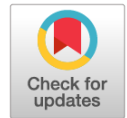


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Forensic Bone Proteomics: Novel Biomarkers and Technologies for Estimating the Postmortem Interval (A Review)

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ABSTRACT

Bone proteomics is a rapidly evolving field in forensic medicine aimed at determining the postmortem interval. Unlike traditional approaches, this method enables quantitative and molecular-level analysis of protein composition changes in bone tissue. Highly degradation-resistant proteins are considered reliable biomarkers for estimating the postmortem interval, providing more accurate and objective results. Mass spectrometry, in combination with modern bioinformatics tools and machine learning technologies, allows for a detailed investigation of postmortem protein degradation processes and the identification of time-dependent molecular patterns. However, environmental factors such as humidity, temperature, soil composition, and microbial activity significantly affect protein preservation in bone tissue, underscoring the need for standardized analytical protocols.

This review summarizes key methods of bone proteomic analysis, prospects for its integration with metabolomics and lipidomics, and the potential of machine learning in postmortem interval estimation. Further research in this field should aim at validating biomarkers, standardizing techniques, and integrating these methods into forensic practice.

The development of forensic bone proteomics opens new possibilities, offering more precise data in complex medico-legal cases.

Keywords: bone proteomics; forensic medicine; mass spectrometry; biomarkers; postmortem interval; PMI; protein degradation; review.

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Судебно-медицинская протеомика костей: новые биомаркёры и технологии оценки давности наступления смерти (обзор)

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АННОТАЦИЯ

Протеомика костной ткани — это быстро развивающееся направление судебной медицины, ориентированное на установление давности наступления смерти. В отличие от традиционных подходов, этот метод позволяет проводить количественный и молекулярный анализ изменений белкового состава костей. Высокоустойчивые к деградации белки рассматривают как надёжные биомаркёры давности наступления смерти, обеспечивающие более точные и объективные результаты. Масс-спектрометрические методы в сочетании с современными биоинформационными подходами и технологиями машинного обучения позволяют проводить детальный анализ процессов деградации белков и выявлять закономерности их изменений в зависимости от времени, прошедшего после смерти. Однако на сохранность белков костной ткани существенно влияют такие факторы окружающей среды, как влажность, температура, состав почвы и микробная активность, что обуславливает необходимость разработки стандартизированных протоколов анализа.

В обзоре рассмотрены ключевые методы протеомного анализа костей, перспективы его интеграции с метаболомикой и липидомикой, а также возможности применения машинного обучения для оценки давности наступления смерти. Дальнейшие исследования в этой области должны быть направлены на валидацию биомаркёров, стандартизацию методик и их внедрение в судебно-медицинскую практику.

Развитие судебной протеомики костей открывает новые возможности, позволяя получать более точные данные в сложных судебно-медицинских случаях.

Ключевые слова: протеомика костей; судебная медицина; масс-спектрометрия; биомаркёры; давность наступления смерти; ДНС; белковая деградация; обзор.

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法医骨骼蛋白质组学：死亡时间评估的新型生物标志物与技术 (综述)

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摘要

骨骼蛋白质组学是法医学中快速发展的研究领域，主要致力于确定死亡时间。与传统方法不同，该技术可在定量与分子水平上分析骨组织中蛋白质成分的变化。具有高度抗降解性的蛋白质被视为评估死亡时间的可靠生物标志物，可获得更精确、客观的结果。质谱分析技术结合现代生物信息学方法和机器学习技术，使得能够对死亡后蛋白质降解过程进行深入分析，并揭示其随时间推移的变化规律。然而，环境因素，如湿度、温度、土壤成分和微生物活性，对骨组织蛋白的保存具有显著影响，这凸显出建立标准化分析方案的必要性。

本文综述了骨骼蛋白质组学的关键分析方法，探讨了其与代谢组学、脂质组学整合的前景，以及机器学习在死亡时间评估中的应用潜力。未来研究应着重于生物标志物的验证、方法学标准化及其在法医学实践中的推广。

法医骨骼蛋白质组学的发展开辟了新的可能性，使在复杂法医学案件中能够获得更为准确的数据。

关键词：骨骼蛋白质组学；法医学；质谱分析；生物标志物；死亡时间；PMI；蛋白质降解；综述。

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INTRODUCTION

Proteomics is an area of biological research dedicated to the identification and quantitative analysis of the complete set of proteins in a body, as well as studying of their functions and changes caused by external and internal factors [1]. High sensitivity and relative stability of proteins against degradation have stimulated its wide application in forensic medicine, especially in cases of damage or absence of DNA [2, 3].

