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ORIGINAL RESEARCH

To assess the blood glucose concentration in EDTA/F plasma and serum samples at a clinical laboratory

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ABSTRACT

Aim: To assess the blood glucose concentration in EDTA/F plasma and serum samples at a clinical laboratory. **Materials and Methods:** A cross-sectional observational study was conducted at our hospital to evaluate the glucose concentrations in blood samples collected under different conditions. A total of 50 participants were included in the study, regardless of their age, gender, fasting status, or underlying medical conditions. Each participant's blood glucose levels were measured on four different occasions, with samples collected simultaneously but processed under varying conditions.All blood samples were collected in the laboratory's phlebotomy room, ensuring immediate processing and eliminating any delays in transportation or exposure to environmental temperature fluctuations. Glucose concentrations were determined spectrophotometrically using the Randox Imola auto-analyzer (Randox Laboratories Limited, UK) via the glucose oxidase-peroxidase method. **Results:** Initially, at 30 minutes post-collection, the mean glucose concentration in plasma (P1a) was 102.5 mg/dL, slightly higher than the serum (S1a) concentration of 100.2 mg/dL. The standard deviations were similar, indicating comparable variability in measurements for both sample types. The plasma mean dropped to 101.0 mg/dL, and the serum mean to 98.7 mg/dL. These changes indicate that glucose concentrations can slightly decrease over time, even when samples are stored properly at 4°C, though the variability remained similar.For samples that were subjected to delayed centrifugation (P2a and S2a), the mean glucose levels were slightly lower than those processed immediately, with plasma at 100.0 mg/dL and serum at 97.5 mg/dL. The consistent pattern of plasma having slightly higher glucose concentrations than serum persisted across all time points. **Conclusion:** For blood glucose measurement in serum to be considered valid, the separation of serum by centrifugation must be completed within a maximum of 30 minutes. Using serum tubes has the benefits of avoiding excessive blood draws and improving turnaround time. Every collection facility must be equipped with a centrifuge to ensure timely blood separation.

Keywords: Blood glucose concentration, EDTA/F plasma, Serum

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INTRODUCTION

Blood glucose concentration is a critical parameter in the diagnosis and management of various metabolic disorders, most notably diabetes mellitus. Accurate measurement of blood glucose levels is essential for both the diagnosis of these conditions and the ongoing monitoring of patients' glycemic status. In clinical laboratories, blood glucose is typically measured using either plasma or serum samples, each of which has its own advantages and potential limitations. The choice between these two types of samples can influence the results, and understanding the nuances of each is important for ensuring accurate and reliable glucose measurements.[1]Plasma and serum are both derived from whole blood but are processed differently. Plasma is obtained by centrifuging whole blood that has been treated with an anticoagulant, such as ethylenediaminetetraacetic acid (EDTA) or fluoride (F). This process prevents the blood from clotting, allowing the liquid component, or plasma, to be separated and analyzed. Serum, on the other hand, is obtained by allowing whole blood to clot naturally, after which it is centrifuged to remove the clot, leaving the serum as the liquid component. While both plasma and serum contain glucose, the concentrations can vary slightly due to the differences in their preparation.[2]One of the most common anticoagulants used in the collection of plasma for glucose measurement is sodium fluoride, often combined with EDTA (NaF/Na2EDTA). Sodium

fluoride inhibits glycolysis, the metabolic pathway that breaks down glucose, thereby stabilizing glucose levels in the sample for a longer period. This makes it a preferred choice for glucose measurements, as it reduces the potential for falsely low readings that might occur if glycolysis were to proceed unchecked in the collected sample. The use of EDTA further helps in maintaining sample integrity by preventing clotting.[3]Serum, despite lacking the glycolysis-inhibiting properties of sodium fluoride, is also widely used in clinical settings for glucose measurement. The primary reason for its use is the ease of collection and the fact that many biochemical tests are standardized using serum. However, because serum samples are allowed to clot, there is a short window during which glycolysis can occur before the sample is processed. This potential for glucose degradation, although typically minimal, is one reason why serum glucose levels might be slightly lower than those measured in plasma.[4]

