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Original Article

Circulating MicroRNA Profiles as Early Indicators of Breast Cancer Metastatic Potential

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ABSTRACT

Background: Breast cancer outcomes are strongly influenced by metastatic progression, and conventional clinicopathological features may not fully reflect tumor aggressiveness. Circulating microRNAs are stable, blood-based molecules that may provide non-invasive molecular information about metastatic potential. Objective: To compare circulating microRNA expression patterns between localized and metastatic breast cancer patients and assess their discriminatory value as candidate non-invasive biomarkers. Methods: This laboratory-based comparative study included 80 female patients with histologically confirmed breast cancer at a tertiary care hospital in Sialkot, Pakistan. Patients were divided into localized breast cancer (n=40) and metastatic breast cancer (n=40) groups. Clinical and pathological data were recorded. Peripheral blood samples were collected and circulating microRNAs were extracted from serum or plasma. Expression levels of miR-21, miR-10b, miR-155, miR-200c, miR-31, miR-126, and miR-335 were measured using reverse transcription quantitative real-time polymerase chain reaction. Relative expression was compared between groups, and discriminatory performance was assessed using receiver operating characteristic curve analysis. Results: Metastatic cases showed significantly higher expression of miR-21, miR-10b, miR-155, and miR-200c compared with localized cases, while miR-31, miR-126, and miR-335 were significantly reduced ($p<0.001$ for all). miR-10b showed the highest fold increase in metastatic disease, followed by miR-21. Grade III tumors and lymph node-positive cases showed more frequent alterations in miR-21, miR-10b, and miR-155 expression. miR-21 alone showed good discriminatory performance, with an area under the curve of 0.86. A combined microRNA panel showed stronger performance, with an area under the curve of 0.92, sensitivity of 87.5%, and specificity of 85.0%. Conclusion: Circulating microRNA profiles differed clearly between localized and metastatic breast cancer patients. Increased miR-21, miR-10b, miR-155, and miR-200c, together with reduced miR-31, miR-126, and miR-335, formed a candidate non-invasive biomarker pattern associated with metastatic disease. Larger longitudinal studies are needed to validate their prognostic value. Keywords: Breast cancer; metastatic breast cancer; circulating microRNA; miR-21; miR-10b; miR-155; miR-200c; liquid biopsy; prognostic biomarker; Pakistan.

INTRODUCTION

Breast cancer remains one of the leading causes of cancer-related morbidity and mortality among women worldwide, with prognosis largely determined by stage at diagnosis and the presence of metastatic disease (1,2). Although localized breast cancer is often treatable with multimodal therapy,

metastatic spread to distant organs such as bone, liver, lung, and brain substantially worsens survival and increases treatment complexity. In Pakistan, breast cancer represents a major public health burden, and delayed presentation remains common because of limited awareness, socioeconomic barriers, cultural hesitation, and restricted access to timely diagnostic services (3). These challenges highlight the need for accessible biomarkers that may improve risk assessment beyond conventional clinicopathological parameters.

Current prognostic assessment in breast cancer relies on tumor size, histological grade, lymph node status, hormone receptor expression, HER2 status, imaging findings, and clinical stage. These markers are clinically useful, but they do not always fully capture the biological aggressiveness of an individual tumor. Some patients with apparently localized disease may later develop recurrence or distant metastasis, while others with adverse pathological features may follow a less aggressive course. Therefore, additional molecular tools are needed to help identify patients with higher metastatic potential and support more individualized monitoring and treatment planning (4,5).

MicroRNAs are small non-coding RNA molecules that regulate gene expression post-transcriptionally and influence several cancer-related processes, including proliferation, apoptosis, angiogenesis, epithelial–mesenchymal transition, invasion, immune escape, and metastasis (6,7). Because microRNAs can regulate multiple target genes and pathways, altered microRNA expression may reflect important changes in tumor biology. Several microRNAs have been implicated in breast cancer progression: miR-10b has been associated with invasion and metastasis, miR-21 and miR-155 with oncogenic activity and aggressive tumor behavior, and members of the miR-200 family with epithelial–mesenchymal transition and metastatic regulation (8-10). Conversely, miR-31, miR-126, and miR-335 have been described as metastasis-suppressing microRNAs in breast cancer models and clinical studies (9).

