The Effect of Urine Storage for 24 hours on Results of Urinary Leukocytes and Calcium Oxalate

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Abstract

Urine examination is an examination often used and is most commonly done to help establish the diagnosis of a disease. The urine test consists of macroscopic, microscopic examination, and chemical examination of urine. Microscopic tests to look at erythrocytes, leukocytes, epithelial cells, thorax, bacteria, crystals, fungi and parasites. Objective: To determine the effect of urine storage for 24 hours on the results of urinary leukocytes and calcium oxalate in Binawan University staff. Method: This type of research uses primary data with a quantitative descriptive design with an analytical observation approach. This research data uses SPSS Software. This data processing is done descriptively. The tests used were *the Kruskal-Wallis test* and *the Mann-Whitney Test* Results: The frequency distribution of the description of fresh urine leukocyte results was the most 1-5 / LPB with a frequency of 26 people with a percentage of 86.7%. The distribution of the frequency of 24-hour delayed urine results was mostly 1-5 / LPB with a frequency of 29 people with a percentage of 96.7%. The distribution of fresh urine calcium oxalate was the most 1-5 / LPB with a frequency of 23 people with a percentage of 76.7%., and the frequency distribution of 24-hour delayed urine calcium oxalate results was at most 0 / LPB as many as 20 people with a percentage of 66.7%. Conclusion: In leukocyte examination, fresh urine calcium oxalate and delayed 24 hours obtained a p value of 0.00 and the results can be concluded that there is an effect of urine storage for 24 hours on the results of leukocytes and calcium oxalate urine.

Keywords: Leukocytes, Calcium Oxalate, Urine says, Urine Delay

Introduction

A good laboratory must comply with standard operating procedures (SOPs) regarding specimen collection for use by other parts. The guidelines should be reviewed by a laboratory supervisor. The laboratory also needs to establish procedures for specimen handling and

procedures for specimen management (acceptance or rejection of specimens). There are always health workers who have knowledge and skills in health efforts i.e., patients, doctors and paramedics or nurses, transport service personnel, health analysts and laboratory doctors(Permenkes, 2014).

Examination of urine is very important especially in establishing diagonists against leukositaria and crystalluria. Delays in examination result in errors in diagnosis and drug administration that lead to harm patients. Furthermore, the delay also affects the validity of urine sediment results, especially leukocytes, which is an indication of the importance of urinary tract infections(Hardjoeno, 2007). Urine examination is an examination often used and is most commonly done to help establish the diagnosis of a disease. This is because urine samples are easy to get and the examination technique is also not so difficult. So it can be done regularly. The urine test consists of macroscopic, microscopic examination, and chemical examination of urine(Hanifah, 2012).

Sediment in the urine leads to kidney stones, namely calcium oxalate crystals. Calcium oxalate crystals and the presence of predisposion, among others, infection, allow the onset of "urinary stones", namely the formation of kidney stones. Stone formation may be accompanied by crystalluria, and the discovery of crystalluria does not necessarily have to be accompanied by stone formation. This compound is in the form of crystals deposited in tissues that can cause extreme pain(Indrawati et al., 2013).

In previous studies, assessing the stability of urine during 24-hour refrigeration gave false positive results on several urnalysis chemical parameters, namely protein, and false negative results on leukocytes and erythrocytes(Kristal-Boneh et al., 2000).

Due to the lack of research conducted and the lack of literature on the examination of urinary leukocytes and calcium oxalate which was delayed 24, researchers were interested in conducting a study on the effect of urine storage for 24 hours on the results of urinary leukocytes and calcium oxalate on. By using fresh urine and delayed urine without special treatment and stored at room temperature.

The formulation of this problem is: It is not yet known whether there is an effect of urinary storage for 24 hours on the results of urinary leukocytes and calcium oxalate?

