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Case-controlled Study

microRNA-221 and tamoxifen resistance in luminal-subtype breast cancer patients: A case-control study

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ABSTRACT

Background: Around 70% of breast cancers (BCs) are estrogen receptor-α (ERα)-positive. Adjuvant endocrine therapy is used to reduce estrogen levels and inhibit signal transduction through the ER. The anti-estrogen drugs that are most commonly used in endocrine therapy belong to the selective ER modulator (SERM) class and include tamoxifen. Although it has been used for three decades in cases of early-stage and ERα-positive BC, resistance to tamoxifen is a common problem. microRNAs (miRNAs) have a potential role in demonstrating BC resistance to tamoxifen therapy. Hence, there is a need to investigate the expression of miRNA-221 (miR-221) in luminal-subtype BC patients receiving tamoxifen therapy.

Methods: This case-control study investigated luminal-subtype BC patients who had undergone endocrine therapy for at least 1 year. The case group comprised patients with local or metastatic recurrence, and the control group comprised patients without local or metastatic recurrence.

Results: There was a significant difference in miR-221 expression ($p = 0.005$) between the case and control groups. There were no significant differences between the groups that were positive and negative for the progesterone receptor (PR) ($p = 0.25$), had high and low marker of proliferation Ki-67 levels ($p = 0.60$), were positive and negative for lymphovascular invasion ($p = 0.14$), and had stage 2 and stage 3 cancer ($p = 0.25$).

Conclusion: miR-221 expression was higher in tamoxifen-resistant BC cases. miR-221 is a potential biomarker of tamoxifen resistance.

1. Introduction

Breast cancer (BC) is the most common malignancy in women globally. In 2018, the GLOBOCAN database recorded 2 million new cases of BC worldwide, and 626,000 fatalities caused by this disease [1]. In Indonesia, BC is also the most frequent cancer among women [1,2].

Endocrine or hormone therapy to reduce estrogen levels or inhibit signal transduction through the estrogen receptor (ER) can be suitable for some patients. Endocrine therapy includes a class of drugs including selective estrogen receptor modulators (SERMs; e.g., tamoxifen), selective estrogen receptor down-regulators (SERDs; e.g., fulvestrant), and aromatase inhibitors (AIs; e.g., anastrozole) [3–5]. The hormonal agent tamoxifen has been used in many patients; however, resistance to tamoxifen is often encountered. Some patients have shown poor responses to initial therapy, and recurrence has been reported in about 30% after 15 years of follow-up [6–8]. BC cells are often resistant to

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tamoxifen, reducing the success of therapy and resulting in either recurrence or metastatic or advanced-stage disease. BC mortality has often been associated with recurrence and resistance to therapy [4,9].

microRNA-221 (miR-221) in particular has been studied in relation to BC resistance to endocrine therapy. Increased expression of miR-221 has been linked with resistance to tamoxifen and fulvestrant therapy [10,11]. Alteration of cell-cycle processes and evasion of apoptosis are the most likely ways in which miR-221 can provoke resistance to tamoxifen [12]. Important targets that are regulated by miR-221 include the Cip/Kip family signaling pathways (p21, p27, and p57), ERα, and phosphatase and tensin homolog deleted on chromosome 10 (PTEN). Regulation of these signaling pathways leads to increased proliferation and survival of BC cells and decreased apoptosis. miR-221 binds directly to the 3′-untranslated region (UTR) of the *ESR1* gene and silences the expression of the ERα protein [10,11]. By contrast, miR-221 appears to work on other target genes in ER-positive BC, because its expression is significantly higher than in ER-negative BC [13].

Experiments on cultured BC cells showed that suppression of miR-221 and miR-222 expression by anti-miR-221 and anti-miR-222 significantly increased the sensitivity of ER-positive BC cells to tamoxifen and lead to decreased viability. This effect might be related to the increased expression of tissue inhibitor of metalloproteinase-3 (TIMP3) due to decreased expression of miR-221 and miR-222 [14]. These findings could be a reference for anti-miRNA-therapy strategies for BC in the future.

Profiling miR-221 expression in BC, especially the luminal subtypes, may be useful in determining its role in tamoxifen resistance. Expression of miR-221 should be examined not only in the specific tissue but also in the circulating blood. The use of circulating miRNAs as biomarkers has many advantages as they are easy to obtain, their detection is non-invasive and cost-effective, and they are highly stable in body fluids such as serum and plasma [15]. The current study compared the level of plasma miR-221 expression in luminal-subtype BC patients with and without recurrence. We also analyzed the correlation between the expression level of plasma miR-221 and the recurrence of BC with luminal subtypes.

