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Research Article

Variations In Methods For Storing Samples From Diabetes Mellitus Patients On The Results Of Urine Leukocyte Examination

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ABSTRACT

Diabetes mellitus (DM) is often accompanied by both microvascular and macrovascular. One of the common complications in patients with diabetes mellitus is a urinary tract infection. The purpose of this study was to determine the difference between using a 10% formaldehyde preservative and storage at 4°C to delay urine leukocyte examination in diabetic patients. This study employed an analytical observationalmethod using urine samples from patients with diabetes mellitus. A total of six samples were obtained from Siti Fatimah Tulangan 'Aisyiyah Hospital. The research was conducted from May to June 2023. The results indicate that there are differences in the results of urine leukocyte examinations in diabetes mellitus patients based on the variations in sample storage methods.

Keywords: *Delayed, diabetes mellitus, leukocytes, storage, urine.*

INTRODUCTION

Clinical laboratories utilize diagnostic screening examination such as urinalysis. The most standard component of urinalysis is urine sediment analysis (Delanghe & Speeckaert, 2016). Urinalysis functions to assess the state of kidney and urinary tract function. Some parameters that describe the state of the kidneys and urinary tract include erythrocytes, leukocytes, nitrites, protein, and bacteria, which are assesed using the strip test method (Utsch & Klaus, 2014).

Diabetes Mellitus (DM) is a metabolic disease characterized by elevated blood sugar levels due to abnormalities in insulin secretion, insulin action, or both (Sudoyo et al., 2014). A common and typical symptom of diabetes is frequent urination, often accompanied by polyuria (large volumes of urine) and polydipsia(increased thirst) (Tandra, 2013).

Diabetes mellitus (DM) is often accompanied by both microvascular and macrovascular complications. DM patients frequently experience infections, ranging from simple to omplex. The increased risk of microvascular and macrovascular complications grows with the duration of the disease, further elevating the risk of infections. One common complications in patients with diabetes mellitus is a urinary tract infection (Yunir, 2015). DM is sometimes reffered to as the

"Mother of Disease" becuase it leads to many other conditions, such as hypertension, stroke, chronic kidney disease, blindness, and even amputation (WHO, 2016). Additionally, DM is known as "The Silent Killer", as sufferers often do not realize they have the disease until they are already experiencing complications, both acute and chronic (Prasetyani et al., 2017).

Previous research conducted by Humair (2019) on microscopically delayed urine leukocyte examination showed that urine leukocytes per visual field had an average number of 6.00 at minute 0. At minute 120, the number of urine leukocytes per visual field had an average value of 5.50. At the 180th minute, the number of urine leukocytes per visual field had a mean value of 4.25, but no significant changes were detected (Humair, 2019).

Other studies examined the effect of urine storage time at 2-8°C with a delay of 2 hours and 4 hours for urine chemical examination. The study used 50 samples from a population of 100 urine samples per month from outpatients at the East Kalimantan provincial health laboratory. Based on data obtained using the correlation coefficient test (Spearman rank), it was found that the leukocyte examination was influenced by the time factor by 91% while the remaining 9% was influenced by other factors (Kamil et al., 2016).

Urine preservatives generally use 40% formalin for the quantitative assessment of urine sediment elements. However, formalin available on the market typically has a concentration of 37%, so its concentration needs to be adjusted. The type and amount of sediment in the urine can be affected by excessive formalin use (Gandasoebrata, 2013). Another study found that using formalin at concentrations of 10%, 20%, 30%, and 37% in urine samples delayed for 2 hours did not differ much from urine samples examined immediately (within 1 hour). Still, the number of leukocytes decreased slightly due to long storage. It is advisable to use formalin with the smallest concentration of 10% because the examination results are less affected and more significant (Maharani, 2017).

Urine sediment examination also has a close relationship with centrifugation. Preparing urine sediment can be faster but requires accuracy in adjusting the centrifugation speed to optimize results. Faster centrifugation can result in a a greater amount of urine sediment. Research supports that erythrocytes, leukocytes, and epithelial cells in urine rotated at 4500 rpm yield higher average sediment than at 3000 rpm (Hasanah & Puspitasari, 2022). Therefore, the centrifugation speed needs to be carefully considered.

Based on the above statement, the examination of leukocytes in urine can indicate abnormalities in urine can indicate abnormalities in the urinary tract or inflammation of internal organs in DM patients. Additionally, the high volume of urine examinations request at health centers and hospitals often leads to delays in sample examination. Given this context, a study was conducted titled "Variations in methods for storing samples from diabetes mellitus patients on the results of urine leukocyte examination".

MATERIAL AND METHODS

The study was conducted in an analytical observational manner using a cross-sectional approach. The implementation took place at the Clinical Pathology Laboratory of Muhammadiyah Sidoarjo University from May to June 2023. The sample population comprised diabetes mellitus patients undergoing treatment at Siti Fatimah Tulangan 'Aisyiyah Hospital. Samples were obtained from 6 patients, each with repeated treatment 5 times, and selected by purposive sampling. The sample criteria included being female, aged 45-65 years, and having random blood glucose values > 200 mg/dl. The sample design included a control group with immediate examination, a delay

of 4 hours and 6 hours with treatment at at 4°C, and a delay of 4 hours and 6 hours at room temperature with the addition of 10% formaldehyde.

Data was collected through laboratory examinations. The primary data consisted of the microscopic count of urine leukocytes. Data testing utilized the Friedman test with a 95% confidence level and alpha (α=0.05). This research was approved by the Ethics Committee of STIKes Ngudia Husada Madura with No.1667/KEPK/STIKES-NHM/EC/V/2023.

