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Survey the effect of various anticoagulants on plasma biochemistry parameters of sheep in compared with serum sample

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ABSTRACT

The aim of this study was to evaluate and validate the effects of various anticoagulants on the results of biochemical parameters in Sangsari sheep. One hundred and ten healthy sheep of Sangsari Breed in traditional herds of Garmsar City, Semnan Province, Iran were used in this study. Twenty milliliters of blood were taken from each sheep and divided into tubes containing various types of anticoagulants and plain tube for serum harvesting. Sample transferred to laboratory then centrifuged at 1500 RPM for 15 min. no hemolysis was detected in analyzed samples. The concentration of glucose, triglyceride, cholesterol, creatinin, total protein, albumin, calcium, total bilirubin, inorganic phosphorus, the activity of aspartate aminotransferase, magnesium and urea were measured by commercial kits with Using the automated chemistry analyzer. Nonparametric Wilcoxon pair test was performed to compare the differences between serum and plasma. At the end it was found that all of parameters except albumin were significantly decreased in citrated plasma than serum. Glucose, triglyceride, albumin, urea, creatinin, total protein, calcium, magnesium and AST level were significantly lower in EDTA contained samples rather than serum samples. Bilirubin and albumin level were increased significantly in heparin contained samples while urea level was decreased significantly rather than serum samples. In order to correct the dilution effects of sodium citrate anticoagulant, dilution correction index was calculated. After DCI calculation, albumin level was increased significantly in citrated plasma rather than serum samples. recent study showed that serum is preferred for determination of clinical biochemical profiles of sheep, but in emergency cases heparin can be used.

Keywords: Sheep, Plasma, Anticoagulant, Biochemical Parameter, DCI.

INTRODUCTION

An anticoagulants can be used to obtain blood samples that are free of clot for transfusion and analytical works. Anticoagulation is achieved either by calcium chelating (EDTA, citrate and fluoride) or by Thrombin inhibition (heparin) [8]. Serum is preferred specimen for clinical chemical analysis. In a few conditions plasma obtained with an appropriate anticoagulant may be preferred to serum. In addition, whole blood obtained with appropriate anticoagulant is the suitable sample for measuring of some trace elements, ammonia, blood PH and blood gas determination [12]. EDTA is the common anticoagulant for hematological examination. On the other hand, heparin

is the most widely used anticoagulant for biochemical analysis. Sodium citrate is the choice anticoagulant used for coagulation tests. Completion of the coagulation and harvest of serum before centrifugation is time consuming so in emergency situations, plasma is preferred sample for chemical analysis. Furthermore, plasma yield in equal volume of whole blood is always greater than serum [12]. In cases which Complete Blood Count is the only expected test, additional unexpected biochemical tests might be required So it is better to obtain two blood samples for both hematological and biochemical analysis. The effects of various types of anticoagulants on plasma biochemistry were studied in man and various animals [1, 2, 4, 7, 9, 11, 12]. But limited information is existing for sheep plasma biochemistry [5, 8]. The aim of this study was to determine the effects of various anticoagulants on chemical parameters in Sangsari sheep.

MATERIALS AND METHODS

2.1. Study area

The study was performed in the Garmsar city and suburb's, Semnan province, Iran. a region is located in latitude 35° 0' 0" N, longitude 52° 20' 0" E with in area more than of 11,000 km² (<http://tools.wmflabs.org/geohack/geohack>). The region has a average elevation of 856 meters above sea level.

2.2. Sample collection and parameters measured

One hundred and ten healthy sheep of Sangsari breed in traditional herds of Garmsar City, Semnan Province, Iran were used in this study. Twenty milliliters of blood were obtained from from jugular vein of each sheep and divided through tubes, containing different types of anticoagulants (EDTA: 0.1ml of 10% disodium EDTA solution for 5 ml of blood, Sodium Citrate: 0.5 ml Of 3.8% solution for 4.5 ml of blood, Lithium Heparin:100 units for 5 ml of blood) and plain tubes for serum harvesting. Samples were kept on ice flask and transferred to laboratory. Samples were centrifuged at 1500 RPM for 15 min. Serum and plasma was harvested within 90 min. No hemolysis was detected in analyzed samples.

