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RESEARCH ARTICLE

Diagnostic utility of trichorhinophalangeal syndrome type 1 (TRPS1) immunostain in breast carcinoma compared to GATA3

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ABSTRACT

Background: Female breast cancer (BC) has become the most diagnosed cancer and the fifth cause of cancer mortality worldwide. Metastatic breast cancer is a main differential diagnosis for any suspected metastatic carcinoma in women due to its high incidence. It's essential to identify a specific and sensitive marker to diagnose primary breast cancer. TRPS1 is a nuclear transcription factor, belonging to the GATA family with a transcriptional repressor function. The aim of the present study was to assess the diagnostic value of TRPS1 in the detection of BC cases compared to the routinely used GATA3 immunostain. **Material and Methods:** Seventy BC specimens and 80 specimens of non-breast carcinomas known to exhibit morphological similarity to metastatic breast cancer or show GATA3 positivity were evaluated for immunohistochemical expression of TRPS1 and GATA3. **Results:** TRPS1 and GATA3 were positive in 92.9% and 80% of studied BC specimens with a specificity of 96.3% and 80%, respectively. TRPS1 was found to be more sensitive and specific than GATA3 in the detection of BC. **Conclusions:** TRPS1 can be considered as a diagnostic marker of BC since it is more sensitive and specific than GATA3 . The double combination of TRPS1 and GATA3 provided higher sensitivity and specificity in detection of BC.

Keywords:Breast cancer, Diagnostic, GATA3, TRPS1

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INTRODUCTION

Breast cancer comprises the most common cancer in females (38.8%) in Egypt; the National Cancer Institute (NCI) in Egypt reported 28,000 new confirmed cases each year (Saleh et al., 2021).

Breast cancer is considered a heterogeneous group of diseases with highly variable clinical behavior (Provenzano et al., 2018). Metastatic breast cancer affects 20–30% of all cases (Ai et al., 2021). Growing evidence indicates that different subtypes of breast carcinoma display different patterns of metastasis and clinical outcomes (Molnár et al., 2017). It is highly suggestive that early detection and classification of breast cancer may help patients receive appropriate treatment (Khan et al., 2019).

Breast cancer can be categorized according to the expression of ER, PR, HER2 and Ki-67 proliferative index into four molecular subtypes: luminal A (ER+, PR+, HER2-, Ki-67<14%), luminal B (ER+, PR+/-, HER2+/-, Ki-67>14%), HER2-enriched (ER- and PR-/HER2+) and triple-negative (ER- and PR-/HER2-) (Fallahpour et al., 2017).The luminal A subtype shows the best survival among the four molecular subtypes, while HER2 and triple negative subtypes has the worst survival (Hon et al., 2016).

High-grade and triple-negative breast cancer (TNBC) can pose a diagnostic challenge because they may show both nonspecific morphological features and immunostain profiles (Tozbikian & Zynger, 2019). Since TNBC is an aggressive tumor with worse prognosis and high tendency to relapse and to metastasize early compared with other subtypes of breast cancer (Ai et al., 2021; Dawood et al., 2010), The accurate diagnosis of the disease is crucial for ensuring optimal treatment (Penault-Llorca & Viale, 2012).

Immunohistochemistry is used to detect breast origin for primary or metastatic carcinoma and identifying non mammary metastases to the breast. However, no single immunostain is 100% sensitive nor specific (Cimino-Mathews et al., 2013).

Trichorhinophalangeal syndrome type 1 (TRPS1) is gene located in human chromosome 8q23-24 encoding a novel nuclear transcription factor, belonging to GATA family (Hu et al., 2018). TRPS1 has been found to be a critical activator of mesenchymal-to-epithelial transition (MET) during embryonic development in multiple tissues, including cartilage, bone and kidney (Lin et al., 2017) as well as being essential for growth and differentiation of normal mammary epithelial cells (Cornelissen et al., 2020).