Circulating microRNAs are particularly attractive as candidate biomarkers because they are detectable in serum or plasma and remain relatively stable in body fluids (8,9). This makes them suitable for minimally invasive blood-based testing, with potential applications in diagnosis, prognosis, treatment monitoring, and early detection of disease progression. Previous studies have shown that circulating microRNA profiles may differ between patients with primary and metastatic breast cancer and may provide prognostic information in advanced disease (6,8). However, circulating microRNA testing requires careful validation because expression levels may be affected by sample type, pre-analytical handling, normalization methods, treatment exposure, and inflammatory or systemic conditions.

Local evidence from Pakistan on circulating microRNAs in breast cancer remains limited. Some studies have evaluated microRNA panels in triple-negative breast cancer and explored selected circulating microRNAs as non-invasive predictors of breast cancer (11,12). However, there is still insufficient local evidence comparing circulating microRNA expression between localized and metastatic breast cancer patients, particularly in tertiary-care populations where late presentation is common. Establishing such molecular patterns in Pakistani patients may help determine whether internationally reported metastasis-associated microRNAs show similar behavior in the local clinical setting.

This laboratory-based comparative study was therefore conducted to evaluate circulating expression patterns of selected metastasis-related microRNAs in patients with localized and metastatic breast cancer. The study focused on miR-21, miR-10b, miR-155, miR-200c, miR-31, miR-126, and miR-335, and assessed whether their expression profiles differed between disease groups and showed potential discriminatory value as candidate non-invasive biomarkers of metastatic breast cancer. The manuscript's current study design, microRNA panel, and localized-versus-metastatic comparison support this framing as an original laboratory-based comparative biomarker study.

MATERIALS AND METHODS

This observational, laboratory-based comparative study was conducted at a tertiary care hospital in Sialkot, Pakistan. The study compared circulating microRNA expression profiles between patients with localized breast cancer and patients with metastatic breast cancer. Clinical evaluation, staging, sample collection, and laboratory processing were performed through the hospital's clinical and molecular diagnostic facilities. The study included female patients aged 18 years or above with histologically confirmed breast cancer. Participants were divided into two groups according to disease status. Group A included patients with localized breast cancer, defined as disease confined to the breast with or without regional lymph node involvement and no evidence of distant metastasis. Group B included patients with metastatic breast cancer, defined as breast cancer with confirmed distant spread to organs such as bone, liver, lung, brain, or distant lymph nodes.

A total of 80 patients were enrolled, with 40 patients in the localized breast cancer group and 40 patients in the metastatic breast cancer group. A non-probability consecutive sampling technique was used. Eligible patients presenting during the study period were recruited until the required sample size was completed. Female patients with confirmed breast cancer, available clinical staging information, and written informed consent were included. Patients were eligible if they had either localized or metastatic breast cancer based on clinical, histopathological, and radiological assessment. Patients were excluded if they had another known malignancy, severe active infection, autoimmune disease, chronic inflammatory disorder, recent major surgery, or hemolysed blood samples. Patients who had received recent chemotherapy, radiotherapy, or systemic treatment before blood sampling were excluded when treatment-related effects could not be separated from disease-related microRNA expression changes.

Clinical and pathological data were collected using a structured data collection form. Recorded variables included age, marital status, residence, family history of breast cancer, menopausal status, tumor side, tumor size, histological type, tumor grade, lymph node status, estrogen receptor status, progesterone receptor status, HER2 status, triple-negative phenotype, and site of metastasis where present. Disease stage and metastatic status were determined using clinical examination, histopathology, imaging findings, and available laboratory records. Imaging modalities included ultrasound, mammography, computed tomography, bone scan, magnetic resonance imaging, or positron emission tomography where clinically available. Approximately 5 mL of peripheral venous blood was collected from each participant using aseptic technique before initiation of a new treatment session where applicable. Blood samples were collected in appropriate tubes for serum or plasma preparation and transported to the laboratory without delay. For serum preparation, samples were allowed to clot and then centrifuged. For plasma preparation, anticoagulated samples were centrifuged according to standard laboratory protocol. The separated serum or plasma was transferred into clean RNase-free tubes and stored at low temperature until microRNA extraction. Samples showing visible hemolysis were excluded from further analysis.

