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Critical Review on Role of Blood Protein in Forensic Science

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Abstract:

In crime scene, mostly blood, semen, saliva, urine, vaginal fluid, sweat etc. are mostly found in case of sexual assault, theft, burglary, murder, suicide, homicide, road accident etc. these evidence play major role in criminal investigation as they can help to link criminal with crime as act of Locard principal of exchange. This reviewed paper discuss about the different techniques for the identification of blood protein in the body fluids. Also explains the difference between the body fluids on the basis of specific protein present in all body fluids.

Keywords: *Blood Protein, Body Fluids, SDS-PAGE, MALDI-TOF.*

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Introduction

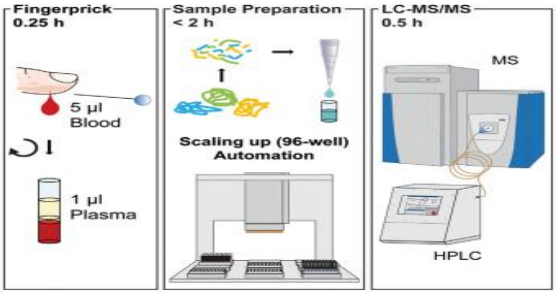
Blood

Blood is a body fluid in humans it is compose of blood cell and blood plasma. Blood cell constitutes 45% of blood fluid. Protein are also called plasma protein which constitutes 55% of blood fluid. It is essentially an aqueous solution containing 92% water 8% blood plasma proteins. Plasma contain verity of protein like albumin globulin fibrinogen. Blood protein serves different function including transport of lipid, hormone, and vitamins, mineral and functioning of immune system, it also acts as enzyme.

Blood Protein

Determination of the protein constituents of human plasma has been an active area of research for several years[1]Proteins are composed of individual units called amino acids[2]. Hundreds of proteins are dissolved in the plasma, Protein identification is an integral part of proteomics research.[3].The analysis of proteins within forensic science is a seldom documented [4]. In crime scene mostly blood semen saliva urine vaginal fluid, sweat etc. are mostly found in case of sexual assault theft burglary murder suicide homicide road accident etc. these evidence play major role in criminal investigation as they can help to link criminal with crime as act of locard principal of exchange[5-7]. The application of protein analyses that are more sophisticated and sensitive than those technique currently utilized within forensic science [8]. Protein studied have maximum used in medical science by measuring the concentration of these proteins, the clinician can obtain information regarding disease states of human [9-16]. Human Serum contains several proteins which have various concentration but bulk of protein is lower molecular weight protein (LMW) [17]. However several protein are present in human serum which mask the expression of lower molecular weight protein (1) especially albumin and immunoglobulin G (IgG) which is present in plentiful amount and does not allowed to express lower weight molecular protein[18]. Each body fluid has different function in our body and it contains different type of protein in different concentration which gives each body fluid as a unique protein identity. This is helpful in forensic science for differentiating one body fluid from others.

Several specific blood protein are Hemoglobin alpha (HBA) Hemoglobin beta (HBB) Beta-spectrin (SPTB) Alpha-spectrin (SPTA1) Band 3 anion transport protein (SLC4A1).there is possibility to determine aged body fluids by MALDI TOF/TOF which also contain specific protein for several year (19) in biomedicine, large-scale analysis of the complete protein composition, or the proteome, of biological samples has been well documented in recent years (8(protein analysis technique have been shown in (figure:1) (20).



