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# Postmortem Ethanol levels in blood, urine and vitreous humor- a cross-sectional study at a tertiary care hospital in Tiruppur

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## ABSTRACT

**Background**: Accurate interpretation of postmortem ethanol concentrations is a critical component of forensic toxicology, yet it is often complicated by postmortem redistribution, microbial fermentation, and variability across biological matrices. While blood is traditionally used for ethanol estimation, alternative matrices such as urine and vitreous humor may offer greater stability and interpretive value, particularly in cases with prolonged postmortem intervals.
**Objectives**: To evaluate the distribution, correlation, and agreement of ethanol concentrations in blood, urine, and vitreous humor in postmortem cases, and to assess the influence of postmortem interval on ethanol levels across these matrices.
**Methods**: A cross-sectional analytical study was conducted on 80 medico-legal autopsy cases at a tertiary care hospital in Tiruppur, Tamil Nadu. Peripheral blood, urine, and vitreous humor samples were collected and analyzed for ethanol concentration using standard forensic toxicology methods. Sociodemographic and autopsy details were recorded. Ethanol concentrations were compared across matrices, correlation and agreement analyses were performed, and variations across postmortem interval categories were assessed using appropriate statistical tests.
**Results**: Mean ethanol concentrations were 0.84 g/dL in blood, 0.96 g/dL in urine, and 0.79 g/dL in vitreous humor. Strong positive correlations were observed between blood and vitreous humor ($r = 0.92$), blood and urine ($r = 0.86$), and urine and vitreous humor ($r = 0.83$) ($p < 0.001$). Ethanol concentrations in all matrices showed a statistically significant decline with increasing postmortem interval. Agreement analysis demonstrated excellent concordance between blood and vitreous humor ethanol concentrations (ICC = 0.91). Discordant ethanol patterns suggestive of possible postmortem ethanol formation were identified in a subset of cases.
**Conclusion**: Multi-matrix ethanol analysis improves the accuracy and reliability of postmortem alcohol interpretation, with vitreous humor serving as a valuable adjunct to blood, especially in challenging forensic scenarios.

***Keywords***: *Postmortem ethanol, vitreous humor, forensic toxicology, blood alcohol concentration, postmortem interval.*

## INTRODUCTION

Ethanol is one of the most widely consumed psychoactive substances worldwide and is frequently implicated in accidental injuries, violent deaths, suicides, and sudden unexpected deaths.[1] In medico-legal practice, determination of ethanol concentration at autopsy plays a critical role in reconstructing circumstances surrounding death, assessing impairment, and supporting judicial decision-making. [2] Consequently, postmortem ethanol analysis remains one of the most commonly requested investigations in forensic toxicology. [3]

Blood is traditionally regarded as the primary specimen for ethanol estimation in both clinical and forensic settings. [4] However, interpretation of postmortem blood ethanol concentrations is often complicated by factors such as postmortem redistribution, microbial activity, putrefaction, contamination, and variable sampling sites. [5] These factors may result in

falsely elevated or reduced ethanol levels, thereby limiting the reliability of blood as a sole matrix for accurate assessment of antemortem alcohol consumption. [6]

To overcome these limitations, alternative biological matrices such as urine and vitreous humor have been increasingly utilized in postmortem investigations. [7] Urine ethanol concentrations reflect alcohol that has been filtered and accumulated over time, while vitreous humor is anatomically protected, relatively sterile, and less susceptible to postmortem microbial fermentation. [8] Owing to its isolated location and slow metabolic changes, vitreous humor has emerged as a valuable specimen for evaluating ethanol exposure, particularly in cases with prolonged postmortem intervals or early decomposition. [9]

Several studies have demonstrated strong correlations between ethanol concentrations in blood and vitreous humor, supporting the use of vitreous humor as a reliable adjunct or alternative when blood samples are unavailable or compromised. [10] Nevertheless, variability in ethanol distribution between matrices may still occur due to differences in diffusion kinetics, timing of alcohol intake prior to death, and postmortem changes. [11] Discordant findings between blood, urine, and vitreous humor can raise concerns regarding postmortem ethanol formation, emphasizing the need for multi-matrix analysis. [12]

