

Ulasan Artikel/Review Article

Advancing Forensic Proteomics in a Developing Forensic System: A Scoping Review of Malaysian Studies in a Global Context

Proteomik Forensik dalam Sistem Forensik yang Sedang Membangun: Satu Tinjauan Skop Kajian Malaysia dalam Konteks Global

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ABSTRACT

Proteomics is increasingly recognised as a complementary tool to DNA analysis in forensic science, particularly for degraded or limited biological evidence. This scoping review aimed to systematically map proteomic studies conducted in Malaysia involving forensically relevant biological samples and to evaluate their alignment with forensic applications. A comprehensive search of PubMed, Scopus, ScienceDirect, and EBSCO Academic databases identified studies published between 2010 and February 2024. Following PRISMA-ScR screening and eligibility assessment, 12 original research articles met the inclusion criteria. These studies primarily investigated hair (n = 5), bodily fluids and tissues (n = 7), with no Malaysian studies identified on bone or fingerprint proteomics for forensic purposes. Most included studies were biomedical in focus; however, they employed mass spectrometry-based methodologies directly transferable to forensic investigations. The review demonstrates that explicitly forensic proteomic research in Malaysia remains limited, despite clear evidence of local technical capacity and expertise. This gap highlights significant opportunities for translational research, population-specific protein databases, and interdisciplinary collaboration to integrate proteomics into routine forensic practice.

Keywords: biological evidence, forensic proteomics, Malaysia, mass-spectrometry, protein biomarkers.

ABSTRAK

Proteomik semakin diiktiraf sebagai alat pelengkap kepada analisis DNA dalam sains forensik, khususnya bagi bukti biologi yang terdegradasi atau terhad. Tinjauan skop ini bertujuan untuk memetakan secara sistematik kajian proteomik yang dijalankan di Malaysia melibatkan sampel biologi yang relevan secara forensik serta menilai kesesuaiannya dengan aplikasi forensik. Pencarian menyeluruh dalam pangkalan data PubMed, Scopus, ScienceDirect dan EBSCO Academic mengenal pasti kajian yang diterbitkan antara tahun 2010 hingga Februari 2024. Selepas proses saringan PRISMA-ScR dan penilaian kelayakan, sebanyak 12 artikel penyelidikan asal memenuhi kriteria kemasukan. Kajian-kajian ini terutamanya meneliti rambut (n = 5), serta cecair dan tisu badan (n = 7), manakala tiada kajian dari Malaysia dikenal pasti berkaitan proteomik tulang atau cap jari untuk tujuan forensik. Kebanyakan kajian yang dimasukkan berfokuskan bioperubatan; namun demikian, kajian-kajian tersebut menggunakan metodologi berasaskan spektrometri jisim yang boleh dipindahkan secara langsung kepada penyiasatan forensik. Tinjauan ini menunjukkan bahawa penyelidikan proteomik yang secara khusus bersifat forensik di Malaysia masih terhad, walaupun terdapat bukti jelas mengenai keupayaan teknikal dan kepakaran tempatan. Jurang ini menonjolkan peluang yang signifikan untuk penyelidikan translasi, pembangunan pangkalan data protein khusus populasi, serta kerjasama antara disiplin bagi mengintegrasikan proteomik ke dalam amalan forensik rutin.

Kata Kunci: bukti biologi, Malaysia, protein biopenanda, proteomik forensik, spektrometri jisim

INTRODUCTION

Globally, forensic proteomics has progressed rapidly, with validated applications in human identification, post-mortem interval estimation, and toxicological screening. However, the adoption of these advances remains uneven across regions. Examining country-specific research landscapes provides insight into structural, technical, and translational challenges faced by developing forensic systems. Within this context, Malaysia serves as a representative case study of an emerging forensic proteomics environment with established analytical capacity but limited forensic implementation.

The handling and analysis of biological evidence recovered from crime scenes constitute a fundamental component of forensic science. Traditionally, forensic identification has been dominated by DNA analysis; however, recent advances in proteomics have introduced new opportunities to complement and, in certain contexts, extend beyond DNA-based approaches (Sacco & Aquila 2023). Proteomics, broadly defined as the large-scale study of proteins, is conceptualised differently across disciplines. Within the forensic context, proteomics may be understood as the application of protein analysis for human identification and investigative reconstruction, particularly when conventional DNA evidence is degraded, limited, or absent.

Forensic proteomic research generally follows two main analytical approaches. The first focuses on identifying and quantifying specific proteins or protein markers within biological materials, such as hair shafts, that may be characteristic of an individual or tissue type. The second approach applies population-based statistical frameworks, whereby the probability of observing particular protein markers is calculated using reference datasets. These probabilities can then be used to assess the likelihood that a questioned sample originates from a specific individual, for example by comparing relative protein abundances in a recovered hair strand against population-level expectations (Tisdale 2016).

Proteins possess several intrinsic properties that make them particularly valuable for forensic investigations. Compared with DNA, proteins are generally more abundant, chemically stable, and structurally complex, and they retain biologically informative signatures linked to their tissue of origin (Aebersold & Mann 2016; Wadsworth et al. 2017). While DNA remains the gold standard for forensic identification, proteins are composed of twenty amino acids, as opposed to the four nucleotides found in DNA, allowing for a greater diversity of molecular variation and potentially richer contextual information (Tisdale 2016). These characteristics

render proteomics especially advantageous when analysing compromised biological evidence.