Statement of the problem

Presumptive tests are not powerful forensic tests and are limited by their cross-reactivity and subjectivity. The purpose of protein analysis are more sophisticated and sensitive than those currently used within forensic science [8]. The test used in forensic science like kastle-Meyer can react with variety of natural occurring substances and can give false positive result. Several confirmatory test suffer drawback like ELISA, which suffer from affinity (sensitivity) antibody specificity (cross reactivity) manufacturing production variability and narrow working range (the Hook effect), which causes result varation in the laboratories[19]. Biochemical test is a destructive technique it destroyed the sample and further DNA analysis is also not possible [21] Another disadvantage is that it is specific for each body fluid [22-27]. Like benzidine test it react with only blood not with other body fluid, for distinguish human and animal blood hexagone Obti test is used [27-29]. From evidentiary sample, analysis of DNA can be done of alleged victim but it does not explain that from which part of body fluid DNA have come either from blood vaginal secretion saliva urine etc. but protein can differentiate each body fluid[30] and it have capability to persist in dried stain and post mortem tissue [31]. Protein consider as more stable and reliable technique for identification [32-33].

Different technique for protein separation

There are several method applied for separation and identify of protein like gel electrophoresis,

immunoelectrophoresis[2] Sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE)[34-35] is commonly used to obtain high resolution separation of complex mixtures of proteins, The method initially denatures the proteins that will undergo electrophoresis, another method is Immunodetection of proteins by western blot[36] , Isoelectric focusing (IEF) , Two-dimensional gel electrophoresis ,Two-dimensional fluorescence difference gel electrophoresis (2-D DIGE), Protein identification by matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry[37]. Software and database search algorithms to analyze spectral data became popular in the analysis of protein as it became increasingly possible to detect very low amounts of peptides and proteins [38].

Forensic Application

Now a day Proteome analysis of plasma is increasingly leading to biomarker discovery of blood protein [8,19] For the generic detection of blood, the protein hemoglobin has served as an excellent diagnostic marker [30, 39, 40] Protein in blood act as biological marker some example of biomarker are Hemoglobin alpha (HBA) Hemoglobin beta (HBB) Beta-spectrin (SPTB) Alpha-spectrin (SPTA1) Band 3 anion transport protein (SLC4A1), aged body fluids could still be identified by MALDI TOF/TOF [19]demonstrating the opportunity to profile and identify multiple blood signatures in bloodstains via a bottom up proteomic approach[37]. The plasma proteome help in profile of proteomic portrait of a person's health state[20]. In forensic science protein profiling of body fluid have been generated and due to which false negative test were also minimized. For example peripheral blood menstrual blood were not distinguish earlier because no such specific test was available but due to protein profiling it can possible[30]. In future technology protein profiling of each body fluid is possible which will be act as biomarker and provide valuable source for identification of body fluid and will be played significant role in forensic science. When protein biomarker technique combined with existing technique such as ABAcard it would be possible to develop low cost assay system that can be used forensic experts(30(. blood dilution factors for mass spectroscopy (MS) demonstrated a sensitivity of 1:100,000, whereas benzidine had a maximum sensitivity of 1:10,000 [8].

Blood protein marker

There are thousands of blood protein which play significant role in forensic science but ideal marker should have the following characteristics: i) it should be unique or highly enriched in the body fluid of interest (hemoglobin in blood is a good example), ii) it should be abundant, as this will increase the sensitivity of the assay as more abundant proteins can be detected in smaller sample volumes, and iii) the peptides from the chosen protein marker should be easily detected[19]. The most abundant proteins present in blood are typically hemoglobin and albumin, although there are a plethora of other proteins that contribute to the plasma and cellular composition of blood (8, 41). C-reactive protein (CRP) is an acute-phase protein that serves as an early marker of inflammation or infection [42-47]. C-reactive protein (CRP) use in forensic autopsy cases [48 49]. C-reactive protein (found in lesser amount as compare to the ante-mortem blood values [50]. Specific blood protein marker are Hemopexin, Histidine-rich glycoprotein, Apolipoprotein , Plasminogen, Transthyretin , Antithrombin-III, Ceruloplasmin, Afamin, Serum amyloid P-component [30] Hemoglobin alpha (HBA) Hemoglobin beta (HBB) Beta-spectrin (SPTB) Alpha-spectrin (SPTA1) Band 3 anion transport protein (SLC4A1) are biomarker of blood[19].

