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Original Article

Exosomal MicroRNA-21 Expression Changes During Neoadjuvant Chemotherapy in Breast Cancer Patients

Rameen Khalid[1], Samira Khaliq[2], Taram Nayab[3], Muhammad Naveed[4], Sehrish Siddiqui[5], Muhammad Abbas Sadiq[6], Nimra Sikander[7]

[1] Forman Christian College University, Lahore, Pakistan
[2] Assistant Professor, Institute of Biochemistry, University of Balochistan, Quetta, Pakistan
[3] MBBS, Fatima Jinnah Medical University, Lahore, Pakistan; Medical Officer, Mayo Hospital, Lahore, Pakistan
[4] The Islamia University of Bahawalpur, Bahawalpur, Pakistan
[5] Senior Research Assistant, Aga Khan University Hospital, Karachi, Pakistan
[6] Relizon Pharmaceuticals, Lahore, Pakistan
[7] MBBS, King Edward Medical University, Lahore, Pakistan

***Corresponding author: Taram Nayab, drtaram6@gmail.com**

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ABSTRACT

Background: Variability in breast cancer response to neoadjuvant chemotherapy highlights the need for minimally invasive biomarkers capable of reflecting treatment effectiveness during therapy. Circulating exosomal microRNA-21 may provide dynamic molecular information because of its stability and association with tumor progression and treatment response. Objective: To evaluate longitudinal changes in circulating exosomal miR-21 during neoadjuvant chemotherapy and examine their association with tumor response. Methods: This parallel-group randomized controlled trial enrolled 72 women with stage II–III breast cancer at a tertiary-care oncology center in Punjab, Pakistan. Participants were allocated equally to protocolized exosome-guided monitoring or routine clinical monitoring. Blood was collected at baseline and before chemotherapy cycles for exosomal miR-21 quantification using quantitative reverse-transcription polymerase chain reaction. Final outcomes were available for 67 participants. Analyses included t-tests, repeated-measures analysis of variance, risk ratios, and Pearson correlation. Results: Post-treatment miR-21 expression was lower in the intervention group than in the control group (1.42±0.39 versus 2.11±0.53; mean difference=−0.69; 95% CI: −0.92 to −0.46; p<0.001). Tumor shrinkage was greater by 15.50 percentage points (95% CI: 6.80–24.20; p<0.001). RECIST response was 84.8% versus 64.7% (p=0.058), and pathological complete response was 39.4% versus 23.5% (p=0.162). miR-21 reduction correlated with tumor shrinkage (r=0.62; 95% CI: 0.45–0.75; p<0.001). Conclusion: Serial exosomal miR-21 measurement reflected treatment-associated molecular changes and was associated with radiological tumor reduction, but larger standardized trials are required before clinical implementation. Keywords: Biomarkers; Breast Neoplasms; Exosomes; MicroRNAs; Neoadjuvant Therapy; Precision Medicine; Treatment Outcome.

EDITORIAL INFORMATION

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INTRODUCTION

Breast cancer is a biologically heterogeneous disease in which patients with apparently similar clinicopathological characteristics may exhibit substantially different responses to systemic therapy. Neoadjuvant chemotherapy is routinely used in patients with locally advanced and selected early-stage breast cancer to reduce tumor burden, facilitate breast-conserving surgery, assess in vivo chemosensitivity, and provide prognostic information before definitive surgical management. Nevertheless, a considerable proportion of patients demonstrate incomplete or poor treatment response, resulting in continued exposure to potentially ineffective chemotherapy and its associated toxicities. Systematic evidence regarding exosome-derived microRNA-21 suggests that this circulating molecular

marker may have diagnostic and predictive value in breast cancer, although its role in longitudinal therapeutic monitoring remains insufficiently established (1,2).

Conventional assessment of neoadjuvant treatment response primarily relies on clinical examination, radiological imaging, and postoperative pathological evaluation. Although these approaches remain central to breast cancer management, structural tumor changes may become apparent only after several treatment cycles, while pathological response cannot be established until surgery. Liquid biopsy offers a complementary, minimally invasive strategy that permits repeated evaluation of tumor-associated molecular alterations during treatment. Circulating microRNAs have attracted particular interest in the neoadjuvant setting because their expression patterns may reflect evolving tumor biology, treatment sensitivity, and the emergence of therapeutic resistance (3,4).

