# The Association between Plasma miRNA-21 Levels with Overall 1-year Survival Rate of Breast Cancer Patients at Various Stages

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## ABSTRACT

***Background:*** *miRNA 21 exhibits an increased expression in breast cancer (BC). However, its relationship with the 1-year survival of breast cancer patients is still disputable and under serious discussion.* ***Methods:*** *Cohort prospective study involving 49 breast cancer patients was done, comprising 26 in early stage and 23 in end-stage. We evaluated miRNA-21 values and observed its association with mortality within 1 year.* ***Results:*** *In general, there was a correlation between the increase in miRNA-21 levels and the mortality rate of breast cancer patients (r = 0.651; p <0.05). In the early stages, the increase in miRNA-21 values was not associated with breast cancer mortality (r= 0.25; p=0.218), but in the later stages, we found that the increase in miRNA-21 had a correlation with mortality (r=0.866; p=<0, 05). In advanced stage, high level of miRNA-21 had high asscociation with mortality rate (HR 17,27 95%CI 7,37-40,69).* ***Conclusion:*** *The increase in miRNA-21 values is associated with the 1-year survival of breast cancer patients.*

***Keywords*****:** *miRNA-21, breast cancer, mortality rate, survival, health.*

## INTRODUCTION

Breast cancer has been noticed as a worldwide health issue with an increasing prevalence in the world with a high mortality rate. Based on GLOBOCAN data (Global Cancer Statistics) 2018, breast cancer ranks first in the incidence of cancer in women with the number of new cases worldwide of 2,088,849 people (24.2%) with a mortality rate of 626,679 people (15%), of which half of the new cases and mortality cases found in Asia.[1] Indonesia came with the data revealing that breast cancer is the second most common malignancy after cervical cancer with a prevalence of 0.5 percent and an estimated number of cases of 61,682 patients, where the highest incidence is found in the Central Java, followed by East Java, West Java and DKI Jakarta.[2] Data on one-year, five-year and ten-year survival rates in breast cancer patients aged 15 to 99 years during the period 2013-2017 in the UK were found to be 95.8%, 85% and 75.9%, respectively, where plans of action are somewhat still needed in increasing breast cancer survival rates.[3]

The examination of minimally invasive circulating biomarkers offers new hope to diagnose breast cancer, monitor progression,

determine prognosis and predict response to treatment, which is a vital part of strategies to reduce breast cancer morbidity and mortality. Several conventional circulating biomarkers that are known and used in monitoring and assessing breast cancer progression are carcino-embryonic antigen (CEA) and carbohydrate antigens (CA) such as (CA15-3). However, the sensitivity values of the biomarkers CEA and CA 15-3 are relatively low, around 22% and 15%, respectively. Due to the low sensitivity of existing biomarkers, new minimally invasive biomarkers with better sensitivity and specificity are required to monitor breast cancer progression.[4-6]

The discovery of microRNA (miRNA) in the last decade has unfolded new opportunities for the detection and prognostication of breast cancer. The advantages of miRNA in the oncogenesis process include various expression patterns associated with the type of cancer, easy detection in biopsies, fine stability in blood and other body fluids, especially in blood, plasma, serum, and saliva so that miRNAs can be used as promising biomarkers.[7,8] Dysregulation in miRNA genes in the form of mutations, deletions, translocations, increased expression, or decreased expression can trigger biological changes in cells, and is believed to have an important role in cancer development.[9] Several literature studies have shown that miRNA-21 has increased expression in breast cancer. MiRNA-21 targets that have been identified are tumor suppressor tropomyosin 1 (TPM1), TIMP3, RECK Cdc25A network of p53, PDCD4, PTEN, and Maspin. MiRNA-21 overexpression correlates with specific breast cancer bio-pathological features, such as advanced tumor stage, lymph node metastases, and poor patient survival, suggesting that miRNA-21 may serve as a molecular prognostic biomarker for breast cancer progression. High miRNA-21 expression had a negative impact on overall survival and recurrence-free survival in breast cancer.[10]

