ORIGINAL RESEARCH REPORT

Glucose Levels of Pleural Effusion Fluid and HER2 Status in Pleural-Metastatic Breast Cancer

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ABSTRACT

Background: Patients diagnosed with breast cancer who have also been affected by distant metastases often suffer from pleural effusion. The prognosis of malignant pleural effusions worsens when glucose levels in the pleural fluid are low. Elevated levels of anaerobic glycolysis due to HER2 overexpression cause breast cancer cells to take in more glucose. **Objective:** This study aimed to identify any correlation between glucose levels in pleural fluid and HER2 status in breast cancer patients. **Material and Method:** In this research, 35 female patients with thoracocentesis pleural metastatic breast cancer participated in an analytical observational study using a cross-sectional design. Patients who had undergone thoracocentesis had their HER2 status determined based on the information included in their medical records in the Integrated Oncology Clinic (*Poli Onkologi Satu Atap* (POSA)) Dr. Soetomo General Academic Hospital, Surabaya, Indonesia. After obtaining data on glucose levels in pleural fluid and HER2 status, statistical analysis was carried out to determine the relationship between the two parameters. **Results:** The statistical test results with a 95% significance level obtained p=0.004 (p<0.05), so it could be statistically said that there was a significant relationship between the glucose level of pleural fluid effusion with HER2 status. The obtained OR values were 10.93 and CI=95% (1.87-63.97), so that it could be interpreted that the low-glucose levels of pleural effusion fluid increase the proportion of patients with positive HER2 compared to not low-glucose levels of pleural effusion fluid. The correlation between the glucose level of pleural effusion fluid and HER2 status was moderately positive (Chi-Square Contingency Coefficient = 0.44). **Conclusion:** Glucose levels in pleural effusion fluid were significantly correlated with HER2 status.

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Highlights

1. Patients with breast cancer who have distant metastases are frequently impacted by pleural effusion.
2. HER2 status was found to be substantially correlated with glucose levels in pleural effusion fluid.

BACKGROUND

Breast cancer is the most often diagnosed malignancy in women globally with in excess of two million new cases in 2020. Based on GLOBOCAN 2020 report, breast cancer has become one of the most commonly diagnosed diseases and the 5th cause of cancer-related fatalities with an anticipated number of 2.3 million newly diagnosed cases worldwide (World Health Organization, 2012; Sung, et al., 2021). Breast cancer is the most prevalent cancer in women, representing 25% of all cancer or 240 among the 100,000 female population. It also holds the second mortality rate after lung cancer, which is 12.9% (Azamjah, et al., 2019). Breast cancer in Indonesia is the most prevalent cancer treated in hospitals. Most of the patients (60-70%) seek treatment at an advanced stage (stage III/IV) (Suzanna, et al., 2012; Panigoro, et al., 2015).

Professionals in the field have long sought a method to categorize the disease in order to identify applicable prognostic and predictive indications due to the complicated and variable manifestation and clinical evolution of breast cancer. Age, menopausal status, size of tumor, lymph nodes involvement, histopathological level, estrogen receptor (ER), and human epidermal growth factor receptor 2 (HER2) status have all been rigorously investigated as indications of clinical course (Fumagalli, et al., 2012). The immunohistochemistry test (IHC) is especially useful in diagnosing certain forms of cancer as it provides an abundance of data regarding the disease to help determine treatment. IHC uses antibodies as probes to detect antigens in tissue fragments or other cell preparation forms. To obtain the best results of an immunohistochemistry examination, many steps must be done, including pre-analytic, analytic, and post-analytic stages. For breast cancer, the standard immunohistochemistry assays are estrogen receptor (ER), progesterone (PR), human epidermal growth factor receptor 2 (HER2), and Ki-67 (Purwanto, et al., 2014; Centers for Disease Control and Prevention (CDC), 2023).

