Case-controlled Study

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Circulating miR-221/222 expression as microRNA biomarker predicting tamoxifen treatment outcome: a case–control study

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Introduction: The high mortality rate in breast cancer (BC) patients is generally due to metastases resistant to systemic therapy. Two causes of systemic therapy resistance in BC patients are circulating miRNAs-221 and miR-222, leading to improved BC cell proliferation, survival, and reduced cell apoptosis. This study investigated the miRNA expression changes associated with cancer cell resistance to tamoxifen therapy and is expected to be clinically meaningful before providing endocrine therapy to luminal-type BC patients who express them.

Methods: This case–control research included individuals with the luminal subtype of BC who had received tamoxifen medication for around one year. Furthermore, the case group contained 15 individuals with local recurrence or metastases, while the control group comprised 19 patients without local recurrence or metastases. Plasma miR-221/222 quantification was performed with real-time PCR using transcript-specific primers.

Results: A significant difference was found in circulating miR-221 expression between cases and controls ($P = 0.005$) but not in miR-222 expression ($P = 0.070$). There were no significant differences between miR-221/222 expression, progesterone receptor, Ki67 protein levels, lymphovascular invasion, and stage. However, receiver operator characteristic curve analyses showed miR-221/222 expressions predictive of tamoxifen resistance ($P = 0.030$) with a sensitivity of 60.00 and a specificity of 83.33%.

Conclusion: The use of circulating miR-221/222 expression can predict relapse as well as resistance to tamoxifen treatment in BC patients, and their testing is recommended for luminal subtype BC patients who will undergo tamoxifen therapy to determine their risk of tamoxifen resistance early, increasing treatment effectiveness.

Keywords: breast cancer, luminal, miR-221, miR-222, tamoxifen

Introduction

Breast cancer (BC) is the most prevalent malignancy in females, comprising 24.5% of all incidences globally[1]. It is also the biggest reason for females related to cancer-related death worldwide[1,2], with an expected 682 000 cases in 2020[1].

HIGHLIGHTS

- The high mortality rate in breast cancer (BC) patients is generally due to metastases resistant to systemic therapy.
- Two causes of systemic therapy resistance in BC patients are miRNAs-221 and miR-222.
- These lead to increased proliferation and survival of BC cells and decreased cell apoptosis.
- Receiver operator characteristic curve analyses showing miR-221/222 expression that was predictive of tamoxifen resistance with sensitivity of 60.00 and specificity of 83.33%.
- The use of miR-221/222 expression can predict relapse as well as resistance to tamoxifen treatment in BC patients.

Currently, there are many modalities of BC therapy available. Surgery remains the main modality for early-stage BC, combined with radiotherapy, chemotherapy, and antiestrogen (hormonal or endocrine) therapy to increase treatment success[3–5]. Targeted therapies have also played an important role in treating BC patients with human epidermal growth factor receptor 2 (HER2)-positive results[6].

The hormone estrogen is important in BC pathogenesis via estrogen receptor (ER) signaling[7]. Antiestrogen therapy in BC patients is based on the expression of estrogen receptor

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alpha (ERα). Based on the immunohistochemical examination, it is known that about 70% of BCs are ERα-positive[8].

Endocrine therapy seeks to lower estrogen levels or block endoplasmic reticulum signal transduction. It comprises a class of medications including selective estrogen receptor modulators (for example, tamoxifen), selective estrogen receptor down regulators (for example, fulvestrant), and an aromatase inhibitor (for example, anastrozole)[9,10].

Tamoxifen has been utilized for more than three decades for patients with early-stage BC who are ER-positive as adjunctive endocrine therapy with the benefit of significantly increased survival[11]. However, tamoxifen resistance is often encountered[12]. This resistance reduces the therapy goals as well as leads to reproduction or metastatic BC. Deaths from BC are often caused by disease recurrence and resistance to therapy[13,14].

Various hypotheses regarding therapeutic resistance have been proposed. Resistance to hormonal therapy can arise de novo early after diagnosis (intrinsic resistance) or during hormonal therapy (acquired resistance). Despite the availability of new, more powerful drugs, endocrine therapy resistance is still the main problem related to BC management[15].

It is believed that changes in the expression of small (16–29 nucleotides) noncoding RNA molecules called microrNA (miRNA) contribute to the development of resistant BC to antihormonal drugs. MicroRNAs reduce the expression of the protein product of their target genes by suppressing the translation process or degrading messenger RNA[16]. Studies on MCF-7 BC cell cultures showed differences in miRNA expression profiles between tamoxifen-sensitive and resistant cells[17]. There are hundreds of miRNA whose expression is believed to differ between sensitive and resistant cells[18].

