Clinical Research

Periodic acid-Schiff and alcian blue immunohistochemistry to detect mucin in mucinous breast carcinoma

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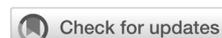
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ABSTRACT

BACKGROUND Detection of mucins has been shown to [...] with several clinicopathological characteristics in patients. Currently periodic acid-Schiff (PAS) and alcian blue staining methods are the histochemistry staining techniques that are frequently used to detect mucin. This study was aimed to evaluate PAS and alcian blue staining in differentiating mucin characteristics between invasive carcinoma of no special type (ICNST) with mucinous degeneration and mucinous carcinoma.

METHODS This cross-sectional study of [...] cases included biopsies of mucinous breast carcinoma and ICNST with mucin degeneration that were histologically verified using hematoxylin and eosin (H&E) staining. The PAS and alcian blue staining were conducted in the Laboratory of Histochemistry, Department of Anatomical Pathology, Cipto Mangunkusumo Hospital. Data were recorded using SPSS software, version 21 (IBM Corp, USA).

RESULTS There were 17 cases of ICNST with mucinous degeneration and 16 cases of mucinous carcinoma with age varied from 27 to 75 years. PAS had sensitivity of 87.5% and specificity of 41.2%. Alcian blue had sensitivity of 43.8% and specificity of 82.4%.

CONCLUSIONS PAS staining method is better than the alcian blue staining method in distinguishing between ICNST with mucinous degeneration and mucinous carcinoma. [...] the limited laboratory, PAS staining alone can be considered to detect mucin.

KEYWORDS alcian blue, mucin, mucinous carcinoma, periodic acid-Schiff reaction

According to the Global Cancer Observatory in 2018, breast cancer accounted for 30.9% of all cancer cases in Indonesia, making this condition the most prevalent form of cancer.[1] The most common malignant breast type is invasive ductal carcinoma, also known as invasive carcinoma of no special type (ICNST), which consists 70–75% of all breast tumor cases.[2] ICNST has nonspecific histopathological features.[2,3] Hence, difficulty arises in its diagnosis, thereby resulting in low-precision management and poor prognosis.[2] Meanwhile, mucinous carcinomas are

a rare pathological entity. These carcinomas must be differentiated from ICNST with mucin degeneration given that the former have a better prognosis due to the lower incidence of lymph node metastasis.[4]

Mucin is a high-molecular-weight glycoprotein, which is composed of a central protein core, that is dispersed throughout the epithelial surface of the gastrointestinal, respiratory, and reproductive tract.[5] This protein can be examined by immunohistochemical analysis, which has been shown to help in differentiating several clinicopathological characteristics, using

specific antibodies.[4] Mucin is preferably combined with hematoxylin and eosin (H&E) staining and panels of markers.[6] Unfortunately, immunohistochemical methods are expensive.[7]

Histochemical methods, such as periodic acid-Schiff (PAS) and alcian blue staining methods, are cheap and frequently used to detect mucin. PAS staining can detect both neutral and acidic mucin, whereas alcian blue staining is more sensitive to acidic and mucopolysaccharide mucins. Therefore, this study was aimed to evaluate the diagnostic performance of PAS or alcian blue and their combination to differentiate ICNST and mucinous carcinoma.

METHODS

This cross-sectional study was conducted using archived breast tissue biopsy specimens. Data were retrieved from Department of Anatomical Pathology, Faculty of Medicine Universitas Indonesia/Cipto Mangunkusumo Hospital from March to August 2014. The subjects of this study were limited to those who underwent mastectomy surgery, which was performed in this hospital. We only included mucinous breast carcinoma and ICNST with mucin degeneration. The biopsies were histologically verified using H&E staining by the senior researcher as mucinous carcinoma and ICNST using H&E staining. All the collected tissues were stained with PAS and alcian blue to analyze the mucin of both cases. Staining was conducted in the Laboratory of Histochemistry, Department of Anatomical Pathology, Cipto Mangunkusumo Hospital.

All of the sections from paraffin blocks of each case underwent deparaffinization process using xylol. Rehydration process was carried out by applying alcohol with decreased concentrations from ethanol 100%, 95%, and 70% and then placing the paraffin blocks in water. Next, the tissues were stained using 1% alcian blue dye (Scy Tech, USA) and washed with clear water. The slides stained with nuclear fast red and rinsed with clear water.[8] For the PAS staining, another set of paraffin blocks were rehydrated using the same method, but they were stained with 1% periodic acid dye (Scy Tech, USA), rinsed, and finally soaked in clear water.