The forensic applications of proteomics are diverse, ranging from individual identification using hair and blood (Parker et al. 2016; Patel et al. 2016) to studying post-mortem processes. Beyond identification, proteomic techniques have contributed to the study of cadaver decomposition processes. Quantitative approaches, including isobaric tags for relative and absolute quantification (iTRAQ) coupled with liquid chromatography–tandem mass spectrometry (LC–MS/MS), have been employed to characterise microbial proteomes involved in decomposition, providing insights into post-mortem changes (Baldrian & López-Mondéjar 2014). Furthermore, predictable patterns of post-mortem protein degradation have been shown to support estimations of the time since death (Kocsmár et al. 2023). Bone proteomics, in particular, has demonstrated value in distinguishing between microbially driven and environmentally induced diagenetic processes, thereby improving models for post-mortem interval (PMI) estimation (Procopio et al. 2021). Such information is crucial for reconstructing sequences of events in forensic investigations. Proteomic analysis has also been extended to fingerprint evidence; despite challenges associated with low protein abundance, sensitive sampling strategies and ageing biomarkers have been successfully developed (Oonk et al. 2018).

The relatively late emergence of proteomics compared with other omics disciplines can largely be attributed to technological constraints, including the complexity of protein structures, data analysis challenges, and the lack of standardised analytical protocols (Al-Amrani et al. 2021). Nevertheless, significant technological advancements particularly in mass spectrometry have driven a rapid evolution in proteomic research capabilities (Aebersold & Mann 2016; Amir et al. 2021; Darrelyn et al. 2021; Tan et al. 2024a; Tan et al. 2024b). These developments have improved analytical sensitivity, increased the volume of recoverable information, and reduced processing times, thereby addressing many early methodological limitations. Continued research remains necessary to fully evaluate the long-term impact of proteomics on human identification and forensic practice.

Despite substantial global progress, forensic-focused proteomic research in Malaysia remains limited. Existing Malaysian studies predominantly concentrate on medical and biomedical applications, even though they involve biological samples frequently encountered in forensic contexts. This disparity highlights a significant research gap between international advances in forensic proteomics and local implementation within Malaysia. Accordingly, this scoping review aims to examine proteomic studies conducted in Malaysia that involve forensically relevant biological

materials and to identify opportunities for expanding forensic applications. By highlighting both existing capabilities and unmet research needs, this study underscores the importance of strategic, localised research efforts and interdisciplinary collaboration to translate existing proteomic capacity into actionable forensic tools within Malaysia.

MATERIALS AND METHODS

STUDY DESIGN

This scoping review was conducted in accordance with the five-stage methodological framework proposed by (Arksey & O'malley 2005), which is widely used to map the extent, nature, and characteristics of research activity within an emerging field. The framework comprises the following stages: (1) defining the research question; (2) identifying relevant studies through clearly defined inclusion and exclusion criteria; (3) systematically searching for and collecting relevant literature; (4) charting the extracted evidence; and (5) collating, summarising, and reporting the findings. The review process was further guided by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR), in accordance with the recommendations of (Tricco et al. 2018), to enhance transparency and methodological rigour.

STEP 1: DEFINING THE RESEARCH QUESTION

The primary research question guiding this review was: *What does the current proteomic literature in Malaysia reveal about local research interest and engagement in forensic proteomics?* To ensure conceptual clarity and consistency during study selection, a formalised definition of *forensic proteomics* was adopted. In this review, forensic proteomics is defined as the application of protein analysis techniques for forensic investigation purposes (Sacco & Aquila 2023). The aim of the review was to map proteomic studies conducted in Malaysia that involve biological samples relevant to forensic contexts, while also drawing on selected global forensic proteomic literature to contextualise local research gaps and highlight areas requiring further investigation within the Malaysian setting.

STEP 2: SOURCES OF DATA AND SEARCH STRATEGY

The literature search strategy was developed in accordance with the review objectives and predefined eligibility criteria. Electronic database searches were conducted in PubMed, Scopus, ScienceDirect, and EBSCO Academic for studies

published between January 2010 and February 2024. The following Boolean search string was adapted for each database syntax:

("forensic proteomics" OR "mass spectrometry-based proteomics" OR "protein biomarker*" OR "protein analysis") AND ("forensic science" OR "body fluid identification") AND ("Malaysia").

In PubMed, Medical Subject Headings (MeSH) and Title/Abstract fields were applied where appropriate. Searches in other databases were restricted to title, abstract, and keyword fields.

The initial search yielded 835 records. After removal of duplicates, 470 unique records remained for title and abstract screening. Seventy-three studies were deemed potentially eligible and retrieved for full-text assessment. Following full-text evaluation against predefined inclusion and exclusion criteria, 12 studies met the eligibility requirements and were included in the qualitative synthesis. To minimise publication bias and identify potentially missed local studies, a supplementary manual search was conducted via Google Scholar using the same keyword combinations. No additional eligible studies were identified beyond those captured in the database search.

STEP 3: STUDY SELECTION AND DATA EXTRACTION

Eligibility for inclusion was independently assessed by the authors against the pre-defined criteria. The study selection was conducted in two distinct phases. In the initial phase, titles and abstracts were screened for relevance, and any records that clearly failed to meet the inclusion criteria were excluded. In the second phase, the full text of the remaining articles was retrieved and examined in detail, with the inclusion and exclusion criteria rigorously re-applied to ensure consistency.

