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ORIGINAL PAPER

Plasma biochemistry of ostrich (*Struthio camelus*): effects of anticoagulants and comparison with serum

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Abstract The effects of various types of anticoagulants on plasma biochemistry were studied in man and various animals, but limited information is existing for ostrich plasma biochemistry. Ten clinically healthy ostrich were blood sampled in different tubes containing each anticoagulant and plain tube for harvesting plasma and serum. The concentrations of glucose, cholesterol, uric acid, creatinine, total protein, albumin, calcium, inorganic phosphorus, and magnesium and the activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and gamma glutamyl transferase (GGT) were measured. The concentrations of glucose, uric acid, total protein, and calcium were significantly lower in citrated plasma than that of serum. For dilution corrected citrated plasma significant differences were only seen for the concentration of uric acid. Most parameters did not show any differences, but significant increase were seen for glucose, total protein, albumin, and phosphorus concentrations when heparin was used as an anticoagulant.

Keywords Anticoagulants · Biochemistry · Ostrich · Plasma · Serum

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Introduction

Evaluation of the ratite hematology and clinical chemistry has become increasingly important in veterinary care of diseased birds. In the past few years, the focus of ratite medicine has shifted from individual care of extremely expensive breeding stock to one concerned primarily with flock oriented production (Green and Blue-Mclendon 2000).

The primary goal of a clinical chemistry laboratory is the correct performance of analytic procedures that yield accurate and precise information, aiding patient diagnosis and treatment. Whole blood, plasma, and serum have been used extensively as measures of nutritional and metabolic status.

In mammals, serum from coagulated blood is the preferred specimen for clinical chemistry analysis. But plasma obtained with an appropriate anticoagulant may be an equally valid specimen and in certain conditions preferable to serum. In addition, whole blood obtained on appropriate anticoagulant is the sample of choice for measurement of trace elements, ammonia, blood pH and blood gas determination (Young and Bermes 1999). Anticoagulants are additives that inhibit the clotting of blood and/or plasma, thereby ensuring that the concentration of the substance to be measured is changed as little as possible before the analytical process (Guder 2001). Anticoagulation is achieved either by the binding of calcium ions (EDTA, citrate and fluoride) or by inhibition of thrombin (heparin).

Heparin is the most widely used anticoagulant for clinical chemistry analysis. On the other hand, EDTA is particularly useful for hematological examination and many blood samples which send to clinical laboratory anticoagulated with EDTA. Sodium citrate solution is widely used for coagulation studies because the effect is easily reversible by addition of Ca^{+2}.

The preferred anticoagulant in ratite hematology is citrate. When comparing citrate values to other values, the effect of 1:9 dilutions must be considered. Heparin interferes with staining and causes marked heterophil clumping. EDTA adversely affects blood from some ratite, causing severe hemolysis or poor cell preservation (Green and Blue-Mclendon 2000). Ostrich erythrocyte tend to lyze in EDTA, rendering a sample worthless in hours post collection for hematology. Heparinized tubes, producing plasma on centrifugation should be standardized as the sample container of choice in birds blood biochemistry (Fudge 2000).

Because harvest of serum requires 15–30 minutes wait for coagulation completion before centrifugation, use of plasma expedites analysis in emergency situations. Furthermore, plasma yield from a given volume of whole blood is always greater than the yield of serum (Young and Bermes 1999). In ratite, plain tubes, which are free of any additives should not be used for chemistry testing, hemolysis during processing can be a significant problem and fibrin jelling of the serum can render the sample worthless for chemistry testing (Fudge 2000). In addition, it is possible to require additional biochemical results, which not previously expected to the initial required hemogram. It is better to obtained another sample for serum harvesting but it is not possible for all patients and also serum is not suitable sample in ratitite for this purpose. Thus, analysis must be performed on plasma anticoagulated with various types of anticoagulants most commonly those obtained for hematological analysis (citrated blood in ostrich).

The effects of various types of anticoagulants on plasma biochemistry were studied in man and various animals (Jones 1985a, b; Young and Bermes 1999; Boyanton and Blick 2002; Stokol et al. 2001; Ceron et al. 2004; Mohri et al. 2007a, b, 2008; Mohri and Rezapoor 2008), and no information's were found for ostrich plasma biochemistry. The purpose of the present study was to determine and compare how the main anticoagulants may affect the results of routine and cost- effective biochemistry in ostrich plasma specimens.

