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Utilization Crystal Violet as Nuclei Dye in Histopathology of Breast Cancer

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Abstract

Microscopic examination of cancer development can be identified through histopathological examination. Hematoxylin–eosin (HE) is the gold standard for staining method. This processing can be obstructed if hematoxylin reagent out of stock in the laboratory. Other reagents are needed that can replace the use of hematoxylin, one of which is crystal violet. This study aims to evaluate the use of crystal violet as a nuclei stain in breast cancer preparations. The research sample consisted of 15 breast cancer preparations taken at the sample bank in the anatomical pathology laboratory of RSUD Dr. Slamet Garut. The results showed that from 15 preparations, it was observed that 4 preparations (27%) stained worth and 11 preparations (73%) stained poorly. Based on the research results, crystal violet crystal violet has a low core coloring ability in breast cancer preparations. From the results of this study, it is recommended to modify the crystal violet reagent by paying attention to the acidity of pH and concentration of crystal violet.

Keywords: Breast cancer, crystal violet, hematoxylin, staining

INTRODUCTION

Breast cancer is the highest cause of death in women. In 2020, globally there were 2.3 million women diagnosed with breast cancer and 685,000 deaths (Sung, 2021). In Indonesia, of the total number of new cancer cases of 396,914 in 2020, the number of breast cancer cases reached 68,858 cases with a percentage reaching 16.6% (Indonesian Ministry of Health, 2022). Data on cancer sufferers who underwent histopathological examination in the anatomical pathology laboratory at RSUD dr. Slamet in 2018, data was obtained, namely 44.9% of breast cancer with a diagnosis of stage I (14 people), stage II (21 people), stage III (34 people), and other diagnoses of 16 people (Rahmawati, 2020).

Increased mitosis involves cell proliferation, which is a marker of pre-cancer and cancer (Levine, 2018). Increased and abnormal mitoses indicate defects in features important for precancer and cancer screening. Identification and quantification of cells undergoing mitosis are used as markers in histological examination to support cancer diagnosis (Kadoo, 2017). Histological examination of which staining

is an important component and is the gold standard for the diagnosis of many pathological diseases (Gurina, 2022). Microscopic examination of cancer development can be identified through histopathological examination.

An important step in histopathological examination begins with tissue handling and tissue staining (Meyerholz, 2018). Standard histopathological staining method using the hematoxylin–eosin (HE) staining method. Hematoxylin is a basic sintetic dye. This dye is used to color the cell nuclei a bluish color while eosin is a pink dye in the cytoplasm (Alturkistani, 2016). The principle of dyeing uses chemical interactions in the binding between dye-tissue . Cell nuclei in negatively charged or anionic tissue, this part will be stained with hematoxylin as basophilic which gives a blackish blue color. Cytoplasmic network components that are positively charged or cationic are more easily colored by eosin dye which is acidophilic with a positive charge and gives a red color (Veuthey, 2014).

Anatomical pathology examination can be obstructed if hematoxylin reagent out of stock in the laboratory. Providing new stock of reagents takes a long time. This is because the availability of hematoxylin is low because the maturation process of hematoxylin takes quite a long time (Rahmawati, 2020). However, examination services in the anatomical pathology laboratory must continue. Therefore, a reagent is needed that can replace the use of hematoxylin in staining surgical biopsies.

Use of other reagents for coloring preparations such as methylene green, toluidine blue, neutral red, methylene blue (Rahmawati, 2020), crystal violet (Tandon, 2016). Löffler's alkaline methylene blue staining is used in histopathological examination of conjunctival epithelial cells. The result is that the neutrophils are blue and the nuclei are colored more intensively (Kiuchi, 2016). Crystal violet can be used to differentiate between pyknotic nuclei, apoptotic cells and mitotic cells (Kadoo, 2017)). The use of crystal violet with a concentration of 1% shows a better picture of mitosis (Tandon, 2016). Crystal violet is a basic dye that is able to color tissue components with a negative charge which will provide color to the cell nuclei (Mahmoud, 2019). However, crystal violet staining for histopathological staining in breast cancer has not been reported. Therefore, researchers conducted research on the effectiveness of crystal violet in staining cell nuclei against breast cancer preparations.

