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Pre-Analytical Stability of Fasting Blood Glucose: A Comparison Between Immediate Analysis and Two-Hour Room Temperature Storage

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ABSTRACT

The stability of blood samples for glucose testing is a critical preanalytical factor. In vitro glycolysis at room temperature can significantly decrease glucose levels, potentially leading to inaccurate diagnostic results. This study aimed to compare fasting blood glucose levels examined immediately and those stored for 2 h at room temperature. This descriptive, cross-sectional study involved 35 serum samples from participants undergoing fasting blood glucose tests at the Sintang Regional Health Laboratory. Each sample was split into two aliquots: one was analyzed immediately after processing (baseline), and the other was analyzed after being stored for 2 hours at room temperature (25-30°C). Glucose levels were measured using the GOD-PAP method with a Microlab 300 spectrophotometer. Statistical analysis was performed using the Shapiro-Wilk normality test and paired T-test to compare the differences in glucose levels between the two groups. The mean glucose level examined immediately was 90.8 mg/dL (SD=5.098), while the level after the 2-hour delay was 85.8 mg/dL (SD=4.451), showing an average decrease of 5.0 mg/dL. The data were normally distributed (p=0.129), and the paired T-test showed a highly statistically significant difference (p<0.001) between the two examination conditions. Storing serum samples at room temperature for 2 h significantly decreased glucose levels. This finding underscores the importance of immediate sample processing for glucose testing to ensure accurate diagnosis. If a delay is unavoidable, sample refrigeration is highly recommended to inhibit the glycolysis process.

Keywords: *Fasting Blood Glucos; Sample Stabilit; Room Temperatur; Glycolysis; Pre-Analytical Factor; GOD-PAP*

ABSTRAK

Stabilitas sampel darah untuk pemeriksaan glukosa merupakan faktor pra-analitik yang kritis. Glikolisis in vitro pada suhu ruang dapat menurunkan kadar glukosa secara signifikan, berpotensi menyebabkan hasil diagnostik yang tidak akurat. Penelitian ini bertujuan untuk menganalisis perbandingan kadar glukosa darah puasa yang diperiksa secara langsung dengan yang disimpan selama 2 jam pada suhu ruang. Penelitian deskriptif dengan desain potong lintang ini melibatkan 35 sampel serum dari subjek yang menjalani pemeriksaan glukosa darah puasa di Laboratorium Kesehatan Daerah Sintang. Setiap sampel dibagi dua: satu aliquot diperiksa segera setelah pemrosesan (baseline), dan aliquot lainnya diperiksa setelah disimpan selama 2 jam pada suhu ruang (25-30°C). Kadar glukosa diukur dengan metode GOD-PAP menggunakan spektrofotometer Microlab 300. Analisis statistik menggunakan uji normalitas Shapiro-Wilk dan uji T berpasangan untuk membandingkan perbedaan kadar glukosa antara kedua kelompok. Kadar glukosa rata-rata yang diperiksa segera adalah 90.8 mg/dL (SD=5.098), sedangkan kadar setelah penundaan 2 jam adalah 85.8 mg/dL (SD=4.451), menunjukkan penurunan rata-rata sebesar 5.0 mg/dL. Data berdistribusi normal ($p=0.129$) dan uji T berpasangan menunjukkan perbedaan yang sangat signifikan secara statistik ($p<0.001$) antara kedua kondisi pemeriksaan. Penyimpanan sampel serum pada suhu ruang selama 2 jam menyebabkan penurunan kadar glukosa yang signifikan. Temuan ini menekankan pentingnya pemrosesan sampel yang segera untuk pemeriksaan glukosa guna menjamin keakuratan hasil diagnostik. Jika terjadi penundaan, pendinginan sampel sangat dianjurkan untuk menghambat glikolisis.

Keywords: Glukosa Darah Puasa; Stabilitas Sampel; Suhu Ruang; Glikolisis; Faktor Pra-Analitik; GOD-PAP

INTRODUCTION

Glucose examination procedures in clinical laboratories are essential for diagnosing and managing diabetes mellitus. The primary method is the measurement of glucose concentrations in venous plasma and hemoglobin A1c (HbA1c) in the blood. These tests provide indicators of a patient's glycemic control and are critical for diagnosis and ongoing management of the disease (1,2)

Glucose laboratory clinics play a vital role in the management and diagnosis of diabetes by offering a range of specialized services such as glucose monitoring, testing, and consultation. These clinics often utilize advanced technology, including continuous glucose monitoring (CGM) devices, to provide accurate and timely data on patients' glucose levels. CGM systems, which continuously measure blood glucose levels day and night, offer valuable insights into glycemic control, enabling both clinicians and patients to make informed treatment adjustments (3).

