The Differences Among the Quality of Pleural Fluid Cytological Smops from Alcohol Fixation, Hair Spray, and NAFS with Papanicolaou Staining

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Abstract. For the manufacture of cytological preparations, the fixation stage was previously carried out to prevent denaturation, prevent cytolysis and ensure. This study aimed to determine the quality of the picture of cytology preparations fixed by several solutions. This research method was carried out using an experimental laboratory method with a posttest only control group design approach to find out the description of cytology preparations that were given alcohol fixative solution, NAFS, and hair spray. The cytology sample used for preparation was pleural fluid. The data obtained then be processed using the Kruskall-Wallis test SPSS. The results of this study were good image quality from hair spray fixation as much as 20%; NAFS fixation was 6.66%; 70% alcohol fixation was 26.6%; and 96% alcohol fixation was 46.6%. As the conclusion of this study, there was a significant difference in the quality of the picture of the cytology preparations in each fixative solution and 96% alcohol fixative solution resulting in a good picture quality of the cytology preparations.

Keywords: Cytology, Fixative Solution, Pleural Fluid

1 Background

Laboratory examination plays an important role in establishing a diagnosis, one of which is cytology examination. If there is interference with the organs, the fluid from the organs will be observed with slides or smear preparations. So that this examination can identify differences in size, shape, relationships between cells, and characteristics of the cell nucleus between normal cells and cells that are experiencing malignancy [1]. Effusions or body cavity fluids are amongst the most commonly submitted samples to the cytology laboratory [2]. The sample used in cytology preparations is in the form of fluids originating from the human body, one of which is pleural fluid. Pleural fluid is fluid contained in a thin membrane that covers it to minimize irritation when the lungs expand and contract [3]. If a disturbance occurs, this fluid will collect in the body (pleura, peritoneal and pericardial) so that a visible or palpable lump appears [4]. Therefore, pleural fluid cytology examination is usually carried out as an initial diagnosis such as malignancy (non-infection) and pulmonary TB (infection)[5].

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The most popular method in making cytology preparations is smears because the procedure is simple, fast, relatively inexpensive, and accepted by the community[6]. Therefore, cytology examination is an early detection service in cancer examination [7]. As in research[8]. shows the cytology method can be detected quickly. In addition, microscopic examination of cytological preparations is commonly used because it has a sensitivity of 90% and a specificity of 96% [9].

In making cytological preparations, a fixation stage is previously carried out which aims to prevent denaturation, prevent cytolysis and ensure that the specimen is strong enough to survive the pre-analytical process, so that the cellular morphology and position of intracellular parts can remain similar to the condition when the cells were still alive[10]. To determine the final results of the effect of fixation, Papanicolou staining was carried out for cytology preparations. Papanicolaou staining method is used to stain cell nuclei and cytoplasm [11].

The fixation stage is in the first place and has an effect on the final result. The results of this fixation will allow the coloring to be clear and of course result in a correct diagnosis [12]. The fixative solution in the procedure for making cytology preparations that is often used is alcohol[10]. However, fixation with alcohol may shrink the tissue, the cytoplasmic qualities of the cytoplasm and nucleus may become less pronounced. As in research [13], so that NAFS began to be in great demand by anatomical pathologists because it is easier to obtain than other fixative solutions. The use of the NAFS fixative solution resulted in as much as 81.5% of pleural fluid cells being able to be seen properly[14]. In addition, the spray method is now starting to be in demand because it does not require a special container and is faster like hair spray. The use of a hair spray fixative solution resulted in 82% good staining for each cell [15]. Therefore, it is necessary to conduct research regarding the differences in the quality of images of cytology preparations using NAFS fixative solution and hair spray and compare them with 70% alcohol and 96% alcohol with Papanicolaou staining.

2 Methods

The research was carried out using an experimental laboratory method with a post test only control group design approach to determine the description of cytology preparations treated with alcohol fixative solution, NAFS and hair spray. This research was conducted at the Integrated Biology Laboratory at Anwar Medika University for sample treatment and observation of results. Meanwhile, samples were obtained and taken from the Faculty of Medicine, Airlangga University, Surabaya. This research was carried out in February-May 2023. The research sample used was a sample of pleural totaling 15 samples. The sample in this study had the following inclusion and exclusion criteria. Inclusion criteria was male or female and the patient had an infection or there was fluid in the lungs. Exclusion criteria in this study were samples other than pleural fluid.