The concentrations of glucose (glucose oxidase method), triglyceride (lipase method), cholesterol (cholesterol oxidase method), creatinine (kinetic Jaffe method), total protein (Biuret method), albumin (Bromcresol green method), calcium (Arsenazo III method), total bilirubin (bili, dichloroanilin method), inorganic phosphorus (phosphomolybdate method), the activity of aspartate aminotransferase (L-aspartate/2- oxoglutarate as substrate), magnesium (mg, Xylidile blue method) and urea (urea, urease/ glutamate dehydrogenase method) were measured by commercial kits (Pars Azmoon Co, Tehran, Iran) using an automated chemistry analyzer (Roche Cobas Mira Plus Chemistry Analyzer, Switzerland). Control serum (Randox control serum, Antrim, UK) was used for controlling the accuracy. Dilution correction was calculated for citrated plasma [(serum amount × 0.1) + citrate plasma amount]. Nonparametric wilcoxon pair test was performed to compare the differences between serum and plasma.

RESULTS AND DISCUSSION

The results showed in table 1. All of parameters except albumin were significantly decreased in citrated plasma in contrast to serum. Glucose, triglyceride, albumin, urea, creatinine, total protein, calcium, magnesium level and activity of AST significantly had lower rate in EDTA contained samples rather than serum. Bilirubin and albumin level were increased significantly in heparinized samples while urea level was decreased significantly rather than serum. In order to correct the dilution effects of sodium citrate anticoagulant, Dilution Correction Index (DCI) was calculated. There were no significant differences between the amount of glucose, triglyceride, cholesterol, urea, total bilirubin, the activity of AST, total protein, magnesium and phosphorus in citrated plasma rather than serum. After DCI calculation, albumin's level was increased significantly after DCI calculation in citrated plasma rather than serum.

Laboratory evaluation of patient is an integral part of diagnostic procedure. In order to analyze the chemical panel, serum should be harvest from blood. In normal conditions there is no problem and serum harvested regularly while in emergency cases hematological and chemical evaluation of patient is necessary and it should be done immediately. Serum harvesting is time consuming so plasma is preferred. In the other hand harvesting of serum might be artifactually effects the results by the risk of fibrin clot interference on automated analyzers [1]. sodium citrate solution 3.8g/dl in a ratio of 1 to 9 parts of blood, is commonly used for evaluating the coagulation tests.

In the present study, the chemical parameters amounts in citrate containing plasma were decreased considerably. It might be due to dilution effect of citrate (1 to 9) when the blood was mixed. The negative effects of citrate solution on human blood chemistry have been shown by Young et al. [12]. Calcium is chelated by Citrate so level of calcium was decreased. Citrate complexes Molybdate so the color yield in phosphor measurement decreased. Citrate inhibits aminotransferase activity and because its molybdate complexes, it decreases the color yield in phosphate measurement and thus produces lower result [12]. In present study all parameter except albumin were decreased significantly in citrated plasma rather than serum.

After DCI calculation, ratio of all parameters except albumin, creatinine and calcium were similar with serum. This is in contrast with other studies. Stokol showed that the reaction between BCG and albumin is inhibited by citrate [11]. In the present study, albumin was increased significantly after DCI calculation but the mechanism is not clear. EDTA induces changes in chemical parameters [1, 5, 9]. In present study, glucose level was increased significantly in EDTA Contained plasma. Our results are agreed with previous studies [5, 10]. The significant decreased level of urea, triglyceride, creatinine, AST, total protein, calcium and magnesium are agreed with previous studies [5, 8, 10]. The significantly lower concentration of calcium & magnesium is due to chelating moiety of EDTA.

In previous studies in dog and human there were no differences between the level of urea , creatinine and total protein in EDTA treated plasma rather than serum. The lowered concentration of these parameters in our study might be due to difference between animals & laboratory conditions.