Circulating microRNA was extracted from serum or plasma using a commercially available microRNA extraction kit according to the manufacturer's protocol. Equal starting volumes of serum or plasma were used for all samples to maintain consistency across study groups. RNase-free tubes, pipette tips, and reagents were used throughout the extraction process. Extracted RNA was assessed for suitability before downstream reverse transcription and quantitative real-time polymerase chain reaction analysis.

Extracted microRNA was converted into complementary DNA using reverse transcription. Specific stem-loop primers or universal reverse transcription primers were used according to the assay system. Quantitative real-time polymerase chain reaction was performed to measure the expression of selected circulating microRNAs associated with breast cancer progression and metastasis. The microRNA panel included miR-21, miR-10b, miR-155, miR-200c, miR-31, miR-126, and miR-335. These markers were selected because of their reported involvement in tumor invasion, epithelial–mesenchymal transition,

metastatic progression, and metastasis suppression. Each reaction was performed in duplicate or triplicate where sample volume permitted. Internal control microRNA or spike-in control was used for normalization. Cycle threshold values were recorded for each target microRNA.

Relative microRNA expression was calculated using the comparative cycle threshold method. The cycle threshold value of each target microRNA was normalized against the internal control to obtain ΔCt values. Relative expression between localized and metastatic breast cancer groups was then calculated. Fold change was used to determine upregulation or downregulation of circulating microRNAs in metastatic breast cancer compared with localized breast cancer. MicroRNAs with higher relative expression in metastatic cases were classified as upregulated, while those with lower relative expression in metastatic cases were classified as downregulated. The expression patterns of individual microRNAs were also assessed in relation to clinicopathological variables, including tumor grade, lymph node status, receptor status, and metastatic site. The primary outcome was the difference in circulating microRNA expression between localized and metastatic breast cancer patients. The secondary outcomes included the association of selected microRNAs with tumor grade, lymph node positivity, receptor status, and metastatic site. The discriminatory performance of individual microRNAs and a combined microRNA panel was assessed for differentiating metastatic breast cancer from localized breast cancer.

Data were entered and analyzed using statistical software. Continuous variables were expressed as mean and standard deviation or median and interquartile range according to data distribution. Categorical variables were expressed as frequencies and percentages. MicroRNA expression levels between localized and metastatic breast cancer groups were compared using an independent samples t-test or Mann–Whitney U test, depending on distribution. Categorical variables were compared using the chi-square test or Fisher's exact test where appropriate. A p-value of less than 0.05 was considered statistically significant. Receiver operating characteristic curve analysis was performed for microRNAs showing significant differences between the two groups. The area under the curve was calculated to assess discriminatory performance. Sensitivity and specificity were determined for selected cut-off values. A combined microRNA panel was evaluated to determine whether multiple markers provided better discrimination between localized and metastatic breast cancer than individual microRNAs.

Quality control measures were followed during sample collection, processing, storage, RNA extraction, and qRT-PCR analysis. Blood samples were processed using a uniform protocol. Hemolysed samples were excluded. RNA extraction and amplification were performed under RNase-free conditions. Duplicate or triplicate reactions were performed where possible, and samples with poor amplification or technical error were repeated when sufficient material was available. The same internal control strategy was applied throughout the study to reduce technical variation. Ethical approval was obtained from the institutional review board or ethical review committee of the tertiary care hospital. Written informed consent was obtained from all participants before blood sample collection. Participation was voluntary, and patients retained the right to withdraw from the study at any stage. Patient confidentiality was maintained by assigning coded identification numbers to samples and clinical records.

