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Original article

Effects of Sodium Fluoride – Potassium Oxalate on Blood Lipid Profile Results and Lipid Stability when using this Anticoagulant

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Abstract: ***Introduction:*** Some previous studies have shown the effect of K_2EDTA, heparin, citrate, kalioxalate on lipid test results [1,2,3], but no studies on sodium fluoride - potassium oxalate. Furthermore, it is also important to ensure sample stability during testing. We performed this study to evaluate the effect of NaF-KOx on lipid results and lipid stability when storing. ***Methods:*** A cross-sectional study was conducted on the residual of 150 pairs of serum and NaF-KOx plasma samples from the patients at the University Medical Center 2. These patients participated voluntarily after signing the consent form. We divided the samples into groups: serum (group 1), unpreserved plasma (group 2); stored plasma for 24 hours at 2-8°C (group 3), and stored plasma for 48 hours at 2-8°C (group 4). All samples were analyzed on an AU480 system – Beckman Coulter. The data would be processed using Stata 10.0. ***Results:*** The results difference between these groups: 2,3,4 compared to group 1 was statistically significant ($p < 0.001$). Total cholesterol, triglycerides, HDL-C, and LDL-C concentrations in group 2 decreased, respectively: 5.83%; 6.77%; 5.12%; 5.96%. We found the lipid test results of group 3 and group 4 also reduced compared to group 1: cholesterol decreased by 5.25%, 5.77%; triglycerides by 6.7%, 6.49%; HDL-C by 5.8, 7.22%; and LDL-C by 4.79%; 5.05%. After 48 hours, cholesterol, HDL-C, and LDL-C concentration continued to reduce, while the difference in triglycerides concentration was not statistically significant. ***Conclusions:*** NaF-KOx anticoagulant reduces lipid test results. We should not use NaF-KOx plasma to measure lipid tests.

Keywords: lipid concentration; NaF-KOx; cholesterol; triglycerides; HDL-C; LDL-C.

1. INTRODUCTION

According to the latest statistical data from the American Heart Association in 2021, cardiovascular disease is the leading cause of death in the world [4]. Currently, there is no way to prevent vascular accidents caused by atherosclerosis, so screening through blood lipid tests to assess the risk of cardiovascular disease is necessary and beneficial for clinical diagnosis. Highly reliable test results bring extremely important for clinical practice. However, for accurate test results, it depends on many factors. According to the study by Abdollahi A et al., most of the errors occurred in the laboratory related to the pre-analytical phase [5], and inappropriate use of anticoagulants is one of the causes leading to errors [6].

In addition, some chemical manufacturers have also recommended suitable samples for each type of test, but in fact, there are some places still using sodium fluoride - potassium oxalate (NaF-KOx) plasma samples to analyze entire biochemical tests. This is possible that they do not have complete data on the effect of anticoagulants for each specific type of test.

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Many authors have studied and compared the results of lipid tests on serum and plasma samples with different anticoagulants. Mean total cholesterol and triglyceride concentrations for serum samples were higher than for EDTA plasma samples, whereas HDL-C was not different between the two kinds of this sample [1]. The study of author Cloey T et al. [2] also showed that cholesterol concentrations in EDTA plasma samples were 4.7% lower than in serum samples.

The advantage of anticoagulants is that they help us separate plasma quickly without waiting for clotting compared to serum. However, an anticoagulant can cause a shift of water from erythrocytes to plasma, such as EDTA can lead to dilution of plasma volume [7]. This situation makes some analyte concentration would have changed. Therefore, cholesterol testing performed on EDTA plasma requires correction by a factor of 1.03 [8]. Enoch OA et al. [3] also determined the effect of K_2EDTA, lithium heparin, and sodium citrate on the lipid tests and compared them with the serum separator tube. The results showed that when using these plasma tubes, the levels of total cholesterol, LDL-C, and triglycerides decreased statistically compared with serum separator tubes ($p < 0.05$), but HDL-C levels were not significantly reduced [3]. For kali oxalate plasma, the study by Eldon and Ronald also showed a decrease in total cholesterol compared to serum [9]. The lipid values when using heparin plasma were also lower than serum: cholesterol levels were 2 to 4.5% lower, triglycerides levels were 3%, and the effect on HDL-cholesterol levels did not decrease significantly [10]. In analyzing metabolic and lipo-proteomic data, Alessia Vignoli et al. [11] showed that lipoprotein concentrations in citrate plasma samples were lower than in EDTA plasma and serum samples.