Using DNA microarrays, TRPS1 is a gene found to be implicated in breast cancer however its role remains uncertain, as both amplification and inactivating mutation have been reported (Cornelissen et al., 2020). Although TRPS1 is being expressed in breast cancer, its sensitivity to different subtypes of breast cancer is still under study.

This study was designed to evaluate TRPS1 immunostain in various molecular types of breast cancer in comparison to GATA3 immunostain. In addition to other tumor types known to exhibit morphological similarity to metastatic breast cancer or show GATA3 positivity. Furthermore, the validity of TRPS1 in the identification of BC as a single marker or combined with GATA3 was investigated.

MATERIALS AND METHODS

Study design and case selection

This is a retrospective study that was carried out at the Pathology Department, Faculty of Medicine, Tanta University, during the period from March 2021 to September 2022. It included 70 cases of primary BC as well as 80 cases of non-BC. The included non-BC cases exhibited either morphological similarity to BC or GATA3 positivity. They were incorporated as follows Urothelial carcinoma (14 cases), lung carcinoma (14 cases), ovarian carcinoma (12 cases), endometrial carcinoma (5 cases), gastric adenocarcinoma (5 cases), colorectal adenocarcinoma (7 cases), hepatocellular carcinoma (5 cases), pancreatic duct adenocarcinoma (5 cases), and renal cell carcinomas (13 cases). The study was approved by the Institutional Research Ethics Committee (approval code: 34492/2/21).

Data collection and histopathologic evaluation

For BC cases, clinical data as well as tumor-related features (tumor size, tumor gross appearance and location) were obtained from the accompanying pathology reports and patients medical records.

BC cases were molecularly classified according to ER, PR, HER2/Neu expression and Ki67 proliferative index into Luminal A, Luminal B, HER2 enriched and triple negative tumors (Tsang & Gary, 2021).

Nottingham Grading System (NGS) was used to grade BC cases into grade I (well differentiated), grade II (moderately differentiated) and grade III (poorly differentiated) tumor (Pradhan et al., 2017). For non-BC cases, hematoxylin and eosin-stained sections and confirmatory immunohistochemical slides were examined to confirm the diagnosis.

Tissue microarray (TMA)

Tissue microarray (TMA) recipient blocks (6×4 arrays) were produced using the TMA builder mold (CAT# TMA-001, Thermo Fisher Scientific, Runcorn, UK). For each studied specimen, representative areas of tumor tissues with appropriate preservation were identified. Areas showing necrosis and those with crushing artefacts were excluded. This is followed by insertion of two tissue cores from areas of interest on paraffin blocks into the holes on the recipient blocks to form TMA Blocks.

Immunohistochemical staining

Sections (5 µm thick) mounted on positively charged slides were left to dry for 30 minutes at 37°C. Deparaffinization and antigen retrieval were carried out using Dako PT Link unit. High pH EnVision™ FLEX Target Retrieval Solutions was used reaching 97°C for 20 minutes. Immunostain was accomplished using Dako Autostainer Link 48. Antibodies included in this study were TRPS1 rabbit polyclonal antibody (PA5-84874 from Invitrogen/ThermoFisher,

Waltham, MA; 1:150) and mouse monoclonal antibody against human GATA3 (L50-823 from Cell Marque, Rocklin, CA; 1:50). Secondary antibody was used (SM805, Envision Flex+ Rabbit(linker), DAKO). Slides were kept in Peroxidase-Blocking Reagent for 5 minutes, incubated with primary antibodies for 20–30 minutes, horseradish peroxidase polymer reagent for 20 minutes and diaminobenzidine chromogen/substrate working solution for 10 minutes. Lastly, counterstaining with hematoxylin was done (Taylor et al., 2013).

