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Effect of urine volume variation on crystal findings in urine sediment examination

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Abstract

Urine sediment examination supports the diagnosis and monitoring of kidney and urinary tract disorders. One pre-analytical factor that can potentially affect the accuracy of urine sediment examination results is variation in urine specimen volume. This study aims to analyze the effect of urine volume variation on crystal findings in urine sediment examination. This study used an experimental design with 30 urine specimens from patients undergoing complete urine examination. Urine specimens were treated in three volume variations, namely twelve milliliters, eight milliliters, and five milliliters, then microscopic examination of urine sediment was performed to identify crystal findings. The data were analyzed using comparative statistical methods to assess differences in crystal findings across urine volumes. The results showed that urine volume variation significantly affected the number of crystals detected, with a tendency for fewer crystals as urine volume decreased. A higher urine volume resulted in greater crystal detection sensitivity than a lower one. These findings emphasize the importance of controlling pre-analytical factors, particularly urine volume, in urine sediment examination. Therefore, clinical laboratories need to establish minimum urine volume standards to improve the reliability of examination results.

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1. Introduction

Urine testing is one of the oldest and most frequently used clinical laboratory tests in medical practice (Ferrão et al., 2024). It provides essential information about kidney function, the urinary tract, and various other systemic disorders (Vassalotti et al., 2025; Zeng et al., 2024). Urinalysis includes physical, chemical, and microscopic examinations that collectively help detect, monitor, and evaluate the course of disease and the response to therapy (Riswanto & Rizki, 2015). Among these three components, microscopic examination of urine sediment is crucial. It allows identification of sediment components, such as cells, casts, microorganisms, and crystals, which can reflect the physiological and pathological conditions of the urinary tract (Lyu et al., 2023; Mongan et al., 2017; Secchiero et al., 2021).

The presence of crystals in urine sediment is one of the critical inorganic elements to consider in microscopic examination (Yacouba et al., 2023). Urine crystals can form due to the influence of urine composition, acidity (pH), solute concentration, and pre-analytical factors related to specimen handling (Subhan et al., 2019). The presence of certain crystals may be physiological, but under certain conditions, they may also indicate metabolic disorders, the risk of urinary tract stone formation, or errors in urine specimen management (Shastri et al., 2023).

The quality of the pre-analytical, analytical, and post-analytical stages greatly influences the accuracy of urine sediment examination results. Several reports state that most laboratory errors, approximately 32–75%, occur at the pre-analytical stage (McPherson & Pincus, 2012). Pre-analytical factors that can potentially affect urine sediment examination results include patient preparation, specimen collection methods and timing, container type, delivery time, and sample preparation processes before microscopic examination (Riswanto & Rizki, 2015).

One of the pre-analytical factors often encountered in laboratory practice is the variation in urine volume received for sediment examination. In general, the recommended urine volume for sediment examination is 7–8 mL to achieve optimal sediment concentration (Gandasoebrata, 2013). However, in certain conditions, such as in pediatric patients, the elderly, or patients with limited urine production, the specimens received are often smaller than the standard. This variation in urine volume can affect centrifugation, sediment concentration, and the detectability of sediment elements, including crystals. Several previous studies have shown that variations in urine volume can affect the results of urine sediment examination. Naid et al. (2015) reported significant differences in urine sediment examination results for leukocytes, erythrocytes, epithelial cells, and bacteria when using different urine volumes. Another study also showed that precipitate volume affects urine sediment examination results, underscoring the importance of volume standards for obtaining accurate, consistent results (Damayanti et al., 2020). Additionally, research by Niawaty et al. (2021) found that differences in urine volume affect the consistency of specific sediment examination results, although not all parameters achieve optimal consistency.

Previous studies have focused more on sediment elements in the form of cells and cylinders, whereas studies on the effect of urine volume variation on crystal findings remain relatively limited. In fact, urine crystals are sediment elements that are sensitive to changes in urine concentration and sample preparation conditions (Strasinger & Di Lorenzo, 2016). This limitation indicates a gap between laboratory examination standards and field practice, particularly in decision-making for specimens with volumes that do not meet standards. Therefore, this study was conducted to examine the effect of urine volume variation on crystal findings in urine sediment examinations. The results of this study are expected to contribute to the field of medical laboratory technology, particularly by improving understanding of the influence of pre-analytical factors on the quality of urine sediment examination results, and by providing a basis for consideration in the management of urine specimens in clinical laboratories.

2. Method

2.1 Research Design and Participants

This study is a quantitative, experimental design study that aims to analyze the effect of urine volume variation on crystal findings in urine sediment examination. The study was conducted under controlled conditions, using microscopic observation of urine sediment after treatment with varying specimen volumes. The study was conducted at the Clinical Laboratory Installation of Buleleng District Hospital in January 2024. The study population included all urine specimens from patients who underwent urine sediment examination at the facility during the study period. Samples were selected using simple random sampling (Subhaktiyasa, 2024), yielding 30 subjects who met the study criteria. The inclusion criteria in this study included adult patients aged ≥17 years, both male and female, who requested a complete urine test. Meanwhile, the exclusion criteria included patients who had difficulty urinating as required for the examination, female patients who were menstruating, patients who were taking medications that could affect urine color, and patients who were unwilling to be research subjects.

