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THE EFFECT OF DELAY IN TEMPERATURE VARIATIONS ON RESULTS OF GLUCOSE TEST IN DM PATIENTS WITH HYPERCHOLESTEROLEMIA

PENGARUH PENUNDAAN DALAM VARIASI SUHU TERHADAP HASIL PEMERIKSAAN GLUKOSA PADA PENDERITA DM YANG MENGALAMI HIPERKOLESTEROLEMIA

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Research Report
Penelitian

A B S T R A C T

Background: *The pre-analytic stage has the most significant contribution to error, which is 60% to 70%. This is an excellent contribution to the dependability of laboratory results. Delays in the examination often occur in the laboratory due to various factors influencing glucose examination outcomes.* ***Purpose:*** *To ascertain the effect of long delays in various temperatures on the results of plasma NaF and serum glucose tests in diabetes mellitus patients who have hypercholesterolemia.* ***Method:*** *Pre-test and post-test design used in a pre-experimental method. In the pretest, plasma NaF and serum glucose levels were checked immediately. In the posttest, the plasma NaF and serum samples were delayed for 4 and 8 hours at the temperature of the refrigerator (4℃) and room temperature (25℃).* ***Result:*** *The results of One-way* ANOVA *test analysis of the plasma NaF glucose level with an immediate examination, a delay of 4 and 8 hours at room temperature (25℃) showed no effect with a Sig. of 0.423 > 0.05. Plasma NaF glucose level with an immediate examination, 4 and 8 hours delay in refrigerator temperature (4℃) showed no effect with Sig. of 0.772 > 0.05. Serum glucose levels with an immediate examination, 4 and 8 hours delay at room temperature (25℃) showed no effect with a Sig. of 0.333 > 0.05. Serum glucose levels with an immediate examination, 4 and 8 hours delay in refrigerator temperature (4℃) showed no effect with a Sig. of 0.604 > 0.05.* ***Conclusion:*** *There was no effect of 4 hours and 8 hours of delay at the temperature of the refrigerator (4℃) and room temperature (25℃) on the results of NaF and serum glucose examinations in patients with diabetes mellitus who have hypercholesterolemia.*

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A B S T R A K

Latar belakang: Tahap pra analitik memiliki kontribusi kesalahan terbesar yaitu 60%-70%, sehingga kontribusi yang diberikan sangat besar untuk keandalan hasil laboratorium. Penundaan pemeriksaan seringkali terjadi di laboratorium disebabkan oleh berbagai faktor yang mempengaruhi hasil pemeriksaan glukosa. **Tujuan:** Mengetahui pengaruh lama penundaan dalam suhu yang bervariasi terhadap hasil pemeriksaan glukosa plasma NaF dan serum pada penderita diabetes melitus yang mengalami *hiperkolesterolemia*. **Metode:** Pra-experimental rancangan menggunakan *pre-test dan post-test design*. Pada *pre-test* dilakukan pemeriksaan kadar glukosa plasma NaF dan serum segera, kemudian pada *post-test* sampel plasma NaF dan serum ditunda selama 4 dan 8 jam dalam suhu ruangan (25°C) dan suhu kulkas (4°C). **Hasil:** Hasil analisis uji *One-way* ANOVA kadar glukosa plasma NaF dengan pemeriksaan segera, penundaan 4 dan 8 jam dalam suhu ruangan (25°C) didapatkan hasil tidak ada pengaruh dengan Sig. sebesar 0,423 > 0,05. Glukosa plasma NaF dengan pemeriksaan segera, penundaan 4 dan 8 jam dalam suhu kulkas (4°C) didapatkan hasil tidak ada pengaruh dengan Sig. sebesar 0,772 > 0,05. Glukosa serum dengan pemeriksaan segera, penundaan 4 dan 8 jam dalam suhu ruangan (25°C) didapatkan hasil tidak ada pengaruh dengan Sig. sebesar 0,333 > 0,05. Glukosa serum dengan pemeriksaan segera, penundaan 4 dan 8 jam dalam suhu kulkas (4°C) didapatkan hasil tidak ada pengaruh dengan Sig. sebesar 0,604 > 0,05. **Kesimpulan:** Tidak ada pengaruh lama penundaan 4 jam dan 8 jam dalam suhu ruangan (25°C) dan suhu kulkas (4°C) terhadap hasil pemeriksaan glukosa plasma NaF dan serum pada penderita diabetes melitus mengalami *hiperkolesterolemia*.

