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Beyond Histology: Genetic and Transcriptomic Insights into the Postmortem Diagnosis of Mechanical Asphyxia

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ABSTRACT

***Background*:** Mechanical asphyxia is a cause of death characterized by external interference with breathing or circulation, leading to critical hypoxia and often rapid demise. Its forensic diagnosis remains one of the most challenging tasks in postmortem practice due to the frequently nonspecific and overlapping nature of external and internal findings. In recent years, advancements in molecular biology have opened new avenues for the objective identification of antemortem hypoxic stress. Gene expression analysis, microRNA profiling, and proteomic approaches now offer promising supplementary tools for detecting the physiological responses associated with mechanical asphyxia at the cellular and subcellular levels.

This review highlights key genetic and transcriptomic markers involved in hypoxia and mechanical asphyxia, including hypoxia-inducible factor 1-alpha (HIF-1α), BNIP3, VEGFA, and various apoptosis and inflammation-related genes such as caspase-3, IL-6, and TNF-α. Additionally, microRNAs—particularly miR-210, miR-208, and miR-499—have demonstrated diagnostic value due to their stability and tissue-specific expression patterns following hypoxic stress. Emerging molecular tools such as digital droplet PCR, next-generation sequencing (NGS), and transcriptome-wide analysis are increasingly being applied to postmortem samples, including lung and cardiac tissues, offering high sensitivity and specificity in detecting gene-level responses to hypoxia.

Conclusion:

Genetic and molecular profiling represents a promising frontier in the forensic diagnosis of mechanical asphyxia. These tools provide insight into antemortem cellular responses that cannot be appreciated through traditional autopsy methods alone. While many of these approaches remain in developmental or validation phases, their integration into forensic workflows has the potential to significantly improve diagnostic accuracy in complex or ambiguous deaths. A multimodal approach—combining macroscopic, histological, and molecular findings—will likely define the future standard in the postmortem assessment of suspected asphyxial deaths.

Keywords: Mechanical Asphyxia, Genetic and Transcriptomic Insights, Postmortem Diagnosis

INTRODUCTION

Mechanical asphyxia, caused by obstruction of airflow or vascular compression, is a frequent cause of death encountered in forensic practice. However, its postmortem diagnosis remains challenging due to the nonspecificity of traditional autopsy findings and overlapping features with other hypoxic or sudden deaths. While histopathology and immunohistochemistry have provided valuable insights into tissue-level changes, recent advancements in genetic and molecular biology offer promising new dimensions in forensic diagnostics.

Molecular forensic pathology focuses on analyzing the biochemical and genetic alterations that occur in response to antemortem

hypoxia, stress, or injury. These include changes in gene expression, microRNA (miRNA) profiles, mitochondrial function, and epigenetic markers. Such molecular events occur early and can provide more sensitive, objective evidence of vital reactions preceding death. Specifically, the expression of hypoxia-regulated genes, pro-apoptotic pathways, and inflammatory mediators may serve as postmortem indicators of mechanical asphyxia [1–3].

In recent years, forensic researchers have identified several promising markers in both lung and cardiac tissues, such as HIF1A, BNIP3, miR-210, and caspases, that are upregulated in asphyxial deaths. Moreover, molecular autopsy—the use of postmortem genetic testing to detect underlying or acquired causes of sudden death—has gained traction, particularly in unexplained or controversial cases [4,5].

This review explores the current knowledge on genomic, transcriptomic, and proteomic markers associated with mechanical asphyxia, focusing on their utility, limitations, and future applications in forensic medicine. By integrating molecular science with traditional autopsy findings, forensic pathologists may move closer to establishing objective criteria for diagnosing asphyxial deaths in both routine and complex medico-legal scenarios.

2. Molecular Pathways Activated in Mechanical Asphyxia

The pathophysiological events of mechanical asphyxia—namely hypoxia, ischemia, and cellular stress—trigger a complex series of molecular responses that are increasingly being explored for forensic diagnostic use. These responses include changes in gene expression, activation of apoptotic signaling pathways, mitochondrial dysfunction, and upregulation of inflammation-related genes. Together, these alterations provide a molecular footprint of hypoxic injury that may help distinguish mechanical asphyxia from other causes of death when histological and external signs are inconclusive.

The hypoxia-inducible factor (HIF) pathway is central to the cellular adaptation to low oxygen levels. Under normoxic conditions, HIF-1α is rapidly degraded. However, during hypoxia, HIF-1α stabilizes and translocates to the nucleus, where it activates the transcription of several genes involved in angiogenesis, glycolysis, erythropoiesis, and cell survival. Notable HIF-1α target genes include BNIP3, which promotes hypoxia-induced apoptosis and autophagy; VEGFA, which enhances vascular permeability; EPO, responsible for stimulating red blood cell production; and GLUT1, which supports anaerobic glucose metabolism [6,7]. Forensic studies have shown that HIF1A gene expression and HIF-1α protein accumulation are significantly elevated in the lungs and myocardium in asphyxial deaths, especially when there is a short survival interval after the onset of hypoxia [8].