A pleural effusion is a huge accumulation of fluid in the pleural space. It might pose a diagnostic difficulty to the treating physician because it may be correlated with illnesses of the lung or pleura or to a systemic disorder (Karkhanis & Joshi, 2012). Pleural effusion occurs between 7-11% of patients with breast cancer. The patients commonly come to the hospital because of shortness of breath and in need of immediate and appropriate treatment. One of the main factors contributing to pulmonary morbidity and mortality is pleural effusion (Jany & Welte, 2019; Krishna, et al., 2023).

Low glucose levels and high lactate dehydrogenase levels (LDH) in a pleural fluid are associated with a poor prognosis of malignant pleural effusion. Overexpression of HER2 should increase the expression of lactate dehydrogenase-A (LDH-A) and Heat Shock Factor-1 (HSF 1), which results in improved glycolysis processes (Ziaian, et al., 2014; Yuan, et al., 2021). Low glucose levels in pleural fluid and HER2 overexpression in lung cancer were significantly correlated. The study revealed significant disparities in pleural fluid glucose levels between the HER2-negative group and the HER2-positive group ($p=0.02$) (Ziaian, et al., 2014). Nonetheless, comparable research on breast cancer has never been conducted. As a result, this study aimed to discover the association between the glucose level in pleural fluid and the status of HER2 in breast cancer patients being treated at Dr. Soetomo General Academic Hospital Surabaya, Indonesia.

OBJECTIVE

This study aimed to determine whether the presence of HER2 in breast cancer patients with pleural metastases was correlated with the presence of glucose in the pleural fluid.

MATERIAL AND METHOD

This was an analytical observational study with a cross-sectional design in 35 female subjects with pleural metastatic breast cancer. Most patients with advanced breast cancer reported shortness of breath owing to pleural effusion upon presenting to the emergency room. Thoracocentesis is one of the methods for controlling pleural effusion (Wiederhold, et al., 2023). In this study, thoracocentesis was performed as a diagnostic and therapeutic method.

Subjects who had performed thoracocentesis were traced to HER2 status through medical record data in the Integrated Oncology Clinic (*Poli Onkologi Satu Atap* ((POSA)). After obtaining data on glucose levels in pleural fluid and HER 2 status, subjects with qualified exclusion and inclusion criteria were analyzed statistically to determine the relationship between the two parameters. IBM SPSS statistic for Windows, version 25.0 (IBM Corp., Armonk, N.Y., USA) was used to examine the data.

In the Clinical Pathology Laboratory, Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, glucose levels in pleural fluid effusion are examined using the GLUC system chemical analysis method and the Flex reactor cartridge. Low pleural glucose levels are <60 mg/dL and a very low pleural glucose levels are ≥60 mg/dL. Patients who have low glucose levels (<60 mg/dL) in their pleural fluid may have a malignant pleural effusion (Fitzgerald, et al., 2019).

HER2 status testing (c-erbB-2, HER/neu) is presently advised for invasive breast cancer (DCIS is not evaluated for HER2). HER2 testing must be performed on paraffine blocks of tissues containing 10% NBF; it cannot be performed on cytological samples (Purwanto, et al., 2014). No anatomical pathology examinations were performed in this study. A score of 0 or 1+ indicates that breast cancer is HER2-negative. A score of 2+ indicates that the breast cancer is borderline, while a score of 3+ indicates that it is HER2 positive (Ahn, et al., 2020).