2. Materials and methods

2.1. Study design and sample size

This case-controlled study has been reported in accordance with the Strengthening the reporting of cohort studies in surgery (STROCSS) guidelines [16] and has been registered with the research registry (no. 7241). The inclusion criteria were as follows: luminal subtype BC patients who had undergone endocrine therapy for at least 1 year, had complete clinical data, and consented to be included in the study. The exclusion criteria for the case group were a history of discontinuous and irregular use of tamoxifen, and receiving inadequate doses of tamoxifen.

Subjects were separated into two groups: case and control. The case group included all subjects with local and metastatic recurrence, while the control group comprised patients without any recurrence.

For the controls, the exclusion criterion was receiving any other systematic therapy besides tamoxifen, such as chemotherapy or targeted therapy. We obtained ethical approval from our institutional review board.

The target population comprised BC patients with luminal subtypes who received tamoxifen hormone therapy in Mitra Keluarga Kemayoran, Royal Taruna, and another networking hospital at Jakarta, Indonesia, and met the inclusion criteria. Patients were recruited using consecutive sampling until the estimated target sample size was reached.

2.2. Patient treatments and assessments

Subjects were recruited from the surgical oncology, breast surgery, or medical hematology-oncology polyclinics in accordance with the Declaration of Helsinki. BC was diagnosed by histopathological examination. The luminal subtypes were determined by the presence of ERα and PR using immunohistochemistry (IHC) staining. Evaluations were performed by pathologists at the Mitra Kemayoran Anatomic Pathology Laboratory, Jakarta, Indonesia.

Subjects were administered endocrine therapy with indications to tamoxifen according to standard protocols of adjuvant therapy for at least 1 year. After informed consent had been given, 5 ml of each subject's blood was drawn in ethylenediaminetetraacetic acid (EDTA), centrifuged, and the plasma was then separated. Serum samples were frozen until RNA isolations were performed.

2.2.1. Sample processing: total RNA extractions

Total RNA was extracted from the plasma using the QIAamp RNA Blood Mini Kit (Qiagen, Hildenburg, Germany) according to the manufacturer's protocols. The samples were eluted in elution buffer and miRNA/total RNA concentrations and purity were measured using nanospectrophotometry.

2.2.2. Sample processing: miRNA cDNA synthesis

The conversion process from miRNA to complementary DNA (cDNA) was performed using a miRNA Reverse Transcription Kit (miScript II RT Kit, Qiagen, Hildenburg, Germany) according to the manufacturer's protocol by the real-time polymerase chain reaction (PCR).

2.2.3. Quantification of serum miR-221

Specific miR-221s were amplified using the miScript SBYR Green PCR Kit (Cat No. # 218073, Qiagen, Germany) and the miScript Primer Assay (Cat No. #218300, Qiagen, Germany). The miR-221 primer sequences that were used in this study were as follows: 5′-CGA GCT ACA TTG TCT GCT GGG T-3′ and 5′-CCG CAG CTA CAT CTG GCT ACT G-3′ as forward primers; and 5′-GTG CAG GGT CCG AGG T-3′ as the reverse primer. miR-221 expression was recorded and analyzed relative to the expression of U6 gene as a housekeeping gene using the following formula: ΔCt miR-221 = Ct miR-221 – CtU6.

2.3. Statistical analysis

The dependent variable in this study was tamoxifen resistance, examined based on tumor size after neoadjuvant administrations, whereas the independent variable was miR-221 expressions. IBM SPSS Statistics version 23.0 (IBM Co., Armonk, NY, USA) was used for statistical analysis. The significance values of miR-221 expression in relation to the other dependent variables, such as tumor size, stage, Ki67, and PR expression, were analyzed using the Mann–Whitney *U* test.

3. Results

3.1. Plasma miR-221 levels in cases compared to controls

The miR-221 expression was analyzed in samples from 15 patients from the case group and 19 patients from the control group. Fig. 1 shows that the mean expression level of miRNA-221 in the case group was 2.378 while that of the control was only 0.03. Statistical analysis using the Mann–Whitney *U* test showed that miR-221 levels were increased significantly in cases compared to controls ($p = 0.005$).

