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Agreement of Urine Sediment Using Shih-Yung Method in Aspirated and Decanted Supernatant Removal Technique

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ABSTRACT

The technique of supernatant removal in urine sediment examination using the Shih-Yung method can be done by aspirating and decanting. The aspirated technique is the recommended technique. The Central Laboratory Installation of Dr. M. Djamil Hospital, Padang used decanted supernatant removal technique. The variety of preanalytical procedures affect the results of urine sediment examination. This study aimed to analyze the agreement of erythrocyte, leukocyte, and non-hyaline cast sediment examination results using the Shih-Yung method in aspirated and decanted supernatant removal technique. This study was an analytical observational study with a cross-sectional design of 37 urine specimens that met the inclusion and exclusion criteria at the Central Laboratory Installation of Dr. M. Djamil Hospital, Padang from July to September 2020. Examination of erythrocyte, leukocyte, and non-hyaline cast sediment using Shih-Yung method with aspirated and decanted supernatant removal technique. Numeric data were analyzed using the Mann-Whitney U test and Cohen's Kappa test for the degree of agreement, p-value < 0.05 was considered significant. The agreement test results for erythrocyte, leukocyte, and non-hyaline cast sediment in aspirated and decanted supernatant removal technique were (Kappa=0.88, p < 0.05), (Kappa=0.83, p < 0.05), and (Kappa=0.86, p < 0.05), respectively. The degree of agreement test results for erythrocyte, leukocyte, and non-hyaline cast sediment using the Shih-Yung method in aspirated and decanted supernatant removal technique were almost perfect and statistically significant. The technique for supernatant removal in urine sediment examination using the Shih-Yung method can be done by decanting.

Keywords: Urine sediment, supernatant removal, Shih-Yung

INTRODUCTION

Urinalysis is an integral part of routine laboratory work.[1] Urinalysis is the third major diagnostic screening test in the clinical laboratory, only preceded by serum or plasma chemistry profiles and complete blood count analysis.[2,3] Routine urinalysis consists of three steps, macroscopic or physical examination, urine chemical examination, and microscopic examination.[4,5] Microscopic examination also known as urine sediment examination, is performed to identify the sediment in the urine. Urine sediment examination is a clinically important step of urinalysis, especially when the sample presents alterations in the physical and chemical steps.[6,7]

Urine sediment examination can be done by three methods, conventional, modified conventional, and automatic using flow cytometry.[8] Manual urine sediment examination is a gold standard in laboratory work for decades.[3] Manual urine sediment examination using bright-field microscopy of unstained centrifuged native urine is still a part of routine work. However, detailed protocols, especially in the preanalytical phase, slightly vary between laboratories because there is no references method for urine sediment examination.[1]

The biggest source of errors in laboratory diagnostics (urinalysis in particular), both preanalytical and postanalytical phase are much more vulnerable.[1,3,5] Several things that need to be considered in examining the urine sediment are: Specimen preparation; Specimen volume; Centrifugation; Sediment preparation; Volume of sediment examination; Urine sediment examination method; and Method of reporting the results.[5,9-11] The results of urine sediment examination are influenced by the method of specimen collection and sediment preparation.[12] The preparation of specimen for examination, principally centrifugation efficiency and residual volume of the sediment, has been shown to be a large source of errors in the preanalytical phase.[1]

The laboratories often modify the preanalytical procedures for urine sediment examination based on the availability of the types of equipments,

consumables, materials, and reagents. The modifications may affect the results of the urine sediment examination. The effect of those modifications needs to be observed before they are implemented in routine practice.[1]

The technique for supernatant removal is one thing that needs to be considered in urine sediment examination. Supernatant removal can be done by two techniques, aspirating and decanting.[5] Perhimpunan Dokter Spesialis Patologi Klinik dan Kedokteran Laboratorium Indonesia (PDS PatKLIn) recommends the aspirated technique for supernatant removal.[13] The Central Laboratory Installation of Dr. M. Djamil Hospital used the Shih-Yung method for urine sediment examination with decanted supernatant removal technique. The Shih-Yung method is a manual method of quantitative urine sediment examination, consists of counting chambers, a plastic pipette, and a plastic tube for centrifugation.[8] The tube has a special diagonal inner hole.[14]

A study in Croatia about preanalytics of urine sediment examination includes the effect of supernatant removal technique on the results of urine sediment using a conventional method. They found that the number of leukocytes was significantly lower when supernatant was removed by aspirating, while there was no statistically significant difference in the number of erythrocyte and non-hyaline cast between the two techniques of supernatant removal.[1]

The technique for supernatant removal in urine sediment examination using the Shih-Yung method has not been widely studied. The agreement of urine sediment examination results in aspirated and decanted the supernatant removal technique has to be proven. Therefore, this study aimed to analyze the agreement of urine sediment results between supernatant removal technique used in the Central Laboratory Installation of Dr. M. Djamil Hospital, Padang and the recommended supernatant removal technique.