Forensic examination employs various methods of proteomic analysis:

- one-dimensional and two-dimensional gel electrophoresis [4, 5];
- stable isotope ratio mass spectrometry [6];
- multidimensional protein identification technology (MudPIT) [7];
- microflow liquid chromatography with tandem mass spectrometry (microflow LC-MS/MS) [8].

The obtained data are processed using bioinformatics, which increases the accuracy and reliability.

Proteomics is particularly relevant in studying biological samples of hair [9], bone tissue [10], or biological fluids [11], where protein biomarkers help determine sex, ethnicity, individual characteristics, and the origin of the samples [12]. Its use in assessing the postmortem intervals (PMI) is of particular importance, especially for skeletal remains [13–16]. Nevertheless, the development of forensic proteomics requires special standards and methodological approaches that consider the specifics of forensic examination [17].

PMI ASSESSMENT AND POSSIBLE LIMITATIONS

PMI determination is key in forensic medicine [18]. Conventional morphological methods are based on the analysis of postmortem changes; however, they largely depend on the environment, and their accuracy is often limited [19].

The rate of postmortem changes is determined by many factors, such as body condition and cause of death, presence of injuries, diseases, fat deposits, as well as the temperature and humidity of the environment. For instance, high temperature and humidity accelerate the onset of postmortem changes, whereas body temperature reduction or clothing slows them down [20–22].

In recent years, biomolecular methods for PMI assessment have been actively implemented, showing greater objectivity and less dependence on environmental factors compared to conventional morphological methods. They are based on the analysis of biological markers. Those are proteins, metabolites, and nucleic acids, changes of which in the postmortem period can be quantitatively recorded under controlled conditions [23]. According to scientific papers, the use of these methods considerably increases the accuracy and reproducibility, and reduces the influence of external factors that complicate PMI assessment [24].

BONE TISSUE PROTEOMICS

Postmortem protein degradation is a sequential, systematic process that considers changes in protein composition in PMI assessment [25]. Death is accompanied by the cessation of metabolic activity, which induces enzymatic and microbial proteins degradation. In turn, the analysis of specific proteins and their breakdown products in various tissues enables PMI determination [26]. Unlike others, this method is more reliable because protein degradation occurs gradually and mostly depends on PMI.

Bone proteomics is the analysis of proteins that are part of bone tissue [27]. This method is used in clinical studies to determine pathological processes in the skeletal system [28]. Besides, it is widely used in forensic medicine, particularly in assessing a person's age using protein profiling [29]. Proteomic methods are far more efficient in the examination of bones, muscle tissues, and biological fluids when DNA analysis is difficult or impossible [30].

The protein composition of bone tissue consists of collagen and non-collagen proteins that form its structure and functional properties [31]. Collagens make up approximately 30% of all proteins in the mammal's body. In turn, 90% of the protein matrix is type I collagen, which provides strength and stability to the bone [32]. A smaller portion is represented by type III [33] and V collagens, as well as the fibril-associated collagens with interrupted triple helices group (the FACIT group), which participate at different stages of its formation [34]. This ratio ensures the normal metabolism and functional state of bone tissue.

In recent years, the analysis of bone tissue proteins has been actively used to assess age by the time of death and PMI [13]. The examination of postmortem changes in the bone proteome increases forensic examination accuracy, facilitates personal identification, and clarifies death circumstances [14]. The bone proteome, or osteome, is considered a promising source of expert information [13], including determining the postmortem submersion interval (PMSI), which is also important for assessing causes of death and personal identification [35].

In turn, PMSI determination requires considering specific factors, including temperature, water currents, and hydrodynamic impact, which substantially alter the rate and nature of postmortem processes compared to terrestrial conditions [35].