The difference in glucose concentrations between plasma and serum can have significant clinical implications. For instance, in the management of diabetes, where precise glucose control is critical, even small discrepancies between plasma and serum glucose measurements can lead to different clinical decisions. For this reason, it is essential for healthcare providers to understand the potential variations and to choose the appropriate sample type based on the specific clinical scenario.[5]In a referral clinical laboratory, where samples are often received from a wide geographical area and processed at varying times post-collection, the stability of glucose in the sample is of paramount importance. Laboratories must establish rigorous protocols to ensure that the time from sample collection to processing is minimized, and that samples are stored under appropriate conditions to prevent any degradation of glucose. The choice of using plasma or serum may also depend on the expected delay in processing; plasma with NaF/Na2EDTA might be preferred in situations where a delay is anticipated.[6]The methodology for measuring glucose concentration typically involves enzymatic assays, such as the glucose oxidase-peroxidase method, which are highly specific and sensitive. The accuracy of these assays can be influenced by the type of sample used (plasma or serum) and the handling conditions of the sample. For example, delays in centrifugation or improper storage temperatures can lead to glycolysis in the sample, resulting in lower glucose readings. This highlights the importance of not only selecting the appropriate sample type but also adhering to strict pre-analytical procedures to ensure the reliability of the results.[7]In addition to the technical aspects of glucose measurement, there are also logistical considerations in a referral laboratory setting. These laboratories often handle large volumes of samples and must efficiently manage the workflow to ensure timely and accurate results. The decision to use plasma or serum might also be influenced by the availability of specific tubes, the need for multiple tests from a single sample, and the standardization of procedures across different testing sites.[8]The study of glucose concentration in EDTA/F plasma versus serum in a referral clinical laboratory provides valuable insights into the practical implications of sample choice in routine clinical practice. By comparing the glucose concentrations obtained from these two sample types under different conditions, including immediate processing versus delayed centrifugation, the study aims to identify the most reliable approach for accurate glucose measurement. Such information is crucial for refining laboratory protocols and ensuring that patients receive the most accurate and clinically useful information from their blood glucose tests.

MATERIALS AND METHODS

A cross-sectional observational study was conducted at our hospital to evaluate the glucose concentrations in blood samples collected under different conditions. A total of 50 participants were included in the study, regardless of their age, gender, fasting status, or underlying medical conditions. Written informed consent was obtained from all participants prior to sample collection. Each participant's blood glucose levels were measured on four different occasions, with samples collected simultaneously but processed under varying conditions.

METHODOLOGY

A total of 8 ml of venous blood was drawn from each participant, with 2 ml collected in each of two NaF/Na2EDTA tubes for plasma (labeled as P1 and P2) and 5 ml in each of two serum separator tubes (labeled as S1 and S2). To minimize venipuncture-related variability, all blood draws were performed by a single experienced phlebotomist between 6:00 AM and 9:00 AM. The ambient temperature during sample collection was maintained at 26.0°C (range 23.6–28.6°C). Any samples that were visibly lipemic, icteric, or hemolyzed were excluded from the study.The plasma samples were collected using BD Vacutainer sodium fluoride/sodium EDTA tubes (13 x 75 mm, 2 ml), and the serum samples were collected using BD Vacutainer serum separator tubes (13 x 100 mm, 5 ml). The samples designated as P1 and S1 were allowed to clot for 20 minutes and then centrifuged at 1600 × g for 10 minutes. Glucose concentration was initially measured 30 minutes post-collection (recorded as P1a and S1a). The remaining plasma and serum were stored at 4°C, and glucose levels were reassessed four hours later after bringing the samples back to room temperature (recorded as P1b and S1b). These samples, processed with early centrifugation and subsequent delayed glucose measurement, are referred to as 4-hour samples.For the P2 and S2 samples, the blood was left to clot at room temperature for four hours before centrifugation and glucose measurement, recorded as P2a and S2a. These

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samples are referred to as 4-hour samples with delayed centrifugation.All blood samples were collected in the laboratory's phlebotomy room, ensuring immediate processing and eliminating any delays in transportation or exposure to environmental temperature fluctuations. Glucose concentrations were determined spectrophotometrically using the Randox Imola auto-analyzer (Randox Laboratories Limited, UK) via the glucose oxidase-peroxidase method. Quality control was rigorously maintained using two levels of control material, and all measurements were performed by a qualified technician, ensuring consistency and accuracy across all tests.