Exosomes are small extracellular vesicles released by normal and malignant cells into biological fluids. They facilitate intercellular communication by transporting proteins, lipids, messenger RNA, and regulatory microRNAs between cells. Their lipid bilayer protects the enclosed molecular cargo from enzymatic degradation, thereby improving its stability in the circulation. Consequently, tumor-derived exosomes may provide a biologically informative and reproducible source of biomarkers for repeated therapeutic assessment. Among the oncogenic microRNAs identified in breast cancer, microRNA-21 (miR-21) has received considerable attention because of its involvement in cellular proliferation, invasion, angiogenesis, resistance to apoptosis, and altered therapeutic responsiveness. Its oncogenic effects are mediated partly through the suppression of tumor-regulatory pathways, including phosphatase and tensin homolog-related signaling and downstream activation of proliferative and survival pathways (5).

Elevated circulating or exosomal miR-21 expression has been associated with tumor progression, advanced disease characteristics, and unfavorable treatment outcomes. Conversely, declining expression during systemic treatment may indicate reduced tumor activity or treatment-sensitive disease. Previous studies have reported associations between circulating small extracellular-vesicle microRNAs and pathological response to neoadjuvant therapy, while broader investigations of circulating microRNAs have demonstrated their potential for predicting clinical response in breast cancer (6,7). However, much of the available evidence is based on observational studies, heterogeneous patient populations, non-standardized laboratory procedures, or comparisons limited to measurements obtained before and after treatment.

The temporal behavior of exosomal miR-21 across consecutive chemotherapy cycles therefore remains inadequately characterized. The existing literature provides limited evidence regarding whether serial miR-21 monitoring offers clinically useful information beyond routine assessment, whether molecular changes correspond with radiological tumor reduction, and whether biomarker-informed monitoring can support individualized treatment evaluation. Differences in specimen-processing methods, sampling schedules, analytical platforms, breast cancer subtypes, chemotherapy regimens, and response definitions have further restricted comparison among studies and delayed translation into clinical practice (8). Addressing these limitations is important within precision oncology, where treatment decisions increasingly incorporate dynamic biological information alongside conventional clinicopathological indicators (9,10).

Accordingly, this study aimed to evaluate longitudinal changes in circulating exosomal miR-21 expression during neoadjuvant chemotherapy in women with stage II–III breast cancer. It further compared protocolized exosome-guided monitoring with routine clinical monitoring and examined the association between changes in exosomal miR-21 expression, radiological tumor response, percentage tumor shrinkage, and pathological complete response. It was hypothesized that a greater reduction in exosomal miR-21 expression would be associated with a more favorable therapeutic response.

MATERIALS AND METHODS

A parallel-group randomized controlled trial was conducted at a tertiary-care oncology center in the industrial and urban core region of Punjab, Pakistan, between September 2025 and February 2026. The six-month study period included participant recruitment, allocation, delivery of the intervention, serial

biological sampling, and follow-up. Each participant underwent an 18-week course of neoadjuvant chemotherapy before definitive surgery. The study setting was selected because it provided centralized chemotherapy services, standardized oncology care, sufficient patient turnover, and facilities for repeated collection and laboratory evaluation of peripheral blood samples.

The target sample size was estimated using the treatment-related difference in circulating microRNA expression reported in a previous interventional breast cancer study. The calculation was based on an anticipated moderate treatment effect and was increased by 10% to account for potential attrition, resulting in a planned sample of 72 participants. Women aged 18–70 years were eligible when they had histologically confirmed stage II or III breast cancer, were scheduled to receive neoadjuvant chemotherapy, and had an Eastern Cooperative Oncology Group performance status of 0–2. Patients were excluded if they had metastatic breast cancer, previous chemotherapy or radiotherapy, another concurrent malignancy, an active inflammatory or autoimmune disorder, severe hepatic or renal dysfunction, pregnancy or lactation, or an inability to attend the scheduled treatment and follow-up assessments.

