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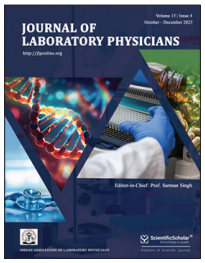

Original Article

Unrevealing the role of miRNA in successful TNBC treatment: A pilot study to explore the chemotherapy drugs for timely treatment of TNBC

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ABSTRACT

Objectives: Worldwide, breast cancer is the most prevalent and common type of cancer. Physical examination and mammography with a range of sensitivities are currently used as screening methods. Triple-negative breast cancer (TNBC) lacks estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER-2) gene expression. MicroRNAs (miRNA) as potential prognostic and diagnostic biomarkers, miRNA 125, 200c, 221, 21, and 34a were selected for study.

Materials and Methods: Here, 25 consenting TNBC patients with negative ER/PR/HER-2 status and compatible history were accrued from the Department of Oncosurgery, All India Institutes of Medical Sciences (AIIMS) Bhopal. Serum from participants and 25 controls was collected for quantitative estimation of miRNA by quantitative real-time polymerase chain reaction. After being treated with epirubicin, capecitabine, and paclitaxel, the MDA-MB-231 cell line's expression of these miRNA subtypes was also examined.

Statistical Analysis: All statistical analyses, pie charts, dot plots, and box-whisker plots were performed using EZR (Easy R), R Commander version 2.7–1. Bar graphs were created using Microsoft Excel 2019 software. Heat map graphics were produced using Graph Prism Version 9.

Results: miRNA125 ($p< 0.0001$) and miRNA21 ($p< 0.05$) were found to be statistically significant. miR125 (ΔCt [cycle threshold] 2.77) was seen to be upregulated and miR21 (ΔCt -1.61) was seen to be downregulated in TNBC patients. Epirubicin treatment caused miR125 to be downregulated, but capecitabine treatment caused miR125 to be upregulated. Paclitaxel was seen to downregulate miR21. All three chemotherapeutic agents were seen to downregulate miR34a.

Conclusion: miRNAs can be developed into a reliable biomarker and prognostic tool with more research. They can also help develop and improve pharmaco-therapeutic strategies.

Keywords: breast cancer, biomarker, tnbc, micrornas, diagnosis, chemotherapy

INTRODUCTION

Breast cancer remains leading form of cancer worldwide affecting large female population. As per clinical data, nearly 2.3 million breast cancer cases were reported/diagnosed in the year 2020 that leads to 0.7 million deaths. GLOBOCAN, 2020, estimated an alarming number of

cases where 7.8 million females alive by the end of year undergone for clinical diagnosis in the last 5 years.[1] Triple-negative breast cancer (TNBC) continues to be the leading cause of cancer-related death in Indian women. In women with fatty breast parenchyma and thick breasts, respectively, conventional mammography screening sensitivity ranges from 98 to 36%.[2] Therefore, for the early diagnosis of breast cancer, the development of minimally invasive diagnostic tools is necessary. At least four clinically significant molecular subtypes of breast cancer are now recognized, including TNBC, human epidermal growth factor receptor 2 (HER-2)-enriched, luminal A, and luminal B.[3] TNBC is a heterogeneous subset of these tumors that is positive for basal cytokeratin ⅝ or estimated glomerular filtration rate but does not express estrogen receptors, progesterone receptors, or HER-2 gene amplification.[4] They are also very aggressive and typically affect young patients.[5]

Neoadjuvant chemotherapy may, in some cases, increase a patient's eligibility for breast-conservation surgery with a decreased risk of local recurrence, but it may also be associated with unfavorable patient outcomes, especially in those who do not have a pathological complete response.[6,7] As a result, while a patient is receiving neoadjuvant chemotherapy, early identification of individuals who are not responding well to treatment enables the treatment to be changed effectively to address avoidable adverse effects of systemic therapy. Therefore, it is necessary to create minimally invasive markers with high specificity and sensitivity for tracking chemotherapy response as well as for spotting cancers that become resistant to systemic therapy.[8] MicroRNAs (miRNAs) have been investigated as potential predictive and diagnostic biomarkers in this regard in the past.[9,10] miRNAs are found in the extracellular space and as circulating molecules in bodily fluids such blood, serum, plasma, urine, tears, saliva, seminal fluid, cerebrospinal fluid, and extracellular fluid, among others.[11] miRNA dysregulation has a negative impact on the prognosis overall because it contributes to the emergence of treatment resistance and all types of cancer.[12,13]

Because of this, evaluating miRNA can help with treatment responsiveness, extensive surveillance of high-risk patients, and identifying patients with metastases. Identifying circulating miRNAs as prognostic indicators in TNBC as a result of neo-adjuvant chemotherapy is the aim of this study. This will be accomplished by comparing the expression of different miRNAs in TNBC patients to that in healthy females. This study aims to understand how chemotherapy exposure affects the expression of different miRNAs in breast cancer cell lines both before and after treatment with particular anticancer drugs.[13,14] Despite the fact that numerous studies have demonstrated that miRNAs can be successfully used for diagnostic and prognostic purposes in TNBC, there have not been many studies in an Indian context that have concentrated on the specific miRNAs expressed in TNBC in the Indian population, which can then be studied for specificity as a noninvasive diagnostic marker. In this study, much emphasis was given on has-mir-125, has-mir-34a, has-mir200c, has-mir-221, and has-mir-21. Additionally, this study also aims to evaluate in vitro expression of miRNAs on breast cancer cell lines and their correlation among drug treatment. Overall, the goal of this study is to analyze the expression of miRNAs and their response toward drugs.