Several studies that have been conducted regarding the relationship of miRNA-21 with breast cancer prognosis had contradictory results. The meta-analysis by Wang et al.[11] on the association of miRNA-21 expression with breast cancer prognosis identified 10 studies involving 1,439 cases, showing that high miRNA-21 expression predicted poor overall survival and disease-free survival and low relapse-free survival in breast cancer patients.[11] Study of Khanbashi et al. (2015) on 27 locally-advanced breast cancer patients, Radojicic et al. (2011) on 49 triple-negative breast cancer patients, and Qian et al. (2009) on 344 breast cancer patients, showed that the results of the analysis of serum and tissue miRNA-21 expression were not correlated with overall survival and disease-free survival.[12-14]

According to the fact that there are contradicting results among several previous studies, further analysis is considered necessary to apply miRNA-21 as a prognostic factor for breast cancer. This study aims to determine the relationship between circulating miRNA 21 levels and overall 1-year survival in breast cancer, where the circulating miRNA-21 parameter used is blood plasma which is more stable and different from previous studies using serum or tissue.

## METHODS

This is a cohort-retrospective study which was conducted at the Oncology Hematology Outpatient Clinic, Airlangga University Hospital, Surabaya, starting from November 2019 through November 2020. Participants taking a part in the study were those who met all of the following criteria: female patients aged 18 and above; breast cancer patients who have not received chemotherapy and radiotherapy; and are willingly involved in the study by signing an informed consent form.

Participants who were excluded from the study were patients who met one or more of the following criteria: breast cancer patients with terminal conditions (ECOG2, Karnofsky, score 50%), patients with a history of other malignancy in the last 5 years, and patients with comorbid diseases such as COPD, heart failure, chronic kidney disease, liver cirrhosis and diabetes mellitus that are not under control. Data were obtained from history taking, physical examination and additional examination.

The expressed value of circulating plasma miRNA-21 in the blood plasma of breast cancer

patients is measured using the quantitative Real Time Polymerase Chain Reaction (qRT-PCR) method. The Hsa-miR-16-5p LNA PCR primer set was used as an endogenous control and a qRT-PCR process was used to calculate the expression value of circulating miRNA-21. Blood samples withdrawal were carried out by the Surabaya Prodia Laboratories and then sent for storage and processing at the Jakarta Prodia Laboratory.

Samples for MiRNA21 examination was gained from 10 cc venous blood of deceased and healthy patients. Centrifugation was carried out at 3000 RPM for 5 minutes to separate the serum and then sent and stored in a container with a temperature of -80C until RNA was able to be extracted. Total RNA extraction, including small RNA, was performed by the Qiagen miRNeasy serum/plasma kit according to the protocol of the product supplier.

The miRNA-21 reverse transcription technique used Taqman TM microRNA reverse transcription kit (Applied Biosystem) and miRNA-21 specific stem-loop primer (Applied Biosystem, assay) for miRNA-21 reverse transcription (RT) reaction. RT primers for small nuclear miRNA-16 (Applied Biosystem) were used as endogenous controls.

### Ethics Approval

This study has been approved by the research ethics committee of Universitas Airlangga Hospital (No: 164/KEP/2019).

### Statistical Analysis

The sample size in this study was calculated by the formula of correlative analytic sample. Furthermore, the number of samples required was approximately 37 patients, but our study obtained 49, consisting of 26 patients with early-stage breast cancer and 23 advanced-stage patients. Statistical analysis was carried out using SPSS version 24.0. (Chicago, IL, USA). Statistical analysis to determine the correlation of the two variables used the Pearson statistical test if the data distribution was normal, otherwise, Spearman test was used. The correlation test results are interpreted based on the p value, the strength of the correlation and the direction of the correlation.

