Effects of heparin, citrate, and EDTA on plasma biochemistry of cat: comparison with serum

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SUMMARY

Possible effects of anticoagulants on feline plasma biochemical analyses have almost not been investigated. The purpose of the current study was to determine how the most frequently used anticoagulants affect the results of routine biochemistry in feline plasma specimens.

Ten clinically healthy cats were blood sampled into tubes containing various types of anticoagulants and plain tubes for harvesting plasma and serum, respectively. The concentrations of glucose, cholesterol, triglyceride, fructosamine, total bilirubin, urea, creatinine, total protein, albumin, calcium, inorganic phosphate, magnesium, iron, sodium, potassium, chloride and the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine kinase (CK) and gamma glutamyl transferase (GGT) were measured with commercial kits and an autoanalyser.

Significantly higher concentration of sodium while significantly lower concentration of potassium were seen when heparin was used as an anticoagulant. In sodium citrate plasma most concentrations of metabolites and enzyme activities were lower than in serum due to a specimen dilution and also inhibitory effects. Most analytes differed when EDTA was used as an anticoagulant with the exception of cholesterol, creatinine, CK, GGT, AST, ALT, inorganic phosphate, total protein, urea, and chloride.

Heparinized plasma could be used for the measurement of most blood biochemical analytes in cat except for Na and K. Other anticoagulants (EDTA and citrate) cause unfavorable changes in blood biochemical analytes in cats. However, EDTA–plasma could be used for measure concentrations of cholesterol, creatinine, BUN, total protein, and chloride and activities of AST, ALT, GGT, CK.

Keywords: Anticoagulants; Cat; Metabolites; Plasma biochemistry; Serum

RÉSUMÉ

Comparaison des résultats d'analyses biochimiques effectuées sur sérum et plasmas obtenus sur héparine, citrate, et EDTA chez le chat.

Les effets possibles des anticoagulants sur les résultats des analyses biochimiques plasmatiques ont été très étudiés chez le chat. Le but de la présente étude était de déterminer l'impact les anticoagulants les plus fréquemment utilisés sur les résultats de biochimie de routine dans les échantillons de plasma félins. Des prélèvements sanguins ont été effectués sur tube sec et en présence de divers types d'anticoagulants pour la récolte de sérum et plasma sur dix chats cliniquement sains. Les concentrations en glucose, cholestérol, triglycérides, fructosamine, bilirubine totale, urée, créatinine, protéines totales, albumine, calcium, phosphate inorganique, magnésium, fer, sodium, potassium, chlorures et les activités de l'aspartate aminotransférase (AST), alanine aminotransférase (ALT), créatine kinase (CK) et la gamma-glutamyl transferase (GGT) ont été mesurées avec des kits commerciaux et un analyseur automatique. Une concentration significativement plus élevée en sodium et une concentration significativement plus faible en potassium ont été observés lors de l'utilisation d'héparine. Dans la plupart des plasmas obtenus sur citrate de sodium les concentrations des métabolites et les activités enzymatiques ont été plus faibles que dans le sérum en raison d'une dilution du prélèvement ainsi que des effets inhibiteurs. La plupart des analytes différaient lorsque l'EDTA a été utilisé comme anticoagulant, à l'exception de cholestérol, créatinine, CK, GGT, AST, ALT, phosphate inorganique, protéine totale, urée et chlorures. Le plasma hépariné pourrait être utilisé pour la mesure de la plupart des analytes biochimiques chez le chat à l'exception de Na et K. Les autres anticoagulants (EDTA et citrate) entraînent des variations dans les résultats. Cependant, l' EDTA peut être utilisé pour mesurer les concentrations en cholestérol, créatinine, urée, protéines totales, et chlorures et les activités de AST, ALT, GGT, CK.

Mots-clés: chat, biochimie, sérum, plasma, anticoagulants, héparine, EDTA

Introduction

The most important aim of a clinical laboratory is the correct performance of analytic procedures that yield accurate and precise results aiding patient diagnosis and treatment. In recent years there has been an increasing effort by international committees and working groups to develop quality standards for the pre-analytical phase including the proper use of anticoagulants in specimen collection.