MiR-221 as well as miR-222 are the most investigated microRNAs concerning endocrine treatment resistance in BC. The enhanced expression of these two miRNAs correlates with tamoxifen and fulvestrant resistance[17,19]. Important targets increased by miR-221 and miR-222 include the signaling pathways of the Cip/Kip family (p21, p27, as well as p57), ER, and the phosphatase as well as tensin homolog (PTEN)[20]. These signaling pathway regulation increases the proliferation as well as the BC cell survival and decreases their apoptosis. Both miR-221 and miR-222 target the estrogen receptor 1 (ESR1) gene[17,19]. MiR-221 expression was substantially greater in ER-positive than ER-negative BC[21]. The increase in miR-221 expression, which is high during cancer cell growth, may be intended to suppress the cyclin-dependent kinase inhibitor p27 gene to trigger cell cycle progression as well as cell proliferation[22]. Another study showed that suppression of miR-221 as well as miR-222 expression greatly improved the ER-positive BC cells sensitivity toward tamoxifen, decreasing their viability. This impact is associated with the improved tissue inhibitor production of metalloproteinase-3 (TIMP3), resulting from the reduced expression of miR-221 as well as miR-222[23]. These results can inform future antimiRNA therapeutic techniques for BC.

MiRNAs are present in tissues and circulating blood[24,25]. The detection of circulating miRNAs as biomarkers have several benefits, including ease of collection, noninvasiveness, cost-effectiveness, and stability in body fluids including serum as well as plasma[26]. Research into miRNA profiling has the potential to produce new therapies. Some studies revealed that the modification of miRNA expression has succeeded in making drug-resistant cancer cells sensitive again[27–30]. This study investigated the miRNA expression changes associated with cancer cell resistance to tamoxifen therapy and is expected to be clinically meaningful before providing endocrine therapy to luminal-type BC patients who express them.

Methods

Design, ethics, and sample size

This research conforms to the Strengthening the Reporting of Cohort Studies in Surgery (STROCCS) Guidelines[31] as well as is recorded through the research registry (approval no. 7241). Patients with luminal subtype BC that had received endocrine treatment for a minimum of per annum, outright clinical information, and consent of participation were included in the study. Individuals with local recurrence and metastases comprised the tamoxifen-resistant (case) group, whereas patients without recurrence or metastases included the tamoxifen-sensitive (control) group. The tamoxifen-resistant group has exclusion criteria such as a history of irregular as well as intermittent tamoxifen use or receipt of insufficient tamoxifen dosages. Acquiring a systemic therapy other than tamoxifen, including targeted therapy or chemotherapy, was an exclusion criterion for the tamoxifen-sensitive group.

All participants were BC patients receiving endocrine therapy with luminal subtype who obtained tamoxifen hormonal therapy at Mitra Keluarga Kemayoran, Royal Taruna Hospital, and other network hospitals in Jakarta, Indonesia, who met the inclusion criteria until the required number of samples was reached. The minimum number of study subjects was required to explore differences in the average miR-221 as well as miR-222 expression on the tamoxifen-resistant and tamoxifen-sensitive groups in response to tamoxifen hormonal therapy.

The Health Research Committee, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia was approved this study. The consent from all participants was given by signing the informed consent form.

Treatment and patient assessment

According to the Declaration of Helsinki, participants were sourced from breast surgery, surgical oncology, and medical hematology-oncology polyclinics. A histopathological analysis is used to diagnose BC. Immunohistochemical staining revealed the presence of ER and progesterone receptor (PR) to identify the luminal subtype. A pathologist from the Mitra Kemayoran Anatomical Pathology Laboratory conducted the evaluation (Jakarta, Indonesia).

According to the normal procedure for adjuvant therapy, patients received endocrine therapy with tamoxifen indications for a minimum of one year. Then, 5 ml of the patient's blood was taken with an EDTA blood collection tube, centrifuged (1000 rpm), and the plasma separated before being frozen until RNA isolation.

Lymphovascular invasion

Lymphovascular invasion (LVI) was determined by the existence of cancer cells in a specific endothelium-lined region. On slides stained with hematoxylin and eosin, it was calculated using surgical specimen boundaries obtained after neoadjuvant chemotherapy. If the outcomes were ambiguous, D2-40 for

lymphatic endothelium, specific markers, and CD34 for the endothelium of all vessels, were utilized to enhance the LVI detection.

Stage

The eighth version of the American Joint Committee on Cancer staging classification was utilized for primary tumor staging[32]. Based on imaging investigations and clinical examinations, we used the clinical stage. The largest dimension of the largest tumor was used to define tumor size.

Ki67 expression analysis

The Dako monoclonal antibody test (MIB-1, 1:100, Catalog No. GA626) was used as the primary antibody test and was applied following the manufacturer's recommendations. Counting at least 500 tumor cells per instance over 5 high-power fields of the slice under the microscope allowed researchers to evaluate the nuclear immunostaining for Ki67. The Ki67 proliferative index was given a high score when more than 25% of the tumor cells were positive and a low score when less than 25% of the cells were positive. The immunostained slides underwent independent evaluation.

