https://doi.org/10.31689/rmm.2024.31.1.57

ORIGINAL PAPERS

Differences in Ca 15-3 and Homocysteine Levels in Patients with Benign Tumor and Breast Cancer

Mathink Toonov ANUGERAWATI[1], Indranila Kustarini SAMSURIA[2], I Edward Kurnia Setiawan LIMIJADI[2], Dwi RETNONINGRUM[2], Banundari RACHMAWATI[2]*

Abstract

More than 25% of women have complaints related to breast lumps during their lifetime and approximately 12% have breast cancer. Cancer antigen 15-3 and homocysteine can be used as biomarkers in breast cancer. Breast cancer produces pro-inflammatory cytokines that can lead to increased CA 15-3 levels and serum homocysteine levels.

Objective: To investigate the differences in CA 15-3 and homocysteine levels between patients with benign tumors and breast cancer.

Methods: Observational analytic study with a cross-sectional approach in 30 benign breast tumor patients and 31 breast cancer patients. Measurement of CA 15-3 levels using enzyme linked immunofluorescent assay (ELFA) and homocysteine using competitive enzyme linked immunosorbant assay (ELISA). Comparative tests used independent T test and Mann-Whitney test. The study was conducted in 2023 at Kariadi Hospital Semarang and Ken Saras Hospital Ungaran.

Results: CA 15-3 levels in the benign breast tumor group had a median of 11.74 (2.00-40.00) U/mL, while the breast cancer group was 18.66 (7.22-168.97) U/mL (p = 0.001). Homocysteine levels in benign breast tumors had a median of 3.05 (0.10-11.50) µmol/L and in the breast cancer group was 9.90 (3.70-17.20) µmol/L (p = 0.0001).

Conclusion: CA 15-3 and homocysteine levels were lower in benign breast tumor patients compared with breast cancer patients.

Keywords: benign breast tumor, breast cancer, CA 15-3, homocysteine.

[1]Resident of Clinical Pathology Department, Faculty of Medicine, Universitas Diponegoro, Jl. Dr. Sutomo 16-18, Randusari 50244, Semarang, Indonesia
[2]Staff of Clinical Pathology Department, Faculty of Medicine, Universitas Diponegoro, Jl. Dr. Sutomo 16-18, Randusari 50244, Semarang, Indonesia

***Corresponding author:**
Banundari RACHMAWATI, Staff of Clinical Pathology Department, Faculty of Medicine, Universitas Diponegoro, Jl. Dr. Sutomo 16-18, Randusari 50244, Semarang, Indonesia.
E-mail: banundaridr@yahoo.com

INTRODUCTION

Breast lumps are complained of in more than 25% of women during their lifetime and approximately 12% of these are breast cancers.[1,2,3] Breast cancer is one of the most common malignancies, with a 10-year survival rate for early non-metastatic stages of over 80%.[4] The incidence rate in Indonesia is estimated at 12/100,000 women.[5]

Cancer antigen 15-3 is a mucin-1 (MUC-1) glycoprotein that is expressed from various epithelial cell types, but is found to be overexpressed in 90% of breast cancers so it can be used as a biomarker in breast cancer.[6,7] Increased expression of MUC1 can be caused by proinflammatory cytokines such as tumor necrosis factor α (TNF-α) and interferon gamma (IFN-γ) which induce MUC1 through the active role of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), transcription factor p65 (p65) and signal transducer and activator of transcription 3 (STAT3).[8]

CA 15-3 levels are higher in breast cancer patients compared to patients with benign breast tumors.[9,10] Increased CA 15-3 levels are also associated with breast cancer severity or stage of disease, tumor size, and significantly increased in breast cancer patients with metastases.[6,10] Increased CA 15-3 levels can be found in all stages of breast cancer, appearing to increase according to the stage. CA 15-3 expression did not differ between benign tumors and breast cancer stages I and II, but was found to be significantly different between benign tumors and breast cancer stages III and IV.[10] The results of a study by Lumachi et al. (2010) obtained results contrary to the previous description, namely increased CA 15-3 levels only correlated with molecular subtypes of breast cancer (P<0.001 and P=0.032). Age, menopausal status, tumor size, nodal status, TNM stage and histology, were not associated with increased serum CA 15-3 levels.[12]

CA 15-3 concentrations in blood can be used for screening, not only for breast cancer but also for other malignancies, including pancreatic, lung, ovarian, colon and liver cancers.[11] CA 15-3 concentrations have also been reported to be elevated in benign liver disease and benign breast disease.[11]

Homocysteine is an amino acid that is an intermediate in the biochemical conversion of methionine to cysteine, in the process of transsulfuration.[13] Elevated plasma homocysteine (homocysteinemia) is associated with various diseases, including cardiovascular disease, neurodegeneration, diabetes, Down syndrome, megaloblastic anemia, and various cancers, including breast cancer.[13]

Hyperhomocysteinemia in cancer patients is caused by an increased need for folate, vitamin B6 and vitamin B12 due to chronic inflammation.[13,14] T cells release large amounts of IFN-γ which stimulates the formation of reactive oxygen species (ROS). Excessive reactive oxygen species (ROS) have damaging effects on folate and B12. Inflammation also spurs the formation of (Interleukin-6) IL-6 which can induce an increase in hepatic pyridoxal 5'phosphate phosphatase activity.[14]

Hyperhomocysteinemia can cause chromosomal damage due to failure during the deoxyribonucleic acid (DNA) repair process, which can then cause cells to transform into malignant cells.[15] Research in breast cancer patients with metastases found higher homocysteine results than in early stage cancer patients and in healthy women.[16] The results of a study by Yoshihara et al. (2013) obtained results contrary to the previous description, namely there was no significant relationship between increased plasma homocysteine and circulating tumor cell cancer markers in breast cancer. Increased homocysteine levels are associated with the administration of therapy, where chemotherapy can increase the risk of thromboembolism.[17]

Based on this description, the researcher wants to prove the difference in CA 15-3 and serum homocysteine levels in patients with benign tumors and breast cancer, this study was conducted in patients with benign tumors and locally advanced breast cancer (stages IIIA and IIIB) and advanced stages (III C and IV) when diagnosed with ages 20-74 years.

