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Correlation of Glucose Transporter-1 (GLUT-1) Immunohistochemical Expression on Benign, Borderline, and Malignant Phyllodes Tumors

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Abstract: Background: Phyllodes tumor (PT) is a rare fibroepithelial neoplasm, wherein constitutes 0.3-1% of all breast tumors. According to the WHO classification, PTs are divided into benign, borderline, and malignant. Malignant transformation is often accompanied by significant metabolic changes. In 1924, biochemist Otto Warburg and his colleagues discovered that cancer cells take up large amounts of glucose and metabolize it via glycolysis to lactic acid, then is called the "*Warburg effect*". High activity of glycolysis is associated with high uptake of glucose in cells. Glucose in the metabolic process requires a specific protein carrier in order to pass through the plasma membrane of cells. This process is regulated by the glucose transporter (GLUT). Abnormal expression of GLUT-1 is associated with aggressiveness of tumor growth and poor prognosis. .

Objective: Analyze the correlation of GLUT-1 expression in benign, borderline, and malignant PTs.

Methods: This research is an analytical study that aims to determine the correlation between expression of glucose transporter-1 (GLUT-1) on benign, borderline and malignant phyllodes tumors at H. Adam Malik Hospital Medan and the Department of Anatomic Pathology USU Medical Faculty with a cross sectional approach. The total number of samples used were 35 samples that met the inclusion criteria and were diagnosed as benign, borderline, and malignant phyllodes tumors by the researcher and reviewed by 2 pathologists. The statistical analysis test used in this study was the Mann Whitney test.

Results: From a total of 35 samples, 22 samples were benign phyllodes tumors, 2 were borderline, and 11 were malignant. All benign phyllodes tumors (100%) showed negative expression of GLUT-1. Two samples with borderline phyllodes tumors showed positive expression of GLUT-1 but were focal. Whereas, all samples (100%) with malignant phyllodes tumors showed strong and diffuse positive expression of GLUT-1. The mean age found in this sample was 44.57 with an age range of 17-62 years.

Conclusions: he results of this study showed a significant correlation ($p<0.001$) between the immunohistochemical expression of GLUT-1 and phyllodes tumor subtypes. by using GLUT-1 we can distinguish features of benign, bordrline and malignant lesions from phyllodes tumors.

Keywords: phyllodes tumor, GLUT-1, metabolism, malignancy

I. INTRODUCTION

Phyllodes tumor is a rare fibroepithelial tumor of the breast.[1] This tumor was first reported by Johannes Muller in 1838 and was named cystosarcoma phyllodes.[2] The incidence of phyllodes tumor is found in 0.3-1% of all primary breast tumors and 2.5% of all fibroepithelial tumors of the breast. Phyllodes tumors are often found in Asian women, especially in the elderly with a mean age of 40-50.[3]

Phyllodes tumor is a well-defined breast tumor, with a prominent intracanalicular pattern with a hypercellular stroma and a leaf-like appearance. In 1982, WHO has classified Phyllodes tumors based on histopathological features into benign, borderline, and malignant.[1,2]

Malignant transformation is often accompanied by significant metabolic changes.[4] The proliferation and development of cancer cells involves changes in metabolic pathways as a result of the continuous demand for energy and nutrients.[5] Research on the involvement of metabolism in the development of tumor cells was first carried out by Otto Warburg, a German scientist and his colleagues who conducted research on various types of tumors. From this study, it was concluded that cancer cells absorb a lot of glucose and produce metabolites in the form of lactic acid, despite the availability of oxygen during metabolism (aerobic glycolysis) which became known as the Warburg effect. In addition, Warburg also stated that it occurs due to mitochondrial dysfunction and is a marker of tumorigenesis.[6-10]

Glycolysis is the process of oxidation of glucose or glycogen to pyruvate and lactate.[11] Glycolysis is the main route of carbohydrate or glucose metabolism that occurs in the cytosol of all cells. This glycolysis process can take place aerobically or anaerobically, depending on the availability of oxygen and the electron transport chain. In cancer cells that grow rapidly, the process of glycolysis also takes place quickly and produces a lot of pyruvate which is then reduced to lactic acid. This results in the formation of a relatively acidic tumor environment, and may have an impact on cancer therapy. The formation of an acidic environment in tumors allows tumor cells to invade easily. Low oxygen tension in the tumor area caused by a lack of blood supply stimulates the formation of HIF-1 (Hypoxia Inducible

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Factor-1). In addition to acting as a transcription factor, HIF-1 also enhances the function of enzymes that control glycolysis.[12]

Glucose in the metabolic process requires a specific carrier protein in order to pass through the cell plasma membrane.[13] This glucose homeostasis process is regulated by the GLUT or solute carrier 2 (SLC2) family of facilitative transport proteins.[14] Regulation of the expression and activity of glucose transporters plays a vital role in the supply of glucose. glucose and other carbohydrates in the active metabolism of cells.[15] In several studies that have been carried out, it was found that increased expression of glucose transporters was associated with the malignant process. Some cancers also show abnormal expression patterns compared to normal tissues.[13]