Literature Review

Definition of Urine

Urine or urine is the waste fluid excreted by the kidneys which will then be removed from the body through the urinalization process. Urinary excretion is necessary to remove waste molecules in the blood filtered by the kidneys. Urine secretion is beneficial for maintaining homeostasis of body fluids. The role of urine is very important in maintaining the body's homeostasis, because part of the disposal of body fluids is through urine secretion. Macroscopic examination is performed to assess color, clarity, odor, specific gravity and pH. Chemical analysis is carried out on proteins, glucose, and ketones. Microscopic examination

to see the presence of urinary sediments such as erythrocytes, leukocytes, epithelial cells, thorax, bacteria, crystals, fungi and parasites(Ariyadi & Sukeksi, 2014).

Leukocytes are often found in normal urinary sediments, but they are few and should not exceed 5 per field. Although all types of WBCs that appear in the peripheral direction can also be found in urine (lymphocytes, monocytes, eusinophils), a common cell is PMN (polymorphonuclear). PMN has the function of phagocytosis, actively motile, and moves amubiodically with pseudopodia(Hasdianah &; Suprapto, 2014).

Calcium Oxalate is in the form of crystals such as sharp needles that embed themselves in tissues and can cause excruciating pain. Calcium oxalate can cause 80 percent of kidney stones in adults. Oxalate can precipitate calcium and form calcium oxalate that cannot be absorbed by the body, resulting in insoluble salt deposits that cause kidney stone disease. In the body, oxalate will compound with calcium to form crystals that will enlarge to form kidney stones(Dhea et al., 2019). Calciumoxalate is very often found in acidic and neutral urine. A common form is crystals shaped like envelopes. These crystals are found in normal urine, especially after eating high doses of carbonic acid or oxalic acid-rich foods such as tomatoes or asparagus(Hasdianah &; Suprapto, 2014). The discovery of calcium oxalate clumps in fresh urine can be associated with the formation of kidney stones, because generally kidney stones are composed of calcium oxalate. The presence of 0 (-/Negative), 1-4 crystals/LPB is declared normal, but if 5-9/LPB (+2/Positive Two), >10/LPB (+3/Positive Three) is declared abnormal(Strasinger &; Di Lorenzo, 2014).

Urinalysis

Urinalysis is a macroscopic, chemical, and microscopic examination of urine. Macroscopic tests include color, clarity, pH, specific gravity, odor, and volume measurements. The microscopic test examined is urine sediment using a microscope. While chemical tests are carried out using dipping cardiches which are carried out manually or using a urine analyzer

Urine Sediment Examination

Urinary sediment is an element that does not dissolve in the urine. Those derived from the blood, kidneys, and urinary tract such as erythrocytes, leukocytes, epithelial cells, thorax, bacteria, crystals, fungi, and parasites. Urine sediment tests or microscopic tests are used to identify sedimentary elements so that they are used to detect kidney and urinary tract abnormalities. In addition, urine sediment tests can also be used to monitor the course of kidney and urinary tract disease after treatment(Maharani et al., 2017).
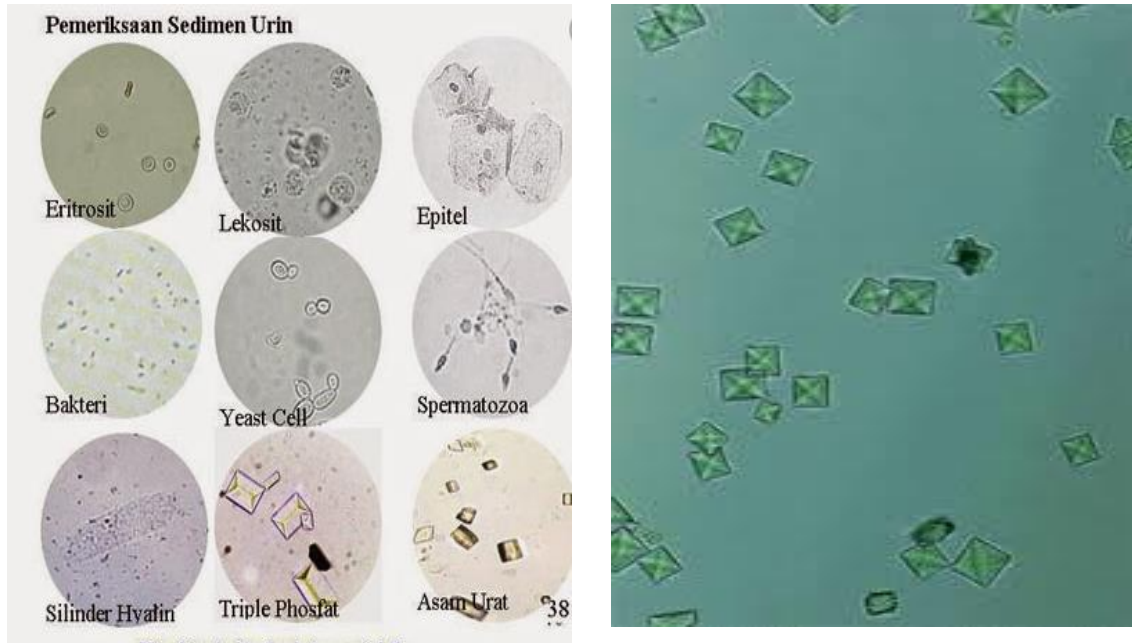
Figure 1. Sedimentation of urine used on microscopic examination

