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Research Notes

Pre-Analytical Stability of Glycemia and Cholesterolemia in Serum Samples After Overnight Storage Under Controlled Freezing Conditions at -20°C

[1]Samia El Mahi, [2]Asmae Tantane and [1]Sanaa Ait Hamou

[1]Department of Biology, Ecology and Environment Laboratory LEE, Faculty of Science Ben Msik, Université Hassan II de Casablanca, Morocco
[2]Department of Biochemistry and Hematology, National Institute of Hygiene, Ministry of Health and Social Protection, Rabat, Morocco

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Corresponding Author:
Samia El Mahi
Department of Biology, Ecology and Environment Laboratory LEE, Faculty of Science Ben Msik, Université Hassan II de Casablanca, Morocco
Email: samia.elmahi-etu@etu.univh2c.ma

Abstract: The process of analysis comprised three phases: the pre-analytical, analytical, and post-analytical phases. Understanding the stability of biochemical analytes is of crucial importance. A study was conducted at the biochemistry and hematology laboratory in the National Institute of Hygiene in Rabat, Morocco. This study, that involved 160 individuals (both patients and healthy subjects), aimed to determine the stability of two biochemical parameters, glycemia and cholesterolemia in their serum. Serum samples were stored overnight at -20°C. During the study, blood samples were collected from fasting volunteers using plain serum tubes. Simultaneously, a questionnaire was completed to assess the subject's health status. Upon arrival at the laboratory, the collection tubes were sorted and then centrifuged to obtain the serum, which was subsequently analyzed using the Cobas C311 system. After that, they were stored overnight at a temperature of -20°C and reanalyzed the following day. Statistical tests showed that blood glucose and cholesterolemia remained stable after being frozen and stored overnight at -20°C. This study concluded that glycemia and cholesterolemia maintain their stability under the chosen conditions.

Keywords: Analytical Process, Pre-Analytical Phase, Stability, Cholesterolemia

Introduction

Medical tests are divided into three phases: the pre-analytical phase, analytical phase, and post-analytical phases (Aakre *et al.*, 2013). Among these, the analytical phase is the most tightly controlled; however, it is actually the pre-analytical phase that represents the greatest source of error, as it is influenced by multiple factors that are difficult to regulate (Lima-Oliveira *et al.*, 2017). Such factors affect not only the stability of analytes but also the reliability of test results, potentially leading to erroneous diagnoses. These sources of variability may be biological, such as age, sex, ethnicity, and diet, or environmental, including temperature, geographic location, and humidity (Ellervik and Vaught, 2015).

In this context, a study was carried out in the Biochemistry and Hematology Laboratory at the National Institute of Hygiene in Rabat with the aim to determine if glycemia and cholesterol can maintain their stability overnight at -20°C.

The selection of these two biochemical parameters is based on their close physiological relationship. Diabetes and hypercholesterolemia frequently coexist, and research has shown that insulin plays a central role in regulating several key enzymes involved in lipoprotein metabolism (Chahil and Ginsberg, 2006). The interaction between glycemia and cholesterolemia is largely mediated by insulin resistance, which leads to hyperglycemia and, in turn, alters cholesterol metabolism (James, 2002). Under normal conditions, insulin promotes muscular glucose uptake and suppresses adipocyte lipolysis; however, this regulatory function is impaired in individuals with diabetes (James, 2002).

Materials and Methods

This cross-sectional study was conducted between December 1, 2023, and April 1, 2024, and included 160 individuals, both healthy and ill, who requested glycemia and cholesterol testing.

Given that in any research, ethical rules must be respected and announced to the participants, this study

was conducted in accordance with INH Ethics Committee. All participants were informed of the purpose of the study, the nature of the questionnaire and the anonymity and confidentiality of each person during this study.

The sample size of 160 patients was determined thought a power analysis for a single-group study. Given an estimated standard deviation of 10 units and clinically margin of error of 1.5 units, the required sample size was approximately 171 sample. To maintain feasibility while still ensuring adequate statistical power, a slightly lower sample size of 160 patients was adopted.

