

## Differences in Urine Protein Levels of Hemodialysis Patients With Acetic Acid and Lemon Juice Examination

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### ABSTRACT

**Background and Objectives:** Urine protein examination is a routine examination performed to determine kidney function. The principle of urine protein examination is that the protein in urine is denatured by heating and adding acid. Lemon fruit has a high acid content and lemon has similar properties to acetic acid, namely properties as a weak acid with a pH of 3.0. The direction of this research is to understand the comparison of urine protein using lemon juice and acetic acid solution reagents.

**Methods:** This research uses experimental techniques because urine is treated directly by testing using a 6% acetic acid solution (control) and lemon juice solution.

**Results:** the examination results using lemon juice solution are the same as the examination results using 6% acetic acid solution (control), using the Wilcoxon test, the Asymp.sig (2-tailed) value is 1.000. With the provisions of the test  $>0.05$ ,  $H_0$  is accepted and  $H_a$  is rejected, so testing the difference in urine protein levels of hemodialysis patients with acetic acid and lemon juice examination can be carried out.

**Conclusion:** The results of urine protein examination with acetic acid (control) and lemon juice as an alternative reagent. There is no difference in the results of urine protein examination using 6% acetic acid reagent and lemon juice.

**Keywords:** Acetic acid 6%; Hemodialysis; Lemon; Urine protein.

### Introduction

Urine is the residual fluid excreted by the kidneys which will then be excreted from the body through the process of urination

(Rodan et al., 2018). Urine excretion is necessary to remove residual molecules in the blood filtered by the kidneys and to maintain body fluid homeostasis. However,

some species use urine as a means of olfactory communication. Urine is filtered in the kidneys, carried through the ureters to the bladder, and finally discharged out of the body through the urethra (Gold et al., 2018). One substance that is still useful for the body that is often found in urine is protein. The presence of protein in the urine indicates a leak in the glomerulus. The glomerulus is a part of the nephron that functions to filter various metabolic waste substances. Under normal conditions, protein will not pass through the glomerulus but will go directly to the efferent artery and return to the heart. Leakage and damage to the glomerulus will cause some substances that are still useful for the body to be wasted, one of which is protein. The presence of protein in urine can simply be detected using the acetic acid test (Laskowski et al., 2019).

Urine protein examination is now quite effective in determining the presence of impaired renal function and can be used as a good biomarker for chronic kidney disease. Urine protein examination is a routine examination performed to determine kidney function. Protein can enter the urine if there is damage to the glomerulus and tubular (Purwati et al., 2022).

Patients who have severely decreased kidney function require hemodialysis therapy. Hemodialysis is a high-level technology as a substitute for kidney function to remove metabolic waste or certain toxins from human blood circulation such as water, sodium, potassium, hydrogen, urea, creatinine, uric acid, and other substances through a semi-permeable membrane as a blood separator and dialysis fluid in an artificial kidney where diffusion, osmosis, and ultrafiltration processes occur (Garcia-Aloy et al., 2020).

The principle of the 6% acetic acid method urine protein examination is that the protein in the urine is denatured by heating and

adding acid, the degree of turbidity is directly proportional to the concentration of protein in the urine (He et al., 2021). The 6% acetic acid method urine protein examination has the advantage of being quite sensitive because as much as 0.004% protein can be expressed using this method, there are also disadvantages, namely if dilute urine that has a low specific gravity cannot be examined using this method because it causes false negative results (Garcia-Aloy et al., 2020b).

Urine protein examination is a screening examination to determine kidney function. Proteinuria is the presence of protein in the urine, in normal conditions there is no high concentration in the urine. In its metabolism in the human body, only very little protein is filtered through the glomerulus (Schwab et al., 2018).