The mechanism of uptake of glucose molecules from extracellular to intracellular is through the process of facilitated diffusion. This process does not require energy (ATP), so molecules can cross the plasma membrane directly with their own concentration gradient. Facilitated diffusion allows polar and charged molecules such as carbohydrates, amino acids, nucleosides, and ions to pass through the plasma membrane.[16]

Glucose transporter 1 (GLUT-1) is a member of the GLUT family. GLUT-1 is generally undetectable in normal epithelial tissue and benign tumors, but is expressed in most malignant/malignant tumors. However, there are some normal tissues that are positively expressed with GLUT-1, such as erythrocytes, endothelial cells, placenta, peripheral nerve perineurium, renal tubules, and blood brain barriers.[17,18]

II. MATERIAL AND METHODS

This research is descriptive analytic with a cross sectional approach, which was carried out at the Department of Anatomical Pathology, Faculty of Medicine, Universitas Sumatera Utara and the Pathology Unit of the H. Adam Malik Central General Hospital Medan, starting from October 2020 to July 2021.

Samples that met the inclusion criteria and were diagnosed with benign, borderline, and malignant phyllodes tumors were stained with hematoxylin and eosin, then the paraffin block was re-cut for GLUT-1 (*Bioenzy GLUT1 Polyclonal 100 u)* immunohistochemical staining. GLUT-1 expression was positive if stained brown on the membrane and cytoplasm of tumor cells.

Differences in GLUT-1 expression in each phyllodes tumor subtype were statistically analyzed using the Mann Whitney test. Statistical analysis was performed using SPSS software.

III. RESULTS

Samples were selected randomly from medical record data, and obtained a total of 35 samples. From 35 samples of phyllodes tumors obtained, 22 were benign (62,9%), 2 were borderline (5,7%), and 11 were malignant (31,4%). Based on the expression of GLUT-1 in phyllodes tumor subtypes, all benign phyllodes tumors were negatively expressed, borderline was focally positive, and malignant was diffusely positive.(Fig.1) The results of statistical analysis using the Mann Whitney test showed a significant correlation (p=0.001) between the immunohistochemical expression of GLUT-1 and the phyllodes tumor subtype.(Table-1)

Table 1. **Correlation of GLUT-1 Immunohistochemical Expression with Phyllodes Tumor Subtypes**

GLUT-1 expression	Phyllodes tumor subtypes			p
	Malignant	Borderline	Benign	
Positive	11 (84,6)	2 (15,4)	0	<0,001[a]
Negative	0	0	22 (100)	

[a]*Mann Whitney*

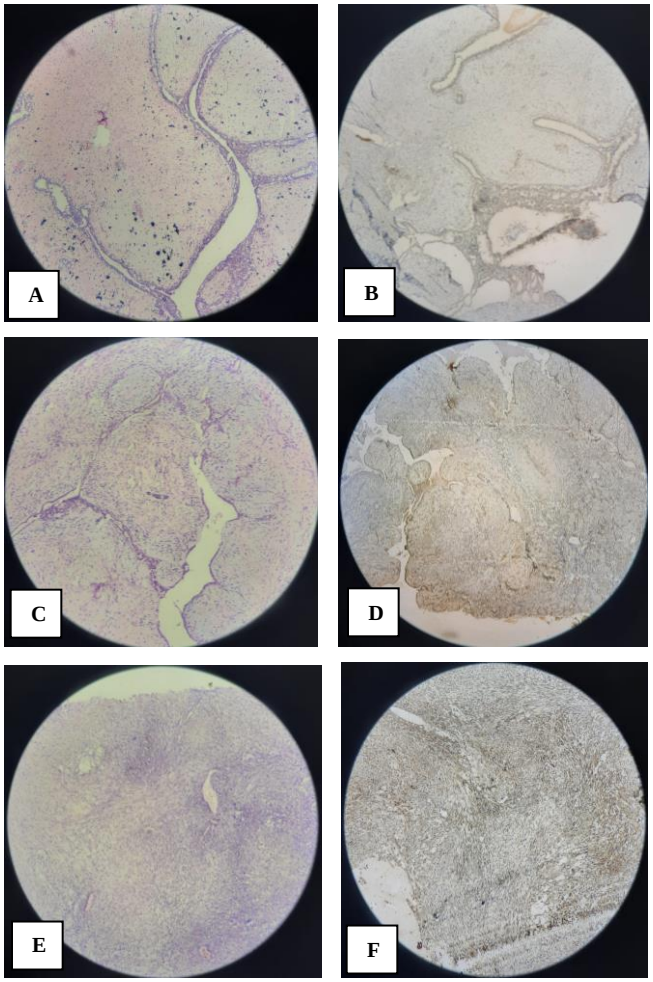

Figure 1. A. benign phyllodes tumor (HE, 100x), B. negative expression of GLUT-1 in benign phyllodes (100x), C. borderline phyllodes tumor (HE, 100x), D. focal positive expression of GLUT-1 in borderline phyllodes tumor (100x), E. malignant phyllodes tumor (HE, 100x), F. strong and diffuse expression of GLUT-1 in phyllodes malignant tumors (100x)