MATERIALS AND METHODS

Study Design

After scrutinizing 100 breast cancer patients, 25 samples (n = 25) of serum from patients with TNBC who had not yet received treatment and 25 serum samples from healthy, normal women were obtained from the All India Institute of Medical Sciences (AIIMS), Bhopal, outpatient department (OPD). The patients were chosen at random as they arrived at the hospital's Onco-surgery/Radiotherapy OPD. Collected blood was centrifuged at 3,000 g for 15 minutes at 4°C after standing at room temperature for at least 30 minutes. Isolated serum was then aliquoted (500 µL) into 1.5 mL Eppendorf tubes and kept at 80°C until RNA extraction.

Cell Culture and Cell Growth Assay

The National Centre for Cell Science, Pune provided the human breast cancer cell lines MDA-MB-231, which were cultured in Eagle's modified essential medium at 37°C with 5% CO_2. In 96-well plates, MDA-MB-231 and MDAMB-231/epirubicin, capecitabine, and paclitaxel were plated. Cell viability was assessed at 0, 24, 48, and 72 hours using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) reagent (Keygen Biotech, Nanjing, China). By measuring the absorbance at 490 nm on enzyme-linked immunosorbent assay reader, cell viability was determined (BioTek).

Colorimetric MTT (Tetrazolium) Assay

MTT was used to assess the impact of three anticancer drugs, namely epirubicin, capecitabine, and paclitaxel, on the TNBC cell line MDA-MB-231. In 90 L of culture media, 5 103 MDA-MB-231 cells were seeded onto 96-well microtiter plates, and the plates were incubated overnight. The three anticancer drugs were applied to the cells in varied quantities for 24 hours. Each well received 10% v/v of the MTT solution, which was produced at 5 mg/mL in sterile phosphate buffered saline, and was then incubated at 37°C for 4 hours. To fully solubilize the formazan crystals, dimethyl

sulfoxide was added, and the micro-plate reader was used to measure absorbance at 570 nm (BioTek). The number of reagents required to block 50% of cellular proliferation following a 48-hour treatment period is known as the half maximal inhibitory concentration (IC50). Epirubicin's IC50 was determined to be 74 μM, capecitabine's IC50 to be 85 μM, and paclitaxel's IC50 to be 24μM.

Reverse Transcriptase PCR

The total RNA from breast cancer cell lines was extracted using Trizol method. Using the miRNAs sequence (Assay Technology), distinct miRNA primers for each of the five miRNAs were designed and synthesized, and then each miRNA's unique annealing temperature was determined (Table 1). Using an avian myeloblastosis virus reverse transcriptase kit that is commercially available from GCC Biotech, 500 ng of total RNA was reverse transcribed. The reaction mixtures were then incubated at 16°C for 30 minutes, 42°C for 30 minutes, 85°C for 5 minutes, and then held at 4°C. Before usage, the produced cDNA was kept at -20°C.

Quantitative Real-Time

PCR Using the Sybr green matrix kit (Bio-Rad) and 20 ng cDNA per 20 μL reaction, real-time polymerase chain reaction (PCR) was performed individually for sera and cell lines with their respective normal controls. The real-time (RTPCR) cycles included 40 cycles of 95°C for 1 minute. and 58°C for 1 minutes, followed by predenaturation at 1 cycle of 95°C for 3 minutes. Every action, including the no-template controls, was performed twice. Fixed threshold settings were used to obtain the resulting Cq values. To maintain the same efficiency across all miRNA analyses, we have always employed the same concentration of cDNA. Based on the threshold cycle (Ct) value, the twofold change in miRNA expression for each breast tumor sample in comparison to the normal control was computed using the following formula: relative quantification (RQ) = $2 - {}^{\Delta\Delta CT}$. miRNA expression was standardized to serum volume because U6 and 5S rRNA are destroyed in serum samples and there isn't a consensus housekeeping miRNA for the RT-quantitative PCR investigation of serum miRNAs.

Table 1: microRNA (miRNA) primer sequence

miRNA	Primer sequence
miR 34a-5p	5′-TGCGCTGGCAGTGTCTTAGCTG-3′
miR 221–5p	5′-GCACCTGGCATACAATGTAGA -3′
miR 125–5p	5′-TCCCTGAGACCCTAACTTG-3′
miR 200c-3p	5′-TAATACTGCCGGGTAATGATGGA-3′
miR-21–5p	5′-GCCCGCTAGCTTATCAGAC-3′
U6	5′- GCTTCGGCAGCACATATACTAAAAT -3′

Statistical Analysis

All statistical analyses, pie charts, dot plots, and box-whisker plots were performed using EZR (Easy R), R Commander version 2.7–1. Bar graphs were created using Microsoft Excel 2019 software. Heat map graphics were produced using Graph Prism Version 9. Using the Kolmogorov–Smirnov test for normal distribution, the normality of a continuous variable was evaluated. Mann–Whitney U test without parameters. The statistics were provided together with the median and interquartile range using the U test to determine the statistical significance between the two groups (interquartile range [IQR]). Statistical significance was defined as a p-value less than 0.05 ($p = 0.05$). To assess the predictive potential, receiver operating characteristic curve analysis and area under the curve (AUC) calculation were used.

