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Short communication

Post mortem aqueous humor analysis in sheep as index of *ante mortem* serum biochemistry profile

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ABSTRACT

We investigated the usefulness of the concentration of total proteins, albumin, creatinine, calcium (Ca), magnesium (Mg) and the activities of aspartate aminotransferase (AST) and gamma-glutamyltransferase (GGT) in aqueous humor in estimating the *ante mortem* levels of the same biochemical analytes in sheep blood. A blood sample was taken *ante mortem* and aqueous humor was collected 12 h *post mortem* from 63 sheep. Linear Regression Analysis was run to determine the equations predicting the serum value of each parameter based on the value determined in aqueous humor for those parameters that were significantly correlated. A strong relationship was found for Mg and creatinine concentration as well as for AST and GGT activities in aqueous humor and serum. Aqueous humor analysis of creatinine, Mg, AST and GGT levels can be used for the estimation of the corresponding concentration in blood, while, total proteins, albumin and Ca levels are of limited value.

1. Introduction

In veterinary medicine, interpretation of the biochemical profile is a significant diagnostic aid and in conjunction with history evaluation and physical examination or necropsy provides the clinician with valuable information to reach a final diagnosis (Braun et al., 2010). However, *post mortem* biochemical analysis of blood can be unreliable unless samples are collected immediately after death and prior to autolysis. Aqueous humor seems to be better preserved for some time after death and is easily accessible and ready to use for analysis without requiring centrifugation. Aqueous humor is formed as a result of diffusion, ultrafiltration, and active secretion and its content is higher in ascorbate, lactate and chloride and lower in protein compared to blood (Edwards et al., 2009).

In sheep practice, during necropsy blood biochemistry values are unknown to the clinician and this poses limitations to diagnostic efficacy. Therefore, alternative samples for estimating *ante mortem* blood values warrant investigation. Studies have been conducted in dogs, cats (Appleby et al., 1990; Hanna et al., 1990), cattle (Hanna et al., 1990; Lane and Lincoln, 1985; McCoy et al., 2001b; McCoy et al., 2001c) and pigs (Drolet et al., 1990) using values of biochemical parameters in aqueous humor collected *post mortem* to estimate *ante mortem* serum chemistry profiles. There are, however, limited data on sheep, with the exception of the correlation between the concentrations of magnesium (Mg) (Scott et al., 1995) and 3-OH butyrate (McCoy et al., 2001a) in aqueous humor and blood.

Among the biochemical parameters selected to be tested in the present study, the concentration of total protein and albumin are used for the detection of disorders in synthesis, one of the major liver functions, the activity of aspartate aminotransferase (AST) and (gamma-glutamyltransferase (GGT) for the detection of liver damage, creatinine for renal function while calcium (Ca) and Mg for the detection of two important metabolic disorders in sheep, hypocalcemia and hypomagnesaemia, respectively (Braun et al., 2010). Also in contrast to previous research (McCoy et al., 2001a), we used Atomic Absorption Spectrometry (AAS), the method of choice for Mg measurement (Rosol and Capen, 1997).

The aim of this study was to evaluate the potential of the *post mortem* aqueous humor concentration of total protein, albumin, creatinine, Ca, Mg and the activity of AST and GGT after death to predict their corresponding *ante mortem* values in sheep blood.

2. Materials and methods

Prior to the onset of the experiment the minimum required sample size was calculated using Power analysis in Medcalc software. The

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minimum desired correlation coefficient for detection that would be of biological significance in this study was set at 0.5. Using a Type 1 error at 0.05 and Type II error at 0.2 (desired power = 0.8) the minimum required size was calculated at 29 samples.

A total of 66 sheep were randomly selected from a number of 348 sheep from 11 different farms (6 from each farm) that were about to be slaughtered on the sampling day at the local slaughterhouse.

The blood sample was collected from each sheep just before slaughter in a plain tube (BD, Franklin Lakes, NJ, USA) and allowed to clot. The blood samples and the heads of the animals were transferred to the Diagnostic Laboratory of the Clinic of Medicine, University of Thessaly. After low-speed centrifugation at 1600*g* for 10 min, serum was inspected for visual abnormalities (hemolysis, clots). Finally, 63 sera were used in the study and 3 were excluded due to hemolysis. Serum was separated into two aliquots and was kept at 4 °C pending analysis. The heads of the animals were kept in ambient conditions until aqueous humor sampling.

Aqueous humor was gently aspirated from both eyes 12 h after slaughter with a syringe using a 21-gauge needle inserted horizontally just under the cornea into the anterior chamber. Attention was given to avoiding fine needle aspiration of the iris tissue. Aspirated aqueous humor samples from both eyes were pooled and placed in two plain tubes without anticoagulant.

One aliquot of each sample of aqueous humor and serum was transferred to the diagnostic Laboratory Faculty of Veterinary Medicine, Aristotle University of Thessaloniki for measuring calcium and magnesium by AAS (Perkin-Elmer AAnalyst−100 spectrophotometer, Norwalk, Connecticut).