## RESULTS

The characteristics of 49 breast cancer patients are presented in **Table 1**. Of the 49 breast cancer patients, 26 patients were in the early stages, consisting of 5 at stage 1 and 21 at stage 2. Meanwhile, of 23 patients with breast cancer in the advanced stage, 11 at stage 3 while 12 at stage 4.

**Table 1.** Characteristics of breast cancer patients.

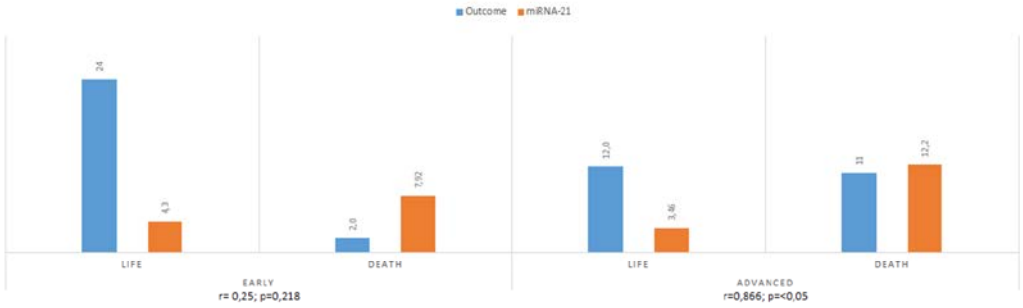
| Variables | n | % |
|---|---|---|
| Age (Mean/SD) | 51.95 | 10.21 |
| Stage | | |
| Early (n,%): | 26 | 53.06 |
| - 1A | 4 | 8.16 |
| - 1B | 1 | 2.04 |
| - 2A | 9 | 18.37 |
| - 2B | 12 | 24.49 |
| Advanced (n,%): | 23 | 46.94 |
| - 3A | 3 | 6.12 |
| - 3B | 5 | 10.20 |
| - 3C | 3 | 6.12 |
| - 4 | 12 | 24.49 |
| Menarche | | |
| - Premenopause | 25 | 51.02 |
| - Menopause | 24 | 48.98 |
| Comorbid | | |
| - HT | 13 | 26.53 |
| - DM | 5 | 10.20 |
| - Thallasemia | 1 | 2.04 |
| Chemotherapy | | |
| - Adjuvan | 23 | 46.94 |
| - Neoadjuvan | 14 | 28.57 |
| - Palliative | 12 | 24.49 |
| Performance status (ECOG) | | |
| - 0 | 32 | 65.31 |
| - 1 | 15 | 30.61 |
| - 2 | 2 | 4.08 |
| Estrogen receptor | | |
| - Neg | 14 | 28.57 |
| - Pos | 23 | 46.94 |
| Progesteron receptor | | |
| - Neg | 19 | 38.78 |
| - Pos | 18 | 36.73 |
| Human Epidermal Growth Factor Receptor (HER-2) | | |
| - Neg | 17 | 34.69 |
| - Pos 1 | 4 | 8.16 |
| - Pos 2 | 4 | 8.16 |
| - Pos 3 | 11 | 22.45 |
| Ki67 (mean) | 50% | |

