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The Influence of Postponing the Examination Period on the Results of Urinalysis of Urine Sediment in Patients with Suspected Urinary Tract Infections

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ABSTRACT

Urinalysis monitors kidney and urinary tract health. Accuracy in urinalysis stages is crucial for reliable results. However, delays in specimen analysis often affect this accuracy. This study investigates the impact of examination delays on Urine Sediment Urinalysis results (leukocytes, erythrocytes, and epithelium) in suspected Urinary Tract Infection patients. The employed research method is a static group comparison where the study participants are divided into two groups: the experimental group, which receives treatment, and the control group, which does not receive treatment. The treatment group consists of urine samples that are delayed for 2 hours and those that are delayed for 3 hours. Microscopic examination of urine sediment is conducted using the Shih-Yung method. The study showed average leukocyte counts at 1, 2, and 3 hours were 19.44/HPF, 18.78/HPF, and 17.67/HPF, respectively. Average erythrocyte counts at these times were 9.0/HPF, 8.56/HPF, and 7.89/HPF. Average epithelial cell counts were 11.56/LPF, 9.89/LPF, and 9.33/LPF. The Wilcoxon test indicated a significant effect ($p<0.05$) on urinalysis results between <1 hour and the 2-hour delay for erythrocyte sediment and epithelium. Urinalysis results for <1 hour were also significantly different ($p<0.05$) from the 3-hour delay for leukocytes, erythrocytes, and epithelium.

KEYWORDS

Epithelium, Erythrocytes, Leukocytes, Shih-Yung, Urinary Tract Infection (UTI)

INTRODUCTION

Urinary tract infections (UTIs) continue to present a significant public health concern. According to the World Health Organization (WHO), UTIs rank as the second most prevalent infections in the human body, following respiratory tract infections, with an annual incidence of

8.3 million cases (Humair, 2019). The prevalence of UTIs in Indonesia is reported to affect 5-15% of the population, translating to approximately 90-100 cases per 100,000 individuals each year (Widiyastuti & Soleha, 2023). These infections arise when the concentration of bacteria reaches a level of 105 CFU/mL or greater, particularly within the bladder and urethra. Common symptoms associated with UTIs may encompass suprapubic pain, dysuria, incontinence, hematuria, as well as abdominal or back pain (Maulani & Siagian, 2022).

Supporting laboratory tests for the diagnosis of infections associated with kidney function, including urinary tract infections (UTIs), necessitate urinalysis. This assessment aims to monitor the condition and functionality of the kidneys and urinary tract. Urinalysis can be performed through macroscopic examination of urine, microscopic evaluation of urine sediment, and the utilization of dipstick technology (Hadijah et al., 2022). Urine sediment comprises elements dissolved in urine that derive from the kidneys and urinary tract. The analysis of urine sediment aims to detect and identify both organic constituents (leukocytes, erythrocytes, epithelial cells, cylinders, and bacteria) and inorganic constituents (crystals, amorphous materials, and fatty substances) in urine. The examination of urine sediment holds clinical significance and is regarded as the gold standard for diagnosing and managing patients with UTIs and renal disease (Niawaty et al., 2020).

Urine sediment analysis requires fresh specimens collected in sterile, tightly closed containers upon arrival at the laboratory. Examination should be conducted immediately, ideally within one hour of collection. Delays between urination and analysis can compromise the accuracy of the examination results. If testing cannot be performed right away, urine specimens should be analyzed within a maximum of four hours or stored in a refrigerator at a temperature of 2–4ºc. However, in practice, urine samples often reach the laboratory no longer fresh and may have been collected several hours earlier. Clinicians frequently encounter challenges in sending samples promptly, leading to many results not aligning with the patient's clinical condition. Furthermore, the high volume of specimens and the lack of adequate laboratory personnel increase the risk of delaying the examination of urine samples (Pinontoan et al., 2023).

Urine storage can cause changes in urine characteristics that affect examination results. Several studies have stated that a delay in examination of > 2 hours at room temperature leads to differences in the number of leukocytes and erythrocytes in urine (Dewanti et al., 2019; Anwar & Jais, 2021). However, other studies have indicated that a delay of <4 hours at room temperature does not significantly alter the amount of urine sediment, particularly leukocytes, erythrocytes, and epithelial cells (Niad et al., 2014). The results of the study by Tena et al. (2021) demonstrated

that there was no effect of delaying the examination of alkaline pH urine samples on urine leukocyte sediment following a 3-hour delay at room temperature.