RESULTS

Patient Demographics

Here in this study, 50 people in all were enrolled in the trial, of which 25 (the test group) had TNBC and 25 (the control group) were otherwise healthy people. In contrast to the healthy control group, which had a mean age of 41.68 years (standard deviation [SD]: 14.25 years), the test group had a mean age of 50 years (SD: 11.72 years) (Supplementary Figure S1). Here, 13 patients (52%) and 12 patients (48%) from the test group of this study's participants were from rural areas, whereas all 25 of the healthy controls were from metropolitan areas. Among the patients enrolled in the test group, 11 (44%) were premenopausal women and 14 (56%) were postmenopausal women (Supplementary Table S1). Supplementary Figure S2 summarizes IC 50 values of anticancer drugs used in this study: a. Epirubicin, b; paclitaxel and c; capecitabine. Seven (28%) of the 25 TNBC patients recruited in this study had a confirmed family history of breast cancer, compared with 18 (72%) of the patients who had no such history (Supplementary Table S2).

Risk Factors and Comorbidities

As the result showed in Supplementary Table S3, three (12%) of the 25 TNBC patients participating in the trial disclosed a history of cigarette use, but no one disclosed a history of alcohol use. Of the 24 TNBC patients, 24 (96%) disclosed a history of pregnancy. Further, 4 (16%), 13 (52%), and 8 (32%) of the test group members had body mass indices (BMIs) of 18.5 to 22.9, 23 to 24.9, and less than 25, respectively. Eight (32%) had diabetes mellitus, five (20%) had hypertension, and two (8%) had hypothyroidism. Nine patients (36%) and 16 (64%) of the patients, respectively, were found to have

hemoglobin levels below 12 mg/dL. As per data summarized in the Supplementary Table S4, only 3 (12%) of the patients in the test group had lesions in their right breast, compared with 22 (88%) who had involvement in their left breast. Eleven (44%) had stage II, 3 (12%) had stage III, and 11 (44%) had stage IV, according to the American Joint Committee on Cancer. There were 25 TNBC patients (100%) who were all involved in the lymph nodes. Eight (32%) of the patients who were enrolled in the test group experienced metastases, while 17 (68%) did not.

Expression Profile of miRNAs

Here in this study selection of miRNAs for their expression profile analysis associated with the TNBC patients was performed. Previous studies have demonstrated role of mir-34a,[15] miR125,[16–18] miR-21,[19,20] miR221,[21] and mir-200[22–24] miRNAs in the development of TNBC. The miRNA mean cycle threshold (Ct) levels were calculated independently for patients and controls (Supplementary Table S5, S6). By deducting the D Ct values of the controls from the Ct values of the cases, the Ct value was computed. miR125 had a Ct of 2.77, miR34a a Ct of -1.31, miR200c a Ct of 0.71, miR21 a Ct of 1.61, and miR221 a Ct of -0.88 (Tables 2 and 3). The difference between cases and samples for miR125 (median: 36.62, IQR: 3.365) and miR21 (median: 36.285, IQR: 2.7225) was found to be statistically significant ($p<$ 0.0001 and $p<$ 0.005, respectively). For miR34a, miR200c, and miR221, the difference between cases and samples was found to be not statistically significant (Table 4).

Table 2: Circulating miRNAs differentially expressed in the plasma of TNBC cases compared with healthy controls in PCR analysis

miRNA	Mean Ct (Control)	Mean Ct (Cases)	Δ Ct
miRNA 125	39.7024	36.9388	2.77
miRNA 34a	37.28	38.598	—1.31
miRNA 200c	13.1792	13.8828	—0.71
miRNA 21	35.5008	37.1144	—1.61
miRNA 221	28.1516	29.0376	—0.88

Ct, cycle threshold; miRNA, microRNA; PCR, polymerase chain reaction. *p*-Value<0.05.

Table 3: Mann–Whitney U test: Statistical identification of microRNA (miRNA) that was differentially expressed between cases and controls in the set

miRNA	Median	Interquartile range (IQR)	*p*-Value
miRNA 125	38.62	3.365	<0.0001****
miRNA 34a	39.315	2.945	0.764
miRNA 200c	30.06	1.49	0.159
miRNA 21	36.285	2.7225	<0.05*
miRNA 221	27.565	1.8375	0.0859

p-Value<0.05, miRNA: micro RNA, IQR: Interquartile range

Evaluation of the Diagnostic Potential of miR-125, miR-34a, miR-21, miR-200c, and miR221 in the Serum of TNBC Patients

MiR125 was seen to have an AUC of 0.698 (95% confidence interval [CI]: 0.548–0.847), miR34a had an AUC of 0.524 (95% CI: 0.362–0.686), miR21 was seen to have an AUC of 0.962 (95% CI: 0. 913–1.000), miR200c had an AUC of 0.616 (95% CI: 0.447–0.785), and miR221 had an AUC of 0.642 (95% CI: 0.486–0.799). Euclidean hierarchical complete linkage clustering based heat map showed in Figure 1A for the selected 5 circulating miRNA (miR21, miR 221, miR200c, miR125, and miR34a). Additionally, a comparison of expression profile of miR21, miR 221, miR200c, miR125 and miR34a associated with the breast cancer precisely TNBC shown in Figure 1B to E. After epirubicin treatment, a striking downregulation of miR125 was observed; however, capecitabine and paclitaxel showed no comparable downregulation changes. Capecitabine increased the expression of miRNA 125.

