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REVEALING THE ROLE OF PROTEINS IN THE FIELD OF FORENSIC INVESTIGATIONS

Muhammad Kaleem[1*], Arooj Munawar[1], Afshan Maqbool[2]

[1]Department of Forensic Science, The Islamia University of Bahawalpur, Pakistan
[2]Akbar Institute of Medical Sciences, Bahawalpur, Pakistan

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ABSTRACT

Forensic science, a multidisciplinary domain, employs a myriad of techniques to elucidate criminal investigations and legal disputes. Proteins, among the diverse array of biomolecules, have ascended as indispensable entities, playing a cardinal role in forensic applications. This review aims to offer an exhaustive overview of the importance and applications of proteins in forensic science, with an emphasis on their critical function as biomarkers and molecular tools. In the sphere of forensic identification, proteins have demonstrated their worth in determining the identity of individuals, both alive and deceased. The analysis of human DNA is one of the most renowned protein-based applications. Although DNA profiling is the gold standard, advancements in proteomics have ushered in supplementary methods such as protein profiling and peptide-based identification. These protein-centric techniques provide alternative avenues for forensic specialists, particularly when DNA samples are degraded or absent. Proteins have also been extensively utilized in crime scene analysis, where their presence can divulge crucial clues about the transpired events. Bodily fluids such as blood and saliva contain specific proteins that assist in identifying the offender or the victim. Moreover, the examination of gunshot residue, hair, and other trace evidence involves the detection of distinct protein markers, contributing to the reconstruction of the crime scene and enhancing the evidentiary value of the findings. Postmortem investigations have reaped substantial benefits from the incorporation of protein analysis techniques. The determination of the time of death, identification of the cause and manner of death, and differentiation between ante-mortem and postmortem injuries have been rendered more accurate through the study of protein degradation patterns and postmortem alterations. These insights are invaluable in delivering accurate testimonies and pivotal evidence in court proceedings. The emergence of advanced technologies, such as mass spectrometry and immunoassays, has broadened the scope of protein applications in forensic science. Mass spectrometry facilitates rapid and sensitive detection of proteins in minute quantities, proving particularly beneficial in the analysis of trace samples. Immunoassays leverage the specificity of antibodies to detect and quantify target proteins, enabling the identification of key biomarkers with high precision. In conclusion, proteins have surfaced as vital contributors in the realm of forensic science, offering a diverse array of applications in forensic identification, crime scene analysis, and postmortem investigations. The ongoing advancements in proteomics and related technologies promise to further augment the capabilities of these protein-centric approaches, ensuring their sustained utility in the quest for justice and truth.

Key Words: Proteins; Post Mortem Interval; Techniques; Biological Samples; Forensic Investigations; Blood; hemoglobin.

[*]**Corresponding Author.** E-mail: kaleemyaseen322@gmail.com

INTRODUCTION

A multidisciplinary subject called forensic science is essential to the investigation of crimes and court cases. Its main goal is to examine and analyze physical evidence in order to determine the truth regarding criminal events. The use of proteins as crucial tools in the resolution of complex criminal cases is one of the important developments in forensic science that has occurred as a result of technological and scientific advances over time. Proteins are intricate proteins that are present in all living things and are frequently referred to as the "building blocks of life." They perform a number of crucial jobs within cells, tissues, and organs, and because of their many functions, they are great tools for forensic scientists trying to decipher

crucial information from the evidence gathered at crime scenes. A new area of investigation has been made possible by the study of proteins in forensic science, which offers novel perspectives on the identities of the perpetrators, the victims, and the circumstances of the crime. In this essay, we examine the crucial function that proteins play in forensic science and examine how they can be used to identify people, ascertain the cause and timing of deaths, analyze bodily fluids, and comprehend the dynamics of crime scenes. We intend to shed light on the crucial part that proteins have played in influencing the current forensic investigation landscape by looking at these important components [1].

INDIVIDUAL IDENTIFICATION BASED ON PROTEINS

Proteins are essential for identifying people from small biological samples discovered at crime scenes, especially those with genetic differences like Human Leukocyte Antigens (HLAs) and Short Tandem Repeats (STRs) [2]. By providing great sensitivity and specificity in connecting suspects to the evidence, the advent of modern protein profiling techniques, including mass spectrometry and gel electrophoresis, has revolutionized forensic identification. Proteins are frequently used by forensic scientists to determine the postmortem interval and determine the cause of death in circumstances involving suspicious deaths. Understanding the sequence of events leading to the victim's death and reconstructing the crime scene are made easier by changes in protein breakdown patterns and postmortem biochemical processes. Blood, saliva, semen, and sweat are just a few of the biological fluids that have been discovered at crime scenes that can be identified using forensic proteomics. These flexible identifications play a key role in connecting suspects to the crime and can be extremely useful in cases involving sexual assault. Trace levels of proteins at crime scenes can reveal important details about what happened during the commission of a crime. The chronology of events can be reconstructed by forensic professionals by examining blood splatter patterns, contact DNA, and other biological clues, which helps the detectives comprehend the dynamics of the crime scene.