METHODS

Study Design and Subject Recruitment

This study was an analytical observational study with a cross-sectional approach to prove differences in serum CA 15-3 and homocysteine levels in patients with benign tumors and breast cancer. Subjects of this study were female patients with complaints of breast lumps suspected of benign breast tumors and breast cancer stages III and IV who came to the surgical clinic of Dr. Kariadi Hospital and Ken Saras Hospital to confirm the diagnosis with pathological anatomy examination and had not received therapy (surgery, chemotherapy, radiotherapy, and other cancer therapies) from February 2023 to May 2023. The inclusion criteria were female patients who were diagnosed by PA examination as benign tumors (epithelial and stromal) and

stage III and IV breast cancer, were aged 20-74 years, had a normal serum creatinine and serum glucose. This study excluded patients with a history of cancer other than breast tissue, history of systemic heart and cardiovascular disease, history of hypertension, history of cirrhosis, history of tuberculosis, history of SLE, history of endometriosis, pregnant or breastfeeding, history of oral contraceptive use, history of metformin use.

All research subjects were asked for their consent by signing a written informed consent before the study. This research protocol was approved by the Health Research Ethics Commission of Central General Hospital Dokter Kariadi Semarang (No. 1411/EC/KEPK-RSDK/2023 dated February 3, 2023).

Data collection

Baseline data were collected through anamnesis (obsessional status, menstrual history, history of contraceptive use, history of medication use, history of previous diseases, especially history of cancer other than breast tissue, history of heart and systemic cardiovascular disease, history of hypertension, history of hepatitis cirrhosis, history of tuberculosis, history of SLE), physical examination (temperature, blood pressure, pulse rate, and respiratory frequency), and medical records (previous medication and laboratory data). Venous blood from the subjects was drawn before the scheduled surgery or biopsy.

Diagnosis of benign tumors and breast cancer

Benign tumors (epithelial and stromal) and stage III and IV breast cancer were diagnosed based on the results of anatomical pathology examination and breast cancer staging based on the TNM criteria of the UICC.

CA 15-3 and homocysteine serum level test

Five ml of venous blood was collected, serum was separated (centrifuged at 3,000 rpm for 15 minutes) and stored at -80°C until the time of examination. CA 15-3 levels were measured using a VIDAS machine, using the ELFA method. Homocysteine levels in serum were examined by enzyme-linked immunosorbent assay (ELISA) method using HCY ELISA kit (Wuhan Fine Biotech Co., Ltd., Wuhan, China).

Analysis Data

This study aims to prove the difference in CA 15-3 and homocysteine levels between patients of benign tumors and breast cancer. The study subjects were divided into two groups based on the results of anatomical pathology examination and breast cancer stage based on TNM criteria from UICC, namely the benign tumor group and breast cancer. Data was processed using the SPSS program. Normality of data distribution of both groups was analyzed by Shapiro Wilk test. Normal data distribution continued with independent t-test while abnormal data continued with Mann Whitney test. Significance at p value <0.05 with 95% confidence interval range.

The study was conducted from February 2023 to May 2023. This study involved 65 subjects who met the inclusion and exclusion criteria from 75 patients with complaints of lumps in the breast. The study subjects were then divided into 2 groups, 34 benign tumor patients and 31 breast cancer patients. During data processing, 4 extreme values were obtained from the benign tumor group and 3 extreme values from the breast cancer group. Extreme data were excluded from statistical analysis. The final data used 61 samples. The distribution of study subject characteristics is presented in Table 1.

Table 1. Data characteristics of research subjects

Variable	Benign breast tumor group (n=30)		Breast cancer group (n=31)		*p*
	Mean ± SD	Median (min-maks)	Mean ± SD	Median (min-maks)	
Age (years)	41,83±9,966		50,97±9,22		
Menarche (years)#		13 (11-14)		12,65 (11-14)	
Menstrual status					
Still menstruating	76,47%		38,7%		
Menopause	23,53%		62,3%		
BW (Kg)#		61,00 (53-76)		60,00 (40-76)	0,804 M
Hb (gr/dl)	12,87±1,23		12,73±1,26		0,647†
MCV#		90,35 (60,90-98,7)		87,70 (75,40-101,10)	0,328 M
Ureum (mg/dL)#		17,0 (10,00-35,00)		21,00 (11,00-38,00)	0,311M
Creatinine (mg/dL)#		0,86 (0,50-1,90)		0,86 (0,5-1,9)	0,834M

BW, body weight; HB, Hemoglobin; MCV, Mean Corpusculum Volume; SD, standard deviation; min, minimum; max, maximum; #Normal data distribution; *significant (p < 0.05); p, difference test value; MMann Whitney; †independent t-test;

The results of histochemical examination in the breast cancer group are presented in Table 2, with the most ER negative 12 patients (38.7%), PR negative 19 patients (61.3%), Her2 negative 11 patients (35.5%) and KI67 ≥20% as many as 27 patients (87.1%).

Table 2. Data of immunohistochemical examination results in breast cancer group

Variable	Examination Result	Number of patients (Percentage)
ER	Negatif	12 (38,7%)
	<20%	4 (12,9%)
	20%-50%	5 (16,1%)
	50%-80%	5 (16,1%)
	>80%	5 (16,1%)
PR	Negatif	19 (61,3%)
	<20%	2 (6,5%)
	20%-50%	3 (9,7%)
	50%-80%	5 (16,1%)
	>80%	2 (6,5%)
HER2	Negatif	11 (35,5%)
	Positif 1	10 (32,3%)
	Positif 2	1 (3,2%)
	Positif 3	9 (29%)
Ki67	<20%	4 (12,9%)
	≥20%	27 (87,1%)

ER, *estrogen receptor*; PR, *progesterone receptor*; HER2, *human epidermal growth factor receptor 2*; Ki67, *marker of proliferation* Ki-67.