It was observed that paclitaxel downregulated miR200c, miR21, and miR221 (Figure 1A–E). It has been observed that miR34a expression is downregulated by all three chemotherapy drugs. Epirubicin was reported to induce the highest amount of cell death after anticancer chemotherapeutic therapy (Figure 3). Paclitaxel was shown to have the second-highest rate of cell death following anticancer chemotherapeutic therapy (Figure 4). Capecitabine caused quite minimal cell death after anticancer chemotherapeutic therapy (Figure 4 and Table 4). Additionally, Supplementary Table S7 summarizes miRNAs expression and twofold change of MDA-MB-231 after treatment with anticancer chemotherapeutic agents according to their respective IC50 dose for 24 hours (Supplementary Figure S2). A differential expression profile of miR21, miR221, miR200c, miR125 and miR34a was reported in MDA-MB-231 cell lines after the treatment of anticancer chemotherapeutic agents (Figure 2B).

This study reported higher expression of miR21, miR221, miR200c, miR125, and miR34a with epirubicin treatment in cell line system MDA-MB-231 (Figure 4B).

DISCUSSION

The purpose of this study was to assess the expression of miRNA in TNBC in patients receiving care at a tertiary care facility in central India. Even though there have been improvements in care strategies for breast cancer; it continues to be the biggest contributor to cancer-related deaths in

Table 4: miRNA expression before and after treatment with anticancer agents in MDA-MB-231 cell line (Ct values)

	U6	mir200c	mir21	mir221	mir34a	mir125
Control	18.53	28.3	31.6	22.7	11.5	28.42
Epirubicin (Epi)	24.16	27.9	31.8	23.21	38.5	36.09
Capecitabine (Cap)	23.23	28.15	32	29.9	35.8	28.91
Paclitaxel (Pac)	11.73	29.9	32.8	26.2	24.2	19.3

Abbreviations: Ct, cycle threshold; miRNA, microRNA.

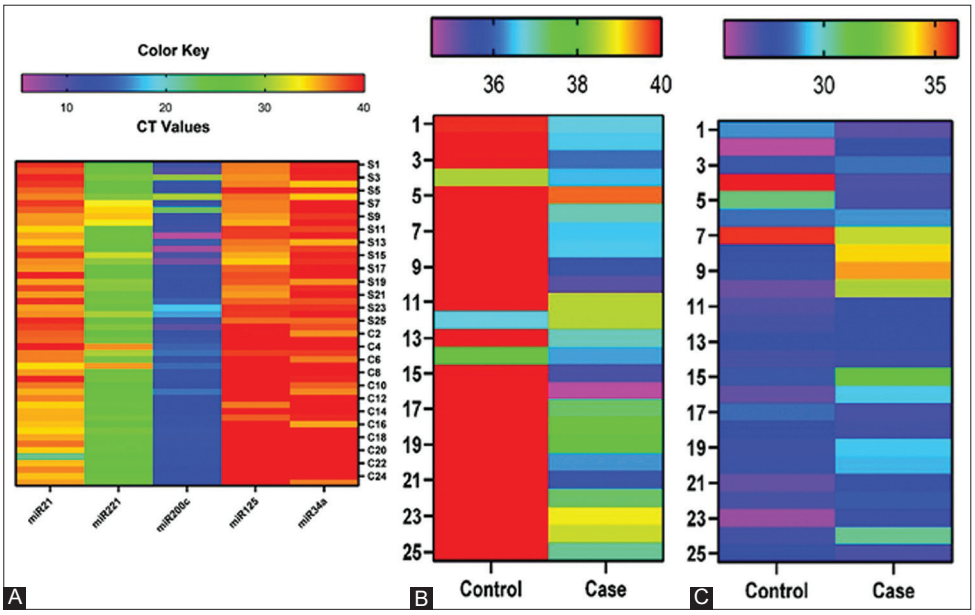


Figure 1: (A) Heat map: Euclidean hierarchical complete linkage clustering based on the selected 5 circulating microRNA (miRNA), (B) Heat map of miRNA 125 of triple-negative breast cancer (TNBC) patient samples and normal healthy control serum samples. (C) Heat map of miRNA 221 of TNBC patient samples and normal healthy control serum samples. Ct, cycle threshold.