Hormone receptor expression analysis

Analyses of the ER and PR were conducted as defined[33]. Primary antibodies for ER (clone 6F11, 1:200) as well as PR (clone 16, 1:800) were used to immunostain diagnostic core biopsies (both Novocastra Laboratories Ltd.). Furthermore, the 'Quickscore' approach[34] was used to grade the stained slides. Cancer cases with a score of 0–3 were considered negative, and those with a score of 4–18 (the maximum) were considered positive. Decisions regarding adjuvant endocrine therapy were based on this evaluation. All malignancies were then further evaluated utilizing the 'Allred' approach, where instances scoring greater than 2 were considered as positive[8], and the ASCO/CAP guidelines[35] for ER as well as PR expression (1% cutoff).

Total RNA extraction

The extraction of total RNA from plasma was carried out using the QIAamp RNA Blood Mini Kit (Qiagen) following the manufacturer's instructions. RNA samples were eluted with the elution buffer as well as quantified using a nano spectrophotometer.

miRNA cDNA synthesis

Following the manufacturer's instructions, miRNA cDNA was generated with the miRNA Reverse Transcription Kit: miScript II RT Kit (Qiagen).

Plasma miR-221/222 quantification

The *miR-221/222* transcripts were subjected to real-time quantitative PCR (qRT-PCR) using the miScript SYBR Green PCR Kit (Qiagen) as well as the miScript Primer Assay (Qiagen). Forward primer sequences for miR-221 and miR-222 were 5′-CGA GCT ACA TTG TCT GCT GGG T-3′ and 5′-CCG CAG CTA CAT CTG GCT ACT G-3′, respectively, in a 94°C cycle for 10 min. The reverse primer sequence used to amplify miR-221 and miR-222 was 5′-GTG CAG GGT CCG AGG T-3′. The cycle was repeated 32 times at 54°C (30 s).

Statistical analysis

This study's dependent variable was tamoxifen resistance, which was measured by the growth of the tumor following neoadjuvant therapy, while miR-221/222 expression was the independent variable. For all statistical studies, IBM SPSS Statistics v.23.0 (IBM Company) was utilized. Utilizing the Mann–Whitney *U* test and receiver operator characteristic (ROC) curves, the relevance of miR-221/222 expression on other dependent variables, including tumor size, stage, and Ki67 and PR expression, was evaluated.

Results

Comparison of miR-221/222 expression in tamoxifen-resistant and sensitive groups

In the tamoxifen-resistant group, miR-221 expression was considerably higher compared to the tamoxifen-sensitive group ($P = 0.005$). However, miR-222 expression was not significantly different in the tamoxifen-sensitive group compared to the tamoxifen-resistant group ($P = 0.070$; Fig. 1).

Correlation of plasma miR-221 as well as miR-222 expression with tamoxifen resistance and other clinicopathological variables

We investigated correlations between miR-221/222 expression and several BC markers, including PR and Ki67 protein levels, LVI, and stage (Table 1). However, we found no significant changes in miR-221/222 expression associated with these clinical pathology variables.

Nevertheless, Figure 2A shows that miR-221 expression is statistically (2.02 vs. 0.75; $P = 0.250$) greater in PR-negative ($n = 9$) patients than in PR-positive ($n = 15$) patients. Similar to miR-222, the expression was slightly but not substantially greater in PR-negative than in PR-positive patients ($P = 0.190$; Fig. 2B).

MiR-221 expression was nonsignificantly greater in high Ki67 patients than in low Ki67 patients ($P = 0.600$), with respective mean values of 1.59 and 0.99 (Fig. 2C). In contrast, miR-222 expression was marginally higher in low Ki67 individuals than in high Ki67 patients. ($P = 0.560$; Fig. 2D).

Figures 2E and F present the results of miR-221 expression in 11 LVI-positive as well as 15 LVI-negative patients. Moreover, LVI-positive patients carried higher miR-221 expression than LVI-negative patients, with mean values of 2.23 as well as 0.69, respectively, albeit nonsignificantly ($P = 0.140$; Fig. 2E). Conversely, LVI-negative patients had higher miR-222 expression than LVI-positive patients, again nonsignificantly ($P = 0.190$; Fig. 2F).

Figure 2G depicts the expression of miR-221 in patients classified according to their stage. Patients in stage III expressed miR-221 at a nonsignificantly greater level than those in stage II ($P = 0.140$). Despite no statistically significant difference in stage II as well as stage III patients, miR-222 expression was greater in the former ($P = 0.330$; Fig. 2H).