Kata kunci:
Suhu penundaan, Lama penundaan, Glukosa, Plasma NaF, Serum

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INTRODUCTION

Diabetes mellitus is a disease that can cause various complications. In patients with diabetes mellitus, metabolic disorders are strongly associated with increased morbidity and mortality, causing various acute and chronic complications (Permana, 2017). Diabetes mellitus can also cause damage to body systems, including nerves, kidneys, and cardiac (Kasimo, 2020). Cholesterol levels that increase beyond normal values (*hypercholesterolemia*) in people with diabetes mellitus can occur due to insulin resistance. Insulin resistance causes lipolysis in adipose tissue and blood fat to increase (Noviyanti *et al*., 2015). Therefore, early detection of diabetics and control of glucose levels is essential to prevent complications due to diabetes mellitus.

The glucose examination is one of the most critical standards for diagnosing diabetes and monitoring glucose level control, so the examination procedure must be considered (Agung *et al*., 2017). A glucose examination can be done using a glucometer and a spectrometer. Blood glucose examination materials can be used with serum, plasma, and capillary blood (Mariady *et al*., 2013). The specimens obtained must comply with the requirements, one of which is that the specimen does not undergo hemolysis (Sujono *et al*., 2016).

Glycolysis is the primary metabolic pathway for glucose (Yuliana, 2018). Glycolysis can be initiated outside the body after taking blood specimens. Without the addition of glycolysis inhibitors, the components in the specimen can cause glucose to decrease (Putra *et al*., 2015). Specimens for glucose testing are prone to glycolysis, which results in a decrease in glucose, in which case anticoagulants are required to inhibit glycolysis. The use of anticoagulant Sodium Fluoride (NaF) for glucose examination is often used because it can prevent glycolysis (Nurhayati *et al*., 2017). The anticoagulant NaF can inhibit glucose metabolism by reducing the activity of phosphoenol pyruvate and urease enzymes to maintain glucose stability in the specimen (Yuni *et al*., 2019). This shows that using NaF anticoagulant is suitable for checking glucose levels.

The pre-analytic stage has the largest error contribution, 60%-70% because the pre-analytic stage is difficult to control. The pre-analytic stage includes a critical stage, where patient preparation, collection, and handling of specimens contribute significantly to the reliability of laboratory results. Laboratory examinations must comply with reference standards in the form of guidelines, instructions, and fixed procedures to avoid variations that can affect the quality of the examination. At the pre-analytic stage, the accuracy of the procedure is essential to obtain a specimen that is genuinely suitable for examination (Siregar *et al*., 2018). Cases of delayed examination still often occur in public and private clinical laboratories. The delay in laboratory examinations occurs due to various factors, thus affecting the results. Specimens that have been taken must be examined immediately. This is because the stability of the specimen can change (Santi *et al*., 2011). Therefore, the pre-analytic stage must be given more attention to maintain the reliability and quality of the examination results. The results of research conducted by Agung *et al*. (2017) said that the effects of glucose in serum and plasma NaF after a delay of 4 and 8 hours statistically showed a significant decrease in serum samples but no significant reduction in plasma glucose. Research by Santi *et al*. (2011) showed no statistically significant change in serum samples at 0 and 4 hours of storage at 2-8°C and 25-28°C.

The phenomenon that occurs in the field of decreased glucose levels in samples can be caused by delayed examinations due to the shortage of laboratory staff, inadequate transportation from the place of specimen collection to the laboratory, and lack of reagents and tools (Agung *et al*., 2017). In addition, other factors include the number of examination samples that are too many, errors of medical laboratory personnel (human error), and the occurrence of the examination process being delayed due to waiting for public services and patient sampling to be completed so that the examination can be carried out at once. In connection with this, it is necessary to research the effect of delay in temperature variations on the results of glucose tests in diabetes mellitus patients with *hypercholesterolemia*.

MATERIAL AND METHOD

This type of research was pre-experimental and used the pre-test and post-test design. In the pretest, the plasma NaF glucose and serum were checked immediately (0 hours). Then in the posttest, the plasma NaF and serum glucose samples were checked for 4 hours and 8 hours at the temperature of the refrigerator (4℃) and room temperature (25℃). Data collection techniques are primary data obtained directly during data collection and the results of laboratory examinations and interviews are used as supporting data.