Serum from coagulated blood is the common specimen for clinical chemistry analysis. However, plasma mixed with an appropriate anticoagulant may be an equally valid specimen and, in certain conditions preferable to serum. In addition, whole blood collected with an appropriate anticoagulant is the specimen of choice for blood pH and blood gas determination and measurement of ammonia and some trace elements [13].

A number of different anticoagulants are used in routine medical and veterinary practice depending on the analytes to be quantified. Heparin is the most widely used anticoagulant for clinical chemistry analysis. EDTA and sodium citrate are also used, mainly for hematology and coagulation tests. Harvesting serum requires leaving the blood specimen resting for at least 30 minutes in order to complete coagulation before centrifugation except when coagulation activators are added. Thus use of plasma is often preferable in emergency situations as it expedites analysis. Furthermore, plasma yield from a given volume of whole blood is always greater than the yield of serum [13]. On occasion, biochemical analyses, not previously indicated for the initial required hemogram, may be required. In this situation, it is better to obtain another

specimen for serum harvesting but this is not always possible for all patients. Thus, analysis must be performed on plasma anticoagulated with various types of anticoagulants, most commonly EDTA [2].

The effects of various types of anticoagulants on plasma biochemistry have been studied in human and various animals [1,2,5-13]. As previously reported in dog, differences in albumin, ionized calcium and potassium were found between serum and heparinized plasma. In addition, significantly lower concentrations of electrolytes, fructosamine and albumin were found when EDTA was used. Most analytes and enzymes had 10–15% lower concentrations in citrated-plasma than serum [2]. In other animal species, the effects of anticoagulants on plasma biochemistry results were different from reports in dog and human. For example, in horses, significantly lower concentrations of urea, total bilirubin, ALT and CK activities were seen when heparin was used as an anticoagulant [8] or in cattle, lower concentrations of urea, creatinine, and total protein, and higher concentrations of bilirubin were seen when heparin was used as anticoagulant [10]. Thus, it seems that specific studies are necessary for different species of animals.

According to the authors knowledge there is not published articles concerning to the effects of anticoagulants for feline plasma biochemistry. The purpose of the current study was to determine how the most frequently used anticoagulants affect the results of routine biochemistry in cat plasma specimens.

Materials and methods

Ten clinically healthy cats (domestic short hair), 5 males and 5 females, aged 1-3 years were used in the current study. All cats weigh between 2.5 to 4.1 kg (median 3.1 kg) and selected from two shelters in Mashhad city (northeast of Iran). All cats were fasted for 12- 14 hours prior to sampling. The methods of study were approved by ethical committee of Faculty of Veterinary Medicine and Ferdowsi University of Mashhad.

Blood specimens were collected from the jugular vein after mild physical restraint by a veterinary technician (without sedation) by disposable syringe and 23G needle. 8 ml of blood were taken from each cat and divided into tubes containing appropriate concentrations of anticoagulant which prepared by authors (EDTA: 0.04 ml of 10% dipotassium EDTA solution for 2 ml of blood, sodium heparin: 0.01ml (40 units) for 2 ml of blood, sodium citrate: 0.25 ml of 3.2% solution per 2 ml of blood) and also plain tube for serum harvesting. All specimens were transferred on ice to laboratory within one hour of collection and centrifuged at 1800g for 10 min (Jouan, C 412, France) providing serum and plasma, which were frozen at -18C until analysis. No hemolysis was detected in any of the measured specimens after separation of serum or plasma.

The concentrations of glucose (GLU). cholesterol (CHOL). triglycerides (TRIG). total bilirubin (BIL). urea (UREA). creatinine (CREA). fructosamine (FRUC), total proteins (TP). albumin (ALB). iron (Fe), calcium (Ca). inorganic phosphate (Pi). Magnesium (Mg), and the activities of creatine kinase (CK), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma glutamyl transferase (GGT) were measured by commercial kits (Pars Azmoon, Tehran, Iran) using an auto analyzer (Biotecnia, BT 1500, Rome, Italy). The concentrations of sodium (Na), potassium (K) and chloride (Cl) were measured by direct ion-specific electrode method (Starlyte III, Alfa Wassermann, NJ, USA). The methods of measurements and tests characteristics are summarized in Table I. Control serum (Randox control sera, Antrim, UK) was used for controlling the measurement accuracy. Measurements were made in the same run.