RESULTS

A total of 80 female patients with histologically confirmed breast cancer were included in the study. Of these, 40 patients had localized breast cancer and 40 had metastatic breast cancer. The age of patients ranged from 29 to 71 years. The mean age was 47.8 ± 9.6 years in the localized group and 52.4 ± 10.8 years in the metastatic group. Patients with metastatic breast cancer were significantly older than those with localized disease ($p = 0.047$). Most patients were from Sialkot city and surrounding rural areas. The baseline demographic and clinicopathological characteristics of the two groups are shown in Table 1. Patients aged ≥50 years were more frequent in the metastatic group than in the localized group, although the difference was not statistically significant. Postmenopausal status was also more common among metastatic cases. Invasive ductal carcinoma was the most frequent histological type in both

groups, occurring in 34 patients (85.0%) with localized breast cancer and 36 patients (90.0%) with metastatic breast cancer. Grade III tumors were significantly more common in the metastatic group than in the localized group, affecting 25 patients (62.5%) and 11 patients (27.5%), respectively ($p = 0.002$). Lymph node positivity was also significantly higher among metastatic patients, occurring in 32 patients (80.0%) compared with 15 patients (37.5%) in the localized group ($p < 0.001$). Hormone receptor and HER2 status did not differ significantly between the two groups. ER positivity was observed in 25 patients (62.5%) in the localized group and 20 patients (50.0%) in the metastatic group. PR positivity was present in 22 patients (55.0%) and 17 patients (42.5%), respectively. HER2 positivity was recorded in 9 localized cases (22.5%) and 13 metastatic cases (32.5%). Triple-negative phenotype was observed in 8 localized cases (20.0%) and 12 metastatic cases (30.0%).

Table 1. Baseline demographic and clinicopathological characteristics of localized and metastatic breast cancer patients

Variable	Localized breast cancer, n=40	Metastatic breast cancer, n=40	p-value
Mean age, years	47.8 ± 9.6	52.4 ± 10.8	0.047
Age <50 years	23 (57.5%)	16 (40.0%)	0.118
Age ≥50 years	17 (42.5%)	24 (60.0%)	0.118
Premenopausal	21 (52.5%)	15 (37.5%)	0.179
Postmenopausal	19 (47.5%)	25 (62.5%)	0.179
Invasive ductal carcinoma	34 (85.0%)	36 (90.0%)	0.499
Invasive lobular carcinoma	4 (10.0%)	3 (7.5%)	0.692
Other histological type	2 (5.0%)	1 (2.5%)	0.556
Grade I	5 (12.5%)	1 (2.5%)	0.201
Grade II	24 (60.0%)	14 (35.0%)	0.025
Grade III	11 (27.5%)	25 (62.5%)	0.002
Lymph node positive	15 (37.5%)	32 (80.0%)	<0.001
ER positive	25 (62.5%)	20 (50.0%)	0.260
PR positive	22 (55.0%)	17 (42.5%)	0.263
HER2 positive	9 (22.5%)	13 (32.5%)	0.315
Triple-negative phenotype	8 (20.0%)	12 (30.0%)	0.302

Among the 40 patients with metastatic breast cancer, bone was the most frequent metastatic site, observed in 18 patients (45.0%). Liver metastasis was present in 10 patients (25.0%), followed by lung metastasis in 8 patients (20.0%) and brain metastasis in 4 patients (10.0%). The distribution of metastatic sites is shown in Table 2.

Table 2. Distribution of metastatic sites among patients with metastatic breast cancer

Metastatic site	Metastatic breast cancer, n=40
Bone metastasis	18 (45.0%)
Liver metastasis	10 (25.0%)
Lung metastasis	8 (20.0%)
Brain metastasis	4 (10.0%)

The relative expression levels of selected circulating microRNAs are presented in Table 3. Metastatic breast cancer cases showed significantly higher expression of miR-21, miR-10b, miR-155, and miR-200c compared with localized cases. In contrast, miR-31, miR-126, and miR-335 were significantly lower in the metastatic group. The highest fold increase was observed for miR-10b, which showed a 2.70-fold increase in metastatic breast cancer compared with localized breast cancer. miR-21 showed a 2.31-fold increase, followed by miR-155 with a 2.09-fold increase and miR-200c with a 1.73-fold increase. Among the downregulated markers, miR-126 and miR-335 each showed a fold change of 0.44, while miR-31 showed a fold change of 0.54. All seven microRNAs differed significantly between the two groups ($p < 0.001$).