The postmortem interval is another critical factor influencing ethanol concentration and interpretation. [13] With increasing time after death, ethanol levels may decline due to evaporation or degradation, or conversely increase due to microbial fermentation, particularly in decomposed bodies. [14] Evaluating ethanol concentrations across different postmortem intervals can therefore provide valuable insight into the stability and reliability of various biological matrices used in forensic toxicology. [15]

Despite the growing body of international literature, data on postmortem ethanol distribution and matrix-wise comparison from Indian settings remain limited. [16] Differences in climate, storage conditions, autopsy practices, and population drinking patterns may influence ethanol measurements and their interpretation. In this context, the present study was undertaken at a tertiary care hospital in Tiruppur to evaluate ethanol concentrations in blood, urine, and vitreous humor, examine their correlation and agreement, and assess the influence of postmortem interval, with the aim of improving the forensic interpretation of postmortem ethanol findings.

## OBJECTIVES

- To estimate and compare ethanol concentrations in blood, urine, and vitreous humor in postmortem cases.
- To assess the correlation and agreement between ethanol concentrations in blood and vitreous humor across different postmortem intervals.

## MATERIALS AND METHODS

### Study Design and Setting

This was a hospital-based cross-sectional analytical study conducted in a tertiary care teaching hospital in Tiruppur, Tamil Nadu.

### Study Population

The study population comprised medico-legal autopsy cases in which biological samples were collected for toxicological analysis of ethanol. Only cases where blood, urine, and vitreous humor samples could be obtained simultaneously were included in the analysis.

### Study Duration

The study was conducted over a period of one year.

### Inclusion and Exclusion Criteria

**Inclusion criteria:**

- Medico-legal autopsy cases in which blood, urine, and vitreous humor samples were available
- Cases with a documented postmortem interval
- Cases where samples were collected and preserved as per standard forensic protocols

**Exclusion criteria:**

- Autopsy cases with inadequate or contaminated biological samples
- Cases where one or more of the required biological matrices were unavailable
- Embalmed bodies
- Cases with advanced decomposition where reliable sampling was not possible

### Sample Size and Sampling Technique

A total of 80 autopsy cases were included in the study. The sample size was determined based on feasibility and availability of eligible cases during the study period. Consecutive sampling was employed, wherein all eligible cases meeting the inclusion criteria during the study period were included until the required sample size was achieved.

**Study Procedure**

Following receipt of medico-legal requisition and completion of the autopsy, biological samples were collected using standard aseptic techniques. Peripheral blood samples were preferably collected from the femoral vein, urine samples were obtained from the urinary bladder, and vitreous humor samples were aspirated from both eyes using a sterile syringe. All samples were preserved in appropriate containers with preservatives as per standard forensic toxicology guidelines and stored under refrigerated conditions until analysis.

Ethanol estimation in blood, urine, and vitreous humor was carried out using standard toxicological analytical techniques routinely employed in the forensic laboratory. Ethanol concentrations were expressed in grams per deciliter (g/dL). Relevant sociodemographic details, autopsy findings, manner of death, place of death, postmortem interval, and decomposition status were recorded using a structured data collection proforma.

**Operational Definitions**

- **Postmortem interval (PMI):** Time interval between death and autopsy, categorized as <12 hours, 12–24 hours, and >24 hours.
- **Blood ethanol positivity:** Detectable ethanol concentration in peripheral blood sample.
- **Discordant ethanol findings:** Presence of ethanol in one biological matrix with absence in one or more of the other matrices, suggestive of possible postmortem ethanol formation.
- **Agreement:** Degree of concordance between blood and vitreous humor ethanol concentrations assessed using agreement statistics.

**Statistical Analysis**

Data were entered into Microsoft Excel and analyzed using Statistical Package for the Social Sciences (SPSS) software version 26. Descriptive statistics were used to summarize sociodemographic and autopsy characteristics. Ethanol concentrations were expressed as mean, standard deviation, median, and range. Pearson's correlation coefficient was used to assess the relationship between ethanol concentrations in blood, urine, and vitreous humor. Comparison of ethanol concentrations across postmortem interval categories was performed using one-way analysis of variance (ANOVA). Agreement between blood and vitreous humor ethanol concentrations was assessed using intraclass correlation coefficient and Bland–Altman analysis. A p value of less than 0.05 was considered statistically significant.