RESULT

This research included 35 participants with pleural effusion symptoms and immunohistochemistry findings for pleural effusion fluid collection. The data found that the average age of the subjects was 52.14 ± 9.29. The average Body Mass Index (BMI) was 26.88 ± 2.35. The immunohistochemistry was recorded, and the result of estrogen receptors was positive in 23 (65.7%) and negative progesterone receptors in 12 (34.3%) subjects. Positive progesterone receptors were found in 20 (57.1%) and negative progesterone on 15 (42.9%) subjects. The data of Ki67 found positive in 24 (68.6%) and negative in 11 (31.4%) subjects. The result of HER2 was obtained as follows: negative (-) in 13 (37.14%) subjects, positive 1 (+1) in 6 (17.14%) subjects, positive 2 (+2) in 6 (17.14%) subjects, and positive 3 (+3) in 10 (28.57%) subjects. The HER2 data were grouped into positive HER2 16 (45.71%) subjects and negative HER2 19 (54.29%) subjects. In this study, the subjects were tested for blood, glucose, and serum leukocyte examination to rule out the presence of empyema or diabetes mellitus. The result of mean serum glucose was 121.71 ± 29.71 and the mean serum leukocyte result was 7.84 ± 6.43.

Table 1. The patient's data and glucose level of pleural effusion fluid.

Characteristics	Glucose level of pleural effusion fluid		Significances (p)
	Not low	Low	
Total (patient)	24 (68.6%)	11 (31.4%)	-
Age (year)	Mean: 51.17 SD: 9,26	Mean: 54.27 SD: 9,40	Not significant (p = 0.366)
BMI (kg/m2)	Mean: 26.84 SD: 2,46	Mean: 26.96 SD: 2,20	Not significant (p = 0.943)
ER (Estrogen receptor)	Positive: 16 (66.67%) Negative: 8 (33,33%)	Positive: 7 (63.6%) Negative: 4 (36,4%)	Not significant (p = 0.863)
PR (Progesterone receptor)	Positive: 15 (62.5%) Negative: 9 (37,5%)	Positive: 5 (45.4%) Negative: 6 (54,5%)	Not significant (p = 0.351)
Ki67	Positive: 17 (70.8%) Negative: 7 (29,2%)	Positive: 7 (63.6%) Negative: 4 (36,4%)	Not significant (p = 0.675)
HER2 level	-: 12 (50%) +1: 5 (20.8%) +2: 5 (20.8%)	-: 1 (0.09%) +1: 1 (0.09%) +2: 1 (0.09%)	Significant (p = 0.001)

	+3: 2 (8.3%)	+3: 8 (72.73%)	
HER2 status	Positive: 7 (29.2%) Negative: 17 (70,8%)	Positive: 9 (81.8%) Negative: 2 (18,2%)	Significant (p = 0.004)
Glucose serum level	Mean: 125.58 SD: 28,28	Mean: 113.27 SD: 31,96	Not significant (p = 0.261)
Leukocyte serum	Mean: 8.61 SD: 7,65	Mean: 6.17 SD: 1,33	Not significant (p = 0.303)
pH pleural fluid	8	8	Not significant (p = 1.000)
Pleural fluid protein	Mean: 4.25 SD: 0,27	Mean: 4.07 SD: 0,81	Not significant (p = 0.507)

The result of the pleural examination showed a mean pleural fluid glucose level of 106.49 ± 47.8, an effect of pH of 8 pleural effusion fluid in all subjects, and a mean fluid protein of pleural effusion 4.2 ± 0.5. The data showed a grouping of whether the subjects had low-fluid pleural effusion glucose level or not. In addition, a statistical analysis was performed to find the p-value of all data to show a relationship between the data possessed by the subjects and the glucose level of pleural effusion fluid.

In Table 1, the p-value has been searched using statistical analysis to show the relationship between the patient's data and fluid glucose level in pleural effusion among pleural metastatic breast cancer patients. It appeared that only HER2 status had a significant difference between the low-fluid pleural effusion glucose level group and not low-fluid pleural effusion glucose level with p-value = 0.004 ($p<0.05$).

Table 2. The relationship between the glucose level of pleural effusion fluid and HER2 status.

