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Comparison of Blood Glucose Examination Results Using Serum and Plasma Sodium Floride (NaF) in Hyperglycemia Samples With Examination Delay Time of 30 Minutes and 1 Hour

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ABSTRACT

Blood glucose level examination until now often cannot be done immediately after the sample is taken. Glycolysis in the blood can occur in vitro, thus affecting the examination results. The addition of anticoagulants such as NaF aims to inhibit the in vitro glycolysis process. Several studies have described the effects of anticoagulants in maintaining blood sugar levels in examination samples. However, the results presented still have some differences. This study aims to determine the difference in blood glucose levels using serum and plasma NaF in hyperglycemia samples with a delay of 30 minutes and 1 hour. The type of research used in this study is analytical observational with a cross-sectional approach that is a comparative study. The population in this study were all outpatients at the Muhammadiyah Hospital in Surabaya. This study was conducted at the Muhammadiyah Hospital Laboratory in Surabaya, during the study in March-April 2024. The results of this study obtained blood glucose levels using either serum or NaF plasma in hyperglycemic and non- hyperglycemic samples examined with a delay of 30 minutes and 1 hour. The Greenhouse-Geisser Sig value was 0.000 (<0.05) which means there is a real (significant) difference.

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1. INTRODUCTION

In an effort to prevent errors in the laboratory examination process that can affect the examination results, it is necessary to pay attention to several error factors that often occur which are divided into 3 stages, namely pre-analytical, analytical, and post-analytical [2] . The pre-analytical stage includes the examination request form, patient preparation, specimen collection and receipt, specimen handling, and sample preparation for analysis. Analytical stage covering preparation reagent or media, pipetting reagent And incubation samples , examination, and reading of results. As well as the post-analytical stage, namely reporting results [7]

Glucose examination is a parameter for screening Diabetes Mellitus (DM). Abnormal blood glucose levels marked with improvement or decline glucose levels in in the blood [6] . Hyperglycemia is a condition in which blood glucose levels in the body increase beyond normal limits, which occurs due to the inability of the pancreas to produce enough insulin or the body's inability to use the insulin produced properly [5] . Hyperglycemia is one of the early signs that someone has diabetes mellitus. People with hyperglycemia really need accurate blood glucose tests to determine further monitoring and treatment steps [1].

In overcoming the decrease in blood glucose levels due to delayed examination, plasma samples containing sodium fluoride (NaF) anticoagulant are used in gray-capped tubes which have been proven to minimize glycolysis [4] . NaF anticoagulant functions as an antiglycolytic which can prevent sugar metabolism in blood samples. The advantage of storing blood samples in NaF tubes compared to ordinary clotting tubes is that in NaF tubes, blood samples can be centrifuged immediately without having to be left [9].

Previous research conducted by [3] was to test stability. glucose levels blood in serum and NaF plasma with a 2-hour examination time, delayed 4 hours and 8 hours, the average serum glucose levels obtained at 2-hour, 4-hour, and 8-hour examinations were 98.00 mg/dL, 93.07 mg/dL, and 83.73 mg/dL. The average plasma glucose levels at the examination before 2 hour, 4 hours, and 8 the time is 103.93 mg/dL, 98.73 mg/dL, 91.40 mg/dL. So it is concluded that there is a difference in serum glucose levels And plasma NaF with delay examination. In his research The average percentage difference in serum and plasma glucose levels was also discussed, which were 5.71%, 6.08%, and 9.16% respectively in the examinations before 2 hours, 4 hours, and 8 hours .

In contrast to the study that examined the significance of the effect of adding additive compounds to the tube in the form of NaF compounds compared to *Serum Separator Tube gel* (SST), the results of the study obtained an average blood glucose value examined before 2 hours, namely in the NaF Oxalate tube of 147.93 mg / dl while in the SST tube it was 135.53 mg / dl. In the second check, the average blood glucose value was obtained after being delayed for 8 hours, namely in the NaF Oxalate tube of 145.67 mg / dl, while in the SST tube it was 137.87 mg / dl. So it is concluded that there is no significant difference between serum and plasma NaF glucose levels and there is no significant correlation between the level of glucose decrease and the length of examination time before 2 hours and a delay of 8 hours (Putra et al., 2018) .

