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## The Differences of Salivary and Urine Acetone Levels in **Diabetes Mellitus Patients**

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Abstract. Ketone objects (acetone, beta-hydroxybutyric, acetoacetic acid) are compounds produced by the body from the breakdown of fatty acids (lipolysis) on the pathway of lipid metabolism. Synthesis of ketones can be caused by insulin hormone disorders such as those experienced by people with diabetes mellitus (DM). Such conditions trigger an increase in lipolysis in adipose tissue which results in the release of free fatty acids as a substrate for the process of ketogenesis in the liver. Acetone as a product of ketogenesis will enter the kidney to be excreted with urine, and if the amount is excessive, then some will accumulate into the saliva. The purpose of this research was to determine differences in salivary and urine acetone levels in DM patients. The type of research is analytic with the cross-sectional approach. Samples were taken using a sequential random sampling technique as many as 18 DM patients, both men and women aged 40-65 years old. The data collection technique was by conducting a direct examination of salivary and urine acetone which measured using spectrophotometric methods. The results showed an average salivary acetone level of 18.9 mg/L, while the mean level of urine acetone was 555.6 mg/L. Mann-Whitney statistical test showed a significance value of 0.000 (P <0.05), so it was concluded that there were significant differences between salivary acetone levels and urine acetone levels. Urine samples with smaller acetone concentrations are able to give positive results, while salivary samples will show positive results when acetone levels in urine are very high.

Keywords: Acetone, Saliva, Urine.

## 1. Introduction

Diabetes mellitus (DM) is one of the most common diseases in Indonesia. Based on the 2013 Basic Health Research data by the Ministry of Health, more than 12 million people were suffering from DM. Central Java Province is one of the regions with an increase in the prevalence of DM patients reaching 152,075 cases, where the highest DM patients were 5,919 in Semarang City.

DM is a metabolic disease caused by absolute or relative insulin deficiency and decreased insulin sensitivity, resulting in an increase in blood glucose levels or hyperglycemia [2], due to carbohydrate metabolic processes not running properly [1].

The most normal body energy produced from carbohydrate metabolism reaches 80%. Carbohydrates consumed will be broken down in the body into glucose by involving various digestive enzymes. The unavailability of glucose in the body because insulin dysfunction in DM patients can trigger an increase in lipolysis in adipose tissue, resulting in the release of fatty acids to be converted into energy through a process of fat metabolism [3].

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The use of fat as an energy source will produce ketones in the form of acetoacetic acid, betahydroxybutyric acid and acetone. Acetoacetic acid and beta-hydroxybutyric acid are used as metabolite fuels for skeletal and cardiac muscles and can meet some of the brain's energy needs, whereas acetone is a toxin-based waste product so the body will excrete it with urine [4]. DM Patients with high acetone levels will emit a distinctive aroma of breath resembling the smell of nail polish which indicates ketone contamination in saliva [7].

Acetone examination in both urine and salivary samples can be used as a parameter to strengthen the diagnosis of DM with acute complications such as diabetic ketoacidosis which measured using spectrophotometric methods [6]. This study aims to determine differences in salivary and urine acetone levels in DM patients.

#### 2. Method

The type of research is analytic with the cross-sectional approach. The population of the study was DM patients, both men and women aged 40-65 years old. The sampling used sequential random sampling technique that met the inclusion and exclusion criteria. The sample of the study consisted of 18 people based on the Slovin formula [9]. The data collection technique was by conducting a direct examination of salivary and urine acetone which measured using spectrophotometric methods. The specimens were taken in a fasting state. The data from the examination were tested for normality using the Shapiro-Wilk test, and followed by the Mann-Whitney test.

#### **3. Results And Discussions**

The optimization was carried out using 50 ppm acetone standard, 1,100 ppm and 2,000 ppm with a wavelength of 530, 540, 550, 560 and 570 nm. The results of absorbance readings are as in Table 1.