Then, dehydration was performed to eliminate the water content in both slides of PAS and alcian blue staining using alcohol with increased concentrations of ethanol (70%, 95%, and 100%). Next, the slides were

cleaned using xylol, mounted by dripping the mounting agent (Entellan, Germany) sufficiently, and covered with a glass cover.[8] The positive result for PAS was the red-pink color glycogen or carbohydrate, whereas the negative result was a pink-reddish color. Meanwhile, the positive result for alcian blue staining was the blue color of mucin acid, whereas the negative result was a color other than blue color.[8]

Tumor node metastasis (TNM) staging was conducted based on the Union for International Cancer Control staging system.[9] All data were retrieved from archival history in the database. Data were recorded using SPSS software, version 20 (IBM Corp, USA). Sensitivity and specificity were calculated. Positive and negative predictive values were not calculated given that the prevalence of mucinous breast carcinoma and ICNST with mucin degeneration were not comparable with that of the general population. This study has been reviewed and approved by the Ethics Committee of the Faculty of Medicine, Universitas Indonesia (No: /H2.F1/ET/2014).

RESULTS

During the study period, 33 cases of both types of breast carcinoma were included. There were 17 cases of ICNST with mucinous degeneration and 16 cases of mucinous carcinoma. The age of breast cancer patients was varied from 27 to 75 years (Table 1).

Figure 1 shows the results using the PAS and alcian blue staining to detect mucins in ICNST with mucinous degeneration and mucinous carcinoma. Most of the cases were positive to PAS staining but not to alcian blue staining (Table 2).

PAS staining method had a higher sensitivity that was 87.5% rather than its specificity, contrary to alcian blue which had a higher specificity that was 82.4%. Comparison of diagnostic values between PAS and alcian blue in detecting mucin are shown in Table 3.

DISCUSSION

This study was conducted to compare the diagnostic performance of PAS, alcian blue, and their combination to differentiate between ICNST with mucin degeneration and mucinous carcinoma. In the diagnosis of biopsy, the pathologist usually only uses morphological data. If any difficulties exist, the pathologists will attempt to use further staining with

Table 1. Clinical and histopathological characteristics comparison of ICNST with mucin degeneration and mucinous carcinoma

Characteristics	ICNST with mucin degeneration (n)	Mucinous carcinoma (n)
Age (years)		
<50	9	11
≥50	8	5
Tumor size		
1	2	1
2	2	4
3	4	5
4	9	6
Nodes		
0	2	2
1	5	6
2	6	4
3	4	4
Metatasis		
0	5	6
1	7	4
unknown	5	6

ICNST=invasive carcinoma of no special type

Table 2. Comparison of positive results using PAS and alcian blue mucin staining on ICNST with mucinous degeneration and mucinous carcinoma

Histopathological feature	ICNST with mucin degeneration (n)	Mucinous carcinoma (n)
PAS, positive	14	10
Alcian blue, positive	7	3
PAS and alcian blue, positive	7	3
PAS or alcian blue, positive	14	10

PAS=periodic acid-Schiff; ICNST=invasive carcinoma of no special type

Table 3. Comparison of diagnostic values in detecting mucin between PAS and alcian blue mucin staining methods

Diagnostic value	PAS	Alcian blue	PAS or alcian blue
Sensitivity	87.5%	43.8%	87.5%
Specificity	41.2%	82.4%	41.2%

PAS=periodic acid-Schiff
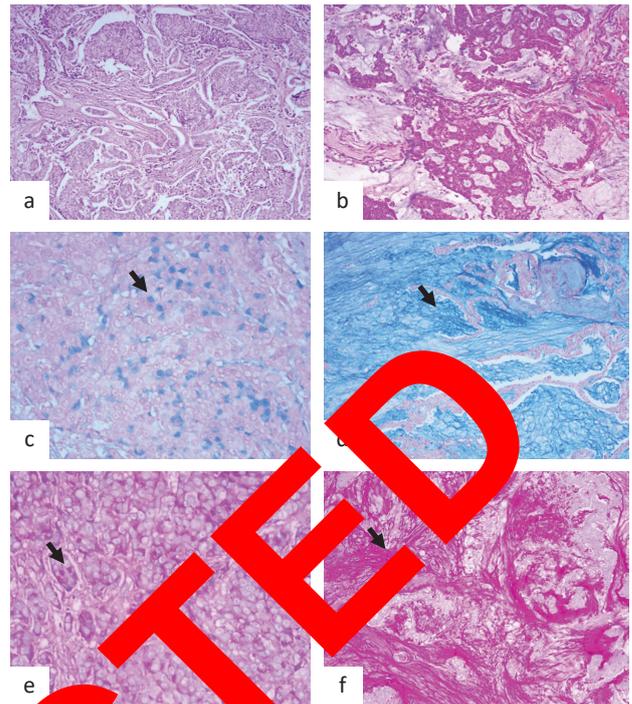
Figure 1. Histochemistry staining using periodic acid-Schiff (PAS) and alcian blue in breast cancer histology of invasive carcinoma of no special type (ICNST) with mucinous degeneration and mucinous carcinoma. (a and b) ICNST with mucinous degeneration and mucin carcinoma in hematoxylin and eosin (H&E) staining (10× magnification); (c) ICNST with alcian blue staining (10× magnification), the blue color (black arrow) respresents the cytoplasm; (d) mucinous carcinoma with alcian blue staining (40× magnification), the blue color (black arrow) indicates mucin; (e) ICNST with PAS staining (40× magnification), the red color (black arrow) denotes the cytoplasm; (f) mucinous carcinoma with PAS staining (10× magnification), the red color (black arrow) shows mucin and is similar to alcian blue staining