The examination was conducted at the Regional Health Laboratory of Sumenep Regency, East Java, in April 2022. The study population was diabetes mellitus patients with *hypercholesterolemia* who were examined at the Regional Health Laboratory of Sumenep Regency. The sample criteria were people with diabetes mellitus who had *hypercholesterolemia*, had an age range of 30–70 years, and were willing to have their blood drawn. The sample size obtained using *Frederer's* formula calculation is five samples for every eight treatment groups, so the total number of research subjects is 40.

The test materials used are plasma NaF and serum separated. Glucose examination was carried out using the GOD-PAP method enzymatically colorimetrically using a photometer 5010 V5+. Data obtained from the results of plasma NaF and serum glucose examinations were managed using *One-way* ANOVA to determine whether or not there was an effect.

RESULT

Blood glucose examination must have good quality. A good level of accuracy and precision influences good quality. Based on the results of the effect of the length of delay in varying temperatures on the results of the plasma NaF glucose examination in people with diabetes mellitus who have *hypercholesterolemia*. The results are shown Table 1.

The plasma NaF glucose examination results with the delayed examination (Figure 1) tend to show lower values than those of the examination of plasma NaF glucose levels for immediate examination.

The results of plasma NaF glucose examination with immediate examination obtained (Table 2) an average value of 345.80 mg/dl, plasma NaF glucose which was delayed 4 hours at room temperature (25°C) obtained an average value of 325.60 mg/dl, plasma NaF glucose which was delayed 8 hours at room temperature (25°C) got an average value of 306.20 mg/dl, plasma NaF glucose which was delayed 4 hours in refrigerator temperature (4°C) earned an average value of 334, 20 mg/dl, plasma NaF glucose which was delayed 8 hours in a refrigerator temperature (4°C) obtained an average value of 323.20 mg/dl.

Table 1. Result of plasma NaF glucose level examination

No	Code of sample	Cholesterol levels (mg/dl)	Plasma NaF glucose levels (mg/dl)					
			Room temperature (25°C)			Refrigerator temperature (4°C)		
			Immediately (0 hours)	4 hours	8 hours	Immediately (0 hours)	4 hours	8 hours
1	LA (53 th)	248	382	349	317	382	358	343
2	JH (56 th)	236	394	371	345	394	388	379
3	ST (63 th)	224	359	342	335	359	345	337
4	HO (50 th)	251	322	313	297	322	320	312
5	AW (53 th)	218	272	253	237	272	260	245
Mean			345.80	325.60	306.20	345.80	334.20	323.20
SD			49.55	45.57	42.79	49.55	48.18	49.84

Table 2. Result of serum glucose level examination

No	Code of sample	Cholesterol levels (mg/dl)	Serum flucose levels (mg/dl)					
			Room temperature (25°C)			Refrigerator temperature (4°C)		
			Immediately (0 hours)	4 hours	8 hours	Immediately (0 hours)	4 hours	8 hours
1	LA (53 th)	248	370	340	302	370	343	327
2	JH (56 th)	236	378	352	342	378	368	353
3	ST (63 th)	224	348	338	319	348	341	333
4	HO (50 th)	251	322	300	278	322	304	288
5	AW (53 th)	218	269	237	222	269	242	236
Mean			337.40	313.40	292.60	337.40	319.60	307.40
SD			43.99	46.96	45.89	43.99	49.02	46.35

Table 3. Result of test *One-way* ANOVA

Variable	Sig. value	Description
Plasma NaF glucose level with immediate examination, 4 hours delay and 8 hours at room temperature (25°C)	0.423	No effect
Plasma NaF glucose level with immediate examination, 4 hours delay and 8 hours in refrigerator temperature (4°C)	0.772	No effect
Serum glucose level with immediate examination, 4 hours and 8 hours delay at room temperature (25°C)	0.333	No effect
Serum glucose level with immediate examination, 4 hours and 8 hours delay in refrigerator temperature (4°C)	0.604	No effect