| | | |
|---|---|---|
| Vital sign | | |
| - Systolic Pressure (mean, SD) | 128.38 | 15.09 |
| - Diastolic Pressure (mean,SD) | 78.14 | 9.59 |
| - Pulse (mean, SD) | 85.88 | 8.64 |
| - Respiration Rate (mean, SD) | 19.08 | 1 |
| - Temperature (mean,SD) | 36.19 | 0.91 |
| Physical status: | | |
| - Height (cm) | 152.84 | 4.88 |
| - Weight (kg) | 57.03 | 11.18 |
| - Body Surface Area (BSA/$m^2$) | 1.51 | 0.16 |
| Laboratory | | |
| - Hb (g/dl) (mean, SD) | 12.24 | 1.28 |
| - WBC ($10^3$/L) (mean, SD) | 7.75 | 2.62 |
| - PLT ($10^3$/L) (mean, SD) | 344.16 | 100.01 |
| - AST (u/L) (mean, SD) | 26.57 | 16.7 |
| - ALT (u/L) (mean, SD) | 28.35 | 29.83 |
| - Basal Urea Nitrogen (mg/L) (mean, SD) | 10.77 | 4.12 |
| - Creatinine (mg/L)(mean, SD) | 0.69 | 0.17 |
| - miRNA-21 | 6 | 5.35 |
| - CEA | 5.63 | 6.74 |
| - CA 15-3 | 68.62 | 141.95 |
| Outcome: | | |
| - Life (n,%) | 36 | 73.47 |
| - Death (n,%) | 13 | 26.53 |

Based on the breast cancer stages, the number of pre-menopausal patients were dominantly found (57.69%) in the early stages compared to the menopausal ones, while at an advanced stage, menopausal patients were more frequent than premenopausal ones (56.52%). Hypertension seemed to be the first-ranked comorbidity both in the early and advanced stage patients. MiRNA, CEA, and CA 15-3 values in advanced stages had higher mean values. There were 2 deaths in the early stage, while in the late stage there were 11 deaths. Eleven cases of death at an advanced stage consisted of 3 cases of death at stage 3 (1 in case 3A and 2 in case 3B) and 8 cases of death occurred at stage 4. Overall the increase in miRNA-21 values correlated with the death of breast cancer patients ($r = 0.651$ ; $p<0.05$). Nonetheless, in the early stages, the increase in miRNA-21 did not show any correlation ($r = 0.25$; $p = 0.218$). Otherwise, miRNA-21 was correlated with death in the advanced stages ($r = 0.866$; $p = <0.05$). (**Table 2**).

**Tabel 2.** The comparison between early and advanced stage and the association between death and miRNA-21 in breast cancer patients.

| | Stage | | | | Overall (n=49) | |
|---|---|---|---|---|---|---|
| | Early (n=26) | | Advance (n=23) | | | |
| Menarche | | | | | | |
| - Menopause | 11 | 42.31 | 13 | 56.52 | 24 | 48.98 |
| - Premenopause | 15 | 57.69 | 10 | 43.48 | 25 | 51.02 |
| Comorbid | | | | | | |
| - HT | 9 | 34.62 | 4 | 17.39 | 13 | 26.53 |
| - DM | 2 | 7.69 | 3 | 13.04 | 5 | 10.2 |
| - Thalasemia | 1 | 3.85 | 0 | 0.00 | 1 | 2.04 |
| Ca Mammae marker | | | | | | |
| - miRNA-21 (mean,SD) | 4.57 | 2.83 | 7.62 | 6.94 | 6 | 5.35 |
| - CEA (mean, SD) | 3.43 | 3.09 | 8.12 | 8.73 | 5.63 | 6.74 |
| - CA 15-3 (mean, SD) | 17.60 | 4.66 | 128.91 | 3.52 | 68.62 | 142 |
| Outcome | | | | | | |
| - Life (n,%) | 24 | 92.31 | 12 | 52.17 | 36 | 73.47 |
| - Death (n,%) | 2 | 7.69 | 11 | 47.83 | 13 | 26.53 |
| Correlation with Mortality rate | | | | | | |
| - miRNA-21 (r; pvalue) | 0.25 | 0.218 | 0.866 | <0.05 | 0.651 | <0.05 |




| | Stage | | | |
|---|---|---|---|---|
| | Early | | Advanced | |
| | Life | Death | Life | Death |
| Outcome | 24 | 2,0 | 12,0 | 11 |
| miRNA-21 | 4,3 | 7,92 | 3,46 | 12,2 |

**Figure 1.**

As seen on **Figure 1**, it appears that in the advanced and early stages, the mean miRNA values were lower in the surviving patients and higher in the deceased patients.