Mendeley Reference Manager was employed to organise the retrieved literature and to identify and remove duplicate records. Any disagreements regarding the eligibility of specific studies were resolved through discussion with the second author (N.H.H.) to reach a consensus on the final selection. For each study that progressed to the full-text review, relevant data were systematically extracted into a charting form to facilitate analysis. The following information was captured:

- **Bibliographic details:** Author(s) and year of publication.
- **Sample characteristics:** The type of forensically relevant biological sample analysed (e.g., hair, blood, saliva, tissue).
- **Methodological approach:** The primary proteomic technique employed (liquid chromatography–tandem mass spectrometry (LC-MS/MS), two-dimensional gel electrophoresis).

- **Study focus and key findings:** The principal aim of the research and its main outcomes.
- **Forensic relevance:** An assessment of the study's direct applicability or methodological contribution to forensic science, based on the adopted definition of forensic proteomics.

Following duplicate removal, 470 records remained for title and abstract screening. Seventy-three studies met the preliminary eligibility criteria and were retrieved for full-text assessment. After detailed evaluation against predefined inclusion and exclusion criteria, 12 studies were deemed suitable for inclusion in the qualitative synthesis. Reasons for exclusion at full-text stage included non-forensic application, absence of proteomic methodology, review-only articles, and insufficient validation data. This extracted data provided the basis for the narrative synthesis and was later summarised in Table 1 to present an overview of the included studies categorised by biological sample type, year, and research focus.

STEP 4: INCLUSION AND EXCLUSION CRITERIA

Studies were eligible for inclusion if they met all of the following criteria:

- original research articles published between January 2010 and February 2024;
- conducted wholly or partially in Malaysia, or involving Malaysian institutional affiliation;
- employed proteomic techniques, including mass spectrometry-based or gel-based protein analysis;
- analysed biological samples with established forensic relevance (e.g., hair, bodily fluids, tissues, bone, fingerprints); and
- provided sufficient methodological detail to assess proteomic workflows.

Studies were excluded if they:

- were review articles, conference abstracts, editorials, or opinion papers;
- did not involve proteomic analysis;
- focused exclusively on non-biological or non-forensic materials;
- lacked full-text availability; or
- contained insufficient methodological information to permit evaluation.

RESULTS

SEARCH OUTCOME

The systematic database search identified a total of 835 records across PubMed, Scopus, ScienceDirect,

and EBSCO Academic. Following the removal of duplicate records, titles and abstracts of the remaining studies were screened for relevance. This process resulted in 59 articles undergoing full-text assessment. After applying the predefined inclusion and exclusion criteria, 12 studies were deemed eligible for inclusion in this scoping review. The study selection process and reasons for exclusion at each stage are summarised in the PRISMA-ScR flow diagram (FIGURE 1).

CHARACTERISTICS OF INCLUDED STUDIES

The 12 included studies reflect proteomic research conducted in Malaysia between 2010 and 2024 involving biological materials of potential forensic relevance. These studies were categorised according to biological sample type, research focus, and analytical methodology, as summarised in TABLE 1.

Hair samples constituted the most frequently investigated biological material within a forensic-oriented framework. Several studies focused on methodological optimisation for protein extraction and profiling from human hair shafts, alongside the identification of keratin-derived peptide signatures with potential relevance for individual differentiation. In contrast, no Malaysian studies applying proteomic analysis to bone or fingerprint samples for forensic purposes were identified during the review period.

Studies involving bodily fluids and tissues such as blood, plasma, saliva, urine, and soft tissues were more numerous but were predominantly situated within medical or biomedical research contexts. These investigations primarily aimed at biomarker discovery, disease characterisation, or optimisation of proteomic workflows rather than forensic application. Nevertheless, the biological matrices examined are routinely encountered in forensic casework, and the analytical approaches employed are directly transferable to forensic investigations.

TRENDS IN METHODOLOGICAL APPROACHES

Across all included studies, mass spectrometry-based proteomic techniques dominated the analytical landscape. Liquid chromatography–tandem mass spectrometry (LC–MS/MS) was the most commonly employed platform, often complemented by gel-based separation methods such as two-dimensional gel electrophoresis. The consistent use of these high-resolution analytical techniques indicates a well-established technical capacity for advanced proteomic analysis within Malaysian research institutions.

TABLE 1 Proteomic analysis on biological samples in Malaysia by year and type of study

