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Effect of Blood Sample Tubes on Diagnosis of Gestational Diabetes in Pregnant Women Undergoing OGTT

OGTT Yapılan Gebelerde Kan Örnek Tüplerinin Gestasyonel Diyabet Tanısına Etkisi

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Öz

Gebelikte diagnostik oral glukoz tolerans testi (OGTT) yapılması gestasyonel diyabetin erken tanısı için önemlidir. Hiperglisemi hızlı bir şekilde saptanabilir, böylece anne ve fetüs korunabilir. Bu çalışmada OGTT uygulanan gebelerde kan örneği tüpleri ile glukoz stabilitesi arasındaki ilişkinin araştırılması amaçlanmıştır.Yöntemler: OGTT (75g) yapılan 20 gönüllü gebenin glukoz yüklemesi sonrası 120. dakikada kan örnekleri alındı. Serum için VACUETTE CAT Serum Separator (Clot Activator Tube) kullanıldı; Plazma için VACUETTE FC Mix tüpü (Na2 EDTA, sodyum florür, sodyum sitrat) ve VACUETTE FE (Sodyum Florür/K3 EDTA) kullanıldı. Bu tüplerin üçü de 0. saat, 2. saat ve 4. saat olmak üzere üç farklı zamanda santrifüjlendi. Tüm örnekler 0., 2., 4. saatlerde santrifüj edilerek glukoz değerleri ölçüldü. Sadece VACUETTE FC Mix tüplerinde glukoz değerleri arasında istatistiksel olarak anlamlı fark bulunmadı ($p>0.05$). Ancak, bu farklı zaman aralıklarında serum ve florürlü tüplerde istatistiksel olarak anlamlı fark bulundu ($p<0.01$). VACUETTE FC Mix'e alınan kanların 4. saatte bile santrifüj edilmeden bekletildiğinde glukoz değerlerinin düşmediği ve bu nedenle rutin kullanımının faydalı olacağı gösterildi. Ancak VACUETTE CAT serum ayırıcı ve VACUETTE FE'de glukoz değerlerinin stabil olmadığı saptanmıştır.

Anahtar Kelimeler: Gebelik, Gestasyonel Diyabet, Glikolizis, Glukoz, Oral Glukoz Tolerans Testi

Abstract

Performing a diagnostic oral glucose tolerance test (OGTT) during pregnancy is important for early diagnosis of gestational diabetes mellitus. Hyperglycemia can be detected quickly, and mother and the fetus can be protected. This study aimed to research the relationship between blood sample tubes and the stability of glucose in pregnant women undergoing OGTT. Blood samples were taken at the 120th minute after glucose loading from 20 pregnant volunteers who underwent OGTT (75g). VACUETTE CAT Serum Separator (Clot Activator Tube) was used for serum; VACUETTE FC Mix tube (Na2 EDTA, sodium fluoride, sodium citrate) and VACUETTE FE (Sodium Fluoride/K3 EDTA) were used for plasma. All three of these tubes were centrifuged at three different times i.e. 0. hour, 2. hour, and 4. hour. All samples were centrifuged at 0., 2., and 4. hours, and glucose values were measured. No statistically meaningful difference was found between their glucose values in only VACUETTE FC Mix tubes ($p>0.05$). However, a statistically meaningful difference was found at these different time intervals in serum and fluoride tubes ($p<0.01$). It was shown that when the blood sampled into VACUETTE FC Mix was kept without centrifugation even at the 4. hour, their glucose values did not decrease and therefore the routine use of them would be beneficial. However, it was found that the glucose values were not stable in VACUETTE CAT Serum Separator and VACUETTE FE.

Keywords: Pregnancy, Gestational Diabetes, Glycolysis, Glucose, Oral Glucose Tolerance Test

Introduction

Gestational diabetes mellitus (GDM) is glucose intolerance with first recognition or diagnosis during pregnancy. Blood glucose levels are low or normal in general after giving birth (1). GDM occurs in 7% of pregnant women. Usually, GDM develops in connection with the placenta hormones blocking the insulin's effects (increasing the insulin's resistance) after the 24th week of pregnancy (2). If the regulation of blood glucose is disrupted in pregnant women, it might cause negative outcomes to emerge in both them and their children, especially in mothers who had suffered diabetes before getting pregnant.