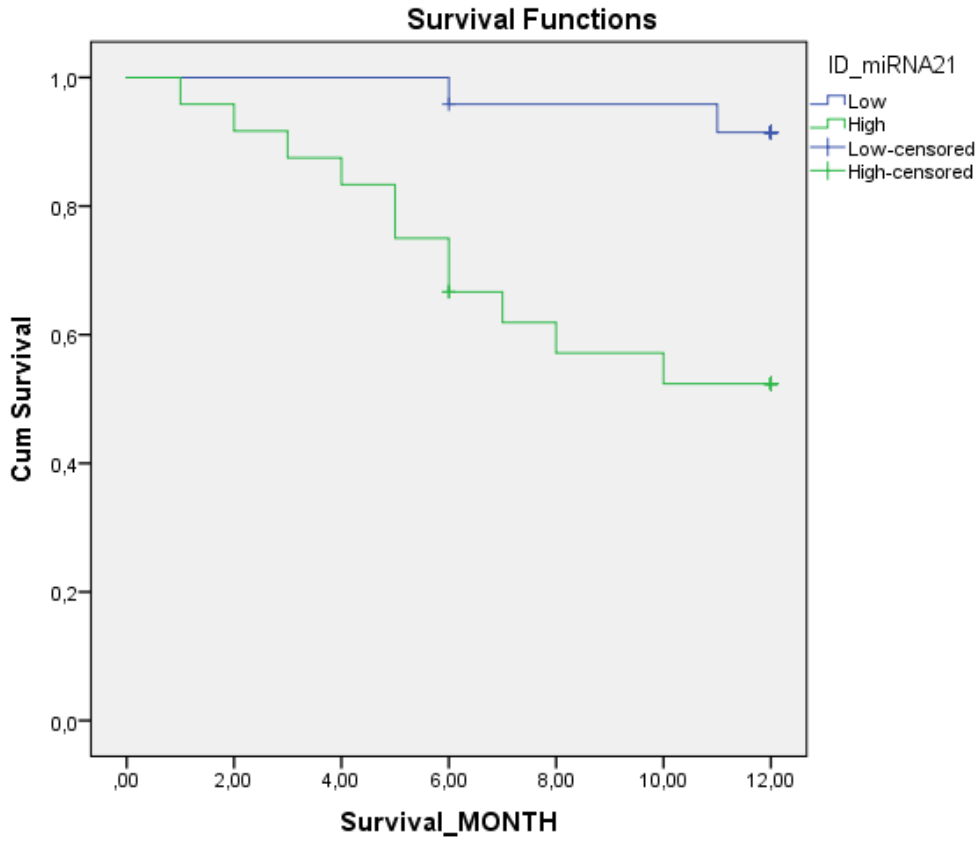



**Figure 2**. Graphic survival mRNA-21 level in 1 year

**Table 3.** One year survival breast cancer according miRNA21 level

| mRNA21 | One year survival | | | |
|---|---|---|---|---|
| | Low mRNA | | High miRNA | |
| | HR | 95% CI | HR | 95% CI |
| Overall | 0.18 | 0.11-0.31 | 5.5 | 3.2-9.46 |
| Early stadium | 0.766 | 0.33-1.8 | 1.31 | 0.55-3.07 |
| Advance stdium | 0.058 | 0.03-0.14 | 17.27 | 7.33-40.69 |

According to **Figure 2**, it appears that low level of miRNA21 indicates better survival which is statistically significant ($p<0.05$). Meanwhile, high levels of miRNA21 represented a statistically significant acceleration of death within 1 year in all cases, particularly in advanced stages (HR 5.5; 95%CI (3.2-9.46); HR 17.27; 95% CI : 7.33-40.69).