The presence of protein in urine can be caused by glomerular disease, tubular disease, non-renal disease, urinary tract disease, increased production of filterable protein, renal vein thrombosis, and pre-eclampsia (pregnancy) (Raikou & Kyriaki, 2019). To detect the presence of protein in the urine, laboratory examination must be carried out, one of which is the heating method using 6% acetic acid. Giving acetic acid to reach the protein's isoelectric point. Heating causes denaturation and precipitation (Aziz & Kamran, 2019).

Urine protein examination reagents use finished chemicals or are made by officers in the laboratory which are relatively expensive. So other materials are needed that can be used as an alternative to urine protein examination reagents, namely by using plants or other natural ingredients that are relatively cheap and easy to find (Kanno & Kanda, 2019).

### **Objective**

To understand the comparison of urine protein using lemon juice and acetic acid

solution reagents.

### Method

The type of research in this activity is experimental research because urine is treated directly by testing using a 6% acetic acid solution (control) and lemon juice solution (Lestari, 2020). This experimental and comparative research is a research method used to determine the comparison of lemon juice and acetic acid solutions as urine protein examination reagents. in the category of ordinal measurement scale.

The variables used in this study are independent variables and dependent variables. The independent variables in this study are 6% acetic acid reagent and lemon juice solution added to urine samples as urine protein examination reagents. The dependent variable in this study is the urine protein level seen from the turbidity if the urine is positive for protein after adding 6% acetic acid reagent and lemon juice.

The sample in this study is urine obtained from hemodialysis patients of dr. Slamet Garut General Hospital has met the inclusion criteria that have been set. The samples used in this study were 30 samples. (Garcia-Aloy et al., 2020a), argue that for comparative causal research, the minimum sample is 30 subjects per group.

The tools used in the research that will be done are urine pots, drop pipettes, test tubes, tube racks, beaker glasses, spiritus lamps, pens, matches, knives, orange squeezers, 5 ml and 10 ml volume pipettes, and ball fillers. The materials used in this study were protein-positive urine samples, lemon fruit, 6% acetic acid, and pH paper.

Data collection in this study was carried out by semi-quantitative examination of urine protein. Covering proteinuria samples obtained from hemodialysis patients and lemon fruit (*Citrus limon*) were selected as research materials and then brought to the

Clinical Chemistry Laboratory of the Health Analyst Department of STIKes Karsa Husada Garut, then made a solution of lemon juice as an alternative reagent for urine protein examination. Which begins with the treatment of lemon squeezed first, then urine protein examination using 6% acetic acid solution (control) and lemon juice solution, prepare 5 ml of urine and enter the urine into a test tube, then drip 5 drops of lemon juice reagent and for control drip 5 drops of acetic acid reagent. After that, heat it on a spiritus lamp until it boils for 30 seconds. Note the presence or absence of turbidity in the top layer. If there is turbidity, it may be caused by protein.

Interpretation:

-: No turbidity (score=0)

+: There is turbidity but does not appear grainy (score=1)

++: Turbidity is present and appears grainy (score=2)

+++: Extremely turbid with granular clumps (score=3)

++++: Turbidity is thick and clumpy. (score=4)

To determine the difference in urine protein levels with lemon juice and acetic acid reagents, the data obtained from the study were analyzed with Categorical Comparative (Oto et al., 2020). Categorical comparative is to compare or contrast two treatment methods, and other cases. Categorical comparisons were made with differences in urine protein levels with lemon juice and acetic acid reagents (Medina et al., 2020). The data obtained will be analyzed with the Wilcoxon test. Wilcoxon test is used to analyze the results of paired observations of two data whether different or not. If the value of Asymp. sig. (2-tailed) is smaller than  $<0.05$ , then  $H_0$  is rejected and  $H_a$  is accepted. Conversely, if the Asymp. sig. (2-tailed) is greater than  $> 0.05$ , then  $H_0$  is

accepted and  $H_a$  is rejected (Hosseini et al., 2019).