Important functions of proteins in forensic science

Due to their distinctive properties and prevalence in biological evidence, proteins play a significant role in forensic research [3]. Protein analysis is a tool that forensic scientists employ to examine evidence, identify people, and conduct a variety of criminal investigations. Proteins play the following important roles in forensic science:

Recognizing bodily fluids

Identification of body fluids is a critical component of forensic science because it can provide important details about a crime scene, a victim, or a suspect. Since proteins are prevalent in bodily fluids and can serve as biomarkers for particular fluid types, they are important in this process. Hemoglobin, a protein that is present in red blood cells and is in charge of delivering oxygen, is found in blood. The Kastle-Meyer test, the luminol test, or immunological techniques that focus on particular hemoglobin antigens can all be used to identify it. Blood serum contains a variety of proteins, including albumin, globulins, and fibrinogen. These proteins can be found using immunological and electrophoretic methods to identify blood. The enzyme alpha-amylase, which is present in saliva, aids in the first digestion of carbohydrates. This enzyme can be used to detect the presence of saliva. Cystatin proteins can be used to identify saliva and are found in it [4]. PSA or prostate-specific antigen, is a protein that the prostate gland creates. It is frequently employed to determine whether semen is present in forensic materials. Semenogelin is a significant protein found in semen, it can also be used to identify semen. Human Spermatozoa Antigen (HSA)is a protein that can be used to detect sperm-containing vaginal fluid. It is located in the acrosome of sperm cells. Uromodulin, also known as the Tamm-Horsfall protein, is a glycoprotein that is present in large quantities in urine and can be used to detect it. To identify these proteins, forensic scientists employ a variety of procedures, including immunological ones like the enzyme-linked immunosorbent test (ELISA), Western blotting, or more sophisticated ones like mass spectrometry. It's crucial to remember that forensic analysis typically entails examining many markers to improve identification precision [5].

In criminal investigations, protein-based body fluid identification can be extremely helpful since it can be used to prove the existence of particular bodily fluids at a crime scene, connect suspects to victims, or offer proof for the reconstruction of events. However, because the outcomes of their investigations might have a big impact on judicial procedures, forensic scientists must follow stringent guidelines and make sure that their work is accurate and reliable.

DNA testing

In forensic science, nuclear DNA is analyzed using PCR and short tandem repeat (STR) analysis. This process is known as DNA profiling. Proteins can be engaged in a number of biological activities related to DNA and are essential for the proper operation of cells and organisms. The investigation of postmortem interval (PMI), which is the period of time following a person's passing, may be one possible use of proteins in forensic research. In forensic investigations, estimating PMI is crucial in order to establish timelines of occurrences and exclude suspects. After

death, some proteins decay in a predictable way, and researching these patterns may reveal important details about the postmortem period. One method to measure the amount of time since death is to look at the changes in particular proteins in different tissues. However, this field of study was only just getting started in 2021, therefore it might not be widely used in routine forensic processes [6].

Forensic Serology

A subfield of forensic science, forensic serology deals with the identification and evaluation of body fluids, particularly blood, in criminal investigations. Because they are plentiful in human fluids and can be useful for identifying people and understanding the circumstances of a crime, proteins play a vital role in forensic serology. Blood typing is one of the most fundamental uses of protein analysis in forensic serology. Blood contains a variety of proteins, including the Rh factor and ABO blood group antigens. Using these proteins as a guide, forensic investigators can identify a person's blood type. When blood stains are discovered at a crime scene, blood typing can be useful in cases to help eliminate or include suspects in criminal investigations. To distinguish between various kinds of physiological fluids, forensic serologists can identify particular enzymes in bodily fluids, such as amylase and pepsin in saliva. This can assist in determining whether a stain is made up of blood, saliva, semen, or another fluid, helping to reconstruct what happened at a crime scene. It is frequently the initial step in the DNA analysis procedure, even though DNA profiling is the industry standard for person identification in forensic investigations. In order to liberate the DNA for examination, this extraction technique often entails dissolving cell membranes and proteins. Specific proteins that are present in physiological fluids can be employed for more accurate identification. For instance, the protein known as prostate-specific antigen (PSA), which is found in semen, can be utilized to detect the presence of semen stains and provide proof of sexual assault or other related crimes. Over time, proteins are subject to degradation, which may affect their capacity to offer insightful data. To prevent protein degradation and protect the integrity of the evidence, forensic serologists use a variety of preservation and storage methods, such as freezing or refrigeration.