No	Biological Sample	Author (s)	Year	Study Type	Study Title
1	Hair	(Mohamed Nasir et al. 2020)	2020	Proteomic Analysis	Potential use of human hair shaft keratin peptide signatures to distinguish gender and ethnicity
2		(Wong et al. 2019)	2019	Analysis method development	Development of high-performance two-dimensional gel electrophoresis for human hair
3		(Wong et al. 2016)	2016	Analysis method development	A high-yield two-hour protocol for extraction of human hair shaft proteins
4	Bodily Fluid & Tissues	(Rehiman et al. 2022)	2022	Blood proteomes	Fibrinogen isoforms as potential blood-based biomarkers of Alzheimer's disease using a proteomics approach
5		Oothuman et al. (2021)	2021	Urine proteomes	Optimised Preparation of Urine Samples from Acute Meliodosis Patients for In-Solution Proteomic Studies
6		(Al-Wajeeh et al. 2020)	2020	Tissues Proteomes	Comparative proteomic analysis of different stages of breast cancer tissues using ultra high performance liquid chromatography tandem mass spectrometer
7		(Mohamed Bakrim et al. 2019)	2019	Plasma proteomes	Proteomic profiling of Young Adults with Acute Myocardial Infarction
8		(Ibrahim 2017)	2017	Blood proteomes	Serum proteomic analysis of patients with bone tumours using gel-, Lectin- and mass spectrometry based strategies
9		(Rahim et al. 2015)	2015	Saliva proteomes	Can saliva proteins be used to predict the onset of acute myocardial infarction among high-risk patients?
10		(Wan-Ibrahim et al. 2015)	2015	Tissue proteomes	Biomarkers for bone tumours: discovery from genomics and proteomics studies and their challenges
11		(Ab-Rahim et al. 2016)	2016	Blood proteomes	Proteome Comparisons between Pre-Chemotherapy and Post-Chemotherapy Serum of Metastatic Osteosarcoma Patients Reveals Potential Novel Biomarker
		(Ellias et al. 2012)	2012	Saliva proteomes	Proteomic analysis of saliva identifies potential biomarker for orthodontic tooth movement
12		(Ellias et al. 2012)	2012	Saliva proteomes	Proteomic analysis of saliva identifies potential biomarker for orthodontic tooth movement

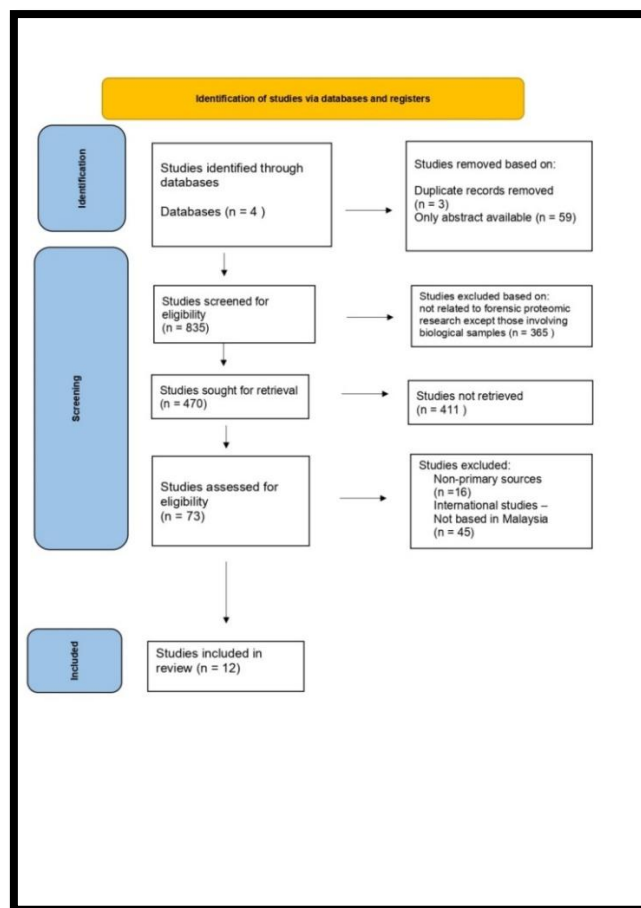


FIGURE 1 PRISMA-ScR flowchart and reasons of studies exclusion

TABLE 2 Comparison of GVP analysis and traditional DNA analysis

GVP Analysis	Comparison	Traditional DNA Analysis
GVPs are present in any part of biological sample regardless of condition (Parker et al. 2016)	Presence	DNA can be absent in degraded and compromised biological samples (Parker et al. 2016)
Proteins have higher chemical stability (Porto et al. 2011)	Resistance	DNA have limited chemical stability (Porto et al. 2011)
Around 60 000 possible GVP alleles in an individual cell (Plott et al. 2020)	Abundance	Trace DNA are typically found in minute biological samples which is less than 100 pg (Van Oorschot et al. 2010)

Temporal analysis of the included studies suggests a gradual increase in proteomic research activity over the past decade, with a more pronounced growth observed in recent years. This trend reflects broader global developments in proteomic technologies, including improvements in instrumental sensitivity, data acquisition, and bioinformatics support.

FORENSIC RELEVANCE AND RESEARCH GAPS

Despite the increasing volume of proteomic research, explicit forensic applications remain limited. Only a small subset of studies directly addressed forensic questions, while the majority were aligned with biomedical or translational

research objectives. This reveals a clear disparity between methodological capability and forensic implementation.

Notably, several biological sample types of high forensic importance particularly bone and fingerprints were entirely absent from Malaysian proteomic research within the scope of this review. This contrasts with international forensic proteomics literature, where these matrices are increasingly explored for human identification, post-mortem interval estimation, and activity-level reconstruction.

Overall, the results indicate that while forensic proteomics in Malaysia is still in an early developmental stage, the foundational infrastructure, expertise, and methodological competence required for forensic translation are

already in place. The principal limitation lies not in technical capacity but in the lack of targeted forensic validation studies and application-driven research.

DISCUSSION

Here is a glimpse into the growing interest of forensic proteomics in Malaysia by various researches on common biological samples in crime scenes.