METHODS

This study was an analytical study with a cross-sectional design. The study was conducted in the Central Laboratory Installation of the Dr. M. Djamil from July to September 2020. The study population was urine specimens that were sent to the Central Laboratory Installation of Dr. M. Djamil for routine urine testing. The inclusion criteria were morning urine that was sent to the laboratory less than two hours after collection, urine volume of more than 20 mL, blood and/or leukocyte in dipstick results showing one or two positive. The exclusion criteria were blood and/or leukocyte in dipstick results showing negative or positive three because it was difficult to count the sediment one by one clearly in counting chamber when blood and/or leukocyte in dipstick results showing positive three. Dipstick test using DIRUI H-500 urine analyzer together with DIRUI H-11 reagent. The minimum sample size was 35 samples, determined by the formula for agreement test using the Cohen's Kappa test.

Ten mL of urine were poured into each centrifugation tube. The first tube for aspirated supernatant removal technique and the second tube for decanted technique. Both tubes were centrifuged for five minutes at 1500 RPM. For the first tube, the supernatant was removed by an aspirated technique using a one mL pipette from the Shih-Yung kit. The pipette attached to the tube wall over the inner hole was used to aspirate the supernatant off. The supernatant in the second tube was removed by decanted technique, the supernatant was poured off gently. The residual volume after supernatant removal in both techniques was 0.6 mL. One drop of Sternheimer-Malbin stain was added and resuspended. One drop of residual volume was dropped to the counting chamber using a different pipette.[5]

The urine sediment examination was carried out using the Shih-Yung method. Switching the microscope to phase-contrast by lowering the condenser and opening the aperture diaphragm and the field diaphragm. The counting chamber was examined under a low power field (LPF) with the 10x objective to detect cast and to ascertain the general composition of the sediment, followed by High Power Field (HPF) with the 40x objective.[5,15] Cast was examined with 100x magnification in six small squares and reported per Low Power Field (/LPF), while erythrocytes and leukocytes were examined with 400x magnification in two small squares and reported per High Power Field (/HPF).[14] Sediment in both techniques was examined by three different persons. The results of the three examiners were averaged, reported semi-quantitatively.[16] Urine sediment was interpretated based on the criteria from PDS PatKLIn in Pemeriksaan Laboratorium Urine Rutin book. Erythrocytes and leukocytes interpretation were negative for less than one cells/HPF, positive (1+) for 1-4 cells/HPF, positive (2+) for 5-9 cells/HPF, positive (3+) for 10-19 cells/HPF, positive (4+) for 20-29 cells/HPF, positive

(5+) for 30-49 cells/HPF, positive (6+) for 50-99 cells/HPF, and positive (7+) for 100 or more cells/HPF. Non-hyaline interpretation was negative for 0 cells/LPF, positive (1+) for 1-9 cells/LPF, positive (2+) for 10-29 cells/LPF, positive (3+) for 30-99 cells/LPF, positive (4+) for 100-999 cells/LPF, and positive (5+) for ≥ 1000 cells/LPF.[17] The study was approved by the Health Research Ethics Committee of Dr. M. Djamil Hospital, Padang No. 325/KEPK/2020.

Statistical analysis was performed using SPSS version 24. Numeric data were analyzed using the Mann-Whitney U test and Cohen's Kappa test for the degree of agreement. Kappa had values ranging from 0-1. The closer to the value 1, the greater the level of agreement. Interpretation of Kappa values, 0.00-0.20 indicated slight agreement, 0.21-0.40 fair agreement, 0.41-0.60 moderate agreement, 0.61-0.80 substantial agreement, and 0.81-1.00 indicated almost perfect agreement, p-value < 0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

This study involved 37 samples of urine specimens, 12 (32.4%) specimens were obtained from male patients and 25 (67.6%) specimens from female patients. The mean age of patients undergoing routine urinalysis was 44.68 (18.31) years.

The mean of erythrocyte sediment in the aspirated supernatant removal technique was 11.14 (12.28) cells/HPF and 9.86 (11.60) cells/HPF in decanted supernatant removal technique. The mean of leukocyte sediment in the aspirated supernatant removal technique was 11.81 (11.01) cells/HPF and 10.57 (10.56) cells/HPF in decanted supernatant removal technique. The mean of a non-hyaline cast in the aspirated supernatant removal technique was 2.43 (5.97) cells/LPF and 2.05 (5.34) cells/LPF in decanted supernatant removal technique. The mean for erythrocyte, leukocyte, and non-hyaline cast sediment using the Shih-Yung method in the aspirated supernatant removal technique was higher than decanted technique. There was no statistically significant difference in erythrocyte, leukocyte, and non-hyaline cast sediment between both supernatant removal techniques (p=0.614, p=0.603, and p=0.649, respectively) (Table 1).