Assessment of the immunostain

TRPS1 and GATA3 positive staining was identified as brownish nuclear staining for both markers, whereas cytoplasmic or membranous staining were considered nonspecific. For markers scoring, percentages of positive tumor cell were considered regardless of the staining intensity. Receiver operator characteristic (ROC) curve was plotted to identify the best TRPS1 cut-off point for diagnosis of BC. The percentage located close by the point that provides maximum sensitivity and specificity was selected as the cut-off point. GATA3 were considered positive when the percentage of stained tumor cells was 10 or more (Luo et al., 2019).

Statistical analysis

Data were statistically analyzed using the Statistical Package for the Social Sciences software version 25. Categorical variables were expressed as frequencies whereas numerical variables were expressed as mean ± SD. Accuracy, specificity, sensitivity, positive and negative predictive values (NPVs) were used to assess diagnostic values of the tested markers. The histopathologic diagnosis was considered the gold standard.

ROC curve was used to select the best cut-off point for TRPS1 through assessing the diagnostic values of different percentages of TRPS1 expression. ROC curve for GATA3 was performed to compare the validity of both markers. Areas under the ROC curve (AUC) of each marker were calculated; higher AUC values indicate better test performance (Fan et al., 2006).

RESULTS

Clinicopathologic characteristics of BC cases

This study included 70 primary BC cases all of which were females with a mean age of 50.84 ± 10.72 years. Most of studied cases presented with single mass (53/70; 75.7%) measuring less than 5 cm in the greatest dimension (67.2%). Almost half of the cases had left breast mass (37/70; 52.9%). The most common histopathological variant among the studied BC cases was IDC constituting 44.3% (31/70) and grade II was the prevalent histologic grade (34/70; 48.6%). Lymphovascular and perineural invasion were detected in 44 and 37 cases representing 62.9%, and 52.9% respectively. Associated carcinoma in situ (CIS) was detected in 31.4% (22/70). Only 12 cases (17.1%) had skin and nipple invasion. Regional lymph node metastasis was detected in 58.6% represented as 14.3% for N1, 24.3% for N2, and 20% for N3. Clinicopathologic data of the studied cases are listed in Table 1.

TRPS1 and GATA3 immunostain results

Expression of TRPS1 and GATA3 in the studied primary BC cases

TRPS1 and GATA3 were detected in various subtypes of BC cases as illustrated in Table 2. TRPS1 was identified as nuclear staining in 65 (92.9%) while GATA3 nuclear expression was positive in 56 (80%) primary BC cases. Representative images of TRPS1 and GATA3 expression in primary BC are demonstrated in Figure 1.

In relation to molecular subtypes, both markers showed similar high expression in luminal and Her2 enriched subtypes; TRPS1 was expressed in all included 38 luminal cases (100%) and 14 HER2 enriched cases (82.4%) while GATA3 was expressed in 37 luminal cases (97.4%) and 15 cases of HER2 enriched subtype (88.2%). In triple negative cases, TRPS1 showed higher positivity than GATA3; 86.7% were positive for TRPS1 (13/15) and only 4 cases (26.7%) were GATA3 positive.

Expression of TRPS1 and GATA3 in the studied non-BC cases

TRPS1 was detected in only 3 (3.7%) non-BC cases; 2 cases of lung squamous cell carcinoma and one case of ovarian serous carcinoma.

Table 1. Patients' characteristics

	N (%)
Age (years)	
< 50	27 (38.6%)
≥ 50	43 (61.4%)
Laterality	
Right breast	24 (34.3%)
Left breast	37 (52.9%)
Bilateral	9 (12.9%)
Multiplicity	
Single	53 (75.7%)
Multiple	17 (24.3%)
Tumour size	
≤2 cm	30 (42.9%)
>2- ≤5cm	25 (35.7%)
>5cm	15 (21.4%)
Histopathological types	
Invasive ductal carcinoma	31 (44.3%)
Invasive lobular carcinoma	15 (21.4%)
Mucinous carcinoma	7 (10%)
Tubular carcinoma	6 (8.6%)
Mixed ductal and lobular carcinoma	6 (8.6%)
Metaplastic squamous cell carcinoma	5 (7.1%)
Histopathological grade	
Grade I	11 (15.7%)
Grade II	34 (48.6%)
Grade III	25 (35.7%)
Lymphovascular invasion	
Present	44 (62.9%)
Absent	26 (37.1%)
Perineural invasion	
Present	37 (52.9%)
Absent	33 (47.1%)
Associated carcinoma in situ	
Present	22 (31.4%)
Absent	48 (68.6%)
Skin and nipple invasion	
Present	12 (17.1%)
Absent	58 (82.9%)
Lymph node metastasis	
N0	29 (41.4%)
N1	10 (14.3%)
N2	17 (24.3%)
N3	14 (20%)
Molecular classification	
Luminal A	20 (28.6%)
Luminal B	18 (25.7%)
HER2-enriched	17 (24.3%)
Triple negative	15 (21.4%)