Microscopic examination aims to determine the number of erythrocytes, leukocytes, epithelial cells, cylinders, parasites, crystals, spermatozoa, and bacteria in the urine. Erythrocytes, leukocytes, and epithelial cells can be reported as mean numbers in readings of 10-15 large field of view/LPB (400x). The principle of examination of urine sediment is that it contains trace elements of metabolism in the body. Some of these elements are normally excreted with urine, but some are excreted under certain circumstances. The element can be separated from the urine by means of a dicentrifuge. The element will precipitate and the precipitate is viewed under a microscope(Hinestroza, 2018).

To maximize urine sediment readings, the tools used should be in good condition such as glass, objects, and microscopes. Dirty glass objects cannot be used for urine sediment readings due to the presence of feces or fungi that resemble blood cells. Microscopy is a major tool in urine sediment research. The microscope used must be with a clean lens free of dust or fungi because it can affect the field of view during the examination of urine sediments under a microscope(Gopala, 2016).

Examination of fresh urine sediment with a total specimen volume of 10 mL, has stability at room temperature for 1 hour. If urine is left for a long time then the bacteria will multiply a lot so that it can decompose NH_3 (ammonia) which is alkaline. In alkaline conditions, the pH in the urine will increase. This can affect the sediment component in the urine into rapid lysis so that its amount will be reduced. Factors that affect the examination of sediment in urine are the presence of kidney abnormalities, delays in examining urine sediment because it can result in changes in sediment content by bacteria, time in the screening process and the speed of centrifuge playback of urine sediment examination(Gopala, 2016).