Most studies about specimen stability had conducted with serum samples [12,13]. The serum is often noticed as the gold standard because it contains no additives. A serum tube has a gel that can separate the blood cells, improve analytes stability and make separation easier [11]. The stability of analytes in serum and plasma samples is the most important in a clinical laboratory, especially if there is a delay in testing or storing samples for future study [14]. Banfi G et al. showed that when stored the samples at room temperature and 4-8°C, the stability of cholesterol and triglycerides in serum and citrate, EDTA, and heparin plasma were similar, while HDL-C stored at 4-8°C was more stable than room temperature [15]. For NaF-KOx plasma samples, we have not seen any studies investigating the stability related to blood lipid testing, but most of them are related to blood glucose testing.

Sodium fluoride - potassium oxalate (NaF-KOx) anticoagulant known as a glycolysis inhibitor. Ernest B and Walter G had demonstrated the effect as well as the stability of blood glucose concentration in NaF-KOx plasma samples, the results showed that with a suitable rate of anticoagulant, NaF-KOx was used to collect samples for glucose testing [16]. If sodium fluoride anticoagulant plasma and serum samples were extracted within 1 hour, there was no difference in glucose concentrations, and these plasma samples were also stable when stored at 4°C for 7 days [17]. However, some studies had also shown that the NaF-KOx was not effective for inhibiting glycolysis during the first 1-4 hours [18,19]. Thereby, we found that most of the studies about NaF-KOx anticoagulants were related to blood glucose concentration without knowing whether it affected other biochemical parameters or not.

In addition, laboratory technicians can re-use samples previously taken from a patient in cases: delaying in the analysis process, confirm or re-check test results, or need to add new tests missed [14]. These can affect the test results because the stability of some biochemicals can change following the storage conditions. The sample stability under suitable storage conditions helps the test results to increase their reliability [20]. Depending on the type of substance for each test, the stability of the sample under storage conditions would be different. We should analyze specimens as soon as well to bring valid results for clinical diagnosis [21].

We carried out this study to evaluate the influence and the stability of the blood lipid tests when storing NaF-KOx plasma samples at 2-8°C after 24 and 48 hours. We measured lipid tests after storage at 2-8°C because this was a commonly surveyed condition for short-term preserving specimens in the laboratory [22]. Selvakumar C also recommended that we should analyze the samples in the laboratory within 24 hours of collection to ensure valid results [23]. Most of the previous studies showed no significant changes in the blood lipid tests when storing serum samples at 4-8°C, lipid levels were stable for at least three days [15, 20, 21], but no data on NaF-KOx plasma samples. Therefore, we preserved plasma samples 24 hours before measuring lipid tests to determine if they were stable or not. We further investigated 48 hours after storage to determine how the concentration of lipid tests tends to change every 24 hours. These results can help laboratories be more careful when using the samples for analysis after storage and improve the test quality in the pre-analysis phase.

2. MATERIALS AND METHOD

2.1. Study design and participants

In a cross-sectional descriptive study, the residual of 150 pairs of serum and NaF-KOx plasma samples were collected from patients who joined the physical examination at the University Medical Center Branch 2, after completing the tests there. All patients voluntarily participated in this study.

The blood samples were collected and tested within 2 hours. We collected 150 excess samples of each type from the same code patient. We divided these samples into groups: serum samples (group 1), unpreserved NaF-KOx plasma samples (group 2), stored NaF-KOx plasma samples for 24 hours at 2-8°C (group 3), and stored NaF-KOx plasma samples for 48 hours at 2-8°C (group 4).

