Section 1. Clinical Medicine

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THE STABILITY OF GLUCOSE AND TRIGLYCERIDE ANALYSIS IN GEL TUBES AT 2–8 °C

Abstract

Introduction: Studies have shown that there are important, significant relationships between the values of different analytes with different physical variables such as temperature, measurement time, and centrifugation time. In this paper, the relationship that may exist between the concentration values of two analytes such as blood glucose and triglycerides with the storage temperature of the samples has been studied.

This study aimed to determine the analyte stability of venous blood samples in serum gel tubes stored at 2–8 °C.

Methods*:* 30 healthy adult volunteers take part in the study. Blood was collected in tubes with gel (Clot activator). All samples were allowed to clot at room temperature for 30 min. They were centrifuged CAPP CENTRIFUGE at 3500 RPM and 2 analytes were analyzed: Triglycerides and Fasting Blood Glucose. These values were determined as control values. Serums were stored at 2–8 °C. Measurements were repeated after 1 week.

Results*:* 18 men and 12 women participated in the study. Triglycerides control values range from 54–149 mg/dl average (86.58mg/dL).

Fasting blood glucose values were in the range of 77–91.8 mg/dl, with an average value (of 80.23 mg/dL).

After keeping the serum at a temperature of 2–8 °C for 1 week, the following results were obtained:

- Triglyceride values ranged from 60.3–117 mg/dl, mean of value (92.25 mg/dl);
- Glucose values ranged from 53–124.4mg/dl on average (83.7 mg/dL).

Conclusions: There is a statistically significant relationship between the storage temperature of the sample and the concentration of the Triglyceride analyte (p – 0.00), while for the comparison of Glucose control and Glucose values after one week, they are not statistically significant (p = 0.2).

Keywords: Gel tube, Triglyceride, fasting blood glucose, temperature.

Introduction

Laboratory examinations have an important role in clinical diagnoses. This has made some tests part of the check-up routine. Tests such as fats and blood sugar have a role both in the diagnosis and in the prognosis of a certain pathology, so often for these tests, there is a need to repeat the measurements.

Most biochemical tests are performed on serum samples, that is, in gel tubes (Clot activator) Some studies show that these tubes maintain the stability of the values without the need to divide the samples into other tubes, but there are also studies that prove the opposite. The purpose of this study is precisely to see the stability of the values of the analyte *Glycemia* and *Triglyceride* in the tubes with gel but exposed to the temperature variable

2. Materials and methods

The study involved 30 (thirty) volunteers, who were healthy adults, not anemic, and they were not on an anticoagulant therapy. Twelve female and 18 men volunteers between the age of 19 and 22 took part of this study. Five milliliters of venous blood were drown from each volunteer using a vacuum machine and gel tubes (Clot activator)

2.1 Collection and processing of samples

Each volunteer underwent a phlebotomy procedure for blood collection. Blood was collected from forearm veins using a 21G vacuum system. All samples were identified and homogenized at least five times. Each sample was allowed to be set for 30 minutes at 18–25 °C. All tubes were centrifuged at 1500g/3500 RPM for 10 minutes at 18–25 °C.

2.2 Analysis of samples

All tubes were evaluated for hemolysis, icterus, and lipemia. The CYANSMART device (ELITEK) was used to analyze the samples. Triglyceride and glucose reagents were kept at a temperature of 18––25 °C for 15–20 minutes. After each test, a calibration and control procedure was performed.

The principle of the Glycemic measurement method is: ***Glucose oxidase***

METHOD & PRINCIPLE [5]

Enzymatic / PAP - End Point.

$$\text{Glucose} + O_2 \xrightarrow{\text{Glucose oxidase}} \text{Gluconic acid} + H_2O_2$$

$$2H_2O_2 + \text{Phenol} + \text{4-Aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + 4H_2O$$

Figure 1. The principle of the glucose method

Manual procedure

- Wavelength 505 nm;
- Optical path: 1cm;
- Sample/ Reagent ratio: 1:100;
- Temperature: 37 °C

Read against reagent blank.