HER2 status	Glucose level of pleural effusion fluid				p	OR
	Not low (>60mg/dl)		Low (≤60mg/dl)			
	Qt (n)	Qt (%)	Qt (n)	Qt (%)		
Positive	7	29.25	9	81.80	0.004	10.93
Negative	17	70.85	2	18.20		
Total	24	100	11	100		

(CI 95% = 1.87-63.97) Coefficient Contingency Chi-Square = 0.44)

Table 2 shows that statistical tests with a 95% significance level revealed p=0.004 ($p<0.05$), so there is a significant relationship between glucose levels of pleural effusion fluid with HER2 status. The obtained odds ratio (OR) revealed 10.93 and CI=95% (1.87-63.97). It can be interpreted that the low glucose level of pleural effusion fluid increases the proportion of patients with positive HER2 compared not-low glucose level of pleural effusion fluid. The correlation between the glucose level of pleural effusion fluid and HER2 status was moderately positive (Chi-Square Contingency coefficient = 0.44).

DISCUSSION

This study analyzed 35 breast cancer patients with pleural effusion symptoms, with an average age of 52.14 ± 9.29 years. A statistical test was conducted to determine the relationship between glucose levels and characteristics, including age, BMI, estrogen receptors, progesterone receptors, Ki67, pH, protein levels, and HER2 status. HER2 status examination revealed the following results: negative (-) in 13 (37.14%) subjects, positive 1 (+1) in 6 (17.14%) subjects, positive 2 (+2) in 6 (17.14 %) subjects, and positive 3 (+3) in 10 (28.57%) subjects. Panjwani et al., (2010) performed a FISH examination in 200 paraffin blocks of patients with HER2 2+ and obtained results that 66.6% of patients with HER2 +2 showed gene amplification that could be categorized in the positive HER2 group. The HER2 data were grouped into positive HER2 16 (45.71%) subjects and negative HER2 19 (54.29%) subjects.

This study showed that the relationship between low glucose levels in pleural fluid is associated with a poor prognosis of malignant pleural effusion. In malignant pleural effusions, excessive utilization of

glucose in the pleural cavity rather than translocation glucose generates low glucose levels in the pleural effusion (Skok, et al., 2019). Ziaian et al., (2014) reported a significant association between low glucose levels in pleural fluid and overexpression of HER2 in lung cancer. The study showed significant differences in pleural fluid glucose levels between the negative HER2 group and the positive HER2 group.

Rapid growth and proliferation are typical signs of tumors. To survive, tumor cells must adopt all existing mechanisms to meet their energy needs. Increasing demand for energy also requires a higher level of nutritional metabolism (Pavlova & Thompson, 2016). In normal cells, this is achieved through increased mitochondrial oxidative phosphorylation, the main cellular ATP source. In contrast to cancer cells, Otto Warburg's observations show that tumor cells tend to use glycolysis which is greater than the phosphorylation of glucose mitochondria, as their primary energy supply and apply this inefficient pathway even in aerobic conditions (Liberti & Locasale, 2016).

The tendency to use glycolysis in producing ATP in tumor cells can be caused by 1) glycolysis that produces ATP faster, even though it is less efficient in the long term than oxidative phosphorylation, 2) parts of carbon intermediates from glycolysis that can be used for various biosynthetic pathways to maintain tumor growth to remain constant (Li, et al., 2011).

Breast cancer can have up to 25-50 copies of the HER2 gene and an increase in HER2 protein as high as 40-100 times that results in 2 million receptors expressed on the surface of tumor cells. HER2 amplification also correlates significantly with the disease's pathological stage, the number of axillary KGB nodules, and possible metastasis (Iqbal & Iqbal, 2014).

Overexpression of HER2 will lead to increased glucose uptake, lactate production, and decreased oxygen use in breast cancer cells. This also causes an increase in the expression of LDH-A and its activity which causes an increasing HSF 1 (a transcription factor that plays a role in the regulation of eukaryotic cells against heat shock), which results in an increased glycolysis process (Farhadi, et al., 2022).