3.2. Correlation of plasma miR-221 level with resistance to tamoxifen and other clinicopathological variables

This study examined the relationship of miR-221 expression to several markers in BCs, including progesterone receptor (PR) and Ki67 protein expression, lymphovascular invasion (LVI), and stage (Table 1).

miR-221 expression was higher in PR-negative patients than PR-positive patients; however, statistical analysis of the data from nine

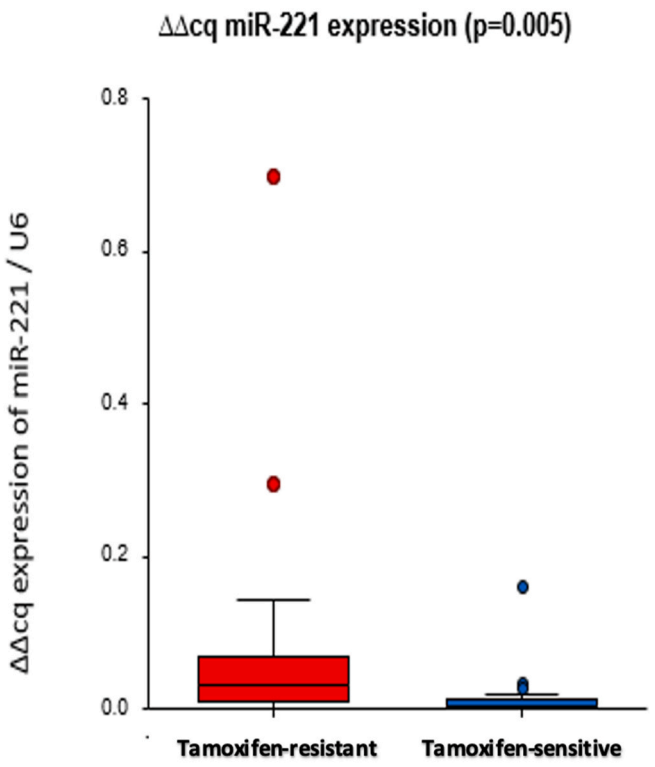

Fig. 1. The miRNA-221 Expression between case and control groups. The difference of miRNA- 221 expression between the two groups was analyzed using Mann-Whitney test (p = 0.005).

PR-negative patients and 15 PR-positive patients, with mean values of 2.02 and 0.75, respectively, showed that the difference in expression was not statistically significant ($p = 0.25$) (Fig. 2A).

miR-221 expression was also higher in Ki67-positive patients compared to Ki67-negative patients (Fig. 2B); however, statistical analysis of the data from 11 Ki67-patients and 12 Ki67-negative patients, with mean values of 1.59 and 0.99 respectively, again showed that the difference in expression was not statistically significant ($p = 0.60$).

miR-221 expression data from 11 LVI-positive patients and 15 LVI-negative patients showed that the former had higher levels compared to the controls, with means of 2.23 and 0.69, respectively; however, this difference was not statistically significant ($p = 0.14$) (Fig. 2C). miR-221 expression was higher in stage 2 patients compared to stage 3 patients, according to a comparison of data from 11 stage 3 patients and 12 stage 2 patients, with means of 2.18 and 0.49, respectively; once again, this difference was not statistically significant ($p = 0.14$).

Based on the receiver operating characteristic (ROC) curve, with a cut-off value of ≥0.01, miR-221 could predict tamoxifen resistance with a sensitivity of 82.35% and a specificity of 71.43%, and an area under the curve (AUC) value of 0.75 with a 95% confidence interval (CI) of 0.52–0.88. miRNA-221 was significantly correlated with tamoxifen resistance ($p = 0.001$) (Fig. 3).

4. Discussion

4.1. BC treatment

BC treatment options depend on the disease stage, histopathology, and molecular classification [17]. In general, early-stage BC patients are suitable to undergo surgery to remove the primary tumor. According to the indications, they can then undergo additional treatment, such as radiotherapy, chemotherapy, or hormone therapy. The classification of BC subtypes using molecular markers could be useful for identifying the optimal treatment for patients. Hormone-receptor expression profiles in BC are also important to determine sensitivity to therapeutic drugs and determine appropriate therapeutic strategies [17,18].

The expression levels of ERs, PRs, and human epidermal growth factor receptor 2 (HER2) could determine the choice of adjuvant therapy for patients [19]. Estrogen is a hormone that promotes the growth of some BCs. Patients with ER + BC should receive adjuvant therapy to control its effects on any cancer cells that were not removed by surgery. Adjuvant therapy reduces the risk of recurrence or spread of cancer cells. Blocking the effect of estrogen and decreasing the estrogen level are two of the main approaches used in hormone receptor-positive BC treatment. Anti-estrogen drugs can block the effect of estrogen on cancer cells without decreasing the amount produced by the ovaries [20]. Tamoxifen is the most commonly used antiestrogen drug for adjuvant BC therapy and is usually referred to as endocrine/hormone therapy. Administration of tamoxifen, for 5 years as adjuvant therapy after surgery decreases the chance of recurrence of ER + BC [8,20,21].