In our study albumin and total protein were increased while urea concentration was decreased significantly. The results of urea concentration was similar with Laborde et al. , Morris et al. but was in contrast with Mohri et al. [7, 8, 9].

Table. Mean values of biochemical parameters in Sheep (max - min) *P <0.05 ; **P <0.01

Parameters	Serum	EDTA plasma	Heparinized plasma	Citrated plasma	Citrated plasma (dilution corrected)
Glucose (mmol/l)	4.14 (2.86-5.55)	4.54 (3.02-5.83) *	4.3 (3.21-5.24)	3.52 (3.22-4.39) *	3.91 (3.61-4.84)
Triglyceride (mmol/l)	0.55 (0.39-0.756)	0.48 (0.38-0.64) *	0.52 (0.39-0.7)	0.48 (0.38-0.68) *	0.53 (0.39-0.77)
Cholesterol (mmol/l)	1.61 (1.32-2.02)	1.59 (1.24-2.04)	1.53 (1.24-1.88)	1.49 (1.21-1.86) *	1.56 (1.34-2.06)
Albumin (g/l)	45.2 (41-50)	43 (38-51) *	46.9 (42-51) *	43.8 * (41-47)	48.1 (44.2-52.3) *
Urea (mmol/l)	8.53 (4.57-10.99)	8.12 (4.75-10.43) **	8.14 (4.9-11.02) **	8.09 (4.96-10.72) **	8.61 (5.39-11.74)
Total Bilirubin (µmol/l)	4.87 (4.32-5.18)	4.92 (3.26-5.41)	5.11 (4.67-5.29) *	4.69 (3.72-5.1) *	5.17 (4.82-5.63)
Creatinine (µmol/l)	124.21 (115.12-132.87)	106.11 (99.79-111.77) **	119.43 (112.81-127.81)	99.8 (94.12-103.91) **	107.62 (102.14-110.63) *
AST (IU/L)	129.7 (109.3-148.2)	117.9 (101.6-139.9) *	131.2 (112.8-149.3)	109.6 (97.7-130.4) **	134.4 (100.4-142.6)
Total protein (g/l)	69.9 (64.7-71.4)	67.3 (63.9-69.9) *	71.3 (65.9-73.2)	64.7 (60.6-66.1) **	69.2 (66.8-70.4)
Calcium (mmol/l)	2.47 (2.21-2.62)	0.09 (0.07-0.01) **	2.42 (2.18-2.59)	1.96 (1.27-2.08) **	2.12 (1.57-2.29) **
Magnesium (mmol/l)	1.12 (0.78-1.37)	0.34 (0.32-0.35) **	1.09 (0.84-1.29)	0.89 (0.77-1.1) *	1.09 (0.81-1.41)
Phosphorus (mmol/l)	2.21 (2.11-2.39)	2.19 (2.07-2.26)	2.19 (2.02-2.22)	1.72 (1.61-1.89) *	2.14 (2.81-2.39)

Heparin has been generally recommended as the most suitable anticoagulant for plasma biochemical measurements [8, 12]. Although serum and heparinized plasma specimens are considered equivalent for many assays, differences in results between these two sample types have been reported for several chemistry analytcs [6]. In present study in Garmsar region, Iran albumin & urea level were decreased significantly in heparinized plasma. An artifactual increase in albumin in heparinized plasma, compared with serum and other anticoagulants, using a bromocresol green assay (BCG) was recently described in canine [2, 11] and sheep samples [5]. This difference is partly due to the combination of heparin and fibrinogen [11]. Significant increase of total protein and glucose amounts by significant

decrease in urea concentration were reported in sheep heparinized plasma in contrast to serum [5, 8]. In this study total bilirubin was increased significantly in heparinized plasma. It might be due to interference of heparin with reagents. In this condition the reaction solution appears cloudy, the reagent decreased transmittance, absorbance increased, resulting in the determination of total bilirubin value increased.

CONCLUSION

The results of present study showed that serum is preferred for determination of clinical biochemical profiles of sheep, but in emergency cases, heparin can be used.

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