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Correlation between the Pre-Analytical Stage and Quality of Breast Histopathology Specimen

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ABSTRACT

The histopathology preparations procedure in the Anatomic Pathology Laboratory consists of three stages: pre-analytic, analytical and post-analytic. Errors in the pre-analytic process can affect the quality of histopathology preparations, thereby affecting diagnosis and patient management. The length of fixation and the large size of tissue lamellae are variables in the pre-analytic process that influence the quality of histopathological preparations. The aim of this study is to determine the relationship between the length of fixation and the size of large tissue lamellae on the quality of histopathological preparation. A cross-sectional study was conducted on medical record data of histopathological examination of breast tissue examined at the Barokah Palembang Anatomical Pathology Laboratory between January - December 2022. There were 37 cases of histopathological specimens that met the inclusion criteria, of which 3 cases were underfixed, 13 cases were overfixed and the rest were normal. One case from the underfixation, 20 cases from the normal fixation and 3 from the overfixation group were included in the good specimen quality category. The Spearman test showed that there was a significant relationship between normal fixation and good specimen quality (p=0.00217). Of the 37 research subjects, there were 18 subjects who were categorized as good lamellation. When related to specimen quality, good lamellation is associated with good specimen quality (p=0.02161). The correct fixation and lamellation during the pre-analytic phase are associated with good specimen quality.

1. Introduction

Histopathology preparations procedure in the anatomical pathology laboratory consists of three stages: pre-analytical, analytical, and post-analytical stage. Fixation becomes the first step in the pre-analytical stage where the tissue fixation with several chemical and physical agents are utilized to prevent morphological changes, distortion, or tissue decomposition.1 Fixations are required to maintain the tissue to be as similar as possible as the living tissues. Furthermore, fixation also aims to prevent bacterial growth, harden the tissue, and allows easier staining. To ensured that the fixative fluid penetrates the entire large tissue, a lamellation process is carried out when the tissue is cut without breaking it through 0.5 - 1 cm parallel incisions.[1,2]

Ten-percent buffered neutral formalin (BNF) has been widely used as a routine fixative over the last decade. This fluid is advantageous because it has a neutral pH of 7, easy to use and obtain, and has a long shelf-life.[3] Some factors influence the quality of fixation, including the length of fixation, temperature, concentration, and osmolarity of the fixative fluid.[4] Duration of fixation influences the tissue stabilization process. Very long fixation can cause excessive bonding between the fixative fluid and the tissue, causing the tissue to become brittle; however, too short fixation causes inadequate tissue penetration of the fixative fluid.[5]

The aim of this study is to determine the

relationship between good fixation and the morphological quality of breast histology preparations. Good fixation includes the fixation material, duration, and lamellation in large tissues.

2. Methods

This is a cross-sectional comparative study of medical record data from histopathological examination of breast tissue examined at the Barokah Palembang Anatomical Pathology Laboratory between January – December 2022. The time taken from the breast tissue sampling surgery until it reached the laboratory processing section was calculated as the time fixation using 10% formalin fixative. The fixation time is considered adequate when the time is in accordance with the guidelines from the College of American Pathologists and the National Society for Histotechnology, or between 6 - 72 hours.6 The fixation time is categorized as bad fixation (under-fixation or over-fixation) when the fixation time is beyond 6-72 hours. Tissue with a diameter more than 2 cm requires lamellation; the lamellation is considered good when the lamellation result has a thickness between 0.5 – 1 cm in parallel.2 Interpretation of the histology preparations was carried out by two pathologists (NFK and KM). The preparations were categorized into good and bad; "bad fixation" is when the blue color in the cell nucleus was not clear, the red color in the cytoplasm was not clear, and lysis was found in more than 5% of the field of view. On the other hand, "good fixation" is when the blue color of the nucleus and the red color of the cytoplasm is clear and lysis is less than 5% of the field of view (Table 1).3,7 The Spearman statistical test was carried out to determine the correlation between variables.

Tabel 1. Preparation quality criteria.

Parameter	Good	Poor
Blue color at nucleus	Clear	Not clear
Red color at cytoplasm and interstitial	Clear	Not clear
Tissue lysis: ● Piknosis ● Karyorrhexis ● Karyolysis ● Nuclear loss ● Cell edema or swelling ● Intracytoplasmic vacuolation ● Putrefaction ● Loss of tissue arrangement	< 5% field view	≥ 5% field view

3. Results

We collected 37 histopathological specimens that met the inclusion criteria: 3 cases were underfixed, 21 cases were appropriate fixation, and 13 cases were overfixed. Table 2 shows one case from the underfixation group, 20 cases from the normal fixation group, and 3 cases from overfixation met the good quality specimen category. Spearman test shows a significant relationship between normal fixation and good specimen quality (p=0.00217). There is a significant relationship between normal fixation and good fixation quality. From 37 specimens collected, we found 18 subjects with good lamellations. Statistical analysis shows that good lamellation is associated with good specimen quality (p=0.02161). Figure 1 shows a histography of research subjects.