	Blank	Calibration	Test
Reagent R	1000 µl	1000 µl	1000µl
Distilet water	10 µl	–	
Standart/ Calibrator	–	10 µl	–
Sample	–	–	10 µl

Mix and read the absorbances (A) after an incubacion of 10 minutes

METHOD [3]

Enzymatic-colorimetric. End point.

PRINCIPLE [3]

Enzymatic determination of triglycerides according to the following reactions :

$$\text{Triglycerides} + H_2O \xrightarrow{\text{Lipoprotein Lipase}} \text{Glycerol} + \text{Fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerol kinase}} \text{Glycerol-3-Phosphate} + \text{ADP}$$

$$\text{Glycerol-3-Phosphate} + O_2 \xrightarrow{\text{GPO}} \text{DHA-P} + H_2O_2$$

$$H_2O_2 + \text{4-AAP} + p\text{-Chlorophenol} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine}$$

GPO = Glycerol-3-phosphate oxidase
DHA-P = Dihydroxyacetone-P
4-AAP = Amino-4-antipyrine

Figure 2. The principle of the Triglicerid method

The principle of the method of measuring triglycerides vs Triglyceride Oxidase. Both spectrophotometric methods are END points.

Manual procedure

- Wavelength 505 nm;
- Optical path: 1cm;
- Sample/ Reagent ratio: 1:100;
- Temperature: 37 °C;

Read against reagent blank.

	Blank	Calibration	Test
Reagent R	1000 µl	1000 µl	1000µl
Distilet water	10 µl	–	
Standart / Calibrator	–	10 µl	–
Sample	–	–	10 µl

Mix and read the absorbances (A) after an incubacion of 10 minutes

All samples were measured at the beginning and their values were considered control values. Afterward, the gel tubes were stored in a refrigerator at a temperature of 2–8 °C. After 1 week, these tubes were left at room temperature for 10–15 min, re-centrifuged and re-measured, maintaining the same calibration and control conditions.

Figure 3. Semi-automatic biochemistry analyzer CYANSmart

3. Results of the study

All measurement values were entered into the SPSS version 21 program and processed. The statistical analysis showed that there is a statistically significant relationship between the concentrations of triglycerides and the temperature variable.

Table 1. – Indicates the statistical relationship between Tg concentrations. According to the statistical processing, we have a statistically significant relationship (P = 0000) between the concentration of Tg control and the concentration of Tg after 1 week at a temperature of 2–8 °C

Paired Samples Test									
		Paired Differences					**t**	**df**	**Sig. (2-tailed)**
		Mean	**Std. Deviation**	**Std. Error Mean**	**95% Confidence Interval of the Difference**				
					Lower	**Upper**			
Pair 1	**The concentration of Tg control Tg concentration after 1 week**	–5.67000	7.87594	1.43794	–8.61092	–2.72908	–3.943	29	0.000