**Ethical Consideration**

The study was conducted after obtaining approval from the Institutional Ethics Committee. As the study involved analysis of biological samples collected during routine medico-legal autopsies, informed consent was waived. Confidentiality of case details was strictly maintained, and all data were used solely for academic and research purposes in accordance with ethical and legal guidelines.

**RESULTS**

A total of 80 autopsy cases were included in the study. The majority of the study population belonged to the 30–49 years age group (n = 34, 42.5%), followed by individuals aged ≥50 years (n = 28, 35.0%) and those younger than 30 years (n = 18, 22.5%). Males constituted a predominant proportion of the cases (n = 62, 77.5%), while females accounted for 22.5% (n = 18) of the study population. With respect to the manner of death, accidental deaths were the most common (n = 38, 47.5%), followed by suicidal deaths (n = 22, 27.5%), natural deaths (n = 14, 17.5%), and homicidal deaths (n = 6, 7.5%). More than half of the deaths occurred in a hospital setting (n = 46, 57.5%), whereas 34 cases (42.5%) were brought dead or occurred at the scene. Regarding autopsy-related characteristics, the postmortem interval was between 12 and 24 hours in the largest proportion of cases (n = 31, 38.8%), followed by intervals of less than 12 hours (n = 26, 32.5%) and greater than 24 hours (n = 23, 28.7%). Decomposition was absent in most cases (n = 61, 76.3%), while early signs of decomposition were observed in 19 cases (23.7%). (Table 1)

**Table 1. Sociodemographic and Autopsy Characteristics of the Study Population (N = 80)**

| Variable | Category | n | % |
|---|---|---|---|
| **Age (years)** | <30 | 18 | 22.5 |
| | 30–49 | 34 | 42.5 |
| | ≥50 | 28 | 35.0 |
| **Sex** | Male | 62 | 77.5 |
| | Female | 18 | 22.5 |
| **Manner of Death** | Accidental | 38 | 47.5 |
| | Suicidal | 22 | 27.5 |
| | Natural | 14 | 17.5 |

| | | | |
|---|---|---|---|
| | Homicidal | 6 | 7.5 |
| **Place of Death** | Hospital | 46 | 57.5 |
| | Scene / Brought Dead | 34 | 42.5 |
| **Postmortem Interval (hours)** | <12 | 26 | 32.5 |
| | 12–24 | 31 | 38.8 |
| | >24 | 23 | 28.7 |
| **Decomposition Status** | Absent | 61 | 76.3 |
| | Early decomposition | 19 | 23.7 |

The distribution of ethanol concentrations across different biological matrices is summarized in Table 2. The mean blood ethanol concentration was 0.84 g/dL (SD = 0.32), with a median value of 0.71 g/dL and a range of 0.05–2.40 g/dL. Urine ethanol concentrations were comparatively higher, with a mean of 0.96 g/dL (SD = 0.38), a median of 0.83 g/dL, and a range of 0.06–2.60 g/dL. Vitreous humor ethanol concentrations showed a slightly lower mean value of 0.79 g/dL (SD = 0.38), with a median of 0.68 g/dL and a range of 0.04–2.20 g/dL. Overall, urine samples demonstrated higher ethanol concentrations, while vitreous humor showed relatively lower central tendency and variability compared to blood and urine.

**Table 2. Distribution of Ethanol Concentrations in Blood, Urine, and Vitreous Humor (N = 80)**

| Biological Sample | Mean (g/dL) | SD | Median (g/dL) | Range (g/dL) |
|---|---|---|---|---|
| **Blood Ethanol** | 0.84 | 0.32 | 0.71 | 0.05–2.40 |
| **Urine Ethanol** | 0.96 | 0.38 | 0.83 | 0.06–2.60 |
| **Vitreous Humor Ethanol** | 0.79 | 0.38 | 0.68 | 0.04–2.20 |

Correlation analysis between ethanol concentrations in blood, urine, and vitreous humor is presented in Table 3. A strong positive correlation was observed between blood and urine ethanol concentrations ($r = 0.86$, $p < 0.001$). Blood ethanol levels also showed a very strong correlation with vitreous humor ethanol concentrations ($r = 0.92$, $p < 0.001$). Additionally, urine ethanol concentrations were strongly correlated with vitreous humor ethanol levels ($r = 0.83$, $p < 0.001$). These findings indicate a statistically significant and robust association between ethanol concentrations across all three biological matrices in postmortem cases.