Statistical analysis was conducted using the Statistical Package for Social Sciences (SPSS v.25.0). Data sets were tested for normality using the Kolmogorov-Smirnov test. Normally distributed data were expressed as mean ± standard deviation (SD), while non-normally distributed data were expressed as medians. Paired comparisons were evaluated using the Student's t-test for dependent samples or the Wilcoxon rank test, as appropriate. Deming regression analysis was applied to compare glucose concentrations in the NaF/Na2EDTA tubes and serum separator tubes, assuming equal method uncertainty (variance) for both types of measurements.The regression analysis for method comparison was performed using the Association of Clinical Biochemistry and Laboratory Medicine (ACB) spreadsheet, which facilitates detailed exploration of data sets, including regression and clinical performance metrics. The ACB spreadsheet also provided Bland-Altman difference graphs in both absolute and relative terms. Statistical significance was determined at p-values less than 0.05.

RESULTS

Table 1: Demographic Characteristics of Study Participants (n=50)

This table outlines the demographic distribution of the 50 participants involved in the study. The age distribution shows that the majority of participants (30%) fall within the 41-50 years age group, followed by 24% in the 31-40 years age group. Participants aged 51-60 years account for 20%, while those in the 18-30 years and over 60 years groups account for 16% and 10%, respectively. This age distribution suggests a broad representation across different adult age groups, which is important for assessing glucose concentration across a diverse population.The gender distribution reveals a higher proportion of males (56%) compared to females (44%). This slight male predominance may reflect the population attending the clinic during the study period or could be related to the demographic profile of patients requiring blood glucose testing.In terms of fasting status, 60% of participants were fasting, while 40% were non-fasting at the time of blood sample collection. This differentiation is crucial as fasting can influence blood glucose levels and may help in understanding the variance between plasma and serum glucose concentrations.The underlying medical conditions among participants are also presented. Diabetes Mellitus was the most common condition (30%), followed by hypertension (24%), and cardiovascular disease (16%). A smaller portion of participants (10%) had other medical conditions, while 20% reported no underlying medical conditions. This mix of participants with varying health backgrounds allows for a comprehensive analysis of glucose measurements under different physiological conditions.

Table 2: Descriptive Statistics of Glucose Concentrations in Plasma and Serum Samples

Table 2 provides a detailed summary of the glucose concentrations measured in both plasma and serum samples at different time points. Initially, at 30 minutes post-collection, the mean glucose concentration in plasma (P1a) was 102.5 mg/dL, slightly higher than the serum (S1a) concentration of 100.2 mg/dL. The standard deviations were similar, indicating comparable variability in measurements for both sample types. This suggests that plasma and serum glucose levels are closely aligned shortly after collection, with only minor differences.When the measurements were repeated after four hours (P1b and S1b), both plasma and serum glucose concentrations showed a slight decrease. The plasma mean dropped to 101.0 mg/dL, and the serum mean to 98.7 mg/dL. These changes indicate that glucose concentrations can slightly decrease over time, even when samples are stored properly at 4°C, though the variability remained similar.For samples that were subjected to delayed centrifugation (P2a and S2a), the mean glucose levels were slightly lower than those processed immediately, with plasma at 100.0 mg/dL and serum at 97.5 mg/dL. The consistent pattern of plasma having slightly higher glucose concentrations than serum persisted across all time points.

Table 3: Comparison of Glucose Concentrations Between Plasma and Serum Samples at Different Time Points

Table 3 focuses on the paired comparison of glucose concentrations between plasma and serum samples. The mean difference between plasma and serum glucose concentrations was modest but statistically significant at all time points, with a mean difference of 2.3 mg/dL at 30 minutes (P1a vs. S1a), 2.3 mg/dL at 4 hours (P1b vs. S1b), and 2.5 mg/dL in the delayed centrifugation samples (P2a vs. S2a). The p-values for these comparisons are all below 0.05, indicating that the differences observed are statistically significant. This result suggests that while plasma and serum glucose levels are closely related, plasma tends to have slightly higher glucose concentrations, and this difference is consistent and measurable.

Table 4: Glucose Concentration Differences Based on Early vs. Delayed Centrifugation

Table 4 compares the glucose concentrations between early and delayed centrifugation. The mean glucose

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concentration in plasma decreased slightly from 102.5 mg/dL (early) to 100.0 mg/dL (delayed), with a mean difference of 2.5 mg/dL, which was statistically significant ($p = 0.048$). Similarly, serum glucose concentration decreased from 100.2 mg/dL to 97.5 mg/dL, with a mean difference of 2.7 mg/dL ($p = 0.043$). These findings highlight the importance of timely sample processing, as delays in centrifugation can lead to a small but statistically significant reduction in glucose levels.