A total of 160 blood samples were collected from fasting volunteers in plain tubes, accompanied by the completion of a questionnaire. All individuals requesting glycemia and cholesterolemia tests, whether healthy or sick and of varying ages, were included in the study, except for infants. Upon arrival at the laboratory, the collection tubes were sorted and centrifuged at 4000 rpm for 10 minutes to obtain serum. The serum was then transferred into Eppendorf tubes, one per participant. Immediately after aliquoting, the samples were analyzed using the Cobas C 311 system.

Glycemia and cholesterolemia concentrations were measured on the first day after the daily laboratory routine for a baseline value. The next day, after an overnight freeze at -20°C, which lasted between 15 and 19 hours, another analysis was performed. The freezer used for sample storage was equipped with a control thermometer to ensure stable and accurate temperature monitoring throughout the study. The different times (from blood collection to analysis), dosage values, and information taken from the questionnaire are recorded in an Excel database.

Statistical analyses were conducted using SPSS version 22. The Pearson correlation test was applied to examine associations between two continuous quantitative variables. Subsequently, a simple linear regression model was performed with a 95% confidence interval, after confirming that the assumptions for regression analysis were met. In addition, a Student's t-test was used to compare parameter values across different temperature conditions.

Results

To assess the stability of blood glucose and cholesterolemia, two analytical variables were taken into account: time and temperature. A descriptive study was first conducted, including 160 individuals with a mean age of 51 and a standard deviation of 13.84, ranging from 14 to 92 years. Women represented the majority of participants (84%), while men accounted for 16%. In terms of transport time, 93% of cases were delivered to the laboratory in less than 2 hours. The results showed that blood glucose levels remained stable in 97% of cases. In contrast, total cholesterol levels were stable in 57.5% of cases and showed variation in 42.5%.

The simple linear regression model (95% confidence interval) confirmed a positive linear relationship of moderate intensity between freezing duration and glycemia values ($r = 0.403$; p-value $= 0.000 < 0.05$) (Table 1). This indicates that as the freezing time increases, glycemia values tend to remain more stable. The coefficient of determination (R^2) was 14%, with a significant F-test ($F = 5.888$, $p < 0.01$), demonstrating that freezing time accounts for a meaningful portion of the variation in glycemia. Overall, the analysis supports a statistically significant relationship between freezing duration and glycemia stability.

The regression analysis ($t = 3.241$; $p = 0.002$) indicates that freezing time is significantly associated with the stability of glycemia (Table 1).

According to Pearson's correlation test, there is a moderate positive linear relationship between cholesterolemia values and freezing duration (Table 1). In other words, longer freezing times are associated with greater stability of cholesterolemia. The coefficient of determination (R^2) was 8%, with a significant F-test ($p < 0.01$), confirming the statistical significance of the model.

Application of the ANOVA test revealed a statistically significant relationship between freezing time and cholesterolemia values ($p < 0.01$) (Table 1). Similarly, regression analysis ($t = 2.643$; $p = 0.009$) confirmed that freezing time is the primary factor associated with cholesterolemia stability.

However, the magnitude of this variation does not appear to carry major clinical implications when considered against the standard reference ranges for glycemia and cholesterolemia. Overall, these findings suggest that, within our study population, time and temperature exert no clinically significant effect on glycemia or cholesterolemia levels.

Table 1: Results of the statistical study

Variable	Pearson Correlation		Linear Regression		ANOVA		Student	
	r	p-value	R^2	Sig F	F	p-value	t-test	p-value
Glycemia	0.403	<0.05	0.140	<0.01	5.888	<0.01	3.241	0.002
Cholesterolemia	0.281	<0.05	0.076	<0.01	3.483	<0.01	2.643	0.009

Discussion

In this article, we studied the impact of overnight storage at freezing temperature on the stability of two biochemical parameters: glycemia and cholesterolemia, using samples from 160 patients.