Predictive ability of miR-221/222 expression for predicting tamoxifen resistance and recurrence

Figure 3A shows a ROC curve for miR-221 expression and its ability to expect tamoxifen resistance, with sensitivity at 82.35, specificity at 71.43%, as well as area under the curve

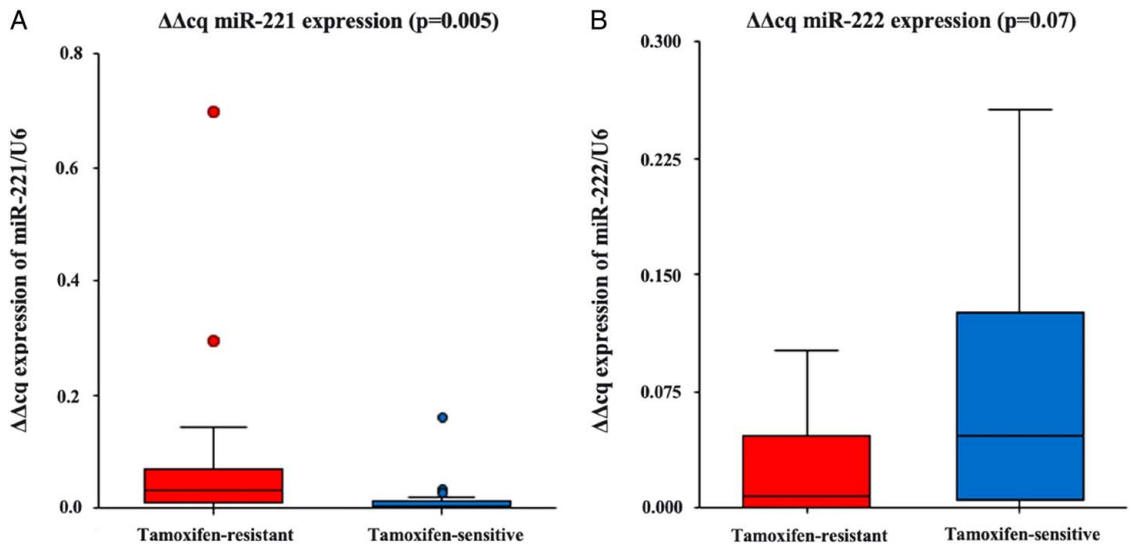

Figure 1. MiR-221/222 expression between tamoxifen-resistant and -sensitive groups. (A) miR-221 ($P = 0.005$). (B) miR-222 ($P = 0.070$).

(AUC) of 0.75 (95% CI: 0.52–0.88). Moreover, miR-221 expression was also significantly correlated with tamoxifen resistance ($P = 0.001$).

Similarly, using a ROC curve (Fig. 3B) with a cutoff value of 0.01, miR-221 expression was predictive of recurrence with sensitivity at 70, specificity at 72.73%, as well as AUC at 0.68 (95% CI: 0.40–0.84). Moreover, miR-221 expression was greatly correlated with recurrence ($P = 0.040$).

Additionally, using a ROC curve (Fig. 3C) with a cutoff value of 0.02, miR-222 expression was predictive of tamoxifen resistance with sensitivity at 66.66, specificity at 61.11%, as well as AUC at 0.67 (95% CI: 0.45–0.81). Moreover, miR-221 expression was greatly correlated with tamoxifen resistance ($P = 0.030$).

Finally, using a ROC curve (Fig. 3D) with a cutoff value of 0.01, miR-222 expression was predictive of recurrence with sensitivity at 76.00, specificity at 80.00%, as well as AUC at 0.74 (95% CI: 0.46–0.88). Moreover, miR-221 expression was significantly correlated with recurrence ($P = 0.010$).

Table 1

Correlation between miR-221 and miR-222 expression and the other variables.

			P	
Clinicopathological variable		***n***	***miR-221***	***miR-222***
Group	Tamoxifen-resistant (case)	15	0.005	0.070
	Tamoxifen-sensitive (control)	19		
PR	Negative	9	0.250	0.520
	Positive	15		
Ki67 Proliferation	High	11	0.600	0.560
	Low	12		
LVI	Negative	15	0.140	0.190
	Positive	11		
Stage	Stage II	12	0.140	0.330
	Stage III	11		

LVI, lymphovascular invasion; PR, progesterone receptor.

Considering the joint expression of miR-221 as well as miR-222, they were significantly correlated with tamoxifen resistance ($P = 0.030$; Fig. 3E). Using a ROC curve with a cutoff value at 0.56, we found miR-221/222 expression predictive of tamoxifen resistance with a sensitivity of 60.00, specificity of 83.33%, and AUC of 0.77 (95% CI: 0.56–0.89). Similarly, miR-221/222 expression was significantly correlated with recurrence ($P = 0.030$). Using a ROC curve (Fig. 3F) with a cutoff value of 0.53, miR-221/222 expression was predictive of recurrence with sensitivity at 80.00, specificity at 88.23%, as well as AUC at 0.80 (95% CI: 0.55–0.91).

Discussion

Approximately 50% of BC patients fail to respond to tamoxifen therapy[10,36], worsening their clinical outcomes. In a meta-analysis of 10 645 patients with ER-positive BC, administering tamoxifen reduced recurrence by 50% (relative risk [RR] = 0.53, $P < 1.00 \times 10–5$) at 0–5 years of use and 39% (RR = 0.53 = 0.61; $P < 1.00 \times 10–5$) at 10 years. After 10 years of tamoxifen administration, almost no decrease in relapse was observed (RR = 0.97). Therefore, the maximum length of tamoxifen therapy is currently set at 10 years[10,37].

