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TIME-DEPENDENT CHANGES IN TRIGLYCERIDE LEVELS IN STORED DIABETIC SERUM: ANALYZING IMMEDIATE AND DELAYED TESTING

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Abstract

Background: Managing diabetes effectively requires regular monitoring of blood glucose and lipid profiles, including triglycerides. However, laboratory delays due to additional specialist-requested tests can impact timely diagnosis and treatment adjustments. **Objective:** This study aims to explore how storage duration at specific temperatures affects triglyceride levels in diabetic patients' serum, providing critical insights into the reliability of delayed laboratory analysis. **Methods:** Employing an intact-group comparison pre-experimental design, this study analyzed serum triglyceride levels from diabetic patients immediately after collection and after 4 and 8 hours of storage at 2º-8ºC. Forty samples were scrutinized. Normality tests using Shapiro-Wilk revealed non-normal distribution for all three conditions (immediate, 4-hour, and 8-hour; Sig < 0.05). The Friedman Test, a non-parametric statistical method, was used to detect differences, with significance set at 0.05. **Results:** The statistical analysis showed a significant variance in triglyceride levels between immediate and delayed testing conditions (Asymp.Sig = 0.001). Despite these significant differences, the mean triglyceride level changes remained within clinically acceptable limits, ensuring the reliability of triglyceride measurements even with delayed processing. **Conclusion:** This study highlights that while triglyceride levels in serum stored for 4 and 8 hours at 2º-8ºC show statistically significant differences from immediate testing, these variations are within permissible clinical ranges. The impact of these findings is substantial for clinical practice, as they suggest that delayed triglyceride analysis up to 8 hours post-collection does not compromise diagnostic accuracy. This flexibility can enhance laboratory workflows, reduce the pressure for immediate testing, and potentially improve patient management by allowing for comprehensive and timely testing as requested by specialists.

INTRODUCTION

The International Diabetes Federation (IDF) estimates that, in 2019, at least 463 million people aged 20-79 worldwide had diabetes. In Indonesia, the number of individuals with diabetes has surged over the past decade, reaching 19.47 million in 2021. Regular or periodic check-ups for blood glucose levels and treatment monitoring are essential for diabetic patients. However, laboratory examination delays often occur due to additional specialist-requested tests, including lipid profile assessments such as triglyceride measurement.

Elevated triglyceride levels in type 2 diabetes mellitus (T2DM) patients are linked to impaired insulin function, which disrupts fat metabolism. Napitupulu (2020) reported that 63% of T2DM patients exhibit increased triglyceride levels. Dyslipidemia, characterized by elevated triglycerides and decreased HDL cholesterol, is common among diabetics, while LDL cholesterol levels may remain normal or slightly elevated.

Efficient hospital and laboratory services require that samples need not be retaken. However, several factors can delay examinations: shift changes in hospitals (8-hour working shifts), human error, failure to inform about additional tests, and laboratory equipment malfunctions. According to the Clinical Chemistry Examination Guidelines (1792/MENKES/SK/XII/2010), factors such as contamination, exposure to sunlight,

temperature, and the metabolism of living cells can affect specimen stability. Given these challenges, this study investigates the stability of serum triglyceride levels in diabetic patients immediately after collection and after 4 and 8 hours of storage at 2º-8ºC. Understanding these effects is crucial for ensuring accurate and reliable triglyceride measurements despite potential delays.

METHOD

This study utilizes primary data and employs an intact-group comparison pre-experimental design. This design involves dividing the subjects into two groups: the experimental group and the control group. Observations are conducted twice on each research subject—before the experiment (pre-test) and after the experiment (post-test). The intact-group comparison, also known as the static group comparison design, is a type of pre-experimental research design. The experimental group consists of diabetic patients' serum samples stored at 2º-8ºC for 4 and 8 hours. The control group comprises serum samples analyzed immediately after collection to serve as the baseline measurement. A significant confounding variable in this study is the lipase enzyme, which can hydrolyze triglycerides and potentially affect their levels in the serum samples. Forty diabetic patients were recruited for the study, and their serum samples were collected using standard phlebotomy techniques. The samples were then divided into three aliquots: one for immediate analysis, one stored for 4 hours, and another stored for 8 hours at 2º-8ºC. All samples were handled with care to prevent contamination and degradation.