The stability of blood samples for glucose testing is a critical preanalytical factor, as it directly influences the accuracy of diagnostic results and the effective management of conditions such as diabetes mellitus. This stability is primarily governed by three key factors: time, temperature, and the handling of the sample. Even short delays between blood collection and processing can lead to significant glucose degradation through glycolysis, artificially lowering the measured concentrations. The rate of degradation is highly dependent on storage temperature, and maintaining consistent, cool conditions is essential to slow the metabolic process. Furthermore, proper sample handling is paramount, as vigorous shaking or improper mixing can cause hemolysis, which, in turn, interferes with the accurate measurement of glucose concentration. Therefore, a strict protocol controlling all these variables is necessary to ensure the integrity of the results (4,5)

Storage temperature plays a pivotal role in the stability of glucose in blood samples. According to a study evaluating the stability of 65 analytes, including glucose, delayed centrifugation of blood stored at room temperature can lead to significant instability, affecting the accuracy of glucose measurements (6).

When blood samples are collected, glucose levels can decrease over time due to continued glycolytic activity by cells within the sample, particularly red blood cells. This is primarily because these cells continually metabolize glucose to maintain their energy requirements, even after blood has been drawn and isolated in a test tube. Glucose uptake is initiated by transporters such as GLUT1 and GLUT4, which facilitate the movement of glucose into cells (7).

Once inside, glucose undergoes several enzymatic transformations through the glycolysis pathway, where it is ultimately broken down into pyruvate, producing ATP and NADH. This series of reactions helps reduce the glucose levels in blood samples over time (8)

Glycolysis is subject to regulation by various factors, including hormonal signals. Insulin, for example, facilitates glycolysis, thereby decreasing blood glucose levels. In contrast, hormones such as glucagon and epinephrine can inhibit glycolysis and promote gluconeogenesis, thereby increasing glucose levels when necessary (9)

Understanding the ongoing glycolytic process in blood samples is crucial in clinical contexts, especially when diagnosing and managing metabolic diseases such as diabetes, in which blood glucose regulation is disrupted. Thus, knowledge of glycolysis not only aids in the efficient management of glucose levels but also assists in strategizing proper therapeutic interventions (10,11).

In daily practice within healthcare facilities, limitations in refrigeration equipment or challenges during the transport process often make it impossible to examine blood samples immediately or refrigerate them promptly (12). Consequently, samples are frequently stored at room temperature for a certain period before being analyzed in the laboratory. This situation raises concerns about the accuracy of glucose test results, as glucose in blood samples is known to potentially decrease in concentration in vitro due to ongoing glycolysis.

Therefore, empirical evidence is required regarding the extent of decrease in glucose levels after samples are stored for 2 hours at room temperature. This study aimed to analyze the significant difference between glucose levels examined immediately (baseline) and glucose levels from the same samples examined 2 hours after being stored at room temperature to provide clear recommendations regarding the safe window for examination under real-world conditions in healthcare facilities.

METHODS

Research Design

This study employed a descriptive design with a cross-sectional method to analyze fasting blood glucose levels in the serum, comparing immediate checks with those delayed by 2 hours.

Population and Sample

The study population included all individuals who underwent fasting blood glucose testing at the Sintang Regency Regional Health Laboratory during a designated one-month period. A total of 35 participants were included in the study sample, which was drawn from this population during standard working hours.

Sampling Techniques

The sampling technique employed in this study was accidental (convenience) sampling. This non-probability method involved selecting participants based on their availability and willingness to participate during the data collection period. Sampling was conducted over a one-month period during the laboratory's operational hours. The study subjects were patients who presented for fasting blood glucose testing and met the following criteria: inclusion criteria comprised adult patients who had fasted for 8-12 hours prior to testing; exclusion criteria included patients who were non-fasting, had consumed caloric beverages, or were unable to provide informed consent.

Procedures

Blood Collection

Blood collection for fasting glucose analysis was performed via venipuncture of the median cubital vein. The standardized protocol involved arm immobilization, tourniquet application, and antisepsis of the puncture site with 70% alcohol. Following successful venipuncture and blood aspiration, the tourniquet was released, the needle was withdrawn, and hemostasis was achieved by applying pressure with dry cotton and a bandage

Serum Separation Procedure

Post-collection sample processing involved transferring blood to serum separation tubes. After clot formation, the samples were centrifuged, and the clarified serum was aliquoted using a micropipette. To evaluate the preanalytical storage effects, a split-sample protocol was implemented: one aliquot was analyzed immediately (baseline), while a paired aliquot was stored at room temperature (25 – 30^{o}C) for two hours before identical testing.