RESEARCH METHOD

This research was carried out experimentally in the cytohistotechnology laboratory of STIKes Karsa Husada Garut

using 15 samples of breast cancer preparations taken randomly from the sample bank in the anatomical pathology laboratory at Dr. Slamet Garut. In this study, the control group will stain cell nuclei with hematoxylin-eosin staining and the experimental group will stain cell nuclei with crystal violet-eosin staining.

Tissue handling is carried out through several processes including dehydration, clearing, impregnation, embedding, cutting, grossing and coloring. The dehydration process uses alcohol with graded concentrations starting from 70%, 80%, 95% (I), 95% (II) alcohol and ethanol. The dehydration process was carried out in the oven at a temperature of 65^{o}C - 70^{o}C for 45 minutes. The clearing process using xylol (I) and xylol (II) solutions is each carried out in an oven at a temperature of 65^{o}C - 70^{o}C for 45 minutes. Impregnation using liquid paraffin is carried out in the oven at a temperature of 60^{o}C - 70^{o}C for 45 minutes. Embedding the tissue into a paraffin mold accompanied by no. The specimen is then stored in the refrigerator for 2 hours until completely solidified. Gross cutting is carried out using a microtome with a thickness of 10 microns so that a flat surface is obtained, after which fine cuts are carried out using a microtome with a thickness of 4 microns so that the desired cut is obtained in the form of tissue bands. Mounting is carried out by attaching tissue tape to a glass object in a water bath at a temperature of 40^{o}C to obtain histopathological preparations that are ready to be stained.

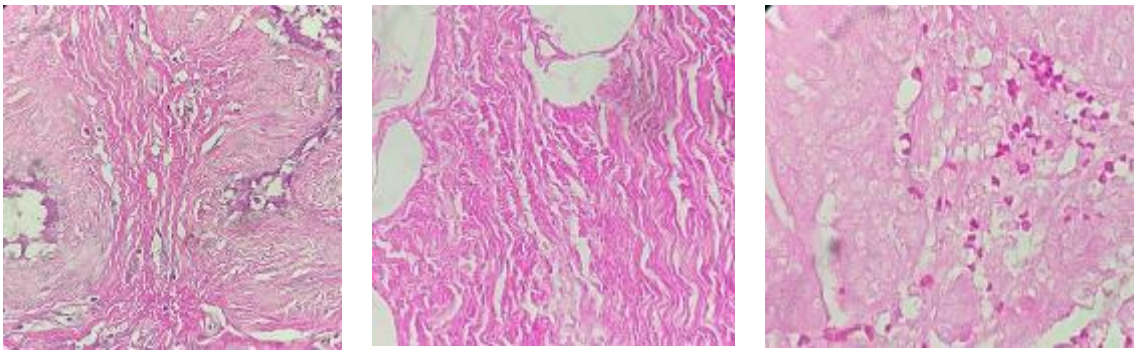
Histopathology preparations for the control group used the Hematoxylin Mayers staining method while the experimental group used crystal violet-eosin staining. Staining preparations for breast cancer with several stages of deparaffinization, rehydration, staining with the main dye hematoxylin, dehydration, second dye eosin. Staining of breast cancer preparations for the experimental group used crystal violet dye with the following composition: 2 grams of crystal violet, 0.8 grams of ammonium oxalate, 20 mL of 95% alcohol, and 80 mL of distilled water. (Kadoo, 2017).