Breast cancer group consisted of grade 1 with 3 (9.7%) patients, mean CA 15-3 level was 12.38±5.87 U/mL, mean homocysteine level was 8.63±1.95 µmol/L. Grade 2 with 20 (64.5%) patients, mean CA 15-3 level 42.67±49.28 U/mL, mean homocysteine level 10.34±3.37 µmol/L. Grade 3 with 8 (25.8%) patients, mean CA 15-3 level 42.10±38.31 U/mL, mean homocysteine level 8.63±3.73 µmol/L (Table 3).

Table 3. Breast cancer group characteristics data

Variable	CA 15-3 level (U/mL)	Homocystein Level (µmol/L)
	Median (min-maks)	Median (min-maks)
Breast cancer stage		
Stage III (stage IIIA and IIIB) = 26(83,87%)	16,72(7,22-142,15)	9,90(3,70-14,90)
Stage IV = 5(16,13%)	110,37(19,04-168,97)	8,90(3,80-17,20)
Breast cancer grade		
1 = 3(9,7%)	10,15(7,96-19,04)	9,50(6,40-10,00)
2 = 20(64,5%)	18,08(7,22-168,97)	9,90(3,70-17,20)
3 = 8(25,8%)	20,97(9,81-110,37)	8,10(3,80-14,90)

Analysis of differences in serum CA 15-3 and homocysteine levels in benign tumor and breast cancer patients using Mann Whitney test is shown in Table 4.

Table 4. Differences in CA 15-3 and homocysteine levels in patients with benign tumors and breast cancer

Variable	Benign breast tumor group (n=30)	Breast cancer group (n=31)	*p*
	Median (min-maks)	Median (min-maks)	
CA 15-3 level (U/mL)#	11,74 (2,00-40,00)	18,66 (7,22-168,97)	0,001*ᴍ
homocysteine level (µmol/L)#	3,05 (0,10-11,50)	9,90 (3,70-17,20)	0,0001*ᴍ

min, minimum; max, maximum; #Normal data distribution; *significant ($p < 0.05$); p, difference test value; ᴍMann Whitney

The results of data processing showed differences in CA 15-3 levels in the benign tumor and breast cancer groups (p = 0.01, Table 4) and there were also significant differences in homocysteine levels in the benign tumor and breast cancer groups (p = 0.0001, Table 4).

The box plot of the difference between CA 15-3 levels and homocysteine levels in patients with benign tumors and breast cancer is shown in Figure 1 and Figure 2.

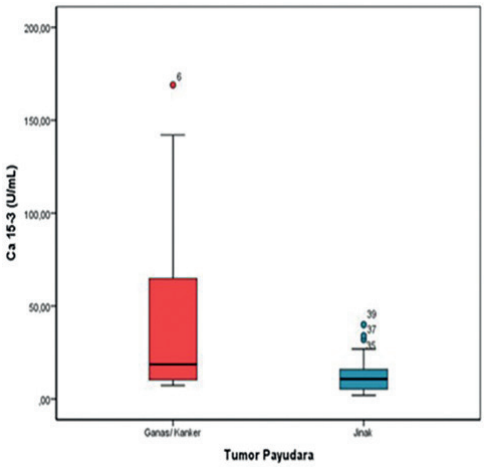

Figure 1. Box plot graph of CA 15-3 levels in benign tumor and breast cancer patients.

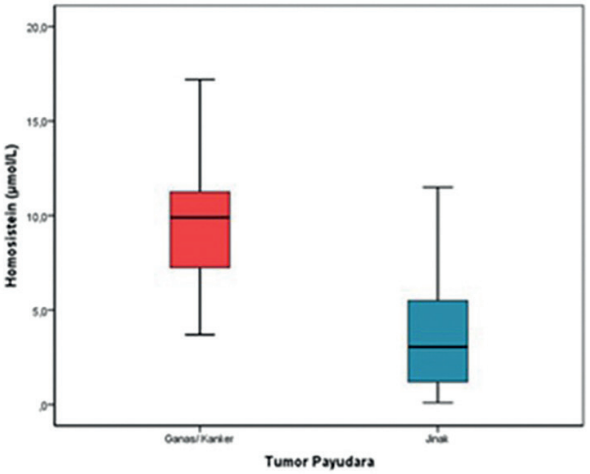

Figure 2. Box plot graph of homocysteine levels in patients with benign breast tumors and breast cancer.

DISCUSSIONS

Characteristic Data

This study included 61 subjects divided into 30 subjects of benign breast tumor group and 31 subjects of breast cancer group. The mean age of the subjects in this study for benign breast tumors was 40.97 ± 10.23 years, while for the breast cancer group was 50.41 ± 9.09 years. Research by Angrit et al. (2019) found that benign breast tumors are more common in women of childbearing age, peaking between the ages of 30 and 50 years.[18] The average age of female patients in this study is in accordance with the American Cancer Society (2019) which states that the average age of female patients when diagnosed with breast cancer in 2012-2016 was 62 years.[19] Anders et al. (2009) added that the incidence of breast cancer during 2010-2018 increased among women aged 20-49 years.[20]

Research by Sun et al. (2017) showed a scheme of risk factors for mammary carcinoma in the form of a pyramid. Age (aging factor) is at the bottom of the pyramid, followed by family history, reproductive factors, estrogen effects, and lifestyle at the top.[21] Age is the most important risk factor for breast cancer because the incidence of breast cancer increases with age. Age over 50 years increases the risk of breast cancer.[22] Research by Krusinska et al. (2019) shows 80% of breast cancer cases occur in post-menopausal women with almost 50% of cases diagnosed at the age of 50-69 years.[23]