Diabetes or prediabetes is diagnosed by performing fasting plasma glucose (FPG), oral glucose tolerance test (OGTT), and glycosylated hemoglobin Alc (HbAlc) measurement (2). As a principle, OGTT is used for diagnostic purposes during pregnancy or in epidemiologic studies where the blood glucose levels are uncertain (4). Thus, the high glucose level in mothers' plasma is found out on time and precautions can be taken to prevent the harmful effects of hyperglycemia on the fetus (3). International Association of the Diabetes and Pregnancy Study Groups suggests performing OGTT with 75g glucose for two hours (2). At present, there is no full consensus to employ the single-stage diagnosis approach instead of the two-stage test to diagnose GDM. Any of the two-stage diagnosis approach (preliminary screening test with 50g glucose followed by OGTT using 100g glucose for three hours) or the single-stage diagnosis approach (OGTT using 75g glucose) can be employed (3).

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In this study, a 75g oral glucose tolerance test was performed on the volunteer pregnant women, and samples were taken at the 120th minute. A plasma glucose test performed in a laboratory is the basic tool to diagnose diabetes mellitus, impaired fasting glucose (IFG), and/or impaired glucose tolerance (IGT), especially for screening, and to diagnose GDM in cases where HbAlc cannot be used (5). So, according to international guidelines, plasma glucose measurement must be accurate precise, and final in terms of patient classification following the international guidelines (5).

For glucose measurements, plasma must be separated as soon as possible from the cells. Sometimes this may not be possible, in which case a tube containing a glycolysis inhibitor such as sodium fluoride should be used for sampling (6). If a sample is processed after a delay, determination of glucose may not be accurate (7). It is a known fact that if blood samples are kept without centrifugation, their glucose concentration will gradually reduce (8). It was reported that plasma glucose samples which are not centrifuged immediately, reduce 5% to 7% in vitro per hour due to glycolysis (5). American Diabetes Association (ADA) and the National Academy of Clinical Biochemistry (NACB) recommend that to minimize in vitro glycolysis, the sample tube should be immediately placed in ice water and the plasma should be separated from the cells within 30 minutes, or if this is not possible, a sample tube containing a rapid glycolysis inhibitor should be used, e.g. citrate buffer (5).

In conclusion, measured concentrations of glucose highly depend on such pre-analytic variables as the type of phlebotomy tubes used and the time between phlebotomy and analysis (9). This study focused on vacuum tubes containing different anticoagulants for collecting venous blood samples, and their effect on the stability of glucose.

Material and Method

Twenty pregnant women who were loaded with 75g glucose for OGTT to assess gestational diabetes at Istanbul Bakırkoy Dr. Sadi Konuk Training and Research Hospital were included. This study was performed after the Ethics Committee approved it (protocol no: 2022/170, decision no: 2022-09-23) and informed consent was obtained from each subject. Research involving human subjects complied with all relevant national regulations, and institutional policies and is following Helsinki Declaration (as revised in 2013).

The same personnel collected nine venous blood samples from each subject 120 minutes after loading 75g glucose. 3 VACUETTE CAT Serum Separator Clot Activator Tubes (SST) were used to obtain a serum sample; 3 VACUETTE FC Mix tubes (Na2 EDTA, sodium fluoride, sodium citrate) and 3 VACUETTE FE Sodium Fluoride/K3 EDTA tubes were used for plasma. In VACUETTE FC Mix tubes; to ensure optimal glucose stabilization, the tubes were inverted 10x directly after blood collection. 3 tubes (SST, Mix and EDTA) were centrifuged at 1,800g and 20°C for 10 minutes at the 0. hour, 3 tubes at 2. hour, and 3 tubes at 4. hour.

Glucose was measured with hexokinase glucose-6-phosphate dehydrogenase method using AU5800 Series Chemistry Analyzers (Beckman Coulter, USA). Internal quality control was performed on a minimum of two levels of glucose by using Beckman Coulter control materials.

VACUETTE CAT Serum Separator Tube-454243, VACUETTE FC Mix Tube-454513, and VACUETTE FE Sodium Fluoride/K3 EDTA-454221 tubes are manufactured by Greiner Bio-One (Kremsmünster, Austria).