This study focused on luminal-subtype BCs since most patients were given adjuvant therapy in the form of tamoxifen, which was related to the positive ER expression. We excluded subjects who received therapy other than tamoxifen from the control group to avoid any bias that might occur in the study results. For the case group, we excluded subjects with a history of discontinuous and irregular use of tamoxifen or patients who had received inadequate doses of tamoxifen to ensure that all subjects received a relatively similar dose of tamoxifen.

4.2. The importance of therapy predictive markers

Currently, up to 50% of cases of BC fail to respond to tamoxifen therapy [22]. Resistance occurs in some patients receiving tamoxifen therapy, resulting in recurrence and worsening disease. A meta-analysis of 10,645 patients with ER + BC concluded that tamoxifen administration in the first 0–4 years was able to reduce the recurrence rate by 50% (relative risk [RR] = 0.53; $p < 0.00001$). At 10 years, the relapse rate had fallen by about 39% (RR = 0.61; $p < 0.00001$). However, after 10 years there was almost no further reduction in relapse (RR = 0.97), so there appeared to be no benefit to continuing therapy after this time point [23].

Table 1
Correlation of miR-221 and other variables.

		N	Mean	SD	SE	T	CI 95%	*p*-value	
Sample group	Case	15	2.38	3.13	0.81	2.14	0.64	4.11	0.005
	Control	19	0.02	0.04	0.01	2.10	0.00	0.03	
PR	Negative	9	2.03	3.94	1.31	2.31	−0.10	5.06	0.25
	Positive	15	0.75	1.25	0.32	2.14	0.06	1.44	
Ki67 proliferation	High	11	1.59	2.42	0.73	2.23	−0.04	3.22	0.60
	Low	12	1.00	2.90	0.84	2.20	−0.85	2.85	
LVI	Negative	15	0.70	1.46	0.38	2.14	−0.11	1.50	0.14
	Positive	11	2.24	3.60	1.09	2.23	−0.18	4.66	
Stage	Stage 2	12	0.50	0.80	0.23	2.20	−0.01	1.00	0.14
	Stage 3	11	2.19	3.73	1.12	2.23	−0.32	4.69	