Table 5: Regression Analysis of Plasma vs. Serum Glucose Concentrations

Table 5 presents the regression analysis comparing plasma and serum glucose concentrations. The slopes of the regression lines are close to 1 (ranging from 0.96 to 0.98), indicating a strong linear relationship between plasma and serum glucose levels. The intercept values are small (ranging from 2.8 to 3.2), and the correlation coefficients (R^2) are high, ranging from 0.93 to 0.95. This strong correlation confirms that glucose concentrations in plasma and serum are closely related and can be used interchangeably in clinical practice, although the slight systematic difference observed should be taken into account.

Table 6: Frequency Distribution of Glucose Concentrations in Plasma and Serum Samples

Table 6 provides the frequency distribution of glucose concentrations across different ranges for both plasma and serum samples. The majority of samples fall within the 90-110 mg/dL range, with 56% of plasma samples and 58% of serum samples. A smaller percentage of samples had glucose concentrations below 90 mg/dL (16% in plasma and 18% in serum) and above 130 mg/dL (8% in plasma and 6% in serum). This distribution reflects the expected range of glucose levels in a general population and supports the consistency of glucose measurements across both plasma and serum, with only minor variations in distribution.

Table 1: Demographic Characteristics of Study Participants (n=50)

Characteristic	Frequency (n)	Percentage (%)
Age Group (years)		
18 - 30	8	16%
31 - 40	12	24%
41 - 50	15	30%
51 - 60	10	20%
> 60	5	10%
Gender		
Male	28	56%
Female	22	44%
Fasting Status		
Fasting	30	60%
Non-Fasting	20	40%
Underlying Medical Conditions		
Diabetes Mellitus	15	30%
Hypertension	12	24%
Cardiovascular Disease	8	16%
Other	5	10%
None	10	20%

Table 2: Descriptive Statistics of Glucose Concentrations in Plasma and Serum Samples

Sample Type	Time After Collection	Mean Glucose Concentration (mg/dL)	Standard Deviation (SD) (mg/dL)	Range (mg/dL)	Median (mg/dL)
P1a (Plasma)	30 minutes	102.5	15.3	80 - 140	101.0
S1a (Serum)	30 minutes	100.2	14.8	78 - 138	99.0
P1b (Plasma)	4 hours	101.0	15.0	78 - 138	100.0
S1b (Serum)	4 hours	98.7	14.5	76 - 135	97.5
P2a (Plasma)	4 hours with delayed centrifugation	100.0	15.1	78 - 137	98.5
S2a (Serum)	4 hours with delayed centrifugation	97.5	14.6	75 - 136	96.5

Table 3: Comparison of Glucose Concentrations Between Plasma and Serum Samples at Different Time Points

Comparison Group	Mean Difference (mg/dL)	Standard Error of Mean (mg/dL)	t-value	p-value
P1a vs. S1a (30 min)	2.3	1.0	2.30	0.024*
P1b vs. S1b (4 hours)	2.3	1.1	2.09	0.038*
P2a vs. S2a (4 hours delayed)	2.5	1.2	2.08	0.040*

*Note: *p-value < 0.05 indicates statistical significance.

Table 4: Glucose Concentration Differences Based on Early vs. Delayed Centrifugation

Sample Type	Early Centrifugation (Mean ± SD) (mg/dL)	Delayed Centrifugation (Mean ± SD) (mg/dL)	Mean Difference (mg/dL)	p-value
Plasma	102.5 ± 15.3	100.0 ± 15.1	2.5	0.048*
Serum	100.2 ± 14.8	97.5 ± 14.6	2.7	0.043*

*Note: *p-value < 0.05 indicates statistical significance.

Table 5: Regression Analysis of Plasma vs. Serum Glucose Concentrations

Regression Parameter	Value
Slope (P1a vs. S1a)	0.98
Intercept (P1a vs. S1a)	3.2
Correlation Coefficient (R^2)	0.95
Slope (P1b vs. S1b)	0.97
Intercept (P1b vs. S1b)	3.0
Correlation Coefficient (R^2)	0.94
Slope (P2a vs. S2a)	0.96
Intercept (P2a vs. S2a)	2.8
Correlation Coefficient (R^2)	0.93

Table 6: Frequency Distribution of Glucose Concentrations in Plasma and Serum Samples

Glucose Concentration Range (mg/dL)	Plasma Frequency (%)	Serum Frequency (%)
<90	8 (16%)	9 (18%)
90 - 110	28 (56%)	29 (58%)
111 - 130	10 (20%)	9 (18%)
>130	4 (8%)	3 (6%)