Based on the analysis of the literature review conducted, it can be observed that there are inconsistencies among several previous studies. This underlies the researcher's interest in conducting a study that aims to determine whether there is an effect of the delay in examination time on the results of urine sediment urinalysis (leukocytes, erythrocytes, and epithelium) in patients suspected of UTI. The study was carried out at the Prodia Kendari Clinic, with delays in urine examination time of 2 hours and 3 hours at room temperature.

LITERATURE REVIEW

Urine is a waste fluid excreted by the kidneys and subsequently eliminated from the body through the process of urination. This process is crucial for removing waste molecules from the blood, which are filtered by the kidneys to maintain the homeostasis of bodily fluids. Typically, freshly excreted urine appears clear to slightly cloudy, exhibiting a yellow hue due to the pigments urobilin and urochrome. In general, urine is comprised of water and various dissolved metabolic wastes, including urea, dissolved salts, and other organic materials (Putri et al., 2023).

Urine examination in the laboratory can provide a comprehensive overview of the performance of the kidneys and urinary tract, as well as the function of various organs in the body, including the liver, pancreas, and bile ducts, among others. A complete urine examination conducted in the laboratory is referred to as urinalysis. Urinalysis involves the examination of urine specimens through physical, chemical, and microscopic analyses. The primary purpose of urinalysis is to detect abnormalities or infections within the urinary tract. This examination can also be utilized to monitor disease progression and the body's response to treatment (Arfaizah et al., 2022).

Microscopic examination of urine sediment involves the analysis of insoluble elements within the urine, including erythrocytes, leukocytes, epithelial cells, crystals, bacteria, fungi, and parasites, which may originate from the blood, kidneys, and urinary tract (Hadijah et al., 2022). The optimal urine specimen for microscopic examination is concentrated urine, with a specific gravity of 1.023 or higher. Such concentrated urine is most effectively obtained using morning urine as the specimen for analysis. According to the Clinical and Laboratory Standards Institute (CLSI), urine sediment examinations must be conducted within two hours following specimen collection. In cases of delayed analysis, urine preservatives may be applied or the specimens can be stored at a temperature ranging from 2 to 8 degrees Celsius. This measure is crucial for

maintaining the stability of organic and inorganic elements, which can influence various urine parameters (Dolscheid-pommerich et al., 2016).

The manual methodology for the quantitative examination of urine sediment utilizing the Shih-Yung (S-Y) system has been successfully developed. Within the S-Y system, both the volume of urine employed and the corresponding equipment, as well as the centrifugation process, have been standardized. The Shih-Yung method was originally formulated by Shih-Yung Medical Instrument in Taipei, incorporating a medium field consisting of 81 small boxes with a depth of 0.01 mm. In this method, urine undergoes an initial centrifugation, after which the resultant sediment is transferred to the counting chamber, and the quantitative results regarding the number of sediment elements obtained are reported per microliter of urine (Perdani, 2019).

The S-Y method comprises an S-Y counting chamber, a graduated centrifuge tube, a sediment dropper, and a sediment dye. The S-Y counting chamber is constructed of acrylic and features four medium fields, each with an area of 4 x 1 mm^2, which consists of 24 small boxes with a height of 0.05 mm. These small boxes facilitate the reading of urine sediment, thereby enhancing clarity during subsequent microscopic observation. Additionally, a 1 ml plastic dropper and a 12 ml plastic tube with a cap are utilized in this method. In the S-Y method, urine is subjected to centrifugation, and the resulting sediment is transferred into the counting chamber, permitting a quantitative report of the sediment elements per microliter of urine (Perdani, 2019).

METHOD

Type, time, and place of research. The type of research is pre-experimental, involving a treatment of delaying urine specimen examination for 2 hours and 3 hours. The control group, without treatment, consists of examinations carried out immediately, without any time delay. The research was conducted at the Prodia Kendari Clinic, with the research period spanning from February to June 2023.

Population and sample. The population in this study consisted of patients with suspected UTIS who underwent routine urine examinations, specifically random urine tests. The sample size was determined using the Federer formula, which indicated that the minimum number of repetitions required is 9. For this study, the sample size was calculated by multiplying the number of treatments by the replication (N = t x r), resulting in a total of 27 samples. Sample collection was conducted using a purposive sampling technique, based on specific considerations made by the researcher. Inclusion criteria include: 1) Urine from patients with an initial diagnosis of suspected UTI who underwent routine urine examinations; 2) Urine specimens collected within 1 hour, with

recording and collection carried out by sampling officers. Exclusion criteria include: 1) Urine from check-up patients without UTI complaints; 2) Urine specimens collected with a volume of less than 12 mL.