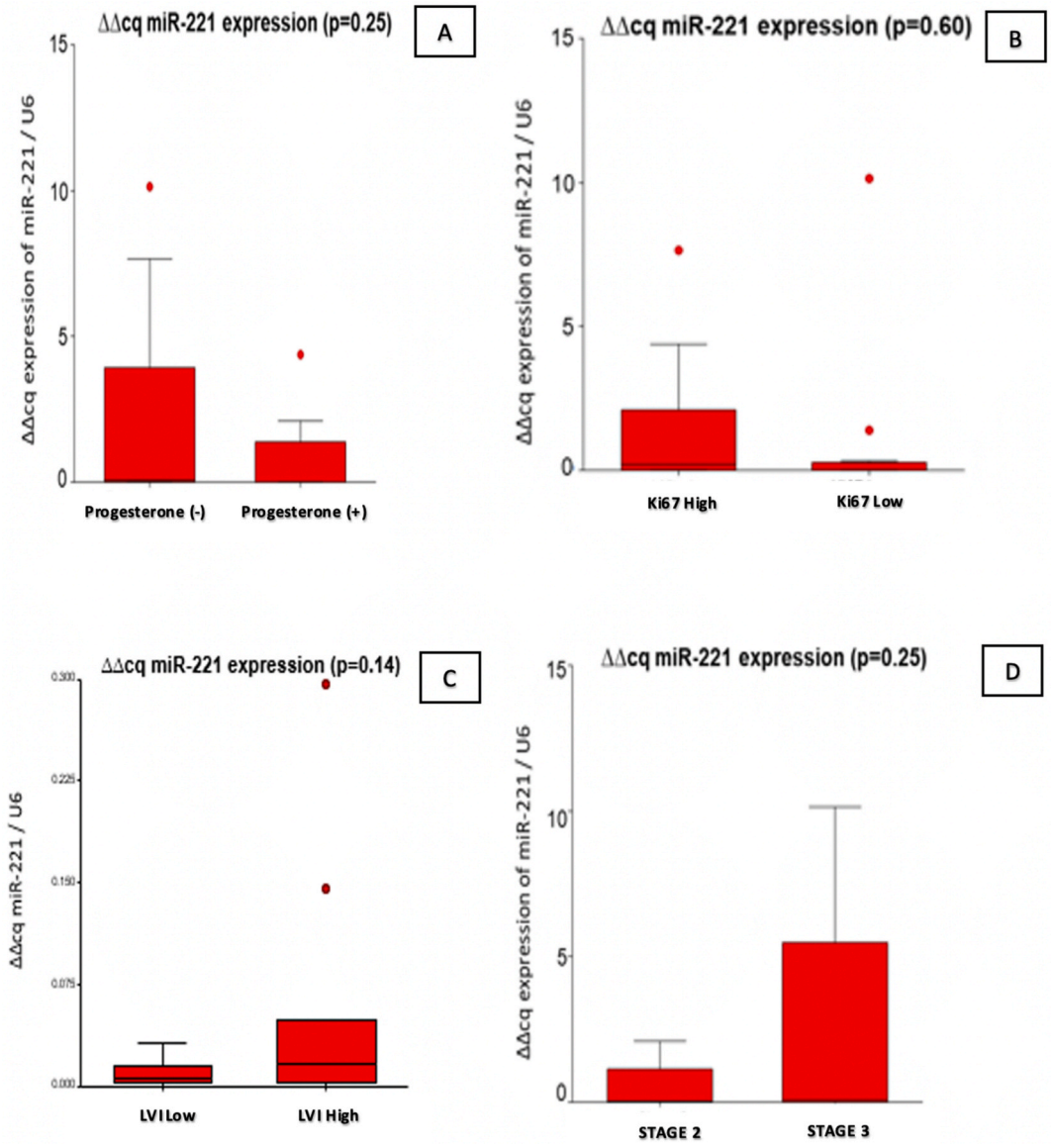

Fig. 2. Correlations between miR-221 and other variables.

The resistance that arises in tumor recurrence is a complicated clinical problem. Resistance to hormone therapy can occur at any time between diagnosis and the start therapy (primary resistance) or after therapy (secondary resistance). The European School of Oncology (ESO) and the European Society of Medical Oncology (ESMO) provided the most recent definition of resistance: primary resistance can be defined as relapse during the first 2 years of adjuvant endocrine therapy, or progressive disease (PD) within 6 months of first-line endocrine therapy for metastatic BC; while secondary (acquired) resistance can be defined as a relapse while on adjuvant endocrine therapy but after the first 2 years, or a relapse within 12 months of completing adjuvant endocrine therapy, or PD > 6 months after starting endocrine therapy for metastatic BC [23,24].

Among our consecutively collected samples, there were 15 cases of local or metastatic recurrence after adjuvant therapy with tamoxifen for at least 1 year. These results indicate that primary resistance occurs even in the first year of tamoxifen administration to the patient. This needs to be a concern, especially when considering the continuation of therapy and disease progression in patients. Furthermore, it is important to detect possible causes of resistance in patients using biomarkers as early as possible.

Various factors may cause tamoxifen resistance; therefore, determining the molecular mechanisms of tamoxifen resistance is important for enabling more appropriate therapy strategies in the future. Cellular properties, such as exosomes, cytokines, and soluble receptors, have been identified as important factors involved in resistance development [25]. Resistance to hormone therapy might involve the interaction between ER and growth factor receptor (GFR), mutations in the *ESR1* gene, epigenetic changes, and other mechanisms [6].

It will be useful to set up a diagnostic approach that can predict tamoxifen sensitivity and the development of tamoxifen resistance. Biomarkers are also required to predict the resistance phenotype and to generate alternative treatment options in order to prevent ineffective treatment for patients and to avoid failure of antiestrogen therapy using tamoxifen [26]. One of the epigenetic factors involved in the mechanism of tamoxifen resistance is miRNA regulation [27]. Some miRNAs consistently either become tumor suppressors by suppressing oncogenes or conversely become oncogenes (onco-miRs) by suppressing tumor-suppressor genes. However, it appears that some miRNAs can act as both onco-miR and onco-suppressor-miRs depending on the cellular pathway [28]. Elevation or depression of miRNA expression in cancer has been proposed as a promising biomarker to predict the progression

