ORIGINAL ARTICLE

Alteration in Organic Elements of Sediment in Delayed Examinations of Alkaline pH Urine Sample using Conventional Method

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ABSTRACT

Introduction: Delayed examinations of alkaline pH urine samples will affect xamination results quality for the microscopic urine sediment elements. The organic elements of urine sediment in alkaline and aqueous (hypotonic) urine in delayed examination will cause the cells in urine sediment to be damaged quickly within 2 hours after specimen collection. Purpose: The aim of the study was to evaluate the effects of delayed examination in alkaline pH urine samples (pH> 7.5) using conventional method on alterations in organic elements of urine sediment. The type of research was a laboratory experiment. **Methods:** Six urine samples from adult males and females inpatients with alkaline pH were performed immediate urine sediment examination, delayed examination within 1 hour, 2 hours and 3 hours after urine collection using conventional methods at room temperature in the clinical pathology laboratory Prof. Dr.W. Z. Johannes, Kupang. **Results:** The repeated anova test results (p> 0.05) revealed that delayed examinations of alkaline pH urine samples did not affect changes in leukocyte organic element of urine sediments. Friedman test results (p <0.05) revealed that delayed examinations of urine samples of alkaline pH might affect changes in erythrocyte and epithelial organic elements of urine sediments in 3 hours delay. **Conclusion:** The results of this study showed that there was no effect of delayed examination of alkaline pH urine samples using conventional method on organic elements of leukocyte urine sediments, but altered the examination results for erythrocyte and epithelial organic elements of urine sediments in 3 hours delay at room temperature.

Keywords: Delayed examination, Conventional method, Organic elements of urine sediment

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INTRODUCTION

Urine is a concentrated liquid that contains little water and various metabolic waste products that are removed from the body through urination process. If urine is not removed from the body, metabolic waste products will accumulate and might cause damage to organ systems and might lead to various diseases (1). Urinalysis is a diagnostic screening that is often performed in urology cases that include macroscopic, chemical, and urine sediments analysis (2). Urinalysis is also a part of routine checkup to help detect abnormalities in urinary system (3) and describe the state of other organs such as pancreas (glucose urine), liver, ducts and gallbladder (urobilinogen, urobilin and bilirubin). Urine sediment analysis is a part of routine urine tests (4).

Urine is the final product of urinary system (tractus urinarius) consisting of kidneys, ureters, bladder (vesica urinaria) and urethra, through filtration process by glomerulus, and secretion and absorption processes by tubules (4). Urine production process begins with filtration of large amounts of protein-free fluid from glomerular capillaries to Bowman capsule. Filtration products from glomeruli then go through re-absorption process, and the remaining liquid (filtration product) will be forwarded to kidney tubules to undergo absorption

process (Figure 1). the next step is secretion process, where kidney tubules might secrete or add substances to filtration product during cell metabolism, forming large amounts of acid (5).

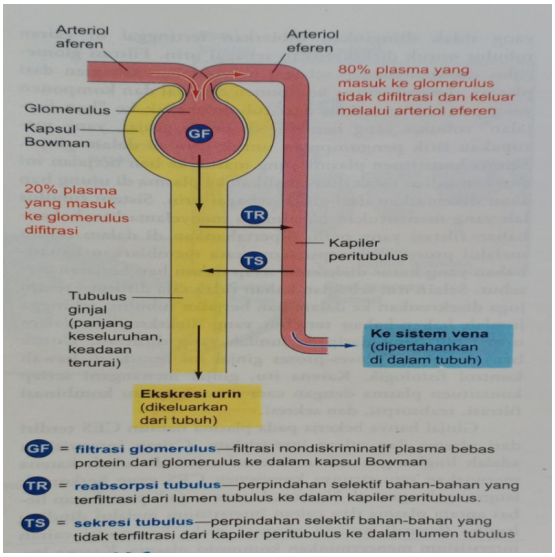

Figure 1 : Mechanism urine formation; stages filtration of the *glomerolus*, stages reabsorption Tubulus and stage Secretion in the kidneys (15).