The tools needed in this research include sample pots, tissue, pastic, centrifuge tube, centrifuges, object glass, cover glass, manual staining set (jar and rack). Material needed in this research include pleural fluid from research subject, 80% alcohol,

70% alcohol, 50% alcohol, Harris hematoxylin, 96% alcohol, Orange G, eosin, xylol, entelan, NAFS, hair spray. Data collection was obtained from primary data using pleural fluid samples from patients. The collection technique was carried out by observing images of preparations that are fixed with four solutions. The data was presented in the form of tables and graphs. The data results were obtained from evaluating the observation of the preparation image with 400x magnification in 5 fields of view using a microscope[16]. Then the data was tested by Kruskall Wallis to see if there were significant differences using SPSS[17].

3 Results

The results of research on the quality of pleural fluid cytology preparations fixed with Hair Spray, NAFS, 70% alcohol and 96% alcohol using the smear method after assessment and continued with grouping the quality of the preparations by total microscopic quality scoring of the preparations showed on Table 1.

Table 1. Quality Image Assessment of Various Solution Fixative

Solution fixative	Microscopic Image							
	Good		Less good		Not good		Total	
	n	%	n	%	n	%	n	%
Hair spray	3	20	4	26.6	8.3	5.5	15	100
NAFS	1	6.66	4	26.6	10.6	66	15	100
70% alcohol	4	26.6	8	53.3	3	20	15	100
96% alcohol	7	46.6	4	26.6	4	26.6	15	100

Based on Table 1 for preparations using hair spray, 20% of the results were good, 26.6% were less good; and not good as much as 53.3%. In preparations using NAFS, 6.6% of the results were good, 26.6% were poor; and not good as much as 66.6%. In preparations using 70% alcohol, good results were obtained as much as 26.6%; not good as much as 53.3%; and not good as much as 20%. In preparations using 96% alcohol, good results were obtained as much as 46.6%; not good 26.6%; and not good as much as 26.6%. The percentage value is obtained from the number of assessment results grouped according to category. The observation results in Figure 1 show that there are visual differences in each preparation. Figure (A) shows that the blue color in the nucleus is clear and the red color in the cytoplasm is not clear. Figure (B) shows the blue color of the cell nucleus and less clear red color of the cytoplasm. Figure (C) shows that the blue color of the cell nucleus is less clear and the red color of the cytoplasm is clearly visible. All three groups can still be diagnosed. Figure (D) shows the blue color of the cell nucleus and the red color of the cytoplasm and the cell

nucleus is clear. The statistical results used the Kruskal Wallis test to determine the differences in each treatment. The p-value shows 0.024 which means that there is a significant difference in the quality of the picture of the preparation in each fixative solution.

