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EFFECT OF DELAYING SERUM SEPARATION ON THE RESULTS OF BLOOD SUGAR FASTING EXAMINATION

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Abstract

Blood sugar fasting measurement is crucial for the diagnosis and management of diseases caused by carbohydrate metabolism disorders. One factor influencing the accuracy of blood glucose test results is the timing of serum separation. Delays in serum separation result in glycolysis, which can decrease glucose levels by 10 mg/dL per hour. The purpose of this study was to determine the effect of delayed serum separation on blood sugar fasting test results using time intervals without delayed separation, a 1-hour delay, and a 2-hour delay. The research used a simple experiment. The sample size for this study was 30 individuals, with three treatment categories no separation delay, a 1-hour delay, and a 2-hour delay, resulting in a total of 90 samples. The sampling technique used in this study was accidental sampling. Based on the results of the Kruskal-Wallis test, a p-value of 0.000 was obtained, which indicates that there is an effect of delaying serum separation on the results of blood sugar fasting examinations using a serum separation time range without delaying separation, a 1-hour delay in separation, and a 2-hour delay in separation. The average blood sugar fasting level in samples without separation delay was 91 mg/dL, after a 1-hour separation delay the average blood sugar fasting test results decreased to 76 mg/dL and at a 2-hour separation delay the blood sugar fasting test results decreased again to 65 mg/dL. Delayed serum separation greatly affects the results of blood sugar fasting tests, it is known that the longer the delay in serum separation results in a decrease in blood sugar fasting test results.

Keywords: Blood glucose, Delay, Separation time

1. INTRODUCTION

Clinical laboratories are a key component in establishing a disease diagnosis. Various tests performed by laboratory personnel can result in errors at the pre-analytical, analytical, and post-analytical stages. Errors are most likely to occur in the pre-analytical stage, where 60% to 70% of laboratory errors, including issues with sample identification and suitability, are caused by

errors during this stage (Azizah, 2023). One of the examinations that requires caution in every stage, especially in the pre-analytical stage, is the blood glucose examination (Sunita, 2021).

Blood glucose serves as an energy source to support tissue and cell activity. Blood glucose tests include blood sugar fasting (Nuchter), blood sugar random, and blood sugar postprandial (Yana & Yuliana, 2021). Blood sugar fasting test is crucial for diagnosing and treating conditions caused by abnormalities in carbohydrate metabolism. Glucose is essential for the survival of cells that are still actively metabolizing energy, such as muscle cells and blood cells. Blood sugar fasting testing must be performed accurately and promptly (Putra et al., 2012).

Cells that actively utilize glucose even outside the body are blood cells, both red and white blood cells. This use of glucose for blood cell metabolism is what causes blood glucose levels to decrease, even after a blood sample has been taken and is outside the body. Serum samples are often used for testing because their glucose levels are more consistent. The serum separation process is carried out no later than two hours after the blood draw. Serum that meets the examination criteria is serum that is not red and cloudy (lipemic) (Departemen Kesehatan RI, 2008).

Kiswari (2014) stated that to ensure accurate and precise test results, laboratory staff must supervise and pay attention to every step of the specimen handling process. Specimen examination should be conducted within 45 minutes to 1 hour after sample collection. Delaying serum separation results in decreased blood glucose levels. Most of the blood glucose is used for blood cell metabolism. Delayed separation risks microbial contamination of the sample. Blood glucose levels in serum samples are stable at 2-8°C for 12 hours (Rahmatunisa et al., 2021).

Based on the results of Azizah's research (2023), it shows that the average results of glucose examinations carried out by immediate centrifugation, 1 hour delay, 2 hours, 3 hours and 4 hours are 78.1 mg/dL, 75.2 mg/dL, 71.8 mg/dL, 66.9 mg/dL and 65.1 mg/dL where the results indicate that there is an effect of variations in serum separation delay time on glucose examination results. According to another study conducted by Trisyani et al., (2020), a significant decrease was obtained in samples that experienced a 1 hour delay with a decrease in blood glucose levels of 4-6% per hour.

Research conducted by Santi et al., (2011) showed that the group of serum samples stored at a temperature of 2–8 °C had no effect on the group of serum analyzed directly, and the group stored for 4 hours had no effect on the group of serum stored at a temperature of 25–28 °C. According to another study conducted by (Sunita, 2021) the results showed that there was no significant effect on the delay of samples separated within 2 hours and 4 hours.