The staining results were analyzed under a microscope with 400x magnification. Optimal quality staining as the core shows bluish purple with the presence of cormatin threads (Bendzinski, 2020) and suboptimal quality if the core is weakly colored and appears pale (Chapman, 2019). The staining quality assessment was classified as "good" if the nuclei was clearly stained and the chromatin was clearly visible; "worth" if the nuclei are stained but less clear and the chromatin is less clearly visible, and classified as "bad" if the nuclei are not stained (Rahmawati, 2020).

RESEARCH RESULT AND DISCUSSION

RESULT

The results of the observations showed that in the control group using hematoxylin-eosin staining all 15 preparations were observed (100%) as good. Meanwhile, in the experimental group with crystal violet-eosin staining, out of 15 preparations, it was observed that 4 preparations (27%) were worth stained and 11 preparations (73%) were not well stained. Staining of breast cancer preparations using hematoxylin-eosin (HE) can be seen in Figure 1 (a) and using crystal violet-eosin in Figures 1 (b) and 1 (c).



(a) (b) (c)

Figure 1. Staining of cell nuclei using hematoxylin-eosin (a) and crystal violet-eosin (b), (c) on breast cancer preparations with 400x magnification.

The results of staining cell nuclei in breast cancer preparations were at score 3 with good staining quality, namely those that had been stained with hematoxylin-eosin as a control, including at score 3 having good staining quality, namely cell nuclei were clearly visible with rough chromatin, cell sizes varied (from large to small). The use of hematoxylin eosin staining shows the presence of mitotic cell nuclei (observed cell nuclei with 3 lobes in one cell) as in Figure 1 (a). Figure 1(b) The staining results have quite good staining quality, namely the cell nuclei is colored but it is not clearly visible, meaning that the shape of the cell nuclei is visible but not from the crystal violet staining results but rather the outline of the cytoplasm so that there should be division of the cell nuclei which has 3 lobes but only Only 1 lobe is visible. The results of staining cell nuclei in breast cancer preparations had poor staining quality, characterized by cell nuclei not being stained, cell nuclei mitosis and chromatin not being observed, as in Figure 1(c).

DISCUSSION

Crystal violet is a triphenyl methane dye used as a histology stain (Al-Khikani, 2022). Crystal violet is also often used to stain peptidoglycan in prokaryotic bacterial cells in Gram staining, which is a type of alkaline staining (Kadoo, 2017) so that it

can color acidic tissue components . Crystal violet is an alkaline dye that is able to color tissue components with a negative charge which will give color to the cell nuclei (Mahmoud, 2019). Staining cell nuclei is very important in histological staining because the structure of cell nuclei often changes if an abnormality occurs in the organ. With this staining, it is easy to recognize tissue structures if the tissue is stained with other structures that are not stained.

In previous research conducted by (Pathak, 2016) it was stated that the breast cancer that often occurs is invasive carcinoma of no special type (NST) in as many as 75% of patients with microscopic characteristics, namely the presence of mitotic cell nuclei that are clearly visible and often prominent. However, in this study, the results of 15 preparations were shown, 4 preparations (27%) were observed where the nuclei was colored but less clear and the chromatin was less clearly visible and observable. In addition, 11 of the 15 preparations (73%) were observed if cell nuclei without color. Crystal violet had low ability to color the nuclei in breast cancer preparations.

In this study, crystal violet had low effectiveness for staining cell nuclei, characterized by staining results that underwent mitosis, namely cell nuclei that had 3 lobes in the control, but in the experiment only 1 small lobe was observed, this can be influenced by the composition of the dye used. different between control and experiment. The composition of hematoxylin for staining cell nuclei contains several ingredients that are strong bases, such as potassium or ammonium alum, sodium iodate, chloral hydrate. Meanwhile, crystal violet with a concentration of 1% does not add any strong alkaline ingredients to increase the pH. Crystal violet is added with a strong acid solution, namely 8.7 mL of 1 N HCl which is dissolved in 90 mL of distilled water from a concentration of 35-37% then mixed with a weak base of 0.8 gram ammonium oxalate in 80 mL of distilled water with the aim of maintaining the pH at basic region (>7) (Kadoo, 2017). Meanwhile, to make the crystal violet solution in an alkaline condition, that is by adding 0.1 M NaOH then adding a buffer to maintain the pH value using a phosphate buffer for a pH value of 7-9 (Bahrami, 2020).