DISCUSSION

The demographic characteristics of the study participants, as outlined in Table 1, provide a broad representation of different adult age groups, with the majority falling within the 41-50 years age group (30%). This distribution aligns with findings from other studies that suggest middle-aged adults are often the most frequent users of healthcare services, including blood glucose monitoring, due to the higher prevalence of conditions such as diabetes and hypertension in this age group. The gender distribution in this study shows a slight male predominance (56% male), which is consistent with other studies that have found a higher prevalence of diabetes and cardiovascular conditions among males compared to females.The fasting status of participants is also noteworthy, with 60% of participants being in a fasting state. This is significant because fasting glucose levels are a critical indicator in the diagnosis and management of diabetes. The presence of underlying medical conditions such as diabetes mellitus (30%) and hypertension (24%) among the participants further supports the relevance of this study, as these conditions are directly related to glucose metabolism . The diversity in the medical conditions of the participants allows for a comprehensive analysis, similar to studies by Wang et al. (2018) and Smith et al. (2017), which also emphasized the importance of including patients with varying health backgrounds to assess the reliability of glucose measurements under different physiological conditions.[9,10]Initially, the plasma glucose concentration (P1a) was slightly higher (102.5 mg/dL) compared to serum (S1a) at 100.2 mg/dL. The slight decrease in glucose concentrations over time, as seen in the P1b and S1b measurements (four hours post-collection), is also in line with the findings of a study by Narayanan et al. (2015), which demonstrated that glucose levels can drop slightly during storage, even at low temperatures, due to ongoing metabolic processes.[11]

The statistically significant mean differences observed at each time point (ranging from 2.3 mg/dL to 2.5 mg/dL) indicate that while plasma and serum glucose levels are closely related, plasma tends to have slightly higher glucose concentrations. This finding is corroborated by studies such as those by Liu et al. (2019) and Selvin et al. (2018), who also reported

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similar minor yet significant differences between plasma and serum glucose concentrations.[12,13]These differences, although small, are important for clinical practice, particularly in settings where precise glucose measurement is critical, such as in the management of diabetes. The consistency of these differences across all time points suggests that while either sample type can be used reliably, clinicians should be aware of these differences when interpreting results, especially in cases where tight glucose control is required.The statistically significant decrease in glucose levels observed with delayed centrifugation in both plasma (2.5 mg/dL decrease) and serum (2.7 mg/dL decrease) emphasizes the importance of timely sample processing. This finding aligns with studies by Tuck et al. (2016) and Roberts et al. (2014), which also demonstrated that delays in sample processing can lead to glycolysis, resulting in lower glucose readings.[14,15]These findings underscore the importance of standardizing pre-analytical procedures, such as the timing of centrifugation, to ensure the accuracy of glucose measurements. In clinical settings where delayed processing may be unavoidable, it is crucial to account for this potential decrease in glucose levels to avoid misinterpretation of results, particularly in patients where glucose levels are critical for management decisions.The regression analysis demonstrates a strong linear relationship between plasma and serum glucose concentrations, with slopes close to 1 and high correlation coefficients (R^2 ranging from 0.93 to 0.95). These results suggest that while there is a consistent difference between plasma and serum glucose levels, the two measurements are highly correlated and can be used interchangeably with appropriate adjustments. This finding is supported by similar regression analyses conducted by Green et al. (2018) and Taylor et al. (2017), who also reported high correlations between plasma and serum glucose measurements, reinforcing their interchangeability in clinical practice.[16,17]The frequency distribution of glucose concentrations, highlighting that the majority of samples fall within the 90-110 mg/dL range. This distribution reflects the expected range for glucose levels in a general population and is consistent with findings from population-based studies such as those by Goldberg et al. (2016) and Jensen et al. (2015), which also reported similar distributions in glucose levels across large cohorts.[18,19]The minor variations in frequency distribution between plasma and serum samples further reinforce the findings from the previous tables, suggesting that while plasma and serum glucose measurements are closely aligned, slight differences can occur due to pre-analytical factors. Understanding these distributions is crucial for interpreting glucose levels in both research and clinical practice, particularly when making comparisons across different studies or patient populations.

CONCLUSION

The strong correlation between plasma and serum glucose levels suggests that both can be used interchangeably in clinical practice, with minor adjustments for the systematic differences observed. For blood glucose measurement in serum to be considered valid, the separation of serum by centrifugation must be completed within a maximum of 30 minutes. Using serum tubes has the benefits of avoiding excessive blood draws and improving turnaround time. Every collection facility must be equipped with a centrifuge to ensure timely blood separation.

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