Despite the fact that forensic serology has been a useful tool in criminal investigations, modern DNA analysis methods for person identification, such as PCR and STR analysis, have essentially superseded conventional protein-based serological procedures. Modern forensic science uses DNA profiling as the principal tool for determining identity since it provides a better level of specificity and sensitivity. However, in some circumstances, protein analysis still contributes to the identification of body fluids and provides additional data.

Fingerprint Enhancement

In the past, forensic scientists have used a variety of physical and chemical techniques to enhance the clarity and visibility of latent fingerprints left at crime scenes. Applying finely powdered materials (such as black, white, or magnetic powder) to the fingerprint residue to adhere to it and make it visible against the surface it was deposited on. Applying chemical reagents that react with the fingerprint residue to make it more noticeable. For instance, latent prints are frequently created on porous surfaces using ninhydrin. Cyanoacrylate fuming's a method for creating fingerprints on non-porous surfaces by using superglue (cyanoacrylate)Applying a dye solution to a surface to make fingerprints more noticeable. Lifting and visualizing fingerprint impressions from surfaces using electrostatic charges. Although many instances have seen success using these conventional ways, research is still being done to find more sophisticated and efficient methods [8]. After my previous update, it's probable that innovations in the usage of proteins or other biomolecules for fingerprint improvement have taken place [23]. Researchers and forensic professionals are constantly looking for new technology and procedures to increase forensic evidence and fingerprint identification.

Forensic Anthropology

Forensic anthropology is a discipline of study that applies anthropological ideas to the examination of human remains for legal reasons, frequently in the context of criminal investigations and the identification of unidentified persons. Despite the fact that DNA analysis is frequently employed in forensic investigations, other biomolecular methods, such protein analysis, can also be very useful in forensic anthropology. Using different methods and procedures, forensic anthropologists can extract, examine, and interpret protein data from skeletal remains or other biological samples. Among the crucial aspects of forensic anthropology involving proteins are Proteomic analysis and this is the comprehensive examination of proteins in a living organism or material. Proteomic methods can be used by forensic anthropologists to locate and measure the presence of particular proteins in human remains. This can reveal information about the person's age, sex, and perhaps even some medical issues or genetic markers [22]. The human body's specific proteins experience predictable changes over time. Forensic anthropologists can determine an individual's age at the time of death by examining the amounts of particular proteins in samples of bone or teeth. The sex of a person can be ascertained by looking at proteins

linked to sexual dimorphism, which refers to biological variations between males and females. Proteomic studies can help with identification when the deceased persons identify is unclear. Forensic anthropologists can assist in establishing identity by comparing protein profiles from the remains with potential reference samples, such as family members [21]. Post-mortem intervals also referred to as the time since death, can be calculated using the breakdown of specific proteins in human remains. The ability to pinpoint the time period during which a crime may have occurred depends on this information. Environmental elements like temperature, humidity, and the presence of scavengers can have an impact on proteins. Forensic anthropologists can better evaluate the circumstances of a death and the postmortem handling of the remains by comprehending the protein changes brought on by Taphonomic processes. While protein analysis can yield insightful data, it is crucial to keep in mind that it is frequently combined with other forensic methods, such as DNA analysis, osteological examination, and facial reconstruction, to build a thorough portrait of the subject and support the investigation. Additionally, while handling human remains and examining biological samples for legal purposes, forensic anthropologists are required to adhere to strict regulations and ethical considerations [7].

Forensic Toxicology

Forensic toxicology is the scientific discipline that deals with the analysis of drugs, poisons, and other toxic substances in biological samples taken from living or deceased individuals. The aim of forensic toxicology is to determine the presence, concentration, and effects of these substances in order to understand their role in accidents, crimes, or deaths. While traditional forensic toxicology mainly focuses on analyzing small molecules, such as drugs and their metabolites, proteins have gained increasing attention in recent years as potential biomarkers for toxicological investigations. Proteins are large, complex molecules that play crucial roles in various biological processes. Changes in protein expression or modifications can provide valuable insights into the effects of toxic substances on the body [9,61]. There are some ways forensic toxicologists utilize proteins in their investigations. It's important to note that utilizing proteins in forensic toxicology requires specialized techniques and equipment, as proteins are much larger and more complex than small molecules typically analyzed in traditional toxicology. Mass spectrometry, immunoassays, and various other proteomic methods are commonly employed for protein analysis in forensic settings.

