

H185 The Use of High-Mobility Group Protein (HMGB1) to Determine Time Since Death: An Autopsy-Based Study

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Learning Overview: After attending this presentation, attendees will be informed regarding the importance of Enzyme Linked Immune-Sorbent Assay (ELISA) utility in the evaluation of time since death. The goal of this presentation is to update attendees regarding the use of HMGB1 as an easy, cheap, and reliable marker to determine time since death.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing a new method to determine time since death, which can be utilized even at the grass root level.

Determining time since death is one of the most important aspects of forensic work. Since time immemorial, time since death has been estimated using various techniques. The earlier standards included checking livor mortis, hypostasis, rigor mortis, signs of decomposition, forensic entomology, etc.¹ These standards are of a subjective nature and depend upon non-scientific aids such as gross observation for their recording. Since these were never accurate, the quest for better methods led to the development of postmortem biochemistry, especially in the vitreous humor.^{2,3} The major shortcoming of this method was that the results were applicable only for a short postmortem interval, merely a few hours.^{4,5}

A group of non-histone nuclear proteins with high electrophoretic mobility were discovered in 1973 and were termed High Mobility Group (HMG) proteins. These include three superfamilies, HMGB, HMGN, and HMGA. Out of these, HMGB1 is the most abundant as well as the most well-studied protein. HMGB1 is an important protein that plays a critical role as a DNA chaperone, chromosome guardian, autophagy sustainer, and protector from apoptotic cell death. HMGB1 is released by eukaryotic cells upon necrosis. This property has been made use of in estimating Postmortem Interval (PMI) in animal models using Wistar rats.^{6,7}

This presentation is part of an ongoing study on the determination of time since death using serial estimation of HMGB1 in blood, liver, spleen, and brain tissues harvested during routine medicolegal autopsy. The selection criteria for the cadavers is in-hospital death undergoing medicolegal autopsies, and those who do not have any condition that increases tissue necrosis/cell death. The blood and tissue samples are removed at the time of the autopsy and further subjected to extraction of serum and tissue supernatant (in phosphate-buffered saline) at regular time intervals of zero, three, six, and nine hours. The levels of HMGB1 in serum and tissue supernatant are being assessed using a commercially available ELISA kit. The initial results have shown positive correlation between the HMGB1 levels and time since death. The results of various tissues, such as liver, brain, spleen, and blood, and their correlation will be presented. The use of ELISA as the method for analysis makes it a cheap, rapid, and easily available procedure that can be utilized even in the busy mortuaries of developing countries as well as in the peripheral centers where facilities for advanced research and analysis are not available.

Reference(s):

1. Verica P., Janeska B., Gutevska A., Duma A. Post Mortem Cooling of the Body and Estimation of Time Since Death. *Soudni lekarstvi / casopis Sekce soudního lékařství Cs lekárske společnosti J Ev Purkyne*. 2007;52(4):50-6.
2. Siddamsetty A.K., Verma S.K., Kohli A., Puri D., Singh A. Estimation of Time Since Death From Electrolyte, Glucose and Calcium Analysis of Postmortem Vitreous Humour in Semi-Arid Climate. *Medicine, Science, and the Law*. 2014;54(3):158-66.
3. Bortolotti F., Pascali J.P., Davis G.G., Smith F.P., Brissie R.M., Tagliaro F. Study of Vitreous Potassium Correlation With Time Since Death in the Postmortem Range From 2 To 110 Hours Using Capillary Ion Analysis. *Medicine, Science, and the Law*. 2011;51 Suppl 1:S20-3.
4. Chandrakanth H.V., Kanchan T., Balaraj B.M., Virupaksha H.S., Chandrashekar T.N. Postmortem Vitreous Chemistry—An Evaluation of Sodium, Potassium and Chloride Levels in Estimation of Time Since Death (During the First 36 h After Death). *Journal of Forensic and Legal Medicine*. 2013;20(4):211-6.
5. Kanz F., Reiter C., Risser D.U. Citrate Content of Bone for Time Since Death Estimation: Results From Burials With Different Physical Characteristics and Known PMI. *Journal of Forensic Sciences*. 2014;59(3):613-20.
6. Kikuchi K., Kawahara K.I., Biswas K.K., Ito T., Tancharoen S., Shiomi N., et al. HMGB1: A New Marker for Estimation of the Postmortem Interval. *Experimental and Therapeutic Medicine*. 2010;1(1):109-11.
7. Kang R., Chen R., Zhang Q., Hou W., Wu S., Cao L., et al. HMGB1 in Health and Disease. *Molecular Aspects of Medicine*. 2014.

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