Hormonal factors are important risk factors for breast cancer incidence after age.[24-26] Increased

exposure to the hormone estrogen increases the risk of breast cancer. Factors associated with exposure to estrogen hormones include early menarche, menopause after age 55, age of first childbirth more than 35 years, number of parities, breastfeeding, use of oral contraceptives and or hormone replacement therapy for more than 5 years.[27-29] The risk of mammary carcinoma is associated with age of menarche <12 years.[27-29] The subjects of this study only 13.3% had menarche before 12 years. Most of the subjects were menopausal (62.3%). Post-menopausal women have a 4.18 times higher risk of developing breast cancer.[27] Age at menarche and menopause affect the duration of exposure to reproductive hormones, but interaction with genetic and environmental factors is required for breast cancer to occur.[21,29]

The median age of menarche in the subjects of this study was 13 (11-14) years. The risk of breast cancer increases with earlier menarche and later menopause.[30] The risk of breast cancer is about 20% higher among those who started menstruating before the age of 11 years compared to those who started menstruating at the age of 14 years or older. Women who go through menopause at age 55 or older have about 12% higher risk compared to those who go through menopause at age 50-54.[30] The increased risk may be due to longer lifetime exposure to reproductive hormones and is more strongly associated with hormone receptor positive (HR+) breast cancer than other subtypes.[19]

Hormone receptor (HR) screening serves the purpose of guiding treatment selection in breast cancer patients. Estrogen receptor (ER) status plays an important role in making clinical decisions and predicting outcomes for invasive breast cancer patients.[31] In this study, 38.7% of breast cancer patients were ER-negative and 61.3% were ER-positive.

Progesterone receptor modulates the action of estrogen receptor α (ERα) in breast cancer, which is an ER target gene that is upregulated, and its expression is dependent on estrogen. Progesterone receptor is also an important prognostic biomarker in breast cancer, especially in HR+ breast cancer. High PR expression is more often observed in tumors with a better initial prognosis than tumors with a poor initial prognosis.[32] In accordance with this study, where only 38.7% of breast cancer patients were found to have positive PR, while 61.3% were negative. A hallmark of precancerous lesions and mammary tumors is an increased proportion of ER/PR-positive cells that proliferate during breast carcinogenesis and then switch from paracrine to autocrine mode of regulation by steroid hormones. Cells expressing ERα and the proliferation-associated antigen Ki-67 increase during breast tumorigenesis.[32]

The overall frequency of positive human epidermal growth factor receptor 2 (HER2) expression found in breast cancer in all previous studies was approximately 22%, with a range of 9% to 74%. In current practice, the HER2 positive rate is <20%, with most researchers currently reporting that the true positive rate ranges from 15% to 20%.33 In this study, 35.5% of breast cancer patients had HER-2 negative, 32.3% HER-2+, 3.2% HER-2+, and 29%% had HER-2++. Human epidermal growth factor receptor 2 is a membrane tyrosine kinase and when activated affects cell proliferation and survival. HER2 amplification is the main pathway of HER2 overexpression and is the main driver of tumor progression in breast cancer. Overexpressed HER2 is an important therapeutic target. The 2007 American Society of Clinical Oncology (ASCO) guidelines have also stated that HER2 should be evaluated in every invasive breast cancer, either at diagnosis or recurrence to guide therapy.[33]

In breast cancer progression, Ki-67 expression is strongly associated with cancer proliferation and is an indicator of prognosis and outcome. Ki-67 expression levels are also useful for informing treatment decision-making in some cases, so routine measurement of Ki-67 is now widely performed during pathological tumor evaluation. The Ki-67 proliferation index provides a precise measurement of the proliferation potential of breast cancer cells.[34] In this study, Ki-67 <20% was found in 12.9% and Ki-67 >20% in 87.1% of the breast cancer patients. Despite its widespread use in histopathological evaluation, inconsistencies in scoring methodology, lack of gold standard guidelines, and varying uptake of multigene panels incorporating Ki-67 negatively impact the reliability and standardization of this biomarker in clinical practice.[34]

The combination of frequently used pathological markers ER, PR, and HER2 is used to classify tumors into intrinsic subtypes. Semiquantitative evaluation of Ki-67 and PR is helpful for further determination of luminal subtypes. Breast cancers are grouped into 4 molecular subtypes according to their mRNA gene expression levels: luminal, her2-enriched, basal-like/triple-negative breast cancer, and normal breast-like. Further studies divided the luminal group into two subgroups (Luminal A and B).[34]

Immunohistochemical examination plays an important role in determining the choice of adjuvant therapy, namely chemotherapy or hormonal therapy. Hormonal therapy is given in hormonal positive cases. Hormonal therapy can be given in stages I to IV. Cancer cases with luminal A (ER+, PR+, Her2-) the main adjuvant therapy option is hormonal instead of chemotherapy. Hormonal therapy with tamoxifen should take precedence over aromatase inhibitors especially in menopausal and Her2- patients. Anti-Her2 targeted therapy should only be given in cases with Her2-positive immuohistochemical examination. The anti-Her2 regimen of choice is herceptin, preferably in cases that are early stage and have a good prognosis.[5]

Subjects in the breast cancer group in this study were divided into stage III with 26 people (83.87%) and stage IV with 5 people (16.13%). The mean value of CA 15-3 was lower in stage III group = 28.29±32.10 U/mL compared to stage IV group = 28.29±32.10 U/mL, while the mean value of Homocysteine had no difference between stage III group = 9.57±2.77 µmol/L and stage IV = 10.56±6.02 µmol/L.