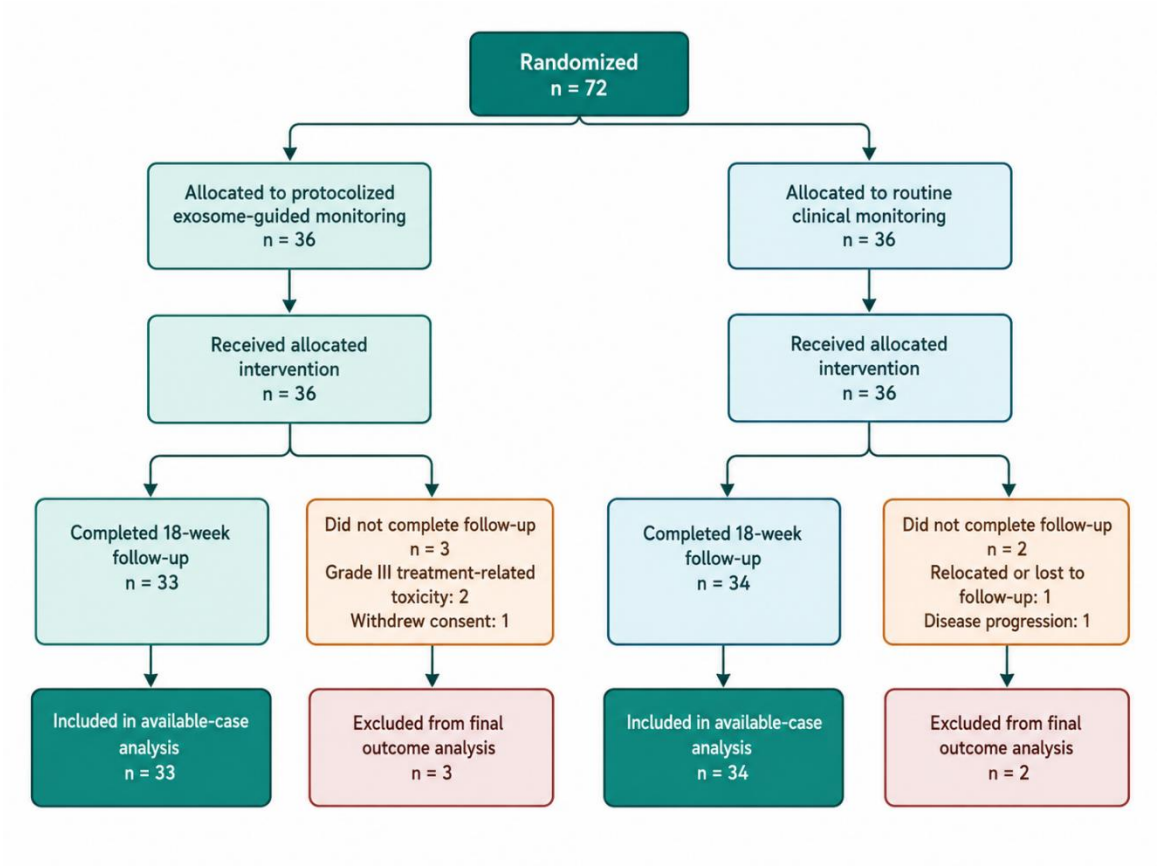

Figure 1 CONSORT Flowchart

Eligible participants were enrolled and randomly allocated in a 1:1 ratio to protocolized exosome-guided monitoring or routine clinical monitoring. An independent researcher prepared the allocation sequence using a computer-generated randomization procedure. Allocation concealment was maintained through sequentially numbered, sealed, opaque envelopes, which were opened only after enrolment. Because clinicians in the intervention group received serial biomarker reports, participants and treating oncologists could not be blinded to group allocation. Laboratory personnel responsible for exosome processing and miR-21 quantification and investigators conducting the statistical analysis remained blinded to the assigned groups.

All participants received neoadjuvant chemotherapy in accordance with the oncology center's institutional treatment protocols. Participants allocated to the intervention group additionally underwent protocolized exosome-guided monitoring. Peripheral venous blood was collected before initiation of chemotherapy and immediately before each scheduled chemotherapy cycle. Circulating exosomes were isolated from the blood samples, and exosomal microRNA was evaluated using quantitative reverse-transcription

polymerase chain reaction to determine the relative expression of miR-21. Samples from both groups were obtained at equivalent assessment points and processed using the same laboratory workflow by blinded personnel.