CONCLUSION

Based on research results, crystal violet crystal violet has low nuclear coloring ability in breast cancer preparations. From the results of this research, it is recommended to modify the crystal violet reagent by paying attention to the acidity level of pH and crystal violet concentration.

REFERENCE

Al-Khikani F, Ayit A. (2022). The Antibacterial Action of Safranin and Gentian Violet. Rambam Maimonides Med 31;13(3):e0018. doi: 10.5041/RMMJ.10475. PMID: 35921487; PMCID: PMC9345769.

Alturkistani HA, Tashkand FM, Saleh ZMM. (2016). Histological Stains: A Literature Review and Case Study. Glob J Health Sci 8;72–79.

Bahrami A, Amiri MJ, Bagheri F. (2020). Optimization of crystal violet adsorption by chemically modified potato starch using response surface methodology. Pollution, 6(1): 159-170.

Bendzinski ECM, Chu K, Johnson JI, Brous M, Copeland K, Bolon B. (2020). Optical density-based image analysis method for the evaluation of hematoxylin and eosin staining precision. J Histotechnology;29–37.

Chapman CM. (2019). Troubleshooting in the histology laboratory. J Histotechnology 42;137–149

Gurina TS, Simms L. Histology, Staining. (2022). In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishinvailable from: https://www.ncbi.nlm.nih.gov/books/NBK557663/

Indonesian Ministry of Health. (2022). Most Common Breast Cancer in Indonesia, Ministry of Health Targets Equalization of Health Services. Jakarta.

Kadoo P, Dandekar R, Kulkarni M, Mahajan A, Kumawat R, Parate N. (2017). Correlation of mitosis obtainned by using 1% crystal violet stain with Ki67LI in histological grades of oral squamous cell carcinoma. J Oral Biol Craniofacial Res. 8;234–240

Kiuchi K. (2016). Rapid alkaline methylene blue supravital staining for assessment of anterior segment infections. Clin Ophthalmol 10;1971–1975.

Levine M.S,Holland A.J (2018) The impact of mitotic errors on cell proliferation and 673 tumorigenesis. Genes Dev. 32, 620-638.

Mahmoud H. Abu Elella, Magdy W. Sabaa, Eman Abd ElHafeez, Riham R. Mohamed. (2019). Crystal Violet Dye Removal Using Crosslinked Grafted Xanthan Gum. S0141-8130(19)31902-6.

Meyerholz DK, Beck AP. (2018). Principles and approaches for reproducible scoring of tissue stains in research. Laboratory Investigation, Volume 98, Issue 7:844-855,

Pathak, R., Jha, A., Neupane, P., Chalise, S., & Basnyat, A. (2016). Histopathological evaluation of carcinoma of breast. *Journal of Pathology of Nepal*, *6*(11), 922–927.

Rahmawati T, Arpiyadi Y, Mamay. (2020) Utilization of 1% of Methylene Blue in Staining Histopathological Preparations at Anatomic Pathology Laboratory. Ina J Med Lab Sci Tech 2(1): 93-100

Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bry F. (2020). Global cancer statistics: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2021: 71: 209-249.

Tandon A, Singh NN. Brave, VR Sreedhar G. (2016) . Image analysis assisted study of mitotic figures in oral epithelial dysplasia and squamous cel carcinoma using differential stains. J Oral Biol Craniofacial Res 6;18–23

Veuthey, T., Georgina Herrera, G, Dodero, V.I. (2014) .Dyes and stains: From molecular structure tohistological application. Front in Biosci;19: 91–112.