Table 2. Distribution of subjects based on length of fixation and lamellation.

	Histological specimen quality		*p value*
	Good	Poor	
Duration of fixation			
Underfixation	1	2	0.00217
Normal fixation	20	1	
Overfixation	3	13	
Lamellation			
Good	17	1	0.02161
Poor	7	12	

4. Discussion

Histopathological examination is gold standard in the diagnosis of a lot of diseases. The accuracy of histopathological diagnosis is supported by the quality of the preparation which is influenced by various factors, including fixation. Tissue fixation, part of the pre-analytical stage, is a physiochemical process in which cells or tissue are chemically fixed to prevent autolysis, decay, and degradation. Furthermore, fixation also aims to maintain the tissue structure as similar as possible to when it was alive.[4]

The 10% neutral buffered formalin (BNF) is one of the most common fixative fluids in the anatomical pathology laboratories today. This fluid will penetrate the tissue while also simultaneously exert bactericidal activity to prevent bacterial growth. Formalin in the tissue will bind to form macromolecules through cross-linking reactions with various amino acids in the cytoplasm.8 Tissues larger than 2 cm requires lamellation to allow for adequate formalin penetration.9 The fixative fluid requires time to penetrate through the tissue; this time varies depending on the size of the tissue, types of the fixative fluid, and temperature.

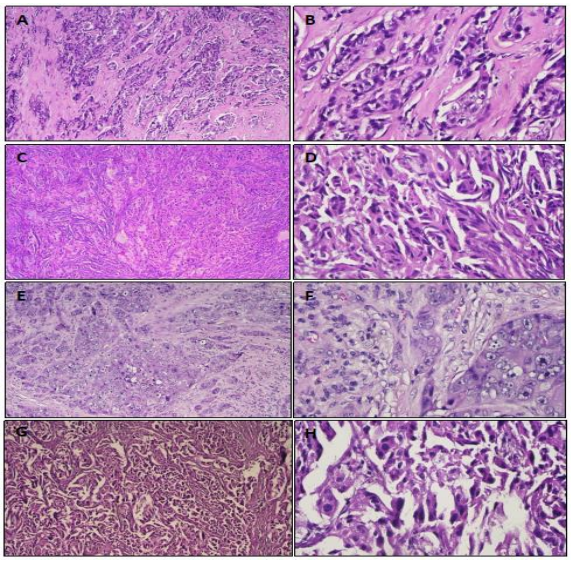

Figure 1. Histological features of subjects. A and B. Histopathological images originating from well-fixed and lamellated tissue. The color appears blue in the nucleus and red in the cytoplasm. Good tissue structure; C and D. Histopathological images originating from underfixed tissue. Lysis is observed as pyknosis and karyorrhexis; E and F. Histopathological images of preparations derived from overfixed tissue. Features of the nucleus and cytoplasm are less clear. Visible vacuolization of the cytoplasm and abnormal tissue structure; G and H. Histological features of large, poorly lamellated tissues. The tissue structure appears to be poorly organized along with various areas of lysis.

Our study shows that a fixation time based on the recommendations of the College of American Pathologists and the National Society for Histotechnology, or between 6 - 72 hours at room temperature, is associated with good histopathological specimen quality. Our finding is similar with Octary et al., showing that the 6- 16 hours of fixation results in satisfactory specimens.[3] Similarly, our study is in line with Chung et al. showing that 24-hours of fixation results in the best-quality specimen.[5] At normal room temperature, the average penetration rate of 10% formalin fixative is around 0.5 mm/hour. Higher temperature of the fixation room results in the faster penetration rate of the fixative fluid; conversely, lower room temperature will slow down the penetration of the fixative fluids. Likewise, hard tissue such as bones require longer time to allow for fluid penetration.1 Fixation for more than 72 hours can result in artifacts, poor staining, and the stronger bond between formalin and cytoplasmic amino acids; all of these factors may result in the reduced accuracy of immunohistochemical examination. On the other hand, inadequate fixation time may result in the tissue autolysis.[6,10]

Our study also shows that the lamellation in accordance with the guidelines of the College of American Pathologists and the National Society for Histotechnology (between 0.5 - 1 cm) is associated with good specimen quality. This result is also is in accordance with recommendations from the Royal College of Pathologists of Australasia suggesting lamellations at a distance of 5 mm.[10]

5. Conclusion

The 10% BNF fluid at room temperature with a duration of 6 - 72 hours results in good tissue fixation. Lamellations are necessary when the tissue is large (more than 2 cm). Good lamellations and fixation time results in good quality of breast tissue histopathological specimens.

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