Materials and methods

The study was conducted at the Binalood ostrich farm near Mashhad, northeast of Iran. In this farm entire steps of breeding from egg to slaughter were performed. Birds were totally confined in openshed housing. Ten clinically healthy ostrich (*Struthio camelus subspecies camelus*, 5 male and 5 female) with mean age of 6 months and mean weight of 42.5 kg were used in the present study. Blood samples were collected from the wing vein (Green and Blue-Mclendon 2000) by disposable syringe and 16 G needle after restraint of birds in handling box using head cover (Kreibich and Sommer 1995) in December 2007. From each bird, 20 ml of blood were taken and divided into glass tubes containing appropriate amounts of anticoagulants (lithium heparin: 100 units for 5 ml of blood, sodium citrate: 0.5 ml of 3.8% solution per 4.5 ml of blood) and into plain tube for serum harvesting. The tubes containing anticoagulants were manually prepared. All samples were transferred, on ice, to the laboratory (approximate transfer time: 45 minutes) and centrifuged at 1,800×g for 10 min providing serum and plasma, which were frozen at - 20°C until analysis. No macroscopic hemolysis was detected in any of the samples after separation of serum or plasma.

The concentrations of glucose (glucose oxidase method), cholesterol (cholesterol oxidase method), uric acid (trinder method), creatinine (kinetic Jaffe method), total protein (Biuret method), albumin (Bromcresol green method), calcium (Arsenazo III method), inorganic phosphorus (phosphomolybdate method), magnesium (Xylidile blue method), and the activity of aspartate aminotransferase (L-aspartate/2-oxoglutarate as substrate), alanine aminotransferase (L-alanine/2-oxoglutarate as substrate), alkaline phosphatase (P-nitrophenylphosphate as substrate), and gamma glutamyl transferase (L-gamma-glutamyl-3-carboxy-4-nitroanilide as substrate) were measured by commercial kits (Pars Azmoon, Tehran, Iran) using an auto analyzer (Biotecnica, TARGA 3000, Rome,

Italy). For all measured parameters, dilutional correction was calculated for citrated plasma [(serum amount×0.1) +citrated plasma amount].

Control serum (Randox control sera, Antrim, UK) was used for ensuring measurement accuracy. The within-run CV of all measured parameters was between 2.5% and 10%.

The SPSS software, version 13 (SPSS Inc, Chicago, USA) was used for data analysis. Based on the results of a Kolmogorov-Smirnov normality test a parametric paired t- test was performed to compare differences between serum and different types of plasma. For all measured parameters, the mean ± standard error (SE) were determined and presented. $P \leq 0.05$ was considered as significant.

Results

The results of measurements and statistical comparisons are shown in Table 1.

The concentrations of glucose, uric acid, total protein, and calcium were significantly lower in citrated plasma than that of serum. For dilution corrected citrated plasma significant difference was only seen for the concentration of uric acid.

Most parameters did not show any difference, but significant increase was seen for glucose, total protein, albumin, and phosphorus concentrations when heparin was used as an anticoagulant.

Discussion

In the past, issues concerning serum analyte measurement and stability were a major concern because serum was the preferred specimen for most clinical pathology laboratories. However, some laboratories are switching to plasma to increase the turnaround time and to avoid the risk of fibrin clot interference on automated analyzers, especially those with sample probe without clot detection ability (Boyanton and Blick 2002). Since, serum is not suitable sample for blood clinical chemistry tests in ratite (Fudge 2000) and most blood samples for hematology were taken on citrate (Green and Blue-Mclendon 2000), thus, plasma must be considered as a major specimen for clinical chemistry tests.

It should be noted that, because the authors could not find articles or related information concerning to the effects of different anticoagulants on plasma biochemistry of birds and especially ostrich, thus, used available data from other animal species for comparison.

Sodium citrate solution at a concentration of 3.4–3.8 g/dL in a ratio of 1 part to 9 parts of blood, is generally used for the analysis of factors related to haemostasis because its effect is easily reversible by the addition of ionized calcium (Young and Bermes 1999). In the present study, citrate produced a decrease in some parameters compared to serum. However, most of these decreases could be attributed

Table 1 Mean ± SE of measured metabolites, minerals, and enzymes in serum and various types of ostrich blood plasma

Parameters	Serum	Heparinized plasma	Citrated plasma	Citrated plasma (dilution corrected)
Glucose (mmol/L)	12.99±0.37	13.51±0.42*	11.83±0.48*	13.13±0.50
Cholesterol (mmol/L)	2.03±0.15	2.07±0.24	1.78±0.12	1.99±0.13
Uric acid (mmol/L)	0.64±0.04	0.62±0.05	0.51±0.05*	0.57±0.05*
Creatinine (μmol/L)	30.06±0.88	35.36±5.3	27.40±0.88	30.94±0.88
Total protein (g/L)	40.0±2.00	42.0±1.00*	35.0±1.0*	39.0±1.0
Albumin (g/L)	17.0±1.0	20.0±1.0*	15.0±1.0	17.0±1.0
Calcium (mmol/L)	2.78±0.1	2.98±0.1	2.48±0.1*	2.75±0.1
Inorganic phosphorus (mmol/L)	5.88±0.39	6.07±0.36*	6.20±0.49	6.78±0.49
Magnesium (mmol/L)	1.32±0.08	1.36±0.08	1.23±0.12	1.36±0.12
AST (IU/L)	342.1±25.2	348.5±25.6	320.1±26.5	354.3±28.7
ALT (IU/L)	7.5±1.9	8.3±1.7	5.6±0.3	6.4±0.5
ALP (IU/L)	482.5±45.8	462.8±39.4	492.0±63.4	544.0±73.3
GGT (IU/L)	1.9±0.7	2.8±0.7	2.5±0.7	3.0±0.8

