RESEARCH ARTICLE

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Comparative Analysis of Modified Liquid-Based Cytology and CytoRich Red Preparation in Assessment of Serous Effusion for Cancer Diagnosis

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Abstract

Objective: We aimed to compare the cytomorphological diagnosis in serous effusion and quality of background between modified liquid-based cytology (modified-LBC) and CytoRich Red (CRR) preservative. **Methods:** We used an experimental study design: 110 fresh serous effusions were received from 50 cases negative for malignant effusions and 60 cases positive for malignant effusions. All fresh serous effusions were processed using both the CRR solution and the modified-LBC preparation. Blind sample slides were interpreted for cytomorphological diagnosis and the quality of background by 2 cytotechnologists. **Result:** All cases had the same diagnosis irrespective of the method. There was no statistically significant difference in the cytological diagnosis between the CRR and modified-LBC preparations ($p>0.999$). The quality of the background smear for the CRR preparation was clean (54%), moderate in 42%, and poor in 4%. By comparison, the modified-LBC preparation was clean in 46%, moderate in 47%, and poor in 7%. The difference between the quality of background smears between the two methods was not statistically significant ($p= 0.527$). **Conclusion:** There was no statistically significant difference in the diagnosis or quality of background between CRR and modified-LBC preparations. The serous effusion specimen prepared by modified-LBC solution was less expensive than CRR. The modified-LBC could be an alternative preparation when commercial preparations are too expensive.

Keywords: Modified liquid-based cytology- cytorich red- cytology- effusion

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Introduction

The appearance of malignant cells in an effusion is a common complication of malignancy in the pleural, pericardial, or peritoneal space. The detection of malignant cells in the serous effusion indicates a more advanced stage of cancer. The most common causes of pleural effusions are lung cancer, followed by breast cancer. Ovarian cancer is the most common cause of peritoneal effusion, which is detected at diagnosis in two-thirds of cases. By contrast, cancer indicated by pericardial effusion is less common and is detected in only about 2-30% of cancer patients at the time of autopsy (Naylor, 2008). The cytological diagnosis of serous effusion is an important method for the diagnosis of benign and malignant cells, especially when other tests are not available. The accuracy of a cytological diagnosis is thus critical to determining the prognosis and treatment of the patient (Kim et al., 2010). The cytological diagnosis for pleural, pericardial, and peritoneal effusion is an effective method, which not only gives a correct result but is suggestive of the primary origin of cancer. A sensitivity of 52-84% and a specificity of 89-92% of cytomorphological diagnosis has been reported (Motherby et al., 1999; Sen et al., 2015).

The cytological methods of both the direct smear method and the liquid-based cytological method can be used for effusion cytological diagnosis. The direct smear method and liquid-based cytological method are widely used for the preparation of gynecological and non-gynecological cytology samples (Sharma et al., 2016). Other researchers have reported that despite the greater cost, the liquid-based cytological preparation resulted in (a) a cleaner background smear, (b) good cell distribution, (c) well-preserved cytomorphology, (d) reduced screening time, (e) well-preserved cells in solution for longer storage time, and (f) decreased air-dry artifacts better than direct smear preparation (Veneti et al., 2003; Gabriel et al., 2004; Nandini et al., 2012; Sigurdsson, 2013).

Several commercial fixative solutions have become popular choices for liquid-based fixation, including: CytoRich Red (CRR), CytoRich Blue (BD Diagnostics), ThinPrep, and CellPrepPlus. CytoRich Red preservative solution (BD Diagnostics) is the most commonly used around the world, including Thailand. CRR is composed

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of alcohol, 0.2% formalin, and non-toxic demulcents. The buffer is able to lyse red blood cells, to solubilize many proteins, and to fix cells and small tissue fragments. CRR yields a clean background smear, which preserves both diagnostic cells and cellular immunocytochemistry (Weidmann et al., 1999; Davis-Devine et al., 2003). The current study aimed to develop a modified liquid-based cytology technique for effusion cytology. The diagnostic results were based on cytomorphology and the quality of the background smear, and provided a comparison between the CRR solution and a modified-LBC preparation.