Working procedure: The stages of this study comprise pre-analytical, analytical, and post-analytical phases. The pre-analytical phase involves collecting urine specimens, specifically midstream urine, following established procedures, ensuring a sufficient volume of approximately 30 mL or meeting the mark on the urine container. The time of urine collection is recorded, and it is confirmed that the urine reaches the laboratory immediately after collection, ideally within 1 hour. The laboratory room temperature is maintained between 20 and 25°c. The analytical phase entails examining urine sediment using the S-Y (Shih-Yung) method. A sample of up to 12 ml of urine is placed in a tube fitted with a cap. The urine is then centrifuged for 5 to 10 minutes at speeds of 1500 to 2000 rpm. After centrifugation, the upper layer of urine is discarded, leaving 0.5 to 1 ml of sediment, to which one drop of Sternheimer-Malbin dye is added for resuspension. The sediment is then squeezed with a dropper, and one drop is placed into the counting chamber. Examination is performed under a microscope at 10X magnification to identify epithelial cells (HPF) and at 40X magnification for erythrocytes and leukocytes (lPF), with the number of cells counted. This examination is conducted no later than 1 hour after urine collection, with attention to the timing. The sediment is then allowed to sit at room temperature for 2 hours without special treatment. After exactly 2 hours and again at 3 hours, the sediment is re-examined. The post-analytical phase involves recording the findings for each sediment examination at three different time points.

Data processing and analysis. The data obtained were subjected to descriptive statistical analysis by being entered into a computer for further examination. The data collected in the study were tested for normality, and then parametric tests were conducted using paired t-tests if the data were normally distributed, or Wilcoxon non-parametric tests if they were not. In this study, statistical calculations were performed using SPSS Statistics software version 23.

RESULT AND DISCUSSION

In this study, microscopic examination of urine samples of suspected UTI patients was carried out with variations in examination time, namely being examined immediately or <1 hour, delayed for 2 hours, delayed for 3 hours. The types of sediment examined included leukocyte sediment, erythrocyte, and epithelial cells. The results of the examination of the average amount of urine sediment with variations in the delay in the processing time are tabulated in Table 1.

Table 1. Average quantity of urine sediment with a delay in examination time

No	Sediment type	Delay in processing time	Number of samples	Average result	SD (Standard Deviation)
1	Leukocyte	<1 hour	9	19.44	22.628
		2 hours	9	18.78	22.944
		3 hours	9	17.67	22.170
2	Erythrocyte	<1 hour	9	9.00	18.540
		2 hours	9	8.56	18.290
		3 hours	9	7.89	17.062
3	Epithelial cells	<1 hour	9	11.56	10.795
		2 hours	9	9.89	10.240
		3 hours	9	9.33	10.428

The results of the normality test using the Shapiro-Wilk method on urine sediment leukocytes, erythrocytes, and squamous epithelium yielded a significant value of <0.05. Based on these findings, it can be concluded that the data is not normally distributed. Following these results, the next difference analysis performed is the Wilcoxon signed-rank test.

Table 2. Results of the normality test on urine sediment data using the Shapiro-Wilk test

Parameters of urine sediment	Examination time	Statistic	Df	Sig.
Leukocytes	1 hour	.596	9	.000
	2 hours	.613	9	.000
	3 hours	.601	9	.000
Erythrocytes	1 hour	.506	9	.000
	2 hours	.492	9	.000
	3 hours	.504	9	.000
Squamous epithelium	1 hour	.870	9	.123
	2 hours	.793	9	.017
	3 hours	.788	9	.015

Based on the results of the Wilcoxon test (Table 3), it is understood that if the significance value is greater than 0.05, then H0 is accepted, and if the significance value is less than 0.05, then H1 is accepted. Consequently, the results of the immediate examination difference test (<1 hour) compared with 2 hours indicated that the results of leukocyte sediment had no significant effect, whereas erythrocyte and epithelial sediments exhibited a significant effect. Meanwhile, the results of the immediate examination difference test (<1 hour) compared with 3 hours demonstrated that the results of leukocyte, erythrocyte, and epithelial sediments showed a significant difference (p <0.05).

Table 3. Wilcoxon statistical test results regarding the effect of urine sediment time delay.

No	Parameters of urine sediment	Examination time	Asymp.Sig.(2-tailed)
1	Leukocyte	<1 hour – 2 hours	0.063
		<1 hour – 3 jam hours	0.011
2	Erythrocyte	<1 hour – 2 hours	0.046
		<1 hour – 3 hours	0.039
3	Epithelial sediments	<1 hour – 2 hours	0.031
		<1 hour – 3 hours	0.017

In this study, urinalysis was conducted by analyzed urine sediment elements, including leukocytes, erythrocytes, and epithelial cells. Urinalysis is a laboratory examination used to detect patients with urinary tract infections and monitor the progression of kidney and urinary tract disease. This study performed the urine sediment test using the Shih-Yung (S-Y) method. This method offers improved accuracy and precision, and helps to reduce contamination, as the centrifuge tube, counting chamber, and pipette are single-use, thereby yielding better results (Ahmahdaly, 2012).