histochemistry or/and immunohistochemistry. In a normal tissue, mucin exhibits specific histochemical patterns in tissues and cells. However, these patterns may change in pathological conditions. In adenocarcinoma, mucin expression could be disturbed, including the increased, decreased, or aberrant expression of several mucin glycoproteins.[10,11] The PAS staining technique is sensitive in detecting neutral mucin and acidic mucin that contains sialic acid. Meanwhile, the alcian blue staining technique is sensitive in detecting sulfomucin and sialomucin.[12] In this study, both PAS and alcian blue staining methods demonstrated varied positivities toward ICNST with mucin degeneration and mucinous carcinoma.

Badowska-Kozakiewicz et al[13] performed histopathological examination of ICNST in 691 patients. The age of patient was ranged between 30–81 years old (mean [standard deviation] of age = 60.47 [5.07]),

which showed that most of the study patients affected by ICNST were older than those included in this study. The TNM staging revealed that most of the tumors were assessed with T2, N0, and M0, in contrast to this study in which many patients were already on advanced stage. This result might be caused of the low awareness to breast cancer screening in the community in Indonesia. Dumitru et al[14] showed that the mean age of patients with mucinous carcinoma was 62.3 years, in contrast to this study which showed mucinous carcinoma in younger patients. They also stated that most cases had T2, N0, and M0, and showed similar results with our study.

Fagare[11] showed that more acidic mucin was detected in adenocarcinoma of the breast, colon, ovary, and lungs compared with neutral mucin. A similar result was reported by Ali et al,[10] who observed more acidic mucin and sulfomucin in mucinous adenocarcinoma. They also demonstrated that more acidic mucin was detected in 13 of 33 samples that were positive with the PAS staining method, and 6 were positive with alcian blue staining. This result is similar with the finding of this study, with 13 out of 16 mucinous carcino... showing positive result in PAS staining. The sensitivi... of the PAS staining was also higher than that of alcia... blue staining. However, in alcian blue s... ... both ICNST with mucin degeneration and ... ... carcinoma showed positive results.

Battles et al[15] showed that ... ... an blue ... PAS was more sensitive to mucin s... ... han mucic... mine; despite the wide varia... ... among ... ...es, one case was uniformly nega... ... e. By contrast, ... mucicarmine showed negative r... ...lts in ... ree patients with primary extramammary Paget... ...ase. Th... study also showed that the s... ... ...ity of ... ... alcian blue staining reached ... ...5%, ... ich indicates both PAS and alcian blue are ... ...ve to ... ... ucin differentiation. Contrary to the resea... ... by Micke et al[16] which showed that the mucin staining by alcian blue and PAS exhibit high specificity but low sensitivity for adenocarcinoma in lung cancer when the cut-off for positivity was defined as 10+ cytoplasmic mucin inclusions in a 1 mm TMA core. Another study by Jastrzebski et al[17] showed that alcian blue had a sensitivity of 88% and a specificity of 100% for showing mucinous production in differentiating mucoepidermoid carcinoma of conjunctiva and squamous cell carcinoma of the conjunctiva. This result had a same result with this study which alcian blue had a higher specificity than sensitivity.

The limitation of this study was the number of similar cases of mucinous carcinoma and ICNST with mucin degeneration. A limited number of cases of mucinous carcinoma was also found in the archival data in Department of Anatomical Pathology, Cipto Mangunkusumo Hospital. Although ICNST cases occurs often, this study only used data of patients who underwent surgical biopsy in this hospital. Hence, the number of paraffin block that we could use was limited. Furthermore, the positive and ... ...ative predictive values could not be counted g... ...n that ... sample did not represent the prevalence ... eneral p... ...lations.

In conclusion, the ... ...topa... ...logical ...method of PAS staining used in ... ...e hist... ...logica... ...ermination of mucin expression ... ... a ... ...her sensitivity and better performance ... ...so, t... ...origin... ...the acidic mucin produced ... ...e identifie... ...g PAS staining method. In the lim... ...ed se... ...g laboratory, PAS staining alone can be ... ...dered to d... ...ct mucin.

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