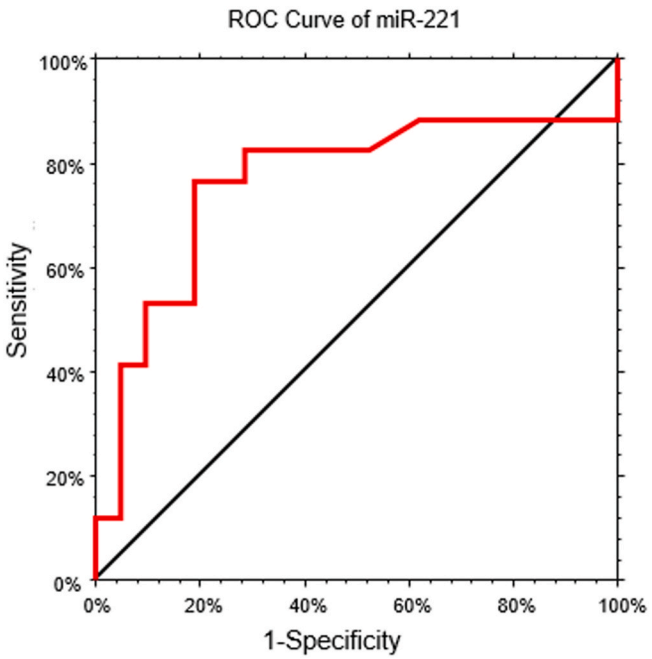

Fig. 3. ROC curve of miR-221 potency to predict tamoxifen resistance.

of the disease and therapy.

4.3. miRNAs as biomarkers of tamoxifen resistance

The exact mechanisms that induce drug resistance remain unknown. There is growing evidence for roles of exosomal miRNAs in tumor progression, starting from initiation, and continuing through proliferation, angiogenesis, migration, invasion, metastasis, eventually resulting in drug resistance [29]. miRNAs are essential regulators of gene expression and contribute significantly to cell behavior. To date, miRNA expression has also been found to change in patients in response to tamoxifen therapy. A study demonstrated increased levels of miR-98 and miR-21 upon E2 treatment in MCF-7 cells [30]. miRNAs can also be used as prognostic markers for BC patients, for example in miR-34a [31] and miR-187 [32].

miRNA expression profiles were previously screened by Manavalan et al. who found multiple miRNAs deregulated in tamoxifen-resistant cells compared to the sensitive control cells. miR-15a, miR-16, miR-320, miR-451, miR-214, miR-342, miR-873, miRNA-375, miR-378a-3p, and miR-574-3p were found to be downregulated in tamoxifen-resistant cells, meanwhile, upregulation of miRNA expression was identified in miR-101, miR-221, miR-222, miR-301, and miRNA-C19MC clusters [33].

Particularly for tamoxifen resistance, miR-375 [34], miR-221/222 [35], miR-200 [36], and miR-519a [37] have been proposed as potential biomarkers for the response. Exosomal miRNAs-221/222 released from tamoxifen resistant (TamR) cells can enter sensitive cells, leading to tamoxifen resistance in the sensitive cells by targeting P27 and Erα [35]. Reduction or loss of PTEN has been implicated in BC progression and drug resistance. PTEN was proven to be a target gene for miR-21 and miR-221 [38]. Some miRNAs are dysregulated in hormone-resistant BC and regulate specific genes in growth-promoting, apoptosis-resistant, epithelial-to-mesenchymal transition (EMT) pathways, which may result in tamoxifen resistance [27]. We determined that micro-RNAs could be used as circulating biomarkers and predictors for tamoxifen sensitivity by initially examining the level of expression of miR-221 in the plasma of BC patients.

4.4. miR-221 as a candidate endocrine-therapy predictor

miR-221 and miR-222 are encoded as a tandem gene cluster located on the X chromosome (Xp11.3) [39,40]. These genes are located on the same seed sequence and separated by 726 nucleotide bases. Under normal conditions, miR-221 and miR-222 are known to regulate a variety of important physiological vascular processes, such as angiogenesis, neointimal hyperplasia, vascular wound healing, vascular aging, and atherosclerotic vascular remodeling [41]. This gene cluster has been extensively studied in various malignancies in humans [39,42]. miR-221/miR-222 can act as an onco-miR in epithelial tumors and as an onco-suppressor-miR or onco-miR in hematopoietic malignancies [43].

In addition to ER, several important proteins and signaling pathways are regulated by miR- 221/miR-222 including the Cip/Kip family (p27Kip1) [44], tissue inhibitor metalloproteinase 3 (TIMP3), Forkhead box O3 (FOXO3A), the p53 upregulated modulator of apoptosis (PUMA), and PTEN [11]. miR-221/miR-222 has been reported to regulate the post-translational mechanism that alters the expression of 4-integrin and other targets in luminal BC so that it behaves more aggressively [45]. Overexpression of miR- 221 can cause drug resistance through deregulation of multiple signaling pathways including β-catenin and transforming growth factor-β (TGF-β) [46]. Cell-cycle inhibitor p27Kip1, which antagonizes cell death and promotes hormone-independent cell growth, was also targeted by miR-221/miR-222 in TamR BC cells [47].

