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Original Research

Biochemical Analysis of Synovial Fluid, Cerebrospinal Fluid and Vitreous Humor at Early Postmortem Intervals in Donkeys

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Abstract

Biochemical analysis of body fluids after death is a helpful tool in veterinary forensic medicine. Synovial fluid, cerebrospinal fluid (CSF) and vitreous humor are easily accessible and well preserved from contamination. Five donkeys (*Equus africanus asinus*) aged 1 - 2 years old were subjected to the study. Samples (Synovial fluid, CSF and vitreous humor) were collected before death (antimortem) and then at 2, 4, 6, 8, 10 and 12 hours postmortem. Samples were analyzed for glucose, chloride, sodium, magnesium, potassium, enzymes and total protein. Synovial fluid analysis showed that glucose concentration started to decrease at 6 hours postmortem, while magnesium level increased with time. Other parameters were more stable. CSF analysis showed several changes related to time after death as the decrease in glucose and sodium levels, and the increased levels of potassium, magnesium, calcium and total protein. Vitreous analysis revealed a reduction in glucose level and increased potassium and magnesium concentrations. The present study concluded that biochemical analysis of synovial fluid, vitreous humor and CSF can help in determination of time since death in donkeys. This study recommend using CSF for determination of early post-mortem intervals.

Keywords: Biochemical; Cerebrospinal; Donkey; Postmortem; Synovial; Vitreous humor

Introduction

Postmortem chemical changes start to occur in the body immediately or shortly after death and progress until the body disintegrates. Each change has its own time factor and rate. These changes occur in various body fluids including blood, cerebrospinal fluid (CSF) and vitreous humor; so determination of the chemical abnormalities could help forensic pathologists to ascertain time since death more precisely (Aggrawal *et al*., 1983). Amongst these the most widely used method is the estimation of potassium concentration in vitreous humor (Lincoln and Lane 1985; Coe, 1989; Ahi and Garg 2011).

Recently most of forensic studies have been concentrated on the biochemical changes that occur after death in body fluids like CSF, vitreous humor and blood. All these fluids showed time related changes after death (Arikeri *et al*., 2013).

Synovial fluid is isolated in a well protected compartment which was hardly used for postmortem chemistry. Only few studies of medico-legal interest on synovial fluid have been published and studies on the distribution of various analytes which are helpful in postmortem chemistry are missing (Madea *et al*., 2001).

The eye is isolated and well protected so that vitreous humor is normally preserved, relatively stable, less susceptible than other body fluids to rapid chemical changes and contamination, easily accessible and its composition is quite similar to that of aqueous fluid, cerebrospinal fluid and serum; thus it is suitable for many analyses to estimate postmortem intervals (Saugstad and Olaisen, 1978).

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In veterinary field, CSF and vitreous humor a are commonly used for postmortem biochemical analysis and determination of postmortem interval, but the use of synovial fluid is rare in animals.The goal of the present study was to estimate the early postmortem changes in the biochemical constituents of synovial fluid, CSF and vitreous humor in donkey.

Materials and methods

The current study was carried out in the Veterinary Teaching Hospital, Assiut University, Assiut, Egypt, during February 2013, where the environmental temperature ranged between 20ºC and 25ºC.

Animals

Five donkeys (*Equus africanus asinus*) aged 1 - 2 years old were subjected to this study. Synovial fluid, CSF and vitreous humor were collected from donkeys and then animals were euthanized by overdose of chloral hydrate and magnesium sulfate injection.

Samples were collected immediately before death (antimortem) then at 2, 4, 6, 8, 10 and 12 hours postmortem.

Synovial fluid was collected from the carpal and tarsal joints using 2", 20g sterile needle and sterile syringe. CSF samples were collected through the atlanto-occipital puncture using a 5", 18g spinal needle. Vitreous humor was collected from the eye through puncture of the sclera at the outer canthus with a 2", 25 g needle. The auriclo-palpebral nerve block was performed firstly using lidocaine Hcl 2%, in addition to the topical analgesia of the cornea using xylocaine spray 10%.

The study time did not exceed 12 hours postmortem because there were no facilities to keep dead animals more than this time and we preferred that all samples should be collected from the whole carcass.

Preparation of Samples

All samples were centrifuged directly after collection at 3500 rpm for 15 minutes; the supernatant was transferred to eppendorf tubes and stored at -20ºC till analysis. Biochemical analyses of samples were done at the end of the experiment.