ELISA USED TO DIFFERENTIATE BLOOD AND PERSONS

The Enzyme-Linked Immunosorbent Assay (ELISA) has been a fundamental technique in immunology for over a century, providing a robust method for the detection of specific constituents in blood samples. As highlighted by Cattaneo, ELISA has proven pivotal in identifying various proteins, including human albumin, Immunoglobulin G (IgG), and beta thrombo-globulin in bloodstains. Moreover, it has been employed to evaluate the stability of proteins in desiccated blood samples. In modern applications, Horseradish Peroxidase (HRP) is commonly utilized in ELISA due to its distinctive capacity to catalyze the formation of colored products. The absorbance of these colored products can be quantitatively assessed using Ultraviolet-Visible (UV-Vis) spectroscopy, facilitating precise measurements and interpretations, thereby augmenting the accuracy and reliability of ELISA. The amalgamation of HRP and UV-Vis-spectroscopy has considerably enhanced the capabilities of ELISA, cementing its status as an essential instrument in immunological research and diagnostics. This technique is employed to distinguish between aged and fresh blood samples. In most instances, desiccated blood samples are found at crime scenes, which can be collected and analyzed using this technique to determine whether the blood is old or fresh, and to differentiate whether it belongs to a human or another animal. Proteins such as hemoglobin, an oxygen-carrying protein present in blood, can also be used to differentiate blood samples. This can further be used to distinguish between individuals, whether they are identical or different, solely based on this protein present in our blood, known as hemoglobin and there are many other proteins which can be used in forensic science and these are enzymes, hormones and keratins [76].

ANALYSIS OF HAIR AND FIBER FOR PROTEINS

Hair loss is a natural process and we lose 50 to 100 hairs every day. In violent situations, such as crimes, hair may be pulled out and left at the scene. Hair is often found at crime scenes and can play an important role in solving cases [26, 27]. Hair has two parts: the hair shaft (the visible part) and the root (the part inside the skin). The shaft has three layers that help identify different populations. The root of the hair is located in the hair follicles from which the hair grows. Follicles have three growth phases, anagen (active growth), catagen (transitional phase) and resting phase (resting phase). When hair is removed in the anagen phase, some follicular tissue may also be removed, but the hair follicle still has the ability to grow new hair [28,29]. Forensic medicine is used to analyze hair

morphology and determine the origin, species and identity of body parts. Something in a person's hair can also indicate their occupation. However, this method had limitations and could lead to false positive results [30]. Currently, hair is typically analyzed for DNA or protein. Forensic scientists classify hair growth stages and choose the appropriate analysis method. Although anagen hairs are best for DNA analysis, most hairs found at crime scenes are in the telogen phase and have little cellular material. Telogen hair can be classified into three types based on the material attached to the hair root. In forensic science, most hairs found at crime scenes are type 1 telogen hairs that are not suitable for standard DNA (STR) genotyping. Therefore, scientists often use mitochondrial DNA (mtDNA) or protein analysis [31,32]. mtDNA has a higher copy number and is more stable than nuclear DNA (nuDNA), making it a good alternative. It is especially useful for tracing maternal lineage. There are three methods for analyzing mtDNA. Genotyping hypervariable regions, control regions or the entire mitochondrial genome. mtDNA analysis of even small pieces of hair has a high success rate. However, it is more expensive, more labor-intensive and less individual-specific than STR genotyping [33,34]. Recent studies have shown that hair contains large amounts of fragmented nuDNA. New techniques are being developed to recover small fragments of DNA from hair, including the use of specific extraction protocols, construction of single-stranded libraries, and shotgun sequencing [35]. Protein analysis methods have also improved. Nonsynonymous single nucleotide polymorphisms can be inferred from protein sequences for individual identification. Proteins can help identify body fluids, tissues, species, and even fingerprints [36,37,38]. In summary, hair analysis is an important part of forensic genetics. In very important cases, valuable genetic information can be obtained. The field continues to advance, particularly in DNA and protein analysis techniques [26,39]. Forensic scientists have developed different techniques to study hair because the human body has a large number of hairs, approximately 5 million, and hairs are exchanged when two people come in contact. Forensic scientists examine hair to determine whether it is of human (as opposed to animal or synthetic) source, whether the source is male or female, the race of the source, and whether the source is poisonous. Examples include whether the person was sexually active. Have you ever been assaulted or drugged? These determinations can be made using basic analytical techniques such as conventional microscopy. However, although several techniques have been developed to provide more accurate information about the origin of retrieved or retrieved hair, only a few are admissible in court. Ion microbiology is the process of measuring the number and mass of ions released from a hair sample as a result of exposure to ion radiation. The theory behind using ionic microorganisms to identify is that two hairs from the same source have similar levels of a specific ion, but hairs from different sources contain less of a specific ion. Its amount varies [40]. One of the key elements of forensic science is the study of hair and fibers, and proteins are important in this field. Hair and fibers are often found at crime scenes and can be very helpful in connecting suspects, victims, and crime scenes. One of the factors tested to aid in criminal investigations is the protein content of hair and fibers. Hair analysis shows that keratin, a type of protein, makes up the majority of human hair. The structure of the hair consists of the fibrous protein keratin, which gives it strength and durability. By examining the protein composition of hair, forensic experts can determine whether the hair at a crime scene is human or another animal species. Hair analysis can also reveal a person's racial or ethnic background, but this method is less accurate. Looking at the hair shaft can also tell if the hair has been chemically treated (dyed). It may be important in certain situations. Like hair, fibers are also composed of different proteins depending on the nature of the hair. Where did they come from? Synthetic fibers such as nylon and polyester are made from polymers produced from petrochemicals, while natural fibers such as wool and silk are mainly made from protein. The proteins in the fibers can be examined by forensic experts to determine whether they are of animal or synthetic origin. This helps reduce the scope of clothing and other materials used in crimes. Insights from fiber analysis can reveal the potential movement of fibers between suspects, victims, and victims, thereby pinpointing the facts or presence of a suspect in a specific location. It can also be used to confirm or deny. and crime scene [25]. Despite its value, it is important to remember that fiber and hair analysis has limitations. Because hair and fibers may be shared by many people or obtained from multiple sources, it is impossible to identify specific individuals by their use. In addition, contamination and subsequent migration of hairs and fibers can complicate the interpretation of findings [24]. If necessary, the forensic doctor will also perform DNA analysis.