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Research Method

The type of study is quantitative descriptive with an analytical observation approach to see the changes that occur in the treatment given by the researcher in this case to see the effect of urine storage for 24 hours on changes in leukocyte and calcium oxalate urine values. The study was conducted at the Clinical Pathology Laboratory of Binawan University in March – August 2023.The population is the entire object of research or the object studied.[16] The population in this study was Binawan University staff with many samples of 30 people taken with *Non-probability* sampling technique *purposive sample* approach. *Purposive sampling* is sampling based on the purpose of collection that is adjusted to the inclusion criteria and exclusion criteria in the study. The conceptual framework in this study has two research variables, namely Independent Variables (Free Variables) and Dependent Variables (Dependent Variables). In this study, the independent variables are fresh urine and delayed urine. In this study, the dependent variables are leukocytes and calcium oxalate.
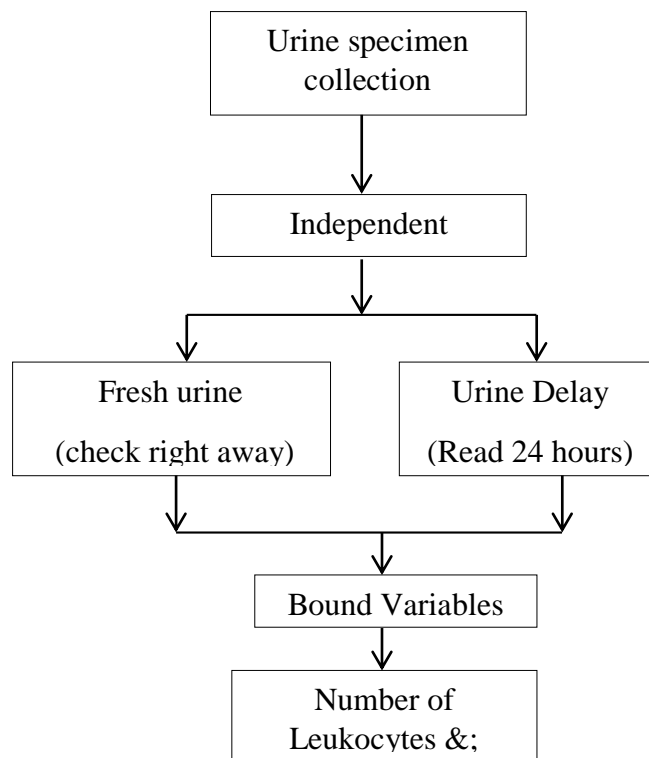
From the framework of the concept above, it explains that there is a treatment carried out to observe leukocytes in urine specimens. Where urine that must be examined immediately is delayed for 24 hours and observed on leukocytes, whether there is an effect of results between leukocytes and calcium oxalate which is examined directly with delayed urine.

Result/Findings

This research was conducted at Binawan University, East Jakarta. The study was conducted by researchers at the Pathology Laboratory of Binawan University who was

supervised by the person in charge of the laboratory. The sample of this study used urine samples on binawan staff as many as 30 samples by shelter using midstream urine.

Research on the effect of urine storage for 24 hours on changes in leukocyte and calcium oxalate values of urine seen is the average of the values obtained by small field of view / LPK or large field of view / LPB. The sediments obtained are observed and documented in the form of pictures. The data was tested by statistical analysis using the SPSS program. Research data processing is carried out descriptively. The statistical tests used are the *Wallis Kruskal Test* and the Whitney Mann Test

Univariate analysis

Gender

Table under Respondents' Gender by Gender at Binawan University in August 2020

Table 4.1. Gender

Gender	Frequency (people)	Percent (%)
Male	19	63,3
Woman	11	36,7
Total	**30**	**100**

Based on the table above, respondents with more male sex, namely 30 respondents examined for urine sediment, there were 19 men (63.3%) and 11 women (36.7%).

Age

The table below shows the Characteristics of Respondents Based on Age at Binawan University in August 2020

Characteristics of Respondents Based on Age at Binawan University in August 2020
Table 4.2 Characteristics of Respondents Based on Age

Age	Frequency (person)	Percent (%)
25 – 35	16	53,3
36 – 46	6	20,0
47 – 57	7	23,3
58 – 68	1	1
Total	**30**	**100**

Based on the above, respondents can be seen based on the age of respondents aged 25-35 years as many as 16 people (53.3%), ages 36-46 years as many as 6 people (20%), ages 47-57 years as many as 7 0rang (23.3%) and ages 58-68 years as many as (1%).

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Table 4.3. Overview of Fresh Urine Leukocyte Results

Results of fresh urine leukocytes	Frequency (people)	Percent(%)
1-5/LPB	26	86,7
5 – 10/LPB	4	13,3
Total	30	100

From the table above which shows the frequency distribution of fresh urine leukocyte examination results in study respondents who have characteristics with results of 1-5 / LPB as many as 26 people (86.7%), 5-10 / LPB as many as 4 people (13.3%).