We collected all the samples at the same time. The inclusion criteria for our serum and plasma samples were: no hemolysis, not too yellow, and no turbidity. The exclusion criteria were: the residual samples with insufficient volume, turbidity, too yellow, or hemolysis. The serum samples are considered the gold standard for comparing results obtained with plasma samples.

We calculated the sample size according to the compare two means (using mean and standard deviation) formula:

$$n \geq \frac{\left(Z_{1-\alpha/2} + Z_{1-\beta}\right)^2 \left(\sigma_1^2 + {\sigma_2^2}/{r}\right)}{(\mu_1 - \mu_2)^2}$$

With: $\alpha = 0.05$, $\beta = 0.1$, $\mu_1 = 0.65$, $\sigma_1 = 0.03$, $\mu_2 = 0.64$, $\sigma_2 = 0.02$.

μ_1, μ_2, σ_1, σ_2: the mean and standard deviation of the triglyceride test when measuring serum and EDTA samples groups (according to Enoch OA et al.'s study [3]). So the sample size of each group is equal to or more than 137. We used 150 samples as the sample size for each group.

2.2. Data collection

We analyzed blood lipid tests (total cholesterol, triglycerides, HDL-C) from serum and plasma samples immediately after collecting and storing for 24, 48 hours at 2-8°C on the same automatic biochemical machine (AU480 – Beckman Coulter) at the Center for Quality Control of Medical Laboratory - University of Medicine and Pharmacy, Ho Chi Minh City.

LDL-C concentrations were calculated based on the Friedewald equation (LDL-C = TC - HDL-C - (1⁄5) * TG, Friedewald, 1972).

We collected all of the results for processing and analysis. Quality control methods were performed according to the protocol of the chemical company [24,25,26,27].

2.3. Data analysis

We processed the study data by Stata 14.0 and Microsoft Excel 365 software. We used The Paired Sample T-Test to compare lipid concentration from NaF plasma samples to serum samples. The results were statistically significant when $p < 0.05$.

3. RESULTS

Comparison of lipid concentrations in groups: serum samples (group 1), NaF-KOx plasma samples (group 2), NaF-KOx plasma stored for 24 and 48 hours at 2-8°C (groups 3, 4)

Table 1. Comparison of lipid concentrations in groups: serum samples (group 1), NaF-KOx plasma samples (group 2), NaF-KOx plasma stored for 24 and 48 hours at 2-8°C (groups 3, 4)

Values	Groups	Results			
		Cholesterol	**Triglyceride**	**HDL-C**	**LDL-C**
Mean ± SD (mg/dL) (SD: standard deviation)	1 *	209.26 ± 39.94	115.65 ± 61.76	52.71 ± 10.68	133.42 ± 32.88
	2 *	197.06 ± 36.91	107.81 ± 57.21	50.01 ± 10.16	125.46 ± 30.57
	3 *	198.26 ± 37.37	107.90 ± 56.59	49.65 ± 10.20	127.02 ± 31.12
	4 *	197.18 ± 37.21	108.14 ± 56.96	48.90 ± 10.18	126.68 ± 30.78
Mean difference between the 2 groups (mg/dL)	Group 2 vs Group 1	12.2	7.84	2.7	7.96
Average percentage difference between the 2 groups (**) (%)	Group 2 vs Group 1	↓ 5.83 %	↓ 6.77%	↓ 5.12%	↓ 5.96%
	Group 3 vs Group 1	↓ 5.25%	↓ 6.7%	↓ 5.8%	↓ 4.79%
	Group 4 vs Group 1	↓ 5.77 %	↓ 6.49%	↓ 7.22%	↓ 5.05%
P-value	Group 2,3,4 vs Group 1	< 0.001	< 0.001	< 0.001	< 0.001
	Group 3,4	< 0.001	0.614	< 0.001	0.0357