The breast cancer group in this study was divided into grade 1 of 3 people (9.7%), grade 2 of 20 people (64.5%) and grade 3 of 8 people (25.8%). The mean value of CA 15-3 was lower in the grade 1 group = 12.38±5.87 U/mL compared to grade 2 = 42.67±49.28 U/mL and grade 3 = 42.10±38.31 U/mL, and there was no difference in the mean values in the grade 2 and grade 3 groups. There was no difference in the mean value of homocysteine between grade 1, 2, and 3 groups.

Differences in CA 15-3 Levels in Patients with Benign Breast Tumors and Breast Cancer.

This study found a significant difference between CA 15-3 levels in the benign breast tumor and breast cancer groups (p=0.001). CA 15-3 levels in the benign breast tumor group had a median value of 11.74 (2.00-40.00) U/mL statistically lower than the breast cancer group 18.66 (7.22-168.97) U/mL. The results of this study are in accordance with research conducted by Z.M. Hashim (2014) who found a significant increase (P<0.05) in breast cancer women with a mean value of 29.02±1.79 U/ml compared to controls, a mean value of 8.92±0.48 U/ml and women with benign tumors, a mean value of 13.78±1.24 U/ml.[36] Research conducted by Zhang et al. (2022) which obtained the average results of CA 15-3 levels in breast cancer of 11.80 ± 6.60 U/mL.[37] Research conducted by Gaughran et al. (2020) found that CA 15-3 is the tumor marker that is most often increased in all subtypes of breast cancer and associated with breast cancer metastasis.[38]

Cancer antigen 15-3 is an antigen expressed on breast duct epithelial cells in both benign and malignant breast tumors.[39] CA 15-3 antigen represents MUC1 which is often overexpressed in malignant cells, such as in breast cancer. This carbohydrate epitope in breast cancer is antigenically distinct from the epitope present in normal breast cells.[40]

Mucin 1 has two subunits N-terminus and C-terminus, which play different roles in normal and diseased states. The C-terminus in tumors is localized within the cell and is more involved in different signaling pathways that influence and regulate tumor growth, survival, invasion, migration and apoptosis.[41]

Mucin 1 is encoded by a gene located on the long arm of chromosome 1 at position 21, a region that is frequently mutated in breast cancer cells. Overexpression of MUC1 in cancer is caused by increased gene dosage and transcription levels, and by loss of posttranscriptional regulation. Studies on epigenetic regulation have shown that methylation of H3-K9 and CpG islands in the MUC1 promoter (close to the transcription start site; -174 to -182 bp) causes transcriptional repression. In contrast, acetylation of H3-K9 accommodates MUC1 expression. Thus, demethylation of CpG and H3-K9, and acetylation of H3-K9 in the 5′ flanking region led to increased expression of MUC1 in cancer cells.[8,42] Increased expression of MUC1 can also be caused by proinflammatory cytokines such as TNF-α and IFN-γ) which induce MUC1 through independent actions of NF-κB, p65 and STAT3.[8]

CA 15-3 levels in this study were found to be significantly higher in the breast cancer group compared to the breast tumor group, in accordance with the theory presented by Kabel et al. (2017), namely an increase in CA 15-3 levels in breast cancer cases, along with an increase in tumor progressivity such as tumor size and continued nodal stage.[11] Cancer antigen 15-3 is a high molecular weight mucin, and MUC-1-based glycoprotein markers can be used to monitor response to treatment, increased serum concentrations provide an early indication of progression in some breast cancer patients.[43]

Cancer antigen 15-3 supports tumor invasion and metastasis through mitogen-activated protein kinase signaling pathway and decreased e-cadherin. Elevated

levels of CA15-3 will predict poor prognosis with increased risk of metastasis.[44] The European group on tumor markers has recommended CA 15-3 levels be used to assess prognosis, early detection of disease progression, and treatment monitoring in breast cancer. The American Society of Clinical Oncology (ASCO) and the National Comprehensive Cancer Network (NCCN) do not currently recommend the use of serum CA 15-3 for breast cancer screening and treatment guidance, which may be partly due to conflicting conclusions from other studies, which found low positive serum tumor marker levels in breast cancer. A study by Wu et al. (2014) found that CA 15-3 levels were elevated in 12.3% of breast cancer cases.[45-47]

Previous research conducted by Park et al. (2012) showed that CA15-3 levels were associated with tumor load indicators including tumor size and lymph node status and patients with locally advanced breast cancer showed significantly higher CA 15-3 levels.[48-49] Shao et al. (2015) also showed that higher CA 15-3 levels were found in patients with larger tumor size, further spread to axillary lymph nodes and increased TNM stage. Elevated CA 15-3 levels are associated with tumor load and higher levels may indicate increased likelihood of systemic metastasis.[47] The study by Sang et al. (2013) showed that elevated levels of tumor markers were more frequently observed in patients with metastatic breast cancer than primary breast cancer, and patients who had elevated levels of tumor markers before surgery also showed increased recurrence.[50]

Differences in Homocysteine Levels in Breast Benign Tumor and Breast Cancer Patients.

This study found a significant difference between homocysteine levels in the benign breast tumor and breast cancer groups (p=0.0001). Homocysteine levels were statistically found in the benign breast tumor group to have a lower median value than the breast cancer group.