Examination Procedure

Quantitative analysis of blood glucose was performed using the glucose oxidase/peroxidase (GOD-PAP) method with Human Glucose GOD-PAP reagents. Spectrophotometric measurements were conducted using a Microlab 300 spectrophotometer, which was configured with optimized parameters, including a wavelength of 546 nm and a 1 cm optical path length, while the instrument's integrated thermostat maintained a constant reaction temperature of 37°C. Prior to the measurement of all patient samples, a reagent blank was used to calibrate the spectrophotometer, establishing a reliable baseline to ensure analytical accuracy and precision of the results.

Table 1. examination procedure

Reagent	Blank	Cal/std	Test
Glucose Reagent	1000 ul	1000 ul	1000 ul
Standar/ Calibrator	-	10 ul	-
Sampel	-	-	10 ul

Absorbance (A) was recorded after 10 min of incubation.

Reading Result Technique

Fasting blood glucose levels were quantified in serum samples using the GOD-PAP method. A comparative analysis was conducted by measuring the analyte concentration in two sample sets: one was analyzed immediately post-processing, and the other was analyzed following a two-hour incubation period at room temperature.

Data Processing

The data were processed in a sequence of steps to ensure the quality. First, during data editing, all patient information was checked for accuracy and completeness, and any issues were corrected. Next, each Blood Sample was assigned a unique code (Blood Sample 1–35). The coded data were entered into a computer database for analysis. Finally, the data were cleaned by carefully rechecking for entry errors or inconsistencies to ensure that the dataset was valid and reliable for analysis.

Data Analysis

Data were analyzed descriptively to describe the fasting blood glucose levels in serum samples taken immediately and those delayed for 2 hours. The results are reported in nominal terms only. This was followed by a description of the fasting blood glucose levels in serum samples taken immediately and those delayed for 2 hours in room temperature

RESULT

This study was conducted at the Sintang Regional Health Laboratory for sample testing, with 35 samples. Based on the results of the examination, the following data were obtained

Table 2 Examination result

	Direct	2 hours
N	35	35
Mean	90.8	85.8
Median	91	86
Std dev	5.098	4.451
Minimum	81	78
maximum	99	95

As shown in Table 2, blood glucose levels were measured in 35 samples under two conditions: immediately after processing and after a 2-hour delay. The results showed that glucose levels were consistently lower after a 2-hour delay. The average level decreased from 90.8 mg/dL to 85.8 mg/dL. The data also showed less variation after the delay, with values ranging from 78-95 mg/dL compared to 81-99 mg/dL in the immediate measurements.

Table.3 Statistic Test

Tes	p-value
Normality	0.129
Paired T-Tes	< 0.001

Based on Table 3, the statistical analysis confirms that the data are reliable, as evidenced by a passed normality test ($p=0.129$), indicating consistent and predictable patterns in glucose level changes, while the highly significant Paired T-Test result ($p<0.001$) provides strong evidence that the observed decrease in blood glucose levels following a 2-hour delay represents a genuine effect rather than random variation.

DISCUSSION

The impact of preanalytical sample handling is significant in achieving reliable and accurate results. Changes in sample handling, such as delays in centrifugation or inappropriate storage conditions, can lead to variations in the measured levels of blood constituents. For instance, maintaining a consistent temperature control is essential. It has been observed that the handling temperature of the blood samples can significantly influence the measurement outcomes of various components. Protocol deviations, such as delayed centrifugation or storage at room temperature, can cause considerable changes in analyte levels, potentially affecting diagnostic accuracy (13).

Glycolysis is a metabolic pathway that converts glucose to pyruvate, generating energy in the form of ATP and NADH. It involves a series of enzymatic reactions that primarily occur in the cytoplasm of cells. Glycolysis begins with the phosphorylation of glucose by hexokinase, a rate-limiting enzyme, leading to a series of steps that ultimately results in the production of pyruvate, ATP, and NADH. This process is crucial for energy production, especially in cells where oxygen availability may be limited, serving as a primary source of ATP under anaerobic conditions (14,15).