Urine sediment analysis is very useful for diagnosis and therapeutic evaluation, especially in urinary tract infections patients and other kidneys and urinary system diseases (6) by accurately measuring urine components such as blood cells, cells from male reproductive tract, organism cells from outside the urinary tract, cylinders, or crystals (7). An appropriate urine sediment analysis should be performed when urine sample is still fresh (less than 1 hour), without preservatives addition, or within 2 hours after urination process. If urine sample is stored for too long it will cause alkaline state (pH > 7.5) of urine (8). Preparation of examination specimen is one of errors that often occur at pre-analytic stage. Inappropriate preparation, such as prolonged storage of urine samples, is one source of error that might affect examination results. According to Riswanto and Rizki (8) if alkaline state (pH> 7.5) urine is stored for too long or there is a delay of analysis, it will cause bacterial proliferation which increases turbidity due to amorphous material deposits, and might reduce quality of urine sediment analysis. Hypotonic urine at alkaline state (pH> 7.5) triggers cells in urine sediment to absorb a lot of water and then swell, which damage them within 2 hours after urine specimen's collection.

Urine sediment analysis in hospitalized patients at clinical laboratory of Bhayangkara Hospital Tk.III Kupang, East Nusa Tenggara are often delayed. Analysis delay occurs due to several technical issues such as limited number of analysts during shift changes, blackouts, and delay of specimen's arrival to the laboratory. This causes an average of 1 to 2 hours delay.

MATERIALS AND METHODS

In clusion of Participants

This study was an experimental study with one group, pre-test and post-test design, which is a type of study that conducts experimental activities (9). The purpose of this study was to observe changes that occur by measuring data that were collected during the first observation (pre test), and changes after examination delay (post test) where urine sediment organic elements in alkaline urine samples (pH> 7.5) was observed during 1 hour, 2 hours, and 3 hours delay using conventional methods. This study was conducted at Clinical Pathology Laboratory of Prof. Dr. W. Z. Johannes Regional General Hospital, Kupang using morning urine samples of adult male and female inpatients with an alkaline pH of 6 at Clinical Pathology Laboratory of Prof. Dr. W. Z. Johannes Regional General Hospital. Kupang in 2017.

Selection and Examination of Morning Alkaline Urine Samples

pH analysis of morning urine sample; with 10 ml midstream urine was conducted directly using a urine pH strip that was dipped quickly (less than 1 second) into the patient's urine. The strip was drained by touching one side of the strip with a tissue, then urine pH reading was conducted in less than 1 minute by comparing the color changes that occur in the dry reagent contained in the strip with the color table attached to the dipstick label. The next step was to record observation results and collecting urine samples with an alkaline pH (pH> 7.5) (8).

Analysis of Urinary Sediment Organic Elements

pH analysis of morning urine sample was conducted directly using a urine pH strip that was dipped quickly (less than 1 second) into the patient's urine. The strip was drained by touching one side of the strip with a tissue, then urine pH reading was conducted in less than 1 minute by comparing the color changes that occur in the dry reagent contained in the strip with the color table attached to the dipstick label. The next step was to record observation results and collecting urine samples with an alkaline pH (pH> 7.5) (8).

Analysis of Urinary Sediment Organic Elements

Analysis of urine sediment organic began with pouring 10 ml of urine specimen into a centrifuge tube then centrifuge it with a speed of 1500 rpm for 5 minutes. Afterwards, the top part of the fluid was discharged from the tube with one quick movement. The next step was to use a pasteur pipette for dropping ± 2 drops of sediment onto an object slide and then covered it with a glass cover, then observed the specimen under a microscope with a small objective lens (10X) to calculate the average epithelial count and a large

objective lens (40X) to calculate the average erythrocyte and leukocyte counts (10).

Statistical Analysis

The collected data were analyzed using Repeated Measures Anova parametric statistical test, then the data was presented in tabular form and were performed a statistical analysis using a computer program named Statistics Social Programming (SPPS). The results of the study were first conducted with a distribution and normality test data using Shapiro-Wilk test at 95% confidence level (p α = 0.05). Normality test results for erythrocyte sediments revealed abnormal distribution (p <0.05), thereby the data were analyzed using Friedman's nonparametric statistical tests at 95% confidence level (p α = 0.05). Leukocyte and epithelial sediments were normally distributed and homogeneous (p> 0.05), thereby the data were analyzed using Repeated Measures Anova parametric statistical test (11).