Mitochondrial dysfunction plays a pivotal role in the cellular demise associated with prolonged oxygen deprivation. As mitochondrial membranes become permeable, cytochrome c is released into the cytoplasm, triggering caspase-9 and subsequently caspase-3 activation. This intrinsic apoptotic pathway also involves the upregulation of pro-apoptotic proteins like Bax and the downregulation of anti-apoptotic proteins such as Bcl-2 [9]. Caspase-3, in particular, has been shown to be upregulated in myocardial tissues of individuals who succumbed to mechanical asphyxia, reflecting the activation of programmed cell death mechanisms [10].

Inflammatory and oxidative stress responses are also hallmarks of mechanical asphyxia, particularly in deaths that involve an agonal phase. These responses are mediated by a variety of cytokines and transcription factors, including interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), inducible nitric oxide synthase (iNOS), and the NF-κB pathway [11]. These molecules are typically expressed when there is a delay between the onset of hypoxia and death, allowing time for cellular transcription and translation. Their presence in postmortem tissue can indicate that the individual experienced a period of physiological struggle and survived long enough for an inflammatory cascade to begin [12].

MicroRNAs (miRNAs) have recently emerged as promising forensic biomarkers due to their stability, tissue specificity, and responsiveness to environmental changes such as hypoxia. Among these, miR-210 is the most well-established hypoxia-inducible miRNA and is regulated by HIF-1α. It plays roles in mitochondrial metabolism and cell survival and has been repeatedly shown to be upregulated in mechanical asphyxia [13]. Other relevant miRNAs include miR-21, which modulates apoptosis and fibrosis; miR-1246, a marker of stress and immune signaling; and cardiac-specific miRNAs such as miR-499, miR-208, and miR-1, which reflect myocardial damage. These molecules can be extracted from postmortem tissue, blood, or other body fluids and analyzed using techniques like reverse transcription quantitative PCR (RT-qPCR), offering a sensitive molecular means of detecting hypoxic injury [14,15].

Finally, epigenetic changes such as DNA methylation and histone modifications are being investigated for their potential forensic applications. Hypoxia has been shown to alter methylation patterns in promoter regions of several genes involved in apoptosis and inflammation. While these epigenetic modifications have not yet been validated for routine forensic use, they may eventually help determine the timing and severity of hypoxic insults, especially in cases involving chronic stress or delayed death. Current evidence in this field remains experimental, but the prospect of using epigenetic signatures as part of molecular autopsy protocols is a compelling direction for future research [16].

3. Genetic and Transcriptomic Markers in Pulmonary Tissues

The lungs are the primary organs affected during mechanical asphyxia, and thus, they are a critical focus in the search for molecular indicators of hypoxic injury. Advances in genetic and transcriptomic profiling have revealed that pulmonary tissues express several genes and non-coding RNAs in response to hypoxia and mechanical stress. Identifying and validating these changes postmortem can enhance the diagnostic accuracy in suspected cases of asphyxia, especially when traditional histological signs are inconclusive.

One of the most extensively studied molecular markers in the lungs is **HIF1A**, which encodes hypoxia-inducible factor-1α. Elevated mRNA levels of HIF1A in lung tissue have been observed in autopsy samples from individuals who died due to mechanical asphyxia, including smothering, hanging, and drowning [8]. This upregulation reflects the cellular adaptation to oxygen deprivation and can be detected using quantitative real-time PCR (qRT-PCR) or in situ hybridization techniques.

In addition to HIF1A, **BNIP3** (BCL2/adenovirus E1B 19 kDa-interacting protein 3) has emerged as a gene of interest. BNIP3 plays a dual role in promoting apoptosis and autophagy under hypoxic conditions. In forensic samples, its expression is typically elevated in alveolar epithelial cells and bronchial walls of individuals who experienced asphyxial death, suggesting its utility in confirming hypoxia-driven cell stress [7,17].

Other hypoxia-regulated genes expressed in the lung include **VEGFA** (vascular endothelial growth factor A), which is involved in vascular permeability and angiogenesis, and **EPO** (erythropoietin), which promotes erythropoiesis. While these genes are primarily studied in clinical models of hypoxia, their expression patterns have also been noted in postmortem lung tissues, further supporting their diagnostic relevance in forensic pathology [6,18].