All cases of urothelial carcinoma included were negative for TRPS1. While GATA3 nuclear expression was positive in 16 (20%) non-BC cases; 11 cases of urothelial carcinoma, 2 cases of chromophobe RCC, 2 cases of clear cell papillary RCC, and one case of pancreatic duct adenocarcinoma as illustrated in Table 3. Representative images of TRPS1 and GATA3 expression in studied non-BC cases are demonstrated in Figure 2.

Validity of single marker expression in the detection of BC origin

ROC curve analysis of TRPS1 expression [shown in Figure 3] alone provided 92.9% sensitivity, 96.3% specificity and 0.974 AUC (p = 0.000). TRPS1 had 95.6% positive predictive value (PPV), 93.9% NPV and diagnostic accuracy of 94.7%. The ideal TRPS1 cut-off value for distinguishing breast from non-breast cases, in this study, was 12%. Positive GATA3 expression alone described lower sensitivity (80%), specificity (80%) and smaller AUC (0.861). GATA3 had 76.7% PPV, 83.1% NPV and diagnostic accuracy of 80%.

Validity of combined marker expression in the detection of BC origin

Next, we evaluated whether combination of the two markers could improve the identification of a BC origin. It was demonstrated that combining TRPS1 and GATA3 revealed better sensitivity (94.3%), specificity (100%) compared to TRPS1 alone (92.9% sensitivity and 96.3% specificity), GATA3 alone (80% sensitivity and 80% specificity)

DISCUSSION

Primary invasive breast cancer is a group of highly heterogeneous malignancies with different histological features, molecular signatures, immunohistochemical profiles, biological behaviors and prognoses which vary from case to case (Shaoxian et al., 2017). The currently used immune markers for breast tissue namely GATA3, GCDFP15, and mammaglobin have a relatively good sensitivity in ER-positive breast cancer only, but not for TNBC. TNBC is the most aggressive with a high relapse incidence and early metastasis, and the most undifferentiated phenotype; moreover, metastatic poorly differentiated TNBC frequently has no specific tumor marker for its breast origin (Ai et al., 2021).

This study investigated immunohistochemical expression of TRPS1 and GATA3 in breast and non-breast carcinomas. Also, the diagnostic values of TRPS1 and GATA3 were evaluated for each marker and for combined markers.

Table 2. TRPS1 and GATA3 expression in breast carcinoma

	TRPS1		GATA3	
	Positive	Negative	Positive	Negative
Luminal	38 (100%)	0 (0%)	37 (97.4%)	1 (2.6%)
HER2- enriched	14 (82.4%)	3 (17.6%)	15 (88.2%)	2 (11.8%)
Triple negative	13 (86.7%)	2 (13.3%)	4 (26.7%)	11 (73.3%)