Most of the biochemical parameters (albumin, creatinine, AST, GGT) were determined in the Diagnostic Laboratory of the Clinic of Medicine, Faculty of Veterinary Medicine, University of Thessaly using a spectrophotometer (Shimadzu 1601 UV spectrophotometer, Tokyo, Japan), and total protein using a refractometer (American Optical/Leica/TS Meter, Southbridge, Massachusetts).

All analyses were performed within 24 h after aqueous humor sampling. The interval between aqueous humor and serum sample analysis from the same animal and for each parameter was less than 2 h.

Analysis of the data was done using MedCalc software (version 9.2).

Normality of data distribution was assessed with Kolmogorov-Smirnov test. As data for all parameters were normally distributed, Pearson correlation coefficients were determined to evaluate the linear relationship between paired values in serum and aqueous humor. Only for the parameters that were significantly linearly related and with $r > 0.6$ considered as a strong linear relationship (Evans, 1996). Linear Regression Analysis was run to determine the equations predicting the serum value of each parameter based on the value determined in the aqueous humor as well as the coefficient of determination R^2. Based on the equations generated, the serum concentration was calculated from the aqueous humor values and Bland-Altman plots (Bland and Altman, 1986) were created for the two serum values. Paired samples *t*-test were used to evaluate the significance of the differences between the values obtained in serum and the ocular fluid. A value of $P \leq 0.05$ was considered significant in all comparisons.

All procedures were done according to the ethical standards in the Helsinki Declaration of 1975, as revised in 2000, as well as the national law and in compliance with our Institutional Animal Use Ethics Committee.

3. Results

As shown in Table 1 there was a significant linear relationship between the blood serum and the aqueous humor values of all parameters tested ($P < 0.05$) except total protein ($P > 0.05$). However, this relationship was strong only for Mg, creatinine, AST and GGT ($r > 0.6$).

The linear regression equations and the coefficient of determination for these are presented in Table 2. The linear regression plots for creatinine and Mg are shown in Figs. 1 and 2 respectively. Bland-Altman plot analysis revealed that the average bias with confidence interval (CI) between the actual and the calculated serum values for Mg (Fig. 1) was −0.1% (CI: −1%–11.8%), for creatinine (Fig. 2) was 0% (CI: −14.4% − 14.4%), for AST was 2.3% (CI: −38.4%–42.9%) and for GGT was 3.3% (CI: −46.8%–53.45%).

The blood serum activities of AST and GGT and the blood serum concentrations of Mg and creatinine were significantly higher compared to those determined in aqueous humor ($P < 0.001$; Table 2).

Table 1
Correlation coefficient (*r*) and significance (*P*) between the values of total protein (TP), albumin, aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), calcium (Ca), magnesium (Mg) and creatinine obtained in serum and aqueous humor in 63 sheep samples.

Biochemical parameter	*r*	*P*
TP	0.206	0.1164
Albumin	0.357	0.004
AST	0.695	< 0.0001
GGT	0.636	< 0.0001
Ca	0.287	0.0224
Mg	0.959	< 0.0001
Creatinine	0.978	< 0.0001

Table 2
Linear regression equation and coefficient of determination (R^2) between the values of aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), magnesium (Mg) and creatinine obtained in serum (y) and aqueous humor (x) and their mean values (SE) in 63 sheep samples.

Biochemical parameter	Linear regression equation	R^2	Serum	Aqueous humor	*P*
AST (U/l)	y = 1.108 x + 47.69	0.482	106.69 (3.78)	53.24 (2.37)	< 0.001
GGT (U/l)	y = 2.419 x + 24.10	0.404	48.94 (2.04)	10.26 (0.54)	< 0.001
Mg (mmol/l)	y = 1.451 x − 0.14	0.920	1.06 (0.027)	0.83 (0.018)	< 0.001
Creatinine (umol/l)	y = 1.469 x − 0.0002	0.956	0.017 (0.0007)	0.012 (0.0004)	< 0.001

4. Discussion

The most remarkable findings in this study were the high correlation between aqueous humor and serum concentration of Mg and creatinine and to a lesser extent AST/GGT activity. The results indicate that *post mortem* samples can be used with a high degree of confidence to predict *ante mortem* values for these analytes, facilitating the ability to assess hypomagnesemia, renal function, and cholestasis in sheep at necropsy.

The good correlation in Mg results between serum and aqueous humor in our study was similar to that in a previous study (McCoy et al., 2001a). Our use of AAS confirms the accuracy of this finding. Using the regression equation generated from our data the average total bias when comparing the actual and the calculated serum Mg concentration was 0.1% with relatively low range indicating that serum value of Mg can be sufficiently predicted based on the aqueous humor value. A previous study (McCoy et al., 2001a) found that Mg concentration in aqueous humor may increase as much as 60% by 24 h after sampling at ambient temperature. For this reason and to make interpretation of results possible, it was decided in our study to have a fixed time point of 12 h *post mortem* for sampling.

Correlations between serum creatinine concentration and *post mortem* aqueous humor values have been reported in dogs, cats and cattle (Lane and Lincoln, 1985). Our results confirmed this strong correlation and further indicated that aqueous humor creatinine concentration can be used to accurately predict *ante mortem* creatinine