Biochemical analysis

Biochemical constituents were measured in synovial fluid, CSF and vitreous humor using UV visible/spectrophotometer (Optizen 3220 UV, Mecasys Co. Ltd, Korea); reagents kits were supplied by Spinreact (GIRONA- Spain). Biochemical analysis included estimation of glucose, chloride, sodium, calcium, magnesium, potassium, aspartate aminotransferase (AST), alkaline phosphatase (ALP) and total protein levels.

Statistical analysis

Statistical analysis was conducted using SPSS 16.0 for windows (SPSS, Chicago, USA). Data were tested for difference using Post-hoc test, Least Significant Difference (LSD). Statistically significant differences were determined at $p \leq 0.05$. Data were expressed as Mean ± SD.

Results

Post-mortem variations in the biochemical constituents of synovial fluid

Comparing data at post-mortem intervals with those of anti-mortem revealed that synovial glucose level decreased gradually starting at 6 hours post-mortem (45.12±17.98 mg/dl), reaching the lowest level at 12 hours (6.56±2.82 mg/dl),while synovial magnesium level gradually increased after death (1.64 ± 0.37 mg/dl) with the significant changes started from 4 hours post-mortem (5.12±1.07 mg/dl). Synovial sodium level showed a significant increase only at 2 hours post-mortem. Synovial calcium level showed a significant decrease ($P < 0.01$) both at 6 hours and 8 hours post-mortem. Synovial potassium level showed a significant decrease only at 8 hours post-mortem and ALP level showed a significant increase at 10 hours ($P < 0.05$), and decreased significantly at 12 hours post-mortem. All data were presented in Table 1.

Post-mortem variations in biochemical constituents of CSF

Measuring different biochemical constituents in CSF revealed that glucose level was significantly decreased ($P < 0.01$) at 4, 6, 8, 10 and 12 hours post-mortem. There was a significant reduction in

Table 1. Anti-mortem and post-mortem levels of biochemical constituents of synovial fluid in dokeys

	Glucose (mg/dl)	Chloride (mmol/l)	Sodium (mmol/l)	Calcium (mg/dl)	Magnesium (mg/dl)	Potassium (mmol/l)	AST (U/l)	ALP (U/l)	Total protein (g/dl)
Anti-Mortem	45.26±8.82	96.19±19.80	149.42±32.64	4.92±0.60	1.64±0.37	10.06±1.81	75.36±52.05	56.79±29.2	6.11±1.79
Post-Mortem									
2 hours	63.79±22.12	101.0±7.67	209.19±42.76**	3.67±0.72	2.60±0.92	7.98±0.45	67.20±23.11	39.31±23.16	6.49±2.19
4 hours	68.79±33.67*	94.04±18.83	167.88±13.51	7.59±3.21	4.30±1.85**	8.65±2.80	70.11±27.00	71.68±41.17	5.38±2.45
6 hours	45.12±17.98	104.99±22.0	121.10±29.64	9.56±3.09**	4.44±2.13**	8.32±2.63	68.25±28.59	54.59±33.28	3.89±1.10
8 hours	18.81±8.97*	109.43±13.53	146.72±22.34	10.53±2.96**	4.46±1.17**	8.02±2.12*	58.68±16.73	76.55±18.37	5.90±2.67
10 hours	11.84±10.24**	111.70±16.19	157.73±11.74	5.66±3.21	5.27±1.06**	10.23±1.61	75.65±22.16	97.52±21.13*	5.52±1.54
12 hours	6.56±2.82**	106.06±7.13	140.39±19.50	6.93±4.08	5.12±1.07**	9.34±3.30	89.01±34.06	15.40±7.96*	4.89±1.33