For the majority of samples (93%), the transport time to the laboratory was under two hours. Foucher *et al.* (2005) demonstrated that transport time for glycemia analysis should not exceed two hours at room temperature in plain tubes, as glycolysis begins approximately two hours after blood collection. Another study, Foucher *et al.* (2004) successfully demonstrated that the stability of glycemia can be prolonged by using tubes containing antiglycolysant agents (mono-iodoacetate, sodium fluoride). Regarding cholesterol, it can be stable for up to seven hours at room temperature (Foucher *et al.*, 2004).

Statistical analysis revealed a significant relationship between glycemia values and freezing time. Glycemia remained stable for up to 19 hours of storage at -20°C. Similarly, Gislefoss *et al.* (2017) demonstrated that glycemia can be stable after a cycle of freezing-thawing at -20°C.

In systematic review conducted by Mahi *et al.* (2024) glucose can be stable at -20°C for up to 3 month and after cycles of freezing-thawing at -20°C. Another study by Vasanthan and Vinodhini (2022) shows that serum glucose can be stable at 2-8°C for 2h.

Several studies have reported significant variations in glycemia under different storage conditions. A study by Kang *et al.* (2013) found that glucose became unstable after 3 cycles of freezing-thawing at -196°C. Similarly, studies by Tanner *et al.* (2008) and Cuhadar *et al.* (2013) concluded that glucose can be unstable after storing in serum at 15 and 24°C for 8 and 1 h, respectively. In contrast, Taylor and Sethi (2011) and Parra-Robert *et al.* (2016) reported that glucose remained stable at room temperature up to 120 hours.

In the present study, more than half of the cholesterolemia samples remained stable after freezing for 19 hours at -20°C. These findings are consistent with previous reports. Kachhawa *et al.* (2017) concluded that cholesterolemia can be stable after several cycles of freezing-thawing at -20°C. This stability lasted for up to 30 days. Similarly, Comstock *et al.* (2001) also demonstrated that cholesterolemia was significantly stable after three cycles of freezing and thawing under the same conditions. According to Cuhadar *et al.* (2013) and Haslacher *et al.* (2017) further confirmed that cholesterolemia maintains its stablility even after cycles of freeze-thaw and temperature fluctuations. Tanner *et al.* (2008) and Cuhadar *et al.* (2012) also reported that serum cholesterol can be stable up to 3 months after freezing.

Moreover, serum cholesterol has been reported to remain stable at room temperature and under refrigeration for up to 1 week (Abraham *et al.*, 2019; Tanner *et al.*, 2008; Taylor and Sethi, 2011; Cuhadar *et al.*, 2012; Ng and Yeo, 2013; Kang *et al.*, 2013; Parra-Robert *et al.*, 2016). On the other hand, Henriksen *et al.* (2014) discovered that cholesterolemia can be unstable in whole blood collected in lithium heparin tubes and serum separating tubes at room temperature for 10 hours.

Conclusion

This study demonstrated that glycemia and cholesterolemia remained stable after 15 hours of freezing at -20°C, with no significant differences observed between healthy and sick individuals.

This study examined the stability of glycemia and cholesterolemia, providing useful insights for situations requiring repeated analysis on the following day. However, certain limitations should be noted. Only a single freezing condition (-20°C) was tested, which may not reflect variations in sample stability under different storage temperatures. Furthermore, all measurements were performed using a single instrument (Cobas C311), which may limit generalizability. Future studies should investigate the effects of extended storage durations and evaluate results across different analytical platforms to better understand long-term analyte stability.

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Conflict of Interest

The authors declare no conflicts of interest regarding this research work.

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Author's Contributions

Samia El Mahi: Conceived the research idea, conducted the data analysis and drafted the initial manuscript.

Asmae Tantane: Conceived the research idea, conducted the research, contributed to the data

interpretation and revised the manuscript critically for important intellectual content.

Sanaa Ait Hamou: Provided substantial input in the study designed and contributed to the manuscript's revision and finalization.

Ethics

Since the research involved human samples (blood and serum), it was carried out in strict observance of the ethical standards of National Institute of Hygiene, Rabat, Morocco and relevant national and international guidelines. A written informed consent questionnaire was administered to all participants, ensuring their approval to use their blood samples for research purposes. All procedures were performed considering ethical principles to make sure findings were obtained with integrity and reliability.

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