Based on the sample obtained, the age of the youngest phyllodes tumor patient is 17 years, while the oldest age is 62 years. The most common age in this sample was > 40 years as many as 24 samples (68.6%), with a mean age of 44.57.

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IV. DISCUSSION

In this study, the number of final samples with a diagnosis of benign, borderline, and malignant phyllodes tumors that met the inclusion criteria was 35 samples, with details of 22 samples with a diagnosis of benign phyllodes tumors, 2 samples of borderline phyllodes tumors, and 11 samples of malignant phyllodes tumors. From the 35 samples, the youngest age was 17 years and the oldest was 62 years with a mean age of 44.57 years, and the most common age was >40 years as many as 24 people (68.6%). This is in accordance with the literature and research conducted by Fatimah, et al[2] which stated that the average age of patients with phyllodes tumors was between 40-50 years.

Based on the results of the analysis of the expression of GLUT-1 immunohistochemistry in 35 samples of phyllodes tumors, 13 samples were positively expressed and 22 samples were negatively expressed. Thirteen samples that expressed a positive expression consisted of 11 samples of malignant phyllodes tumors, and 2 samples of borderline phyllodes tumors, while 22 samples that expressed negative totality came from benign phyllodes tumors. From the comparison of positive expression results between phyllodes malignant and borderline tumors, there were differences, where the positive expression of GLUT-1 in phyllodes malignant tumors was generally diffuse compared to the borderline type which was more focal. Positive GLUT-1 expression was also found in heterologous components found in phyllodes malignant tumors.

The results of this study are in line with previous studies which made GLUT-1 expression a marker of a benign lesion and a malignant lesion in a neoplasm. Kato, et al[19] in 2007 concluded that the immunohistochemical expression of GLUT-1 can be used to differentiate reactive mesothelium and malignant mesothelioma. In 2011, Legan et al[20] performed an analysis of GLUT-1 expression in benign, pre-malignant, and malignant lesions of the gallbladder, where results were 100% negative for benign lesions, and positive for low-grade dysplasia, high-grade dysplasia and gallbalder's carcinoma. Dura, et al[21] in 2017 also obtained significant results on the expression of GLUT-1 and GLUT-3 in malignant melanoma and melanocytic nevi and concluded that GLUT can be used as a marker to differentiate melanocytic lesions on the skin.

The limitation of this study is that there is no standard cut-off regarding the assessment of GLUT-1 immunohistochemical expression. In addition, the number of samples from each subtype that is not homogeneous in number can cause bias in the results obtained.

V. CONCLUSION

After analyzing the results of this study, we conclude that :

1. The age of most patients with Phyllodes tumor is >40 years.
2. The difference in the immunohistochemical expression of GLUT-1 in each phyllodes tumor subtype can prove the truth of the Warburg hypothesis.
3. There is a relationship between GLUT-1 expression and histopathological subtypes of phyllodes tumors, where the higher the grade of a phyllodes tumor the stronger and more diffuse the expression of GLUT-1.
4. Immunohistochemistry GLUT-1 can be used as a marker to differentiate benign, borderline, and malignant lesions in phyllodes tumors.
5. Immunohistochemistry GLUT-1 can be used as a marker to differentiate benign and malignant lesions in general.

VI. COMPETING INTERESTS

The author has no financial interests relevant to the product or company described in this article.

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VII. ETHICAL APPROVAL

This research has been granted a research permit by the Health Research Ethics Committee of the Universitas Sumatera Utara.

REFERENCES

[1] Rayzah M. Phyllodes Tumors of the Breast: A Literature Review. Cureus 12(9): e10288. (September 07, 2020). DOI 10.7759/cureus.10288

[2] Fatimah, F. Mustokoweni, S. Rahniayu, A. Analisis Ekspresi CD117 dan Ki-67 Pada Tumor Phyllodes Benign, Borderline dan Malignant. Majalah Patologi Indonesia,Vol.27 No.1. 2018

[3] Tse, G. Koo, JS. Thike, AA. WHO Classification of Tumours : Breast Tumours. 5th ed. Lyon. IARC. 2019