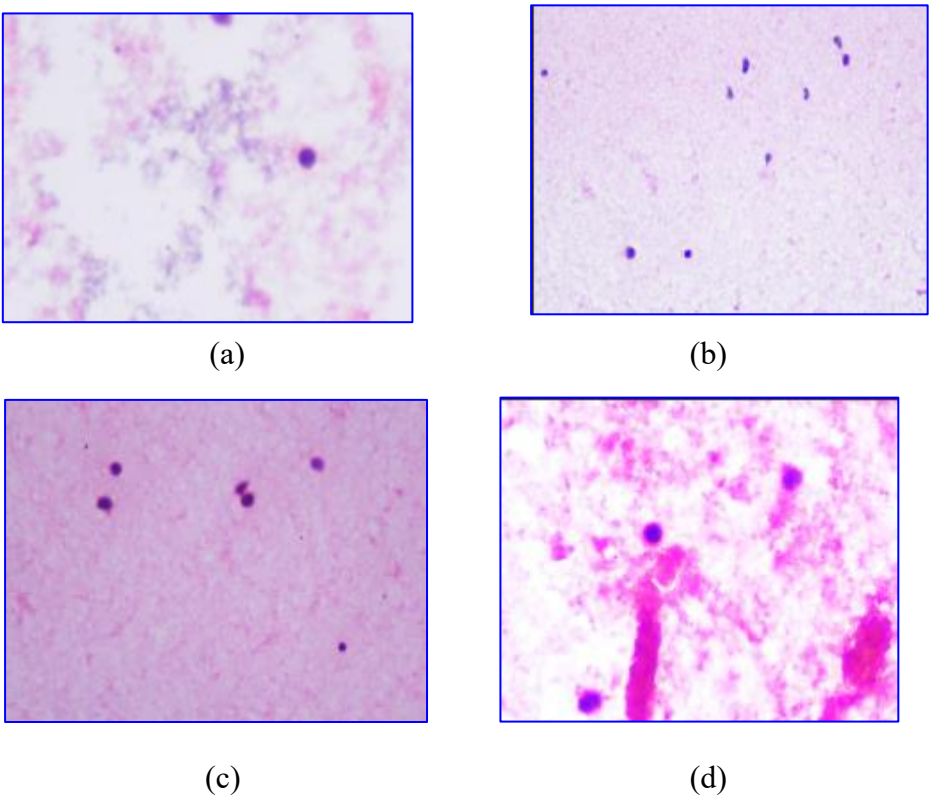

Fig. 1. Observation Results preparation Cytology sample HE Staining Pleural Fluid 400x Magnification. (a) Preparations that use solution fixative hair spray, (b) Preparations that use solution NAFS fixative, (c) preparations that use solution fixative 70% alcohol, (d) Preparations that use solution fixative alcohol 96%.

4 Discussion

Based on Table 1, the quality of the image is good in pleural fluid preparations, namely preparations that use a 96% alcohol fixative solution because the percentage results are the highest with a good assessment of 46.6%, while other fixative solutions have lower percentages as evidenced by microscope observations of the preparations. which used a 96% alcohol fixative solution showed a blue color in the cell nucleus and a red color in the cytoplasm was clearly visible.

The smears that were fixed using NAFS and Hair Spray produced a less bright color but still had a blue color in the cell nucleus, and a red color in the cytoplasm, but from these images it could still be diagnosed. This is consistent with research[18]. That preparations fixed using NAFS produce lower scores than preparations using other fixations. This is due to the high concentration of formalin used in NAFS, namely 10% formalin, thus affecting the number of cells penetrated by formalin, as a result, cells that are not penetrated are also wasted with the supernatant. so that changes occur in the membrane structure which can affect every process in the cell [19]. The results of preparations using hair spray are not in accordance with research [20] which states that the use of fixation with hair spray can minimize contamination from other preparations, due to repeated immersion in alcohol tubes. This is because the previous smear preparation was a dry fixation which could cause cell damage. Other factors are also caused by the too long fixation time, when making smears, delayed staining will cause cell changes and also expired dyes [21]. In addition, the various hair spray content factors can also affect it because it is still left on the glass object. Apart from containing ethanol, hair spray also contains alcohol denat, butane, isobutane, propane, aminomethylpropanol, PEG-12 dimethicone, perfume, Cocamide DEA which can stick to glass objects when hair spray is sprayed. Where these particles are not obtained from the use of pure alcohol fixation [22]. Thus, the fixation of NAFS and hair spray is still not optimal in terms of the quality of preparations in pleural fluid.

In the evaluation of preparations fixed by 70% alcohol it looks still less good than 96% alcohol. This is in accordance with research [23]. That fixation with 96% alcohol provides a good image of cells with bright colors. This is only 2 weak cells from histoscore whereas according to [24]. The results of fixation with 70% alcohol obtained less clear microscopic images, color absorption. the preparation is not good but the color quality of the cell nucleus and cytoplasm can still be seen and can be diagnosed. These poor or unfavorable results are caused by the alcohol experiencing rapid penetration due to evaporation, resulting in shriveled tissue and opaque cytoplasm, so that when staining is not absorbed optimally. Alcohol is rarely used to preserve tissue because it is too brittle and hard. Apart from that, there are other factors such as human error, all of which are still done manually, such as when making smears that are less than optimal and not good which results in many cells being lost or when staining the preparations, it is also not good as a result there are cells that are also washed off. alcohol with a high concentration turned out to be very good at making cell smears [25]. Based on the results of statistical tests there were significant differences in each fixative solution. Thus, 96% alcohol can be used as an alternative in fixing pleural fluid smears.

5 Conclusion

Various fixative solution used for cytological preparation on this research, that were hair spray fixation, NAFS fixation, 70% alcohol fixation, and 96% alcohol fixation. The result of this research showed that there was significant difference of the picture

quality in each fixative solution. Alcohol 0f 96% provided the best quality of the picture for cytological examination. The next research recommendation is tehe selection of a fixative solution that is often used for cytological examinations. The sample used uses vaginal secretions to see the microscopic picture.

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