HAIR

Hair is a commonly encountered biological sample in forensic investigations and is formed through the keratinisation of epidermal keratinocytes (Bragulla & Homberger 2009). Structurally, hair exhibits considerable physical flexibility and resilience, enabling it to withstand harsh environmental conditions more effectively than many other biological materials. This durability is largely attributed to its biochemical composition, which consists predominantly of coiled-coil keratin proteins stabilised by extensive intermolecular disulfide bonds (Bengtsson et al. 2012). These characteristics make hair a valuable source of forensic information, as it can persist at crime scenes long after other biological evidence has degraded.

In forensic practice, hair has traditionally been utilised for human identification and for establishing potential associations between individuals and crime scenes. In addition to genetic information, hair can retain exogenous and endogenous chemical signatures over extended periods. For example, (Aziz et al. 2022) demonstrated that heavy metal exposure can be detected in hair many years after exposure, owing to the long biological half-life of such elements within the hair shaft. These persistent chemical profiles may therefore provide supplementary individualising or contextual information in forensic investigations.

DNA analysis remains the most widely used approach for hair-based identification, particularly when hair roots are present, as mitochondrial DNA can be recovered from the follicular tissue. However, DNA extraction from hair samples is technically challenging and often yields limited results, especially when the hair is fragmented, lacks a root, or has undergone environmental degradation (Landron 2019). In hair samples, several studies reported successful detection of keratin-derived and other structural protein markers despite limited or compromised DNA recovery. The relative resistance of hair shaft proteins to environmental degradation enabled informative proteomic profiling in cases where nucleic acid analysis was incomplete or unsuccessful. These findings suggest that protein-based approaches may provide supplementary evidential value in the examination of degraded hair specimens.

Proteomic studies of hair have also demonstrated the potential to extract information beyond that obtainable from DNA analysis alone. Protein expression patterns and post-translational modifications can reflect biological attributes such as age, sex, and ethnic background, which are not directly encoded at the DNA sequence level. Within the Malaysian context, several studies have contributed to the development of hair proteomics. Notably, a collaborative study between the University of Malaya and a Japanese institution established a high-performance two-dimensional gel electrophoresis protocol to improve protein separation and identification in human hair (Wong et al. 2019). Additional research has demonstrated the feasibility of using hair shaft keratin peptide signatures to distinguish between individuals based on sex and ethnicity (Mohamed Nasir et al. 2020). Earlier methodological work also reported a rapid alkaline-based protein extraction protocol that enhanced protein yield from hair samples (Wong et al. 2016). These studies collectively confirm the technical feasibility of hair proteomics in Malaysia and provide preliminary data on peptide signatures for gender and ethnicity. The logical next step for local forensic research is to expand these pilot studies into larger, population-specific databases to assess the statistical power of such signatures for individual discrimination.

BONES

Bones constitute a highly valuable form of biological evidence in forensic investigations. Owing to their rigid structure and the presence of mineral salts within the osteoid matrix, bones are considerably more resistant to physical, chemical, and biological degradation than most soft tissues (Freckelton 2021). As a result, skeletal remains often persist long after death and are frequently used to establish biological profiles, including age, sex, ancestry, and stature, thereby contributing to the identification of unknown individuals. Bone samples recovered from crime scenes may also provide information about the demographic characteristics of individuals involved in criminal events (Freckelton 2021).

Burnt skeletal remains present particular forensic significance, as arson is commonly employed in attempts to destroy biological evidence. However, exposure to high temperatures causes substantial physical and chemical alterations in bone tissue, which can severely compromise DNA integrity and complicate genetic profiling (Imaizumi 2015). Additional challenges arise from the dense mineralised structure of bone, which necessitates complex extraction procedures and may co-extract inhibitory substances that further hinder DNA analysis (Iyavoo & Goodwin 2022). These limitations highlight the need for alternative or

complementary analytical approaches when dealing with skeletal evidence.

Within skeletal samples, studies highlighted the persistence of collagen and other structural bone matrix proteins across extended post-mortem intervals. The mineralised matrix and cross-linked architecture of bone appear to facilitate long-term preservation of selected protein biomarkers, enabling proteomic characterisation even in environmentally challenged remains. This capacity was particularly evident in samples where conventional DNA profiling yielded limited results. (Choi et al. 2018). Previous studies have demonstrated the utility of bone proteomes in estimating biological age and post-mortem interval (PMI), as well as in differentiating between intrinsic biological changes and environmentally driven diagenetic processes (Procopio et al. 2017; Sawafuji et al. 2017).

To date, no studies have been reported that specifically apply proteomic analysis to skeletal remains within the Malaysian forensic context. Nevertheless, a growing body of local forensic and forensic anthropology research underscores the recognised value of bone as a source of biological information. For instance, age regression models for Malaysian males have been developed using cortical bone histomorphometry (Khan et al. 2018), while population-specific stature estimation studies have employed radiographic measurements of upper limb bones (Ismail et al. 2018). Morphological and morphometric analyses of skeletal features, such as the mental foramen of the mandible, have also been conducted to support forensic identification within local populations (Alias et al. 2017). This existing research ecosystem, focused on skeletal morphology, presents a prime opportunity for interdisciplinary collaboration to integrate proteomic analyses, thereby adding a molecular dimension to traditional osteological methods for identification and PMI estimation.