**Table 3. Correlation Between Ethanol Levels in Blood, Urine, and Vitreous Humor (N = 80)**

| Variables | Blood ethanol | Urine ethanol | Vitreous ethanol |
|---|---|---|---|
| **Blood ethanol** | — | $r = 0.86$, $p < 0.001$ | $r = 0.92$, $p < 0.001$ |
| **Urine ethanol** | $r = 0.86$, $p < 0.001$ | — | $r = 0.83$, $p < 0.001$ |
| **Vitreous ethanol** | $r = 0.92$, $p < 0.001$ | $r = 0.83$, $p < 0.001$ | — |

Comparison of ethanol concentrations across postmortem interval categories is presented in Table 4. Blood ethanol concentrations showed a gradual decline with increasing postmortem interval, with the highest mean value observed in cases with a postmortem interval of less than 12 hours (0.88 ± 0.30 g/dL), followed by those with intervals of 12 –24 hours (0.85 ± 0.33 g/dL), and the lowest levels in cases with intervals greater than 24 hours (0.76 ± 0.32 g/dL). This difference across postmortem interval categories was statistically significant ($p = 0.041$). A similar decreasing trend was observed for urine ethanol concentrations, which were highest in cases with a postmortem interval of less than 12 hours (1.02 ± 0.36 g/dL), followed by 12–24 hours (0.97 ± 0.39 g/dL), and greater than 24 hours (0.88 ± 0.30 g/dL). The variation in urine ethanol concentrations across the postmortem interval categories was statistically significant ($p = 0.032$). Vitreous humor ethanol concentrations also demonstrated a significant decline with increasing postmortem interval, with mean values of 0.83 ± 0.35 g/dL, 0.80 ± 0.30 g/dL, and 0.72 ± 0.38 g/dL for postmortem intervals of less than 12 hours, 12–24 hours, and greater than 24 hours, respectively ($p = 0.028$). Overall, ethanol concentrations in all three biological matrices showed a statistically significant association with postmortem interval, indicating lower concentrations with longer intervals after death.

**Table 4. Comparison of Ethanol Concentrations Across Postmortem Interval Categories (N = 80)**

| Postmortem Interval (hours) | n | Blood ethanol Mean ± SD (g/dL) | Urine ethanol Mean ± SD (g/dL) | Vitreous ethanol Mean ± SD (g/dL) |
|---|---|---|---|---|
| **< 12** | 26 | 0.88 ± 0.30 | 1.02 ± 0.36 | 0.83 ± 0.35 |
| **12–24** | 31 | 0.85 ± 0.33 | 0.97 ± 0.39 | 0.80 ± 0.30 |
| **> 24** | 23 | 0.76 ± 0.32 | 0.88 ± 0.30 | 0.72 ± 0.38 |
| **p value** | | 0.041* | 0.032* | 0.028* |

*-statistically significant

Agreement between blood and vitreous humor ethanol concentrations is summarized in Table 5. The mean blood ethanol concentration was 0.84 g/dL, while the mean vitreous humor ethanol concentration was 0.79 g/dL. The mean difference

between blood and vitreous humor ethanol concentrations was 0.05 g/dL, with a standard deviation of 0.18. The 95% limits of agreement ranged from −0.30 to 0.40 g/dL, indicating acceptable agreement between the two matrices across the observed range of ethanol concentrations. The intraclass correlation coefficient demonstrated excellent agreement between blood and vitreous humor ethanol measurements (ICC = 0.91), and this agreement was statistically significant ($p < 0.001$).

