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Effects of heparin, citrate, and EDTA on plasma biochemistry of sheep: Comparison with serum

M. Mohri *, H. Rezapoor

Department of Clinical Sciences, and Center of Excellence in Ruminant Abortion and Neonatal Mortality, School of Veterinary Medicine, Ferdowsi University of Mashhad, P.O. Box 91775-1793, Mashhad, Iran

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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 sheep plasma biochemistry. Ten clinically healthy Baloochi breed of sheep were blood sampled in different tubes containing each anticoagulants and plain tube for harvesting plasma and serum. The concentrations of glucose, cholesterol, total bilirubin, urea, creatinine, total protein, albumin, calcium, inorganic phosphorus, and magnesium and the activity of aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatine kinase (CK) and gamma glutamyl transferase (GGT) were measured. Except for the amounts of GGT, bilirubin and inorganic phosphorus, other measured parameters were significantly lower in citrated plasma than that of serum. For corrected citrated plasma significant differences were seen for the concentrations of glucose, creatinine, calcium and the activity of ALP.

Most parameters did not show any difference, but significant increase was seen for albumin concentration when heparin was used as an anticoagulant. Using EDTA as anticoagulant caused a significant difference for the concentrations of some of the measured parameters in plasma except glucose, GGT, cholesterol, albumin, bilirubin, CK, and inorganic phosphorus comparing with serum.

Keywords: Anticoagulants; Plasma biochemistry; Sheep

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 processes (Guder, 2001). Anticoagulation is achieved either by the binding of calcium ions (EDTA, citrate and fluoride) or by the inhibition of thrombin (heparin).

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 some trace elements, ammonia, blood pH and blood gas determination (Young and Bermes, 1999).

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 the addition of Ca^{2+}.

Because harvest of serum requires 15–30 min wait for the completion of the coagulation 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 addition, it is possible to require additional biochemical results which not previously expected to the initial required hemogram. It is better to

* Corresponding author. Tel.: +98 511 8796782; fax: +98 511 8763852.
E-mail address: mohri@ferdowsi.um.ac.ir (M. Mohri).

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obtain another sample for serum harvesting, but it is not possible for all patients especially sheep. Thus analysis must be performed on plasma anticoagulated with various type of anticoagulants, most commonly EDTA.

Many laboratories offer packages to investigate common syndromes of sheep for example, illthrift, fatty liver and ruminant energy/protein status packages, but do they contain the most helpful and cost-effective tests? Much useful information can be determined from the history collected during the clinical examination and from examination of other animals in the group. Thus, it may be more cost- effective to choose the individual tests most relevant to the case (Milne and Scott, 2006). The effects of various types of anticoagulants on plasma biochemistry were studied in man and various animals (Jones, 1985a; Young and Bermes, 1999; Boyanton and Blick, 2002; Stokol et al., 2001; Ceron et al., 2004; Mohri et al., 2007a,b), but limited information is existing for sheep plasma biochemistry (Jones, 1985b; Laborde et al., 1995; Morris et al., 2002). 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 sheep plasma specimens.

Ten clinically healthy sheep of Baloochi breed from sheep research center of agricultural school of Ferdowsi university of Mashhad, Iran were used in the present study. Blood samples were collected from jugular vein. Twenty milliliter of blood were taken from each sheep and divided into glass tubes containing of appropriate amounts of anticoagulant (EDTA: 0.1 ml of %10 disodium EDTA solution for 5 ml of blood, 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 also plain tube for serum harvesting.

All samples were transferred, on ice, to laboratory and centrifuged at 1800*g* for 10 min and serum and plasma were harvested within two hours after sampling and refrigerated until measurement (approximately 90 min after harvesting). No hemolysis was detected in any of the samples analysed.