Statistical review

The NCSS (Number Cruncher Statistical System) 2007 (Kaysville, Utah, USA) software was used for statistical analyses. Descriptive statistical methods (mean, standard deviation, median, frequency, minimum, maximum) were utilized to evaluate study data. The normality of distribution for quantitative data was assessed using the Shapiro-Wilk test and graphical examinations. Fasting blood glucose measurements were evaluated based on time and application types using the Repeated Measures with Linear Mixed Model. For within-group comparisons of normally distributed quantitative variables, repeated measures ANOVA was applied, and Bonferroni-corrected pairwise comparisons were used for dual comparisons. For non-normally distributed quantitative variables, within-group comparisons were conducted with the Friedman test, and Bonferroni-corrected Wilcoxon signed-ranks tests were used for pairwise comparisons. Dependent t-tests were used for normally distributed within-group comparisons of quantitative variables, and Wilcoxon signed-ranks tests were used for non-normally distributed variables. Statistical significance was set at $p<0.05$.

Results

At the end of the study, glucose levels were measured in VACUETTE FC Mix plasma, VACUETTE CAT Serum Separator, and VACUETTE NaF (sodium fluoride) /EDTA plasma tubes, in which blood samples were collected 120 minutes after loading glucose (Table 1).

To examine the effects of application types and timing on postprandial blood glucose measurements, GLM modeling was used in repeated measures. The model obtained was found to be statistically significant ($F=22.758$, $p=0.000$). In the model, the effects of both applications and the Time × Application interaction were statistically significant ($p=0.000$). The effect size was determined as 0.577,

and post hoc power was 98.2%. Therefore, all evaluations were conducted in detail within groups, and the results are presented in Table 2.

The average difference of 3.89±2.25 units between the postprandial FC Mix measurement and the gray measurement at baseline (0 hour) in the subjects participating in the study was found to be statistically significant (p=0.001).

The average difference of 6.39±3.01 units between the postprandial FC Mix measurement and the gray measurement at the 2nd hour was also statistically significant (p=0.001).

Similarly, the average difference of 7.47±3.13 units between the postprandial FC Mix measurement and the gray measurement at the 4th hour was statistically significant (p=0.001).

The average difference of 8.61±4.39 units between the postprandial FC Mix measurement and the serum measurement at baseline (0 hour) was found to be statistically significant (p=0.001). The average difference of 18.66±4.66 units between the postprandial FC Mix measurement and the serum measurement at the 2nd hour was statistically significant (p=0.001).

The average difference of 27.85±8.75 units between the postprandial FC Mix measurement and the serum measurement at the 4th hour was also statistically significant (p=0.001).

The average difference of 4.72±3.62 units between the gray measurement at baseline (0 hour) and the serum measurement was statistically significant (p=0.001).

The average difference of 12.27±5.03 units between the gray measurement at the 2nd hour and the serum measurement was statistically significant (p=0.001).

Table 1. Investigation of factors affecting postprandial blood glucose measurements

	Type III Sum of Squares	Mean Square	F	*p*
Application	487.62	487.62	28.55	***0.000*****
Time X Application	1850.89	1850.89	108.37	***0.000*****

*Application: FC mix, Gray tube, and Serum, Time: Baseline (0 hour), 2nd hour, and 4th hour, Repeated Measures with Lineer Mix Model, **p<0.01*

The average difference of 20.37±8.50 units between the gray measurement at the 4th hour and the serum measurement was also statistically significant (p=0.001).

No statistically significant difference was found among the changes in postprandial blood glucose measurements at 0, 2, and 4 hours in the FC Mix group (p>0.05).

In the gray group, statistically significant differences were found among the changes in postprandial blood glucose measurements at 0, 2, and 4 hours (p=0.001). According to the results of pairwise comparisons conducted to determine the differences, the average decrease of 2.57±1.80 units in postprandial blood glucose measurements at the 2nd hour compared to baseline was statistically significant (p=0.001). The average decrease of 4.55±2.45 units in postprandial blood glucose measurements at the 4th hour compared to baseline was statistically significant (p=0.001). The average decrease of 1.97±1.61 units in postprandial blood glucose measurements at the 4th hour compared to the 2nd hour was also statistically significant (p=0.001).