Method of Blood Protein Identification

Electrophoresis

Electrophoresis is the process of moving charged molecules in a solution by applying an electric field. During electrophoresis, mobility is dependent on the charge, shape and size of the molecules [51-53] Arne Tiselius described U-shaped electrophoresis in 1930 and used it to separate serum proteins in 1937 [54]. Four blood serum protein were separated albumin, alpha globulin, beta globulin and gamma globulin [55].

Gel Electrophoresis

Gel electrophoresis is a widely known group of techniques used to separate and identify such as protein based on size form, and isoelectric point, RNA, and DNA. The separation of molecules by electrophoresis is based on the fact that charged

molecules migrate through a gel matrix upon application of an electric field [38, 55].

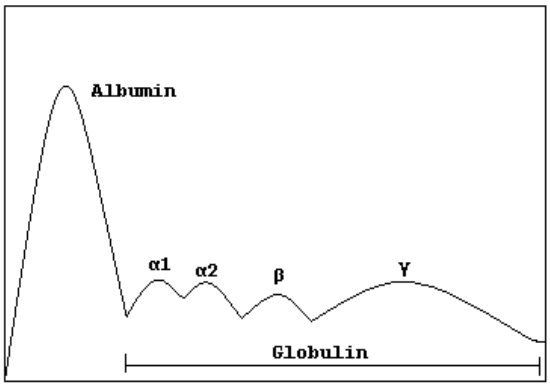


Figure 2: Globulin Schematic representation of a protein by gel electrophoresis of albumin, globulin and its type (Alpha globulins, Alpha 2-globulins, Beta globulins Gamma globulins)

SDS-PAGE Electrophoresis

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) is a well-established analytical method utilised for the denaturation and separation of proteins [8]. It is widely used as a qualitative method, its main function is to separate protein from mixture on the bases of size, it is very beneficial method for monitoring purified protein [56]. it also help to fin d out relative molecular mass of protein [35, 52, 57-58].The reveal proteins does not depend on their antigenicity of blood as this found in immunoelectrophoresis, thus proteins that are not antigens can be detected, with help of SDS-PAGE blood protein of different body fluid can be differentiate. The proteins of human seminal plasma and blood plasma as revealed by SDS-PAGE electrophoresis are compared it is clear that the distribution of proteins in the two physiological fluids is quite distinct, as shown in (figure: 1.4)[60]. Another example are given where different body fluid have separated according to their size given in (figure 3) Upon inspection of the blood separation several specific blood protein were found shown in (figure:1.6). The molecular weight of band 1 was consistent with that of immunoglobulins, which have molecular weights ranging from 150-190 kDa [61]. Band 4 was identified as erythrocyte cytoplasmic protein ubiquitin-activating enzyme molecular weight of 117 kDa [62]Bands 6 and 7 were representative of molecular weights 74 and 68 kDa, which correlate with the molecular weights of prothrombin and transferrin (band 6) and albumin (band 7), all of which are key proteins within human serum [61]. Band 8 molecular weight of 51 kDa coagulation of blood and known as coagulation factor IX, or Christmas factor (63) 9 was 44 kDa actin, a key protein in the cytoskeleton of erythrocytes (61). Band 10 was representative of glycophorin (28kDa).

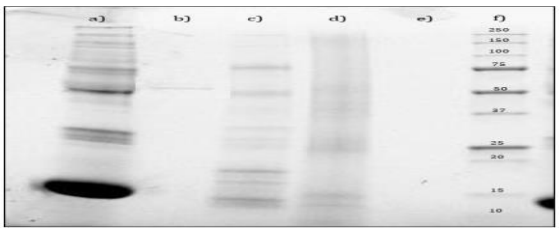


Figure 3: SDS-PAGE separation of neat body fluids; a) blood, b) saliva, c) semen, d) vaginal secretions, e) negative control, f) protein size standard with molecular weights (kDa) (Orphanou CMR., 2015)