RESULTS

Observation and Result of Sample Analysis

The study was conducted at Clinical Pathology Laboratory of Prof. Dr. W. Z. Johannes Regional General Hospital, Kupang. Sample of the study was morning urine with alkaline pH from hospitalized patients with a total of 6 samples that fit inclusion criteria in this study. Afterwards, each sample was analyzed for its urine sediment organic elements, which were erythrocytes, leukocytes, and epithelial, at immediate time and with 1 hour, 2 hours and 3 hours delay using conventional methods. Erythrocyte changes due to delay of alkaline urine analysis using conventional method during immediate analysis, 1 hour, 2 hours and 3 hours delay are illustrated in Table I. Table I reveals that morning urine sediment analysis from 6 samples showed that average erythrocyte count was reduced at an hour delay by 34.0% and continued to decrease at 2 hours delay by 50.4% and during 3 hours delay it reduced by 63.4%. The longer the delay of analysis, the erythrocyte sediment count would reduce more. Erythrocyte changes due to delay of alkaline urine analysis using conventional method during immediate analysis, 1 hour, 2 hours and 3 hours delay are illustrated in Table I.

Table I : Descriptive data of analysis results for erythrocyte count (cell/ 10 large visual field).

Variables	N	Average count	Changes	
			cell / 10 large visual field	%
Immediate erythrocyte	6	30.7		
1 hour delay erythrocyte	6	20.3	-10.4	4.0
2 hours delay erythrocyte	6	15.2	-15.5	50.4
3 hours delay erythrocyte	6	11.2	-19,5	63.4

Changes of leukocyte sediment in urine due to delay of alkaline urine sample analysis using conventional methods during immediate analysis, 1 hour, 2 hours and 3 hours delay are illustrated in Table II. Table II reveals that morning urine sediment analysis from 6 samples showed that average leukocyte count was reduced at an hour delay by 46.2% and continued to decrease at 2 hours delay by 62.6% and by 3 hours the reduce was 73.2%. Changes of epithelial sediment due to delay of alkaline urine sample analysis using conventional methods during immediate analysis, 1 hour, 2 hours and 3 hours delay are illustrated in Table III. Table III. reveals that morning urine sediment analysis from 6 samples showed that average epithelial count was reduced at an hour delay by 32,7% and continued to decrease at 2 hours delay by 56,2% and by 3 hours the reduce was 73,3%.

Table II : Descriptive data of analysis results for leukocyte count (cell/ 10 large visual field).

Variables	N	Average count	Changes	
			cell / 10 large visual field	%
Immediate leukocyte	6	40.7		
1 hour delay leukocyte	6	21.9	-18.8	46.2
2 hours delay leukocyte	6	15.2	-25.5	62.6
3 hours delay leukocyte	6	10.9	-29.8	73.2

Table III : Descriptive data of analysis results for epithelial count (cell/ 10 large visual field).

Variables	N	Average count	Changes	
			cell / 10 large visual field	%
Immediate epithelial	6	88.2		
1 hour delay epithelial	6	59.4	-28.8	32.7
2 hours delay epithelial	6	38.6	-49.5	56.2
3 hours delay epithelial	6	23.5	-64.7	73.3

Analysis of data

Friedman's non parametric test was used for erythrocyte sediment and Repeated Measurement Anova parametric test was used for leukocyte and epithelial sediments. Normality test using Shapiro Wilk test was performed first to determine data distribution, where erythrocytes had abnormal distribution while leukocytes and epithelial had normal distribution. After normality test was carried out, Friedman test was conducted for erythrocyte sediment and Repeated Measurement Anova test was conducted for leukocyte and epithelial sediment with a significance level of P = 0.05. The results

of Friedman non parametric test for erythrocyte changes are illustrated in Table IV. Table IV shows the results of Friedman test for erythrocyte changes due to delay of alkaline urine sample analysis using conventional methods during immediate analysis, 1 hour, 2 hours and 3 hours delay was significant ($p<0.05$). Repeated Anova test results for leukocytes and epithelial changes are illustrated in Table V. Table V also shows significant changes ($p <0.05$) of epithelial due to delay of alkaline urine sample analysis, but there were no significant changes ($p>0.05$) found for leukocytes.

Table IV : Friedman non parametric test results for erythrocyte changes in alkaline urine samples using conventional methods during immediate analysis, 1 hour, 2 hours, and 3 hours delay.

Variable	Time	Sig.[a]
Erythrocyte	Immediate-1 hour delay	0.027
	Immediate-2 hours delay	0.027
	Immediate-3 hours delay	0.028

Table V : Repeated Anova test results for leukocytes and epithelial changes in alkaline urine samples using conventional methods during immediate analysis, 1 hour, 2 hours, and 3 hours delay.