BODILY FLUID AND TISSUE

Bodily fluids such as blood, semen, and saliva are critical sources of evidence in forensic investigations. Identification of these fluids can establish the origin of the sample, aiding in the identification of victims, suspects, and potential third parties. Beyond source attribution, bodily fluids can provide information on individual characteristics including sex, age, and ethnicity (Bhuptani et al. 2018; Shao et al. 2021). Analysis of these fluids may also reveal the presence of drugs, toxins, or other substances of forensic interest, and contribute to the estimation of post-mortem interval (PMI) (Nolan et al. 2020; Wilke 2021).

Proteomic analysis offers several advantages over conventional DNA-based approaches when examining bodily fluids. Across degraded and low-

template samples, included studies consistently demonstrated the detectability of fluid-specific protein biomarkers under conditions associated with reduced nucleic acid quality (Parker et al. 2016). These observations indicate that proteomic assays may retain analytical utility in compromised specimens, particularly in mixed or environmentally exposed samples (Liu et al. 2019). Additionally, proteomic analysis can detect drugs and toxins within biological samples, providing further context to cause-of-death investigations or substance exposure at the crime scene.

In Malaysia, numerous proteomic studies have been conducted on bodily fluids and tissues, although most focus on medical research rather than forensic applications. For example, (Ibrahim 2017) analysed serum proteomes in patients with bone tumours using gel-, lectin-, and mass spectrometry-based strategies. High-performance instrumentation is commonly employed to improve analytical sensitivity and reproducibility, as demonstrated in studies examining breast cancer tissues using ultra-high-performance liquid chromatography coupled with tandem mass spectrometry (Al-Wajeeh et al. 2020). Sample preparation optimisation has also been a focus, as in the study by (Pakeer et al. 2021), which developed enhanced protocols for urine samples from patients with acute melioidosis. Proteomic profiling has additionally been applied in the context of acute myocardial infarction (Mohamed Bakrim et al. 2019), Alzheimer's disease, and other conditions (Rahim et al. 2015; Rehiman et al. 2022; Wan-Ibrahim et al. 2015).

Saliva proteomics has been explored primarily in relation to oral and systemic health. For instance, Rahim et al. (2015) investigated salivary proteomes to predict the onset of acute myocardial infarction, while (Ellias et al. 2012) identified potential biomarkers associated with orthodontic tooth movement. Although these studies are predominantly biomedical, they demonstrate methodological approaches directly applicable to forensic research, indicating that proteomic workflows are already established and feasible within the Malaysian context.

Overall, while local studies of bodily fluids and tissues are currently medical in focus, they provide a foundational platform for future forensic proteomic research. Existing expertise, instrumentation, and analytical methodologies position Malaysian researchers well to translate these techniques to forensic investigations, supporting the development of more targeted studies on fluids and tissues relevant to crime scene analysis. Therefore, while a direct forensic proteomic literature is sparse, the required technical infrastructure, methodological expertise, and experience with relevant sample types are demonstrably present within the Malaysian biomedical research community. Bridging this gap

requires fostering collaborations between forensic practitioners and proteomics laboratories to re-purpose these techniques for forensic question-solving

FINGERPRINT

Fingerprints represent a challenging but forensically crucial sample where proteomics may offer supplemental data. Proteins within fingerprint residue have the potential to carry genetic information via single amino acid polymorphisms (SAPs) (Parker et al. 2016). Fingerprints are a valuable but limited source of information due to how unique they are to each individual in terms of DNA identification (Borja et al. 2019). They have been an important source of suspect identification for years and will continue to be in future. The physical amount of material which is transferred from the skin to solid surface is highly sample limited and contains genetic information (Hefetz et al. 2019). Proteomics analysis serves as a wonderful opportunity to gain more information about this biological sample as protein has the potential to carry genetic information in the form of single amino acid polymorphisms (SAP) (Parker et al. 2016).

A study by (Deininger et al. 2016) developed an in-situ proteomic method to enable multiple blood signatures on blood fingerprints. The blood mapping on fingerprints was predicted to be useful in reconstructing events of bloodshed. A study in China has developed artificial fingerprints samples using advancements in protein sequence identification so that these samples contain an adequate amount of protein (Lesassier et al. 2019). The development of these artificial fingerprints is to further understand proteomic and genomic analysis for forensic purposes.

There is no proteomic analysis conducted on fingerprints in Malaysia till date. However, fingerprints are commonly studied locally in forensic cases. Fingerprint distributions are commonly studied among different demographics such as age, ethnic group and gender to determine significant differences (Darrelyn et al. 2021; Nayak et al. 2010; Seif et al. 2022). With the growing interest of research in other proteomic analysis on other biological samples, there are many interesting potential insights to discover with the proteomic research on fingerprints in Malaysia.

GLOBAL ADVANCES AND THEIR IMPLICATIONS FOR MALAYSIA

GENETICALLY VARIED PEPTIDES (GVPS) CONCEPT

Proteomics can provide more detailed individual identification are due to the amount of the genetic

information in proteins. In fact, some pioneering work in population genetics relied on protein markers (Woerner et al. 2022). GVPs are crucial in enabling more detailed proteomic analysis as a promising biomolecular profiling technique in forensic science (Dahal et al. 2023). Genetically varied peptides (GVPs) refer to peptides that contain single amino acid polymorphism (SAPs). Like all peptides, GVPs are produced by the proteases in the human body but the codes of these proteins are single nucleotide polymorphisms (SNPs) (Chu et al. 2019). A typical genome contains 3.5-4.3 million SNPs at an individual level (Auton et al. 2015). SNPs are caused by DNA mutation, the most common type of genetic variation where nucleotide substitution occurs in the DNA sequence.