Table 4.4 Overview of 24-Hour Delayed Urinary Leukocyte Results

24-hour urinary leukocyte results	Frequency (people)	Percent(%)
0/LPB	1	3,3
1-5/LPB	29	96,7
Total	30	100

From table 4.4 which shows the frequency distribution of 24-hour delayed urine leukocyte examination results in study respondents who have characteristics with 0 results as many as 1 person (3.3%), 1-5 / LPB as many as 29 people (96.7%).

Table 4.5 Overview of Fresh Urinary Calcium Oxalate Results

Fresh Urinary Calcium Oxalate Results	Frequency (people)	Percent(%)
1-5/LPB	23	76,7
5 – 10/LPB	4	13,3
10-15/LPB	2	6,7
20-25/LPB	1	3,3
Total	30	100

From the table above which shows the frequency distribution of fresh urine calcium oxalate test results in research respondents who have characteristics with results of 1-5 / LPB as many as 23 people (76.7%), 5-10 / LPB as many as 4 people (13.3%), 10-15 / LPB as many as 2 people (6.3%), and 20-25 / LPB as many as 1 person (3.3).

Table 4.6 Frequency Distribution of 24-Hour Delayed Urinary Calcium Oxalate Results

Urinary Calcium Oxalate Results Delayed 24 Hours	Frequency (people)	Percent(%)
0/LPB	20	66,7
1 – 5/LPB	7	23,3
5-10/LPB	3	10,0
Total	30	100

From the table below which shows the frequency distribution of 24-hour delayed urine calcium oxalate test results in study respondents who have characteristics with 0 / LPB results as many as 20 people (66.7%), 1-5 / LPB as many as 7 people (23.3%), and 5-10 / LPB as many as 3 people (10.0%).

Bivariate Analysis

Kruskal Wallis Test &; Mann-Whitney Test The Effect of 24-hour Storage on Changes in Leukocyte and Calcium Oxalate Values

Table 4.7Wallis *Kruskal Testing*

	Asymp. Sig.
Leukocyte count	.000
Calcium Oxalate	.000

Based on table 4.7 obtained values *p value* The results of the examination of fresh urine leukocytes and 24-hour urine are 0.000 and the results of calcium oxalate in fresh urine and 24-hour urine are obtained values *p value* = 0.000. From these results, it can be concluded that there is a significant influence on the results of leukocyte and calcium oxalate examination on the sedimentation examination of fresh urine and 24-hour urine. In addition to seeing the influence, researchers also conduct tests*Mann Whitney* To see the difference between the results of leukocytes and calcium oxalate in fresh urine and 24-hour urine. As for the results of statistical testing of tilapia *p value* = 0.002 so it can be concluded that there is a significant difference in leukocytes and calcium oxalate which is immediately examined and delayed for 24 hours. The results can be seen in table 4.8.

Table 4.8 *Mann-Whitney Testing Difference Test Results*

	Leukocyte	**Calciumoxalate**
Asymp. Sig.	.000	.002

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Discussion

Urine examination research has been conducted at Binawan University as many as 30 people who are staff at Binawan University. The results showed that the number of respondents was dominated by men, namely 19 people (63.3%) and 11 women (36.7%). This explains that men are more interested in urine examination than women. In addition, the process of collecting urine is one of the factors that cause women not willing to be respondents. While the characteristics of respondents based on age consist of 30 samples. based on the age of respondents aged 25-35 years as many as 16 people (53.3%), ages 36-46 years as many as 6 people (20%), ages 47-57 years as many as 7 00 (23.3%) and ages 58-68 years as many as (1%).