2.2 Instruments and Data Collection

The instruments and equipment used in this study included sterile urine collection containers, plastic urine centrifuge tubes, centrifuge tube racks, centrifuges, microscope slides, cover slips, and an Olympus CX21 light microscope. The research material consisted of spot urine specimens obtained from patients at the Clinical Laboratory Unit of Buleleng District Hospital. Data collection was carried out in several stages. During the pre-analytical stage, patients were educated on the correct method of urine collection, namely the midstream urine method. Urine specimens collected in sterile containers labeled with the patient's identity were immediately sent to the laboratory for examination. In the analytical stage, each urine specimen was examined for volume, then divided into three treatment groups: 12 mL, 8 mL, and 5 mL. The specimens were then centrifuged at 1500 rpm for 5 minutes. After centrifugation, the supernatant is discarded, and the remaining urine sediment is homogenized. One drop of sediment is then taken, placed on a glass slide, covered with a cover slip, and examined using a microscope with 10× and 40× magnification. The crystal findings are recorded according to each urine volume variation.

2.3 Data Analysis

The results of the urine sediment examination are presented in tabular form to illustrate the distribution of crystal findings by urine volume. Statistical analysis was performed using the One-Way Analysis of Variance (ANOVA) to determine differences in crystal findings across urine volume groups. Data analysis was performed using the Statistical Package for the Social Sciences (SPSS) at the 95% confidence level ($\alpha = 0.05$). Statistical decisions were determined based on the significance value (p-value), where a p-value < 0.05 indicated a statistically significant difference between urine volume variations, while a p-value ≥ 0.05 indicated no significant difference.

2.4 Ethical Considerations

This study was conducted in accordance with the principles of health research ethics. All research subjects were informed of the study's purpose and procedures and voluntarily agreed to participate. The identities and personal data of patients were kept confidential, and the examination results were used solely for research purposes. The validation and verification of examination results were carried out under the supervision of laboratory staff and the Doctor in Charge of the Clinical Laboratory Facility at Buleleng District Hospital.

3. Results and Discussion

3.1 Results

This study involved 30 urine specimens obtained from patients undergoing complete urine tests at the Buleleng District Hospital Clinical Laboratory. The tests were conducted to identify crystals in urine sediment using three specimen volumes: 12 mL, 8 mL, and 5 mL. Based on subject characteristics, among the total samples that showed crystals in the urine sediment, 20 subjects (66.7%) were male, and 10 (33.3%) were female. Crystal findings were most commonly found in the 25–50 years and >50 years age groups, while the <25 years age group showed the fewest crystal findings. The average number of crystals in microscopic examination of urine sediment with varying urine volumes is shown in Table 1.

Table 1. Average crystal results in urine sediment with varying urine volumes

Volume		Quantity	Mean	SD
12 ml	Kristal Kalsium Oksalat	19	11.6842	7.24992
	Kristal Asam Urat	5	22.0000	11.35782
	Kristal Triple Phosphat	3	4.3333	1.52753
	Cystine	2	3.0000	1.41421
	Leusin/Tyrosine	1	2.0000	0
	Total	30	11.7667	9.11113
8 ml	Kristal Kalsium Oksalat	19	9.6842	6.88035
	Kristal Asam Urat	5	20.0000	11.24722
	Kristal Triple Phosphat	3	3.0000	1.00000
	Cystine	2	2.5000	0.70711
	Leusin/Tyrosine	1	1.0000	0
	Total	30	9.9667	8.71575
5 ml	Kristal Kalsium Oksalat	19	6.0000	5.39547
	Kristal Asam Urat	5	15.6000	9.18150
	Kristal Triple Phosphat	3	0.6667	1.15470
	Cystine	2	0.5000	0.70711
	Leusin/Tyrosine	1	0.0000	0
	Total	30	6.5000	7.19075

Table 1 shows that the identified crystals were calcium oxalate (63.3%), uric acid (16.7%), triple phosphate (10%), cystine (6.7%), and leucine/tyrosine (3.3%). Calcium oxalate crystals were the most dominant type of crystal found in all urine volume variations. Microscopic examination revealed differences in the average number of crystals across urine volumes. At a volume of 12 mL, the average total number of crystals was

11.77 ± 9.11 per high-power field (HPF). At a volume of 8 mL, the average total crystal count decreased to 9.97 ± 8.72 per FOV, while at a volume of 5 mL, the average total crystal count was lower, at 6.50 ± 7.19 per FOV. The average calcium oxalate crystal count decreased gradually as urine volume decreased: 11.68 ± 7.25 per LPB at 12 mL, 9.68 ± 6.88 per LPB at 8 mL, and 6.00 ± 5.39 per LPB at 5 mL. A similar decrease was also observed in uric acid crystals, triple phosphate crystals, cystine, and leucine/tyrosine. Next, a difference test was performed using the One-Way ANOVA, with the results shown in Table 2.