Data represented as mean±SD

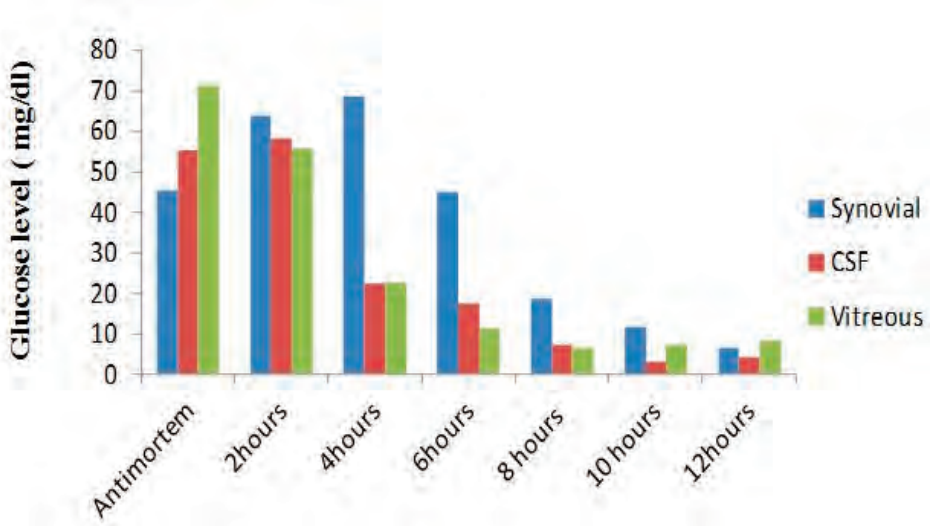

Fig. 1. Changes in glucose level in synovial fluid, CSF and vitreous humor at postmortem intervals in donkeys.

sodium level starting from 4 hours after death. There was a gradual increase in calcium level with significant changes (P <0.01) at 8, 10 and 12 hours post-mortem. Magnesium level in CSF was gradually increased at postmortem intervals with significant changes (P <0.01) at 6, 8, 10 and 12 hours. CSF potassium level was significantly increased starting from 2 hours (P <0.05) and continues till 12 hours (P <0.01) post-mortem.Total protein showed a significant increase at 6 (P <0.05), 8 (P <0.01) and 10 hours (P <0.05) post-mortem. ALP level significantly increased (P <0.01) at 8 hours (Table 2).

Post-mortem variations in biochemical constituents of vitreous humor

Vitreous humor glucose level was gradually and significantly decreased (P <0.01) at all measured post-mortem intervals. There was a significant increase in vitreous humor calcium level at 8 hours (P <0.05) and 10 hours (P <0.01) post-mortem. Magnesium level was significantly increased at 2 (P<0.05), 6 (P<0.05), 8 (P <0.01) and 10 (P <0.01) hours post-mortem. Chloride level showed a significant increase (P <0.01) at 10 hours post-mortem (P<0.01). Sodium level showed a significant increase (P <0.05) only at 2 hours post-mortem. The level of potassium in vitreous humor showed gradual increase starting from 2 hours post-mortem, and increased (P <0.01) significantly at 8, 10 and 12 hours. There was a significant increase in vitreous humor ALP only at 8 hours post-mortem. Total protein was significantly decreased (P <0.05) at 12 hours post-mortem. Data of analyzed parameters were presented in Table 3.

The current data showed similar changes in glucose and magnesium levels in the three studied fluids (synovial fluid, CSF and vitreous humor), On the other hand, potassium level increased in both CSF and vitreous humor after death (Figs. 1, 2 and 3).

Table 2. Anti-mortem and post-mortem levels of biochemical constituents of CSF in donkeys.

	Glucose (mg/dl)	Chloride (mmol/l)	Sodium (mmol/l)	Calcium (mg/dl)	Magnesium (mg/dl)	Potassium (mmol/l)	AST (U/l)	ALP (U/l)	Total protein (g/dl)
Anti-Mortem	55.40±27.22	124.85±23.56	240.61±46.71	4.28±0.74	2.28±0.97	7.28±1.41	24.26±7.22	13.04±3.40	0.32±0.17
Post-Mortem									
2 hours	58.21±30.85	129.32±17.71	259.83±21.17	4.32±0.86	2.98±0.92	15.89±2.63*	18.41±3.94	18.08±3.88	0.56±0.36
4 hours	22.44±4.52**	130.13±23.93	157.06±29.52**	4.62±1.67	5.27±2.51	23.01±7.35**	12.15±1.63	14.70±2.67	0.74±0.24
6 hours	17.72±6.96**	130.66±17.80	157.21±25.67**	6.86±1.53	9.44±4.99**	24.72±7.07**	21.00±11.50	21.85±5.89	1.45±0.98*
8 hours	7.31±4.48**	137.30±26.18	154.95±10.35**	8.91±4.67**	7.59±2.49**	32.06±6.48**	29.90±19.23	38.73±31.58**	1.90±1.50**
10 hours	3.28±1.56**	123.08±12.18	162.46±12.56**	10.38±4.95**	14.87±5.02**	28.82±3.27**	27.29±8.32	25.92±6.75	1.68±1.21*
12 hours	4.16±2.14**	124.02±9.31	170.77±20.33**	10.64±4.06**	14.37±3.03**	29.63±7.69**	22.99±5.54	16.46±6.52	1.16±0.51