## DISCUSSION

The mean age of the subjects was 51.95 ± 10.21 years, with the youngest being 29 years and the oldest being 73. This is in line with a study conducted by Elghourory et al., (2018) on the evaluation of miRNA-21 as a prognostic marker in breast cancer in 50 breast cancer patients with a mean age of 53.4 ± 9.9 years, also with a study by Cuk et al. (2013) who examined circulating plasma miRNA-21 as a marker for early detection of breast cancer in 127 breast cancer patients with a mean age of 56.6 years.[15, 16]

Age somewhat is the major risk of breast cancer, in addition to reproductive factors, heredity and lifestyle factors, because the incidence of breast cancer is strongly associated with the increasing age. Data in 2016 in the United States about 99% of breast cancers were reported at the age of over 40 years, so it is highly recommended to screen women aged 40 and over.[17, 18] Recent research data showed that in East Asian and Southeast Asian, the breast cancer was more commonly found in the younger women than in the older ones. Factors that influence this trend are "westernization" which includes dietary changes (high fat, low vegetables) and environmental factors (physical inactivity, smoking, alcoholism) in the last two decades in Asian countries.[19]

In this study, according to the menarche status, 25 patients were the premenopausal women (51.02%), while 24 were the at the menopause stage (48.97%). This was supported by several previous studies where the percentage of the pre-menopausal group was higher than the menopausal one, such as by Dong et al., (2014) (54.16% and 45.84%), Elghourory et al. (2018) (64% and 36%). Meanwhile, another study by Papadaki et al. (2018) on circulating miRNA-21 as a predictive factor for recurrence in 133 breast cancer patients, stated that the proportion of those being pre-menopausal subjects were 42.9% (n=57), while menopausal ones were 57.1% (n= 76).[15, 20]

Reproductive factors such as menstrual history (early menarche, late menopause) are one of the risk factors for breast cancer. Every one year delay in menopause has been shown to elevate the risk of breast cancer by 3%, while every one year delaying menarche has been shown to reduce the it by 5%. [17, 18] In this study, the percentage of pre-menopause patients was slightly higher than the menopause group, which differred from the European study. This condition is in accordance with the age factor of breast cancer patients in Asia who tend to be younger than European and American countries. Reproductive factors also play a role because Asian women today tend to delay the birth of children, limit the number of children and rarely breastfeed. All of those factors elevate the tendency of breast cancer incidence at a younger age. Menarche status in the two groups of subjects in this study was homogeneous, so the relative risk for breast cancer did not differ.

Of all 49 research subjects, 5 were at the stage 1 (10,2%), 21 were at the stage 2 (42,85%), 10 were at the stage 3 (20,40%), and 13 were at the stage 4 (26,53%). This is congruent with a study by Gao et al. (2013) comparing miRNA-21 with CEA and CA 15-3 in 89 breast cancer patients, which obtained 23.59% stage 1 (n=21), 31.46% stage 2 (n=28), 10.11% stage 3 (n=9), and 34.83% stage 4 (n=31) patients.[5] Research by Zhang et al. (2016) revealed that stage 1 was 10.4% (n=11), stage 2 was 40.5% (n=43), stage 3 was 30.2% (n=32), and stage 4 was 18, 9% (n=20).[8] While Fattah et al. (2018) stated that 20% (n=6) were at, 30% at stage 2 (n=9), 23.33%

at stage 3 (n=7), and 26.66% at stage 4 (n=8).[21] The subjects at stage 1 of breast cancer were at the smallest proportion, while the stage 4 group is second largest proportion. In contrast to other studies, this was due to the fact that breast cancer patients in Indonesia often come at an advanced stage and rarely check themselves routinely for early detection.

The results of this study obtained circulating plasma miRNA-21 expression values using the 2-ΔΔCt formula to calculate relative gene expression in samples using the quantitative Real Time polymerase chain reaction (qRT-PCR). The calculation of Ct (cycle threshold of the fluorescence cycle sample from the qRT-PCR product) was obtained from the difference between Ct of the breast cancer group and Ct of the control group. The calculation of Ct was obtained from the qRT-PCR value results of miRNA-21 as the expression of the gene under study minus the value of the qRT-PCR miRNA-16 (Hsa-miR-16-5p LNA PCR primer set) as the expression of endogenous control genes that were not affected in the study.

