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A SYSTEMATIC REVIEW ON THE BIOCHEMISTRY OF THE VITREOUS HUMOR IN ESTIMATING THE POST-MORTEM INTERVAL

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ADMINISTRATIVE INFORMATION

Support - Not applicable.

Review Stage at time of this submission - The review has not yet started.

Conflicts of interest - None declared.

INPLASY registration number: INPLASY2025120042

Amendments - This protocol was registered with the International Platform of Registered Systematic Review and Meta-Analysis Protocols (INPLASY) on 11 December 2025 and was last updated on 11 December 2025.

INTRODUCTION

Review question / Objective 1. To what extent are vitreous humor biochemical markers reliable in estimating the PMI, and which specific marker demonstrates the strongest correlation with PMI across the literature?
2. What is the reported linear regression equation or standard error of the estimate (SEE) for vitreous humor potassium (K+) concentration, and how strong is its correlation coefficient (R) compared to other analytes (Na+, Cl–, Hx, etc.)?
3. What are the specific confounding factors consistently identified in the literature, and what is their reported impact on the accuracy and precision of PMI estimations using vitreous humor biochemistry?
4. What are the most common limitations and sources of error reported in the literature concerning the use of vitreous humor biochemistry for PMI estimation?

Rationale Discrepancies arise from methodological differences in sampling, storage, analytical techniques, population demographics, and environmental conditions. Moreover, comorbidities such as diabetes, renal impairment, and premortem hypoxia can substantially alter vitreous biochemistry, reducing the universal reliability of biochemical models. There is also a lack of standardized protocols regarding the timing of sampling, preservation methods, and analytical calibration. While potassium remains the most widely used parameter, its variability across studies underscores the need for critical appraisal and consolidation of the evidence base.

The literature demonstrates the promise of vitreous humor biochemistry in PMI estimation, but also reveals methodological inconsistencies and conflicting findings. To date, no universally accepted model or guideline has been established for forensic practitioners. A systematic review is thus essential to critically synthesize existing evidence, evaluate the reliability of individual and

combined biomarkers, and identify gaps requiring further investigation. By doing so, this review will provide an evidence-based framework to strengthen forensic practice and guide future research into standardized biochemical approaches for PMI estimation.

Condition being studied Population (P): Human subjects undergoing postmortem laboratory investigation.
Intervention (I): Quantitative analysis of biochemical markers in the vitreous humor
Comparison (C): Not applicable
Outcome (O): Measures of association and predictive value for PMI estimation.

We will include original online research studies comprising observational, experimental, case-control, cohort, and forensic case series, that report the quantitative analysis of vitreous biochemical markers for PMI estimation. Only human studies (adult and pediatric) will be considered.

METHODS

Search strategy A highly comprehensive systematic search will be executed across four major electronic databases: PubMed, Scopus, Web of Science (WoS) and Embase. The search will integrate controlled vocabulary (e.g., MeSH terms for 'Vitreous Body' and 'Forensic Pathology') and free-text keywords using Boolean operators to maximize the retrieval sensitivity.
• Primary keywords: “vitreous humor”, “vitreous fluid”, “ocular fluid”, “biochemistry”, “post-mortem interval”, “forensic”, “potassium”, “hypoxanthine”, “PMI estimation”, “PMI”, “time since death”.

Comprehensive search strategy employed across major electronic databases :

PubMed : (("Vitreous Body"[MeSH] OR "vitreous humor" OR "ocular fluid") AND ("Postmortem Interval"[MeSH] OR "PMI" OR "time since death") AND ("potassium" OR "hypoxanthine" OR “biochemistry"))

Scopus : (TITLE-ABS-KEY ("vitreous humor" OR "vitreous fluid" OR "ocular fluid") AND TITLE-ABS-KEY ("post-mortem interval" OR PMI OR "time since death") AND TITLE-ABS-KEY (potassium OR hypoxanthine OR biochemistry OR metabolite*))

WoS : TS=("vitreous humor" OR "vitreous fluid" OR "ocular fluid") AND TS=("post-mortem interval" OR PMI OR "time since death") AND TS=(potassium OR hypoxanthine OR biochemistry OR "body fluid chemistry”)

Embase : ('vitreous body'/exp OR 'vitreous humor' OR 'ocular fluid' OR 'vitreous fluid') AND ('postmortem interval'/exp OR 'post-mortem interval' OR PMI OR 'time since death') AND (potassium OR hypoxanthine OR biochemistry OR 'body fluid analysis’/exp)

Google Scholar : "vitreous humor" OR "ocular fluid" AND "post-mortem interval" OR PMI OR "time since death" AND potassium OR hypoxanthine OR biochemistry

To mitigate publication bias, a targeted search will also be conducted for grey literature. This includes searching institutional repositories such as Google Scholar for the first ten pages, looking for relevant theses, dissertations, technical reports, and conference abstracts. We will also conduct backward citation chaining and forward citation chaining. The search will not be restricted by publication date or language during the initial screening.

Participant or population Human subjects undergoing postmortem laboratory investigation.