These SNPs then form single amino acid polymorphisms (SAPs) where the amino acid in the protein sequence undergoes substitution as how nucleotide substitution occurred in the SNPs formation throughout a person's DNA (Rizzato et al. 2017). The correlation of SNPs and SAPs ensures high accuracy of individual identification. The presence of a particular SNP is associated with the presence of a particular SAP as SNPs can sometimes alter the amino acid sequence of a protein (Wu & Zeng 2012) (Table 2).

Table 2

In forensics, GVPs are not only an alternative for human identification but can also be used to determine ancestry and distinguish monozygotic twins (Wu et al. 2017). The correlation of SNPs and SAPs enables GVPs to generate detailed individual profiling from various biological samples such as hair, blood and human saliva (Chu et al. 2019). GVPs contain genetic variations unique to every individual as the probability of two people have the exact same genetic variations are low. GVPs are also useful as it can conduct human identification even when DNA is compromised or absent as proteins are more resilient. Cleaved DNA which are often found in incomplete biological samples at crime scenes are also not suitable for DNA profiling thus making GVPs more effective.

MAIN APPROACH TO GVP USE IN FORENSIC SCIENCE

STANDARD GVP IDENTIFICATION

A group of GVPs are selected if they contain information for human identification. These groups consist of GVPs that correspond with SNPs with population frequencies within 10% to 70% (Zhang et al. 2020). The wide range ensures that these groups of GVPs are highly informative and can be applied to any crime evidence containing protein without much suspect initial information in the case.

TARGETED GVP ANALYSIS

Specific GVPs are targeted which are known to be potentially associated with the individuals involved in a certain crime case. This is conducted in instances where a suspect is known to obtain a certain specific genetic disorder or any likelihood of the data given some hypothesis (Woerner et al. 2022). This can also be used if a suspect's ethnicity is potentially known so that the GVP of the ethnic group is targeted (Huang et al. 2015) as shown in TABLE 2.

CASE EXAMPLES OF BREAKTHROUGH IN FORENSIC PROTEOMICS

The potential of proteomics application in enhancing forensic identification in several cases globally are highlighted as below. A brief review of these breakthrough helps highlight the urgent need to explore forensic proteomic studies in Malaysia to bring impact to the forensic field locally.

DISTINGUISH MONOZYGOTIC TWINS

In forensics generally, physical information provided by biological samples is highly dependant on the quantity and quality. Monozygotic twins are produced from single fertilization as they arise from the same zygote causing perfectly identical genomes and little known about their genomic differences (Jonsson et al. 2021). This is the reason why twins cannot be distinguished by DNA analysis till date, despite the presence of new developments in the recent DNA sequencing technology may potentially distinguish these twins (Rolf & Krawczak 2021).

From the proteomics perspective, it is observed that proteins can be used in distinguish the twins with the presence of genetically varied proteins (GVPs). Current research validates more recent proteomic approaches to extract quantitative genetic information especially using hair samples of monozygotic twins. A study by (Wu et al. 2017) applied protein profiling using protein tryptic digests where results revealed samples from identical twins have fewer protein expression differences than unrelated individuals.

ANALYSIS OF BULLET HOLES ON ORGANS

Usually, bullets examination is conducted with a mechanical or ballistic reconstruction of the crime according to standard procedures. This is conducted to identify the projectile using DNA analysis of the biological sample on the surface. The issue lies in the difficulty to identify the projectile. With proteomic approach, (Dammeier et al. 2016) develops a method to determine biological material on the projectile surface by analysing the tissues involved. Various major bovine organs were

penetrated with projectiles experimentally and validated organ classification accuracy of >99% was achieved.

PEPTIDE TOXICOLOGICAL DETECTION

Proteomics application assists in toxicological detection of biological material inserted into a sample. Current DNA-based methods in forensic are unhelpful in detecting purified protein such as protein toxin. Proteomics methods are used in protein and peptide analyses. Conventional proteomic detection methods include merging proteomic methods with in vitro bioassays such as immunoassays and mass spectrometry-based techniques (Duracova et al. 2018). For instance, ricin is from a family of protein toxins that is crucially detected for food production and security purposes such as doping control (Guan & Robinson 2017; Rasetti-Escargueil & Avril 2023). A study have detected dermorphin which originates from the skin of the South American Phyllomedusa frog (Mizoguchi et al. 2011). There is also several research on the detection of synthetic insulins with suspected usage for homicides.

POTENTIAL BIOMARKERS

This study used mass spectrometry-based techniques to identify differentially expressed proteins as potential biomarkers in the post-mortem diagnosis of drowning. A study at Spain performed a pilot proteomic experiment which identified apolipoprotein A1 (ApoA1) and α -1 antitrypsin as the differentially expressed proteins (Hernández-Romero et al. 2020). Results of the study showed higher ApoA1 levels and lower α -1 antitrypsin among drowned individuals and vice versa. This is a pilot approach and future studies are necessary to affirm the preliminary data.