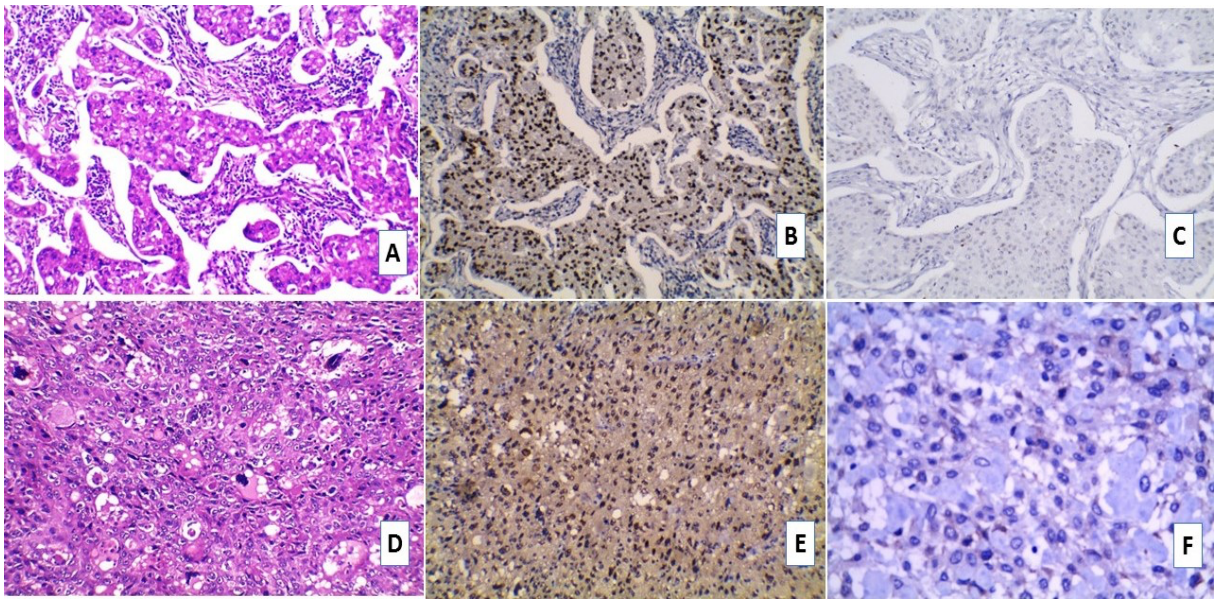

Figure 1. TRPS1 and GATA3 expression in studied breast cancer cases. A: Invasive breast carcinoma with medullary pattern, grade III, triple negative subtype; showing highly dysplastic malignant ductal cells arranged in syncytial sheets separated by fibrovascular stroma heavily infiltrated by mature lymphocytes (H&E x200). B: TRPS1 immunostain of the previous case of invasive breast carcinoma showing positive nuclear expression (x200). C: GATA3 immunostain of the previous case of invasive breast carcinoma showing negative GATA3 staining (x200)**.** D: Metaplastic squamous cell carcinoma, grade III, triple negative subtype (H&E x200). E: TRPS1 immunostain of the previous case of metaplastic squamous cell carcinoma, triple negative subtype showing positive nuclear TRPS1 expression (x200). Note the non- specific cytoplasmic staining. F: GATA3 immunostain of the same case of metaplastic squamous cell carcinoma, triple negative subtype showing negative GATA3 staining (x400).

Table 3. Expression of TRPS1 and GATA3 in non-breast carcinomas.

	TRPS1		GATA3		Total
	positive	negative	positive	negative	
Urothelial carcinoma	0	14	11	3	14
Lung adenocarcinoma	0	7	0	7	7
Lung squamous cell carcinoma	2	5	0	7	7
Ovarian serous carcinoma	1	5	0	6	6
Ovarian mucinous carcinoma	0	6	0	6	6
Endometrial carcinoma	0	5	0	5	5
Gastric adenocarcinoma	0	5	0	5	5
Colorectal adenocarcinoma	0	7	0	7	7
Hepatocellular carcinoma	0	5	0	5	5
Pancreatic duct carcinoma	0	5	1	4	5
Clear cell renal cell carcinoma	0	3	0	6	6
chromophobe renal cell carcinoma	0	4	2	2	4
Clear cell Papillary renal cell carcinoma	0	3	2	1	3
Total	3	77	16	64	80