Extraction of proteins from hair

Structures characterized by keratinization, such as hair, nails, horns, and animal feathers, are mainly composed of protein, accounting for about 80% of their total mass. Human hair proteins are mainly classified into two major groups. One is the tough alpha-keratin that forms the fibrous intermediate strands, and the other is the matrix protein that forms a non-fibrous matrix. Hard alpha keratins can be divided into two subfamilies, each consisting of

several different members. Matrix proteins are usually classified as high sulfur proteins and high tyrosine proteins. Although post-translational modifications of α-keratin have been reported in animal hair, similar modifications of α-keratin in human hair remain largely unknown. Extraction of these proteins for analytical purposes is a major challenge due to their extensive cross-linking through disulfide bonds. Extraction protocols often require reducing the presence of denaturants and S-alkylation under extreme pH conditions, which destabilize protein production and protein hydrolysis. Another method can be protein extraction from the lower cells of the hair follicle. However, this method often provides insufficient protein yields suitable for analysis, and the resulting proteins are not derived from fully nucleated mature structures. In this article, we introduce a new extraction method called Shindaiho, which was developed at Shinshu University. This method allows the easy extraction of protein components from human hair, wool, chicken feathers, mouse hair and human nails without using detergents. This manuscript provides the first evidence of phosphorylated species in human hair alpha-keratin and hard matrix proteins that may have implications for our understanding of hair structure and function [41–49].

Procedure of proteins extraction from hair

Hair samples taken from human subjects were first subjected to a cleaning process using ethanol. Then, excess fats were removed using a 2:1 mixture of chloroform and methanol. Then, degreased hairs were mixed with a solution consisting of 25 mM Tris-HCl (pH 8.5), 2.6 M thiourea, 5 M urea, and 5% 2-mercaptoethanol (2-ME). They were incubated at 50 °C for 1 min. A period between 1 and 3 days. The resulting mixture was filtered and centrifuged at 15,000 g for 20 min at room temperature. The supernatant obtained after centrifugation was used as the hair protein fraction. The pellet was collected, washed with distilled water and used as the extracted hair sample.

The hair protein fraction was dialyzed against 2 L of distilled water for 5-7 changes and then lyophilized and completely dried in a silica gel box. The dry weight of the sample was measured using an electronic scale, and the hair protein fraction and the extracted hair sample (residual fraction) were confirmed. In addition, the Bradford method using the Bio-Rad protein assay was used for protein quantification.