Materials and Methods

The study protocol was reviewed and approved by the Ethics Committee of Khon Kaen University, Thailand. The serous effusions of 110 cases for cytological examination were collected (between August, 2014 and March, 2016) from patients at Srinagarind Hospital. The serous effusions were received from 50 cases negative for, and 60 cases positive for, malignant effusions. All fresh serous effusions were processed using both the CRR solution and the modified-LBC preparation.

Processing of CytoRich Red solution

Ten milliliters of effusion sample was centrifuged at 1,600 rpm for 10 min. The supernatant was discarded and re-suspended with 6-10 ml of CRR solution and incubated for 15 min at room temperature. The sample was mixed with a pasteur pipette then 1 ml of sample/slide transferred to a cytofunnel disposable sample chamber, centrifuged at 1,250 rpm for 10 min by Cytospin, then the slide was dipped into 95% ethanol for at least 15 min. The sample was prepared on 2 positive charge slides and using Papanicolaou staining. The method of preparation of the slides was masked but observed by 2 cytotechnologists using a light microscope.

Processing of modified-LBC

Ten milliliters of effusion sample was centrifuged at 1,600 rpm for 10 min. The supernatant was discarded and the remaining protein pellet washed with 0.9% normal saline solution. The solution was centrifuged at 1,600 rpm for 5 min, then 50% ethanol added to the pellet; a ratio 1:1 before incubating for 15 min at room temperature to allow for blood hemolysis. The specimen was then centrifuged at 1,600 rpm for 5 min and re-suspended with 70% ethanol for fixation. The supernatant was mixed with a pasteur pipette, and 1 ml of sample/slide transferred to a cytofunnel disposable sample chamber. Cytospin smears stained with Papanicolaou were prepared on 2 positive charge slides, then observed by 2 cytotechnologists.

Interpretation and statistical analysis

All 110 serous effusion samples were prepared with CRR and modified-LBC then Papanicolaou staining. The respective diagnosis of the 60 malignancy and 50 benign cases were compared using light microscope by 2 independent cytotechnologists. The diagnoses were classified as either negative or positive for malignancy. The quality of the respective background smear studies was classified into the 3 categories: clean, moderate, and poor. The chi-square test was used to analyze the correlation between diagnosis and the quality of the background smear when using modified-LBC vs. CRR. A p-value < 0.05 was considered significant. All statistical tests were performed using STATA version 10.

Results

A total of 110 serous effusions were prepared by modified-LBC and CRR, including 54 (49.1%) pleural effusions, 50 (45.5%) peritoneal effusions, and 6 (5.4%) pericardial effusions. The effusions came from 57 (51.8%) men and 53 (48.2%) women between 27 and 89 years of age (mean 55.9).

Primary diseases in effusions from clinical diagnosis

Fifty benign effusion samples included 15 pleural effusions cases (30.0%), 29 peritoneal effusions cases (58.0%), and 6 pericardial effusions cases (12.0%). The most common primary disease in benign peritoneal effusions was cirrhosis (50%), in pleural effusions tuberculosis (26.7%), and in pericardial effusions chronic pericarditis and congestive heart failure (Table 1). Sixty malignant effusion samples included 39 pleural effusions cases (65.0%) and 21 peritoneal effusions cases (35.0%). The most common primary disease in malignant pleural effusions in males was lung cancer (15 cases; 38.5%) while it was breast cancer in females (13 cases; 33.3%). The most common primary disease in malignant peritoneal effusions in males was cholangiocarcinoma (4 cases; 19.0%) while it