The storage of glucose, particularly at room temperature, can lead to its degradation via chemical and biological processes. High ambient temperatures facilitate glucose oxidation. For instance, in sugar-based systems, glucose undergoes deep oxidation at room temperature, converting into various acids which indicates its degradation over time (15). Additionally, the stability of glucose can be affected by microbial activity, as seen in coconut water stored at room temperature, where glucose levels decline as microorganisms proliferate(16). The decline in glucose levels in serum left at room temperature for 2 hours can be attributed to several factors related to preanalytical stability. Glucose values decrease due to ongoing cellular metabolism after blood separation into serum. This occurs because red blood cells and remaining leukocytes in serum continue to consume glucose through glycolysis, a process that persists. Room temperature significantly affects serum glucose stability if blood sample processing is delayed. When blood samples are left at room temperature, serum glucose concentration declines due to glycolysis, the enzymatic breakdown of glucose by red blood cells and leukocytes. This process can lead to erroneous laboratory results if not accounted for or if samples aren't processed promptly. Serum glucose levels decrease in whole blood specimens stored at room temperature for extended periods, such as 8 hours or more, due to glycolytic activity. (6).

If the serum remains in contact with cells for a prolonged duration prior to centrifugation, glucose levels may diminish. Research on other analytes indicates that serum biomarkers can maintain stability for specific durations under certain conditions, such as room temperature for up to 24 hours in some instances; however, this stability may not extend to glucose in the absence of glycolysis inhibitors (17). The temperature at which blood is held before processing significantly influences serum glucose levels. Storage at room temperature can result in a reduction of glucose due to metabolic activities within the blood cells. To ensure stability, it is advisable to refrigerate samples at 2-8 °C if there is any delay in processing (13). For other stable analytes, such as miRNAs in glycosylated extracellular vesicles, guidelines recommend the separation of serum within two hours at either 4 °C or 25 °C to prevent degradation, underscoring the importance of timely processing even at room temperature. Once serum is separated, the storage temperature becomes a critical factor in maintaining stability (18).

In clinical chemistry, a delay in glucose examination can lead to a decrease in glucose levels even after centrifugation, primarily due to the preanalytical handling of the samples. Delayed processing of samples can cause alterations in various analyte levels, including glucose, which is often due to ongoing cellular metabolism that continues to consume glucose in the blood after the sample collection. This metabolism persists until the sample is processed, and centrifugation alone is insufficient to halt this process (13).

A research study investigating the stability of various biochemical analytes discovered that glucose is significantly impacted by delays in processing at room temperature. The study highlights that delays in centrifugation and storage at room temperature can lead to instability and inaccurate glucose measurements (6).

It is important to recognize that the stability of glucose can be affected by the type of container and anticoagulant used during sample collection. Ideally, glucose levels should be measured immediately, or the samples should be stored under specific conditions, such as refrigeration or with the use of glycolysis inhibitors, to prevent degradation and ensure accurate results (6)

In the field of clinical chemistry, assessing glucose levels is crucial, especially for managing diabetes. Glucose can be evaluated using different techniques, such as glucose meters and continuous glucose monitoring (CGM) systems. Nevertheless, issues like lipemic interference in samples can compromise the precision of glucose tests. Utilizing high-speed centrifugation is a successful approach to minimizing this interference, particularly in hexokinase-based methods (19).

In diagnosing and managing diabetes, glucose levels can be evaluated through a range of laboratory tests. These tests involve examining glucose concentrations in venous plasma and determining hemoglobin A1c (HbA1c) levels. The guidelines for these assessments have been meticulously reviewed to ensure precise diagnosis and effective diabetes monitoring (1,2).

Continuous glucose monitoring systems are integral in the assessment of blood glucose levels, particularly in non-diabetic contexts or during periods of rapid glucose fluctuation. These systems provide valuable insights, although they may demonstrate variability and discrepancies when compared to conventional blood glucose measurement techniques (20).

To ensure the accuracy of glucose measurements, it is imperative to either process blood samples promptly or employ additives that inhibit glycolysis during storage. This approach minimizes deviations from baseline glucose levels, thereby maintaining the precision of results for clinical assessments or research purposes (6)

CONCLUSION

Based on the research findings, it was concluded that storing blood samples at room temperature led to a statistically significant decrease in glucose levels after 2 hours. The average glucose level dropped from 90.8 mg/dL to 85.8 mg/dL, primarily due to ongoing glycolysis in the blood cells. This finding confirms the vulnerability of glucose test results to preanalytical factors, specifically time and storage temperature. Therefore, to ensure diagnostic accuracy, blood samples for glucose testing should be processed immediately after collection. If a delay is unavoidable, the samples must be refrigerated (2-8°C) to inhibit glycolysis and maintain the integrity of the test results.

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