Based on the statistical analysis of all parameters obtained in the study subjects, it appeared that only HER2 status which had a significant relationship with glucose levels of pleural effusion fluid. However, the result showed that 7 (29.2%) subjects with positive HER2 status had glucose levels of pleural effusion fluid that were not low. Conversely, 2 (18.2%) subjects had negative HER2 status but had low glucose pleural effusion levels. Various factors, including immunohistochemistry examination factors and factors from the subjects, might have caused this.

Strength and limitations

This study can contribute data for future studies, especially in glucose levels of pleural effusion fluid and HER2 status in pleural-metastatic breast cancer. Glucose levels in the pleural effusion fluid are influenced by many factors and correlate with pleural inflammation. Therefore, further research is needed to examine subjects with positive HER2 status but have low pleural effusion fluid glucose levels. Follow-up studies should also be performed to examine subjects with negative HER2 status but have low fluid pleural effusion glucose levels.

CONCLUSION

Glucose levels in pleural effusion fluid correlated significantly with HER2 status. HER2-positive patients have a 0.083x lower glucose level of pleural effusion fluid than HER2-negative patients.

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Conflict of Interest

All authors have no conflict of interest.

Ethic Consideration

This research has been approved by the ethical committee of Dr. Soetomo General Academic Hospital Surabaya, Indonesia, with ethics certification no. 0854/KEPK/XII/2018 on 19-12-2018.

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None.

Author Contribution

MDI contributes to conception and design, analysis and interpretation of the data, drafting of the article, critical revision of the article for important intellectual content, and final approval of the article. DGAS contributes to conception and design, critical revision of the article for important intellectual content, and final approval of the article. HP contributes to conception and design and final approval of the article.

REFERENCES

Ahn, S., Woo, J. W., Lee, K., Park, S. Y. 2020. HER2 status in breast cancer: changes in guidelines and complicating factors for interpretation. Journal of Pathology and Translational Medicine, 54(1): 34-33. doi: https://doi.org/10.4132/jptm.2019.11.03.

Azamjah, N., Soltan-Zadeh, Y., Zayeri, F. 2019. Global trend of breast cancer mortality rate: A 25-year study. Asian Pacific Journal of Cancer Prevention, 20(7): 2015–2020. doi: 10.31557/APJCP.2019.20.7.2015.

Centers for Disease Control and Prevention (CDC). 2023. Immunohistochemistry screening (IHC), CDC. Available at: https://www.cdc.gov/genomics/disease/colorectal_cancer/IHC.htm.

Farhadi, P., Yarani, R., Valipour, E., Kiani, S., Hoseinkhani, Z., et al. 2022. Cell line-directed breast cancer research based on glucose metabolism status. Biomedicine & Pharmacotherapy, 146: 112526. doi: 10.1016/j.biopha.2021.112526.

Fitzgerald, D. B., Leong, S. L., Budgeon, C. A., Murray, K., Rosenstengal, A., et al. 2019. Relationship of pleural fluid pH and glucose: a multi-centre study of 2,971 cases. Journal of Thoracic Disease, 11(1): 123-130. doi: 10.21037/jtd.2018.12.101.

Fumagalli, D., Andre, F., Piccart-Gebhart, M. J., Sotiriou, C., Desmedt, C. 2012. Molecular biology in breast cancer: Should molecular classifiers be assessed by conventional tools or by gene expression arrays?. Critical Reviews in Oncology/Hematology, 84: e58–e69. doi: 10.1016/j.critrevonc.2012.08.003.

IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.

Iqbal, N., Iqbal, N. 2014. Human epidermal growth factor receptor 2 (HER2) in cancers: Overexpression and therapeutic implications. Molecular biology international, 2014: 852748. doi: 10.1155/2014/852748.

Jany, B.,Welte, T. 2019. Pleural effusion in adults—Etiology, diagnosis, and treatment. Deutsches Ärzteblatt international. doi: 10.3238/arztebl.2019.0377.