Tumor recurrence resistance is a complex clinical condition that can appear at any point, from diagnosis through treatment (primary resistance) or after treatment has ended (secondary resistance)[10]. European School of Oncology as well as the European Society of Medical Oncology define primary resistance in metastatic BC as relapse within the first 2 years of adjuvant hormone treatment or progressive disease within the first 6 months of first-line hormonal therapy (European Society of Medical Oncology), whereas secondary (acquired) resistance is described as a recurrence with adjuvant hormonal therapy after the first 2 years, a recurrence within 12 months of cessation of adjuvant hormonal therapy, or an advancement of the metastatic BC more than six months after the initiation of hormonal treatment[10,37]

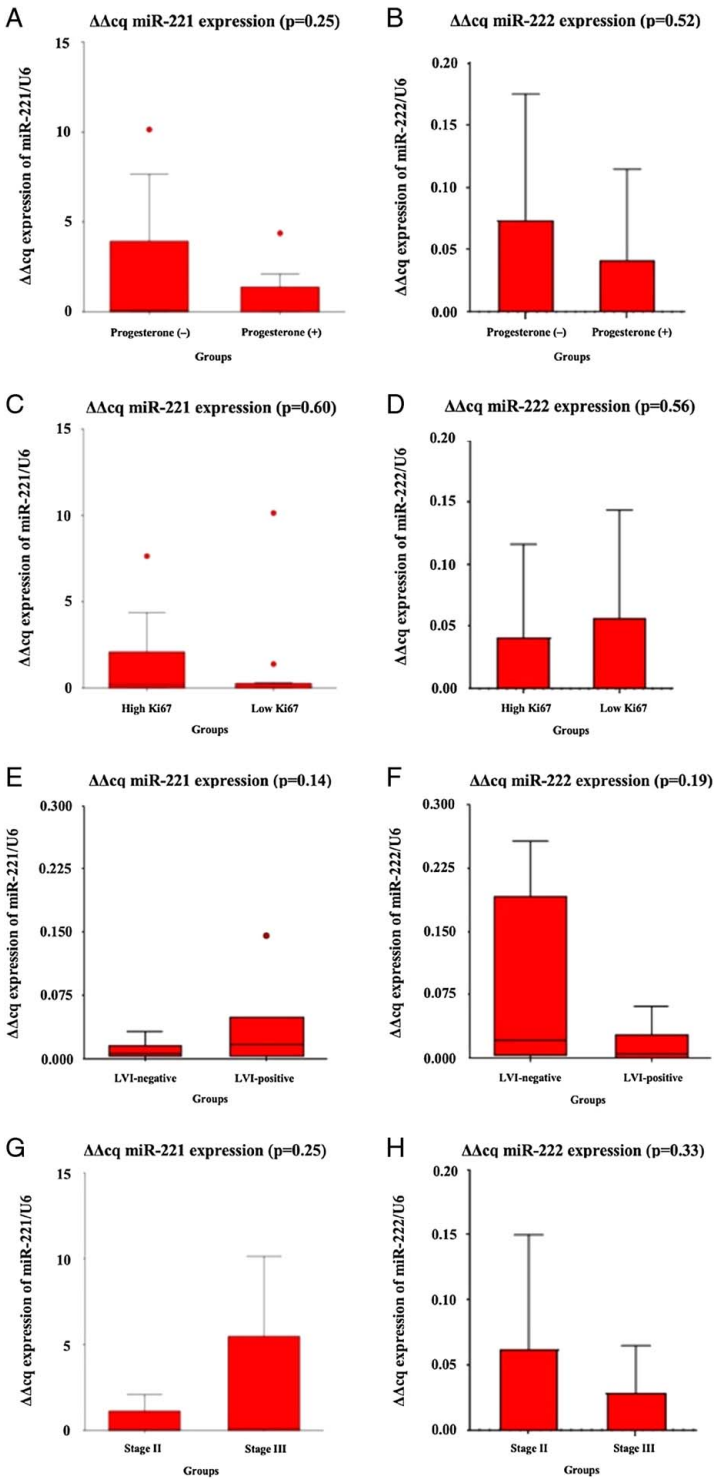

Figure 2. MiR-221/222 expression in patients categorized based on several breast cancer markers. (A) miR-221 and (B) miR-222 expression in progesterone receptor (PR)-negative and PR-positive patients. (C) miR-221 as well as (D) miR-222 expression in high Ki67 and low Ki67 patients. (E) miR-221 and (F) miR-222 expression in lymphovascular invasion (LVI)-positive and LVI-negative patients. (G) miR-221 as well as (H) miR-222 expression in stages II and III patients.

There was evidence of primary resistance in ER-positive BC patients taking tamoxifen as early as the first year of treatment. The relationship between treatment persistence and BC development, especially in luminal-type tumors, necessitates the investigation of this resistance. In addition, it is necessary to determine the possible causes of resistance as early as possible using biomarkers.