Indian women.[14] Locally advanced breast cancer and TNBC are frequently treated with neoadjuvant chemotherapy, but the poor clinical outcome in TNBC has pushed for the development of predictive biomarkers for early detection of TNBC and for the assessment of these biomarkers' responses to chemotherapeutic treatment in TNBC patients because there are so few established prognostic and predictive biomarkers.[25] Twenty-five TNBC patients who had serum samples taken before beginning neoadjuvant chemotherapy, radiation, and surgery made up the test group. Twenty-five healthy women who had no family history of uterine, ovarian, or breast cancer made up the control group. The TNBC patients in this study ranged in age from 26 to 75 years old, with a median age of 50.04 years (SD: 11.72 years). Other research done in India revealed that patients were diagnosed with TNBC on average at 48.41 and 48.8 years old, respectively. Despite the fact that TNBC affects women of various ages, there is no clear association between the prognosis and the age of diagnosis.[26] The most frequent cancer now diagnosed worldwide is breast cancer.1 In this study, 12 patients (48%) came from metropolitan areas, whereas 13 patients (52%) came from rural areas.[27]

Lack of knowledge of breast cancer in the rural population, which is reflected in the high mortality-to-incidence ratio, is another explanation for the discrepancy that skews the incidence of TNBC or breast cancer as a whole toward a more urban population.[28,29] Here, 11 (44%) of the TNBC patients who participated in our study were premenopausal, whereas 14 (56%) patients were postmenopausal. Family history plays a big role in predicting the likelihood of having TNBC.[30] Only 7 (28%) of the TNBC patients in our study provided a positive family history, while 18 (72%) of the patients provided a negative one. This is in contrast to other research' findings, which indicated a high association between a family history of breast cancer and its progression, particularly TNBC.[31,32] It can also predispose to diabetes mellitus and cardiovascular complications. In our investigation, five (20%) of the subjects had known cases of hypertension, while eight (32%) patients were found to have a BMI below

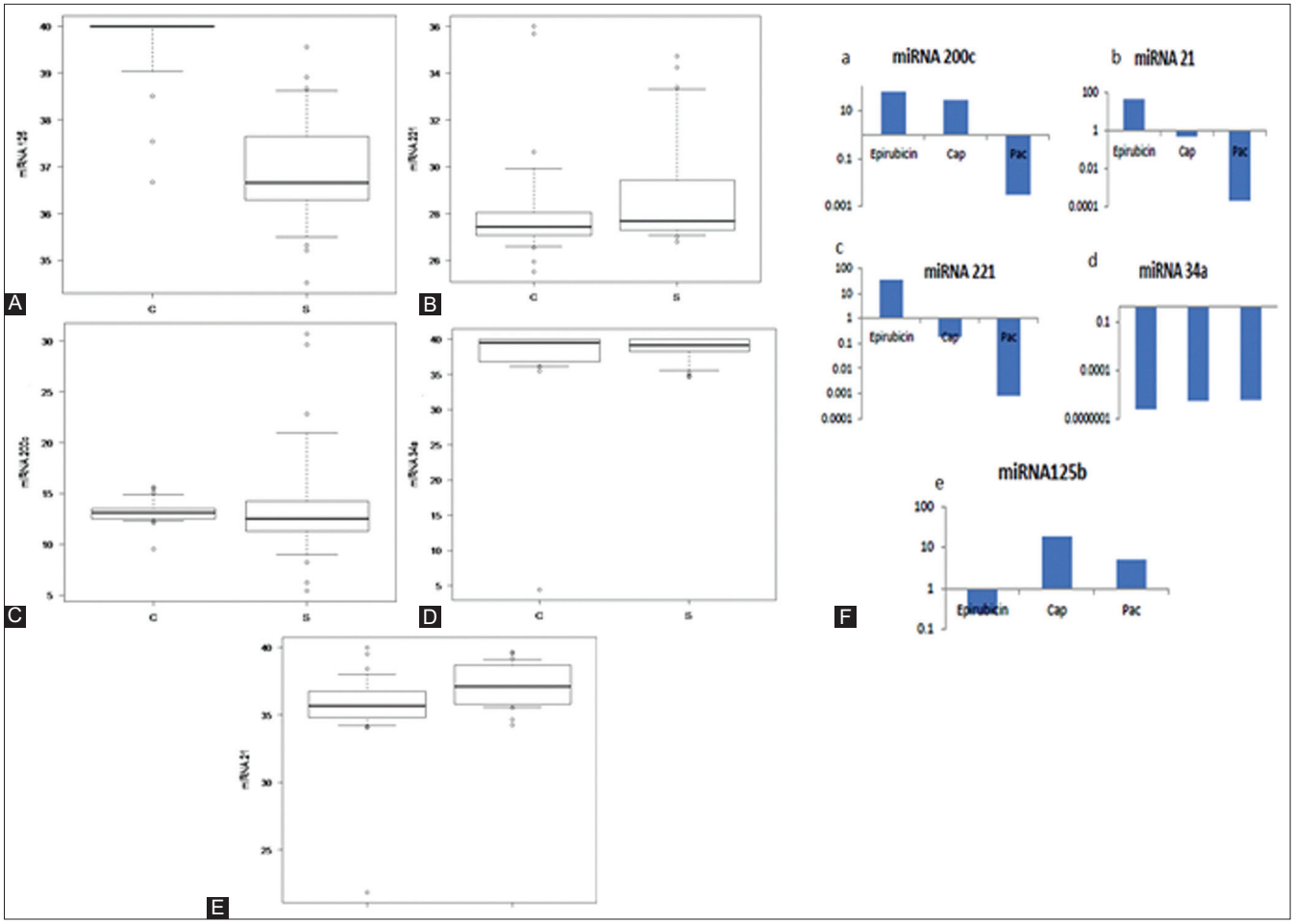
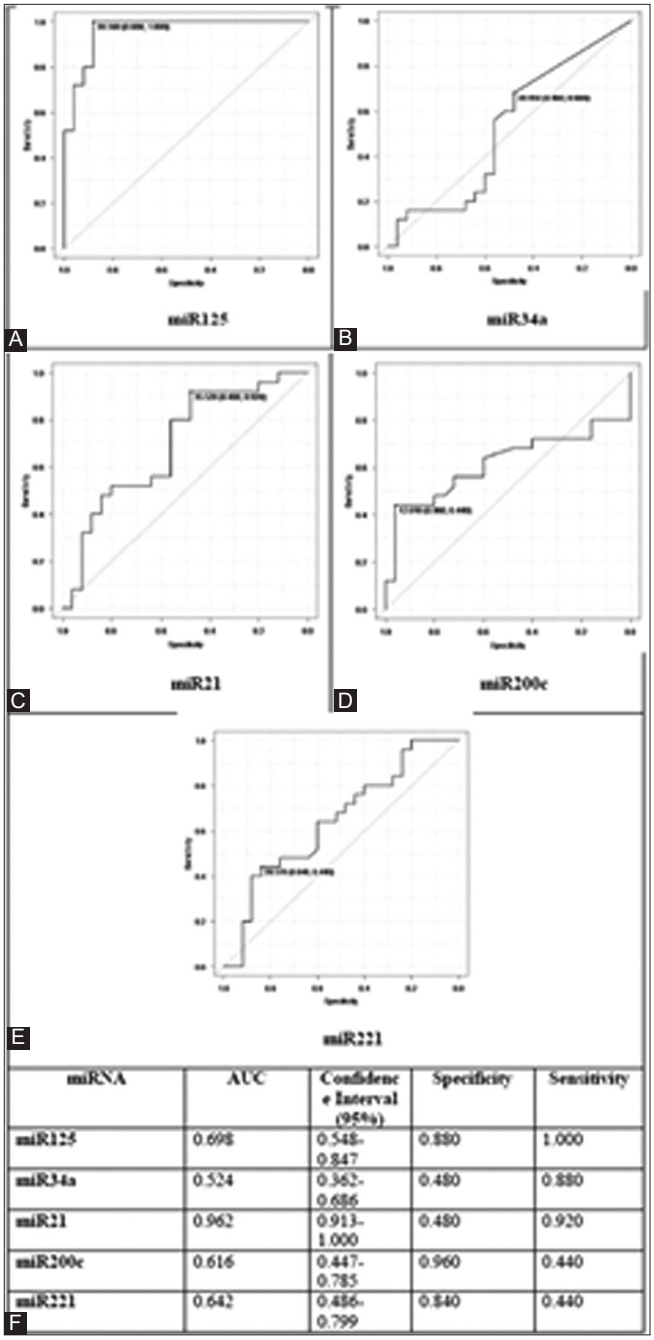