Table 3. Relative expression of circulating microRNAs in localized and metastatic breast cancer

Circulating microRNA	Localized group, mean ± SD	Metastatic group, mean ± SD	Fold change	Expression pattern	p-value
miR-21	2.10 ± 0.62	4.85 ± 1.05	2.31	Upregulated	<0.001
miR-10b	1.45 ± 0.45	3.92 ± 0.88	2.70	Upregulated	<0.001
miR-155	1.62 ± 0.51	3.38 ± 0.79	2.09	Upregulated	<0.001
miR-200c	1.80 ± 0.58	3.12 ± 0.74	1.73	Upregulated	<0.001
miR-31	1.52 ± 0.44	0.82 ± 0.31	0.54	Downregulated	<0.001
miR-126	1.70 ± 0.50	0.74 ± 0.29	0.44	Downregulated	<0.001
miR-335	1.55 ± 0.46	0.68 ± 0.25	0.44	Downregulated	<0.001

Receiver operating characteristic curve analysis was performed for microRNAs showing significant differences between localized and metastatic breast cancer groups.

The ROC findings are summarized in Table 4. miR-21 showed good discriminatory performance, with an area under the curve of 0.86. A combined microRNA panel including miR-21, miR-10b, miR-155, miR-126, and miR-335 demonstrated stronger discriminatory performance, with an area under the curve of 0.92. At the selected cut-off value, the combined microRNA panel showed 87.5% sensitivity and 85.0% specificity for distinguishing metastatic breast cancer from localized breast cancer.

Table 4. ROC curve analysis for distinguishing metastatic from localized breast cancer

Marker or panel	Area under the curve	Sensitivity	Specificity
miR-21	0.86		
Combined microRNA panel: miR-21, miR-10b, miR-155, miR-126, and miR-335	0.92	87.5%	85.0%

Associations between selected microRNAs and clinicopathological features are summarized in Table 5. Patients with grade III tumors showed higher levels of miR-21 and miR-10b than patients with grade I or grade II tumors. Lymph node-positive patients showed increased expression of miR-21, miR-10b, and miR-155. These expression patterns were more prominent among metastatic breast cancer cases.

Among patients with metastatic disease, those with liver and lung metastasis showed slightly higher miR-21 and miR-10b expression compared with patients with bone-only metastasis. This difference was not statistically significant.

Table 5. Summary of microRNA expression patterns according to clinicopathological features

Clinicopathological feature	MicroRNA expression pattern
Grade III tumors	Higher miR-21 and miR-10b expression compared with grade I–II tumors
Lymph node positivity	Increased miR-21, miR-10b, and miR-155 expression
Liver and lung metastasis	Slightly higher miR-21 and miR-10b expression compared with bone-only metastasis
Bone-only metastasis	Lower miR-21 and miR-10b expression compared with liver and lung metastasis
Statistical significance by metastatic site	Not statistically significant

Overall, circulating microRNA expression differed clearly between localized and metastatic breast cancer groups.

Metastatic breast cancer was associated with increased expression of miR-21, miR-10b, miR-155, and miR-200c and decreased expression of miR-31, miR-126, and miR-335. miR-10b showed the highest fold increase among upregulated microRNAs, while miR-126 and miR-335 showed the greatest reduction among downregulated microRNAs. The combined microRNA panel demonstrated stronger discriminatory performance than miR-21 alone, with an area under the curve of 0.92, sensitivity of 87.5%, and specificity of 85.0%.