In the intervention group, serial miR-21 results were made available to the treating oncologists during routine clinical assessments. Biomarker findings were interpreted alongside the participant's clinical condition and conventional treatment-response assessment. When an inadequate molecular response corresponded with clinical evidence of insufficient therapeutic response, optimization of the ongoing treatment was permitted in accordance with the recommendations of the institutional multidisciplinary oncology team. No clinical decision was based solely on an isolated miR-21 result. Participants allocated to the control group received the same neoadjuvant chemotherapy and routine clinical and radiological monitoring. Blood samples were collected at the same time points as in the intervention group, but the miR-21 results were analyzed after completion of neoadjuvant treatment and were not used to guide clinical management.

The primary outcome was the longitudinal change in circulating exosomal miR-21 relative expression from baseline to completion of the 18-week neoadjuvant chemotherapy course. Serial measurements obtained before each chemotherapy cycle were used to evaluate the temporal pattern of biomarker change. Secondary outcomes included radiological tumor response according to Response Evaluation Criteria in Solid Tumors version 1.1, percentage tumor shrinkage, pathological complete response following surgery, and the association between changes in exosomal miR-21 expression and tumor response. Radiological response was categorized according to the established complete-response, partial-response, stable-disease, and progressive-disease classifications. Participants with complete or partial radiological responses were classified as RECIST responders. Pathological complete response was determined following definitive surgery using the postoperative pathological assessment.

Treatment attendance, chemotherapy completion, and adherence to scheduled assessments were monitored through clinical treatment records and attendance logs. Participants who discontinued treatment or did not attend the final assessment were documented with the reason for withdrawal where available. Baseline characteristics were evaluated for all 72 randomized participants. Because five participants did not complete the post-treatment assessment, analyses of the final biomarker and clinical outcomes were performed using the available follow-up data from 67 participants. Thus, the post-intervention analysis represented an available-case analysis rather than a complete intention-to-treat analysis.

Data were analysed using IBM SPSS Statistics version 26.0. Continuous variables were summarized as mean ± standard deviation, and categorical variables were presented as frequencies and percentages. Distributional normality was evaluated using the Shapiro–Wilk test. Independent-samples t-tests were used to compare continuous variables between the intervention and control groups, while paired-samples t-tests evaluated within-group changes between baseline and completion of chemotherapy. Categorical outcomes were compared between groups using tests appropriate to the observed frequencies. Repeated-measures analysis of variance was performed to assess the effects of time, group allocation, and the time × group interaction on serial exosomal miR-21 expression. Between-group effects were reported as mean differences with 95% confidence intervals where available. Pearson correlation analysis was used to examine the relationship between the magnitude of change in exosomal miR-21 expression and percentage tumor shrinkage. All tests were two-sided, and statistical significance was established at $p<0.05$.

RESULTS

Seventy-two women with histologically confirmed stage II–III breast cancer were enrolled and randomly allocated equally to the intervention and control groups, with 36 participants in each group. During the 18-week neoadjuvant chemotherapy period, three participants in the intervention group did not complete follow-up: two discontinued treatment because of grade III treatment-related toxicity, and one withdrew consent. In the control group, one participant was lost to follow-up after relocation, and one discontinued

treatment because of disease progression. Consequently, complete post-treatment data were available for 67 participants, comprising 33 in the intervention group and 34 in the control group. Baseline characteristics were evaluated in the complete randomized cohort, whereas treatment outcomes were evaluated using the available follow-up data.

Table 1. Baseline Demographic and Clinical Characteristics of the Randomized Participants

Variable	Total (N=72)	Intervention (n=36)	Control (n=36)	Group Difference (95% CI)	p-value
Age, years	49.1 ± 9.3	48.8 ± 9.5	49.4 ± 9.2	−0.60 (−5.00 to 3.80)	0.786
BMI, kg/m²	27.3 ± 4.1	27.1 ± 4.3	27.5 ± 3.9	−0.40 (−2.33 to 1.53)	0.681
Stage II	42 (58.3)	22 (61.1)	20 (55.6)	—	0.635
Stage III	30 (41.7)	14 (38.9)	16 (44.4)	—	—
Baseline exosomal miR-21 relative expression	3.48 ± 0.72	3.50 ± 0.74	3.46 ± 0.70	0.04 (−0.30 to 0.38)	0.814

Values are presented as mean ± SD or n (%). Between-group differences for continuous variables represent intervention minus control. Continuous variables were compared using independent-samples t-tests, and disease stage was compared using the Pearson chi-square test. BMI: body mass index; CI: confidence interval; miR-21: microRNA-21; SD: standard deviation.