Figure 2: Box plot: Triple-negative breast cancer patients versus healthy controls: microRNA (miRNA) expression in serum sample. Box-whisker plot showing Ct values and its corresponding *p*-value; (A) miRNA125, (B) miRNA225, (C) miRNA200c, (D) miRNA 34a, and (E) miRNA 21, (F) Representation of miRNA expression and twofold change of MDA-MB-231 after treatment with anticancer chemotherapeutic agents; miRNA200c, miRNA21, miRNA221, miRNA 34a, and miRNA125b.

25. Eight (32%) of the TNBC patients had a history of type II diabetes. Although type II diabetes and insulin resistance are both connected to an increase in the overall incidence of breast cancer, TNBC is less frequently associated with type II diabetes.[33,34] In this study, only three (12%) of the TNBC patients had involvement of the right breast, while 22 (88%) of the TNBC patients had lesion involving the left breast. This is consistent with Perkins et al 2004[35] and other studies[36,37] found that the left breast was more affected than the right breast. Additional investigation into the physiological and immunological pathways may assist to elucidate the issue, even though the precise source of the phenomenon is yet unknown.[38]

According to the clinical staging of the TNBC patients included in this study, 11 (44%) patients were in stage II, 3 (12%) were in stage III, and 11 (44%) were in stage IV. Similar patterns were observed in patients reporting with clinical stage II in studies by Nabi et al[34] and Cornier et al. 2008,[39] although in those studies the trend of patients diagnosed with stage III was noticeably higher, the trend of patients diagnosed with stage IV was noticeably lower. This discrepancy can be explained by the higher proportion of patients from rural areas, who often only seek care when very ill due to ignorance and financial constraints.[40] Patients with TNBC are more likely to have lymph node involvement, and the degree of lymph node involvement affects the course of treatment and the prognosis.41 In this investigation, lymph nodes were involved in all 25 (100%) individuals. Studies by Akhtar et al[27] and Nabi et al[34] also demonstrated tendencies of greater lymph node involvement in TNBC patients. TNBC has a higher propensity for distant metastasis because of its high level of malignancy and invasiveness. This increased propensity manifests itself in organs like the liver, lungs, bones, and portions of the central nervous system, which has a detrimental effect on the patient's prognosis.[40] Eight (32%) of the total patients had distant metastases upon presentation, which is consistent with the proportion of