Gel electrophoresis/two-dimensional electrophoresis (2DE-EP), in this step Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using 8-18% Dahl gel according to the Laemmli method. The proteins in the gel were stained with 0.1% Coomassie Brilliant Blue R250, 10% acetic acid, and 40% ethanol for 1 hour, and then destained with 10% acetic acid and 40% ethanol. Two-dimensional electrophoresis was performed according to the O'Farrell method using amphorin 2% (pH 3.5-10).

The next step is western blot analysis and in this step proteins separated on SDS polyacrylamide gels were transferred onto nitrocellulose membranes in a solution containing 20 mM Tris-glycine (pH 8.3) and 10% methanol. Membranes were blocked with 5% BSA in TBS-T (25 mM Tris-HCl, pH 7.2, 50 mM NaCl, and 0.5% Tween-20) at room temperature and reacted with antibodies against phosphotyrosine, phosphoserine, and phosphothreonine. After extensive washing in TBS-T, membranes were incubated with peroxidase-conjugated secondary antibodies against rabbit immunoglobulins in TBS-T for 1 h at room temperature. Blots were visualized using the Super-Signal CL-HRP substrate system [50,55].

Identification of Phosphorylated Proteins

Using 32P-phosphate, Hyde et al showed the phosphorylation of hyper-keratinized intermediate chains at the ends of human, bovine and sheep hair follicles. This study was conducted with the aim of revealing the post-translational changes of hard alpha-creatin in its mature structure. Using phosphoserine, phosphothreonine and phosphotyrosine antibodies, we found that human hair proteins contain phosphorylated species. Creatine cross-reacted with phosphoserine and phosphothreonine antibodies, with acidic creatine type I showed higher reactivity than creatine type II. Matrix proteins also reacted with phosphoserine antibodies. Absence of cross-reactivity when phosphoserine (0.2 mM) or phosphothreonine (0.2 mM) is added to the primary antigen-antibody reaction mixture indicates the presence of phosphorylated forms of keratin and matrix proteins. However, no reactivity with phosphotyrosine antibodies was observed under the given conditions. Intermediate filament components such as Desmin, vimentin, glial fibrillary acidic protein, and neurofilament are phosphorylated by several protein kinases such as cAMP-dependent protein kinase, protein kinase C, and Ca2+/calmodulin-dependent protein kinase II. This phosphorylation can lead to depolymerization. Epithelial cell keratins, including keratin type I 18 and keratin type II 8, are known phosphoproteins containing phosphoserine and phosphothreonine. Phosphorylation of intermediate chain-associated proteins such as Profilaggrin is also known. During cornification, relative dephosphorylation and proteolysis of Profilaggrin to filaggrin occurs, and the interaction of filaggrin and keratin filaments forms keratin patterns in the cornified epithelium. The biological function of matrix proteins with high serine content (9-23%) in cornification is unknown [56].

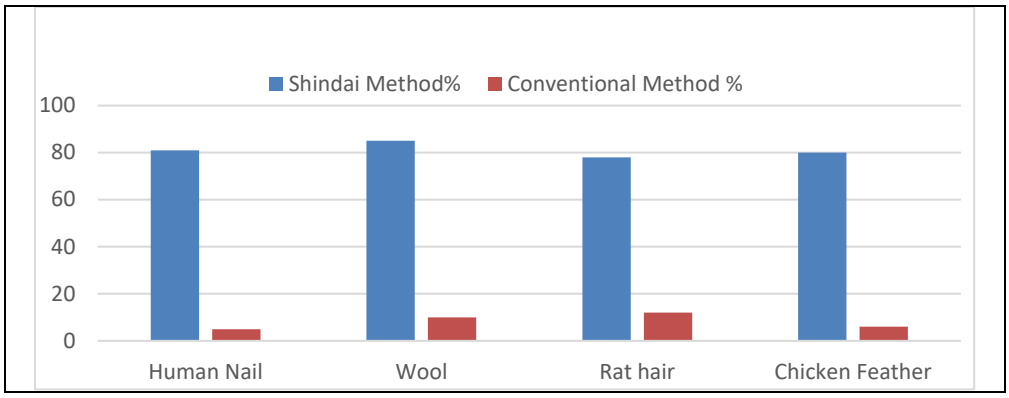

Figure 1: Percentage yield of different samples between conventional and Shindai method

Protein analysis

Protein detection in hair requires different steps such as washing, protein extraction, purification, quantification and data analysis. The most important steps are chemical treatment and trypsin digestion. The length of hair used for analysis is also important. We found that 2 cm of hair is suitable for protein analysis. The amount of protein produced depends on the length of your hair. The amount of keratin (a type of protein) is about the same, but as the length of the hair decreases, the amount of non-keratin decreases drastically. This affects the detection of single amino acid polymorphisms (SAPs) that are important for individual identification. Therefore, the shortest hair shaft length identified is between 0.5 and 2 cm. Using a very sensitive protein extraction method called "direct extraction method", good results can be obtained even with genetic peptides (GVP) that are less than 1 cm in length. When the amount of hair is limited, it is important to extract as much genetic information as possible. We developed a method for the simultaneous extraction of hair proteins and the mitochondrial genome. This method provides valuable two-dimensional information of proteins and mtDNA when working with small amounts of hair samples [57-60].