2. METHOD

The type of research used in this study is analytical observational with a descriptive approach. *cross sectional* comparative study. The population in this study were all outpatients in DM polyclinic of Muhammadiyah Hospital Surabaya. The sample used in this study were outpatients who cameto Poli DM Muhammadiyah Hospital Surabaya to conduct blood glucose level checks on hyperglycemia patients taken using *purposive sampling technique* . Calculated with formula *Lameshow* obtained amount sample minimum Which needed that is as big as 30 sample.

Specimen collection and blood glucose level examination will be carried out at Muhammadiyah Hospital Surabaya. This research will be conducted in March-April 2024. The variables in this study are variation in the delay time of serum and plasma NaF examination which was delayed by 30 minutes and 1 hour and glucose levels blood. Research data were obtained from the results of blood glucose level measurements using serum specimens and NaF plasma in hyperglycemic samples examined immediately, delayed 30 minutes, and delayed 1 hours expressed in mg/dl. Then the data is tabulated into tabular and narrative form. The data The results obtained are presented in table form and then analyzed using the Repeated Measure ANOVA statistical test. use program Statistics Products and Service Solutions (SPSS) version 26.

The tools used in this study were a 5 cc syringe, tourniquet, 70% alcohol cotton, plaster, tissue, blue tip, yellow tip, micropipette, tube rack, serum tube (plain tube), NaF tube, *centrifuge* , Cobas 6000 C-501 and personal protective equipment. The materials used in this study were glucose reagent, serum, and plasma NaF.

3. RESULTS AND DISCUSSION

Presentation of Research Data

Table 1. Results of blood glucose level examination using serum in hyperglycemia samples with examination delay times of 30 minutes and 1 hour.

SERUM				
Hyperglycemia				
No	Sample	Immediately (mg/dl)	30 Minutes (mg/dl)	1 Hour (mg/dl)
1	5830	240	238	230
2	5831	218	210	205
3	5832	288	275	260
4	5833	251	245	235
5	5834	266	240	231
6	5835	253	249	240
7	5836	240	234	230
8	5837	224	220	219
9	5838	254	234	226
10	5839	253	245	240
11	5840	249	236	230
12	5841	219	209	207
13	5842	280	267	243
14	5843	288	279	245
15	5844	276	265	260
16	5845	212	203	200
17	5846	220	216	215
18	5847	225	218	217
19	5848	234	232	228
20	5849	255	247	242
21	5850	243	235	229
22	5851	256	245	229
23	5852	248	234	230
24	5853	241	223	219
25	5854	234	214	208
26	5855	223	220	219
27	5856	253	249	239
28	5857	278	250	249
29	5858	265	260	256
30	5859	237	227	220
	Mean	247.43	237.30	230.03

Table 2. Results of blood glucose level examination using NAF plasma in hyperglycemia samples with examination delay times of 30 minutes and 1 hour.

PLASMA NAF				
Hyperglycemia				
No	Sample	Immediately (mg/dl)	30 Minutes (mg/dl)	1 Hour (mg/dl)
1	5830	243	240	236
2	5831	219	215	210
3	5832	290	280	265
4	5833	254	248	235
5	5834	268	247	240
6	5835	255	238	235
7	5836	245	228	220
8	5837	230	227	226
9	5838	256	250	245
10	5839	258	246	243
11	5840	259	249	239
12	5841	221	220	205

13	5842	288	276	269
14	5843	293	270	266
15	5844	280	264	256
16	5845	225	220	215
17	5846	235	228	227
18	5847	229	223	201
19	5848	240	235	226
20	5849	265	257	246
21	5850	258	245	227
22	5851	270	268	256
23	5852	275	263	247
24	5853	259	255	239
25	5854	247	245	235
26	5855	230	225	219
27	5856	276	270	269
28	5857	287	277	270
29	5858	275	259	260
30	5859	240	238	233
	Mean	255.66	246.86	238.66

3.1. Plasma and Serum Examination For Hyperglycemia

In this study, the characteristics of the research subjects are seen from gender and age, which are presented in the following table:

Table 3. Description of research subjects based on gender

Type Sex			
	Frequency		Percentage (%)
Valid	Women	19	63.3
	Man	11	36.7
	Total	30	100.0

Table 4. Description of research subjects based on age

Statistics Descriptive				
	N	*Min*	*Max*	*Mean*
Age	30	46	65	56.43
Valid N (based on list)	30			

Table 5. Descriptive statistics of serum blood glucose levels with a 30-minute and 1-hour examination delay.