Triglyceride levels in the serum samples were measured using an enzymatic colorimetric method. This method involves the hydrolysis of triglycerides by lipase to produce glycerol and free fatty acids. The glycerol is then oxidized, and the resulting color change is measured spectrophotometrically. Quality control procedures were strictly followed to ensure the accuracy and precision of the measurements. Normality of the data was assessed using the Shapiro-Wilk test, and due to non-normal distribution (Sig < 0.05), non-parametric statistical methods were employed. The Friedman Test was used to detect significant differences in triglyceride levels between the three conditions (immediate, 4-hour, and 8-hour). A significance threshold of 0.05 was set for all statistical tests.

The study was conducted in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments. Informed consent was obtained from all individual participants included in the study. This study has received ethical approval from Pupuk Kaltim Hospital, Bontang with number 383/DIR/RSPKT/VII/2023.

RESULTS AND DISCUSSION

The table 1 presents the mean triglyceride levels and associated statistics for samples examined immediately, after 4 hours of storage, and after 8 hours of storage. The mean triglyceride level measured immediately was 188.75 mg/dL with a standard deviation of 85.581 mg/dL, and the range of values spanned from 78 mg/dL to 399 mg/dL. When triglyceride levels were examined after 4 hours of storage, the mean was slightly higher at 189.78 mg/dL with a slightly lower standard deviation of 83.838 mg/dL, and the values ranged from 78 mg/dL to 394 mg/dL. For samples checked after 8 hours of storage, the mean triglyceride level was 189.68 mg/dL, with a further

reduced standard deviation of 81.16 mg/dL, and the values ranged from 83 mg/dL to 398 mg/dL. The coefficient of variation (CV%) for the triglyceride levels examined after 4 hours of storage was calculated to be 1.96%. This value indicates the degree of variability in relation to the mean triglyceride level, providing a normalized measure of dispersion of the data set. Notably, the mean triglyceride levels remained relatively stable over the different time points, indicating minimal degradation or variation due to the storage conditions over the 8-hour period. This consistency in triglyceride levels suggests reliability in the storage and subsequent measurement process, ensuring accurate and dependable results for clinical or research purposes. Based on statistical tests, it is known that the Asymp.Sig. value is 0.001 <0.05, which means that there is a difference in the average triglyceride levels examined immediately, after being stored for 4 hours and 8 hours at a temperature of 2 ° - 8 ° C.

Table 1: Distribution and Frequention for time Delay

	Mean	Std. Deviation	Minimum Score (mg/dl)	Maximum Score (mg/dl)	Koefisien Variation (CV%)	P-Value
Triglyceride levels examined immediately	188.75	85.581	78	399		0.001
Triglyceride levels examined after 4 hours of storage	189.78	83.838	78	394	1.96	
Triglyceride levels checked after 8 hours storage	189.68	81.16	83	398		

The table 2, provides a detailed analysis of the mean difference percentages in triglyceride levels due to time delays of 4 hours and 8 hours, along with their corresponding upper and lower percentage limits, compared to the Clinical Laboratory Improvement Amendments (CLIA) acceptable performance criterion of 25%. For a 4-hour delay, the mean difference in triglyceride levels was found to be 0.55%, with an upper percentage limit of 1.25% and a lower percentage limit of 0%. This indicates that the maximum observed deviation from the initial measurement was 1.25%, and the minimum was 0%, showing very little variability.

In the case of an 8-hour delay, the mean difference was slightly lower at 0.49%. However, the upper percentage limit decreased to 0.25%, and the lower percentage limit increased to 6.41%. This suggests a greater range of variability in the triglyceride levels after 8 hours, though still within a small range overall. Both time delays showed variations significantly below the CLIA acceptable performance threshold of 25%, indicating that triglyceride measurements remained consistent and within acceptable limits even after extended storage periods. This consistency underscores the reliability and robustness of the triglyceride measurement process over time, supporting its validity for both clinical diagnostics and research applications.

Table 2: Mean Difference between Serum Triglyceride Levels of Diabetes Mellitus Patients at Immediate Examination and After Storing for 4 Hours and 8 Hours at 2°-8°C

Time-Delay	Mean Difference (%)	Upper Percentage (%)	Lower Percentage (%)	CLIA acceptable performance (%)
4-Hours	0.55	1.25	0	25
8-Hours	0.49	0.25	6.41	25

The presented data on triglyceride levels under various storage conditions offers significant insights into the stability and reliability of lipid measurements in clinical practice. The tables demonstrate that triglyceride levels exhibit minimal changes when stored at a temperature of 2°C to 8°C over a short period. This observation is crucial as it validates the integrity of lipid testing protocols that involve storage delays.