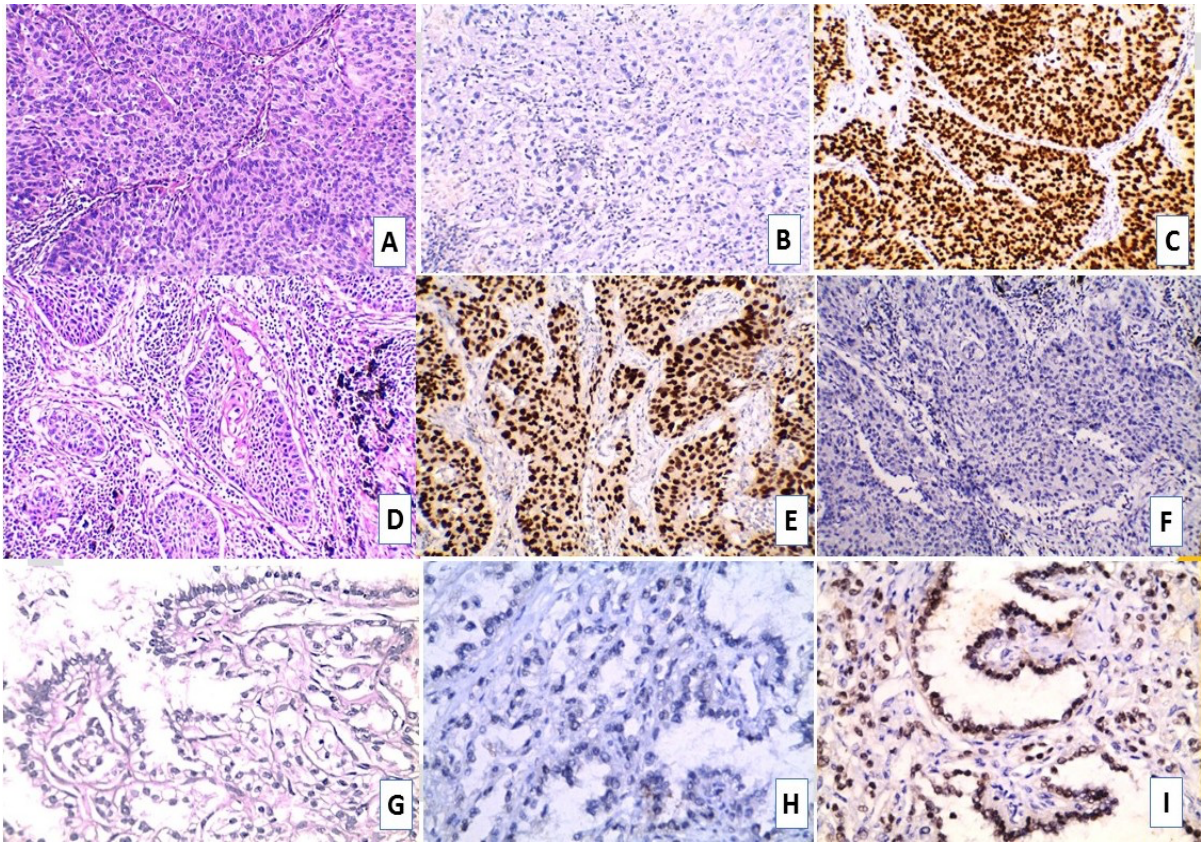

Figure 2. TRPS1 and GATA3 expression in studied non breast cancer cases. A: A case of high-grade urothelial carcinoma (H&E x200). B: TRPS1 immunostain of the previous case of high-grade urothelial carcinoma showing negative expression (x 200). C: GATA3 immunostain of the previous case of high-grade urothelial carcinoma showing positive nuclear GATA3 staining (x200). D: Lung squamous cell carcinoma showing nests of malignant squamous epithelium having peripheral palisading and initial formation of keratin pearl. Areas of anthracosis could be detected (H&E x200). E: TRPS1 immunostain of the previous case of lung squamous cell carcinoma showing positive nuclear staining of TRPS1 (x200). F: GATA3 immunostain of the previous case of lung squamous cell carcinoma showing negative GATA3 nuclear staining (x 200). G: A case of clear cell papillary renal cell carcinoma showing intracystic papillary proliferations lined by clear cells with reversed polarity (nuclei away from the basement membrane) (H&E x400). H: TRPS1 immunostain of the previous case of clear cell papillary renal cell carcinoma showing negative TRPS1 nuclear expression (x400). I: GATA3 immunostain of the previous case of clear cell papillary renal cell carcinoma showing positive GATA3 nuclear expression (x400).

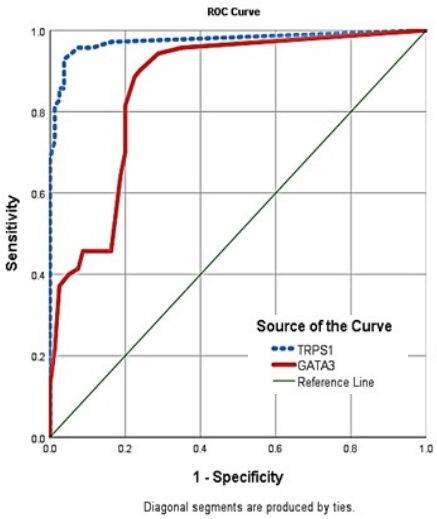

Figure 3. Comparative ROC curve for TRPS1 and GATA3 in breast carcinoma.

From ROC curve analysis, in this study, the optimal TRPS1 cut-off value that afforded the highest sensitivity and specificity for distinguishing breast from non-breast origin was 12%. Whereas, (Parkinson et al., 2022) considered any nuclear staining as TRPS1 positive, so the cut-off point in their study was 1%. These differences may be due to different methods performed to determine cut-off values in these studies.