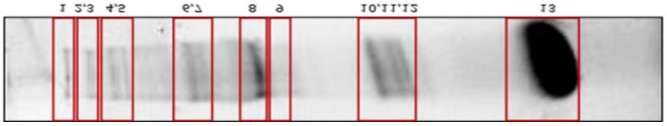


Figure 4: Assignment of the protein separation pattern observed in neat blood

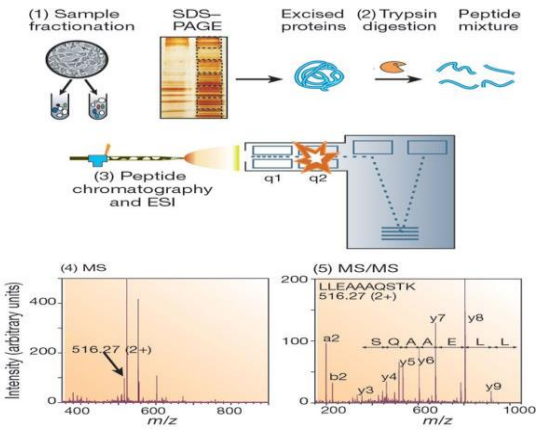
Mass Spectroscopy

Mass spectrometry, as a versatile technique, its main advantage is high sensitivity [64] it help to identify biological traces which are generally found on crime scene [65, 66]. It is an advance and analytical technique that separates molecules based on their mass to charge (*m/z*) ratio which can lead to protein separation and identification. Mass spectroscopy coupled with many technique such as SDS-PAGE , MALDI TOF, gas chromatography (GC) or high performance liquid chromatography (HPLC) [8,19 67]. The fundamental components of a mass spectrometer are summarised in Figure 5 [8]. SDS-PAGE has been demonstrated as a complementary technique to mass spectrometry in the detection and separation of proteins used within biomedicine[68-69] which results in a simpler and more cost effective approach. Therefore, it is for these reasons why the

application of protein analysis involving SDS-PAGE as an investigative biological analysis for forensic evidence is to be explored in body fluid identification and age determinate [70].

MALDI-TOF MS

As technology has evolved, more sensitive and discriminatory techniques, such as MALDI-TOF MS, have been investigated[67, 71]. Which are not protein specific in their application but can identify the numerous proteins present within a sample MALDI-TOF is now used with mass spectroscopy. It as a vaporisation technique, where by the sample of interest is mixed with a matrix which absorbs ultra violet light. The matrix then heats rapidly, causing the matrix and sample to vaporize. It is the amount of time taken for the molecule containing vapor to reach the detector that is quantified. Small and highly charged molecules reach the detector faster than large, low charged molecules. The time at which molecules reach the detector is representative of a mass to charge ratio and gives the process its name, time of flight [8]. Human blood Hemoglobin subunit beta and Hemoglobin subunit alpha were identified [70]. Using a combination of methods, the proteomes of menstrual blood, blood, semen, and saliva were investigated. These included histones, ribosomal proteins, cytokines, and *MMPs*, favored by the mRNA community for menstrual blood [72-73]. Alpha- and beta-hemoglobin, spectrin, and solute carrier family 4 (anion exchanger), member 10 were proposed for blood; α amylase 1, histatin 1, and cystatin SA for saliva; and semenogelins 1 and 2, prostatic acid phosphatase, *MUC6*, and others for semen [74] working representation of MALDI-TOF MS is given in Figure 5(75).



Conclusion

Blood protein identification is playing very important role in criminal identification as, Blood protein is act as a biomarker which can be differentiate mixed body fluid on the basis of specific protein in case of sexual assault, murder, suicide, homicide, etc. Several advance techniques are being used in field of forensic for protein separation and identification. According to this review paper, we concluded that SDS-PAGE is given the best result for separation and MALDI-TOF is the also given the best result for identification of protein because it gives positive result when expert have sample in very minute quantity as well as in degraded condition.

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