RESULTS AND DISCUSSION

Some circumstances can cause delays in urine examination, such as sequential examination processes where the first sample is delayed while waiting for other samples to be collected, the large number of samples requiring examination causing a queue for barcode creation, , insufficient human resources or employees resulting in delayed examinations, and delays in distributing samples from inpatients to the laboratory. Onee weakness of this study is the lack of references regarding sample treatment with the addition of 10% formaldehyde for urine leukocyte examination in patients with diabetes mellitus.

The normality test in this study used the Shapiro-Wilk test, which indicatedthat the population data for urine leukocyte examination with a 6-hour delay was not normally distributed. Therefore, the non-parametric Friedman test was used. The study data included urine leukocyte counts with sample treatment at 4°C and room temperature with the addition of 10% formaldehyde, delayed for 4 hours and 6 hours. The using 6 samples are shown in the following figure:

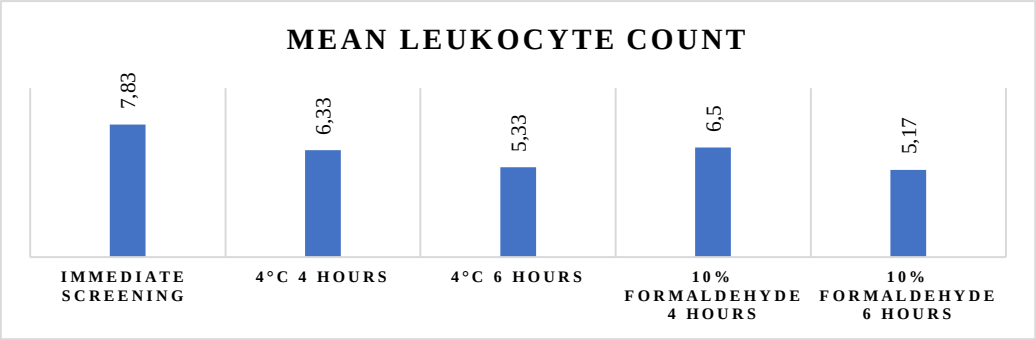

Figure 1. Mean Urine Leukocyte Count and Treatment Mean of Urine Examination

The figure above shows the results of research conducted on the administration of formaldehyde preservatives with 4°C temperature storage for delaying urine leukocyte examination in patients with diabetes mellitus. There is a noticeable difference in the average results. The average for each treatment are as follows: immediate treatment (7.83), treatment at 4°C for 4 hours (6.33), treatment at 4°C for 6 hours (5.33), treatment with the addition of 10% formaldehyde at room temperature 4 hours (6.50), and treatment with the addition of 10% formaldehyde at room temperature 6 hours (5.17).

In the treatment of samples at 4°C for 4 hours, the average value is 6.33, which is less than 6.50 for the treatment with the addition of 10% formaldehyde at room temperature for 4 hours. This indicates that sample treatment with a 4-hour delay is better with the addition of 10% formaldehyde. This finding aligns with research conducted by Parwati (2022), which reports that

formaldehyde preservatives can suppress the growth of decomposing bacteria by reacting with and binding to proteins, an essential elementin blood cellformation, even with a certain delay.

Based on these results, it can be concluded that the treatment of samples at room temperature with 10% formaldehyde preservative is more effective than samples treated at 4°C for 4 hours. Similarly, research conducted by Anugrahatul (2014) comparing the results of complete urine examination that were either immediately examined or delayed for 2 hours at a temperature of 25-27 ° C found changes in urine leukocytes levels. This change can occur because a prolonged delay in urine examination without the addition of preservatives causes the pH of urine to shift to alkaline due to bacterial decompositionof ammonium. Ammonium binds to water, forming ammonium hydroxide, which is alkaline, resultingin an increased pH that affects the leukocyte components in urine, to rapid lysis.

However, in contrast, the sample treated at 4°C for 6 hours received an average value of 5.33, compared to 5.17 for the treatment with the addition of 10% formaldehyde at room temperature for 6 hours. Thus, it can be concluded that the sample treatment stored at 4°C for 6 hours is better than the sample treatment stored at room temperature for 6 hours with the addition of 10% formaldehyde. This result is inversely proportional to the 4-hour delay, where the treatment with the addition of 10% formaldehyde yielded better results.

These results do not align with the research conducted by Parwati (2022), which found that the number of urine leukocytes delayed for 24 hours at room temperature with the addition of formalin increased compared to the immediate examination, with morphology of leukocyte cells shrinking. The difference in results can occur due to the shorter storage time in this study,which was only 4 hours and 6 hours. This shorter duration allows the preservative concentration to still effectively control bacterial growth. Therefore, the average number of leukocytes decrese because it has not been disturbed by bacterial decomposition. The decrease in leukocyte count may occur due to unstable temperature factors, as the samples with 10% formaldehyde were stored at room temperature. Temperature instability outside at room could affect the room temperature, thereby impacting the examination.

Based on the Friedman test, the significance value is 0.002 which is less than 0.05, indicating a significant difference between the administration of formaldehyde with 4ºC temperature storage and the delay in urine leukocyte examination in patients with diabetes mellitus.

CONCLUSION AND SUGGESTION

Based on the analysis of the treatment of samples with formaldehyde preservatives stored at 4 °C on the delay in urine leukocyte examination in patients with diabetes mellitus, there is a noticeable difference in effect. This indicates that there are differences in the results of urine leukocyte examinations in diabetes mellitus patients with variations in sample storage methods. Therefore, medical laboratory technologists need to pay attention to these factors to obtain valid urine leukocyte examination results, especially in diabetes mellitus patients.

Future researchers are encouraged to continue investigating the differences between using formaldehyde preservatives and treating samples at 4 ° C, focusing on the length of delay in urine leukocyte examination in patients with diabetes mellitus.

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