The concentrations of glucose (glu, glucose oxidase method), cholesterol (cho, cholesterol oxidase method), total bilirubin (bili, dichloroanylin method), urea (ure, urease/glutamate dehydrogenase method), creatinine (cre, kinetic Jaffe method), total protein (tp, Biuret method), albumin (alb, bromcresol green method), calcium (ca, Arsenazo III method), inorganic phosphorus (ip, phosphomolybdate method), magnesium (mg, Xylidile blue method) and the activity of aspartate aminotransferase (AST, L-aspartate/2-oxoglutarate as substrate), alkaline phosphatase (ALP, P-nitrophenylphosphate as substrate), creatine kinase (CK, creatine phosphate as substrate) and gamma glutamyl transferase (GGT, 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). Control serum (Randox control sera, Antrim, UK) was used for controlling measurement accuracy. For all measured parameters, dilutional correction was calculated for citrated plasma [(serum amount $\times$ 0.1) + citrated plasma amount]. The within-run CV (analytical precision of the method) of all measured parameters was less than 10%.

Based on the results of Kolmogorov–Smirnov normality test non-parametric Wilcoxon pair test was performed to compare the differences between serum and different types of plasma (SPSS 9 statistical package). Limits of confidence intervals were determined as 25th and 75th percentiles. $p \leqslant 0.05$ was considered as significant.

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

Among various types of anticoagulants used, heparin caused least changes in plasma compared with serum. Albumin concentration was the only parameter that significantly increased in heparinized plasma than serum. Heparin was generally recommended as the most suitable

Table 1
Median (25th–75th percentiles) of measured parameters in serum and various type of plasma

Parameters	Serum	Heparinized plasma	EDTA plasma	Citrated plasma	Citrated plasma (dilution corrected)
Glucose (mmol/l)	4.08 (3.78–4.88)	4.12 (3.91–5.05)	3.92 (3.82–4.86)	3.47 (3.33–4.25)**	3.86 (3.71–4.74)*
Cholesterol (mmol/l)	1.14 (1.00–1.41)	1.11 (1.01–1.40)	1.08 (0.98–1.43)	1.04 (0.86–1.24)**	1.15 (0.96–1.39)
Total bilirubin (μmol/l)	4.96 (4.45–5.30)	5.13 (4.62–5.64)	4.79 (3.25–5.30)	4.62 (3.93–4.96)	5.13 (4.28–5.47)
Urea (mmol/l)	4.97 (3.73–7.31)	5.00 (3.94–7.18)	4.93 (3.71–6.91)*	4.51 (3.37–6.52)**	5.01 (3.75–7.25)
Creatinine (μmol/l)	118.46 (113.15–129.95)	114.92 (103.43–119.34)	104.31 (102.54–107.85)**	96.36 (91.94–99.89)**	108.73 (105.20–112.27)*
Total protein (g/l)	67.9 (65.5–69.7)	70.2 (66.1–72.7)	66.2 (62.9–69.3)*	62.6 (59.2–63.7)**	69.4 (66.0–70.5)
Albumin (g/l)	35.3 (31.3–37.6)	37.3 (35.5–40.1)*	36.0 (32.7–39.3)	31.0 (28.4–33.0)**	34.5 (31.5–36.9)
Calcium (mmol/l)	2.34 (2.14–2.78)	2.38 (2.16–2.60)	0.02 (0.02–0.03)**	1.84 (1.30–2.12)**	2.06 (1.52–2.39)**
Phosphorus (mmol/l)	2.16 (1.85–2.88)	2.15 (2.00–2.81)	2.17 (1.99–2.72)	1.88 (1.73–2.54)	2.10 (1.93–2.80)
Magnesium (mmol/l)	1.02 (0.82–1.36)	1.05 (0.83–1.27)	0.29 (0.27–0.32)**	0.93 (0.77–1.17)*	1.03 (0.85–1.30)
AST (IU/L)	133.5 (113.25–158.5)	131.5 (112.25–159.25)	121.5 (105–151)*	105 (96.13–143.75)**	118.35 (107.53–160.10)
ALP (IU/L)	480 (259–848.5)	493 (260.5-788)	9.23 (5.03–11.99)**	410 (227.5–653.5)**	458.00 (254.30–738.35)*
CK (IU/L)	298.5 (224–375)	324.5 (225–373.75)	296 (233.5–351.75)	253 (209.25–353.75)**	282.15 (231.65–391.25)
GGT (IU/L)	42.6 (37.55–45.08)	39.15 (32.05–41.85)	40.85 (36.2–46.4)	38.05 (34.73–42.33)	42.47 (38.65–47.24)