Table 1. Wavelength optimization results			
	Standard acetone		
Wavelength	concentration		
(nm)	(ppm)		
	50	1,100	2,000
530	0.000	0.110	0.213
540	0.002	0.128	0.229
550	0.005	0.134	0.235
560	0.001	0.125	0.222
570	0.000	0.115	0.213

Table 1 shows the optimum wavelength for determining acetone levels is 550 nm.

ble 2. Optimization results of Sample Stability 11			
	Standard acetone		
Time	concentration		
(minutes)	(ppm)		
	50	1,100	2,000
8	0.001	0.112	0.213
11	0.003	0.138	0.229
14	0.005	0.139	0.235
17	0.002	0.128	0.231

Table 2. Optimization results of Sample Stability Time		
Standard apptore		

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Table 2 shows that the acetone absorbance readings were 50 ppm, 1,100 ppm and 2,000 ppm at 550 nm wavelength incubated for 8, 11, and 14 minutes at room temperature increased, while the standard absorbance with 17 minutes incubation time decreased, so it can be known that the optimum stability time of the sample is 14 minutes.

The determination of the calibration curve was carried out using a standard acetone solution of 50-2000 ppm. The absorbance is read at a wavelength of 550 with an incubation time of 14 minutes. The data of acetone concentration on readable absorbance were processed using a simple linear regression technique so that a line equation was obtained as in Figure 1:



Figure 1. Acetone calibration curve

Figure 1 shows the acetone calibration curve 50 - 2,000 ppm obtained by the linear line equation that is y = 0,0001x + 0.0028 with R square = 0.9956. The linear line equation is used to calculate the acetone levels in salivary and urine samples.

The examination results of the acetone levels in salivary and urine samples as a whole as in Figure 2:



Figure 2. The examination results of acetone levels in salivary and urine samples

Figure 2 shows that of the 18 salivary samples examined, 10 samples were detected containing a number of acetone with the highest levels of 84 ppm (mg/L) while 8 other samples were negative with 0 mg/L. The results of urine acetone examination showed that 15 samples contained acetone with varying levels, the highest was 1,530 mg/L while the other 3 samples were negative. The mean value of salivary acetone is 18.9 mg/L, while urine acetone is 555.6 mg/L. The average difference in acetone levels from these two variables is quite high, namely 536.7 mg/L.

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The normality of the data was determined using the *Shapiro-Wilk* test obtained 0.000 and 0.260, then *Mann-Whitney* test obtained a significance value of 0.000 (p < 0.05), which means that there is a significant difference between salivary acetone levels and urine acetone levels.

Fukao et al. (2004) stated that the synthesis of ketone objects (acetone, beta-hydroxybutyric, acetoacetic acid) could occur when the body experiences severe hunger or because of intrinsic factors caused by insulin hormone disorders such as those experienced by diabetics. Such conditions trigger an increase in lipolysis in fatty tissue, and free fatty acids are formed into the substrate for ketogenesis in the liver [3]. This results in the contamination of blood plasma by ketones. Ketones will circulate to various organs and tissues of the body, one of which is a kidney. Ketones in the kidneys will escape the process of filtration and reabsorption so that they will be excreted with urine. But when excessive ketogenesis occurs, ketones will accumulate to contaminate saliva [8].

The use of urine samples for acetone examination other than blood plasma is preferred because when the process of ketogenesis occurs, the kidneys will immediately excrete acetone with urine so that it can better describe the actual acetone level, while in saliva, acetone will be detected when urine acetone levels are very high. In this study, salivary acetone levels have only been read when urine acetone levels have reached >500 mg/L. This shows the correlation between the two variables, namely the higher the level of urine acetone, the higher the acetone content contained in saliva.



Figure 3. Salivary deproteination process



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## Figure 4. Supernatant screening process

Figure 5. Reading sample absorbance

## 4. Conclusion

Urine samples with smaller acetone concentrations are able to give positive results, while salivary samples will show positive results when acetone levels in urine are very high.

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