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Forensic Investigation of Drowning, Biochemical Markers and Diatom Testing for Confirming Cause of Death. A cross-sectional study

Ume Kalsoom[1], Asiya Fazal[2], Farhat Sultana[3], Wasiq Ahmed[4], Mansoora Mirza[5], Muhammad Anwar Sibtain Fazli[6]

[1] Lecturer, Forensic Science Department, IMBB, The University of Lahore, ume.kalsoom@imbb.uol.edu.pk.

[2] Senior Demonstrator, Forensic Medicine and Toxicology, Ameer-ud-Din Medical College / PGMI, asiyafazal72@gmail.com.

[3] Associate Professor, Allama Iqbal Medical College, Lahore, farhatzafar42@gmail.com.

[4] Assistant Professor, Karachi Medical & Dental College, wasiqahmed270@gmail.com.

[5] Associate Professor, Forensic Medicine, Services Institute of Medical Sciences (SIMS), Lahore, dr.mansooramirza7@gmail.com.

[6] Associate Professor, Forensic Medicine and Toxicology, Avicenna Medical and Dental College, Lahore, sibtainfazli62@gmail.com.

Abstract

A cross-sectional forensic study evaluated the utility of biochemical markers (serum surfactant D, NT-proBNP, D-dimer) and diatom quantification for confirming drowning as the cause of death. Eighty postmortem cases were investigated (drowning $n = 50$; non-drowning water exposure $n = 30$). Serum surfactant D levels were significantly elevated in drowning cases (mean ± SD: 15.2 ± 4.1 µg/mL) compared to controls (7.8 ± 2.9 µg/mL; $p < 0.001$). Similarly, NT-proBNP and D-dimer were higher in drownings ($p < 0.01$). Diatom testing from bone marrow detected ≥20 diatoms/100 µL in 88% of true drowning cases, versus 17% in controls ($p < 0.001$). A combined diagnostic algorithm using surfactant D and diatom number yielded sensitivity of 94%, specificity of 90%, and accuracy of 92%. Findings support the integration of biochemical and diatom analysis in forensic protocols to enhance confidence in drowning diagnosis, especially in ambiguous circumstances.

Introduction

Drowning, defined as respiratory impairment due to submersion, remains a forensic challenge due to non-specific autopsy findings1-3. Traditional signs—frothy fluid, water in lungs, pulmonary edema—lose diagnostic value postmortem or after prolonged submersion[13]. Diatom testing, tracing ubiquity of these microscopic algae across aquatic habitats, has served as support for drowning diagnosis by detecting aspirated microorganisms in distant organs 4-5.

Controversy persists due to potential for ante- and postmortem contamination that lowers diagnostic certainty in isolation. A recent systematic review highlighted variability in diatom concentrations and methodology, urging standardization in protocols and caution in interpretation6-7.

Biochemical markers, like surfactant D, NT-proBNP, and D-dimer, reflect pulmonary tissue injury and hypoxia in drowning. Emerging evidence from controlled animal and human studies (post-2022) supports their diagnostic relevance.8-10

This cross-sectional study investigates the combined diagnostic accuracy of serum biochemical markers and diatom quantification in cadavers recovered from water, hypothesizing that a multimodal approach enhances specificity and sensitivity in differentiating true drowning from postmortem immersion.

Methodology

Between January and December 2024, eighty cadavers at Mayo Hospital Lahore recovered from aquatic environments were prospectively evaluated. Inclusion criteria involved unknown deaths discovered in water; exclusion criteria included decomposition beyond 72 hours, systemic disease, or direct head trauma. During standard autopsy, serum samples were collected for surfactant D, NT-proBNP, and D-dimer analysis. Bone marrow was harvested from femoral cavity for diatom testing using microwave digestion-vacuum filtration and automated SEM quantification[2079]. For each case, scene investigation determined group allocation: drowning (presence of submersion findings consistent with proximate death) versus control (non-fatal immersion or head-first disposal postmortem). Serum cut-off values were established from pooled healthy control data. Diagnostic accuracy metrics were calculated individually and in combination. Data analysis utilized SPSS v27, employing t-tests, chi-square tests, and ROC analysis with $p < 0.05$ indicating significance.

Results

Table 1. Biochemical Marker Comparison

Marker	**Drowning (n=50)**	**Control (n=30)**	**p-value**
Surfactant D (µg/mL)	15.2 ± 4.1	7.8 ± 2.9	<0.001
NT-proBNP (pg/mL)	525 ± 180	340 ± 120	0.002
D-dimer (µg/mL)	2.1 ± 0.8	1.2 ± 0.5	0.005

Biochemical markers were significantly elevated in drowning cases.

Table 2. Diatom Analysis Results

Diatom Count ≥20/100 µL	Drowning	Control	p-value
Positive (≥20)	44 (88%)	5 (17%)	<0.001
Negative (<20)	6 (12%)	25 (83%)	

Drowning cases showed a high prevalence of significant diatom counts.

Table 3. Diagnostic Accuracy of Test Combinations

Diagnostic Model	Sensitivity (%)	Specificity (%)	Accuracy (%)
Surfactant D alone	82	75	80
Diatom test alone	88	83	86
Combined markers + diatom	94	90	92

Combining biochemical markers with diatom analysis produced best overall diagnostic performance.

Discussion

These findings reinforce the diagnostic utility of surfactant D, NT-proBNP, and D-dimer alongside diatom testing in forensic drowning investigations. Surfactant D, indicative of alveolar epithelial injury, showed high discriminatory value consistent with recent translational studies 11-13. The adjunct biochemical data augment standard autopsy findings and aid in early postmortem scenarios.

Diatom analysis detected significant loads in true drowning cases (88%), in line with forensic diatomology best practice14. However, controls exhibited low-level diatom presence (17%), emphasizing risk of false positives from ante- or postmortem environmental exposure. The combined diagnostic algorithm (sensitivity 94%, specificity 90%) aligns with literature recommending multimodal diagnostics, per recent meta-analyses exploring integrative approaches15. Standardized protocols—automated SEM, validated scene-process labs—are critical for accuracy and reproducibility[1],[8].

Study limitations include sample size, possible variability in time of immersion, and marker stability postmortem. Future work should expand biochemical panels (e.g., KL-6, inflammatory cytokines), integrate PCR for algae DNA, and evaluate automated diatom readers for broader application.

In conclusion, integrating quantitative biochemical markers with diatom testing enhances forensic certainty in drowning diagnosis, especially in equivocal cases, and supports refinement of medico-legal standards.

Conclusion

Combining elevated serum surfactant D and moderate-to-high diatom counts in bone marrow provides sensitive and specific confirmation of drowning as cause of death. This multimodal approach significantly improves forensic accuracy over single-method diagnostics and supports its incorporation into standard postmortem protocols.

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