* *Group 1: serum samples*

* *Group 2: NaF-KOx plasma samples*

* *Group 3: NaF-KOx plasma samples, stored at 2-8°C after 24 hours*

* *Group 4: NaF-KOx plasma samples, stored at 2-8°C after 48 hours*

** *Average percentage difference between the 2 groups: | (Xn-X1)/X1) | x 100%*

The results showed that the average percentage difference in cholesterol, triglycerides, HDL-C, and LDL-C concentrations of the NaF-KOx plasma samples (group 2) were 5 to nearly 7% lower than the serum samples (group 1). The mean HDL-C concentration was the least decreased, with a decrease of 2.7 mg/dL (5.12%). The concentration of triglycerides was the most decreased, decreasing by 7.84 mg/dL (6.77%). The difference in cholesterol, triglycerides, HDL-C, and LDL-C concentrations between group 1 and group 2 was statistically significant with $p < 0.001$.

Through Table 1, we found the differences in concentrations of total cholesterol, triglycerides, HDL-C, and LDL-C between the serum sample group (group 1) compared with the NaF-KOx plasma sample groups stored at 2-8°C for 24 hours (group 3) and 48 hours (group 4), were statistically significant, $p < 0.001$.

When we analyzed the plasma samples stored for 24 hours (group 3), and 48 hours (group 4), these data showed the test results of cholesterol, HDL-cholesterol, and LDL-cholesterol from group 4 decreased compared to group 3. This difference was statistically significant ($p < 0.05$), while the difference in triglycerides concentration was not statistically significant (Table 1).

4. DISCUSSION

In this study, we quantified lipid tests for NaF plasma tubes compared to serum tubes. We want to standardize the process of the lipid test for the laboratories and remind laboratory technicians to follow the guidelines of chemical manufacturers when assaying any analytes to get reliable test results.

The results of our study showed that the difference between the serum samples group and the NaF-KOx plasma samples group of most lipid tests has a statistically significant difference ($p < 0.001$). The cholesterol concentration decreased when using NaF-KOx anticoagulant. The mean cholesterol concentration decreased from 209.26 mg/dL to 197.06 mg/dL, and the mean difference between serum and NaF-KOx tubes of 12.2 mg/dL (0.316 mmol/L, down 5.83%). This result is similar to the previous study of Eldon MB and Ronald BM: total cholesterol concentration decreased when using the kali oxalate anticoagulant [9]. According to Table 1, the concentration of triglycerides decreased when using NaF-KOx plasma, and the mean difference between serum tubes and plasma tubes was 7.84 mg/dL (0.089 mmol/L, 6.78% reduction). The results of our study showed that the difference in triglycerides concentrations between serum samples and NaF-KOx plasma samples decreased more (0.089 mmol/L) compared with the study of the author Enoch Odame Anto et al. [3] when using plasma samples of heparin, K_2EDTA, and sodium citrate, respectively 0.05 mmol/L, 0.01 mmol/L, and 0.03 mmol/L.

When using NaF-KOx anticoagulant, the concentration of HDL-C and LDL-C also decreased significantly. The data from Table 1 showed that HDL-C concentration decreased by 2.7 mg/dL (0.069 mmol/L, down 5.12%); LDL-C concentration decreased by 7.96 mg/dL (0.205 mmol/L, down 5.97%). Some authors provided evidence that HDL-C concentrations were similar when using heparin plasma compared with serum samples [3,10]. However, in our studies, when using NaF-KOx plasma for testing HDL-C, the difference between these groups was statistically significant ($p < 0.001$).