Descriptive Statistics					
Group	N	Min	Mom	Average	Std. Dev
Hyperglycemia Serum 30 Minutes	30	203	279	237.30	19,572
1 Hour Hyperglycemia Serum	30	200	260	230.03	15,646

Table 6. Descriptive statistics of plasma blood glucose levels NAF examination delay of 30 minutes and 1 hour

Descriptive Statistics

Group	N	Min	Mom	Average	Std. Dev
Hyperglycemic Plasma 30 Minutes	30	215	280	246.87	18,776
Plasma Hyperglycemia 1 Hour	30	201	270	238.67	19,338

In this study, the blood glucose levels in hyperglycemia samples obtained the average values of serum and plasma NaF respectively with a delay of 30 minutes and 1 hour were in serum of 237.3 mg/dL, 247 mg/dL and in plasma NaF of 230.3 mg/dL, 238.6 mg/dL. Based on these results, the average values of blood glucose levels using serum and plasma NaF began to differ over the delay time of the examination. According to (Nisa, 2020) , in addition to being an anticoagulant, NaF also acts as a blood preservative that inhibits the growth of microorganisms and the work of enzymes, one of which is the enzyme phosphoenol pyruvate and enolase, so that the results of this study proved that blood glucose levels using plasma NaF were more stable compared to serum at a delay of 2 hours to 6 hours.

Hypothesis Test (Analysis of Differences in Blood Glucose Levels Using Serum and Plasma NaF in Hyperglycemic and Non-Hyperglycemic Samples with Examination Delay Times of 30 minutes and 1 hour

Table 7. Normality Test

Normality Test

	Shapiro Wilk		
	Amount	Sig.	Information
Immediate Hyperglycemia Serum	30	.319	Normally Distributed
Hyperglycemia Serum 30 Minutes	30	.640	Normally Distributed
1 Hour Hyperglycemia Serum	30	.669	Normally Distributed
Immediate Hyperglycemia Plasma	30	.382	Normally Distributed
Hyperglycemic Plasma 30 Minutes	30	.363	Normally Distributed
Plasma Hyperglycemia 1 Hour	30	.434	Normally Distributed

Immediate hyperglycemia serum 30 .319 normally distributed, 30-minute hyperglycemia serum 30 .640 normally distributed, 1-hour hyperglycemia serum 30 .669 normally distributed. Immediate hyperglycemia plasma 30 .382 normally distributed, 30-minute hyperglycemia plasma 30 .363 normally distributed, 1-hour hyperglycemia plasma 30 .434 normally distributed.

Data is said to be normal if the sig value > 0.05 and not normal if the sig value < 0.05. Normality test analysis using the Shapiro-Wilk technique, it can be concluded that all data in each group is normally distributed.

The homogeneity test is carried out using the Test of Homogeneity of Variances technique. Data is said to be homogeneous if the sig value is > 0.05 and not homogeneous if the sig value is < 0.05.

Table 8. Homogeneity Test

Homogeneity of Variance Test			
Mark			
Information	**Levene statistics**	**Sig.**	**Serum Examination Results**
Mean	1,520	.224	Homogeneous Variance
Median	1,459	.238	Homogeneous Variance
Median with adjusted df	1,459	.238	Homogeneous Variance
Trimmed Mean	1,529	.222	Homogeneous Variance
Plasma Examination Results			
Mean	.459	.633	Homogeneous Variance
Median	.446	.642	Homogeneous Variance
Median with adjusted df	.446	.642	Homogeneous Variance
Trimmed Mean	.459	.633	Homogeneous Variance

Based on the homogeneity test table above, the sig value is 1,000 (sig > 0.05). So it can be concluded that the data in this study has a homogeneous variance.

The basis for decision making in the Repeated Measures ANOVA test is if the Greenhouse Geisser Sig. value is >0.05 then H0 is accepted and Ha is rejected, whereas if the Greenhouse-Geisser Sig. value is >0.05 then H0 is accepted and Ha is rejected.

Table 9. Significant test of differences in blood glucose level examination results using serum in hyperglycemic samples with a 30 minute and 1 hour examination delay time.

Within-Subject Effect Test			
Measuring : Serum Examination			
Source		Sig.	Information
Inter Group	4582.489	0.003	Significant
In Group	31218.633		
Total	35801.122		

Based on the results of the table above, it is known that the Greenhouse-Geisser Sig. value is 0.003 (<0.05) so H0 is rejected and Ha is accepted, which means that there is a significant difference in the average blood glucose levels from the immediate examination time, 30 minutes to 1 hour. Thus, it can be concluded that there is a difference in blood glucose levels in hyperglycemia samples examined immediately, 30 minutes and 1 hour.