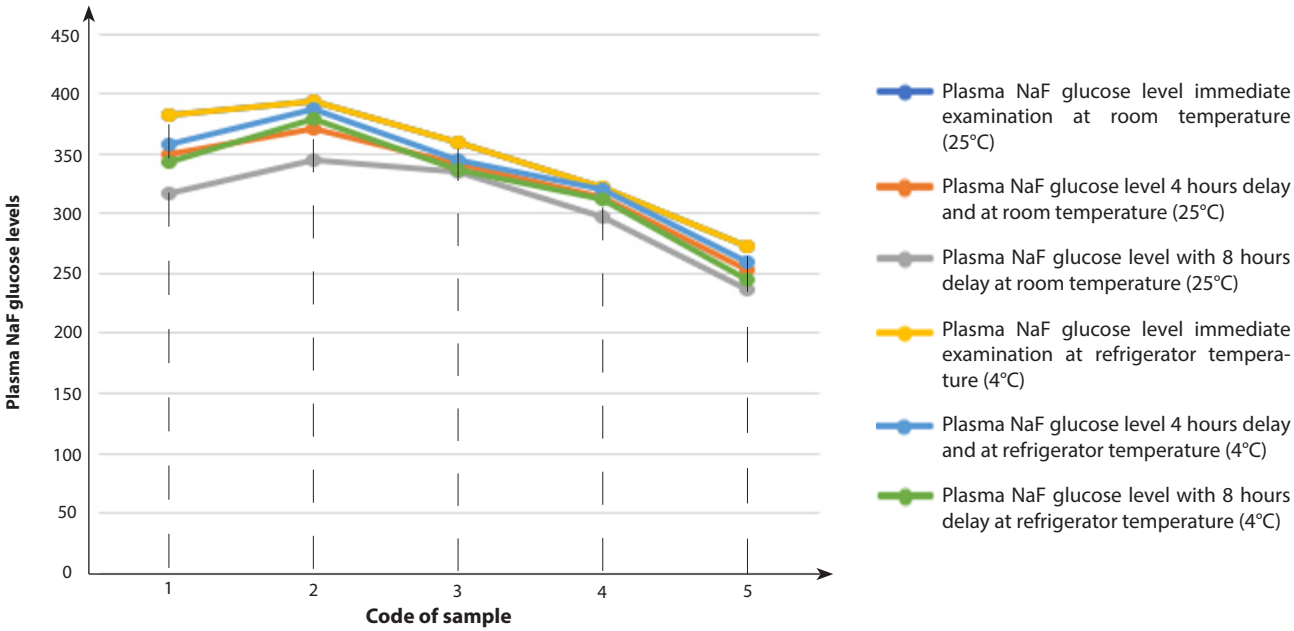

Figure 1. Results of plasma NaF glucose level examination

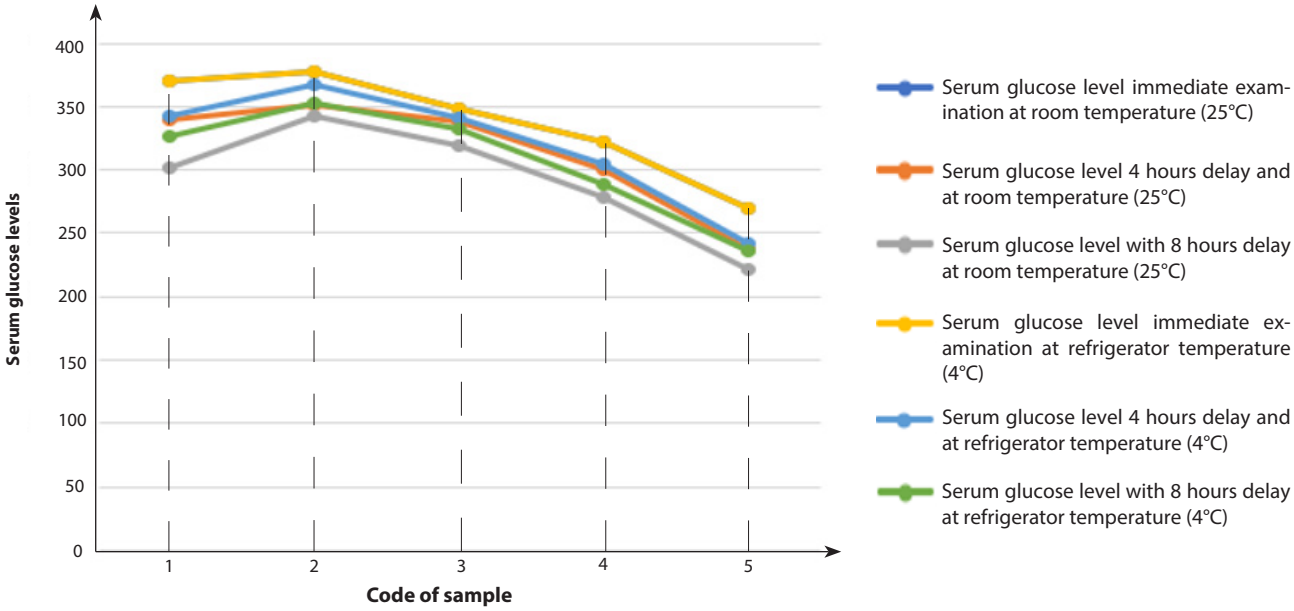

Figure 2. Results of serum glucose level examination

The results of the examination of serum glucose (Figure 2) with a delayed examination tend to show a lower value than the research results from the examination of serum glucose levels which are carried out immediately. The results of serum glucose examination with immediate examination obtained (Table 3) an average value of 337.40 mg/dl, serum glucose which was delayed 4 hours at room temperature (25°C) obtained an average value of 313.40 mg/dl, serum glucose delayed 8 hours at room temperature (25°C) the average value was 292.60 mg/dl. Serum glucose was delayed 4 hours at refrigerator temperature (4°C). The average value was 319.60 mg/dl. Serum glucose which was delayed for 8 hours at a refrigerator temperature (4°C), obtained an average value of 307.40 mg/dl. After obtaining research data regarding the effect of the length of delay in varying temperatures on the results of plasma NaF and serum glucose examinations in people with diabetes mellitus who have hypercholesterolemia, the statistical data analysis technique is the *One-way* ANOVA with a significance criterion of 5%.

Based on the results of the *One-way* ANOVA test, the smallest Sig. value was obtained by serum glucose examination with immediate, 4 hour delay and 8 hour examination at room temperature (*p-value* = 0.333) compared to the Sig. value of NaF plasma at room temperature (*p-value* = 0.423), Sig. value of serum at refrigerator temperature (*p-value* = 0.604), and Sig. value of Naf plasma at refrigerator temperature (*p-value* = 0.772). Thus, the blood glucose examination in each treatment unit showed no effect with a probability value of *p-value* > 0.05.

DISCUSSION

Plasma NaF glucose levels with a delay of 4 hours and 8 hours at the temperature of the refrigerator (4°C) and room temperature (25°C) in diabetic mellitus patients with *hypercholesterolemia* statistically showed that there was no effect. This may be due to the stability of the sample with the addition of NaF anticoagulant for checking glucose levels, which