Modern biomolecular approaches use specific proteins and their post-translational modifications to establish PMI [36, 37]. Biochemical processes after death, including the cessation of anabolic processes, destruction of cellular structures, reduction of enzymatic activity, and cessation of oxygen supply, lead to the formation of metabolites that serve as additional indicators to improve the accuracy of PMI assessment [38].

BONE TISSUE BIOMARKERS IN POSTMORTEM INTERVAL ASSESSMENT

Protein molecules are more resistant to degradation compared to DNA, which in the postmortem period is subject to oxidative damage and fragmentation, thus limiting its use in forensic practice [12]. In this regard, more attention is directed to the search of reliable PMI biomarkers, with proteomic technologies becoming crucial. These methods enable detailed identification and characterization of peptides and proteins in biological samples [39], ensuring reproducibility of results and less dependence on external factors compared to nucleic acid tests [40, 41]. The patterns of protein degradation are closely related to PMI and can be used not only for its assessment, but also for determining the PMSI [3, 42].

Bone Biomarkers and Diagenesis Processes

PMI assessment requires understanding the mechanisms of modification and degradation of the bone matrix after death. Diagenesis represents a sequence of physical and chemical changes in the organic and inorganic tissues of the skeleton [43]. The analysis of diagenesis proteins allows determining the degree of tissue destruction and establishing changes in the proteome with high accuracy [44, 45].

One of the most considerable protein markers is collagen, the main structural component of bone tissue [46]. The ratio of collagen to non-collagenous proteins (Co:NCo) can be used as an indicator of PMI [27]. For more accurate assessment, methods including stereomicroscopy and digital visualization have been developed, surpassing classical spectrophotometric analysis [46]. It should be noted that the data on human and pig bones comparison revealed similar patterns of collagen degradation [47]. However, methods applied to animal samples are to be adjusted for forensic purposes.

Proteomic Analysis and Postmortem Interval Assessment

According to the latest scientific data, the content of certain proteins changes depending on PMI, making them a reliable biomarker. For example, fetuin-A concentration decreases with the time after death increasing, while the content of α 1-antitrypsin and chromogranin-A increases [25]. In addition, there is a decrease in the concentrations of proteins such as myosin (types 2 and 6), lactoferrin, haptoglobin, transferrin, and hemoglobins (A and B) four months after death [48].

Biglycan, a leucine-rich proteoglycan, persists in the tissues for a long time and is a promising PMI biomarker, too [49]. Its resistance to degradation is confirmed by extraction from archaeological samples dating back up to a million years [50]. The biglycan deamination increases with PMI rise, reaching its peak between two and four months after death and continuing to increase later [51].

Omics Technologies in Forensic Medicine

A comprehensive omics approach, which combines proteomics, metabolomics, and lipidomics, enables the highly accurate analysis of molecular changes [7]. Mickleburgh et al. [10] identified 1042 proteins, among which histones (H2A1H, H44), hemoglobin, actin, and vimentin showed the greatest concentration dependence on the age of bone remains. They also noted that the rate of protein degradation is determined by individual differences in the mineral density of bone tissue. Similar conclusions were made by Qi et al. [28], emphasizing the significance of mineral structure and taphonomic conditions for protein preservation. Moreover, some proteins exhibit increased stability due to their ability to bind with mineral components of bone tissue, which protects them from microbial degradation [52].

Proteomic analysis of 16 samples of human femur bones allowed to identify 32 proteins that can be used as PMI biomarkers over 12 years [53, 54].

Thus, proteomic analyses of bone tissue now open new prospects for forensic practice, providing highly accurate PMI assessment. In turn, the development of omics technologies makes the identification of additional biomarkers easier, as well as reducing the influence of external factors and increasing the reliability of expert conclusions.

PROTEINS SUITABILITY FOR ARCHAEOLOGICAL ANALYSIS

Protein molecules are valuable biomarkers for phylogenetic tests and taxonomic identification of ancient bone remains, especially when ancient DNA is insufficiently preserved or completely degraded. Wadsworth et al. [55] show, basing on 19 archaeological bone samples, dated from 4 thousand to 1.5 million years, that proteomic methods enable taxonomic identification even in the absence of ancient DNA. It was established that some proteins, particularly albumin and fetuin-A, bind to the mineral component of bone tissue and demonstrate relative long-term stability [27]. However, their concentration decreases as the samples age. Collagen was the most stable, maintaining a strong bond with the mineral phase of bone tissue; stable collagen chains [α -2(I) and α -1(I)] were found, as well as other proteins—serum albumin, glycoprotein A2HSG, prothrombin, and biglycan [55]. These results support the role of these proteins as potential biomarkers for archaeological and phylogenetic analyses.