The mechanism of resistance to hormonal therapy can occur due to the interaction of ER with growth factor receptors, epigenetic changes, mutations in ESR1, and several other mechanisms[17].

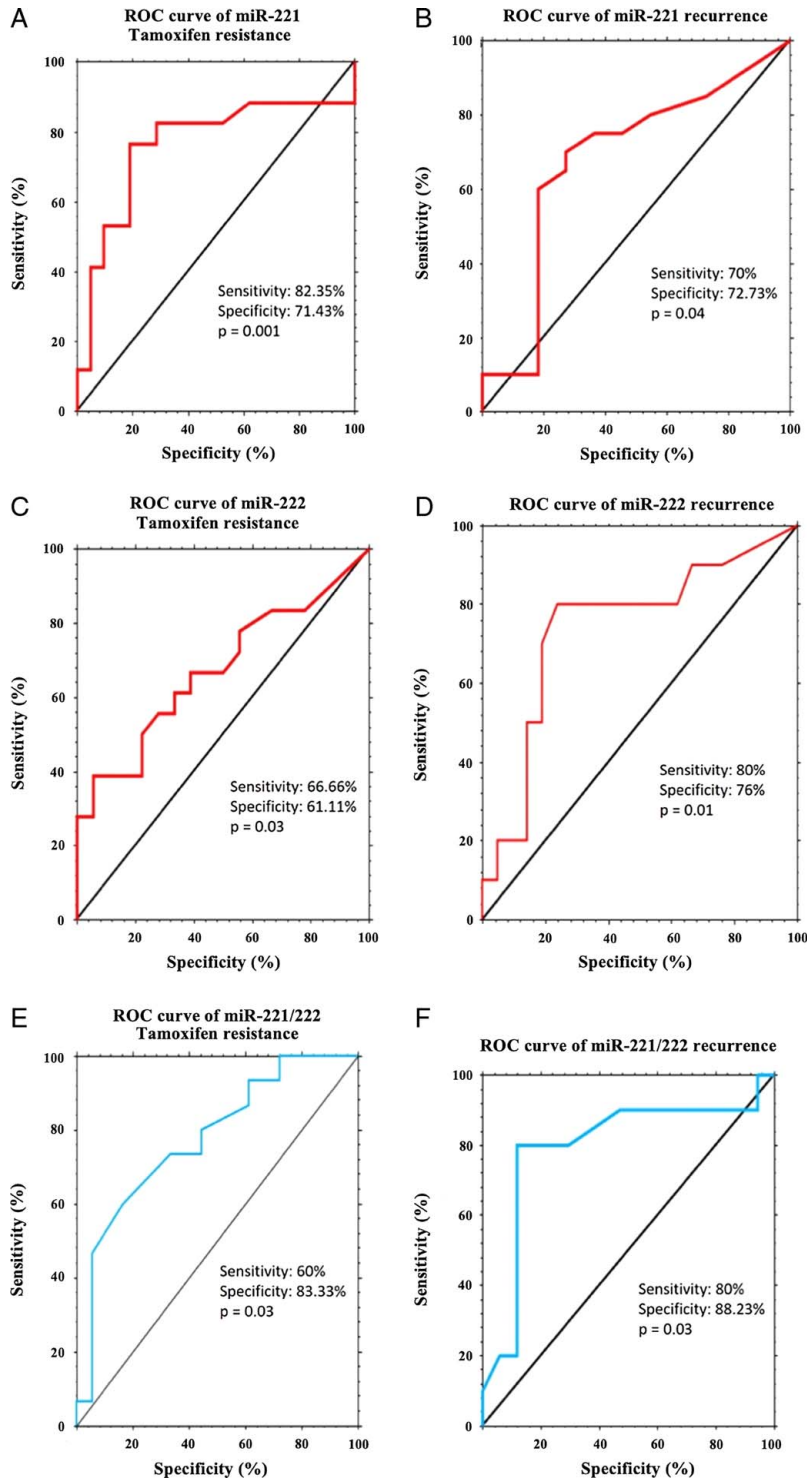

Figure 3. The ability of miR-221/222 expression to predict tamoxifen resistance and recurrence. The ability of miR-221 expression to expect (A) tamoxifen resistance ($P = 0.001$) and (B) recurrence ($P = 0.010$). The ability of miR-222 expression to predict (C) tamoxifen resistance ($P = 0.030$) and (D) recurrence ($P = 0.010$), The joint ability of miR-221/222 expression to expect (E) tamoxifen resistance ($P = 0.030$) and (F) recurrence ($P = 0.030$).

A diagnostic approach can indicate the resistance development to tamoxifen therapy, and biomarker studies are needed to predict the resistance phenotype. Therefore, it is hoped that in the future, alternative therapies will be available to treat patients for whom tamoxifen therapy has failed[38].

