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Postmortem biochemical evaluation in vitrous humor of Indian rhinoceros (Rhinoceros unicornis)

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Abstract
The study of biochemical profile in cadaver/carcass to ascertain the etiology and death occurrence factors, is called thanatochemistry (Postmortem biochemistry).To ascertain the circumstances of death in the wild animals, the biochemical analysis of blood become difficult to interpret because of post-mortem changes in blood and secondly blood was not available. For such cases vitrous humor could be considered a good sample for biochemical analysis. Blood and synovial fluid from the dead Indian rhinoceros (Rhinoceros unicornis) were received for thanatochemistry. The concentration of urea, uric acid and creatinine was found alike the post-mortem blood concentration. These biochemicals in vitrous humor does not vary with the postmortem interval and could consider for assessing the antemortem levels and the corresponding postmortem blood value could be extrapolated.

Keywords: Thanatochemistry, antemortem, vitrous humor, creatinine, urea and uric acid

Introduction
The Indian rhinoceros (Rhinoceros unicornis) also called the Indian rhino, greater one-horned rhinoceros or great Indian rhinoceros. It is a rhinoceros species native to the Indian subcontinent and is listed as Vulnerable on the IUCN Red List. The Indian rhino has a thick grey-brown skin with pinkish skin folds and one horn on its snout. The greater one-horned rhino ("Indian rhino") is the largest of the rhino species. Found in India, Northern Pakistan, Nepal, Bangladesh, and mostly in Assam. Asian rhino horn can be sold for more than twice its weight in gold. The horn is used as a medicine and an aphrodisiac. Medicinal purposes are as a pain reliever and a fever suppressant. In addition to the horn, rhino hide, blood, urine, and dung also have economic value. Post-mortem biochemistry, also called thanatochemistry, has proved useful in forensics for estimating the time since death and assessing the cause of death (Boulagnon *et al.* 2011) [1]. VH is a biofluid that is an aqueous solution of proteins, carbohydrates, electrolytes, and other tiny molecules found in living beings. It is protected from bacterial contamination, autolysis, degradation, and metabolic reactions, making it a helpful tool for its isolated environment. In comparison to blood and other conventionally examined matrices, such as urine, VH is protected after death and is retained longer before deterioration. This is because of the blood-retinal barrier's presence and the minimal vascularization of VH. This bodily matrix seldom ever becomes contaminated by microorganisms. The rate of VH and blood circulation exchange of both organic endogenous small molecules slows down and ion levels drop in the early post-mortem period (Madea & Musshoff, 2007) [3]. The determination of the post-mortem interval (PMI), which might be useful in identifying the cause of death, is made possible by the post-mortem study of VH (Pigaian *et al*., 2020) [2]. The pre-analysis procedures that could affect the outcomes are one of the major analytical issues. Due to high viscosity of VH, there may be a variation between readings of the same electrolyte made with the same analyzer and VH (Blana *et al.*, 2011) [6]. The use of hyaluronidase is one of the primary techniques for decreasing VH viscosity (Garg *et al*., 2004) [7], heating (McNeil *et al*., 1999) [8], liquefying by ultrasonification (Thierauf *et al*., 2009) [5], centrifugation (Madea *et al*., 1989) [9], and dilution (Tagliaro *et al*., 1999) [10]

Materials and Method
Vitreous humor (VH) is a gel-like fluid obtained from the eye of the dead rhinoceros at the time of post-mortem examination at Patna Zoo. All test were done at the Department of Veterinary Biochemistry, Bihar Veterinary College, Patna. 14 gauge one-inch needle on a 3 cc syringe inserted at the dorsal aspect of the eye while angling the needle behind the lens.

VH specimen was stored at −20 °C until analysis. For the analysis of electrolytes, thawed samples was diluted 1:20 with barium solution (as internal standard, 0.02 mM) as per Bocaz-Beneventi *et al*. (2002) [4]. Different analytes (Sodium, potassium, calcium, phosphorus, chloride, urea and creatinine) was analysed as per Thierauf *et al.* (2009) [5]. Specimens were stored at −18 °C. 250- µL aliquots of the thawed sample were treated in the ultrasonic bath for 15 min at 20 °C or centrifuged for 8 min. Analysis of sodium and potassium was performed using an ion-selective electrode; calcium, phosphorus, chloride, urea, and creatinine were determined photometrically.

Result and Discussion

In the whole experiment we found that the concentration of creatinine was similar to the post-mortem blood concentration present in table. but Mitchell *et al*., in 1995 found an elevated level of creatinine values related to PMI. According to several reports by Takata *et al*., in 2015 [14] there is little link between vitreous creatinine content and PMI, and there are differences between natural and unnatural deaths in the post- mortem alteration of creatinine concentration. In 2015 Palmiere *et al.* statistically observed significant differences in creatinine levels between post-mortem serum and VH levels with lower concentrations in VH. These findings imply the diagnostic importance of elevated VH levels of creatinine. The concentration of urea and uric acid was also found to be similar as post-mortem blood concentration shown in table. Pigaiani *et al*. in 2020 [2], measured the levels of urea in post-mortem VH specimens demonstrating and found lower levels than serum. Ultimately, vitreous urea nitrogen is a useful indicator to evaluate the ante-mortem electrolyte imbalance in metabolic disorders that have not yet been characterised (Madea., 2005) [13]. Since both creatinine and urea concentration are quite constant in post-mortem VH collection during autopsy, urea nitrogen (based on measuring the amount of nitrogen) or urea concentration (now quantified by recent regular analytical process) have been determined together with creatinine.

Kidney Function Test

Parameters	Normal Range of Blood Serum (mg/dl)	Vitreous-Humor (mg/dl)
Urea	7.56-13.44	39.73
Creatinine	1.19-1.7	2.23
Calcium	10.5-13.4	4.55
Phosphorus	4.2-6.2	2.49

Electrolytes

Parameters	Normal Range of Blood Serum (mg/dl)	Vitreous-Humor (mg/dl)
Sodium	129-149	141
Potassium	4.3-5.9	4.69
Chloride	89-101	91

Conclusion

Although more research is needed, vitreous humour post-mortem investigation is a crucial tool for forensic purposes, especially for endogenous chemicals. The data and laboratory results may confirm or support the reasons of death and the passing of time. The concentration of urea, uric acid and creatinine of vitreous humour was found similar to the post-mortem blood concentration and these biochemicals in vitrous humor does not vary with the postmortem interval and could consider for assessing the antemortem levels and the corresponding postmortem blood value could be extrapolated.

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