Considering the important role of miR-221 in the process of resistance to tamoxifen, it seems reasonable that it is a candidate biomarker to predict occurrence in BC patients receiving tamoxifen adjuvant therapy. This study examined the expression levels of miR-221 in patients who had relapsed after receiving tamoxifen therapy for at least 1 year; the results showed that miR-221 expression levels were indeed higher in these patients than those who did not relapse. miR-221 was differentially expressed among the two groups, indicating that resistance to tamoxifen may be predicted using miR-221 expression levels in blood serum. The use of miRNA as a biomarker has many advantages, including the relatively non-invasive sampling method, resistance to sample instability during pH changes and freeze–thaw cycles, relatively stable miRNA levels, and easy detection of miRNA expression in the blood.

4.5. Plasma miR-221 levels in case and control groups

The data showed that plasma miR-221 expression levels were higher in the case group compared to the control group. We found that plasma miR-221 had a mean expression leve that was 2.38-fold higher in the case group than in the controls ($p = 0.02$), suggesting that miR-221 was related to BC. Previous studies have shown that serum miR-221 may be used as a diagnostic marker of several cancers, including ovarian and lung, and is related to poor overall survival in malignancy [1,48,49]. In hepatocellular carcinoma (HCC) and liver fibrosis related to hepatitis C infections, miRNA-221 levels were found to be elevated in serum [19,50]. However, serum miR-221 may not be specifically related to cancer; serum miR-221 has been found to be related to the progression of other diseases such as type-2 diabetes [51] and cerebrovascular disease, which is related to atherosclerosis and stroke [52].

4.6. Correlation analysis and ROC curve

Serum miR-221 may be used to predict patient's a resistance towards tamoxifen, as our results showed that it had 71.43% specificity and 82.35% sensitivity. Our findings confirmed that miR-221 had the potential to predict patient resistance towards tamoxifen, with a sensitivity of approximately 86.6%. This predictor should be used together with other clinicopathological factors such as the expression of ER [12]. So far, little evidence has shown that serum miR-221 is related to tamoxifen resistance; therefore, our results may be the first to demonstrate clinical

evidence of such a relationship.

Previously, Miller et al., showed that miR-221 expression levels were found to be increased in Her2/neu2-positive expression BCs compared to Her2/neu negative, which was known related to tamoxifen resistance while suppression of this miR-221 in MCF7 cancer cell lines, increased cell sensitivity towards tamoxifen. In contrast, increased expression of miR-221 also increased cell resistance towards tamoxifen [47]. In parallel to this, Gan et al. demonstrated that down-regulation of miR-221 induced MCF-7 BC cell-line sensitivity towards tamoxifen through upregulation of TIMP3 [53]. Moreover, miR-221 may also be relevant to radiation therapy. For instance, Zhang et al. showed that down-regulation of miR-221 increased radiation sensitivity, through activation of PTEN and Akt, which later led to radiation-induced apoptosis [54].

4.7. Plasma miR-221 levels in the PR+ and PR− groups

This study found that miR-221 expression was expressed at higher levels in PR-compared to PR + patients, although the difference was not statistically significant ($p = 0.25$). As it was described previously that miR-221 was related to resistance against tamoxifen, the higher level of miR-221 in PRnegative patients may also be related to resistance towards tamoxifen. Our findings were in accordance with a previous study conducted by Arpino et al. that demonstrated PR-negative BC to be more resistant to tamoxifen compared to PR-positive BC, although the ER was positive. Moreover, patients who were PR negative were found to have higher expression of HER2, showed more aggressive features, and tended to be more resistant to tamoxifen [55]. Furthermore, miR-221 has been found to be overexpressed and to affect patients' overall survival in triple-negative BCs [56]. Our finding suggested that the PR-negative patients had higher expression of miR-221, which may further explain the resistance against tamoxifen.

4.8. Plasma miR-221 levels in high and low Ki67 proliferation groups

Serum miR-221 was also found to be higher in patients with high Ki67 than low Ki67, although this difference was not statistically significant ($p = 0.60$). Interestingly, a previous study showed that expression of miR-221 in human primary tumors was inversely correlated to Ki67 expression [45]. By contrast, our data suggested that expression of serum miR-221 may not be directly correlated with Ki67 positivity. According to Petriella et al., serum miRNA may vary among different tumors [57].