The randomized groups were comparable at baseline. Mean age was 48.8 ± 9.5 years in the intervention group and 49.4 ± 9.2 years in the control group, corresponding to a mean difference of −0.60 years (95% CI: −5.00 to 3.80; p=0.786). BMI, breast cancer stage, and baseline exosomal miR-21 expression were also similar between the groups, with all baseline comparisons yielding p>0.05.

Table 2. Between-Group Comparison of Exosomal miR-21 Expression at Completion of Neoadjuvant Chemotherapy

Outcome	Intervention (n=33)	Control (n=34)	Mean Difference (95% CI)	Hedges g	p-value
Post-treatment exosomal miR-21 relative expression	1.42 ± 0.39	2.11 ± 0.53	−0.69 (−0.92 to −0.46)	−1.46	<0.001

Values are presented as mean ± SD. The mean difference represents intervention minus control. A negative effect estimate indicates lower post-treatment exosomal miR-21 expression in the intervention group. Hedges g was calculated from the reported group means, SDs, and sample sizes. CI: confidence interval; miR-21: microRNA-21; SD: standard deviation.

At completion of neoadjuvant chemotherapy, mean exosomal miR-21 expression was 1.42 ± 0.39 in the intervention group and 2.11 ± 0.53 in the control group. The intervention group therefore had a mean miR-21 expression level 0.69 units lower than the control group (95% CI: −0.92 to −0.46; p<0.001). The standardized between-group effect was large in magnitude (Hedges g=−1.46).

Table 3. Within-Group and Between-Group Changes in Exosomal miR-21 Expression

Comparison	Baseline	Post-treatment	Difference in Change (95% CI)	Standardized Effect	p-value
Intervention group (n=33)	3.50 ± 0.74	1.42 ± 0.39	−2.08 (−2.28 to −1.88)	−3.71	<0.001
Control group (n=34)	3.46 ± 0.70	2.11 ± 0.53	−1.35 (−1.52 to −1.18)	−2.76	<0.001
Between-group difference in change	—	—	−0.73 (−0.99 to −0.47)	−1.37	<0.001

Values are presented as mean ± SD. Negative change scores indicate reductions from baseline. Standardized within-group effects are based on the mean change divided by the SD of the change score. The standardized between-group effect is Hedges g calculated from the reported change scores. CI: confidence interval; miR-21: microRNA-21; SD: standard deviation.

Both groups demonstrated substantial reductions in exosomal miR-21 expression during chemotherapy. In the intervention group, mean expression declined by 2.08 units from baseline (95% CI: −2.28 to −1.88; p<0.001), whereas the control group demonstrated a mean reduction of 1.35 units (95% CI: −1.52 to −1.18; p<0.001). The reduction was 0.73 units greater in the intervention group than in the control group (95% CI: −0.99 to −0.47; p<0.001), with a large standardized between-group effect (Hedges g=−1.37).

Repeated-measures analysis demonstrated significant effects of time (F=186.8, p<0.001) and group allocation (F=21.4, p<0.001), together with a significant time × group interaction (F=34.7, p<0.001). These

findings indicated that exosomal miR-21 expression changed over the treatment period and that the pattern of change differed between the intervention and control groups.

Table 4. Secondary Clinical and Radiological Outcomes

Outcome	Intervention (n=33)	Control (n=34)	Effect Measure	Effect Estimate (95% CI)	Hedges g	p-value
RECIST responders	28 (84.8)	22 (64.7)	Risk ratio	1.31 (0.98 to 1.75)		0.058
Pathological complete response	13 (39.4)	8 (23.5)	Risk ratio	1.67 (0.80 to 3.51)		0.162
Tumor shrinkage, %	56.8 ± 18.4	41.3 ± 17.2	Mean difference	15.50 (6.80 to 24.20)	0.86	<0.001
Reduction in miR-21 versus tumor shrinkage			Pearson r	0.62 (0.45 to 0.75)		<0.001

