).

Cancer Institute, Cairo University (Donizy et al., 2016).

Triple-negative breast cancer (TNBC) represents 15-20% of all breast cancer (Elston, and Ellis, 2002), which is known to have bad prognosis and poor response to conventional chemotherapy with deprivation of hormonal and herciptin therapy as well (Sung et al., 2018). This leads to the urge of finding new therapeutic agents for this group of patients in particular. A potentially valuable agent is the antiproliferative therapy, that is why this research was done, to evaluate the use of PLK1 inhibitor, being an antiproliferative agent, as a reliable therapy in TNBC patients.

In the current study, PLK1 was expressed in all our studied cases, but with two different scores, high and low. High IRS score was seen in 91.9% while the remaining 8.1% of cases showed low ISR score. This high IRS score was seen in 79.9% of cases with high grade and 97.2% of cases with low grade. These results were statistically significant. Another statistically significant relation was observed between PLK1 overexpression and lymph node metastasis as all cases showing nodal metastasis showed high IRS.

Based on the fact that PLK1 is needed for mitotic entry induced by DNA damage, and based on the fact that PLK1 is a functional key gene in cell proliferation, and since it statistically correlates with an independent prognostic factor like lymph node metastasis (Ueda et al., 2019), we were not surprised by our results that show that PLK1 is a poor prognostic factor. But we were expecting more statistically significant correlations with other factors like LVI, T and M stages and Ki67. This may be attributed to small sample size of our study. Also the prevalence of low grade tumors in our study (73.5%), absence of distant metastasis (M stage) in 75.5% of cases and absence of LVI in also 75.5% of cases which may have affected our results. To our knowledge very few researches have extensively studied the expression of PLK1 in TNBC and most of the available studies were conducted on BC with slight referral to TNBC as one of their observed variants. Never the less, they all agreed that PLK1 is a poor prognostic factor. One of these studies was that done by Sung et al., (2018) who assessed PLK1 expression in five chemoresistant BC and observed its overexpression in tamoxifen-resistant

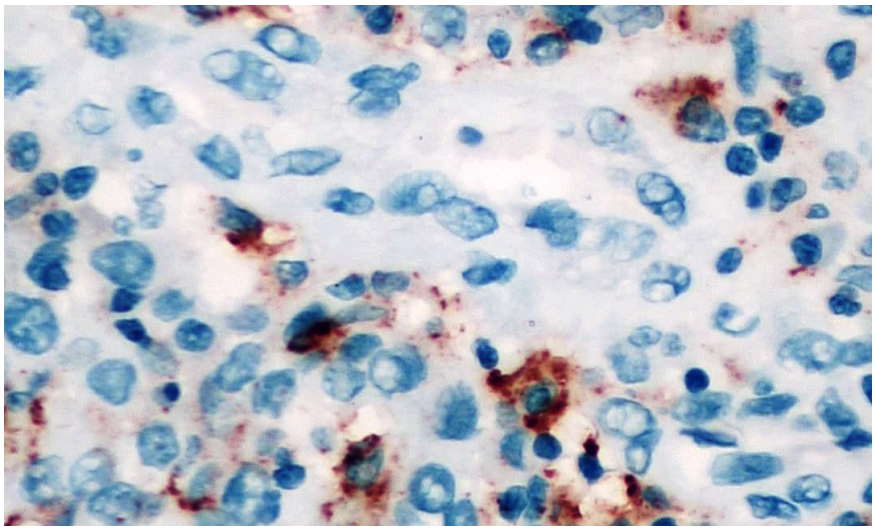

Figure 4. A Case of IBC-NST Showing Positive PLK1 Low Score Expression (x400 Original Power).

BC cells declairing that tamoxifen-resistant BC cells are highly sensitive to Plk1 inhibition recommending its use as an effective therapy in these patients. Also Zhang et al., (2017) concluded the same significant correlation between PLK1 expression and lymph node metastasis indicating worse overall survival for BC patients. Similarly, Ueda et al., (2019), stated that PLK1 was significantly overexpressed in the tissues from TNBC patients compared with the tissues of normal mammary glands and benign breast tumors, moreover they confirmed that PLK1 inhibition eventually triggered apoptosis in TNBC cell. Giordano et al., (2019) in their experimental study concluded that PLK1 inhibition in combination with taxanes (chemotherapeutic agents) shows promising results in treatment of TNBC especially those resistant to chemotherapy encouraging further studies in this field. Unlike our results, a relatively old research done by King et al., (2012) found that PLK1 was under expressed in TNBC (8/33) and that it has a statistically significant inverse relation with Ki67 expression.

From this study we conclude that PLK1 is a promising therapeutic target that is highly expressed in TNBC which should be considered in clinical trials for the treatment of TNBC cases in particular. Our study also indicates that PLK1 expression has a negative prognostic role in TNBC cases and may potentially affect tumor progression. Hoping that this work would encourage other researchers to investigate the effect of PLK1 inhibitors on the treatment TNBC patients, with and without combination with chemotherapy, and how it affects their survival rate and disease free interval.

Author Contribution Statement

None.

Acknowledgments

The authors declare that there is no conflict of interest and received no financial support for this research. The authors solely developed the theory, verified the analytical methods and wrote the manuscript. The material obtained in this study was collected in the form of archived paraffin blocks and clinical data were taken from pathology request sheets designated by numbers, therefore no consent from patients was required. All steps of this research were approved by the ethical committee.

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