The results of this study are in accordance with research conducted by Liu, et al. (2022), where it was found that homocysteine levels in the peripheral blood of breast cancer patients were higher than the control group ($P < 0.05$). Homocysteine levels were also examined in the study and found to be higher in T2-T4 breast cancer patients than Tis-T1 breast cancer patients, and among premenopausal breast cancer patients, homocysteine levels increased in patients with T2-T4 stages ($P < 0.05$), and the mean obtained was 13.40 ± 5.38 in the group of breast cancer patients and 11.54 ± 3.81 in the group of benign tumor patients.[51]

In Gatt et al. (2007) study, the control group had an average plasma homocysteine level of 7.9 μmol/L with a calculated normal range for women (mean ± 2 SD) of 4.9-10.9 μmol/L. The mean total homocysteine level for the early-stage breast cancer group was 9.43 μmol/L with a range of 4.8-35.6 μmol/L with 22.6% having levels above the upper limit of normal. The metastatic group average total homocysteine level was 11.34 μmol/L with 39% of measurements exceeding the normal range.[16]

Other studies obtained different results from this study. Research conducted by Kim et al. (2020), found that there was no statistically significant difference in homocysteine levels between patients with breast cancer with a mean value of 7.8 (6.6-9.4) μmol/L and patients with benign breast tumors with a mean value of 8.2 (6.7-9.9) μmol/L.[52] Research by Houghton et al. (2019), also found that there was no difference in homocysteine levels between groups of breast cancer patients with a mean value of 9.3 (6.0-15.1) and controls with a mean value of 9.2 (6.2-15.0).[53]

Homocysteine is derived from the essential amino acid methionine and serves as a metabolic intermediate of the methionine cycle essential for one-carbon metabolism.[54,55] Methionine is activated by ATP and converted to S-adenosyl methionine (SAM). This process requires 5-methyl-tetra-hydrogen-folate (5-MTHF) and a specialized methyltransferase that uses vitamin B12 as a coenzyme.

He et al. (2021) suggested that homocysteine levels were found to be elevated in the plasma of patients with breast cancer, colorectal cancer, primary hepatocellular carcinoma, and many other malignancies.[56] Homocysteine levels in this study were found to be significantly higher in the breast cancer group compared to the breast tumor group. The results of this study are related to one theory that this may be manifested by strong methionine cycle changes during cancer pathogenesis and associated with facilitated DNA double strand breaks and other mutations. Elevated homocysteine levels have indeed been associated with tumorigenesis through DNA hypomethylation and inactivation of tumor suppressor genes, and it has been noted for several years that plasma homocysteine concentrations are associated with the risk of tumorigenesis.[56]which may be involved in the carcinogenesis of hormone-related cancers, but clinical results of

observational studies are controversial. In this study, we investigated the causal relationships between plasma homocysteine and breast cancer (BRCA

Ferroni, et al. (2009), in their study mentioned that homocysteine levels were significantly higher in patients with breast cancer compared to controls.[57] Hyperhomocysteinemia can increase the inflammatory process through oxidative stress. Homocysteine-induced damage is related to the adhesion of cell molecules, cytokines and chemokines that can contribute to the biological process of cancer. Inflammatory conditions are closely associated with elevated homocysteine levels.[57] Hyperhomocysteinemia in cancer patients is caused by an increased need for folate, vitamin B6 and vitamin B12 due to chronic inflammation.[13,14] T cells release large amounts of IFN-γ which stimulates the formation of reactive oxygen species (ROS). Excessive reactive oxygen species have damaging effects on folate and B12. Interleukin 6 can induce an increase in hepatic pyridoxal 5'phosphate phosphatase activity.[14]

The theory above is also in accordance with research conducted by Hamza et al. (2022) who found that homocysteine levels were more elevated in breast cancer patients undergoing chemotherapy than controls along with malondialdehyde (MDA) levels. Gatt A. et al, (2007) found that an increase in homocysteine triggers the production of OH which is known as the initiator of lauroyl peroxide (LPO) by homocysteine autooxidation. The study conducted by Hamza et al. (2022) referred to previous studies and found that there is a positive association, as increased homocysteine leads to increased free radicals and subsequently increased MDA production.[58]

Naushad et al. (2014), also found an association between hyperhomocysteinemia and hypomethioninemia in breast cancer and other studies showing that there is an association between hyperhomocysteinemia and metastasis and the development of drug resistance in breast cancer cells treated with homocysteine led Naushad et al. (2014) hypothesized that homocysteine may modulate the expression of certain tumor suppressors namely, RASSF1, RARb1, CNND1, BRCA1, and p21, and may affect prognostic markers such as BNIP3 by inducing epigenetic changes.

The high prevalence of hyperhomocysteinemia in metastatic patients, the low SAM/SAH ratio in methionine-dependent cells, the possible role of genetic factors affecting 5-methyl tetrahydrofolate synthesis in the MDP phenomenon, and the role of breast methylomes in breast cancer progression led Naushad et al. (2014) hypothesized that elevated homocysteine may affect cellular methylation of certain important genes that control breast cancer initiation and progression, leading to the conclusion that homocysteine and one-carbon genetic variants affect the epigenetic profile of two important genes, RASSF1 and BRCA1, thus directly affecting breast cancer progression and explaining the MDP phenomenon of breast cancer.[59]

CONCLUSIONS

There are differences in CA 15-3 levels and homocysteine levels between patients with benign tumors and breast cancer, and CA 15-3 levels and homocysteine levels are lower in patients with benign tumors.

Acknowledgment
The authors would like to express acknowledgment to Mrs. Farida for her technical assistance with laboratory work and all participants.

Author contribution
BR and DR were involved in research review, data analysis, script preparation. EL was involved in research planning, measurement, data analysis, and script preparation. All authors were involved in the discussion of the results, the editing process, and approving the final script. There is no conflict of interest in this study.

Ethical Standards
The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law. Informed consent was obtained from all of the patients included in this study.

References

1. Daly C, Puckett Y. New Breast Mass. Natl Cent Biotechnol Inf. 2022;2(1):1–17.
2. Smania MA. Evaluation of common breast complaints in primary care. Nurse Pract. 2017;42(10):9–15.
3. Pangribowo S. Beban Kanker di Indonesia. Pusat Data Dan Informasi Kesehatan Kementerian Kesehatan RI. Jakarta Selatan; 2019.
4. Boghici EM, Parosanu AI, Iaciu IC, Olaru M, Pirlog CF, Orlov-Slavu C, et al. Second Primary Lung Cancer after Breast Cancer: Challenges and Approaches. Med Mod. 2023;30(2):157–60.
5. Kementerian Kesehatan RI. Panduan Nasional Penanganan Kanker Payudara. 2015.