SPSS software, version 20 (SPSS Inc., Chicago, USA) was used for data analysis. Based on the results of Kolmogorov–Smirnov normality test, the skewness and kurtosis of data, non-parametric Friedman test was used for investigate the effects of type of specimen (anticoagulant). Wilcoxon paired tests were performed to compare the differences between serum and different types of plasma. Since the concentrations of Ca, Mg, and K in EDTA-plasma were out of analytical range of kits and method, thus lower (Ca and Mg) and higher (K) limits of the analytical range were used for statistical analysis (Table 1). Results are presented as median and minimum-maximum range. $p \leq 0.05$ was considered as significant.

Results

The median (min-max) of measured analytes in serum and different types of plasma is shown in Table II.

Generally, the effect of specimen (anticoagulant) was significant for most measured analytes except for TRIG, BIL, and CREA concentrations and the activities of AST, ALT, GGT, and CK. In comparing with serum, higher Na concentration and lower K concentration were observed when sodium heparin was used as an anticoagulant. The concentrations of ALB, BIL, CHOL, FRUC, TP, UREA, GLU, Ca, Pi, Cl, and K were significantly lower in citrated plasma than in serum. The concentrations of Na and Fe were higher in citrated plasma than serum.

As compared with serum, the concentrations of ALB, BIL, FRUC, TRIG, GLU, Fe, Ca, Mg, and Na were lower in EDTA-plasma. Potassium concentration was significantly higher in EDTA- plasma as compared to serum.

Discussion

In the past, issues concerning serum analyte measurement and stability were a major concern because serum was the specimen preferred by most laboratories. However, some laboratories are switching to plasma because serum has several inherent problems: an increase in turnaround time

Analytes	Method	Intra assay CV* (%)	Inter assay CV** (%)	Analytical range**
Albumin (g/L)	Bromcresol green	1.12	1.44	2-60
Total proteins (g/L)	Biuret	1.01	1.53	5-150
Total Bilirubin (µmol/L)	Dichloroanylin	2.32	3.49	1.71-513
Cholesterol (mmol/L)	Cholesterol oxidase/PAP (4-aminoantipyrine)	0.61	1.22	0.13-12.93
Triglycerides (mmol/L)	Glycerol 3 phosphate oxidase/PAP (4-aminoantipyrine)	1.82	1.04	0.06-7.91
Fructosamine (µmol/L)	Nitrotetrazolium blue	2.50	4.00	16-800
Glucose (mmol/L)	Glucose oxidase/PAP (4-aminoantipyrine)	1.28	0.84	0.28-22.2
Urea (mmol/L)	urease/glutamate dehydrogenase	3.13	3.87	0.72-72
Creatinine (µmol/L)	Kinetic Jaffe	2.38	0.87	17.68-1326
Calcium (mmol/L)	Arsenazo *III*	0.62	2.48	0.05-4.99
Inorganic Phosphate(mmol/L)	Phosphomolybdate	1.12	1.31	0.06-9.69
Magnesium (mmol/L)	Xylidile blue	0.87	1.12	0.02-2.06
Iron (µmol/L)	Ferene S	1.22	2.19	0.89-89.55
Sodium (mmol/L)	Ion Selective Electrode	1.00	2.00	40-205
Potassium (mmol/L)	Ion Selective Electrode	1.50	1.5	1.5-15
Chloride (mmol/L)	Ion Selective Electrode	1.00	1.5	50-200
AST (U/L)	L-aspartate/2-oxoglutrate as substrate	3.06	1.38	2-300
ALT (U/L)	L-alanine/2-oxoglutrate as substrate	2.62	1.60	4-300
GGT (U/L)	L-gamma-glutamyl-3-carboxy-4-nitroanilide as substrate	1.16	0.85	2-400
CK (U/L)	Creatine phosphate as a substrate	0.70	1.04	2-1015

* Intra assay CV: The biochemical parameters were analyzed ten times in control serum in the same analytical series.
** Inter assay CV, and analytical range were from kits manufacturers.

Table I: Methods of measurements and details of test characteristics.

required because of the time necessary for the clot formation and the risk of fibrin clot interference on automated analyzers, especially those with specimen probes that do not have clot detection ability [1].