Karkhanis, V., Joshi, J. 2012. Pleural effusion: diagnosis, treatment, and management. Open Access Emergency Medicine: 31. doi: 10.2147/OAEM.S29942.

Krishna, R., Antoine, M. H., Rudrappa, M. 2023. Pleural effusion, StatPearls. Available at: http://www.ncbi.nlm.nih.gov/pubmed/30276937.

Li, N., Tan, W., Li, J., Li, P., Lee, S., et al. 2011. Glucose metabolism in breast cancer and its implication in cancer therapy. International Journal of Clinical Medicine, 02(02): 110–128. doi: 10.4236/ijcm.2011.22022.

Liberti, M. V., Locasale, J. W. 2016. The warburg effect: How does it benefit cancer cells?. Trends in Biochemical Sciences, 41(3): 211–218. doi: 10.1016/j.tibs.2015.12.001.

Panigoro, S., Hernowo, B., Purwanto, H., Handjojo., Haryono, S., et al. 2015. Panduan Penatalaksanaan Kanker Payudara. Komite Pananggulangan Kanker Nasional. Available at: http://202.70.136.213/guidelines_read.php?id=2&cancer=1.

Panjwani, P., Epari, S., Karpate, A., Shirsat, H., Rajsekharan, P., et al. 2010. Assessment of HER-2/neu status in breast cancer using fluorescence in situ hybridization & immunohistochemistry: Experience of a tertiary cancer referral centre in India. The Indian journal of medical research, 132: 287–94.

Available at: http://www.ncbi.nlm.nih.gov/pubmed/20847375.
Pavlova, N. N., Thompson, C. B. 2016. The emerging hallmarks of cancer metabolism. Cell Metabolism, 23(1): 27–47. doi: 10.1016/j.cmet.2015.12.006.
Purwanto, H., Handojo, D., Haryono, S. J., Harahap, W. A. 2014. Panduan penatalaksanaan kanker payudara. PERABOI. Available at: https://www.academia.edu/33898762/PANDUAN_PENATALAKSANAAN_KANKER_PAYUDARA.
Skok, K., Hladnik, G., Grm, A., Crnjac, A. 2019. Malignant pleural effusion and its current management: A review. Medicina, 55(8): 490. doi: 10.3390/medicina55080490.
Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., et al. 2021. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer Journal for Clinicians, 71(3): 209–249. doi: 10.3322/caac.21660.
Suzanna, E., Tiarlan, S., Rahayu, P. S., Shalmont, G., Anwar, E., et al. 2012. Registrasi kanker berbasis rumah sakit di Rumah Sakit Kanker "Dharmais" Pusat Kanker Nasional Pusat Kanker, 1993-2007. Indonesian Journal of Cancer, 6(4). doi: http://dx.doi.org/10.33371/ijoc.v6i4.299.
Wiederhold, B. D., Amr, O., Modi P., O'Rourke, M. 2023. Thoracentesis. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing. Available from: https://www.ncbi.nlm.nih.gov/books/NBK441866/
World Health Organization. 2012. Globocan 2012: Estimated incidence, mortality, and prevalence worldwide in 2012. IARC. Available at: https://www.iarc.who.int/news-events/latest-world-cancer-statistics-globocan-2012-estimated-cancer-incidence-mortality-and-prevalence-worldwide-in-2012/.
Yuan, Y., Gao, H., Zhuang, Y., Wei, L., Yu, J., et al. 2021. NDUFA4L2 promotes trastuzumab resistance in HER2-positive breast cancer. Therapeutic Advances in Medical Oncology, 13: 175883592110278. doi: 10.1177/17588359211027836.
Ziaian, B., Saberi, A., Ghayyoumi, M. A., Safaei, A., Ghaderi, A., et al. 2014 Association of high LDH and low glucose levels in pleural space with HER2 expression in non-small cell lung cancer. Asian Pacific Journal of Cancer Prevention, 15(4): 1617–1620. doi: 10.7314/APJCP.2014.15.4.1617.