[4] Wilde, L. Roche, M. Vidal, M.D. Tanson, K. Philp, N. Curry, J. et al. Metabolic Coupling and the Reverse Warburg Effect in Cancer, Implications for Novel Biomarker and Anticancer Agent Development Metabolic Coupling in Cancer. *Seminars in Oncology*. 2017. http://dx.doi.org/10.1053/j.seminoncol.2017.10.004

[5] Lucia, C. Esteban, L. Fajas, L. Cell Cycle Regulators in Cancer Cell Metabolism. *BBA - Molecular Basis of Disease* 1866.165715. 2020. https://doi.org/10.1016/j.bbadis.2020.165715

[6] Millan, S. Brooks, G.A. Reexamining Cancer Metabolism : Lactate Production for Carcinogenesis Could Be The Purpose and Explanation of The Warburg Effect. Oxford University Press. *Carcinogenesis*, Vol.38 119-133. 2017. doi: 10.1093/carcin/bgw127

[7] Zielinski, D. Jamshidi, N. Corbett, A. et al. Systems Biology Analysis of Drivers Underlying Hallmarks of Cancer Cell Metabolism. Sci Rep 7, 41241. 2017. https://doi.org/10.1038/srep41241

[8] Corbet, C. Feron,O. Cancer Cell Metabolism and Mitochondria: Nutrient Plasticity for TCA Cycle Fueling. BBA - Reviews on Cancer. 2017. doi:10.1016/j.bbcan.2017.01.002

[9] Potter, M. Newport, E. Morten, K.J. The Warburg Effect : 80 years on. Biochemical Society Transactions. 2016. doi : 10.1042/BST20160094

[10] Jones, W. Bianchi, K. Aerobic Glycolysis : Beyond Proliferation. *Front.Immunol*. 6:227. 2015. doi: 10.3389/fimmu.2015.00227

[11] Chatterjea, M.N. Shinde, R. *Textbook of Medical Biochemistry*. 8th Ed. New Delhi. Jaypee Medical Publisher. 2012

[12] Rodwell, V.W. Bender, D.A. Botham, K.M. Kennely, P.J. Weil, P.A. *Harper's Ilustrated Biochemistry*. 31st ed. California. McGraw-Hill Education. 2018

[13] Barron, C.C. Bilan, P.J. Tsakiridis, T. Tsiani, E. Facilitative Glucose Transporter : Implication for Cancer Detection, Prognosis and Treatment. *Elsevier Inc*. 2015. http://dx.doi.org/10.1016/j.metabol.2015.10.007

[14] Patching, S.G. Roles of Facilitative Glucose Transporter GLUT1 in [18F] FDG Positron Emission Tomography (PET) Imaging of Human

Diseases. Journal of Diagnostic Imaging in Therapy, 2015; 2(1): 30-102. https://dx.doi.org/10.17229/jdit.2015-0301-014

[15] Macheda, M.L. Rogers, S. Best, J.D. Molecular and Cellular Regulation of Glucose Transporter (GLUT) Protein in Cancer. Journal of Cellular Physiology. 202:654-662. 2016. doi: 10.1002/jcp.20166

[16] Cooper GM. The Cell: A Molecular Approach. 2nd edition. Sunderland (MA): Sinauer Associates; 2000. Transport of Small Molecules. Available from: https://www.ncbi.nlm.nih.gov/books/NBK9847/

[17] Lu, K. Yang, J. Li, D-C. He, B-S. Zhu, M-D. Zhang, L-F. et al. Expression and Clinical Significance of Glucose Transporter-1 in Pancreatic Cancer. Oncol Lett 12, 243-249. 2016. doi: 10.3892/ol.2016.4586

[18] Abdou, A.G. Eldien, M.S. Elsakka, D. GLUT-1 Expression in Cutaneous Basal and Squamous Cell Carcinomas. Int Jou Sur Oncol, 23(6) 447-453. 2015. doi: 10.1177/1066896915589968

[19] Kato, Y. Tsuta, K. Seki, K. Maeshima, A.M. Watanabe, S. Suzuki, K., et al. Immunohistochemical Detection of GLUT-1 Can Discriminate Between Reactive Mesothelium and Malignant Mesothelioma. *Modern Pathology*. 2007. *20*, 215-220. doi: 10.1038/modpathol.3800732

[20] Legan, M., Tevžič, Š., Tolar, A. et al. Glucose Transporter-1 (GLUT-1) Immunoreactivity in Benign, Premalignant and Malignant Lesions of the Gallbladder. Pathol. Oncol. Res. 17, 61–66 (2011). https://doi.org/10.1007/s12253-010-9281-7

[21] Dura, M. Nemejcova, K. Jaksa, R. Bartu, M. Kodet, O. Ticha, I. et al. Expression of GLUT-1 in Malignant Melanoma and Melanocytic Nevi : an Immunhistochemical Study of 400 cases. *Pathol.Oncol.Res*. 2017 https://doi.org/10.1007/s12253-017-0363-7

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