Based on the results of a field survey, sometimes the MCU (Medical Check Up) sample collection has been carried out several hours before arriving at the laboratory, where the average time taken from the MCU (Medical Check Up) implementation from the sample collection process to the laboratory takes around 2 until 3 hours. Whereas according to theory the serum fragmentation process is carried out no later than two hours after blood collection (Departemen Kesehatan RI, 2008). This can occur due to several factors such as the workload of officers, where sampling officers take samples in large quantities so that they cannot be immediately examined

back to the laboratory. In addition, it is also influenced by the time-consuming sample delivery process, insufficient centrifuge capacity and the presence of blood sugar fasting samples in the laboratory for examination so that they cannot be carried out at the same time (immediately after the blood sample is frozen).

The results of different studies, combined with the results of field surveys conducted where the average time taken from the implementation of MCU (Medical Check Up) starting from the sample collection process to the laboratory takes around 2 hours to 3 hours, even exceeding the ideal time limit of two hours according to the guidelines of the Departemen Kesehatan RI, 2008, which has the potential to cause inaccurate glucose test results and can lead to underdiagnosis of conditions such as diabetes mellitus or pre-diabetes. These varying results indicate the need for further research to understand the effect of delayed serum separation on blood sugar fasting test results using a range of serum separation times without delayed separation, delayed separation of 1 hour and 2 hours. This research is important to inform that delayed serum separation can result in inconsistencies in blood glucose test results with the actual patient's condition.

The main objective of this study was to determine and analyze the effect of delayed serum separation on blood sugar fasting test results. This is important because delays in the pre-analytical stage, particularly in the serum separation process, can cause a decrease in glucose levels due to glycolysis activity by blood cells. This decrease has the potential to produce inaccurate results and directly impact diagnostic interpretation, especially in the early detection of metabolic disorders such as diabetes mellitus. Through this study, it is hoped that valid data can be obtained regarding changes in glucose levels due to delayed serum separation within certain time periods (0 hour, 1 hour, and 2 hours), and provide a scientific basis for improving standard operating procedures in laboratories, especially in Medical Check Up (MCU) activities that often face time and logistical constraints.

2. METHOD

The type of research used is a simple experiment. The simple experiment conducted in this study was to determine the effect of serum exposure on blood sugar fasting test results by providing a treatment to the research subjects. Sampling and examination processes were carried out at the Ananta Clinical Laboratory in March-April 2025. The population used was all MCU (Medical Check Up) patients who underwent blood sugar fasting tests. The sample used in this study was 30 people with three treatment categories no separation delay, delayed for 1 hour, and delayed for 2 hours, resulting in a total of 90 samples. The sampling technique in this study was accidental sampling or convenience sampling, which is a non-probability sampling method. The researcher did not determine any specific criteria other than those established for the study, and respondents were drawn spontaneously until the required sample size was reached. This process can be stopped when the sample size is sufficient for the planned analysis. The independent variable in this study was the serum separation delay, which was divided into no separation delay, a 1-hour delay, and a 2-hour delay. The dependent variable in this study

was the results of blood sugar fasting examination. The tools used in this study were a red-capped vacutainer tube (plain), a Hettich EBA 200 centrifuge, a Cobas C111 apparatus, a micropipette, a blue tip, a yellow tip, a cuvette, a flash vacutainer needle, a holder, a plaster cast, a coolbox, a tourniquet, and a timer. The materials used were a 70% alcohol swab, ice gel, a blood sugar fasting sample, and a glucose reagent (Glucose HK (GLUC2)). The data obtained were presented in tabular form and then analyzed using statistical tests using the Statistical Product and Service Solutions (SPSS) version 26 program. The data were tested to see whether there were differences in the effect of serum separation treatment by providing separation treatment using a time span without separation delay, 1 hour separation delay and 2 hour separation delay using the Kruskal-Wallis Test.

3. RESULT AND DISCUSSION

3.1 RESULT

Table 1.1 Results of blood sugar fasting examination on serum samples without time extension

	ıber of respondents	rcentage (%)
ʒlycemia	3 person	10
	27 person	90
Total	30 person	100

Based on Table 1.1 blood sugar fasting tests on serum samples without delayed separation were performed, with the majority of respondents (90%) falling into the normal category. The average blood sugar fasting test on serum samples without delayed separation was 91 mg/dL.