Variable	Time	Sig.[a]
Leukocyte	Immediate-1 hour delay	0.522
	Immediate-2 hours delay	0.440
	Immediate-3 hours delay	0.338
Epithelial	Immediate-1 hour delay	0.024
	Immediate-2 hours delay	0.038
	Immediate-3 hours delay	0.038

DISCUSSION

The study was conducted at Clinical Pathology Laboratory of Prof. Dr. W. Z. Johannes Regional General Hospital, Kupang in June to July 2017. The sample used in this study was morning urine samples because morning urine has concentrated urine sedimentary elements, therefore making it an appropriate sample for microscopic examination. Control of this study was examination that was conducted in less than 1 hour, without preservatives, or no later than 2 hours after urination process. Riswanto & Rizki (8) stated that if urine specimens are in an alkaline (pH> 7.5) and hypotonic state with prolonged storage time and delayed analysis, it will trigger bacterial proliferation which increases turbidity due to amorphous material deposits and might reduce the quality of microscopic urine sediments (erythrocytes, cylinders) analysis within 2 hours after specimen collection.

Riswanto & Rizki (8) stated that microscopically, erythrocytes in urine could not absorb dyes. In hypotonic urine, erythrocytes will absorb a lot of water, which causes its swelling and eventually erythrocyte will undergo lysis, releasing hemoglobin from cell membrane which causes the erythrocyte to appear empty or known as “ghost cells” because it could easily disappear if it is not immediately analyzed, therefore reducing the quality of microscopic analysis results. The result erythrocyte changes in alkaline urine samples using conventional methods due to delayed analysis in accordance to a previous study conducted by Rivana Ariyadi (12) which concluded that there was a significant effect of 1 hour, 2 hours and 3 hours delay to erythrocyte count in hematuria urine sediment. Research conducted by Haba (13) also reported the effects of 3 hours of analysis delay to erythrocyte count in urine sediment.

Riswanto & Rizki (8) stated that microscopically, leukocyte in alkaline and hypotonic urine will experience damage (lysis) and lose its nuclei component after 2-3 hours at room temperature or if immediate analysis is not performed. Therefore, it is very important to do a microscopic examination immediately, which is 1 hour after urination process. The result of this leukocytes changes in alkaline urine samples using conventional methods due to delayed analysis shows that delay of analysis up to 3 hours at room temperature did not affect the number of leukocytes. Delayed urine sediment analysis might trigger nuclei swelling and induces leukocyte damage. According to Yayuk Kustiningsih (14), there was an effect of urine storage time at room temperature to leukocyte count in diabetes mellitus patients. This study result is different from our study, where we found no significant effect occurred in up to 3 hours delay. Leukocyte is 1.5-2 times larger compared to erythrocyte, and has an important role in immune system because of its ability to fight infection. Under certain conditions such as low density and diluted state (hypotonic), leukocytes will transform into glider shape and will absorb a lot of water and then swell. However, cytoplasmic granules on leukocytes show brown movement in cytoplasm, therefore leukocytes do not easily undergo lysis.

The result of epithelial changes in alkaline urine samples using conventional methods due to delayed analysis shows that delay of analysis up to 3 hours at room temperature significantly affected epithelial count. The results of this study differed from a study conducted by Boby E. Haba, (13) which reported effects of delay in urine sediment analysis on erythrocytes and bacteria counts, but did not affect leukocyte and epithelial counts. The results of this study were different from our study, where we found a significant difference in up to 3 hours delay. This is because microscopic epithelial might have single nuclei, has larger size compared to leukocytes, has variable shapes, cell layers, and types depending on its place of origin at genitourinary system. Low density and diluted (hypotonic) urine in alkaline state (pH> 7.5) will induce epithelium to absorb a lot of water and then swell, induces nuclei component

loss and then experience damage quickly, reducing epithelial count during urine analysis.

CONCLUSION

The conclusion of this research is there is no effect on leukocyte changes in urine sediments, but there are effects on erythrocyte and epithelial changes in urine sediments due to delay of alkaline urine sample analysis using conventional methods with a delay of examination time of up to 3 hours. Analysis of leukocyte counts in urine sediment can be delayed up to 3 hours at room temperature, but it is still recommended to carry out urine analysis in less than 1 hour. Analysis of erythrocytes and epithelial counts in urine sediments is recommended to be carried out as soon as possible at room temperature.

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