Ntasi et al. article [56], dedicated to examine the impact of environmental conditions on the preservation of protein molecules, deserves special attention. The authors conducted a proteomic analysis of the bone remains of victims of the eruption of Mount Vesuvius (79 AD), found in Herculaneum and Pompeii. Despite the higher temperature exposure in Herculaneum, the greatest protein degradation was found in the bones from Pompeii. These samples showed

reduced non-collagenous protein content, pronounced deamination, and evident chemical changes in the collagen structure. The authors described specific molecular forms that appeared from chemical modifications due to external factors and proposed to call them “diagenetiforms.”

Proteomic analysis of ancient bone samples considerably enables phylogenetic and archaeological investigations. Investigating the preservation of protein molecules and their modifications enables the identification of ancient species; reconstruct environmental conditions and postmortem changes, opening new perspectives for archaeology, forensic science, and related areas.

PROTEOMIC ANALYSIS OF BONE TISSUE IN INVESTIGATION OF THE CORPSE DECOMPOSITION IN AQUATIC ENVIRONMENT

Proteomic analysis of bone tissue enables to examine the processes of body decomposition in aquatic environments and can be used as a reliable tool for assessing PMI and PMSI. Thus, Mizukami et al. [16] established a correlation between the composition of the bone proteome and the mouse corpses' PMSI, in various types of aquatic environments:

- tap water,
- sea saltwater,
- pond water, and
- chlorinated water.

In the early stages of decomposition, universal proteins predominate in bone tissue, easily depleted due to high solubility and lack of strong binding with hydroxyapatite. During the first week, the preservation of muscle tissue around the bones ensured an increased content of muscle proteins, which characterized short-term postmortem changes [16].

Further stay of corpses in water led to the proteomic profile change: bone and blood proteins predominated, indicating other mechanisms of their release and stabilization during long exposure [16]. Protein extraction according to previously described protocols [53, 54] allowed to identify the muscle protein aldolase as a potential marker for PMSI. In addition, such characteristics as the degree of deamidation of blood coagulation factor VII and the decrease in the concentration of peptidylprolyl isomerase A are diagnostically valuable [16], what leads to an expansion of the range of forensic indicators.

The use of specific peptides with different deamidation as new biomarkers to determine PMSI duration and the type of aquatic environment is also of utmost importance. Unlike previously described terrestrial changes in biglycan, the deamidation of fetuin-A peptides [16] and IGFBP-5 (insulin-like growth factor-binding protein 5) [57] increased considerably with the prolonged PMSI. Moreover, one of the fetuin-A peptides showed a much higher level

of deamidation in pond water compared with other types of aquatic environments [16], which notably expands the list of establishing biomarkers for identification of specific decomposition environments.

Thus, bone tissue proteomics has enormous potential and is becoming an important tool in modern forensic medicine, which helps to identify key biomarkers to determine the conditions and duration of bodies in various environments. Considerable progress in this area was achieved in the second half of the 20th century thanks to the development of mass spectrometry technologies and methods for identifying protein molecules, which marked the beginning of a new stage in forensic investigations and forensic practice [58].

NEW METHODS OF PROTEOMIC ANALYSIS OF BONE TISSUE FOR PMI ASSESSMENT

The development of forensic proteomics requires the integration of modern analytical approaches aimed at analysing the composition and post-translational modifications of bone proteins [12]. The greatest potential is in comprehensive strategies that combine mass spectrometric proteomic analysis with other omics technologies, including genomics, transcriptomics, and metabolomics. This comprehensive approach provides the opportunity to study in more details the molecular processes occurring in skeletal tissues after the death [59].

One important component to drive development of this field is the creation of specialized bioinformatics solutions that ensure the processing of high-volume proteomic data and the identification of specific proteins reflecting postmortem changes [60]. To ensure the comparability of results between different laboratories, it is necessary to standardize the procedures for preparing bone samples and formal analysis, which is of utmost importance for the formation of generally accepted protocols [12].