The results of leukocyte and calcium oxalate examination on fresh urine and 24-hour delayed urine found a significant influence in which on 24-hour delayed urine there was a change in the results of leukocytes and calcium oxalate (Khan et al., 2024). The decrease in the number of urinary leukocytes occurred due to the urine storage of previous researchers by Kustiningsih, Cahyono, and Rahmiati in patients with Diabetes 2016. A decrease in the number of leukocytes occurs due to temperature factors, high temperatures will cause faster bacterial growth so that enzymes in the urine are increasingly catalyzed (Hu et al., 2024). It is capable of damaging leukocyte cellsin the urine.[18]There is a significant difference between leukocyte results in fresh urine and delayed urine for 24 hours with a p value of 0.000.

In fresh urinary calcium oxalate results there is no decrease, a decrease in calcium oxalate results occurs in urine delayed examination with various treatments there is a decrease in calcium oxalate results of varying magnitude. From the results of non-parametric statistical tests, the results of fresh urine calcium oxalate and 24-hour delayed urine were obtained with statistical testing of nila *p value* = 0.002 . Roche (2002), DiaSys (2000) stated that the stability of urinary calcium oxalate stored at room temperature is 2 days, while if stored on a refrigator it is stable within 4 days.

The formation of urinary calcium is related to the concentration of various salts in the urine related to food metabolism (Chattaraj et al., 2023). The kidneys play a role in the excretion of metabolites and the maintenance of homeostasis, where the end products of metabolism are found in high concentrations in the urine. This leads to the deposition of crystals (Zheng et al., 2023). Calcium oxalate crystals belong to abnormal urine crystals (Majmundar et al., 2023). Calcium oxalate is most commonly observed in urine in which urine has many forms of variation such as envelopes, monohydrates (Choudhary et al., 2023)(Choudhary et al., 2023). The formation of calcium oxalate in the urine is influenced by the consumption of foods containing calcium and phosphorus, citric acid and uric acid and the habit of drinking less than 1.5 liters / day (Krisna, 2011; Russari, 2016).

In urine examination, many methods can be used to detect substances contained in urine. One examination method that is often used for urine examination is by using microscopic methods. Microscopic examination of urine requires a sample that matches the needs of the examination (Elshal et al., 2023). The factors that affect urine results are time, temperature, storage container. Morning urine is a type of sample that is suitable for urine sediment examination because its condition shows in accordance with the patient's condition. To examine urine sediment, the sample is first accommodated in a clean urine container, then the

urine sample is poured into a tube of 5-10 ml to be centrifuged at a speed of 3000rpm for 15 minutes. Urine that has been centrifuged, then dripped quickly on a glass object and examined for sediment under a microscope with a magnification of 40x (large field of view / LPB), the results of urine sediment found are calculated at 10 fields of view then the results are averaged. Research conducted on centrifugation speed 3000rpm for 15 minutes.

Conclusion

The conclusion of this study is the influence of leukocyte and calcium oxalate results on fresh urine and 24-hour delayed urine with a p value of < 0.05.There is a significant difference between the results of leukocytes and calcium oxalate in fresh urine and 24-hour urine with a p value of <0.05, The results of fresh urine leukocyte examination in study respondents who have characteristics with results of 1-5 / LPB as many as 26 people (86.7%), 5-10 / LPB as many as 4 people (13.3%), the results of 24-hour delayed urine leukocyte examination in study respondents who have characteristics with 0 / LPB as many as 1 person (3.3%), 1-5 / LPB as many as 29 people (96.7%), The results of fresh urine calcium oxalate examination in research respondents who have characteristics with results of 1-5 / LPB as many as 23 people (76.7%), 5-10/LPB as many as 4 people (13.3%), 10-15/LPB as many as 2 people (6.7%), and 20-25/LPB as many as 1 person (3.3%). and the results of 24-hour delayed urine calcium oxalate examination on study respondents who had characteristics with results of 0 / LPB as many as 20 people (66.7%), 1-5 / LPB as many as 7 people (23.3%), and 5-10 / LPB as many as 3 people (10.0%).

Declaration of conflicting interest

In this study there was no conflict that caused the researchers to differ in making decisions.

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