F

miRNA	AUC	Confidence Interval (95%)	Specificity	Sensitivity
miR125	0.698	0.548-0.847	0.880	1.000
miR34a	0.524	0.362-0.686	0.480	0.880
miR21	0.962	0.913-1.000	0.480	0.920
miR200c	0.616	0.447-0.785	0.960	0.440
miR221	0.642	0.486-0.799	0.840	0.440

Figure 3: (A) Evaluation of the diagnostic potential of microRNA-125 (miRNA-125), (B) miRNA-34a, (C) miRNA-21, (D) miRNA-200c, and (E) miRNA 221 in the serum of triple-negative breast cancer patients. (F) The therapeutic potential of miRNA-125, miRNA-34a, miRNA-21, miRNA-200c, and miRNA 221 was examined via determination of specificity and sensitivity of miRNAs in serum. AUC, area under the curve.

patients with advanced clinical stages.

Circulating miRNAs are an additional, if not a substitute, diagnostic technique for detecting breast cancer early, which opens the door for a better prognosis.[41] This is due to their distinctive expression profiles and exceptional stability in plasma. A work by Guo et al has shown the prospect that certain circulating miRNAs can be employed as better diagnostic biomarkers for malignant lesions of the breast in comparison to traditionally used tumor markers like C15– 3 and CEA.[42] Twenty-five women with TNBC who were receiving OPD-based treatment and 25 healthy women were enrolled in this study.[43] Data on their ages, racial, and ethnic backgrounds, menopausal status, and family history of breast cancer, history of pregnancies, addictions, BMI, and comorbid conditions like hypertension, diabetes mellitus, and hypothyroidism were collected.[44] The expression of a total of five miRNAs—hsa-mir-125, hsa-mir-34a, hsa-mir-200c, hsa-mir-221, and hsa-mir-21—in the serum samples of each patient was assessed.[45]

miRNA 125 ($p< 0.0001$) and miRNA 21 ($p< 0.05$) were found to be statistically significant among the five miRNAs examined, with miR 125 (ΔCt 2.77) being observed to be upregulated and miR 21 (ΔCt -1.61) being seen to be downregulated in patients with TNBC, respectively.[46–48] The miRNA 125 family plays a variety of roles by targeting various areas critical in biological processes like the maintenance of normal homeostasis and proliferation of immune cells, altering cell differentiation, and apoptosis, which can contribute to tumorigenesis.[49] This is done through the process of post-transcriptional regulation of gene expression. The findings of Wang et al[50] revealed elevated miR125b levels in breast cancer patient blood samples that were inversely correlated with the grade of the tumor and the stage of lymph node metastasis, among other findings. Similar to this, Nie et al study revealed that TNBC patients had higher levels of miR125b in their blood.[51] In our study, we found that the miR125 was significantly elevated in TNBC patients, which is consistent with the results previously indicated.

It is widely known that miR21 plays a pro-oncogenic effect.[52] By inhibiting apoptosis, miR21 has been found to be upregulated in several solid tumors, including tumors of the gastrointestinal tract, prostatic, lung, and neuroendocrine systems.[53] In stark contrast to previously reported findings, miR21 was shown to be downregulated in our study (Ct -1.61, p 0.05). Further analysis is necessary because the precise mechanism responsible for the clarification of this finding remains unclear. We also looked at three other miRNAs: miR221 (may be tumor suppressor or oncogenic depending on the tumor),[54] miR200c (a circulating miRNA that prevents TNBC from spreading),[55] and miR34a (whose downregulation indicates a poor prognosis).[56] MiR221 was downregulated (ΔCt -0.88), as was miR200c (ΔCt -0.71) and miR34c (ΔCt -1.31), but these changes were not statistically significant ($p = 0.086$, $p = 0.16$, and $p = 0.76$, respectively). By calculating the AUC, ROC curve analysis can be used to evaluate a possible diagnostic tool's discriminatory capacity