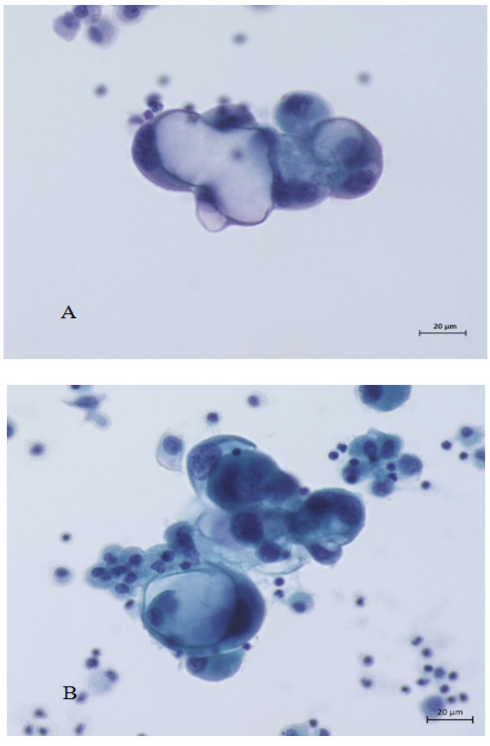

Figure 1. Metastatic Adenocarcinoma of Lung in Pleural Effusion, A. CRR preparation. B. Modified-LBC. Both A and B showed clusters of well-preserved malignant cells with eccentric nuclei, hyperchomatic nuclei, somewhat irregular nuclear membranes and secretory vacuoles in the cytoplasms (Pap staining x400).

Table 1. Primary Diseases and Types of Benign Effusions Included in Study

Final diagnosis with Pap stain	Primary disease	Pleural effusions	Peritoneal effusions	Pericardial effusions	Total
Negative for malignancy	Cirrhosis	3	21	1	25 (50.0%)
	Tuberculosis	9	0	0	9 (18%)
	Chronic peritonitis	0	0	2	2 (4.0%)
	Congestive heart failure	0	0	2	2 (4.0%)
	Other benign conditions	1	3	1	4 (8.0%)
	Bone cancer	1	0	0	1 (2.0%)
	Stomach cancer	0	1	0	1 (2.0%)
	Hepatocellular carcinoma	0	1	0	1 (2.0%)
	Breast cancer	1	0	0	1 (2.0%)
	Cholangiocarcinoma	0	1	0	1 (2.0%)
	Bladder cancer	0	1	0	1 (2.0%)
	Ovarian cancer	0	1	0	1 (2.0%)
	Total	15	29	6	50

was ovarian cancer in females (8 cases; 38.1%) (Table 2).

Comparison between CRR and modified-LBC preparations

All 110 serous effusion samples were prepared with CRR and modified-LBC and stained with Papanicolaou. The diagnostic results of the respective 60 and 50 malignant and benign serous effusion cases were compared by light microscope by 2 independent cytotechnologists. The results of the diagnoses were classified as either negative or positive for malignancy. All cases, whether malignant or benign, had the same diagnostic results; so there was no statistically significant difference in diagnosis ($p>0.999$) between the two methods of preparation (Table 3). Figure 1 illustrates how clusters of malignant cells prepared by either CRR or modified-LBC, retained well-preserved morphological features. Metastatic adenocarcinoma of the lung in pleural effusion showed clusters of well-preserved malignant cells with eccentric nuclei, hyperchomatic nuclei, somewhat irregular nuclear membranes, and secretory vacuoles in the cytoplasms.

The quality of the background was classified as: clean, moderate, or poor. In the CRR preparation, the background was clean in 54% (59/110), moderate in 42% (46/110) and poor in 4% (5/110). By comparison, in modified-LBC, the background was clean in 46% (51/110), moderate in 47% (52/110), and poor in 7% (7/110). The p value was 0.527, indicating there were no significant difference between

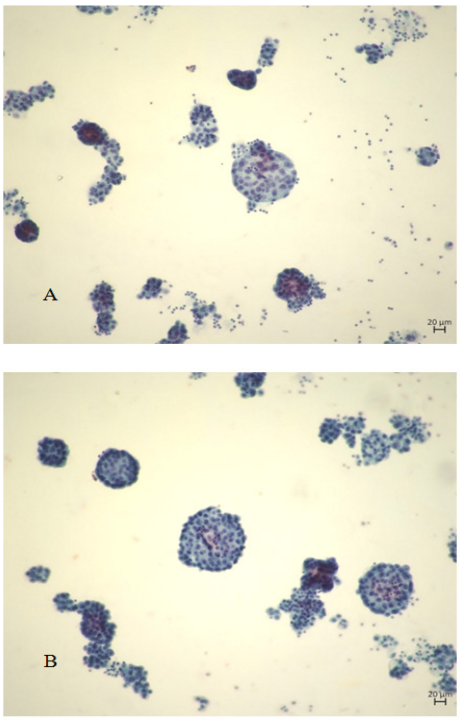

Figure 2. Comparison of Background Smear in Bloody Effusion, A: CRR preparation, B: Modified-LBC. Both A and B showed well-preserved morphological features with a clean background by both methods (Pap staining x100).