Values are presented as n (%) or mean ± SD. Risk ratios compare the intervention group with the control group. The 95% CI for the correlation coefficient was calculated using Fisher's z transformation. RECIST responders included participants with complete or partial radiological responses. CI: confidence interval; miR-21: microRNA-21; RECIST: Response Evaluation Criteria in Solid Tumors; SD: standard deviation. The proportion of participants achieving a complete or partial radiological response was numerically higher in the intervention group than in the control group (84.8% versus 64.7%). Based on the reported event counts, the risk ratio was 1.31 (95% CI: 0.98 to 1.75), and the recalculated Pearson chi-square comparison did not reach the conventional threshold for statistical significance (p=0.058). Pathological complete response occurred in 13 of 33 participants in the intervention group and 8 of 34 participants in the control group. Although this represented an absolute difference of 15.9 percentage points, the corresponding risk ratio of 1.67 had a wide confidence interval that included the null value (95% CI: 0.80 to 3.51; p=0.162).

Mean tumor shrinkage was 56.8 ± 18.4% in the intervention group compared with 41.3 ± 17.2% in the control group. The between-group mean difference was 15.50 percentage points (95% CI: 6.80 to 24.20; p<0.001), representing a large standardized effect (Hedges g=0.86). The magnitude of reduction in exosomal miR-21 expression was positively correlated with percentage tumor shrinkage (r=0.62; 95% CI: 0.45 to 0.75; p<0.001), indicating that greater biomarker reductions were associated with greater radiological reductions in tumor size.

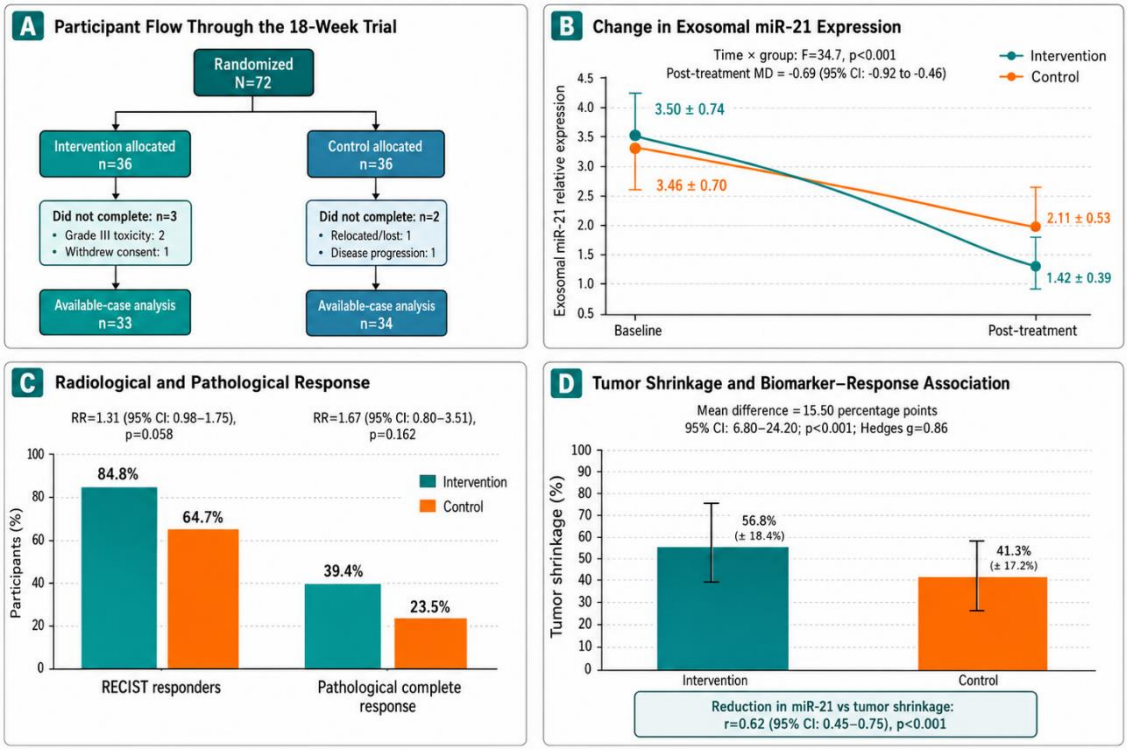

Figure 2 Exosomal miR-21 dynamics and treatment response during neoadjuvant chemotherapy. Panel A presents participant progression from randomization to the available-case outcome analysis. Panel B shows baseline-to-post-treatment changes in exosomal miR-21 relative expression. Panel C compares radiological response and pathological complete response between the groups. Panel D presents percentage tumor shrinkage and the association between miR-21 reduction and tumor shrinkage. Error bars represent standard deviations. Among 72 randomized participants,