In the serum group, statistically significant differences were found among the changes in postprandial blood glucose measurements at 0, 2, and 4 hours (p=0.001; p<0.01). According to the results of pairwise comparisons conducted to determine the differences, the average decrease of 10.12±4.74 units in postprandial blood glucose measurements at the 2nd hour compared to baseline was statistically significant (p=0.001; p<0.01). The average decrease of 20.20±7.86 units in postprandial blood glucose measurements at the 4th hour compared to baseline was statistically significant (p=0.005; p<0.01). The average decrease of 10.08±6.15 units in postprandial blood glucose measurements at the 4th hour compared to the 2nd hour was also statistically significant (p=0.005; p<0.01) (Figure 1).

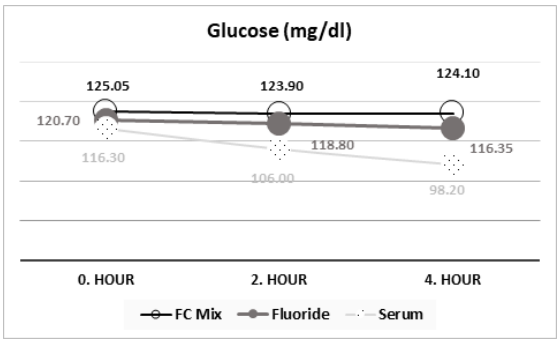

Figure 1. Glucose median levels of three different tubes at 0., 2., and 4. hours

A statistically significant relationship was found among the samples taken at the 120th minute after a 75 g loading dose (p=0.001). According to the results of pairwise comparisons conducted to determine differences, postprandial blood glucose measurements in the serum group were significantly lower than those in the FC Mix and Capillary groups (p=0.003; p=0.026) (Table 3).

The average difference of 3.85±2.68 units between the postprandial FC Mix measurement and the gray measurement was found to be statistically significant (p=0.003).

Table 2. Evaluation of the difference between fc mix and serum measurements over time

Satiety Measurements		[1]FC Mix	[2]Gray tube	[3]Serum	Test Value	[1,2]Difference	[1,2]Test Value; *p*	[1-3]Difference	[1-3]Test Value; *p*	[2,3]Difference	[2,3]Test Value;*p*
0. Hour	*Min-Max (Median)*	65.6-231.8 (125.05)	57.9-229.8 (120.7)	56.6-227.8 (116.3)	***F:57.23***	3.89±2.55	t:6.810	8.61±4.39	Z:-3.920	4.72±3.62	Z:-3.921
	Ort±Sd	132.45±40.93	128.56±40.62	123.84±40.24	***[b]0.001***		***[a]0.001***		***[d]0.001***		***[d]0.001***
2. Hour	*Min-Max (Median)*	65.6-237.3 (123.9)	55.8-227.3 (118.8)	23.3-217.2 (106.0)	F:192.16	6.39±3.01	t:9.498	18.66±4.66	t:17.878	12.27±5.03	t:10.905
	Ort±Sd	132.38±41.61	125.98±41.03	113.71±38.92	***[b]0.001***		***[a]0.001***		***[a]0.001***		***[a]0.001***
4. Hour	*Min-Max (Median)*	62.4-233.5 (124.1)	53.6-223.4(116.35)	48.5-202 (98.2)	F:156.96	7.47±3.13	t:10.672	27.85±8.75	t:14.223	20.37±8.50	t:10.712
	Ort±Sd	131.48±41.36	124.01±40.91	103.63±38.36	***[b]0.001***		***[a]0.001***		***[a]0.001***		***[a]0.001***
Test Value		F:1.458	F:52.590	Chi.Square:40.000							
	p	***[b]0.245***	***[b]0.001***	***[c]0.001***							
0-2 Hour		***1.000***	***0.001***	***0.001***							
0-4 Hour		***0.613***	***0.001***	***0.005***							
2-4 Hour		***0.445***	***0.001***	***0.005***							

*[a]Paired Samples Test, [b]Repeated Measures Test, [c]Friedman's Test, [d]Wilcoxon Test, **p<0.01*