Data represented as mean±SD

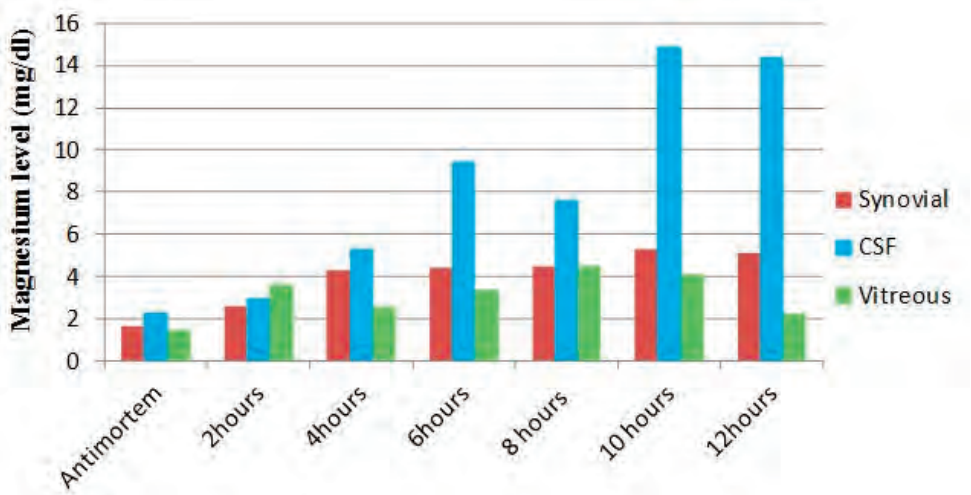

Fig. 2. Changes in magnesium levels in synovial fluid, CSF and vitreous humor at postmortem intervals in donkeys.

Discussion

Postmortem analysis of synovial fluid is not common in the veterinary field; this study could use synovial fluid, CSF and vitreous humor of donkeys to explain the early biochemical changes after death and its relation to post-mortem intervals in the three fluids. Post-mortem Biochemical changes in synovial fluid after death were studied in human (Madea *et al*., 2001; Sheikh, 2008; Tumram *et al*., 2011). In the present study glucose level decreased at 6 hours and continued to show sharp decrease, this result is similar to that reported by Madea *et al*. (2001) and Tumram *et al*. (2011); who reported that glucose level decreased after death. In the current study, potassium level did not change during the twelve hours after death, this result disagree with that reported by Madea *et al*. (2001); Tumram *et al*. (2011) and Arikeri *et al*. (2013) in human, this may be related to species variation between human and equine or may be due to the short time after death implemented in the present study.

CSF postmortem analysis showed promising results that several parameters were changed with increased postmortem interval including glucose, sodium, calcium, potassium, magnesium and total protein. Reduction in glucose level and increased potassium levels in CSF in the present study were in parallel with those obtained by Karkela (1993) and Singha *et al*. (2002) in human, who attributed the increased in potassium to its rapid release from the cells immediately after death and in canines as reported by Ashry (2004), who obtained the same result after analysis of CSF of dogs. Sodium level was reduced in CSF after death this result disagreed with Ashry (2004), who reported that sodium level increased after death in CSF of dogs. Calcium level increased gradually and significantly after death in CSF, which agreed with similar study by Schoning and Strafuss (1980a) in dogs.

In this study, post-mortem vitreous potassium concentration was significantly increased. This

Table 3. Anti-mortem and post-mortem levels of biochemical constituents of vitreous humor in donkeys.