There are several studies on biomarker identification in the determination of the post-mortem interval (PMI). There is also a study that also utilises mass spectrometry for protein markers for the estimation of post-mortem interval. Initially, degradation of skeletal muscle during post-mortem is used to estimate the time since death in the early post-mortem phase. This study utilises an unbiased protein analysis to gain information on systemic post-mortem protein alterations. The differentially expressed proteins selected, eEF1A2 and GAPDH portrays intra- and interspecies degradation behaviour and are potentially used as biomarkers for PMI estimation (Choi et al. 2019). A similar study was also conducted highlighting different proteins such as PDLIM7, TPM1 and ATP2A2 as useful proteins for potential PMI estimation (Battistini et al. 2023).

STRENGTHS, LIMITATION AND RECOMMENDATION

This scoping review provides a structured and comprehensive mapping of the current landscape of proteomic research in Malaysia with relevance to forensic science. By employing a systematic methodology guided by the PRISMA-ScR framework, the review offers a clear and reproducible account of existing local research activity and identifies a critical nexus between advanced technical capacity and untapped forensic application.

Several limitations should be acknowledged. The primary constraint is the scarcity of studies with an explicit forensic focus, which necessarily limits the breadth of application-specific findings that can be discussed. While a thorough literature search was conducted, it remains possible that some relevant studies, particularly older publications not fully digitised or in lesser-known local journals, were not captured. Furthermore, by concentrating the analysis on biological sample types, the review may not encompass all proteomic research with potential forensic relevance, such as studies on microbial communities associated with decomposition.

To address these gaps and capitalise on the identified opportunities, a set of targeted recommendations is proposed. These are derived directly from the review's findings, which highlight a strong foundational proteomics infrastructure awaiting forensic translation.

First, strategic funding initiatives should encourage and facilitate collaborative projects between forensic science institutions and established biomedical proteomics laboratories. Such partnerships are essential to pivot existing expertise towards forensic problem-solving. Second, research efforts should prioritise the development and validation of standardised protein extraction and analysis protocols optimised for forensically challenging samples prevalent in the local context, such as environmentally degraded hair, burnt bone, or aged fingermarks. Finally, exploratory work should begin to establish population-specific databases of protein markers, including genetically variant peptides (GVPs), using the high-resolution mass spectrometry platforms already available within the country. This would provide the statistical framework necessary to move proof-of-concept studies towards validated forensic tools. By implementing these recommendations, future research can build directly upon the foundation outlined in this review, thereby accelerating the integration of proteomics into the Malaysian forensic science toolkit and enhancing investigative capabilities.

Despite the demonstrated analytical robustness of proteomic approaches, their routine implementation within Malaysian forensic

laboratories remains limited. Several factors may contribute to this translational gap. First, mass spectrometry-based platforms require substantial capital investment, specialised instrumentation, and highly trained personnel, which may exceed the operational capacity of standard forensic laboratories. Secondly, forensic casework prioritises validated, court-tested methodologies such as Short Tandem Repeat (STR) profiling, which benefit from established accreditation frameworks and judicial acceptance. Proteomic workflows, although analytically promising, have yet to achieve equivalent standardisation and admissibility benchmarks within the Malaysian legal context. Additionally, forensic laboratories often operate under resource constraints and heavy case backlogs, limiting opportunities for the integration of novel research-based methodologies. Finally, the absence of nationally endorsed validation guidelines specific to forensic proteomics may contribute to institutional reluctance towards implementation. Addressing these structural and regulatory barriers will be essential for bridging the gap between academic proteomic research and operational forensic practice in Malaysia.

CONCLUSION

This scoping review reveals that while forensic proteomics is an emerging global field with demonstrated utility, focused research within Malaysia remains nascent. The analysis highlights a distinct dichotomy: a scarcity of studies with explicit forensic aims, yet a strong and growing local capacity in advanced proteomic techniques, largely situated within biomedical research. This existing expertise, applied to forensically relevant sample types like blood and hair, forms a robust foundation for translation. Proteins offer significant advantages for forensic science, particularly their stability and abundance in degraded evidence. The ongoing evolution of proteomic technology, including more sensitive mass spectrometers and improved bioinformatics tools, continues to expand its potential. For the Malaysian context, these advancements present a clear opportunity. Future innovations, alongside strategic collaborations between forensic practitioners and proteomics laboratories, are essential to bridge the identified research gap. Such efforts would enable the development of localised, reproducible workflows, transforming proteomic analysis from a promising complementary tool into a practical asset within the Malaysian forensic landscape, ultimately enhancing investigative capabilities.

ABBREVIATIONS

ApoA1	Apolipoprotein A1
ATP2A2	ATPase sarcoplasmic/endoplasmic

	reticulum Ca ²⁺ transporting 2
DNA	Deoxyribonucleic Acid
eEF1A2	Eukaryotic Translation Elongation Factor 1 Alpha 2
GAPDH	Glyceraldehyde-3-Phosphate Dehydrogenase
GVP	Genetically Varied Peptides
PDLIM7	PDZ and LIM domain 7
PMI	Post-mortem Interval
PRISMA-ScR	Preferred Reporting Items for Systematic Reviews and the Meta-Analysis extension for Scoping Reviews
SAP	Single Amino Acid Polymorphisms
SNP	Single Nucleotide Polymorphisms
TPM1	Tropomyosin 1

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