In the present study, citrate produced lower concentration of most analytes than serum. Most of these effects could be attributed to a dilution (1:9) effect when the blood was mixed with the liquid anticoagulant. In addition, production of stable salts with calcium and other divalent metals could have contributed to the lower concentrations of these analytes in our work. However, it seems that citrate has a more negative effect on ionized than on total calcium concentration. In addition, citrate inhibits aminotransferase activity and because it complexes molybdate, it decreases the color yield in phosphate measurements and thus produces low results [13]. However; these changes were not observed in the present study. Perhaps feline aminotransferase is not susceptible to citrate inhibition and this change could be species-specific. Higher concentration of Na in citrated plasma than serum was related to addition of sodium citrate to blood as previously reported in dog [2]. Given the adverse effects of citrate, citrated plasma is not the appropriate specimen for feline plasma biochemistry.

Heparin has been generally recommended as the most suitable anticoagulant for plasma. In the present study, there were not any significant differences between serum and heparinized plasma for most measured analytes except Na, and K. Higher concentration of Na in heparinized plasma was due to addition of sodium salt of heparin to blood. Increased K concentration in serum compared to heparinized blood has been attributed to K release from platelets and leukocytes during coagulation process. Our results are consistent with those from a previous study in dogs [2]. Using Li-heparin eliminates the adverse effect of Na or K- heparin on measurement of these electrolytes and Li-heparin is better choice for plasma biochemical analyses.

Analytes	Serum Median(range)	Na-Heparin		K2-EDTA		Na-Citrate		*Effect of anticoagulant* P value
		Median (range)	*P* vs serum	Median (range)	*P* vs serum	Median (range)	*P* vs serum	
Albumin (g/L)	41.5 (33-46)	40.5 (33-45)	NS	38.0 (34-43)	0.045	35.0 (27.0-40.0)	0.005	*<0.001*
Total proteins (g/L)	85.5 (67.0-97.0)	83.0 (77.0-94.0)	NS	79.0 (69.0-86.0)	NS	72.0 (44.0-85.0)	0.007	*<0.001*
Total Bilirubin (µmol/L)	29.9 (28.2-31.3)	29.6 (27.4-32.8)	NS	28.9 (27.9-29.8)	0.021	28.9 (28.0-30.3)	0.008	*0.070*
Cholesterol (mmol/L)	3.4 (2.3-5.1)	3.0 (2.4-5.2)	NS	3.2 (2.0-4.6)	NS	2.4 (1.6-4.4)	0.005	*<0.01*
Triglycerides (mmol/L)	1.1 (0.4-1.9)	0.9 (0.4-1.7)	NS	0.7 (0.4-1.7)	0.028	0.7 (0.3-1.9)	NS	*0.053*
Fructosamine (µmol/L)	185.5 (138-240)	191.0 (169-200)	NS	169.0 (125-189)	0.007	158.5 (109-177)	0.009	*<0.01*
Glucose (mmol/L)	5.6 (4.5-7.5)	5.5 (4.9-6.4)	NS	5.1 (4.5-7.5)	0.028	4.9 (3.1-6.4)	0.007	*<0.05*
Urea (mmol/L)	16.2 (11.4-21.1)	14.3 (13.2-20.0)	NS	14.8 (12.9-20.7)	NS	13.4 (8.9-19.3)	0.046	*0.010*
Creatinine (µmol/L)	107.9 (60.1-177.7)	107.9 (64.5-184.8)	NS	113.2 (52.2-160.9)	NS	102.6 (38.0-162.7)	NS	*0.082*
Calcium (mmol/L)	2.8 (2.6-3.0)	2.8 (2.5-2.9)	NS	*<0.05*	0.008	2.2 (2.0-3.8)	0.007	<0.001
Inorganic Phosphate (mmol/L)	1.9 (1.2-2.6)	2.1 (1.1-2.5)	NS	1.7 (1.1-2.3)	NS	1.5 (1.0-2.0)	0.005	*0.001*
Magnesium (mmol/L)	0.9 (0.6-1.1)	0.9 (0.8-1.0)	NS	<0.02	0.005	0.8 (0.7-1.6)	NS	*<0.001*
Iron (µmol/L)	21.8 (17.9-35.6)	21.5 (20.2-27.2)	NS	9.2 (6.8-13.4)	0.005	27.0 (23.5-31.2)	0.037	*<0.001*
Sodium (mmol/L)	151.0 (119.0-155.0)	154.5 (151.0-163.0)	0.028	127.0 (115.0-131.0)	0.007	166.0 (160.0-182.0)	0.008	*<0.001*
Potassium (mmol/L)	4.4 (3.9-4.9)	3.7 (3.2-4.7)	0.013	*>15*	0.000	3.0 (2.2-3.1)	0.007	*<0.001*
Chloride (mmol/L)	119.5 (104.0-123.0)	121.0 (118.0-127.0)	NS	115.0 (112.0-118.0)	NS	103.5 (78.0-117.0)	0.005	*<0.001*
AST (U/L)	29.5 (23.0-40.0)	36.0 (25.0-87.0)	NS	31.0 (20.0-116.0)	NS	33.0 (21.0-42.0)	NS	*0.299*
ALT (U/L)	64.0 (28.0-100.0)	42.0 (26.0-285.0)	NS	79.0 (38.0-434.0)	NS	58.0 (28.0-82.0)	NS	*0.147*
GGT (U/L)	2.0 (0.0-9.0)	2.5 (0.0-7.0)	NS	3.0 (0.0-5.0)	NS	2.0 (0.0-6.0)	NS	*0.969*
CK (U/L)	347.5 (102-869)	415.5 (138-1021)	NS	171.5 (95-671)	NS	261.0 (136-819)	NS	*0.099*