Table 2. Primary Diseases and Types of Malignant Effusions Included in Study

Final diagnosis with pap stain	Primary disease	Pleural effusions	Peritoneal effusions	Pericardial effusions	Total
Positive for malignancy	Lung cancer, AC	24 (61.5%)	3 (14.3%)	0	27 (45.0%)
	Breast cancer, AC	13 (33.3%)	0 (0.0%)	0	13 (21.7%)
	Ovarian cancer, AC	0 (0.0%)	8 (33.1%)	0	8 (13.3%)
	Bile duct cancer, AC	0 (0.0%)	5 (23.8%)	0	5 (8.3%)
	Endometrium cancer, AC	0 (0.0%)	1 (33.33%)	0	1 (1.6%)
	Unknown	2 (5.1%)	4 (19.0%)	0	6 (10.0%)
	Total	39	21	0	60

AC, adenocarcinoma)

Table 3. Comparison of Background Smear and Diagnostic between CytoRich Red and Modified-LBC Preparation

No	Parameter	Variables	CytoRich-Red N= 110 (100%)	Modified-LBCN= 110 (100%)	Statistical analysis
1	Diagnosis	Positive for malignancy	60 (56%)	60 (56%)	p value†
		Negative for malignancy	50 (44%)	50 (44%)	> 0.999
2	Background	Clean	59 (54%)	51 (46%)	p value†
		Moderate	46 (42%)	52 (47%)	= 0.527
		Poor	5 (4%)	7 (7%)	

† Chi-square test, p value < 0.05

the CRR and modified-LBC preparation (Table 3). Figure 2 shows a comparison of background smears for bloody effusion prepared by CRR and modified-LBC; morphological features are well-preserved and the background is clean by both methods.

Discussion

Serous effusion is a liquid originating from body cavities. We studied the cytopathology of cellular components from various such effusions in patients with a history of a clinical diagnosis (Table 1). Usually, the cytopathologic diagnosis for serous effusion is the gold standard for diagnosis whether or not there is a cancer metastasis at the body cavity (Fashoyin-Aje et al., 2014). Nance et al., (1991) reported that surgical biopsy is less sensitive than effusion cytology for detecting serosal malignancy: that is 45 vs. 71 for detecting biopsy vs. effusion cytology, respectively. Biopsy of focal lesions on the serous surface may be missed, leading to false negatives. Malignant cells, however, exfoliate and accumulate in effusion from all surfaces of the serous cavity and sometimes effusions represent the entire serous cavity.

We compared the quality of a background smear. All effusions were prepared by fixative CRR solution and modified-LBC. We found that the respective CRR preparation resulted in a clean, moderate, and poor background in 54% (59/110), 42% (46/110) and 4% (5/110) of the time. By comparison, the respective modified-LBC preparation resulted in a clean, moderate, and poor background in 46% (51/110), 47% (52/110), and 7% (7/110) of the time. The p value was 0.527 which indicates a non-statistically significant difference between the CRR and modified-LBC preparations; however, the modified-LBC solution was much less expensive than CRR. Our aim was to develop a method(s) for reducing the costs of specimen preparation without compromising quality as compared to using CRR solution. As such, we determined that by this method we can reduce the cost of specimen preparation 75 times. A previous study resulted in 92% clean backgrounds for effusion sample slides using automation CytoRich Red solution system (Dadhich et al., 2016). The clean background in the current study was 54%, which might be due to a difference in the preparation system. If proteinaceous material and/or red blood cells are found on the background of the slide, the malignant cell is obscured and it is difficult to identify abnormal cells. A poor smear background was found in 7% of the preparations, which was likely the effect of alcohol precipitating proteins from the effusion samples.

In conclusion, the preparation method for modified-LBC solution was less expensive than CRR: Modified-LBC solution was 75 times less expensive than the CRR solution: cell preservation and smear background results were as good as CRR. The modified-LBC preparation could be an alternative laboratory method when commercial preparations are unaffordable.

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