Transcriptomic studies have also highlighted the importance of **microRNAs** in hypoxia response. In lung tissue, **miR-210** has been consistently elevated in deaths involving mechanical asphyxia [13]. As a downstream target of HIF-1α, miR-210 modulates mitochondrial metabolism and cellular adaptation to low oxygen levels. It is considered one of the most promising miRNA biomarkers for antemortem hypoxic stress and can be detected from paraffin-embedded tissues or frozen samples.

Furthermore, **miR-21** and **miR-1246** have been implicated in regulating inflammation and apoptosis in pulmonary tissues. Elevated expression of these miRNAs has been detected in alveolar cells of individuals who died due to smothering and ligature compression. These molecular signals may complement traditional histology and help confirm that lung tissue was functionally active and experiencing injury before death [14,15].

In experimental models and human autopsies, differential expression of **inflammatory genes**, including **IL6**, **TNF**, and **NFKB1**, has been demonstrated in asphyxial deaths. These cytokines are involved in the recruitment of immune cells and the generation of oxidative stress. Their detection via RT-qPCR or immunohistochemistry suggests a systemic inflammatory response secondary to hypoxia and supports their inclusion in a diagnostic molecular panel for asphyxial deaths [11,12].

The combination of upregulated hypoxia-responsive genes, microRNAs, and inflammatory mediators in the lungs provides a robust molecular signature of mechanical asphyxia. These findings are most useful when interpreted alongside histological features such as alveolar hemorrhage, edema, and emphysema. Although further validation and standardization are needed, pulmonary transcriptomic analysis holds great promise for improving diagnostic confidence in forensic cases involving suspected asphyxia.

4. Genetic and Transcriptomic Markers in Cardiac Tissues

In mechanical asphyxia, the heart experiences profound hypoxic and ischemic stress, especially during episodes involving chest compression or vascular obstruction. These physiological insults activate several gene networks within myocardial tissue, reflecting both cellular survival responses and pathways leading to irreversible injury. As such, exploring transcriptomic and genetic alterations in the heart has become a growing focus in forensic molecular pathology, with the goal of identifying reliable indicators of antemortem hypoxia and cardiac stress.

One of the most consistent markers is **HIF1A**, which, as in the lungs, is strongly upregulated in hypoxic myocardium. Elevated levels of HIF1A mRNA and HIF-1α protein have been detected in autopsy heart tissues from asphyxial deaths, suggesting that myocardial cells were metabolically active and responding to oxygen deprivation prior to death [8]. These findings are most reliable in cases with an agonal phase of several minutes, allowing for transcriptional activation.

The **BNIP3** gene also plays a key role in cardiac hypoxia. In the heart, BNIP3 induces mitochondrial dysfunction, autophagy, and apoptosis under oxygen-deficient conditions. Studies have shown that BNIP3 expression increases significantly in subendocardial myocytes and vascular endothelium following mechanical asphyxia, and its detection may support histologic findings such as contraction band necrosis or interstitial hemorrhage [17].

Additionally, several cardiac-specific **microRNAs (miRNAs)** have been investigated for their potential forensic utility. Notably, **miR-1**, **miR-133a**, **miR-208**, and **miR-499** are abundantly expressed in cardiac tissue and are rapidly released into the circulation in response to myocardial injury. These miRNAs are involved in regulating calcium handling, contractility, apoptosis, and cell survival. Elevated levels of **miR-208** and **miR-499** have been found in myocardial samples from asphyxial deaths and may serve as indicators of cardiac hypoxia or stress-induced injury [14,19].

Furthermore, expression of **caspase-3** and **cytochrome c**, markers of mitochondrial-mediated apoptosis, is increased in cardiac tissue during lethal hypoxia. These proteins are activated in response to mitochondrial outer membrane permeabilization, a process triggered by prolonged oxygen deprivation. Postmortem detection of caspase-3 mRNA or protein via RT-PCR or immunohistochemistry may indicate that the death process involved ongoing apoptosis in myocardial cells [10,20].

Inflammatory genes, particularly **IL6**, **TNF**, and **ICAM1**, are also upregulated in the heart during hypoxic stress. These genes facilitate leukocyte recruitment and vascular activation and may be particularly relevant in cases of traumatic or prolonged asphyxia where

systemic inflammation develops [11,12]. Their expression can be used to differentiate asphyxial deaths from sudden cardiac arrest due to arrhythmia, which may not elicit a similar transcriptional response.

Though more research is needed to validate and standardize these cardiac molecular markers, preliminary evidence strongly supports their role as objective indicators of hypoxic myocardial injury. When interpreted in conjunction with histological signs such as contraction band necrosis and congestion, cardiac gene expression profiles provide powerful supportive evidence in the postmortem diagnosis of mechanical asphyxia.