Initially, triglyceride levels measured immediately had a mean value of 188.75 mg/dL, with a standard deviation of 85.581 mg/dL, indicating a broad range of values (78 to 399 mg/dL). After 4 hours of storage, the mean value slightly increased to 189.78 mg/dL, with a standard deviation of 83.838 mg/dL, and a range of 78 to 394 mg/dL. At 8 hours, the mean was 189.68 mg/dL with a further reduced standard deviation of 81.16 mg/dL, and the range was 83 to 398 mg/dL. This consistency in mean values and decreasing standard deviations suggests that triglyceride levels are relatively stable under these storage conditions. The coefficient of variation (CV%) for triglyceride levels after 4 hours of storage was calculated to be 1.96%. This low CV% indicates high precision and minimal dispersion of the data around the mean, reflecting consistent results across multiple measurements. Such precision is crucial in clinical diagnostics, where even minor variations can impact clinical decisions. This finding aligns with the results of studies like those by Guder et al. (2001), which also reported minimal degradation in triglyceride levels within the first 72 hours of storage at 4°C. From a statistical perspective, the significant Asymp. Sig. value of 0.001 (<0.05) implies that there are statistically significant differences in mean triglyceride levels immediately after collection and after storage for 4 and 8 hours. However, the practical significance of these differences is minimal, as indicated by the slight changes in mean values and low CV%.

The second table offers a detailed analysis of mean difference percentages due to time delays in triglyceride measurements. For a 4-hour delay, the mean difference was 0.55%, with upper and lower limits of 1.25% and 0%, respectively. For an 8-hour delay, the mean difference was 0.49%, with upper and lower limits of 0.25% and 6.41%, respectively. Both time delays had variations well below the CLIA acceptable performance criterion of 25%, indicating robust measurement stability. This finding is significant as it confirms that triglyceride measurements remain within clinically acceptable limits even after 8 hours of storage. These results underscore the reliability and robustness of the triglyceride measurement process, supporting its use in both clinical diagnostics and research. In comparison, studies like Miller et al. (2011) and Smith et al. (2013) have shown that improper storage conditions, such as higher temperatures or prolonged storage, can lead to significant changes in triglyceride levels, adversely affecting the accuracy of results. This highlights the importance of adhering to recommended storage protocols to maintain sample integrity.

The implications for clinical practice are substantial. Laboratories can confidently store serum samples for up to 8 hours without significant changes in triglyceride levels, allowing for greater flexibility in sample processing. This is particularly beneficial in high-volume settings where immediate processing of all samples may not be feasible. Additionally, it ensures that diagnostic results are reliable even if there are delays in sample transportation or processing, thereby improving patient care and diagnostic accuracy. Furthermore, the demonstrated stability of triglycerides under controlled storage conditions supports the feasibility of remote sample collection and transportation for centralized testing. This can expand access to diagnostic services,

particularly in remote or underserved areas, by allowing samples to be collected locally and transported to centralized laboratories for analysis.

In future examinations, maintaining the recommended storage temperature of 2°C to 8°C will be crucial to preserve the stability of triglycerides in serum samples. The findings from this study reinforce the importance of proper sample handling and adherence to storage protocols to ensure accurate and reliable test results. This will ultimately enhance patient care and clinical outcomes by providing dependable data for diagnostic and therapeutic decision-making. In conclusion, the study provides valuable evidence supporting the stability of triglyceride measurements in serum samples stored under controlled conditions for up to 8 hours. This consistency is essential for ensuring the accuracy and reliability of lipid testing in clinical practice, thereby enhancing the overall quality of patient care. The findings also underscore the need for stringent adherence to storage protocols to maintain sample integrity and support the feasibility of remote sample collection and transportation for centralized testing.

CONCLUSION

The study demonstrates that triglyceride levels in serum samples remain stable when stored at 2°C to 8°C for up to 8 hours, with minimal variations observed. The mean triglyceride levels showed negligible differences immediately, after 4 hours, and after 8 hours of storage, all remaining well within the Clinical Laboratory Improvement Amendments (CLIA) acceptable performance criteria. The coefficient of variation (CV%) was low, indicating high precision in measurements. These findings underscore the reliability of triglyceride measurements over short-term storage, supporting flexible sample processing in clinical settings. Proper adherence to recommended storage protocols ensures accurate and consistent results, enhancing patient care and diagnostic accuracy.

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