

BIOCHEMICAL ANALYSIS OF VITREOUS HUMOR AS A POTENT SOURCE FOR ESTIMATION OF POSTMORTEM INTERVAL IN BUFFALOES

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SUMMARY

The present study was conducted to establish an initial data that could be of further use to analyze the time of death in animals during post-mortem examinations along with other traditional methods. Potassium and sodium ions were estimated in freshly collected vitreous humor from 20 pairs of eyeball of Murrah buffaloes and estimation was done by spectrophotometer at required wavelength. The statistical analytes revealed that there was a linear rise in potassium concentration of vitreous humor with increasing death interval, however, sodium concentration showed discontinuous progression with increasing death time interval. Thus potassium levels in vitreous humor for determining death time interval in buffaloes are useful along with other traditional methods used in post-mortem examination to estimate the time of death.

Keywords: Buffalo, Potassium, Sodium, Vitreous humor

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For several decades, vitreous humor has been used for post-mortem biochemical investigations, as it has been described that vitreous humor is hardly contaminated even in late post-mortem intervals. Analyses have been made in particular with regard to the diagnosis of various diseases presenting changes of the concentrations of electrolytes and chemical parameters. Furthermore, studies on the estimation of the post-mortem interval have been performed on vitreous humor and have been used for the clarification of forensic issues in humans (Devgun and Dunbar, 1986). The most investigated post-mortem analyses in vitreous humor are potassium (Tumram *et al.*, 2014) and sodium (Blana *et al.*, 2011) along with some other ions like chloride, calcium, magnesium, phosphate, urea, creatinine and lactate. In particular, due to largely differing results of various studies and only few systematic investigations, post-mortem biochemical analyses of vitreous humor have hardly been applied in animal practice. It was reported by some authors that the gained concentrations of the analytes depend on the measuring apparatus, and few authors reported the influence of the pre-analytical treatment on the observed concentrations (Coe and Apple, 1985). The vitreous humor consists of gel-like collagen fibrils and fluid. The resulting high viscosity makes a sample pre-treatment advisable (Gagajewski *et al.*, 2004).

Due to paucity of literature in case of animals, the present study was conducted to establish an initial data that could be of further use to analyze the time of death in animals during post-mortem examinations along with other traditional methods.

Vitreous humor analysis: A total of 20 pairs of eyeballs

from adult physically healthy Murrah buffaloes of both the sexes were collected from the government authorized Tangra slaughter house, Kolkata, West Bengal from January 2016-18. Vitreous humor (1.5- 2 ml) of fresh samples was collected aseptically from each eyeball by 20-gauge hypodermic needle. Collected samples were immediately centrifuged at 3500 rpm for 10 min. The separated supernatant was collected and stored in tubes without preservatives, frozen at -20°C. The samples were analyzed in spectrophotometer with Sodium and Potassium kit (Coral Clinical systems) measured at 630nm and 530nm respectively as per the brochure attached with the kit. The samples were analyzed from 0-36 hrs in 6 hourly intervals and in each hour four samples were analyzed. The results were analyzed on the basis of the optical density value.

All the collected data were statistically analyzed by paired- T test and the significance (P value) were recorded at 5 % level of significance by using IBM SPSS statistic 20 software.

Vitreous body: It was a jelly-like transparent substance, found between the retina and the crystalline lens. It was in close contact with the pass optica retinae and was slightly adherent to lens capsule. In front there was a deep cavity, the fossa hyaloidea which fits the posterior surface of the lens. After fixation, it appeared as a frame work of delicate fibrils, the vitreous stroma whose interstices were filled with clear watery fluid, the vitreous humor. The surface was covered by a condensation of the stroma termed as the hyloid membrane.

Biochemical analysis of Vitreous Humor: There was a linear rise in potassium concentration with increasing

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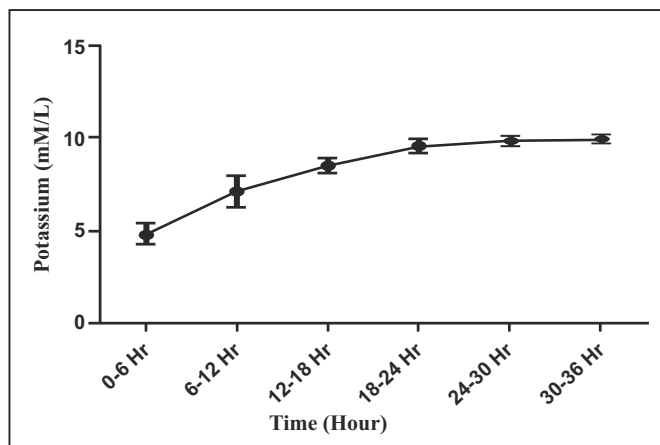


Fig.1. Changes in potassium level in vitreous humor at different time intervals (h)

death interval (Fig. 1). The study indicated that potassium levels in vitreous for determining death interval are useful and can afford a good method of determining the death interval along with other traditional methods. Similar observations were recorded by Tumram *et al.* (2014) in human. On the contrary, the sodium concentration of vitreous humor was also measured but the results, although significant with time interval, showed discontinuous progression at different intervals (Fig. 2). Therefore, the sodium concentration of vitreous humor to determine the death interval might not be used to detect the time of death.