Analyzing TRPS1 expression in primary breast carcinoma cases, the current study demonstrated that TRPS1 was detected in 65 (92.9%) primary breast carcinoma specimens and only in three (3.8%) non-breast carcinoma specimens. As a result, TRPS1 provided 92.9% sensitivity, and 96.3% specificity. This was in agreement with (Ai et al., 2021). who reported similar sensitivity and specificity of about 91%, and 95.8% respectively. On the other hand, (Parkinson et al., 2022) showed higher TRPS1 sensitivity and specificity of 98.6% and 99.4%, respectively.

Moreover, different studies described TRPS1expression on the cell blocks of cytological materials with varying results. (Wang et al., 2022) showed similar TRPS1 sensitivity (92%), although (Abdelwahed et al., 2022) and (Rohra et al., 2022) reported higher TRPS1 sensitivity (96.7% and 98.2%, respectively). This result shows that cell blocks of cytological material can be used as surrogates for tissue biopsies. In the current study, TRPS1 showed positive immunoreactivity in 100% of luminal cases, 82.4% of HER2 enriched cases and 86.7% of TNBC cases. Similar to our findings, (Ai et al., 2021) who reported TRPS1 positivity in 98% of luminal subtype, 87% of HER2 enriched, and 86% of TNBC.

(Rohra et al., 2022) described TRPS1 expression on the cell blocks of cytological material in known cases of BC and reported TRPS1 positivity in 98% of luminal cases, 87% of HER2 enriched, and 86% of TNBC cases.

These results indicated that TRPS1 is a marker for both breast carcinoma with luminal differentiation and BC with basal differentiation (Ding et al., 2022). The Cancer Genome Atlas Database found that TRPS1 mRNA is highly expressed in all breast cancer subtypes (luminal, HER2, and basal-type) (Radvanyi et al., 2005).