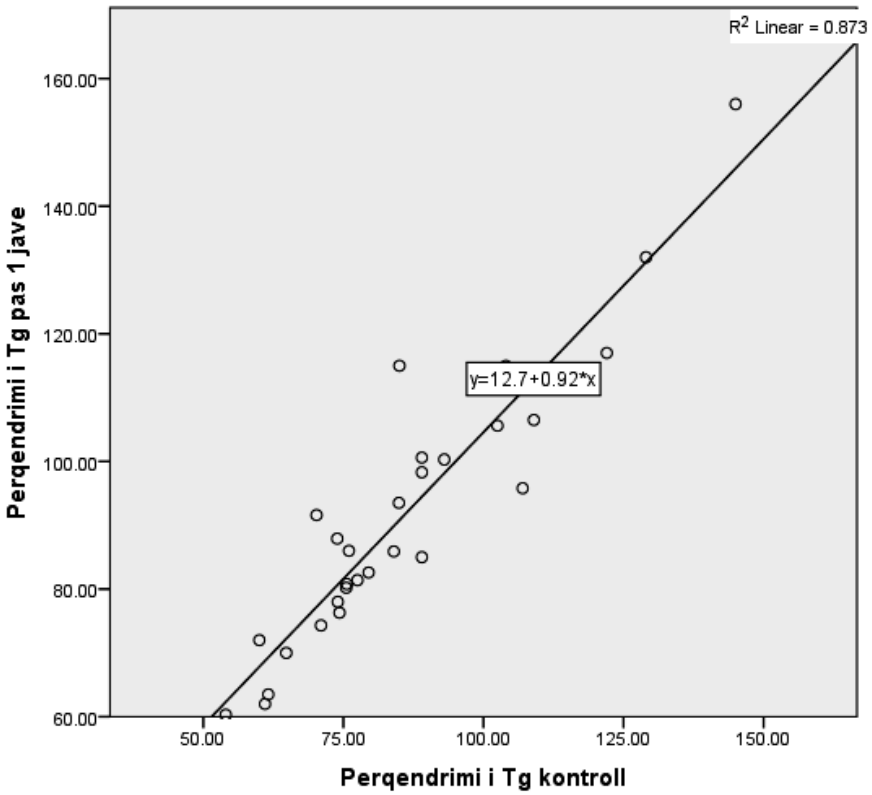

Figure1. Shows the scatter blot of the relationship between the Tg concentration in a 1-week time difference at a temperature of 2–8 ◦C. As can be seen from the graph obtained from SPSS VERSION21, there is a linear distribution of values, which shows that there is a positive statistical relationship between the concentration of triglyceride and the temperature variable

Additionally, it was demonstrated that there is no correlation between glucose concentrations and temperature in the region 2–8 ˚C. The linearity is **R = 0.873** and the equation obtained from excel is **y = 12.7 + 0.92 x.** It was demonstrated that there is no correlation between glucose concentrations and temperature in the region 2–8 ˚C.

Table 2. – Shows the statistical relationship between fasting glucose concentrations. According to the statistical analysis, there is no statistically significant relationship (P=0.2) between the control glucose concentration and the glucose concentration after 1-week storage at a temperature of 2–8 ◦C

Paired Samples Test									
		Paired Differences					**t**	**df**	**Sig. (2-tailed)**
		Mean	**Std. Deviation**	**Std. Error Mean**	**95% Confidence Interval of the Difference**				
					Lower	**Upper**			
Pair 1	**Glucose concentration after 1 week – control glucose concentration**	3.46933	15.71995	2.87006	–2.40059	9.33926	1.209	29	0.237