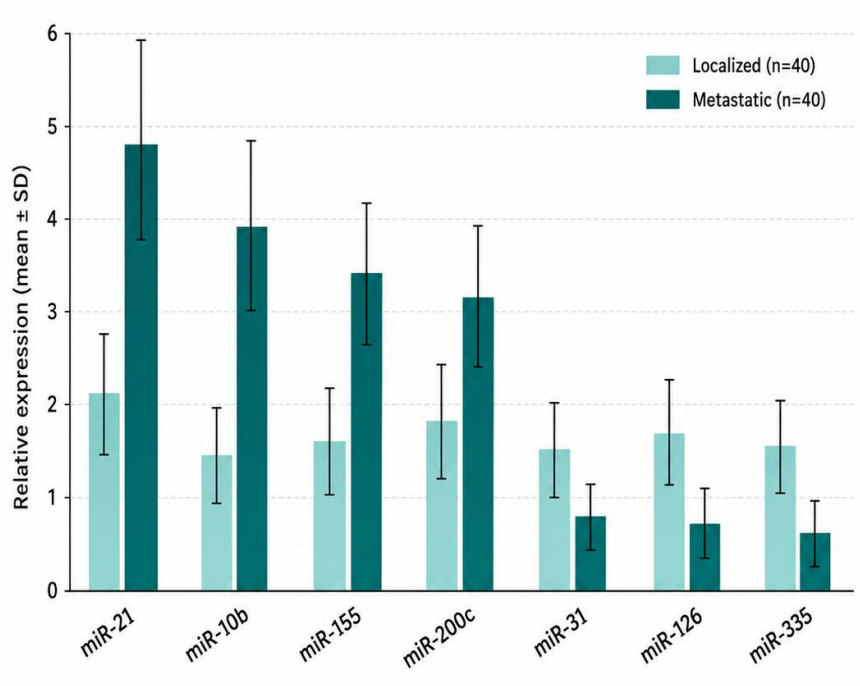

Figure 1. Comparative Expression of Circulating microRNAs in Localized and Metastatic Breast Cancer

Figure Description: Grouped bar chart showing the relative expression levels of selected circulating microRNAs in localized and metastatic breast cancer patients. Bars represent mean relative expression ± standard deviation for localized cases (n=40) and metastatic cases (n=40). Metastatic breast cancer showed increased expression of miR-21, miR-10b, miR-155, and miR-200c, while miR-31, miR-126, and miR-335 were reduced compared with localized disease. All listed microRNAs differed significantly between groups ($p < 0.001$).

DISCUSSION

The present study demonstrated distinct circulating microRNA expression patterns between localized and metastatic breast cancer patients. Metastatic disease was associated with increased expression of miR-21, miR-10b, miR-155, and miR-200c, while miR-31, miR-126, and miR-335 showed reduced expression. The combined microRNA panel showed stronger discriminatory performance than an individual marker, suggesting that a multi-marker circulating microRNA profile may better reflect metastatic disease status than a single microRNA. These findings support the potential role of circulating microRNAs as non-invasive molecular indicators of metastatic potential in breast cancer (13).

The upregulation of miR-21 in metastatic breast cancer is biologically relevant because miR-21 is widely recognized as an oncogenic microRNA involved in tumor growth, invasion, apoptosis resistance, and disease progression. In this study, miR-21 expression was markedly higher in metastatic cases than in localized cases, and it also showed good discriminatory performance on ROC analysis. This pattern suggests that elevated circulating miR-21 may reflect a more aggressive tumor phenotype. However, because miR-21 may also be altered in other malignancies and inflammatory conditions, its clinical value is likely to be stronger when interpreted as part of a broader microRNA panel rather than as an isolated marker (14,15).

miR-10b showed the highest fold increase among the upregulated microRNAs in metastatic breast cancer. This finding is consistent with its known association with tumor invasion, migration, and metastatic behavior. Increased miR-10b expression in metastatic cases indicates that this microRNA may be closely linked with the biological processes that allow breast cancer cells to invade surrounding tissue and spread to distant organs. Its higher expression in patients with aggressive disease features, including

grade III tumors and lymph node positivity, further supports its relevance as a candidate marker of metastatic behavior (16).

The increased expression of miR-155 in metastatic cases also supports its role in aggressive breast cancer biology. miR-155 has been linked with inflammation-associated oncogenesis, immune modulation, tumor proliferation, and progression in several cancers, including breast cancer. In the present study, higher miR-155 expression was observed in metastatic disease and was also more common among lymph node-positive patients. This association suggests that miR-155 may reflect both tumor aggressiveness and the inflammatory or immune-related components of cancer progression (17).

miR-200c was also increased in metastatic breast cancer. The role of the miR-200 family in metastasis is complex because these microRNAs are involved in epithelial–mesenchymal transition and metastatic colonization. Although miR-200 family members may suppress early epithelial–mesenchymal transition in some biological contexts, increased circulating miR-200c in advanced disease may reflect tumor burden, cell shedding, or active metastatic involvement (18). In this study, the rise in miR-200c among metastatic patients indicates that it may serve as a circulating marker of advanced disease activity rather than a simple directional regulator of metastasis.