can be stable at a temperature of 20-25°C for three days, a refrigerator temperature (4°C) for seven days, and a temperature of -20°C for three months. Various factors, including contamination by chemical reagents and germs, metabolic activity by living cells, evaporation, the influence of temperature, and exposure to sunlight, influence the stability of the sample.

Sample stability for checking glucose levels must be maintained following the required sample handling instructions (Siregar *et al*., 2018). The study's results align with prior research (Yuni *et al*., 2018), which showed that plasma NaF glucose at a temperature of 15-25°C was not affected after 3 and 24 hours of delay. This is because the sample added with NaF anticoagulant can inhibit glycolysis in the sample during the examination delay time. By reducing the activity of the enzymes urease and phosphoenol pyruvate, NaF's antiglycolytic property prevents the process of glucose metabolism. But incorrect use of additives can affect the results of the examination. The additives used must meet the requirements that they do not interfere with or change the level of the substance to be examined (Nurhayati *et al*., 2017).

Another factor that affects plasma NaF glucose levels is the process of separating plasma from other blood components during the delay in the examination. Blood samples that have been taken must be immediately separated using a centrifuge, and the plasma must be directly transferred to the sample cup. The delay in examination of the sample results in the process of glucose metabolism by the blood cells in the sample until a separation process occurs using a centrifuge because physiologically, the cell will try to maintain its life by using glucose as energy obtained through the glycolysis process even though the blood sample has been taken or is outside the body (Moe *et al*., 2018). Delayed specimen preparation will affect glucose examination because glucose is at risk of being used by cells in specimens that require an energy source (Sholikin, 2018). Glucose levels can drop due to contamination by bacteria due to the use of equipment and handling of unsterile specimens (Fahmi *et al*., 2020).