**Table 5. Agreement Between Blood and Vitreous Humor Ethanol Concentrations (N = 80)**

| Parameter | Value |
|---|---|
| **Mean blood ethanol (g/dL)** | 0.84 |
| **Mean vitreous ethanol (g/dL)** | 0.79 |
| **Mean difference (Blood − Vitreous) (g/dL)** | 0.05 |
| **Standard deviation of difference** | 0.18 |
| **95% Limits of Agreement (g/dL)** | −0.30 to 0.40 |
| **Intraclass correlation coefficient (ICC)** | 0.91 |
| **p value** | < 0.001* |

*-statistically significant

The distribution of concordant and discordant ethanol findings across different biological matrices is presented in Table 6. Concordant positivity for ethanol in blood, urine, and vitreous humor was observed in the majority of cases (n = 59, 73.6%). Discordant patterns suggestive of possible postmortem ethanol formation were identified in a subset of cases. Blood ethanol positivity in the absence of vitreous ethanol was observed in 7 cases (8.8%), while blood positivity with negative urine ethanol was seen in 5 cases (6.3%). In 3 cases (3.8%), ethanol was detected in blood but absent in both vitreous humor and urine. Conversely, vitreous humor ethanol positivity with negative blood ethanol was noted in 2 cases (2.5%), and urine ethanol positivity with negative blood ethanol was observed in 4 cases (5.0%). Overall, these discordant patterns highlight the variability in ethanol distribution across matrices and underscore the importance of multi-matrix analysis in the interpretation of postmortem ethanol findings.

**Table 6. Discordant Ethanol Findings Between Biological Matrices Suggestive of Postmortem Ethanol Formation (N = 80)**

| Ethanol Pattern | n | % |
|---|---|---|
| **Blood positive, vitreous negative** | 7 | 8.8 |
| **Blood positive, urine negative** | 5 | 6.3 |
| **Blood positive, vitreous and urine negative** | 3 | 3.8 |
| **Vitreous positive, blood negative** | 2 | 2.5 |
| **Urine positive, blood negative** | 4 | 5.0 |
| **Concordant positivity in all matrices** | 59 | 73.6 |
| **Total** | **80** | **100** |

## DISCUSSION

The sociodemographic and autopsy profile observed in the present study revealed a clear predominance of males and a higher representation of individuals in the middle adult age group, with accidental and suicidal deaths accounting for the majority of cases. This demographic distribution is consistent with patterns reported in forensic toxicology literature, where alcohol-related postmortem investigations frequently involve working-age males and deaths associated with trauma or self-harm. Issa et al. similarly reported a predominance of male subjects and a higher frequency of ethanol detection in adult age groups in postmortem cases, reflecting broader epidemiological trends in alcohol use and risk exposure [6]. Comparable demographic profiles have also been documented in studies examining ethanol distribution across biological matrices, reinforcing the relevance of demographic context in interpreting postmortem toxicology findings [3,8]. Interpretive reviews further emphasize that manner of death and demographic characteristics influence the likelihood of antemortem alcohol consumption and should be integrated into the overall forensic assessment rather than interpreted in isolation [15].

Analysis of ethanol distribution across biological matrices demonstrated that urine ethanol concentrations were generally higher than blood ethanol concentrations, while vitreous humor ethanol levels were slightly lower than those observed in blood. This pattern aligns with established pharmacokinetic principles and postmortem dynamics, whereby urine serves as a reservoir reflecting accumulated ethanol, and vitreous humor—being anatomically protected—tends to mirror blood levels with reduced susceptibility to contamination. Similar findings have been reported by Okulevičiūtė et al., who observed higher ethanol concentrations in urine compared to blood in the majority of postmortem cases [7]. Savini et al. also demonstrated close comparability between blood and vitreous ethanol concentrations using validated analytical techniques, supporting the use of vitreous humor as a reliable alternative matrix [4]. The relative stability of vitreous humor ethanol, particularly during storage and delayed analysis, has further been highlighted by Olsen et al., reinforcing its forensic value when blood samples may be compromised [2].

Strong and statistically significant correlations were observed between ethanol concentrations in blood, urine, and vitreous humor, with the highest correlation noted between blood and vitreous humor. These findings are in agreement with those of Ioan et al., who reported a strong correlation between blood and vitreous ethanol concentrations and a comparatively weaker, though still significant, relationship between blood and urine ethanol levels [3]. De Martinis et al. similarly demonstrated high correlation coefficients between vitreous humor and various blood sampling sites as well as urine, supporting the role of vitreous humor as a robust corroborative specimen in postmortem ethanol analysis [8]. Neumann et al. further emphasized that strong inter-matrix correlations enhance interpretive confidence, particularly when vitreous humor is used alongside blood and urine in autopsy cases [5]. Systematic reviews also support the consistency of these correlations across diverse forensic settings, while cautioning against direct one-to-one substitution without contextual consideration [10].