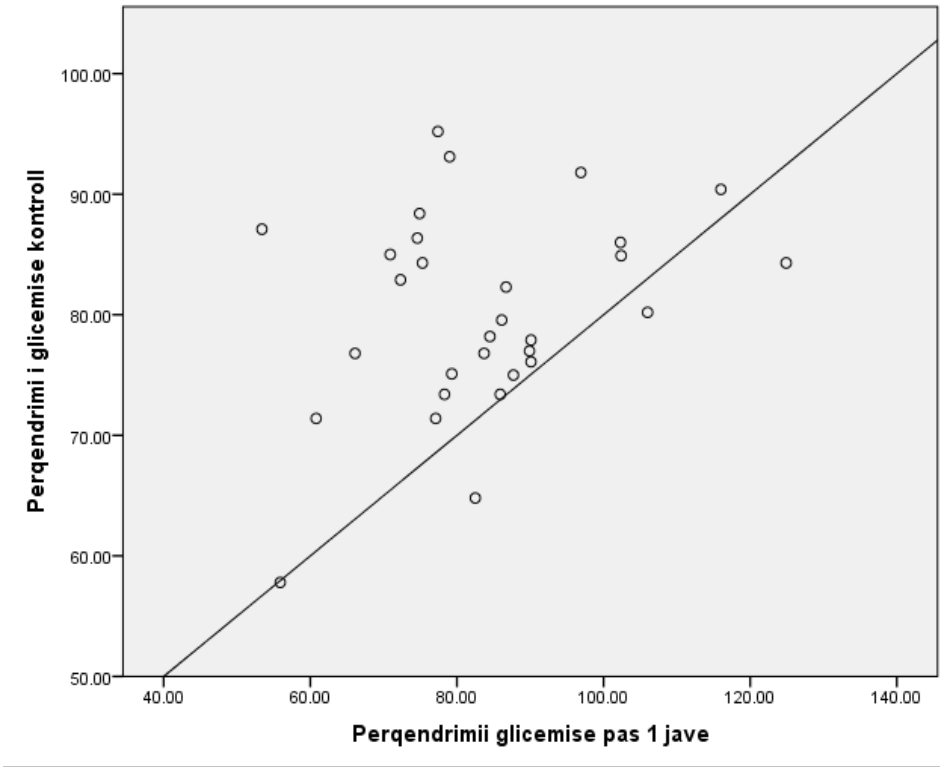

Figure. 2 shows the scatter blot of the relationship between the end of Glycemia in a time difference of 1 week at a temperature of 2–8 °C. As can be seen from the graph obtained from SPSS VERSION21, there is a non-linear distribution of values, which shows that there is no positive statistical relationship between the concentration of glucose and the temperature variable, so the concentration changes. This means that the gel tube does not adequately maintain the glucose concentration

4. Discussions

From the results of the work, different interpretations and opinions arise which will be analyzed in the following works for the measurement of analytes. The results of the work showed the statistical relationship that existed between the temperature variable and Triglycerides, and the lack of a statistically significant relationship between the glucose concentration and the temperature variable, which makes us analyze what would happen if maintaining the same measurement procedures but extending The time the samples stay in the temperature range of 2–8 °C.

It is known that the gel tubes can maintain the stability of the analytes for up to a week, at a temperature of 2–8 (various studies), and in the case of the study, the gel preserved the triglyceride value while the glucose value did not, and this is related to the effect of glycolysis because this is a basic process of cellular energy production, a process which is stimulated by the temperature factor. the effect of the decrease in glucose concentration as a result of this phenomenon is slower as the temperature decreases. in this study, it was observed that the separating gel of the tube does not inhibit the process of glycolysis and as a result, the glycemic values changed. The change in blood glucose values may also have come as a result of the presence of blood elements in the serum. (number of leukocytes always interfere in this avoidance of glucose concentration values).

The second discussion is related to the use of a tube with sodium fluoride, to evaluate if, under the same analysis conditions, these obtained statistical relationships are preserved, which will provide valuable information regarding the obtained values (of blood, serum) and analytes).

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References:

1. Boyanton B. L., Blick K. E. Stability studies of twenty-four analytes in human plasma and serum. Clin Chem – 48. 2002.– P. 2242–7.
2. Ono T., Kitaguchi K., Takehara M., Shiiba M., Hayami K. Serum-constituents analyses: effect of duration and temperature of storage on clotted blood. Clin Chem – 27. 1981.– P. 35–8.
3. CLSI document H 18-A3. Procedures for the Handling and Processing of Blood Specimens; Approved Guideline. 3rd edn. 2004.
4. Zhang D., Elswick R. K., Miller G., Bailey J. L. Effect of serum-clot contact time on clinical chemistry laboratory results. Clin Chem – 44. 1998.– P. 1325–33. 8 Westgard Biological Variation Database and Quality Specifications for Imprecision, Bias and Total Error. 4th edn. 2006.
5. Boyanton B. L. Blick K. E. Stability studies of twenty-four analytes in human plasma and serum. Clin Chem – 48. 2002.– P. 2242–7. Crossref. PubMed. ISI.
6. Rehak N. Chiang B. Storage of whole blood: effect of temperature on the measured concentration of analytes in serum. Clin Chem – 34. 1988.– P. 2111–4 Crossref. PubMed. ISI.
7. Murphy J. M. et al. Effects of transportation and delay in processing on the stability of nutritional and metabolic biomarkers. Nutr Cancer – 37. 2000.– P. 155–60 Crossref. PubMed