In this study, 15 patients were PR-positive, and 9 were PR-negative. Our statistical analyses found a difference in miR-221 expression between these two groups, with average values of 0.75 and 2.02, respectively. However, there was no statistically significant difference in expression ($P = 0.250$). Nevertheless,

miR-221 expression was higher in patients not expressing PR than in those who expressed PR. Since miR-221 has been connected to tamoxifen resistance[39,40], increased miR-221 expression in PR-negative patients may predict their insensitivity to the drug. These results are in line with those of several prior research. Arpino *et al*. demonstrated that despite having an ER-positive status, patients with PR-negative BC were more resistant to tamoxifen than those with PR-positive BC. Moreover, patients with PR-negative BC were proved to have improved HER2 expression, which is clinically more aggressive as well as tends to be better resistant to tamoxifen treatment[41]. Moreover, the miR-221 expression was reported to be greatly elevated and to influence the overall survival of patients with triple-negative BC[42]. These findings indicate that PR-negative BC patients have higher miR-221 expression, providing predictions about the presence of tamoxifen resistance. While the expression of miR-222 was found to be higher in our PR-negative group than in the PR-positive group, the discrepancy in the expression was not significant ($P = 0.190$).

The miR-221 expression was greater in the high Ki67 group (11 patients) than in the low Ki67 group (12 patients), with mean values at 1.59 as well as 0.99, respectively. The change was; however, not statistically significant ($P = 0.600$). Intriguingly, recent research[43] has demonstrated that the expression of miR-221 in primary human tumors is inversely linked with the expression of Ki67. Consistent with this observation, miR-222 expression was higher in our study among patients with low Ki67 than among patients with high Ki67, with average values of 1.59 and 0.99, respectively. However, this dissimilarity was not significant. ($P = 0.560$). Therefore, it can be concluded that the findings of this study indicate that miR-221/222 expression may not be directly associated with Ki67 expression, consistent with the findings of Musgrove *et al*.[44] who reported that miRNA expression varies among tumors.

We showed that LVI-positive patients ($n = 11$) had higher miR-221 expression than LVI-negative patients ($n = 15$), with average values of 2.23 as well as 0.69, respectively. This dissimilarity was insignificant ($P = 0.140$). In contrast, LVI-negative patients expressed more miR-222 than LVI-positive patients. The difference was not statistically significant ($P = 0.190$). LVI is more prevalent in nonluminal subtypes of BC, regardless of ER status, and is associated with unfavorable prognostic characteristics[45]. Vladimir *et al*. examined the relationship of the expression of five miRNAs as well as the LVI status in 80 matched BC samples. They discovered that miR-221 expression was only marginally lower in LVI-positive individuals, and the contrast in expression was not statistically significant, leading them to conclude that miR-221/222 expression and LVI status are unrelated[46].

Regarding the BC stage, miR-222 expression was observed to be greater in stage III patients than in stage II patients, with mean values of 0.49 and 2.10, respectively. This contrast was; however, not statistically significant ($P = 0.330$). miRNA expression is linked to numerous clinicopathological aspects of cancer, including tumor stage, receptor expression, as well as patient survival[47]. In cases of ER-positive BC, tamoxifen therapy is administered, particularly in the early stages of the disease, but it can also be administered at later stages. The average expression of miR-221 was greater in stage III patients than in stage II patients. The difference was; however, not statistically significant ($P = 0.140$). Consistent with these findings, a prior investigation of 86 BC tissues found no significant connection between the miR-221/222 expression as well as lymph node status, tumor size, and histological grade, including cancer stage[48]. Another study discovered a statistically significant difference in the expression of miR-221 at stages II and III versus healthy controls ($P = 0.028$ and $P = 0.016$, respectively). This study investigated miR-221 expression using serum from BC patients at different stages, concluding that circulating miR-221 levels could potentially be a noninvasive biomarker for monitoring therapy response and predicting recurrence in BC[49].

Our qRT-PCR results show that miR-221 expression was significantly different in tamoxifen-resistant and tamoxifen-sensitive patients. In the resistant group, MiR-221 expression was 237-fold higher in tamoxifen-resistant (mean $CT = 2.37 \pm 0.80$) than in tamoxifen-sensitive (mean $CT = 0.018 \pm 0.08$) patients ($P = 0.005$). However, miR-222 expression showed a non-significant increase in tamoxifen-sensitive patients compared to tamoxifen-resistant patients ($P = 0.070$). Nevertheless, the joint expression of miR-221/222 expression was predictive of resistance to tamoxifen therapy ($P = 0.003$).