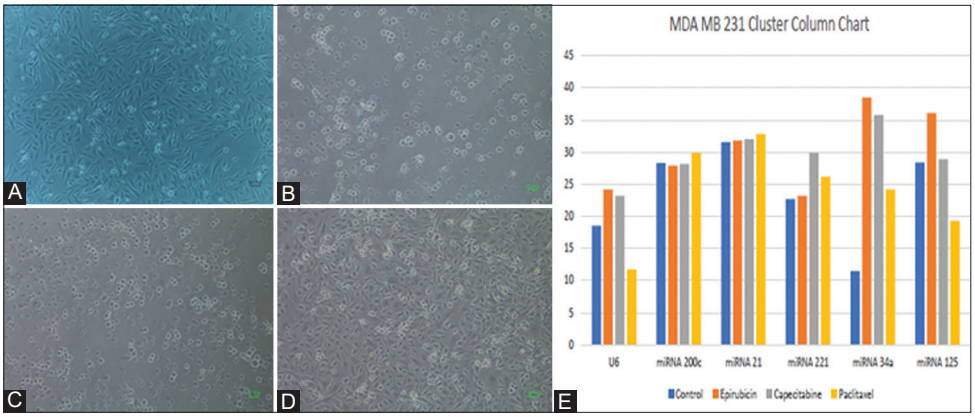


Figure 4: (A) MDA-MB-231 cell phenotype under the exposure of chemotherapeutics; Microscopic image of MDA-MB-231 cell line pre-treatment with anticancer chemotherapeutic agent. (B) Microscopic image of cell death observed in MDA-MB-231 cell line following treatment with epirubicin. (C) Microscopic image of cell death observed in MDA-MB-231 cell line following treatment with paclitaxel. (D) Microscopic image of cell death observed in MDA-MB-231 cell line following treatment with capecitabine. (E) MDA-MB-231 cluster chart.

between cases and control data.[56] With a specificity of 0.880 and a sensitivity of 1.000, miR125 in this investigation shown good predictive ability and had an AUC of 0.698 (95% CI: 0.548–0.847), which is consistent with the findings of Fang et al.[57] Since miR21, an oncogenic miRNA, was observed to be downregulated, a conclusion inferred from this data may be incorrect. MiR21 had an AUC of 0.962 (95% CI: 0.480–0.920), which also demonstrated a strong predictive power. Therefore, more analysis is necessary for the situation. In comparison to miR125, the AUC of miR34a, miR200c, and miR221 did not show a strong predictive power.

Monoclonal antibodies, one of the most effective therapeutic methods now available, are ineffective in TNBC tumors due to the lack of receptors, making the use of nonspecific cytotoxic medicines a necessity.[58] The development of chemotherapy resistance in TNBC is largely influenced by changes in the number and structure of chromosomes, as well as gene deletions or mutations like those in JAK2 and PTEN, immune evasion, and activation of pro-oncogenes such PIM1.[59] Pharmacotherapeutic result in TNBC patients can be improved by composite markers that can extrapolate chemotherapy efficacy while also predicting triple-negative malignant lesions.[60] The expression of the five miRNA subtypes was assessed on the MDA-MB-231 TNBC cell line after treatment with the three chosen anticancer agents, namely epirubicin, capecitabine, and paclitaxel; in study by Wang et al,[61] and miR21 was found to be a potential biomarker for the assessment of response to neoadjuvant chemoradiotherapy. After epirubicin treatment, a striking downregulation of miR125 was observed; however, capecitabine and paclitaxel showed no comparable downregulation changes. Epirubicin may have a better therapeutic effect when miR125 is downregulated because miR125 is oncogenic, but capecitabine may have a worse therapeutic outcome when miR125 is up-regulated.[62]

Similar to how paclitaxel was observed to downregulate miR200c, miR21, and miR221, whereas downregulation of miR200c, a tumor suppressor,[63] may negatively impact the treatment outcome and result in disease progression or chemoresistance. In this investigation, it was discovered that all three chemotherapy drugs reduced the activity of miR34a, a tumor suppressor[64] that may cause chemoresistance. As one of the "first of its kind" research to be undertaken in the institute and in India, there is still a wealth of knowledge to be learned about the patterns of miRNA expression in the Indian population.[65] Evaluation of the circulating miRNA status in TNBC patients in conjunction with assessment of the chemotherapeutic drug's effect on the identified significant miRNAs may offer valuable insight in predicting the prognostic and pharmacotherapeutic outcome, which can be crucial in the formulation of a chemotherapeutic strategy tailored to each patient's needs while minimizing the unfavorable therapeutic and prognostic outcomes. For this hypothesis to be established and validated to improve patient care, more research is necessary.

CONCLUSIONS

Due to their aggressive proliferation, high local infiltration, and potential for metastasis, TNBCs are a significant cause of neoplasm-related morbidity and mortality in women. Due to the dearth of receptors, targeted therapy is impractical, forcing nonspecific cytotoxic chemotherapy that raises morbidity. In this investigation, TNBC patients from central India had the expression of circulating miRNAs evaluated.

We can confirm that miRNAs are trustworthy biomarkers as a screening and prognosis prediction tool based on blood tests that can support the already existing diagnostic modalities. These miRNAs can aid in the formulation and improvement in the pharmacotherapeutic strategy by forecasting pharmacotherapeutic response and the possible development of chemo-resistance. We next assessed whether these miRNAs were upor downregulated in response to three widely used chemotherapeutic anticancer drugs. This study provides some insight into the possibility of a relationship between the measurement of circulating miRNA expression and chemotherapeutic response, which may aid in the development of customized pharmacotherapies for the best possible patient care. However, more research is required to establish and validate circulating miRNAs as compound biomarkers.

Declaration

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Ethical approval

Ref No.-IHEC-LOP/2019/EF0111 by, Institutional Human Ethics Committee, All India Institute of Medical Sciences (AIIMS) Bhopal, Madhya Pradesh, India.

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Conflict of Interest

None declared.

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