The mean expression of circulating plasma miRNA-21 in the breast cancer group was 6.00±5.35 with a median of 4.43 (min-max:1.11-32.22). According to the clinical stage in the breast cancer group, the mean was 4.31 ±0.97 and the median was 4.75 (min-max: 2.88-5.35) at stage 1, 4.62 ±3.13 and the median was 3.68. (min-max: 1.18-11.79) at stage 2, 5.40 ± 4.27 and median 3.83 (min-max: 1.11-14.12) at stage 3, and 9.32 ± 8.21 and median 5.77 (min-max: 3.18-32.22) at stage 4. In line with Fattah et al. (2018), it was found that the expression of circulating miRNA-21 in the stage 1 breast cancer group was 1.6 (1.2-1.9), at stage 2 it was 2.6 (2.3-3.0), at stage 2 it was 2.6 (2.3-3.0). 3 was 3.5 (3.2-4.8), and in stage 4 it was 7.4 (5.9-8.7) (p= < 0.001).[22] This result was also in accordance with the research of Abdulhussain et al. (2017) who examined the relationship between serum and tissue miRNA-21 levels with PDCD4 expression in breast cancer patients in Iraq, where circulating miRNA-21 in the stage 1 breast cancer group was 0.3±0.29, stage 2 was 3.4±0.35, stage 3 was 3.57±0.44 and stage 4 was 4.26.[23]

The measurement of circulating plasma miRNA-21 levels was performed using the qRT-PCR technique. The sample was obtained from venous blood since the levels were more stable than those from breast tumor tissue. Blood plasma was more preferred than serum due to the occurrence of erythrocyte hemolysis during the blood coagulation process which releases miRNA. Blood plasma was stable in an RNAse-rich environment in the bloodstream, in high and low temperature changes, in high and low pH conditions, in repeated freezing-thawing processes, and in long-term storage.[24, 25] MiRNAs were protected by microparticles such as exosomes or bound to the AGO2 protein which was part of the RNA-induced silencing complex. This study used the Hsa-miRNA-16-5p LNA PCR primer set as an endogenous control and carried out a duplicate process in the qRT-PCR process to further improve the accuracy of circulating miRNA-21 expression values. This study used miRNA-16 as an endogenous control, which was consistently expressed in all study groups and was not affected by breast cancer status, and this is also consistent with several studies using miRNA-16 as a cancer normalizer.[21]

In the breast cancer group, higher miRNA-21 levels are detected due to over-expression (up regulation) of the miR-21 gene which further escalates the release of miR-21 in the circulation and acts on several tumor suppressor genes such as P53 Network and Cdc25A which manage cell proliferation; TPM1, TIMP, RECK which have roles in cell invasion and metastases; and PTEN, PDCD4, FasL which regulate apoptosis. The whole process of increasing miRNA-21 expression and inhibition of tumor suppressor genes would trigger breast cancer. miRNAs expression in the breast cancer group significantly increased as the stage got more advanced. The positive interrelation of miRNA-21 and 155 exosomal plasma and tissue miRNAs signify the role of these miRNA cargoes in tumor metastasis and explain the invasiveness of cancer cells through the activation of TIMP3/miRNA-21 expression.

Survival is one of the benchmarks in assessing the mortality and morbidity rate of a

disease in an area, and the survival rate is related to risk factors as well as treatment and prevention plans. In recent years, the mortality rate for breast cancer had down-trended in developed countries. In Indonesia, despite relatively-low survival rate of breast cancer patients, early diagnostic tool and more advanced treatment modalities are currently under the development.