* $p < 0.05$.
** $p < 0.01$.

anticoagulant for plasma biochemical measurements (Young and Bermes, 1999) although in previous reports significant differences in selected parameters were found between heparinized plasma and serum (Thorensen et al., 1992; Mohri et al., 2007a,b). An artifactual increase in albumin in heparinized plasma, compared with serum and other anticoagulants, using a bromcresol green assay (BCG) was recently described in canine (Stokol et al., 2001; Ceron et al., 2004) and sheep samples (Laborde et al., 1995). This difference is partly due to the combination of heparin and fibrinogen (Stokol et al., 2001). Significant increase of total protein and glucose amounts with significant decrease in urea concentration were reported in sheep heparinized plasma in comparing with serum (Laborde et al., 1995; Morris et al., 2002) but the present study do not confirm these reports.

Citrate caused significant decreasing in the levels of measured parameters except GGT activity, bilirubin and inorganic phosphorus levels. However, most of the decrease could be attributed to a dilution (1–9 parts) effect when the blood was mixed with the liquid anticoagulant. The negative effect of citrate solution described in human blood plasma on parameters susceptible to its chelating properties such calcium or AP, could have contributed to the decrease observed in these analytes in our work. In addition, citrate inhibits aminotransferase activity and because it complexes with molybdate, it decreases the color yield in phosphate measurements; thus producing lower results (Young and Bermes, 1999). Citrate also could have inhibited the reaction of albumin with BCG (Stokol et al., 2001). However, the exact cause of this result is not clear. In the present study, comparing of the amounts of measured parameters in dilution corrected citrated plasma with serum suggested significant differences for some parameters (glucose, creatinine, calcium, and the activity of ALP). These differences suggested that the decreased amount of parameters in citrated plasma were not only attributed to dilution of sample by liquid citrate. Other mechanisms such as inhibition of chromogen reagents, and inference with other assay reactions may causes these differences.

Changes in some metabolites, minerals, electrolytes and enzymes induced by EDTA were described in man, dog, cattle, horse, and sheep (Laborde et al., 1995; Guder, 2001; Boyanton and Blick, 2002; Morris et al., 2002; Ceron et al., 2004; Mohri et al., 2007a,b). In the present study, EDTA induced significant decreases in ALP activity. Decreases in ALP activity caused by EDTA have been previously described in humans, dogs, horse, and ruminants (Jones, 1985a,b; Young and Bermes, 1999; Ceron et al., 2004; Mohri et al., 2007a,b). Change of optimum pH for ALP activity during measurements (Myers and Pierce, 1972) and the chelating properties of EDTA may have influenced results, since ALP is a zinc-dependent, magnesium-activated enzyme (Jones, 1985b; Guder, 2001). In accordance with our result, a significant change in AST activity was described in EDTA-treated plasma samples from sheep, horse, and cattle (Jones, 1985b; Mohri et al., 2007a,b), but other studies showed no variation in AST activity with dog and cattle (Ceron et al., 2004; Jones, 1985a). The significant decrease in activity of AST could be attributed to the chelating effect of EDTA.