Table 10. Significant test of differences in blood glucose level examination results using NaF Plasma in hyperglycemia samples with a 30 minute and 1 hour examination delay time.

Within-Subject Effect Test			
Measuring : Inspection			
Source		Sig.	Information
Inter Group	4336.800	.006	Significant
In Group	34684.800		
Total	39021.600		

Based on the results of the table above, it is known that the Greenhouse-Geisser Sig. value is 0.006 (<0.05) so H0 is rejected and Ha is accepted, which means that there is a significant difference in the average blood glucose levels from the immediate examination time, 30 minutes to 1 hour. Thus, it can be concluded that there is a difference in blood glucose levels in hyperglycemia samples examined immediately, 30 minutes and 1 hour.

Based on the results of the hypothesis test, it is known that the Greenhouse-Geisser Sig. value is 0.003 (<0.05) in the hyperglycemic and non-hyperglycemic serum sample groups, so H0 is rejected and Ha is accepted, which means that there is a significant difference in the average decrease in blood glucose levels from the immediate examination time, 30 minutes, to 1 hour using serum or plasma in both hyperglycemic samples. In the hyperglycemic serum group, sequentially in the immediate examination time, 30-minute delay, and 1 hour, there was a decrease in blood glucose levels from 255.66, 246.86 and 238.66.

Blood glucose levels begin to decrease. This occurs due to the activity of blood cells. According

to (Sugiah et al., 2023) , storing samples at room temperature will cause a decrease in blood glucose levels of approximately 1-2% per hour. Blood samples contain erythrocytes, leukocytes, platelets, and also the activity of bacterial cells that will maintain their life, so that the sugar in the blood sample is used as a food source. The enzymatic reaction process of these cells is interrupted, with the addition of NaF, so that these cells cannot use the glucose in the blood sample as a food source. The decrease in blood sugar levels can thus be inhibited (Fernandez et al., 2013). In this study, the results of serum and plasma NaF glucose levels were found to differ even though they were taken from the same research subjects at the same time.

In the NaF plasma group, hyperglycemic samples were sequentially immediate examination time, 30 minute delay, and 1 hour there was a decrease in levels blood glucose with an average of 255.66, 246.86, so that the decrease was 238.66.

Sodium Fluoride is anticoagulant Which can guard level blood glucose by preventing the process of glycolysis so that blood glucose levels can stable (Butt et al., 2018) . Proven from results study level glucoseblood using NaF plasma that has been done, the decrease is greater happen on serum Good on sample hyperglycemia. According to (Lippi et al., 2018) , delayed sample storage can be stable at a temperature of 15-25 ^{o}C.or temperature room during 24 O'clock And temperature

4 ^{o} C stable during 10 day If using anticoagulants. However, in this study, the results of the examination were still found a decrease between blood glucose levels using plasma NaF on sample hyperglycemia

Results this research opposite with research which is conducted by (Ridefelt et al., 2014) , where there was no effect of the length of storage of NaF plasma on Temporary blood glucose levels are checked immediately, stored for 3 and 24 hours temperature 15-25 ^{o} C. However results study This in line with study by (Van Den Berg et al., 2015) which states that anticoagulants cannot stop in totality of glucose breakdown (glycolysis). Thus, in the long term time Which long, glucose concentration can reduce to zero

3.2. Sub section 2

Proper citation of other works should be made to avoid plagiarism. When referring to a reference item, please use the reference number as in or for multiple references. The use of ”Ref...” should be employed for any reference citation at the beginning of sentence. For any reference with more than 3 or more authors, only the first author is to be written followed by *et al* . (eg in). Examples of reference items of different categories are shown in the References section. Each item in the references section should be typed using 8 pt font size.

4. CONCLUSION

This study compared blood glucose test results using serum and sodium fluoride (NaF) plasma in hyperglycemic samples with examination delays of 30 minutes and 1 hour. The findings demonstrated a significant difference in blood glucose levels between immediate testing, 30-minute delay, and 1-hour delay in both the serum and NaF plasma groups. Statistical analysis using the Repeated Measures ANOVA test indicated that the Greenhouse-Geisser significance value for serum was 0.003 (<0.05) and for NaF plasma was 0.006 (<0.05), confirming a statistically significant decrease in glucose levels over time. This decline is attributed to cellular activity within the blood samples. However, NaF plasma exhibited superior stability in preserving glucose levels compared to serum, likely due to its antiglycolytic properties.

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