Table 1.2 Blood sugar fasting test results on serum samples that experienced a 1-hour separation delay

Category	ıber of respondents	ercentage (%)
ʒlycemia	19 person	63,3
	11 person	36,7
Total	30 person	100

Based on Table 1.2 blood sugar fasting tests performed on serum samples that were separated by a 1-hour delay were found to be predominantly in the mild hypoglycemia category, with 19 respondents (63,3%). The average blood sugar fasting test result for the 1-hour delayed-separation serum samples was 76 mg/dL. In the 1-hour delayed-separation serum samples, the blood sugar fasting test result decreased by 15 mg/dL compared to the test result for the unseparated serum samples.

Table 1.3 Blood sugar fasting test results on serum samples that experienced a 2-hour separation delay

Category	ber of respondents	ercentage (%)
Iypoglycemia		13,3
ɜlycemia	22 person	73,3
	4 person	13,3
Total	30 person	100

Based on Table 1.3, blood sugar fasting tests were performed on serum samples that experienced a 2-hour separation delay, and the dominant results were found in respondents who fell into the mild hypoglycemia category, namely 22 people (73,3%). The average blood sugar fasting test result in serum samples that experienced a 2-hour separation delay was 65 mg/dL. In serum samples that experienced a 2-hour separation delay, the blood sugar fasting test result was found to decrease by 26 mg/dL compared to the test result in serum samples without a separation delay.

The results of the effect of delayed serum separation on blood sugar fasting test results using the Kruskal-Wallis Test obtained a p-value of 0.000. This indicates that the p-value <0.05 means that H0 is rejected and Ha is accepted. This indicates that there is an effect of delayed serum separation on blood sugar fasting test results using a serum separation time range without delayed separation, a 1-hour delayed separation, and a 2-hour delayed separation.

3.2 DISCUSSION

Based on the results of blood sugar fasting tests on serum samples without delayed separation, 27 (90%) individuals had normal results and 3 (10%) had mild hypoglycemia. These results indicate that those who underwent blood sugar fasting tests on serum samples without delayed separation predominantly had normal results, with an average blood sugar fasting test result of 91 mg/dL. This is because blood sugar fasting tests were performed using serum samples from respondents undergoing a medical check-up (MCU) and not those with diabetes mellitus.

A medical check-up (MCU), specifically a blood sugar fasting test, is typically performed as an initial screening for diseases such as diabetes mellitus. Initial screening is also considered a crucial step in the prevention and early detection of non-communicable diseases. The screening process can help identify various risk factors and recognize early symptoms of a disease (Lima et al., 2020).

The finding of mild hypoglycemia in 3 individuals (10%) during blood sugar fasting testing without delayed serum separation may be due to several factors, such as the length of fasting time. According to Nugraha & Badrawi (2018), the recommended fasting time for blood glucose testing is 10-12 hours. During blood sugar fasting levels drop, leading to decreased insulin secretion, which in turn increases the activity of counter-insulin hormones, glucagon and catecholamines, which promote glycogen breakdown. Excessive fasting can lead to reduced glycogen reserves and can lead to decreased blood glucose levels (Soelistijo et al., 2019).

Another factor that can influence blood sugar fasting test results is the respondent's age. Wulandari (2020) explains that a person's glucose intolerance increases with age. In the elderly, glucose intolerance is often associated with conditions such as being overweight, lack of exercise or decreased physical activity, decreased muscle mass, having other diseases, and use of medications. As a person ages, insulin production decreases and insulin resistance increases. In addition to increasing glucose intolerance, age can also influence decreased glucose levels, especially in the elderly, due to several factors such as decreased kidney function, decreased appetite, irregular eating patterns, and decreased sensitivity to symptoms of low blood glucose.

Based on the results of blood sugar fasting tests on serum samples delayed by 1 hour, 11 (36,7%) had normal results and 19 (63,3%) had mild hypoglycemia. These results indicate that blood sugar fasting tests on serum samples delayed by 1 hour resulted in a dominant increase in mild hypoglycemia, with 19 (63,3%) having a mean blood sugar fasting test result of 76 mg/dL after a 1-hour delay.

These results align with research conducted by Trisyani et al., (2020) that compared blood glucose levels in samples subjected to varying separation delay lengths. They found a significant decrease in samples subjected to a 1-hour delay, with a 4-6% decrease in blood glucose levels per hour. Therefore, this underscores the importance of proper preanalytical steps, particularly sample preparation.