ESTIMATING THE POST-MORTEM INTERVAL (PMI)

The postmortem interval (PMI) is the period of time between the moment of a person's death and the finding or examination of their body. In forensic investigations, estimating PMI is essential for reconstructing the sequence of events that led to a person's death and creating an event timeline. While there are several ways to estimate PMI, using proteins as indicators has drawn more attention lately. Protein levels and degradation patterns postmortem alterations can offer useful information for PMI estimate [10]. Forensic medicine faces a major challenge in accurately determining postmortem interval (PMI). The first 24 hours after death provide a relatively accurate estimate of PMI based on observable body changes such as body temperature, liver death, and morbidity. However, estimating PMI at later stages is less accurate and remains a complex problem for forensic investigators. Chemical changes in corpses are a promising area for PMI research. Proteomics is particularly useful for identifying PMI biomarkers because it can detect and quantify subtle differences between large numbers of proteins. For example, Hunsaker et al used two-dimensional gel electrophoresis to find that almost all brain proteins in mice had constant expression up to 4 hours after death. There are many other scientists who also used this technique to detect the degradation of specific proteins. The researchers also found that the use of protein metabolites such as urea nitrogen and creatinine as internal standards can significantly improve the accuracy of PMI estimation in combination with potassium ions. With the advent of metabolomics, there is renewed interest in estimating PMI by screening metabolites in cadaver tissues. Currently, many researchers are trying to use new techniques, especially metabolomics, to investigate postmortem chemical changes and estimate PMI. For example, Kaczynski et al used GC-MS-based metabolomics to identify potential metabolite biomarkers of PMI at 48 h postmortem. Du T et al used untargeted LC-MS-based metabolomics. We scan a large number of defined peaks from mouse skeletal muscle within 168 h of death and select a large number of PMI biomarkers. Quantification of specific metabolites has been used to estimate PMI after 120 hours and there is a significant correlation between these metabolites and PMI [62-67].

APPLICATIN OF PROTEINS

At the end of life, your body undergoes a series of changes at the molecular level. The production of new proteins stops, but the degradation of existing proteins continues. Certain types of RNA molecules, such as

microRNAs, are particularly unstable and degrade rapidly after death. By comparing the concentration of these unstable RNA molecules with the concentration of proteins, scientists can estimate the time elapsed since death. Proteins undergo various post-mortem modifications such as cleavage, denaturation and deamidation. These changes are influenced by environmental factors such as temperature and humidity. By studying these changes in specific protein or peptide markers, scientists can estimate postmortem interval (PMI). Certain proteins in the body break down in a predictable way over time, acting as a so-called molecular clock. One of these molecular clocks is amino acid racemization. A postmortem process known as aspartate racemization occurs in a predictable manner in certain proteins and can be used to estimate PMI [20]. Modern proteomics technologies enable the analysis of a wide range of proteins present in post-mortem samples. By comparing the abundance and degradation patterns of different proteins between samples with known and unknown PMI, researchers can build statistical models to estimate PMI. In addition to these protein-based methods, the presence and activity of certain insects on decomposing remains may also provide clues about PMI. For example, certain species of flies have predictable life cycles, and their growth stages indicate how long it has been since they died [11]. Although these protein-based methods are promising for PMI estimation, it is important to remember that this is an early research area and has several limitations. Factors such as individual differences, environmental conditions, and the nature of death can all affect the accuracy of these estimates. As research advances in this area, the combination of multiple strategies and markers may improve the accuracy and reliability of these techniques. However, before these techniques can be applied in real-world forensic investigations, they must be carefully evaluated and fine-tuned by forensic experts and researchers. This is an important step to ensure the validity and reliability of these techniques from a forensic perspective. These are exciting times in the field of forensic science, as these new technologies open up new opportunities to understand postmortem processes and provide more accurate PMI estimates. This could have important implications for criminal investigations and our understanding of death itself [19].