Unlike the traditional markers, TRPS1 showed high positivity in TNBC. Concerning TRPS1 expression in different histopathological variant of triple negative molecular subtype, the recent study revealed TRPS1 positivity in 81.8% of IDC, and 100% of metaplastic squamous cell carcinoma. Also, (Parkinson et al., 2022) performed TRPS1 on different types of metaplastic breast carcinomas and revealed TRPS1 positivity in 100% of metaplastic squamous cell carcinoma cases. Moreover, (Ai et al., 2021) divided TNBC cases into 2 groups; metaplastic and non-metaplastic with reported TRPS1 sensitivity 86% in each group.

Concerning GATA3 expression in primary breast carcinoma cases, in the present study, GATA3 was detected in 56 (80%) primary breast carcinoma specimens and in 16 (20%) non-breast carcinoma specimens as well. GATA3 showed 80% sensitivity and 80% specificity. (Ni et al., 2018) reported similar GATA3 sensitivity of 80.3% but higher specificity of 99%. The higher specificity result is due to GATA3 staining of limited types of non-breast carcinoma with excluding urothelial carcinoma. While (Miettinen et al., 2014) revealed higher sensitivity of 96 and lower specificity of 71%.

The current study showed that most of the cases with GATA3 immunopositivity were of luminal subtype (97.4%), followed by HER2 enriched (88.2%), and only a small percentage of TNBC cases (26.7%). These results were in agreement with (Ai et al., 2021) who reported higher and stronger GATA3 immunopositivity in luminal (95%) than HER2 enriched (88%) and TNBC (21%). Also, (Shaoxian et al., 2017) reported similar GATA3 immunoreactivity in luminal (98%) and TNBC (20%) with lower percentage of positivity in HER2 enriched BC (68%).

Furthermore, (Lu et al., 2019) reported similar GATA3 immunopositivity in luminal (98%), and HER2 enriched (82%) molecular subtypes, with higher GATA3 positivity in basal-like triple negative molecular subtype (46%). GATA3 is known as an ER-associated gene so it is considered as a luminal not a basal marker (Cakir et al., 2017). This could explain the

highest GATA3 immunoreactivity in luminal BC and the lowest percentage of immunopositivity in TNBC.

The difference in reported GATA3 positivity in breast carcinoma could be attributed to the differences in antibody clone and dilution used in different studies, the threshold used to define GATA3 positivity (cutoff value), and the type of specimen (Asch-Kendrick & Cimino-Mathews, 2016). Two monoclonal antibodies to GATA3; clone L50-823 (used in this study) and clone HG3-31. Strong nuclear labeling is obtained with clone L50-823 that is easy to interpret (Miettinen et al., 2014). Regarding GATA3 expression in different histopathological variants of triple negative molecular subtype, the current study revealed 9.1% positivity in IDC cases of triple negative molecular subtype and 60% in cases of metaplastic squamous cell carcinoma. (Yoon et al., 2022) showed 63.2% GATA3 immunopositivity in IDC, and 87.5% positivity in metaplastic squamous cell carcinoma.

In the current study, combination between TRPS1 and GATA3 provided higher sensitivity and specificity (94.3% and 100%, respectively). (Du et al., 2022) reported higher sensitivity of combined TRPS1 and GATA3 in BC cases of 96.3%. (Yoon et al., 2022) stated that combined TRPS1 and GATA3 achieved higher sensitivity in different types of TNBC cases of 93.8%. From the previous results, TRPS1 would help identification of metastatic carcinomas of breast origin especially those that are morphologically similar to other metastatic body organs' carcinomas.

CONCLUSIONS

TRPS1 is a highly sensitive and specific marker to confirm breast origin and can be used as a great diagnostic tool, especially for TNBC. Combination of TRPS1 and GATA3 offered higher sensitivity and specificity so that application of both markers in clinical practice will achieve better results.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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No funding was received for conducting the study.

DATA AVAILABILITY

Data are available with corresponding author upon request.

AUTHOR CONTRIBUTION

All authors contributed equally and approved the manuscript.

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