Urine sediment examination requires urine to be fresh and placed in a tightly closed or uncontaminated container. The examination should be conducted as promptly as possible, ideally within one hour of collection, to prevent changes in its components. Delaying the examination can indeed lead to errors, resulting in the expected outcomes being inconsistent with the patient's clinical condition. Sedimentary elements in urine start to break down after two hours, and if left at room temperature for an extended period, cell lysis will occur, causing the urine to become alkaline (Dolscheid-pommerich et al., 2016).

Based on the results of the study in Table 1, the average results of leukocyte sediment examinations at 1 hour, 2 hours, and 3 hours were 19.44/HPF, 18.78/HPF, and 17.67/HPF, respectively. The average number of erythrocyte sediments at 1 hour, 2 hours, and 3 hours was 9.0/ HPF, 8.56/ HPF, and 7.89/ HPF, respectively. Meanwhile, the average number of epithelial cells at 1 hour, 2 hours, and 3 hours was 11.56/ LPF, 10.89/ LPF, and 9.33/LPF, respectively. The data above indicate a decrease in the results of the examination of leukocytes, erythrocytes, and epithelium in urine sediment from the 1-hour examination to those delayed for 2 hours and 3 hours.

The examination results in data were statistically analyzed to determine whether there was a difference in the results of urinalysis of urine sediment examined immediately or within 1 hour, compared to those examined after a 2-hour delay. Additionally, we sought to establish whether there was a difference in the results for sediment examined immediately or within 1 hour, as compared to those delayed for 3 hours. The results of the Wilcoxon test indicated a significant difference in the urinalysis results for urine sediment examined immediately or within 1 hour versus those examined after a 2-hour delay for erythrocyte and epithelial sediments ($p < 0.005$). However, no significant difference was observed for leukocyte sediment between specimens examined immediately and those delayed for 2 hours. Furthermore, the Wilcoxon test results demonstrated a difference in the urinalysis of urine sediment examined immediately or within 1 hour versus that examined after a 3-hour delay ($p < 0.05$) for all three types of urine sediments analysed, namely leukocytes, erythrocytes, and epithelium.

These results support research by (Ahmahdaly, 2012), who conducted urine stability testing by delaying the examination time in the refrigerator. The results showed that delaying the examination of the urine specimen produced false-positive results for the protein urine sediment parameter. Meanwhile, the delay in examination caused false negative results for the leukocyte and erythrocyte urine sediment. Additionally, research by Dewanti et al., (2019) which analysed the effect of delaying urine examination on the number of leukocytes, also showed a significant difference when compared to urine that was examined immediately. Based on the statistical results, it is evident that there is a difference in the results of leukocyte sediment examination between specimens examined immediately and those delayed for 3 hours.

In this study, it was found that on average there was a decrease in the number of erythrocytes, leukocytes, epithelial urine sediments. This is following the statement from (Niad et al., 2014), which states that the elements formed in urine (sediment) will be damaged within 2 hours. According to (Humair, 2019), delaying urine specimen examination for more than 2 hours

can cause a decrease in the quality of the examination results, especially the number of erythrocyte cells. In addition, the delay will also affect the decrease in the quality of leukocyte results because these cells are easily lysed/broken. In practice, urine specimen examination for urinalysis must be carried out as soon as possible. If a delay must be made, the specimen can be stored in the refrigerator at a temperature of 2- 4^0 °C.

Some weaknesses and obstacles identified during the research include that the urine sediment studied consisted of only three types: leukocytes, erythrocytes, and squamous epithelium. The researchers did not examine other sediment elements such as cylinders, crystals, and others. Therefore, it is hoped that future researchers can investigate the examination delay of all these sediment elements. Another limitation is that the research was solely conducted on urine stored at room temperature, without comparing it to urine examined after being refrigerated. From this limitation, it is anticipated that further research can be conducted in the future.

CONCLUSION

Based on the study's findings, it may be concluded that discrepancies exist in the results of urinalysis of urine sediment examined either immediately or within a timeframe of less than one hour, compared to those evaluated after a two-hour delay concerning erythrocyte and epithelial sediments. However, no significant differences are observed for leukocyte sediments. Furthermore, following a three-hour delay in the examination, considerable differences are noted when compared with urine specimens analyzed immediately regarding leukocyte, erythrocyte, and epithelial sediments.

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