In contrast to the upregulated microRNAs, miR-31, miR-126, and miR-335 were reduced in metastatic breast cancer. This downregulation is important because these microRNAs have been described as metastasis-suppressing molecules. Reduced miR-31 may be associated with loss of control over invasion and migration, while lower miR-126 and miR-335 expression may indicate weakened suppression of metastatic spread. The reduced expression of these microRNAs in metastatic cases suggests that metastatic progression may involve both activation of oncogenic microRNAs and loss of metastasis-suppressive microRNA activity (19).

The combined microRNA panel performed better than miR-21 alone, with a higher area under the ROC curve and strong sensitivity and specificity for distinguishing metastatic from localized breast cancer. This finding is clinically meaningful because breast cancer metastasis is a multi-pathway biological process. A single marker is unlikely to fully capture the complexity of tumor dissemination, whereas a panel including both upregulated and downregulated microRNAs may provide a more balanced molecular signature. The inclusion of miR-21, miR-10b, miR-155, miR-126, and miR-335 in the combined panel reflects both oncogenic activation and loss of metastatic suppression.

The observed relationship between altered microRNA expression and established clinicopathological risk factors further strengthens the findings. Grade III tumors showed higher miR-21 and miR-10b expression, while lymph node-positive cases showed increased miR-21, miR-10b, and miR-155. These patterns are consistent with the clinical understanding that high tumor grade and lymph node involvement are associated with more aggressive disease. The microRNA findings therefore appear to complement conventional prognostic markers rather than replace them (20). In future clinical use, circulating microRNA profiling would be most useful as an additional layer of molecular information alongside tumor grade, receptor status, lymph node status, and imaging findings.

Among metastatic cases, bone was the most frequent site of distant spread, followed by liver, lung, and brain. Patients with liver and lung metastasis showed slightly higher miR-21 and miR-10b expression than those with bone-only metastasis, although this difference was not statistically significant. This pattern may reflect biological variation by metastatic site, but the small subgroup size limits interpretation. Larger studies with site-specific metastatic cohorts would be needed to determine whether particular circulating microRNA profiles are associated with organ-specific metastatic patterns.

The local clinical context is important. In Pakistan, many breast cancer patients present at advanced stages because of delayed diagnosis, limited screening, financial barriers, cultural hesitation, and restricted access to specialist care. In such settings, a blood-based biomarker approach may be valuable

because it is less invasive, repeatable, and potentially suitable for monitoring disease progression. Circulating microRNA testing cannot replace histopathology, receptor testing, or imaging, but it may help identify patients who require closer follow-up, more detailed staging, or more intensive monitoring after validation in larger cohorts.

This study has several limitations. The sample size was modest, and the study was conducted at a single tertiary care hospital, which may limit generalizability. The design was comparative and cross-sectional; therefore, the findings show association with metastatic disease status but do not prove prospective prediction of future metastasis. The study did not include a healthy control group, and treatment exposure may influence circulating microRNA expression if not fully standardized. Pre-analytical factors such as serum or plasma type, sample handling, hemolysis, storage duration, RNA extraction efficiency, and normalization strategy may also affect circulating microRNA measurements. In addition, confidence intervals for ROC estimates and more detailed modeling of the combined panel would strengthen interpretation.

Despite these limitations, the study provides useful evidence that selected circulating microRNAs differ between localized and metastatic breast cancer patients. The combined pattern of increased miR-21, miR-10b, miR-155, and miR-200c with reduced miR-31, miR-126, and miR-335 represents a biologically plausible molecular signature of metastatic disease. The findings support further multicenter and longitudinal validation of circulating microRNA panels for metastatic risk stratification, surveillance, and clinical decision support in breast cancer patients.

CONCLUSION

This study showed distinct circulating microRNA expression patterns between localized and metastatic breast cancer patients. Metastatic breast cancer was associated with increased expression of miR-21, miR-10b, miR-155, and miR-200c, along with reduced expression of miR-31, miR-126, and miR-335. The combined microRNA panel demonstrated stronger discriminatory performance than individual markers, supporting its potential value as a non-invasive biomarker profile for metastatic disease assessment. These findings suggest that circulating microRNA profiling may add useful molecular information to conventional clinicopathological evaluation, although larger multicenter and longitudinal studies are required before routine clinical application.

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