Blood levels can fluctuate during sample collection or storage due to a number of factors, such as length of storage and separation time that can alter protein properties, movement of water into cells that can result in hemoconcentration, evaporation of volatile compounds and cellular metabolic activity. Serum samples undergo significant changes after prolonged direct interaction with blood cells. If not immediately separated, blood glucose is one of the unstable tests in blood samples Kiswari (2014).

Based on the results of blood sugar fasting examination on serum samples that experienced a 2-hour separation delay, normal results were obtained in 4 people (13,3%), mild hypoglycemia in 22 people (73,3%) and moderate hypoglycemia in 4 people (13,3%). These results indicate that blood sugar fasting examination on serum samples that experienced a 2-hour separation delay showed a dominant result of mild hypoglycemia, which increased by 22 people (73,3%) with an average result of blood sugar fasting examination with a 2-hour separation delay of 65 mg/dL and 4 people (13,3%) showed moderate hypoglycemia results. This is in line with research conducted by Agustin (2018), which stated that there was a significant difference between blood glucose levels that were checked immediately and those that were delayed by 2 hours, where at a 2-hour delay, the difference in blood glucose levels decreased by 2.81 mg/dL.

Research results obtained from blood sugar fasting testing show that the longer the delay in serum separation, the higher the number of respondents showing mild hypoglycemia. A 2-hour delay in separation indicates a higher number of respondents showing moderate hypoglycemia, while the number of respondents showing normal results decreases. This is because the cells that actively utilize glucose even outside the body are blood cells, both red and white blood

cells. The use of glucose for blood cell metabolism is what causes blood glucose levels to decrease, even after the blood sample has been taken and is outside the body (Putra et al., 2012).

Based on data analysis using the Kruskal-Wallis test, a p-value of 0.000 was obtained. This indicates a p-value of <0.05, indicating that H0 is rejected and Ha is accepted. Therefore, serum separation with a time interval of no delay, 1-hour extension, and 2-hour extension has a statistically significant effect. This study aligns with research by Putra et al. (2012), which showed a statistically significant difference with a p-value of 0.000 between blood glucose levels measured directly and those with a delay for 1, 2, 3, and 4 hours.

Another study conducted by Azizah (2023) obtained a p-value indicating that there was an effect of varying serum separation delay time on blood glucose levels. Similarly, a similar study conducted by Trisyani et al. (2020) obtained a p-value of 0.003, indicating a statistically significant difference in the hourly decrease in blood glucose levels. In this study, delaying serum separation significantly affected blood sugar fasting test results. It was found that the longer the delay in serum separation, the lower the blood sugar fasting test results. This aligns with the statement of Nur Aini et al. (2022), who stated that delaying serum sample separation for glucose testing results in glycolysis, which can reduce glucose levels by 10 mg/dL per hour in each sample.

Laboratory tests must be conducted in accordance with established procedures to obtain accurate, rapid, and reliable results. Laboratory tests, particularly blood sugar fasting tests, involve three stages, one of which is the pre-analytical stage, which contributes most to laboratory errors. One of the processes included in the pre-analytical stage is specimen management, including separation (centrifugation) (Cahyani & Parwati, 2022).

Blood sugar fasting testing can be affected by several factors, including serum separation (centrifugation), which is the process of using a centrifuge to separate solid particles dispersed in a liquid medium. Serum must be separated from blood cells immediately because red and white blood cells outside the body can break down glucose for metabolism. Delayed centrifugation can result in decreased blood glucose test results. Glycolytic enzymes are found in erythrocytes and leukocytes, which allow glucose to be absorbed and glucose concentrations to gradually decrease (Putra et al., 2012).

Blood sugar fasting measurement is crucial for the diagnosis and treatment of diseases caused by carbohydrate metabolism disorders. The fundamental importance of blood sugar fasting testing is that it must be performed promptly and accurately because glucose is essential for the survival of cells that are still actively metabolizing energy, such as muscle cells and blood cells. Blood cells, both red and white, are the only cells that actively utilize glucose even when outside the body. Glucose levels can decrease even if a blood sample has been taken and is outside the body, because glucose is used for blood cell metabolism (Putra et al., 2012).

4. CONCLUSION

Based on the data on the effect of serum separation on the results of blood sugar fasting examinations, it was concluded that there was an effect of serum separation delays on the

results of blood sugar fasting examinations using a serum separation time range without separation delays, 1 hour separation delays and 2 hour separation delays.

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