4.9. miR-221 expression in LVI+ and LVI– groups

LVI most commonly occurs in non-luminal subtype BCs. However, LVI is associated with protein expression in BC and the presence of LVI is a poor prognostic factor in cancers with either positive or negative ER. Despite no statistically significant difference ($p = 0.14$), our results showed that miR-221 expression in the LVI group was higher than in those with no LVI. In line with these results, Chernyy et al. investigated five miRNAs from 80 paired samples of BC and examined the correlation between miRNA expression and LVI-positive status. They found that miR-221 was only slightly upregulated in the positive LVI group and the differences in miR-221 expression were not statistically significant. It could be concluded that there was no identified association between miR-221 levels and LVI status [58].

Conversely, in colorectal carcinoma, plasma miR-221 with lymph-node metastasis was significantly overexpressed compared to neoplasms without lymph-node metastasis [59].Another study showed that in vitro-forced overexpression of miR-221 in cervical cancer could promote EMT, cell migration and invasion, and lymphatic metastasis in vivo [60]. Upregulation of miR-221 also occurred in 88% (81/92) of gastric cancer tissue samples and was significantly correlated with lymphatic metastasis [61]. Considering the results of our study, it seems that the correlation of LVI status and the expression of miR- 221 need to be further investigated.

4.10. miR-221 expression in stage 2 and 3 BC groups

miRNA expression has been associated with some clinicopathological characters of cancers such as tumor stage, receptor expression, and patient survival [62]. Tamoxifen therapy is given to ER + BC cases. Although generally given in early-stage cases, it can also be given at a more advanced stage. Our study found that the average value of the miR-221 expression in stage 3 patients was higher than in stage 2 patients, although the difference was not statistically significant ($p = 0.25$). Similar to our findings, a total of 86 BC FFPE tissues were analyzed for miR-221 expressions by quantitative RT–PCR and showed no significant association between miR-221 levels and the lympho-node status, tumor size, the histological grade, or the tumor stage in general [11]. Another study found statistically significant differences in miR-221 expression levels between stage 2, stage 3 compared to healthy controls ($p = 0.0279$ for stage 2 and 0.0163 for stage 3). The study examined miR-221 expression from the circulating blood of BC patients in different stages. The authors concluded that circulating miR-221 can serve as a potential non-invasive biomarkers for BC screening and diagnosis [63].

Overexpression of miR-221 in serum samples of HCC patients was correlated with tumor size, cirrhosis, tumor stage, and overall survival rate [64]. In gastric cancer tissue samples, the upregulation of miR-221 was correlated with advanced clinical stage, local invasion, lymphatic metastasis, and poor survival [61]. Sun et al. proved that miR-221 was significantly upregulated in 90% of colorectal cancer samples. This overexpression was positively correlated with an advanced tumor-node-metastasis stage and local invasion [65].

A total of 69 patients with osteosarcoma were included in a previous study to determine miR- 221 expression. The expression level of miR-221 increased along with the increasing tumor stage, and was significantly higher in patients with stages 2B/3 than stage 2A tumors. This study showed that miR-221 was overexpressed in osteosarcoma samples, and was associated with clinical stage and metastasis [66].

There were several limitations to the current study. Firstly, only a small number of samples were included. Research using a larger number of samples from BC patients at various stages is required to verify the possibility of plasma miR-221 for BC screening and diagnosis. Secondly, we recruited only stage 2 and 3 BC patients. It will be necessary to conduct further research that involves stage 1 and 4 BC patients to examine the expression of plasma miR-221 at each stage. Thirdly, due to the limited access of the BC patients to adequate health facilities, molecular markers were not examined in all of the samples that we collected. There is a need for further research to collect the samples that have aligned examination for their molecular markers.

5. Conclusion

Serum miR-221 was expressed at higher levels in patients with local recurrence and metastasis than those without. serum miRNA-221 was not statistically significantly correlated with other clinicopathological variables such as ER, Ki67 expression, LVI, and stage. Furthermore, ROC values suggested that serum miR-221 expression had a high specificity and sensitivity for tamoxifen resistance. These findings suggest that miR-221 is a potential serum biomarker for predicting tamoxifen resistance particularly in BC patients with local recurrence and metastasis.

Provenance and peer review

Not commissioned, externally peer-reviewed.

Conflict of interest

The authors declare that they have no conflict of interests.

Sources of funding

No funding or sponsorship.

Ethical approval

All procedure for human experiment has been approved by our institutional review board.

Consent

The research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. The patients have given their written informed consent on admission to use their prospective data base and files for research work.

Author contribution

MFS, AAI, PR, MNM, and MH initiated and designed the study. IP performed the statistical analysis. NS, WA, HC, and RAN contributed in the data processing. All authors have read and approved the final manuscript.

Registration of research studies

This study has been registered with the Research Registry no. 7241

Guarantor

Alfiah Amiruddin, Muhammad Nassrum Massi, and Andi Asadul Islam.

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