Table 3. Evaluation of measurements on 11 individuals

N=11	**Samples taken at 120 minutes after 75 g loading (0th hour)**	
	Median ±Sd	*Min-Max (Median)*
FC Mix	126.60±51.70	65.6-231.8 (112.60)
Gray	122.75±51.36	57.9-229.8 (108.3)
Serum	119.09±50.81	56.6-227.8 (107.0)
Capillary	136.59±44.34	97-221 (111.5)
Test Value	F:17.945	
p	*[c]0.001***	
Difference	*Median ±Sd*	*P*
FC Mix- Gray	3.85±2.68	*[d]0.003***
FC Mix-Serum	7.51±3.84	*[d]0.003***
Gray-Serum	3.66±3.21	*[d]0.003***
FC Mix- Capillary	9.98±22.17	*[d]0.374*
Gray-Capillary	13.83±22.28	*[d]0.075*
Serum- Capillary	17.50±22.15	*[d]0.026**

*[c]Friedman's Test, [d]Wilcoxon Test,*p<0.05, **p<0.01*

The average difference of 7.51±3.84 units between the postprandial FC Mix measurement and the serum measurement was also statistically significant (p=0.003; p<0.01).

The average difference of 3.66±3.21 units between the postprandial serum measurement and the gray measurement was statistically significant (p=0.003; p<0.01).

The difference between the postprandial capillary measurement and the FC Mix and gray measurements was not statistically significant (p>0.05).

The average difference of 17.50±22.15 units between the capillary measurement and the gray measurement was statistically significant (p=0.026; p<0.05).

Discussion

Although OGTT for pregnant women has been argued in recent years, laboratories must focus only on performing an accurate glucose assay.

The laboratory process is divided into the pre-analytic, analytic, and post-analytic steps. At the pre-analytic phase, it is essential to ensure blood stable for an accurate analysis of glucose concentration. This process involves centrifugation quickly after specimen collection.

It is observed that today certain negative circumstances of the health sector delay the time to centrifuge blood collection tubes after venipuncture and therefore cause the decreased blood glucose levels.

With the increase in the number of city hospitals, daily patient admission is increased and distances between blood collection departments and laboratories are also increased. And in some cases, the use of a pneumatic system becomes almost mandatory. The faults in the pneumatic system used in these large hospitals can cause delays in getting samples to the laboratory.

In our country, family health centers send blood samples to the central laboratories. However, in some family health centers, lack of centrifuge or adjustment problems in the existing centrifuge cause problems that affect sample quality. Incorrect results are obtained from samples that are not centrifuged and not properly centrifuged.

Furthermore, it is a well-known fact that since glucose concentration is reduced in non-centrifuged blood specimens, the risk of incomplete diagnosis of diabetes is limited and values lower than actual values are measured (10).

The purpose of this study was to assess the stability of glucose and to study the glycolysis inhibition in serum or plasma samples using different test tubes for blood samples from volunteer pregnant subjects who underwent OGTT. Blood samples were stored in test tubes containing NaF/EDTA or EDTA, fluoride and sodium citrate and SST, which were centrifuged after a delay or in an insufficient way under controlled delay conditions (up to 240 minutes after the collection) to take under control the pre-analytic step of the glucose test.

Diabetes is a disease characterized by high blood glucose levels and developing chronically. Any kind of diabetes might lead to complications in various parts of the body and increase the risk of early death in general (11). GDM is one of the types of diabetes and is defined as glucose intolerance arising or diagnosed during pregnancy (1). Performing oral glucose tolerance tests on pregnant women for diagnostic purposes is necessary for early diagnosis of gestational diabetes (3).

To diagnose diabetes, measurement of blood glucose must be available in the primary healthcare services. The role played by the control of blood glucose to prevent the development and progression of complications was proven (5).

Glucose concentration may be measured in whole blood, serum, or plasma, but plasma is suggested for diagnosis (6). When glycolysis is not quickly inhibited or blood cells cannot be quickly separated from plasma, the measurement of fasting

glucose in plasma is influenced by the decrease of glucose over time in blood sample tubes (10).

Organizations such as the American Diabetes Association and the World Health Organization have made the following recommendations: glycolysis should be minimized by centrifugation of the sample or by placing the tubes on ice immediately after blood collection and centrifuging within 30 minutes (9). In many laboratories, samples cannot be processed or analyzed within one hour after they are collected.