Intervention Quantitative analysis of biochemical markers in the vitreous humor.

Comparator Not applicable.

Study designs to be included This systematic review will be conducted following the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement to ensure transparency and prevent duplication, the completed protocol will be registered in an international repository (e.g., PROSPERO) prior to the commencement of the literature search.

Eligibility criteria Inclusion Criteria:
We will include original online research studies comprising observational, experimental, case-control, cohort, and forensic case series, that report the quantitative analysis of vitreous biochemical markers for PMI estimation. Only human studies (adult and pediatric) will be considered.
Exclusion Criteria:
Studies will be excluded if they are reviews, editorials, or conference abstracts lacking primary data, if they report biochemical analysis but do not correlate the findings with PMI, or if they are animal studies.

Information sources A highly comprehensive systematic search will be executed across four major electronic databases: PubMed, Scopus, Web of Science (WoS) and Embase. The search will integrate controlled vocabulary (e.g., MeSH terms for 'Vitreous Body' and 'Forensic Pathology') and free-text keywords using Boolean operators to maximize the retrieval sensitivity.

To mitigate publication bias, a targeted search will also be conducted for grey literature. This includes searching institutional repositories such as Google Scholar for the first ten pages, looking for relevant theses, dissertations, technical reports, and conference abstracts. We will also conduct backward citation chaining and forward citation chaining. The search will not be restricted by publication date or language during the initial screening.

Main outcome(s) Measures of association and predictive value for PMI estimation.

Additional outcome(s) Not applicable.

Data management Data will be extracted by two independent reviewers using a standardized, pre-piloted data extraction sheet. Key data points will include:
• Study characteristics: author, year, country, sample size, and specific study design.
• Biochemical markers and methods: markers analyzed, analytical methods used, and sample handling protocols.
• Quantitative results: reported correlation coefficient R, accuracy, sensitivity, specificity, and regression models with the associated Standard Error of the Estimate (SEE).
• Confounding factors: factors considered and their reported impact (e.g., pre-existing conditions, temperature, cause of death).
• Methodological limitations: limitations and sources of error reported by the authors.

Quality assessment / Risk of bias analysis The methodological quality and risk of bias will be independently assessed by two reviewers using the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) tool. This tool is the standard for evaluating the quality of studies where an index test is used to predict a reference standard This framework is more appropriate for predictive correlation studies than generic observational tools.
The risk of bias assessment will be structured around the four core domains of QUADAS-2:
1. Patient selection: Assessing bias concerning the sampling methods, spectrum of subjects (e.g., range of PMIs, cause of death), and whether the reference standard (true PMI) was definitively known or only estimated.
2. Index test (vitreous humor analysis): Assessing bias concerning the analyte measurement, including adherence to a standardized analytical protocol and whether the assay was performed and interpreted blinded to the reference standard result.
3. Reference standard: Assessing bias concerning the gold standard PMI determination and whether it was interpreted blinded to the index test result.
4. Flow and timing: Assessing bias concerning the study's conduct, specifically the time lag between death, vitreous sampling, analysis, and the potential impact of pre-analytical factors.

Studies will be judged within each domain as being of Low Risk, High Risk, or Unclear Risk of bias based on the following criteria:
• Low Risk: The study satisfies the criteria for all key domains, with methods designed to minimize bias in patient selection, index testing, reference standard, and flow/timing.
• High Risk: The study fails to satisfy key criteria or has significant methodological flaws in one or more domains
• Unclear Risk: There is insufficient information reported in the study to permit a reliable judgment on the risk of bias for a specific domain.

Strategy of data synthesis Due to anticipated heterogeneity in the analytical methods, regression equations, and reporting of confounding variables, a blended approach will be used for data synthesis.

The narrative synthesis will focus on the qualitative summary to describe the range of biochemical markers studied, the general trends observed, the nature of confounding factors identified, and the common limitations and sources of error reported in the literature, addressing specific objectives 2 and 3.

If sufficient data are available and deemed statistically homogenous a meta-analysis will be performed to address specific objective 1. Quantitative pooling will focus on standardized, comparable metrics, such as the correlation coefficient (R) for the most frequently studied analytes and measures of diagnostic accuracy such as sensitivity and specificity. A random-effects model will then be utilized to account for clinical and methodological heterogeneity. Statistical heterogeneity will be assessed using the I2 statistic. If the I2 value is high (> 75%), the quantitative pooling will be deemed unreliable, and

the findings will be presented solely through an advanced narrative synthesis, focusing on the reasons for the wide variation.

Subgroup analysis Not applicable.

Sensitivity analysis Not applicable.

Language restriction The search will not be restricted by publication language during the initial screening.

Country(ies) involved Malaysia.

Other relevant information None

Keywords “vitreous humor”, “vitreous fluid”, “ocular fluid”, “biochemistry”, “post-mortem interval”, “forensic”, “potassium”, “hypoxanthine”, “PMI estimation”, “PMI”, “time since death”.

Contributions of each author

Author 1 - Nur Sabrina A Samad - Author 1 drafted the research proposal/ protocol.
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