post-treatment data were available for 33 intervention and 34 control participants. Exosomal miR-21 expression declined from 3.50±0.74 to 1.42±0.39 in the intervention group and from 3.46±0.70 to 2.11±0.53 in the control group, with a significant time × group interaction (F=34.7, p<0.001) and a post-treatment mean difference of −0.69 (95% CI: −0.92 to −0.46). RECIST response was observed in 84.8% versus 64.7% of participants (RR=1.31; 95% CI: 0.98–1.75; p=0.058), while pathological complete response occurred in 39.4% versus 23.5% (RR=1.67; 95% CI: 0.80–3.51; p=0.162). Mean tumor shrinkage was 15.50 percentage points greater in the intervention group (95% CI: 6.80–24.20; p<0.001), and greater miR-21 reduction was moderately associated with greater tumor shrinkage (r=0.62; 95% CI: 0.45–0.75; p<0.001).

DISCUSSION

This randomized study evaluated longitudinal changes in circulating exosomal miR-21 during neoadjuvant chemotherapy in women with stage II–III breast cancer. Exosomal miR-21 expression decreased substantially in both groups, but the reduction was greater among participants receiving protocolized exosome-guided monitoring. The intervention group also demonstrated greater mean tumor shrinkage, while the magnitude of miR-21 reduction was moderately associated with radiological tumor reduction. Although RECIST response and pathological complete response were numerically more frequent in the intervention group, their confidence intervals included the null value and the between-group differences did not reach conventional statistical significance. These findings indicate that serial exosomal miR-21 measurement may reflect molecular changes occurring during treatment, but they do not yet establish its effectiveness as an independent clinical decision-making tool.

The observed decline in exosomal miR-21 expression is biologically plausible because miR-21 is an oncogenic microRNA associated with cellular proliferation, invasion, angiogenesis, resistance to apoptosis, and chemotherapy resistance. Its activity has been linked to suppression of tumor-regulatory pathways, including phosphatase and tensin homolog signaling, with subsequent activation of the phosphoinositide 3-kinase/protein kinase B pathway. Effective chemotherapy may reduce the number and metabolic activity of malignant cells releasing miR-21-enriched exosomes into the circulation. The progressive decline in circulating exosomal miR-21 may therefore reflect decreasing tumor activity and burden rather than functioning solely as a static diagnostic marker. The increasing use of minimally invasive biomarkers for detecting and monitoring breast cancer recurrence further supports investigation of dynamic exosomal markers during treatment (11).

The greater reduction in miR-21 expression in the intervention group was accompanied by a mean tumor shrinkage advantage of 15.50 percentage points. This pattern suggests that molecular monitoring may provide information complementary to routine clinical and radiological evaluation. Nevertheless, biomarker monitoring itself would not be expected to alter tumor biology unless the results led to clinically meaningful treatment modifications. The present findings should therefore be interpreted in relation to the entire monitoring strategy rather than as evidence that measurement of miR-21 directly improved chemotherapy response. Liquid-biopsy and multi-omic approaches are increasingly being investigated for treatment guidance, but their clinical benefit depends on validated thresholds, reproducible assays, and clearly defined actions linked to the biomarker results (12).

The moderate positive correlation between reduction in exosomal miR-21 and percentage tumor shrinkage supports the potential value of miR-21 as a response-associated biomarker. The correlation coefficient of 0.62 indicated that participants with larger molecular reductions generally experienced greater radiological tumor reduction, although the association did not imply that miR-21 change caused tumor shrinkage. Previous investigations have similarly identified circulating exosomal microRNAs as potential predictors of neoadjuvant chemotherapy response, with treatment-sensitive tumors demonstrating different post-treatment expression patterns from resistant tumors (13). The present study extends this concept through serial assessment during the neoadjuvant period rather than relying exclusively on isolated pretreatment and post-treatment measurements.