## Results

The results of urine protein examination in hemodialysis patients with the addition of 6% acetic acid and lemon juice can be seen in Figure 1. By adding acetic acid and lemon juice to urine that is positive for protein, it shows protein coagulation. The results of scoring the amount of protein in urine are presented in Table 1 and followed by a statistical difference test in Table 2.



**FIGURE 1** Results of urine protein examination with the addition of 6% acetic acid (left) and lemon juice (right)

**TABLE 1** Scoring of urine protein examination using 6% acetic acid solution and lemon juice solution

Sample Code	Control (Acetic Acid 6%)	Lemon juice solution
R01	1	1
R02	3	3
R03	1	1
R04	1	1
R05	0	0
R06	1	1
R07	2	2
R08	1	1
R09	0	0
R010	0	0
R011	3	3
R012	2	2

Sample Code	Control (Acetic Acid 6%)	Lemon juice solution
R013	0	0
R014	2	2
R015	0	0
R016	0	0
R017	0	0
R018	3	3
R019	0	0
R020	3	3
R021	3	3
R022	4	4
R023	1	1
R024	0	0
R025	0	0
R026	0	0
R027	3	3
R028	1	1
R029	3	3
R030	2	2

**TABLE 2** The significant difference analysis

Method of protein in urine examination	Z Asymp, Sig (2-tailed)
Acetic acid 6 %	1,000
Lemon juice	

From the significant difference with non-parametric test results above, the Asymp.Sig (2-tailed) value  $> 0.05$  is obtained, so  $H_0$  is accepted and  $H_a$  is rejected. It is stated that there is no difference between the results of urine protein examination using 6% acetic acid reagent and lemon juice.

## Discussion

In the research, the pH of the 6% acetic acid solution (control) and lemon juice solution was measured. The pH measurement results of 6% acetic acid solution and lemon juice solution obtained the same pH measurement results of 3.0. Measurement of pH in the study using pH paper is determined by

comparing the color of pH paper that has been dipped in 6% acetic acid solution (control) and lemon juice solution with the color standard on the pH paper packaging so that it is very dependent on observation.

Examination of urine protein there are two important stages carried out, namely heating and acid addition. The function of heating is to denature the protein contained in the urine so that a white precipitate is formed. The function of adding acid is to approach the isoelectric point of the protein in the urine (Kurniawan, 2014).

From the results of the study, protein levels contained in urine can be measured after adding 6% acetic acid reagent and can be used as a control. Given acetic acid and lemon juice solution there are 19 positive samples of urine protein and 11 negative samples of urine protein presented in Table 4.1 shows the same results between the 6% acetic acid solution group (control) with lemon juice solution. The similarity of these results is due to the similarity of pH between 6% acetic acid lartuan and lemon juice solution which is 3.0 so that pH is very influential on the process of denaturation and formation of protein precipitation in urine.

In this study, there are shortcomings, namely in measuring pH using pH paper whose results are very dependent on the results of observations. Measurement of pH using pH paper The results are not so precise because the stability level of pH paper is also influenced by the shelf life. The use of pH paper in this study was due to limited tools, so researchers used pH paper to measure the pH of a 6% acetic acid solution, the pH of various concentrations of lime juice solution, and the pH of aquadest.

The results of this study are in line with previous research because lemon juice solution can be used as a substitute reagent

for acetic acid. From the test results using the Wilcoxon test, the Asymp. Sig (2-tailed) is 1.000. With the provisions of the test  $> 0.05$ ,  $H_0$  is accepted and  $H_a$  is rejected, therefore testing the difference in urine protein levels of hemodialysis patients with acetic acid and lemon juice examination can be done.

### **Conclusion**

Based on the results of the study, it can be concluded that the results of urine protein examination with acetic acid (control) and lemon juice as an alternative reagent. There is no difference in the results of urine protein examination using 6% acetic acid reagent and lemon juice.

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### **Conflict of Interest**

There was no conflict in the process until the completion of the research.

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