5. Emerging Molecular Tools and Forensic Applications

The integration of molecular technologies into forensic science is rapidly advancing, providing tools that extend beyond traditional morphology and immunohistochemistry. Recent developments in **high-throughput gene expression profiling**, **microRNA panels**, **next-generation sequencing (NGS)**, and **digital PCR** offer promising approaches for the **postmortem identification of mechanical asphyxia**, especially in cases where conventional findings are equivocal or decomposed.

Among the most promising technologies is **microarray-based transcriptome analysis**, which allows for the simultaneous evaluation of thousands of genes. This method has been applied to postmortem lung and cardiac tissues, revealing distinct gene expression signatures in cases of fatal hypoxia compared to sudden cardiac deaths or trauma. Panels that include HIF1A, BNIP3, IL6, VEGFA, and various miRNAs (e.g., miR-210, miR-499) are showing potential for building **molecular autopsy profiles** tailored to asphyxial deaths [21,22].

Digital droplet PCR (ddPCR) is another tool with emerging relevance. Unlike conventional qPCR, ddPCR offers greater precision and is less affected by RNA degradation, making it particularly suited for forensic samples with varying degrees of preservation. It enables quantification of low-copy targets such as miRNAs and hypoxia-related transcripts even in formalin-fixed or autolyzed tissue, increasing its forensic applicability [23].

Next-generation sequencing (NGS) is also being explored to detect postmortem alterations in gene expression, splicing events, or regulatory RNA activity. Although its use remains largely experimental, NGS may eventually enable comprehensive molecular profiling of asphyxia-related deaths, including potential discovery of new markers or gene variants that affect individual susceptibility to hypoxia or reflex cardiac arrest [24].

Other novel approaches include **proteomics** and **metabolomics**, which analyze the downstream products of gene expression. These techniques can detect specific proteins or metabolic byproducts associated with hypoxic stress, apoptosis, or mitochondrial dysfunction. For example, elevated levels of lactate, succinate, or nitrotyrosine in cardiac or pulmonary tissue may reflect ischemic injury related to asphyxia [25].

Importantly, these tools also support the development of **standardized forensic panels** for use in medicolegal autopsies. Combined with histopathology and immunohistochemistry, they could allow for more objective and reproducible determinations of cause and manner of death. In the future, forensic laboratories may implement **integrated workflows** that combine molecular markers from tissue, blood, and body fluids to improve diagnostic accuracy, especially in complex or decomposed cases.

However, these technologies are not without limitations. Challenges include high cost, limited accessibility in many forensic settings, and a need for large-scale validation across diverse populations. Moreover, forensic pathologists must interpret molecular findings within the broader context of the autopsy, scene investigation, and clinical history to avoid overreliance on isolated molecular signals.

Nonetheless, the trajectory of forensic molecular diagnostics is promising. As tools like ddPCR, NGS, and miRNA-based assays become more refined and accessible, their incorporation into routine casework will likely improve the objectivity, specificity, and confidence with which mechanical asphyxia is diagnosed postmortem.

6. Conclusion and Future Directions

The diagnosis of mechanical asphyxia continues to challenge forensic pathologists due to the nonspecific nature of external findings and overlapping internal features with other causes of sudden death. While traditional histopathology and immunohistochemistry offer foundational insights, emerging **genetic and molecular approaches** are reshaping the postmortem investigative landscape. By analyzing changes in gene expression, microRNA profiles, and molecular markers of hypoxia, these techniques provide valuable evidence of antemortem physiological responses to asphyxial stress.

Molecular signatures such as elevated **HIF1A**, **BNIP3**, **caspase-3**, and hypoxia-responsive **microRNAs** (notably miR-210, miR-499, and miR-208) have shown potential in supporting the diagnosis of asphyxial deaths. These markers, when detected in lung and cardiac tissues, reflect cellular efforts to cope with oxygen deprivation and may distinguish mechanical asphyxia from other hypoxic or non-hypoxic causes of death. The growing role of **digital PCR**, **NGS**, and **transcriptome analysis** further enhances this capability, allowing for sensitive, high-resolution profiling of molecular changes even in degraded or formalin-fixed tissues.

Despite these promising developments, several limitations must be addressed before molecular diagnostics can be widely adopted in forensic casework. These include the need for **standardized protocols**, **large-scale population-based validation**, and **forensic-specific reference databases**. Interpretation must also be integrated with conventional autopsy findings, death scene context, and the decedent's medical history to avoid misclassification or overinterpretation of isolated molecular findings.

Looking ahead, the future of forensic pathology lies in a **multimodal approach** that combines gross pathology, histology, immunohistochemistry, and molecular data into a comprehensive diagnostic model. As research progresses and access to molecular tools expands, forensic experts will be better equipped to render more accurate, objective, and scientifically grounded conclusions in complex deaths — particularly those involving suspected mechanical asphyxia.

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