Our CK results agreed with previous reports, which found no differences in CK between EDTA-treated plasma and serum in cattle samples (Jones, 1985a; Mohri et al., 2007b), unlike human and horse EDTA-treated samples showed a decrease in CK activity (Young and Bermes, 1999; Mohri et al., 2007a). Distribution of specific isoenzymes could be responsible for inter-species variations. The significantly lower concentrations of urea, creatinine, and total protein in EDTA-treated plasma than serum are in contrast with previous reports in dog and in humans (Young and Bermes, 1999; Ceron et al., 2004). Significant increase in glucose and significant decrease in triglyceride, cholesterol, and urea concentrations were reported in EDTA-treated plasma of sheep (Laborde et al., 1995; Morris et al., 2002). The exact mechanism of these differences was not clear although the osmotic fluid shift from red cells to plasma (Dubin and Hunt, 1978) and/or differences between species may be contributory factors.

This study strongly supports the use of serum for the determination of clinical biochemical profiles of Baloochi sheep, although more studies into the differences between serums- derived and plasma-derived results for other breeds, under different laboratory conditions, and clinical situations could be helpful.

References

Boyanton, B.L., Blick, K.E., 2002. Stability studies of twenty-four analytes in human plasma and serum. Clinical Chemistry 48, 2242–2247.

Ceron, J.J., Martinez-Subiela, S., Hennemann, C., Tecles, F., 2004. The effects of different anticoagulants on routine canine plasma biochemistry. Veterinary Journal 167, 294–301.

Dubin, S., Hunt, P., 1978. Effect of anticoagulants and glucose on refractometric estimation of protein in canine and rabbit plasma. Laboratory Animal Science 28, 541–544.

Guder, W.G., 2001. The quality of diagnostic samples. Blood Gas News 10, 18–24.

Jones, D.G., 1985a. Stability and storage characteristics of enzymes in cattle blood. Research in Veterinary Science 38, 301–306.

Jones, D.G., 1985b. Stability and storage characteristics of enzymes in sheep blood. Research in Veterinary Science 38, 307–311.

Laborde, C.J., Chapa, A.M., Burleigh, D.W., Salgado, D.J., Fernandez, J.M., 1995. Effects of processing and storage on the measurement of nitrogenous compounds in ovine blood. Small Ruminant Research 17, 159–166.

Milne, E., Scott, P., 2006. Cost-effective biochemistry and haematology in sheep. In Practice 28, 454–461.

Mohri, M., Allahiari, L., Sardari, K., 2007a. Effects of common anticoagulants on routine plasma biochemistry of horse and comparison with serum. Journal of Equine Veterinary Science 27, 313–316.

Mohri, M., Shakeri, H., Lotfollah Zadeh, S., 2007b. Effects of common anticoagulants (heparin, citrate, and EDTA) on routine plasma biochemistry of cattle. Comparative Clinical Pathology 16, 207–209.

Morris, J.D., Fernandez, J.M., Chapa, A.M., Gentry, L.R., Thorn, K.E., Weick, T.M., 2002. Effects of sample handling, processing, and hemolysis on measurements of key energy metabolites in ovine blood. Small Ruminant Research 43, 157–166.

Myers, L.J., Pierce, K.R., 1972. The effect of EDTA-anticoagulant on enzyme activity and pH of blood. Southwest Veterinarian, 287–292.

Stokol, T., Tarrant, J.M., Scarlett, J.M., 2001. Overestimation of canine albumin concentration with the Bromcresol green method in heparinized plasma samples. Veterinary Clinical Pathology 30, 170–176.

Thorensen, S.I., Havre, G.N., Morberg, H., Mowinckel, P., 1992. Effects of storage time on chemistry results from canine whole blood, heparinized whole blood, serum and heparinized plasma. Veterinary Clinical Pathology 21, 88–94.

Young, D.S., Bermes, E.W., 1999. Specimen collection and processing: sources of biological variation. In: Burtis, C.A., Ashwood, A.R. (Eds.), Tietz Textbook of Clinical Chemistry, third ed. W.B. Saunders, Philadelphia, pp. 42–72.