6. Uygur MM, Gümüş M. The utility of serum tumor markers CEA and CA 15–3 for breast cancer prognosis and their association with clinicopathological parameters. Cancer Treat Res Commun. 2021;28.
7. Park S, Ahn HK, Park LC, Hwang DW, Ji JH, Maeng CH, et al. Implications of different CA 15-3 levels according to breast cancer subtype at initial diagnosis of recurrent or metastatic breast cancer. Oncology. 2012;82(3):180–7.
8. Sritama N, Mukherjee P. Muc1: a multifaceted oncoprotein with a key role in cancer progression Sritama. Physiol Behav. 2017;176(12):139–48.
9. Fu S, Yun ZY, Cui MM, Meng H, Qian C, Liu T, et al. Cancer antigen 15-3, platelet distribution width, and fibrinogen in combination to distinguish breast cancer from benign breast disease in non-conclusive mammography patients. Oncotarget. 2017;8(40):67829–36.
10. Fu Y, Li H. Assessing clinical significance of serum CA15-3 and carcinoembryonic antigen (CEA) levels in breast cancer patients: A meta-analysis. Med Sci Monit. 2016;22:3154–62.
11. Kabel AM. Tumor markers of breast cancer: New prospectives. J Oncol Sci. 2017;3(1):5–11.
12. Lumachi F, Basso SMM, Marzano B, Bonamini M, Milan E, Chiara GB. Relationship between hormone receptors, MIB-1 index and serum tumour markers CEA and CA 15–3 in patients with pT1–2 breast cancer. Eur J Cancer Suppl. 2010;8(3):104–5.
13. Miller JW. Homocysteine. Encycl Hum Nutr. 2012;2–4:424–30.
14. Lazzerini PE, Capecchi PL, Selvi E, Lorenzini S, Bisogno S, Galeazzi M, et al. Hyperhomocysteinemia, inflammation and autoimmunity. Autoimmun Rev. 2007;6(7):503–9.
15. Hasan T, Arora R, Bansal AK, Bhattacharya R, Sharma GS, Singh LR. Disturbed homocysteine metabolism is associated with cancer. Exp Mol Med. 2019;51(2).
16. Gatt A, Makris A, Cladd H, Burcombe RJ, Smith JM, Cooper P, et al. Hyperhomocysteinemia in women with advanced breast cancer. Int J Lab Hematol. 2007;29(6):421–5.
17. Yoshihara RN, Teixeira BM, Adami F, Kuniyoshi RK, Alves BCA, Gehrke FS, et al. Circulating tumor cell detection during chemotherapy in patients with breast cancer is not associated with plasma homocysteine levels. Tumor Biol. 2013;34(5):2937–41.
18. Stachs A, Stubert J, Reimer T, Hartmann S. Benign breast disease in women. Dtsch Arztebl Int. 2019;116(33–34):565–73.
19. American Cancer Society. Breast Cancer Facts & Figures 2019-2020. American Cancer Society. Atlanta: American Cancer Society, Inc.; 2019. 1–44 p.
20. Anders CK, Johnson R, Litton J, Phillips M, Bleyer A. Breast Cancer Before Age 40 Years. Semin Oncol. 2009;36(3):237–49.
21. Sun YS, Zhao Z, Yang ZN, Xu F, Lu HJ, Zhu ZY, et al. Risk factors and preventions of breast cancer. Int J Biol Sci. 2017;13(11):1387–97.
22. Indonesia KKR. Keputusan Menteri Kesehatan Republik Indonesia Nomor HK.01.07/MENKES/414/2018 tentang Pedoman Nasional Pelayanan Kedokteran Tata Taksana Kanker Payudara. Jakarta; 2018.
23. Krusinska B, Wadolowska L, Biernacki M, Slowinska MA, Drozdowski M. Serum 'Vitamin-Mineral' Profiles: Associations with Postmenopausal Breast Cancer Risk Including Dietary Patterns and Supplementation. A Case-Control Study. Nutrients. 2019 Sep 18;11(9):2244.
24. Abdulkareem I. A Review on Aetio-Pathogenesis of Breast Cancer. J Genet Syndr Gene Ther. 2013;04(05).
25. Shah R. Pathogenesis, prevention, diagnosis and treatment of breast cancer. World J Clin Oncol. 2014;5(3):283.
26. Kabel AM, Baali FH. Breast Cancer: Insights into Risk Factors, Pathogenesis, Diagnosis and Management. J Cancer Res Treat Vol 3, 2015, Pages 28-33. 2015;3(2):28–33.
27. Sari SE, Harahap WA, Saputra D. Pengaruh faktor risiko terhadap ekspresi reseptor estrogen pada penderita kanker payudara di kota Padang. J Kesehat Andalas. 2018;7(4):461–8.
28. Indrati R. Faktor-Faktor Risiko yang Berpengaruh Terhadap Kejadian Kanker Payudara Wanita. 2015. p. 1–151.
29. Dewi GAT, Hendrati LY. Analisis risiko kanker payudara berdasar riwayat pemakaian kontrasepsi hormonal dan usia. J Berk Epidemiol. 2015;3:12–23.
30. Group C, Cancer B. Menarche , menopause , and breast cancer risk : individual participant meta-analysis , including 118 964 women with breast cancer from 117 epidemiological studies. Lancet Oncol. 2012;13(11):1141–51.
31. Yu K Da, Cai YW, Wu SY, Shui RH, Shao ZM. Estrogen receptor-low breast cancer: Biology chaos and treatment paradox. Cancer Commun. 2021;41(10):968–80.
32. Li Z, Wei H, Li S, Wu P, Mao X. The role of progesterone receptors in meningioma. Dovepress. 2022;16(January):305–14.
33. Krishnamurti U, Silverman JF. HER2 in breast cancer: A review and update. Adv Anat Pathol. 2014;21(2):100–7.
34. Davey MG, Hynes SO, Kerin MJ, Miller N, Lowery AJ. Ki-67 as a prognostic biomarker in invasive breast cancer. Cancers (Basel). 2021;13(17):1–19.
35. Stanisławek A. Breast Cancer—Epidemiology, Risk Factors, Classification, Prognostic Markers, and Current Treatment Strategies— An Updated Review. 2021;1–30.
36. Hashim ZM. The significance of CA15-3 in breast cancer patients and its relationship to HER-2 receptor status. Int J Immunopathol Pharmacol. 2014;27(1):45–51.
37. Zhang Y, Zhao J, Wang Y, Cai W, Zhang X, Li K, et al. Changes of Tumor Markers in Patients with Breast Cancer during Postoperative Adjuvant Chemotherapy. 2022;2022.
38. Gaughran G. The utility of the tumor markers CA15 . 3 , CEA , CA-125 and CA19 . 9 in metastatic breast cancer. 2020;9.
39. Apostolopoulos V, Stojanovska L, Gargosky SE, Muc Á, Muc Á. MUC1 (CD227): a multi-tasked molecule. Cell Mol Life Sci. 2015;72(23):4475–500.
40. Klee GG, Schreiber WE. MUC1 Gene–Derived Glycoprotein Assays for Monitoring Breast Cancer (CA 15-3, CA 27.29, BR): Are They Measuring the Same Antigen? Arch Pathol Lab Med. 2004 Oct 1;128(10):1131–5.
41. Duffy MJ, Evoy D, McDermott EW. CA 15-3: Uses and limitation as a biomarker for breast cancer. Clin Chim Acta. 2010;411(23–24):1869–74.
42. Yamada N, Kitamoto S, Yokoyama S, Hamada T, Goto M, Tsutsumida H, et al. Epigenetic regulation of mucin genes in human cancers. Clin Epigenetics. 2011;2(2):85–96.
43. Sturgeon CM. Tumour markers. In: Clinical Biochemistry: Metabolic and Clinical Aspects: Third Edition. Third Edit. Elsevier Ltd.; 2014. p. 821–43.
44. Yang Y, Zhang H, Zhang M, Meng Q, Cai L, Zhang Q. Elevation of serum CEA and CA15-3 levels during antitumor therapy predicts poor therapeutic response in advanced breast cancer patients. Oncol Lett. 2017 Oct 10;14(6):7549–56.
45. Lee JS, Park S, Park JM, Cho JH, Kim SI, Park B. Elevated levels of preoperative CA 15-3 and CEA serum levels have independently poor prognostic signi fi cance in breast cancer. 2013;1–7.
46. Wu S, He Z, Zhou J, Sun J, Li F, Lin Q, et al. Serum levels of CEA and CA15-3 in different molecular subtypes and prognostic value in Chinese breast cancer. The Breast. 2014;23(1):88–93.
47. Shao Y, Sun X, He Y, Liu C, Liu H. Elevated Levels of Serum Tumor Markers CEA and CA15-3 Are Prognostic Parameters for Different Molecular Subtypes of Breast Cancer. Batra SK, editor. PLoS One. 2015 Jul 24;10(7):e0133830.