Tamoxifen resistance can occur through several mechanisms, including the absence of ERs to bind tamoxifen and the uncontrolled growth of cancer cells, increasing cancer cell survival[50,51]. In this study, miR-221 expression was higher in BC patients resistant to tamoxifen therapy. One important mechanism is the role of miR-221 in degrading ERα messenger RNA, preventing ERα protein translation[52,53]. Mutations in the ESR1 gene decrease ERα synthesis[54]. Alternatively, post-translational variation in ERα and crosstalk with growth factor receptors may cause increased proliferation and survival of cancer cells[55]. Inhibition of miR-221/222 restored the sensitivity of MCF-7 TamR (Tamoxifen-Resistant) cells, the tamoxifen-resistant BC cell lines, to tamoxifen. MiR-221/222 inhibition may relate to ER and PTEN upregulation. Ouyang *et al*. build a synthetic miR-221/222 sponge with eight multi-antisense binding sites (MBSs) for these two onco-miRs. This sponge may be used as a treatment to overcome tamoxifen resistance in MCF-7 TamR cells by restoring ER and PTEN, which will reduce cell growth and migration[39]. In addition, miR-221 and miR-222 increase tamoxifen resistance by targeting p27kip1, cyclin E inhibitors, and repressing Erα directly[56].

Miller *et al*.[17] stated that HER2/neu-positive, primary BC tissue expressed significantly more miR-221/miR-222 and was more resistant to hormone therapy than HER2/neu-negative tissue. HER2/neu overexpression is related with miR-221 expression, and tamoxifen resistance in primary BC tumors is also related with this resistance[54,55]. Ectopic expression of miR-221 rendered MCF-7 stem cells resistant to tamoxifen. These findings also provide a rationale for using specific miRNAs as predictors of tamoxifen resistance in BC.

Based on research by Manavalan *et al*.[57], several miRNAs experienced changes in regulation in cells resistant to tamoxifen than in sensitive control cells, including miR-15a, miR-16, miR-320, miR-451, miR-214, miR-342, miR-873, miR-375, miR378a-3p, and miR-574-3p. Meanwhile, several miRNAs experienced increased expression, including miR-101, miR-221/222, miR-301, and miR-C19MC.

Wei *et al*.[58] have proposed miR-221/222 as potential biomarkers to predict resistance to tamoxifen. Exosomes containing miR-221/222 from TamR can enter tamoxifen-sensitive cells, causing tamoxifen resistance by targeting p27 and ERα, showing that miR-221/222 are associated with tamoxifen resistance in

luminal subtype BC. MiRNAs carried in exosomes can also act as messengers between cells[59,60]. Increases in miR-221/222 effectively reduce the expression of target genes p27 and ERα, increasing tamoxifen resistance in recipient cells[58,61]. Secreted miR-221/222 functions as a signaling molecule to regulate intercellular communication leading to tamoxifen resistance[58].

The findings of this and other studies[17,18,20] indicate BC patients should undergo miR-221/222 testing, particularly patients with luminal subtype BC who will undergo tamoxifen therapy, to predict tamoxifen resistance early and provide treatment efficiency and financing. Nevertheless, the limitations of this study necessitate further research on the circulating expression of miR-221 and miR-222 in tamoxifen-resistant BC patients with larger sample sizes.

Conclusion

MiR-221/222 are microRNAs that affect transcription and translation processes in protein synthesis. Circulating miR-221/222 expression can be used to predict relapse and resistance to tamoxifen treatment in BC patients, and their testing is recommended for luminal subtype BC patients who will undergo tamoxifen therapy to determine their risk of tamoxifen resistance early, increasing treatment effectiveness.

Ethical approval

The study was approved by the Ethical Committees of the Faculty of Medicine, Hasanuddin University (No. UH21040255). We promised that the participants' data were anonymized or maintained with confidentiality, the rights or interests of participants were not invaded, and informed consent was taken from all individual participants.

Consent

The research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. Written informed consent was obtained from the for publication and any accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request.

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NA.

Author contribution

I.P. and A.A.: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, validation, visualization, writing–original draft, writing–review and editing; M.N.M.: conceptualization, data curation, formal analysis, investigation, methodology, resources, supervision, validation, visualization, writing–original draft, writing–review and editing; A.A.I.: conceptualization, investigation, methodology, resources, software, validation, writing–original draft, writing–review and editing; M.Y.P.: conceptualization, data curation, formal analysis, investigation, methodology, resources, validation, writing–original draft, writing–review and editing; N.S.: conceptualization, data curation, formal analysis, investigation, methodology, resources, supervision, writing–original draft, writing–review and editing; N.H.M.L.: conceptualization, data curation, investigation, resources, supervision, writing–original draft, writing–review and editing; M.F.: data curation, formal analysis, investigation, methodology, project administration, resources, software, validation, visualization, writing–review and editing.

Conflicts of interest disclosure

The authors declare that they have no financial conflict of interest with regard to the content of this report.

Research registration unique identifying number (UIN)

1. This study has been registered with the Research Registry no. 7241
2. https://www.researchregistry.com/browse-theregistry#home/registrationdetails/61622a7a5dff7d002090b264/

Guarantor

Muhammad Nassrum Massi.

Provenance and peer review

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Data availability statement

The data that support the findings of this study are available on request from the corresponding author [Muhammad Nassrum Massi]. The data are not publicly available due to [restrictions e.g. their containing information that could compromise the privacy of research participants].

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