Table II: Median (min-max range) of measured metabolites, minerals, and enzymes in serum and various types of cat blood plasma with the results of statistical comparisons. NS: not significant

Changes in some analytes induced by EDTA have been described in man, dog, cattle, camel, horse, and sheep [1,2,4,7,8,10-12]. Significant decreases in AST activity were reported for EDTA- plasma in cattle, sheep, and horse [8,10,11]. Other studies showed no variation in AST activity in dog and camel [2,9]. It is not clear why EDTA causes these different effects on AST activity in different species of animals.

There was no difference in CK activities between the EDTA-plasma and serum. This was in agreement with previous studies in dogs, cattle, and sheep [2,5,10,11]. EDTA-

plasma from horses and humans however have lower CK activity [8,13]. The distribution of specific isoenzymes could be responsible for these differences between the species.

In the present study, the concentrations of Ca, Mg, Fe, and Na in EDTA- plasma were significantly lower in comparison with serum, a finding attributable to the chelating properties of EDTA. Similar results were reported in dog, horse, sheep, and camel [2,8,9,11]. K concentrations were higher than the analyser limit in the EDTA- plasma due to dipotassium EDTA. Phosphate concentration was unaffected by EDTA-plasma similar to reports in horses and sheep [8,11] but contrary to the finding in dogs [2].

Urea and creatinine concentrations in EDTA-plasma did not differ from those in serum. This is consistent with previous reports in dogs and humans [2,13] but differs for cattle, camels, horses, and sheep in which EDTA-plasma resulted in lower concentrations [8-11].

In a previous report [2], EDTA plasma contained lower concentration of FRUC than serum in dogs. The exact mechanism of these differences is not clear although the osmotic fluid shifts from red cells to plasma [3] and/or differences between the species can have contributed.

Lower ALB concentration in feline EDTA-plasma than in serum differs from the situation in dogs [2] but is similar to results in cattle, sheep, horses, and camels [8-11]. Conversely, TP was unaffected in EDTA-plasma in cats as has been reported in dogs, whilst, it is known to be decreased by EDTA in cattle, sheep, and horse [8,10,11]. In accordance with our resulst, no difference was reported in dog for total protein in EDTA plasma and serum [2]. In cat, also lower GLU and BIL concentrations were observed in EDTA- plasma than serum. These results are similar to those obtained in horses [8] but differ from previous reports in dogs, cattle, camels, and sheep [2,9-11].

In conclusion, plasma heparinized with Na-heparin, could be used for the measurement of all blood biochemical analytes except Na and K. If the measurements of Na and K in plasma are necessary then Li-heparin is a better anticoagulant. The other tested anticoagulants (K-EDTA and Na-citrate) caused unfavorable changes in many of the tested analytes. However, EDTA–plasma could be used for measurements of blood AST, ALT, GGT, CK, CHOL, CREA, UREA, TP, and Cl.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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