Serum glucose levels that were delayed 4 and 8 hours at the temperature of the refrigerator (4°C) and room temperature (25°C) in diabetic mellitus patients with *hypercholesterolemia* were statistically shown to have had no effect. This may be due to the stability of the serum-separated sample for checking glucose levels, namely at room temperature or 25°C for 8 hours and at refrigerator temperature or 4°C for 72 hours (Nugraha and Badrawi, 2018). Serum should be separated from other blood components immediately. The delay in examination of the unseparated serum can affect the outcome of the analysis from the examination of glucose levels because hemolysis in blood cells during a prolonged delay results in contamination of the serum sample. Separation prevents activities carried out by blood components in samples that can use glucose as a food source through the glycolysis process (Sacher and McPherson, 2012). Glucose levels in serum can be lower than in plasma because it can consume glucose in the clotting process before the serum is separated (Ramadhani *et al*., 2019).

The study's results align with prior research Santi *et al*. (2011) that shows serum glucose at 2 to 8°C and 25 to 28°C temperatures is insignificant after being delayed for 4 hours. This is because from the beginning of the venous blood collection process until the blood glucose examination with a photometer always uses clean and sterile tools. Using clean and sterile tools helps prevent bacterial contamination of the sample because bacteria can consume glucose as an energy source to sustain life through glycolysis.

Meanwhile, different results were obtained (Sasmita *et al*., 2020). Namely, there was a significant change in plasma NaF glucose levels at room temperature and 2-8°C after being delayed for 6 hours. There was a significant change in serum glucose with a delay of 5 hours at room temperature and 2-8°C. This could be due to serum samples not using anti glycol so that the decrease in glucose levels continues during the storage period and the use of glucose by microorganisms still present in the sample, which causes glucose levels to decrease examination (Apriani and Umami, 2018).

Samples that undergo a delay in examination need to be considered as to the length and temperature of the delay, the type of anticoagulant, the container, and its stability. Delaying the sample at a low temperature will result in a modification of the biochemical reaction. Changes in biochemical reactions will result in disturbances in the balance of cell metabolism, such as a decrease in metabolic rate. The decreased metabolic rate of cells allows less use of nutrients such as glucose. The delay in low temperatures causes glucose levels in plasma and serum samples to be maintained. However,

at low temperatures, the metabolic processes of cells do not completely stop but only slow down (Stoll and Wolkers, 2011). The decrease in glucose in the specimen can be avoided by giving inhibitor substances and can be delayed in an unconscious state after taking blood specimens immediately (Tyas, 2015). Specimens suspended in a cold state in the refrigerator are more stable than specimens suspended at room temperature, which will cause glucose to decrease more quickly (Susiwati, 2018).

Based on the explanation, although there is no statistically significant effect on plasma NaF and serum glucose levels with a delay of 4 and 8 hours at the temperature of the refrigerator (4°C) and temperature of the room (25°C) in diabetic mellitus patients with *hypercholesterolemia*, it is advisable to Blood samples that have been taken must be examined immediately because the stability of the sample can change with the length of the sample delay. When a blood sample has not been tested or undergoes a delay in the examination, a glycolysis process can occur caused by cell components. It can use 5-7% of the glucose present in the specimen (WHO, 2013) . The temperature delay of serum and plasma specimens before separation using a centrifuge also affects the examination, so it needs more attention (Trisyani *et al*., 2020). Sample handling must be carried out according to the requirements because the pre-analytic stage has an error contribution of 60-70%, so it needs to be paid more attention to because the pre-analytic stage has the most significant percentage of errors that dramatically affects the results of the glucose level examination (Siregar *et al*., 2018).

CONCLUSION

The results of research that have been carried out to prove the effectiveness of the length of delay in varying temperatures on the results of plasma NaF and serum glucose examinations in diabetes mellitus patients with *hypercholesterolemia* can be concluded there was no effect of the delay time for 4 hours and 8 hours at room temperature (25°C) on the results of plasma NaF glucose in diabetes mellitus patients with *hypercholesterolemia* with a Sig. value 0.423 > 0.05. There was no effect of the delay time for 4 hours and 8 hours in a refrigerator temperature (4°C) on the results of plasma NaF glucose in diabetes mellitus patients with *hypercholesterolemia* with a Sig. value 0.772 > 0.05. There was no effect of delay time for 4 hours and 8 hours at room temperature (25°C) on serum glucose results in diabetes mellitus patients with *hypercholesterolemia* with Sig. values 0.333 > 0.05. There was no effect of delay time for 4 hours and 8 hours in a refrigerator temperature (4°C) on serum glucose results in diabetes mellitus patients with *hypercholesterolemia* with a Sig. value. 0.604 > 0.05. There was no effect between plasma NaF glucose result and serum glucose results with delay in room temperature (25°C) and refrigerator temperature (4°C).

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