The initial decrease of glucose concentration can be limited by centrifuging or storing the blood sample tubes in an ice/water medium, but blood samples collected in a department or separate institution relatively far from the laboratory lie outside the range of the laboratory. Furthermore, when blood tubes are placed in ice water, the tube label may become unreadable, which may lead to patient identification problems (10).

Md Nahidul Islam and et al. reported that glucose analysis using FC-Mix tube demonstrated a strong correlation WHO specifications when stored at 4°C. When FC-Mix tubes were stored at room temperature, glucose was stable for 4 days. These findings suggest that the FC-Mix effectively inhibits glycolysis and should be introduced into routine clinical practice (12).

Van den Berg and et al. published that, addition of citrate almost completely prevented in vitro glycolysis, but showed a positive bias (0.2 mmol/l) compared to control. This is partly due to a minor decrease in glucose level in control blood, drawn according to the current guidelines. This decrease occurs within 15 minutes, in which glycolysis has been described to be minimal and acceptable. NaF-EDTA-citrate based test tubes provide the best pre-analytical condition available (13).

Nowadays, considerable efforts have been spent to find out efficient protectors against glucose decrease through glycolysis (14).

A conventional approach is to add NaF in combination with anticoagulant potassium oxalate (KOx) to stabilize the glucose concentration in a blood sample tube. The mechanism of the effect of fluoride is based on inhibition of enzyme enolase applying a late effect in glycolytic terms. Therefore, the activity of the glycolytic enzymes located upstream of the enolase is not considerably affected, so they remain active and continuously metabolize the glucose. This explains why fluoride's effect on glycolysis inhibition lasts up to four hours. (10).

The efficiency of such glycolysis inhibitors as sodium fluoride (NaF) combined with KOx in stabilizing blood glucose is limited (10).

Van den Berg and et al. used a new protocol including a new phlebotomy tube type containing a NaF-EDTA-citrate additive and published glucose results, that are 100% identical to the gold standard in laboratory and clinical diagnosis, without the need to further adapt current procedure characteristics such as pre-analytical turn-around time (TAT) (15).

Regulations on gestational diabetes mellitus suggest using only the blood sample tubes that meet the pre-analytic needs of glycolysis inhibition. Regulations on GDM cover possible errors in pre-analytic needs and focus on fluoride's incomplete inhibition of glycolysis. Inhibition of glycolysis only by NaF, in the absence of other additives, begins two hours after blood is collected and will not be completed for four hours (16).

The study performed by Bonetti et al. indicates that blood samples collected into tubes containing a clot activator, lithium-heparin or sodium fluoride are not suitable for glucose measurements (17).

We evaluated the effect of three routinely used collection tubes (SST, mix and EDTA) on the stability of glucose in blood, and found that initial glucose concentration was not significantly different among three tube types. It is reported, that immediate glycolysis inhibition was not achieved in any tube type, and only sodium fluoride was efficient in inhibiting glycolysis in the settings of delayed sample processing (18).

In stabilization studies of blood glucose using not centrifuged blood samples, hexokinase enzyme found to become active only at pH 5.9 or higher, which is located at the start of the glycolytic way (10).

Therefore, acidifying the blood utilizing a citrate buffer will prevent glucose from catabolizing due to glycolysis starting to happen at a much earlier stage and proceeding at a speed faster than that of fluoride (10).

The new generation blood sample tests designed contain three different additives: the first additive lowers the pH level of the blood and therefore helps to inhibit hexokinase enzyme (i.e. citrate): the second additive directly inhibits enolase enzyme (i.e. NaF), and the third additive irrevocably inhibits the coagulation of blood (i.e. ethyl diamine tetra acetic acid-EDTA) (10).

Regulations issued by ADA and NACB suggest that plasma glucose should be analyzed by using gold standard treatment tubes or blood sample tubes containing such quick effect glycolysis as citrate buffer (19). The use of granular glycolysis inhibitor provides a pre-analytic benefit by removing the dilution effect which might be caused by insufficient filling of a tube with a liquid additive (20).