48. Qassem Ali H, Mahdi NK, Al-Jowher MH. The value of CA15-3 in diagnosis, prognosis and treatment response in women with breast cancer. J Pak Med Assoc. 2013;63(9):1138–41.
49. Hashim ZM. The Significance Of CA15-3 In Breast Cancer Patients and its Relationship To HER-2 Receptor Status. 2014;27(1):45–51.
50. Sang J, Seho L, Ji P, Park M, Park B. Elevated levels of serum tumor markers CA 15-3 and CEA are prognostic factors for diagnosis of metastatic breast cancers. 2013;477–84.
51. Liu Z. The Correlation Between Homocysteine , Blood Coagulation and the Breast Cancer Risk and Clinicopathological Characters : A Case Control Study. 2022;1–11.
52. Kim JA, Choi R, Won H, Kim S, Choi HJ, Ryu JM, et al. Serum Vitamin Levels and Their Relationships with Other Biomarkers in Korean Breast Cancer Patients. Nutrients. 2020 Sep 16;12(9):2831.
53. Houghton SC, Eliassen AH, Zhang SM, Selhub J, Rosner BA, Willett WC, et al. Plasma B-vitamins and one-carbon metabolites and the risk of breast cancer in younger women. Breast Cancer Res Treat. 2019;176(1):191–203.
54. Schmidt EE, Eriksson S, Prigge JR, Talago EA, Arne ESJ. homeostasis in the mouse liver. 2015;1–9.
55. Ducker GS, Rabinowitz JD. One-Carbon Metabolism in Health and Disease. Cell Metab. 2018;25(1):27–42.
56. He Q, Yang Z, Sun Y, Qu Z, Jia X, Li J, et al. The Impact of Homocysteine on the Risk of Hormone-Related Cancers: A Mendelian Randomization Study. Front Nutr. 2021;8(August):1–8.
57. Ferroni P, Palmirotta R, Martini F, Riondino S, Savonarola A, Spila A, et al. Determinants of homocysteine levels in colorectal and breast cancer patients. Anticancer Res. 2009;29(10):4131–8.
58. Hamza TA, Muhsin SA, Khalil TT. EVALUATION OF MALONDIALDEHYDE, HOMOCYSTEINE AND ANTIOXIDANT INFLUENCE CHEMOTHERAPY IN BREAST CANCER PATIENTS. Mil Med Sci Lett. 2023 Jun 2;92(2):105–11.
59. Naushad SM, Reddy CA, Kumaraswami K, Divyya S, Kotamraju S, Gottumukkala SR, et al. Impact of Hyperhomocysteinemia on Breast Cancer Initiation and Progression: Epigenetic Perspective. Cell Biochem Biophys. 2014 Mar 10;68(2):397–406.