Identification of Biological Samples

In several disciplines, such as biochemistry, molecular biology, and forensic science, protein identification of biological samples is a standard practice. It entails examining the presence and quantity of particular proteins in a sample to learn more about their makeup or properties. Here is a general explanation of how this procedure operates:

Sample Gathering: The biological sample that is of interest must be collected first. Any biological material, including blood, saliva, tissue, hair, and others, could be present.

In order to separate the proteins from the surrounding biological material, the sample that was obtained needs to be treated. Several methods, including homogenization, centrifugation, and protein extraction, may be used in this [12]. The next step is to separate the proteins depending on their size, charge, or other physical characteristics after getting the protein extract. Gel electrophoresis is a popular technique for this, in which proteins are passed through a gel matrix while being subjected to an electric field in order to separate them according to their charge and size.

Western blotting (Immunoblotting), is a popular method for identifying particular proteins in a sample. The membrane is then treated with certain antibodies that can detect and bind to the protein of interest after the separated proteins from the gel have been put there. Various techniques are used to identify the attached antibodies, and the presence of the protein reveals the identity of the material [68,69].

Mass spectrometry (MS) is a potent method used to identify proteins in a sample. This process involves breaking down the proteins into smaller peptide fragments, which are then subjected to MS analysis. The proteins present in the sample can be identified by comparing the generated mass spectra to protein databases. [70,71]

ELISA is yet another approach that is frequently used to identify and measure proteins. It entails attaching particular antibodies to a solid surface (such as a microplate), and then using colorimetric or fluorescent indicators to find the attached proteins [72,73].

Once the proteins have been identified, information about the sample's origin, health state, or other traits can be learned from their relative quantity and pattern in the sample [74, 75],

Disease diagnosis, biomarker discovery, forensics (e.g., suspect identification based on biological samples), drug development, and biological process monitoring are examples of applications for protein identification. It's vital to remember that based on the sample type, the goal of the research, and the resources available, several particular procedures may be employed to identify proteins [14]. The accuracy and sensitivity of protein identification techniques are also continuously being improved by developments in proteomics and other analytical techniques.

PRESERVATION OF BIOLOGICAL EVIDENCE

The conservation of biological evidence is paramount in disciplines such as forensic medicine, paleontology, and archaeology, with proteins serving a crucial

function in this endeavor. Proteins enhance the stability of biological materials, thereby inhibiting their degradation. They uphold the structural integrity of cells and tissues, constituting the extracellular matrix and cytoskeleton. This aids in preserving the overall architecture of tissues and organs, decelerating their decomposition. Specific proteins, such as Late Embryogenesis Abundant (LEA) proteins, safeguard organisms against freezing and desiccation, preserving cell and tissue integrity even under severe environmental conditions. Certain proteins have the ability to form cross-links with other molecules, including nucleic acids and other proteins, establishing stable networks that resist enzymatic degradation and preserve the original structure of biological materials. The function and stability of proteins are contingent on their proper folding, which bolsters their resistance to degradation by proteases and other enzymes. This resistance diminishes the rate of degradation and prolongs the longevity of biological evidence. Some proteins, like antimicrobial peptides, are components of the innate immune system that defend organisms against infection and inhibit the proliferation of bacteria and other microorganisms that could contribute to the degradation of biological materials. Proteins also find application in cryopreservation, a technique for conserving biological materials by storing them at extremely low temperatures, with certain proteins acting as cryoprotectants that shield cellular structures during the freezing and thawing process. Forensic science is reliant on the preservation of biological evidence for effective analysis and suspect identification in criminal investigations. The appropriate preservation and archiving of biological samples are vital to conserve DNA and other proteins in the evidence. Proteins are indispensable for preserving biological evidence as they contribute to the stability, structure, and resistance to degradation of cellular materials. Comprehending these principles can facilitate the enhancement of data storage and retrieval methods from biological materials in various scientific fields. [15-18]

CONCLUSION

In recent years, remarkable strides in technology and scientific inquiry have propelled forensic science forward, with proteins emerging as indispensable tools for unraveling intricate criminal cases. Proteins, the fundamental constituents of living organisms, exhibit multifaceted roles within cells, tissues, and organs. Their significance lies in their capacity to yield vital insights from evidence collected at crime scenes. By scrutinizing proteins, forensic scientists gain novel perspectives on the identities of both perpetrators and victims, as well as a deeper understanding of the contextual dynamics surrounding criminal acts. Notably, advanced protein profiling techniques, including mass spectrometry and gel electrophoresis, have revolutionized forensic identification, offering heightened sensitivity and specificity in linking suspects to crucial evidence. This paradigm shift underscores the pivotal role of proteins in the pursuit of justice and truth.

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