Bonetti et al administered 75g OGTT to 147 volunteer subjects, 83 of whom were pregnant, and collected samples into tubes with NaF/K3EDTA, NaF/Na2EDTA/citrate liquid form, NaF/K2Ox, and NaF/Na2EDTA/citrate granule form. It was shown that measurement of glucose in tubes containing citrate is more efficient in terms of diagnosing carbon hydrate disorders than in tubes containing NaF (21).

This was confirmed in 2019 by Jamieson et al., who compared plasma glucose stability over time in 501 samples taken during OGTT after 24 weeks of gestation and found that the samples containing citrate as a glycolytic inhibitor offered the best short and long-term stability for glucose levels even compared with fluoride samples placed immediately on ice (22).

In a study, by using tubes containing citrate and NaF, Yağmur et al found that in NaF plasma tubes, the concentration of glucose decreased from 90 mg/dL to 87 mg/dL (–3.3%) within the first four hours and decreased significantly to 82.6 mg/dL (–8.2%) 12 hours later. They also found that the citrate buffer in the glucose tubes prevent the glucose concentration from falling (23).

In a study on volunteer pregnant women, Daly et al used fluoride/EDTA plasma tubes and citrate-added plasma tubes. They kept fluoride/EDTA(FE) tubes at room temperature and in icy water. As a result, they suggest replacing FE tubes with citrate-fluoride-EDTA tubes to measure maternal glucose for diagnosing GDM in the absence of an ice mixture, early cell separation, and analysis (24).

Zhang et al collected venous blood samples from 58 fasting volunteers into NaF/citrate tubes and no additive tubes. They found that after keeping for 10 hours at room temperature, glucose was higher (13.4%) in no additive tubes than in NaF/citrate tubes (2%) (25).

In 2013, Garcia del Pino et al. determined that citric acid immediately inhibits glycolysis. These authors showed that glucose levels in samples with sodium fluoride was significantly lower than with temporally paired citrate tubes (26).

Stapleton et al. performed a study on volunteer pregnant women, and used tubes containing citrate, fluoride/EDTA tubes, and lithium-heparin. They reported that the average concentration of glucose in samples with fluoride/citrate remained stable for 2.5 hours. In addition, that there was a statistically significant difference between glucose levels of samples at hours 0. and 2.5 with fluoride-EDTA and lithium heparin kept at room temperature (27).

Comparable results were reported by Norman et al. evaluating paired fasting plasma glucose samples collected into sodium fluoride and citrate tubes and found higher glucose levels in the samples collected into the citrate tubes (28).

Gambino et al compared tubes containing NaF and sodium oxalate with tubes containing citrate buffer, NaF, and EDTA. As a result, they found that the average concentration of glucose in blood samples with NaF and sodium oxalate decreased 4.6% at 2. hour and decreased 7.0% at 24. hour. Glucose levels decreased 0.3% at 2. hour and decreased 1.2% at 24. hour in samples with citrate buffer, NaF and EDTA. They commented that acidification should singularly replace NaF to obtain an accurate concentration of glucose (29).

Serum data showed that the recommended clotting time of 30 minutes was sufficient to cause significant changes and that prolonged contact with cells triggered glucose consumption. This simply shows that under usual laboratory operating conditions, routine serum tubes are not suitable for estimation of glucose levels accurately. Also, a similar situation applies to NaF/EDTA tubes.

Conclusion

Our results indicate that VACUETTE FC Mix tubes are the most efficient ones in terms of preventing the concentration of glucose from undergoing clinically meaningful changes at room temperature.

EDTA, fluoride, and citrate/citric acid buffer contribute to reliable measurement of the glucose concentration, in cases where the time until centrifugation is prolonged, including transportation and/or storage periods. Stability of glucose is necessary at the pre-analytic phase for diagnosing and treating diabetes. From a clinical point of view, unreliable blood glucose level measurements can cause misdiagnosis, delay in diagnosis and complications Maximum care and supervision are required for the material used in the laboratory, sample stability in all phases (pre-analytic, analytic, etc.).

Stability of glucose is important at the pre-analytic phase, and studies on stability need to be performed in larger numbers at different centers and on more varied groups of patients. The use of tubes containing EDTA, fluoride, and citrate/citric acid buffer looks promising in terms of protection of the stability of glucose.

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Conflict of interest statement

Authors state no conflict of interest.

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