Technological progress in mass spectrometry has had a decisive impact on the development of forensic proteomics of bone tissue. Increased sensitivity and accuracy of quantitative protein analysis has become possible through the use of methods such as matrix-assisted laser desorption/ionization with TOF (time-of-flight)-detector and liquid chromatography combined with tandem mass spectrometry [10]. For the quantitative characterization of the bone proteome, the use of isotopic labeling of amino acids remains a promising direction, allowing conduct high-accuracy analyses of rapid changes in protein composition [61, 62].

Along with the development of experimental methods, computational approaches, including artificial intelligence technologies, are gaining increasing importance for the analysis of mass spectra and prediction of DNA [63, 64]. Targeted proteomic methods, such as single and parallel reaction monitoring, enable highly sensitive determination of bone tissue biomarkers [31].

Thus, forensic bone tissue proteomics is emerging as an interdisciplinary field that combines modern analytical, bioinformatic, and computational approaches. Its further development depends on the standardization of methodologies, the establishment of legally recognized protocols, and the accumulation of large-scale validation data, enabling the integration of these technologies in forensic practice and improving the accuracy of forensic investigations [65].

ADVANTAGES OF USING BONE TISSUE PROTEOMICS IN FORENSIC INVESTIGATION

The application of proteomic analysis of bone tissue in forensic medicine for DNA establishment has certain advantages over traditional methods. One of the most significant benefits is the possibility of investigation without destroying the material, thereby preserving evidence and reusing samples for additional forensic examinations [50].

Forensic proteomics of bone tissue has shown high effectiveness in determining PMI, including scene investigations. Identification of bone tissue-specific proteins and their subsequent quantitative analysis allows assess PMI with greater accuracy than methods based on, for example, morphological changes [66]. According to scientific writings, the analysis requires a minimal amount of bone material, which allows using proteomics not only for PMI assessment but also for determining age at the time of death [10].

Bone tissue contains certain stable protein molecules that persist even in notably degraded samples. This is confirmed by the successful extraction of proteins from archaeological and paleontological remains, demonstrating an exceptional degree of preservation, thereby making bone tissue a valuable source for forensic investigation [67, 68].

A comprehensive approach that combines proteomic methods with metabolomics and lipidomics is of utmost importance. Data integration not only enhances the accuracy of PMI assessment but also extends the time frame of the analysis, significantly improving forensic diagnostic capabilities and strengthening the reliability of expert conclusions [69].

CONCLUSION

Bone tissue proteomics has emerged as a promising tool in forensic practise, primarily in determining PMI, which is a key issue in conducting examinations. Investigating the changes in the protein composition of bone tissue enables a deeper understanding of the mechanisms of their

degradation and using proteomic changes as a “molecular timeline.” This becomes especially valuable in cases where traditional methods of PMI determination are not that informative.

Mass spectrometry was key in the development bone tissue proteomics, providing accurate identification and quantitative assessment of proteins. Currently, several bone biomarkers have demonstrated high diagnostic value. However, their implementation in forensic practice requires reproducibility confirmation and standardized protocols for quantitative analysis.

One of the main limitations of applying bone proteomics remains the variability of postmortem changes, caused by individual body characteristics and exogenous factors, i. e. temperature, humidity, exposure to microorganisms, and environment. These conditions can greatly alter the proteomic profile, which should be considered when interpreting data.

The future development of forensic proteomics will depend on expanding the biomarker panel, integration with genomic and DNA-based technologies, and the development of comprehensive methodological approaches that consider the impact of the environment on protein preservation. Together, it will enhance the accuracy and reliability of expert conclusions and strengthen the role of proteomic analysis in forensic practice.

ADDITIONAL INFORMATION

Author contributions: G.R. Mustafina: data curation, writing—original draft, writing—review & editing; A.A. Khalikov: writing—review & editing; K.O. Kuznetsov: writing—original draft, writing—review & editing; E.M. Nazarova: data curation, writing—review & editing. All the authors approved the version of the manuscript to be published and agreed to be accountable for all aspects of the work, ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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