The results of our study on the stability of samples showed that all of the lipid test results decreased when using NaF-KOx plasma stored at 2-8°C for 24 hours and 48 hours. After 24 hours and 48 hours, the total cholesterol concentration decreased by 5.25% and 5.77%, respectively; triglycerides decreased by 6.7% and 6.49%; HDL-C decreased by 5.8% and 7.22%; LDL-C also decreased by 4.79% and 5.05%. The cholesterol, HDL-C, and LDL-C levels continued to decrease when stored for up to 48 hours. This difference was statistically significant, while the difference in triglycerides was not statistically significant. If there had no difference in lipid concentrations between serum tubes and NaF-KOx tubes, we would use the lipid data from the original NaF-KOx tubes to compare with the results after storage. However, the study proved the opposite, so we made a comparison as in Table 1.

In previous studies, storage at different temperatures was often investigated on serum samples with a duration longer than 48 hours. Most of the results showed no significant changes in the blood lipid tests. For example, the study of Marjani A. showed when serum samples were stored at 4 ± 1°C and 23 ± 1°C for 72 hours, cholesterol and triglycerides concentration were not affected [21]. França CN et al. also concluded that there was no significant change in the lipid results when stored at room temperature for several hours or at -20°C for 1, 4, and 34 weeks [28]. In addition, the author Kift RL et al. also experimentally analyzed and preserved the un-capped serum samples, and showed that the total cholesterol, triglycerides, and HDL-C concentration remained stable for 4 days at 4°C [20].

The process and results of our study about sample stability when testing blood lipid are different from previous studies because we performed on plasma samples. When we analyze any blood test, we should carefully read the protocols from the diagnostic companies because they often recommend anticoagulant plasma samples that are the most suitable for the test. According to aggregated data from package inserts of some diagnostic companies, G. Banfi et al. [15] have shown that most companies accept heparin plasma samples for testing cholesterol, HDL-C, and triglycerides. Some companies have been allowed to use EDTA plasma samples to measure cholesterol and triglycerides, but HDL-C is not [15]. For citrate anticoagulant, cholesterol and triglycerides test can be used, but with certain limitations [15]. When stored at room temperature, heparin, EDTA plasma, and serum samples were stable for 7 days, while HDL-C is stable for up to 2 days; but if stored at 4-8°C, cholesterol, HDL-C, and triglycerides were stable for 7 days [15]. However, through the results of our study, we have demonstrated that using NaF-KOx plasma when analyzing samples before and after storing led to unreliable results for lipid tests. The results of our study provide evidence that can help laboratories avoid using this type of sample for analysis.

In our study, we have not yet investigated the abnormally high concentration range of lipid parameters to evaluate the influence of NaF-KOx anticoagulant on test results. At the same time, due to the limitation of sample volume used for the study, we were not able to investigate other storage temperature conditions and the effect of delaying the analysis time before 24 hours on both types of samples.

Conclusion

The NaF-KOx anticoagulant has influenced blood lipid tests. This effect is statistically significant with $p < 0.01$. The concentration of lipid parameters will be reduced when using this anticoagulant. These samples were not stable when stored at 2-8°C after 24 hours. Therefore, do not use NaF-KOx anticoagulant plasma to measure the blood lipid tests. Follow the instructions on the type of specimen used for each test that the manufacturer recommended.

LIST OF ABBREVIATIONS

NaF-KOx: sodium fluoride - potassium oxalate

EDTA: ethylenediaminetetraacetic acid

K2EDTA: dipotassium ethylenediaminetetraacetic acid

HDL-C: high-density lipoprotein cholesterol

LDL-C: low-density lipoprotein cholesterol

ETHICAL STATEMENT

We have clearly explained to the patients before participating in the study the benefits, purposes, and disadvantages of participating in the study. They had the right not to participate in the study. They participated in our research voluntarily and with written informed consent. The proposal research was reviewed and approved by the Ethics Committee of the University of Medicine and Pharmacy at Ho Chi Minh City (approval number: 967/ HĐĐĐ-ĐHYD).

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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AUTHORS' CONTRIBUTION

Xinh Thi Le, Ngoc Thi My Le contributed to the research idea, study design, analysis and interpretation of the data, and the report writing. All authors have read and approved the manuscript.

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