The results of the 1-year overall survival evaluation in 49 breast cancer patients in this study showed that 36 patients lived (73.46%) while 13 patients (26.54%) died. Based on the clinical stage, in stage 1 all (100%) survived, 19 (90.47%) did at stage 2, 7 (70%) at stage 3, and 5 at stage 4 (38.46%).

The overall 1-year survival rate for breast cancer patients based on research data in England, Denmark and China was 95.8%, 94.4% and 94.91%, respectively. Research data in China also showed that the overall 1-year survival rate when assessed based on clinical stage, the survival rate will decrease in more advanced stage. In this study, the overall 1-survival rate were lower than the data in England, Denmark and China, possibly because breast cancer patients in Indonesia often come at an advanced stage so that the therapy might not be optimal, then affected the survival rate. This was supported by the data from the Indonesian Ministry of Health where the cancer survival rate in Indonesia was still low, and research data from Noorwati et al. (2020) on cancer patients at Dharmais Cancer Hospital stating 77.9% of patients were in stage 3 and stage 4.[2]

Enhanced expression of miRNA-21 in breast tissue is significantly associated with tumor size, histopathological grade, lymph node involvement, tumor invasion to the vasculature and lymphatic flow, visceral metastases, and advanced stage. Increased miRNA-21 expression is also associated with a lower response to therapy and a poorer breast cancer prognosis. This fact is consistent with the oncogenic role of miRNA-21 during proliferation, invasion, metastasis, and inhibition of apoptosis of cancer cells. miRNA-21 targets that have been identified were tumor suppressor tropomyosin 1 (TPM1), TIMP3, RECK Cdc25A network of p53, PDCD4, PTEN, and Maspin. miRNA-21 overexpression correlates with specific breast cancer bio-pathological features, such as advanced tumor stage, lymph node metastases, and poor patient survival, suggesting that miRNA-21 may act as a molecular prognostic biomarker for breast cancer progression.

The relationship between circulating miRNA-21 plasma expression levels and overall 1-year survival of breast cancer in this study was found to have a strong, negative correlation between circulating miRNA-21 plasma expression and overall 1-year survival of breast cancer (r of 0,-651, p-value <0.05). The negative or opposite direction of the correlation means that the higher the expression of circulating miRNA-21 plasma, the lower the overall 1-year survival. Further statistical analysis shown in the Kaplan-Meier graph revealed that high miRNA-21 expression when compared to low miRNA-21 expression had a hazard ratio value of 5.5 with p <0.05. The research of Yan et al., (2008) showed that miRNA-21 expression was significantly associated with poor prognosis in breast cancer patients (p<0.001), with a Hazard Ratio value of 5.476.[10] The research of Papadaki et al., (2018) stated that circulating miRNA-21 was significantly correlated with overall survival (p=0.033), with a Hazard Ratio value of 2.884.[26] While the research by Dong et al., (2014) showed that miRNA-21 was significantly associated with overall survival (p=0.0107), with a Hazard Ratio value of 2.32.[20]

Nevertheless, this study had a number of weaknesses. The research subjects were not homogeneously distributed among stages, that might cause bias. The follow-up monitoring time took only a year; thus it was not able to conclude patients' survival in a longer period. In addition, this study also did not observe other survival metrics such as recurrence period or disease-free survival. This study also did not explore other factors that influence survival such as tumor histopathological profile or hormone receptor status, and only one type of miRNA was assessed.

## CONCLUSION

Our principal finding is that overexpression of miR-21, one of the most significantly altered

miRNAs in BC, is associated with progression and poor prognosis of the patients. Although the precise molecular mechanism surrounding miRNA-21 up- regulation requires further clarification, our data indicate that miRNA-21 may be a good candidate as a molecular prognostic marker. Future study on the role of miRNA-21 in BC progression will no doubt add to the knowledge of this nascent field.

## CONFLICT OF INTERESTS

The authors have no relevant conflict of interest.

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