Radiological response was observed in 84.8% of the intervention group and 64.7% of the control group. Although the absolute difference of 20.1 percentage points was clinically notable, the corresponding risk ratio was 1.31 with a 95% confidence interval of 0.98–1.75 and a recalculated p-value of 0.058. The result should therefore be interpreted as a possible response advantage requiring confirmation rather than a

statistically established treatment effect. Similarly, pathological complete response occurred in 39.4% and 23.5% of the groups, respectively, but the estimate was imprecise and statistically non-significant. Pathological response is influenced by tumor subtype, receptor status, chemotherapy regimen, intrinsic tumor heterogeneity, immune activity, and host characteristics. A single circulating microRNA is consequently unlikely to capture every biological determinant of treatment response. Multimarker exosomal panels may ultimately provide greater predictive accuracy than isolated biomarkers (14,15).

The findings also contribute to the broader development of liquid biopsy in precision oncology. Conventional imaging remains essential for assessing tumor dimensions and detecting disease progression, but measurable structural changes may emerge later than molecular alterations. Serial exosomal analysis could potentially complement imaging by indicating whether tumor-associated biological activity is decreasing during treatment. Exosomes are particularly attractive for repeated monitoring because their lipid membranes protect microRNA cargo from circulating ribonucleases. However, preanalytical variability in blood collection, centrifugation, storage, exosome isolation, RNA extraction, normalization, and quantitative polymerase chain reaction can materially affect measured expression. Standardization of these processes is essential before interinstitutional comparison or routine clinical use can be considered (16,17).

The study had several methodological strengths. Random allocation and concealed assignment reduced selection bias, while blinding of laboratory and statistical personnel limited measurement and analytical bias. Equivalent sampling schedules in both groups reduced the likelihood that differences arose from assessment timing. Serial sampling also permitted evaluation of biomarker dynamics rather than a single endpoint comparison. The inclusion of molecular, radiological, and pathological outcomes provided a multidimensional assessment of treatment response.

Several limitations require consideration. The trial was conducted at a single tertiary-care center and included a relatively small sample, limiting precision and external validity. Five randomized participants lacked final outcome data, and the post-treatment results were based on an available-case analysis rather than a complete intention-to-treat analysis. Differential reasons for non-completion, including toxicity and disease progression, may have introduced attrition bias. The protocol for inadequate molecular response, the threshold used to identify it, and the number and type of treatment modifications arising from biomarker reports were not fully characterized. Consequently, the mechanism through which protocolized monitoring may have influenced outcomes cannot be determined.

Only exosomal miR-21 was evaluated, and the analyses were not stratified by molecular breast cancer subtype, receptor profile, chemotherapy regimen, or other potential response modifiers. These factors may influence baseline microRNA expression and treatment-related changes. The study did not evaluate assay repeatability, interassay variability, laboratory costs, turnaround time, or the feasibility of incorporating serial exosome testing into routine oncology workflows. Follow-up ended after neoadjuvant therapy and surgery; therefore, the relationship of miR-21 change with recurrence, disease-free survival, and overall survival remains unknown. Broader circulating microRNA investigations and subtype-specific liquid-biopsy studies indicate that biomarker performance may differ across biologically distinct breast cancers (18,19).

Future multicenter studies should use larger samples, prespecified molecular-response thresholds, standardized exosome-processing protocols, and clearly defined clinical actions linked to biomarker findings. Analyses should account for tumor subtype, treatment regimen, baseline disease burden, and other prognostic characteristics. A complete intention-to-treat framework with appropriate management of missing data would strengthen causal interpretation. Longer follow-up is also required to establish whether treatment-related miR-21 changes predict pathological response, recurrence, and survival. Comparison of single-miRNA monitoring with multimarker exosomal, genomic, and imaging models may determine whether miR-21 provides independent and clinically useful predictive information.

CONCLUSION

Circulating exosomal miR-21 expression declined during neoadjuvant chemotherapy and demonstrated a moderate association with radiological tumor shrinkage in women with stage II–III breast cancer. Protocolized exosome-guided monitoring was associated with a greater reduction in miR-21 expression and greater mean tumor shrinkage, whereas the observed differences in RECIST response and pathological complete response were not statistically conclusive. Serial exosomal miR-21 measurement may therefore provide complementary information about treatment response, but larger multicenter studies using standardized laboratory procedures, predefined clinical thresholds, complete intention-to-treat analyses, and long-term follow-up are required before routine clinical implementation.

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