*Significant difference with serum ($p < 0.05$)

to a dilution (1:9) effect when the blood was mixed with the liquid anticoagulant. The negative effect of citrate solution described in humans blood serum on parameters susceptible to its chelating properties such calcium, could have contributed to the decreases observed in these analytes in our work. However, it seems that citrate has a more negative effect on ionized than on total calcium. In addition, citrate inhibits aminotransferase activity and because it complexes molybdate, it decreases the color yield in phosphate measurements and thus produces low results (Young and Bermes 1999). Although, these changes were not observed in the present study. Perhaps ostrich aminotransferase is not susceptible to citrate inhibition and this is a species-specific property. The higher level of phosphate in different type of plasma in comparing with serum in ostrich was unusual finding. According to adverse effects of citrate as mentioned previously, citrated plasma is not appropriate sample for plasma biochemistry. In the present study, comparing of the amounts of measured parameters in dilution corrected citrated plasma with serum suggested significant difference only for uric acid concentration. This difference suggested that the decreased amount of parameters in citrated plasma were not only attributed to dilution of sample by liquid citrate but also other mechanisms such as inhibition of chromogen reagents, and inference with other assay reactions may cause these differences. The results of the present study revealed that dilution corrected citrate could be used in clinical chemistry measurements.

Heparin has been generally recommended as the most suitable anticoagulant for plasma biochemical measurements (Young and Bermes 1999; Fudge 2000). Previous report in dogs has been suggested significant differences in selected parameters between heparinized plasma and serum (Thorensen et al. 1992). In our study, serum and heparinized plasma yielded similar results for most of the measured parameters with the exception of significant increase for glucose, total protein, albumin, and phosphorus concentrations.

In accordance with our result, an artifactual significant increase in albumin concentration in heparinized plasma, compared with serum and other anticoagulants, using a bromcresol green assay (BCG) has been described in canine (Stokol et al. 2001; Ceron et al. 2004) and sheep samples (Laborde et al. 1995; Mohri and Rezapoor 2008). This difference is partly due to the combination of heparin and fibrinogen (Stokol et al. 2001). In the study of Stokol et al. (2001), corrected albumin concentrations in citrated plasma samples (with standard and modified BCG method) were not higher than those in serum and were lower than those in heparinized plasma samples, suggesting that fibrinogen alone was not responsible for the overestimation of albumin concentration in heparinized plasma. They believed that fibrinogen explained only 50% of the difference in albumin concentration between heparinized plasma and serum with the standard BCG method and other unknown causes contributed to the observed difference. In heparinized plasma of dog, a sample blank and a reaction time of less than 1 min for albumin measurements using a BCG method was recommended to prevent such artifactual increases (Stokol et al. 2001).

In the present study, the concentration of total protein was significantly higher in heparinized plasma than serum was. This finding is in contrast to previous studies in sheep, cow, camel, horse, and dog which reported non significant increase or significant decrease in the concentration of total protein in heparinized plasma (Ceron et al. 2004; Mohri et al. 2007a, b, 2008, Mohri and Rezapoor 2008), but is consistent with another report for sheep (Laborde et al. 1995). In coagulation process, fibrinogen is consumed and serum does not have fibrinogen. Thus, the concentration of total protein in plasma must be higher than that in serum.

The significant increase in the concentration of glucose and phosphorus in heparinized plasma of ostrich is in contrast with previous reports for other animals (Laborde et al. 1995; Ceron et al. 2004; Mohri et al. 2007a, b, 2008; Mohri and Rezapoor 2008). The exact mechanisms of these alterations are not clear.

In conclusion, Heparinized plasma could be used for measurement of most biochemical parameters of blood plasma of ostrich except glucose, total protein, albumin, and phosphorus. Other anticoagulant (Citrate) cause unfavorable changes in the concentrations of some biochemical parameters of ostrich blood plasma (glucose, uric acid, total protein, and calcium). However, dilution corrected citrated plasma revealed the best results even better than heparinized plasma compared with serum. The statistically significant

differences for some biochemical parameters between serum and plasma with different type of anticoagulants may not have clinically significant difference because most variations are remained in reference range. Thus, it is important to interpret the changes of the amounts of measured parameters in plasma based on the kind of anticoagulant and the level and direction of variation from reference values. In normal subjects with the values of biochemical parameters at low or high normal levels, the smallest changes due to anticoagulant in plasma resulted to abnormal levels and misinterpretation. The results of the present study apply only to the samples from normal ostrich and additional information for animals with abnormal results is necessary.

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