Table 2. Differences in crystal findings in urine sediments with varying urine volumes

Volume variation		Sum of squares	df	Mean square	F	Signifikan
12 mL	Between Groups	938.595	4	234.649	3.994	0.012
	Within Groups	1468.772	25	58.751		
	Total	2407.367	29			
8 mL	Between Groups	842.361	4	210.590	3.869	0.014
	Within Groups	1360.605	25	54.424		
	Total	2202.967	29			
5 mL	Between Groups	635.133	4	158.783	4.592	0.006
	Within Groups	864.367	25	34.575		
	Total	1499.500	29			

Table 2 presents the results of a one-way ANOVA, indicating that variations in urine volume significantly affect the presence of crystals in urine sediment. At a volume of 12 mL, the F value was 3.994; $p = 0.012$, at a volume of 8 mL, $F = 3.869$; $p = 0.014$, and at a volume of 5 mL, $F = 4.592$; $p = 0.006$. All significance values were below the $\alpha = 0.05$ threshold, indicating statistically significant differences in crystal findings between urine volume variations.

3.2 Discussion

The results of the study indicate that variations in urine volume significantly affect the detection of crystals in urine sediment examinations. The average number of crystals decreases as urine volume decreases, with 12 mL producing the highest number, followed by 8 mL and 5 mL. These findings indicate that urine volume is a pre-analytical factor that plays a vital role in determining the quality and sensitivity of microscopic examination of urine sediment.

Urine sediment examination is strongly influenced by particle concentration in the specimen and the effectiveness of the centrifugation process. A larger urine volume allows for the formation of a more representative sediment after centrifugation, thereby increasing the likelihood of detecting sediment elements, including crystals (Gandasoebrata, 2013). Conversely, a smaller urine volume can produce suboptimal sediment, potentially leading to some crystals not being detected microscopically. It is in line with the statement by Riswanto and Rizki (2015), who noted that inconsistencies in specimen volume can affect sediment concentration and the accuracy of urine examination results. Variations in specimen volume and collection methods can lead to differences in sediment concentrations, thereby affecting the reliability of urine test results (Collins et al., 2020).

Calcium oxalate crystals are the most dominant type of crystal found in all urine volume variations in this study. This finding is consistent with the literature, which states that calcium oxalate crystals are the most commonly found crystals in urine sediment, both in physiological and pathological conditions (Strasinger & Di Lorenzo, 2016). The decrease in the number of calcium oxalate crystals as urine volume decreases indicates that volume variation not only affects the overall presence of crystals but also the quantity of the most frequently encountered crystal type.

The results of this study are also in line with those of Naid et al. (2015), which reported differences in urine sediment examination results based on variations in specimen volume, although that study focused more on sediment elements in the form of cells. Damayanti et al. (2020) further reinforce the notion that urine precipitate volume affects sediment examination results, indicating that pre-analytical factors, such as specimen volume, cannot be ignored in microscopic examinations. Therefore, the results of this study support previous findings by emphasizing that urine crystals are also significantly affected by variations in urine volume.

These findings have important implications for clinical laboratories, particularly in the management of urine specimens. In daily practice, laboratories often receive specimens with volumes that do not meet standards due to patient limitations or particular clinical conditions. The results of this study indicate that using smaller urine volumes may reduce the sensitivity of crystal detection, thereby affecting clinical interpretation and diagnostic decisions. Therefore, laboratories need to strengthen adherence to urine specimen acceptance standards and educate patients and health workers about the importance of adequate urine volume. In addition, the results of this study can serve as a basis for clinical laboratories to develop or update standard operating procedures (SOPs) for urine sediment examination, particularly regarding the minimum acceptable specimen volume for microscopic examination. Empirical evidence confirms the need for laboratories to improve the quality of examinations and minimize the potential for pre-analytical errors that can affect the reliability of laboratory results.

4. Conclusion

This study confirms that urine volume variation is a pre-analytical factor that significantly affects crystal findings in urine sediment examination. The findings show that using an adequate urine volume increases crystal detection sensitivity, whereas a too small volume can reduce the quality of results and increase the risk of inaccurate interpretation. These results reinforce the concept that pre-analytical quality control, particularly regarding specimen volume, is an essential determinant in urine sediment examination. These findings contribute to the development of medical laboratory practices by emphasizing the importance of applying minimum urine-volume standards as part of specimen management to support diagnostic accuracy and clinical decision-making. However, this study has limitations, including a relatively small sample size and the omission of other pre-analytical variables, such as pH and urine specific gravity, that can affect crystal formation. Therefore, further research is recommended to involve a larger sample, consider additional pre-analytical factors, and explore the implications of these findings across various healthcare settings to strengthen scientific evidence and support medical laboratory practices.

Conflict of Interest

The authors declare no conflict of interest.

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