	Glucose (mg/dl)	Chloride (mmol/l)	Sodium (mmol/l)	Calcium (mg/dl)	Magnesium (mg/dl)	Potassium (mmol/l)	AST (U/l)	ALP (U/l)	Total protein (g/dl)
Anti-Mortem	71.11±37.32	111.43±11.28	157.70±25.01	4.04±0.77	1.49±0.25	10.95±3.34	22.51±4.25	12.95±4.53	0.79±0.52
Post-Mortem									
2 hours	55.85±16.49**	140.94±41.35*	204.07±48.46*	8.31±1.62	3.59±1.67*	10.40±2.58	19.22±8.08	22.87±10.19	0.80±0.14
4 hours	22.59±19.11**	117.56±17.07	196.62±53.63	5.28±1.51	2.55±1.03	11.63±1.09	13.35±6.74	12.40±3.55	0.64±0.25
6 hours	11.31±8.14**	133.83±10.27	157.48±37.18	6.41±4.98	3.32±1.45*	13.40±1.36	9.62±4.00	10.47±4.09	0.54±0.17
8 hours	6.50±6.29**	127.73±10.14	144.76±15.75	8.00±2.73*	4.46±1.53**	16.88±2.81**	24.38±17.59	38.18±20.18**	0.43±0.30
10 hours	7.06±6.67**	144.05±19.57**	147.36±27.25	11.17±3.85**	4.10±1.12**	15.33±1.92**	17.16±13.08	23.25±14.26	0.59±0.18
12 hours	8.20±2.38**	116.11±8.37	161.09±15.48	5.83±2.69	2.20±0.98	14.86±2.36**	34.83±34.23	11.02±5.99	0.33±0.09*

Data represented as mean±SD

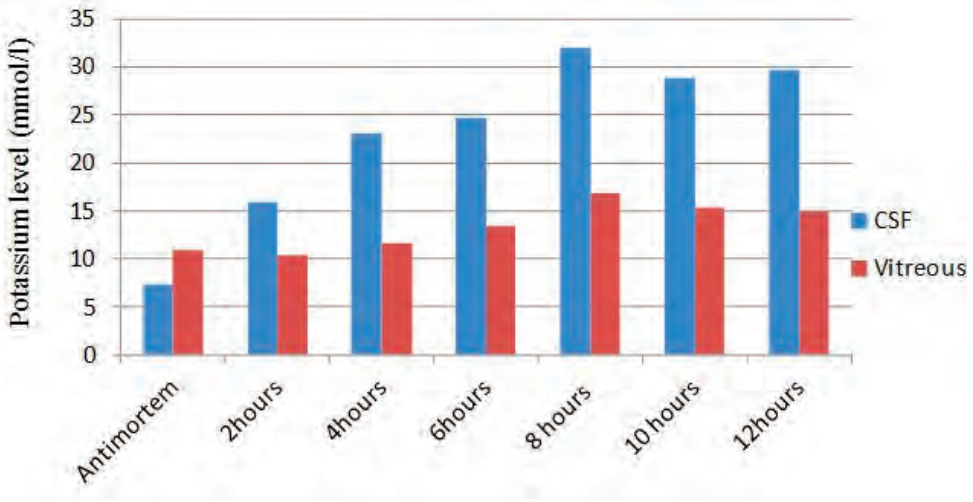

Fig. 3. Changes in potassium concentrations in CSF and vitreous humor at postmortem intervals in donkeys.

finding agreed with previous reports on horses (McLaughlin and McLaughlin 1988) and other species including dog (Crowell and Duncan, 1974; Schoning and Strafuss, 1980b), cattle and pig, which demonstrated that vitreous humor is an excellent sample for post-mortem biochemical analysis (Lincoln and Lane, 1985) and also agreed with studies on human (Henry and Smith 1980, Madea *et al*., 2001; Prasad *et al*., 2003; Ahi and Garg, 2011).

Post-mortem increase in vitreous potassium concentration has been used in forensic medicine and veterinary medicine to estimate time of death (Leahy and Farber 1967; Coe 1972; Henry and Smith, 1980; Schoning and Strafuss, 1980b; James *et al*., 1997; Ahi and Garg, 2011). After death, cell membranes become permeable; potassium immediately begins to diffuse from inside retinal cells out into the vitreous resulting in potassium levels that increased over time. For this reason, vitreous potassium has been used to estimate time of death and postmortem interval. On the other hand, sodium, chloride, creatinine, and urea nitrogen are more stable than potassium, and reflect antimortem levels for up to 120 hours after death (Rose and Collins, 2008).

In the present study, it was observed that glucose level decreased and magnesium level increased in all the studied fluids. However, potassium level increased only in CSF and vitreous humor.

Conclusion

The present study concluded that biochemical analysis of synovial fluid, vitreous humor and CSF can help in determination of time since death in donkeys. This study recommend using CSF for determination of post-mortem intervals.

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