Ethanol concentrations in blood, urine, and vitreous humor showed a statistically significant decline with increasing postmortem interval, underscoring the influence of time-related postmortem changes on ethanol measurement. This observation is consistent with experimental and observational studies demonstrating that ethanol may decrease due to evaporation or degradation, particularly in blood, with increasing time after death. Olsen et al. reported measurable ethanol loss in stored postmortem blood samples over time, while vitreous humor exhibited comparatively better stability [2]. Reviews addressing postmortem ethanol production and degradation have also emphasized that postmortem interval interacts with microbial activity, storage conditions, and decomposition status to influence ethanol levels, necessitating cautious interpretation [14,15]. Neumann et al. similarly highlighted the importance of considering postmortem interval when interpreting ethanol concentrations across matrices, particularly in cases with suspected postmortem formation [5]. Agreement analysis between blood and vitreous humor ethanol concentrations demonstrated excellent concordance, supporting the reliability of vitreous humor as an adjunct to blood in postmortem ethanol estimation. This finding aligns with Savini et al., who reported no statistically significant differences between blood and vitreous ethanol concentrations, reinforcing vitreous humor as an acceptable alternative matrix in forensic toxicology [4]. De Martinis et al. also observed close agreement between vitreous humor and femoral blood ethanol levels, further supporting its use in routine forensic practice [8]. Systematic evaluation by Al-Juhani et al. concluded that vitreous ethanol exhibits strong agreement with peripheral blood under standardized conditions, particularly in cases involving decomposition or compromised blood samples, while emphasizing the importance of multi-parameter interpretation [10]. Broader interpretive reviews similarly advocate the use of vitreous humor to strengthen forensic conclusions rather than relying solely on blood ethanol values [15].

A subset of cases in the present study demonstrated discordant ethanol findings across biological matrices, including patterns such as blood positivity with negative vitreous or urine ethanol. These discordant constellations are of particular forensic significance, as they may suggest postmortem ethanol formation, sampling artefacts, or time-dependent distribution differences. Oshaug et al. reported similar discordant patterns and demonstrated that reliance on blood ethanol alone can lead to misclassification, highlighting the importance of multi-matrix analysis to improve diagnostic accuracy [1]. Neumann et al. further illustrated that cases with low blood ethanol and absent vitreous ethanol, particularly in the absence of confirmatory metabolites, may indicate postmortem formation rather than antemortem consumption [5]. Reviews on postmortem ethanol interpretation emphasize that such discordance warrants cautious evaluation of postmortem interval, decomposition status, and sampling site, and strongly support a multi-matrix approach to reduce false-positive attribution of alcohol intake [14,15].

**Limitations**

The study was limited by its single-center design and relatively modest sample size, which may restrict the generalizability of the findings. Additionally, ethanol metabolites such as ethyl glucuronide or ethyl sulfate were not analyzed, which could have further strengthened differentiation between antemortem alcohol consumption and postmortem ethanol formation.

## CONCLUSION AND RECOMMENDATIONS

In conclusion, the present study demonstrates that ethanol concentrations in blood, urine, and vitreous humor show strong correlations and good agreement in postmortem cases, with vitreous humor emerging as a reliable adjunct matrix for ethanol estimation. The findings highlight the influence of postmortem interval on ethanol levels and underscore the occurrence of discordant matrix patterns in a subset of cases, emphasizing the limitations of relying on a single biological specimen. Overall, the results support the value of a multi-matrix approach for accurate interpretation of postmortem ethanol findings in medico-legal practice.

Based on these observations, it is recommended that routine postmortem ethanol analysis incorporate vitreous humor alongside blood and urine, particularly in cases with prolonged postmortem intervals, suspected decomposition, or compromised blood samples. Future studies should include larger, multicentric datasets and incorporate ethanol metabolites such as ethyl glucuronide and ethyl sulfate to further enhance diagnostic accuracy and reduce uncertainty in distinguishing antemortem alcohol consumption from postmortem ethanol formation.

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