The agreement test result of erythrocyte sediment in aspirated and decanted supernatant removal technique was Kappa=0.88 (p=0.000) (Table 2). The agreement test result of leukocyte sediment in aspirated and decanted supernatant removal technique was Kappa=0.83 (p=0.000) (Table 3). The agreement test results of a non-hyaline cast in aspirated and decanted supernatant removal technique was Kappa=0.86 (p=0.000) (Table 4). The degree of agreement test results of erythrocyte, leukocyte, and non-hyaline cast sediment was almost perfect and statistically significant with Kappa values were (Kappa=0.88, p<0.05), (Kappa=0.83, p < 0.05), and (Kappa=0.86, p < 0.05), respectively.

A previous study in Croatia about pre-analytics of urine sediment examination compared the aspirated and decanted supernatant removal technique. The

Table 1. The difference in urine sediment examination results

Sediment	Aspirated Technique Mean (SD)	Decanted Technique Mean (SD)	p-value
Erythrocyte (cells/HPF)	11.14 (12.28)	9.86 (11.60)	0.614
Leukocyte (cells/HPF)	11.81 (11.01)	10.57 (10.56)	0.603
Non-hyaline (cells/LPF)	2.43 (5.97)	2.05 (5.34)	0.649

Table 2. The agreement of erythrocyte sediment examination results

		Aspirated Technique						Total	Kappa	p
		Negative	1+	2+	3+	4+	5+			
Decanted technique	Negative	3	0	0	0	0	0	3	0.88	0.000
	1+	1	11	1	0	0	0	13		
	2+	0	1	3	1	0	0	5		
	3+	0	0	1	8	0	0	9		
	4+	0	0	0	2	2	0	4		
	5+	0	0	0	0	0	3	3		
Total		4	12	5	11	2	3	37		

Table 3. The agreement of leukocyte sediment examination results

		Aspirated Technique					Total	Kappa	p
		1+	2+	3+	4+	5+			
Decanted technique	1+	10	2	0	0	0	12	0.83	0.000
	2+	1	6	1	0	0	8		
	3+	0	3	6	0	0	9		
	4+	0	1	0	3	0	4		
	5+	0	0	0	0	4	4		
Total		11	12	7	3	4	37		

Table 4. The agreement of non-hyaline cast sediment examination results

		Aspirated Technique			Total	Kappa	p
		Negative	1+	2+			
Decanted technique	Negative	24	0	0	24	0.86	0.000
	1+	2	8	0	10		
	2+	0	1	2	3		
Total		26	9	2	37		

study found that the number of leukocytes in the aspirated supernatant removal technique was significantly lower than in decanted technique (p=0.045), while there was no statistically significant difference in the number of erythrocytes and non-hyaline casts between both supernatant removal technique (p=0.150 and p=0.100, respectively).[1] It was different with this study, which found that there was no statistically significant difference in erythrocyte, leukocyte, and non-hyaline cast sediment between both supernatant removal techniques (p=0.614, p=0.603, and p=0.649, respectively). The difference between these findings can be caused by the difference in equipment and method used for urine sediment examination.

There were several differences between this study and the previous one. First, this study used a centrifugation tube from the Shih-Yung kit that has a special diagonal inner hole, while the previous study used a round bottom centrifugation tube without the inner hole. Second, this study used one mL plastic pipettes from the Shih-Yung kit for aspirated supernatant removal technique, the pipette attached to the tube wall over the inner hole when supernatant was aspirated off, while the previous study used non-standardized disposable plastic pipettes and not recommended adjusted vacuum tools for aspirating the supernatant. Third, this study used the Sternheimer-Malbin stain while the previous study did not. Fourth, this study used the Shih-Yung method while the previous study used the conventional method. The differences in centrifugation tubes, equipment, and supernatant removal technique, and urine sediment examination method can affect the urine sediment results. The limitation of this study was the subjectivity of the urine sediment examination results because it was done manually. Discussion and similarity of perceptions between examiners were conducted before the study to reduce the subjectivity.

CONCLUSION AND SUGGESTION

The agreement test results for erythrocyte, leukocyte, and non-hyaline cast sediment using the Shih-Yung method in the aspirated and decanted supernatant removal technique were almost perfect and statistically significant. The technique for supernatant removal in urine sediment examination using the Shih-Yung method can be done by decanting. Further studies are needed using other types of centrifugation tubes in manually urine sediment examination, and comparing them with the automated method.

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