The mean concentration of potassium at 0-6 hrs interval after death was 4.823 ± 0.567 (mM/L) with the range of 2.79 to 6.55 which was highly significant ($P < 0.05$). The concentrations of potassium were calculated at every 6 hrs interval up to 36 hrs (Table 1). All the values were significant within the time interval. The rise of

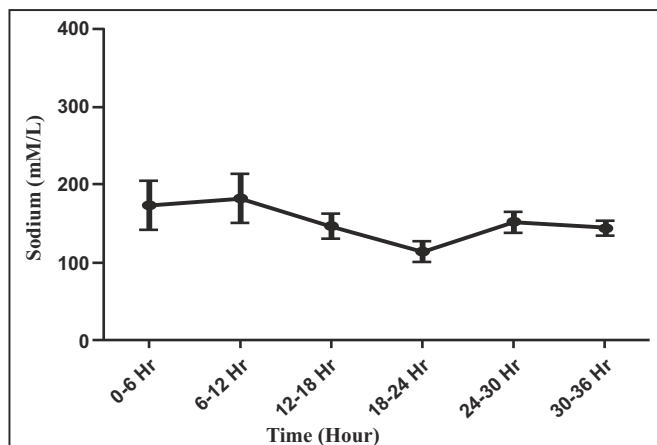


Fig.2. Changes in sodium level in vitreous humor at different time intervals (h)

potassium concentration [K⁺] in vitreous humor had been more concerning accuracy of death time estimation. The vitreous humor of the two ocular bulbs did not lead to significant differences in case of Potassium value. The levels of potassium in vitreous fluid for determining death interval were mostly accurate. These results were in accordance with the findings of Tumram *et al.* (2011), Blana *et al.* (2011) and Tumram *et al.* (2014) in humans.

The mean concentration of sodium at 0-6 hrs interval was 173.7 ± 31.42 (mM/L) with the range of 92.13 to 286.50 which was highly significant ($P < 0.05$). Similarly, the concentration of sodium was also calculated at every 6 hrs interval up to 36 hrs (Table 2). All the values were significant within the time interval, but the results showed discontinuous progression in sodium concentration with increasing death time interval (Graph 2), which was in accordance with the findings of Tumram *et al.* (2011) and

Table 1. Changes in potassium level (mM/L) in vitreous humor at different time interval (hour)

	0-6 Hrs	6-12 Hrs	12-18 Hrs	18-24 Hrs	24-30 Hrs	30-36 Hrs
Range	2.79-6.55	4.09-9.09	7.04-9.68	8.67-10.71	8.83-10.55	9.03-10.38
Median	4.94	7.95	8.5	9.365	10.08	10.2
Mean ± SE	4.823 ± 0.567	7.138 ± 0.845	8.545 ± 0.388	9.61 ± 0.312	9.893 ± 0.251	9.983 ± 0.208
Std. Deviation	1.391	2.071	0.9505	0.7662	0.6169	0.5107
Significant (alpha=0.05)	Yes	Yes	Yes	Yes	Yes	Yes

Mean Values differ significantly at mentioned time interval, where $P < 0.05$

Table 2. Changes in sodium level (mM/L) in vitreous humor at different time interval (hour)

	0-6 Hrs	6-12 Hrs	12-18 Hrs	18-24 Hrs	24-30 Hrs	30-36 Hrs
Range	92.13-286.50	90.07-287.5	100.5-191.8	70.62-144	102.3-185	112.7-172.4
Median	162.4	176.4	147.4	121.2	159.7	148.4
Mean ± SE	173.7 ± 31.42	182.3 ± 32.18	147 ± 15.49	114.1 ± 11.8	151.7 ± 12.5	144.1 ± 9.89
Std. Deviation	76.95	78.83	37.95	28.93	30.61	24.25
Significant (alpha=0.05)	Yes	Yes	Yes	Yes	Yes	Yes

Mean Values differ significantly at mentioned time interval, where $P < 0.05$

Blana *et al.* (2011).

CONCLUSION

There was a linear rise in potassium concentration of vitreous humor with increasing death interval, however, sodium concentration showed discontinuous progression with increasing death time interval. The study indicated that potassium levels in vitreous for determining death time interval are useful and can afford a good method of determining the death interval in animals along with other traditional methods and can be a potent source of information during post-mortem examinations and forensic analysis for wild animals also.

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