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A Systematic Review of Forensic Aspects of Menstrual Blood from Crime Scenes

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Abstract

Menstrual blood, a biochemically unique fluid composed of endometrial tissue, vaginal secretions, and high protein content, has special forensic value in cases of sexual assault and homicide. Unlike peripheral blood, its absence of coagulating substances results in separate degradation processes, making it an important substrate for post-depositional age estimate and fluid distinction. This paper assesses the use of Bradford Assay and SDS-PAGE for measuring and characterising damaged proteins in menstrual blood collected at crime scenes. Samples must be non-invasively collected using sanitary pads and polypropylene containers, processed within two hours, and stored at 4°C to preserve proteomic integrity. Protein quantification can be performed via Bradford Assay using bovine serum albumin standards, while SDS-PAGE enabled molecular weight-based separation and visualization of degradation markers. Emphasising the necessity for portable, affordable diagnostic technologies, the study promotes the practical integration of menstrual blood analysis into forensic processes. Through the integration of biochemical characterisation and forensic application, the study transforms menstrual blood from a peripheral fluid into crucial supporting evidence for forensic coursework.

Keywords: Menstrual Blood; SDS-PAGE; Protein Separation; Protein Degradation; Forensic Proteomics; Bradford Assay; Protein Quantification

Introduction

Proteomic analysis represents an essential advancement in forensic science, offering novel methodologies for the identification and characterization of biological fluids [1]. Among these, blood is a prevalent and critical type of evidence at crime scenes, often serving to establish connections between suspects, victims, and criminal events while facilitating crime scene reconstruction [2]. However, despite extensive research on peripheral blood, the forensic utility of menstrual blood—a biochemically distinct fluid—remains comparatively underexplored. This is especially significant in criminal cases such as sexual assaults, rapes, and homicides, where the differentiation of menstrual blood from other fluids can provide critical investigative insights.

Menstruation is a process, which involves the release of blood and the removal of the endometrial lining from their uterus in Nonpregnant women [3]. From menarche until menopause, the uterus helps prepare the endometrium for embryo implantation [4]. The uterine linings consist of myometrium and endometrium. Myometrium is made up of uterine muscle fibres. The endometrium consists of a simple columnar epithelium and stroma. The endometrium is simple columnar epithelium consists of single-layered, elongated cells on the apical surface [5]. The stroma comprises of connective tissues and spiral arteries. Spiral arteries are small arteries that arise through the endometrium and take from a coil-like structure, which delivers blood to the endometrium.

Composition of Menstrual blood

Menstrual blood contains various pieces of lost endometrial tissue, including gland fragments that contain stem cells/progenitor, epithelial, stromal, and immunological cells; blood cells, clots, cervicovaginal mucus, and secreted substances such as proteins, immune cells, and extracellular matrix debris [6]. Collecting menstrual fluid with a menstrual cup or tampon or sanitary pads is a non-invasive method that allows for direct

measurement of its components. This results in a consistent profile with minimal fluctuation across menstrual cycles. Menstrual fluid, which comprises both peripheral blood and late secretory endometrial tissue, as well as cervicovaginal fluid, may be collected noninvasively during menstruation using menstrual products. Menstrual product-derived endometrial tissue contains specific proteins and stem/progenitor cells that are unique to the endometrial environment and not found in peripheral blood and it is shown in Figure 1

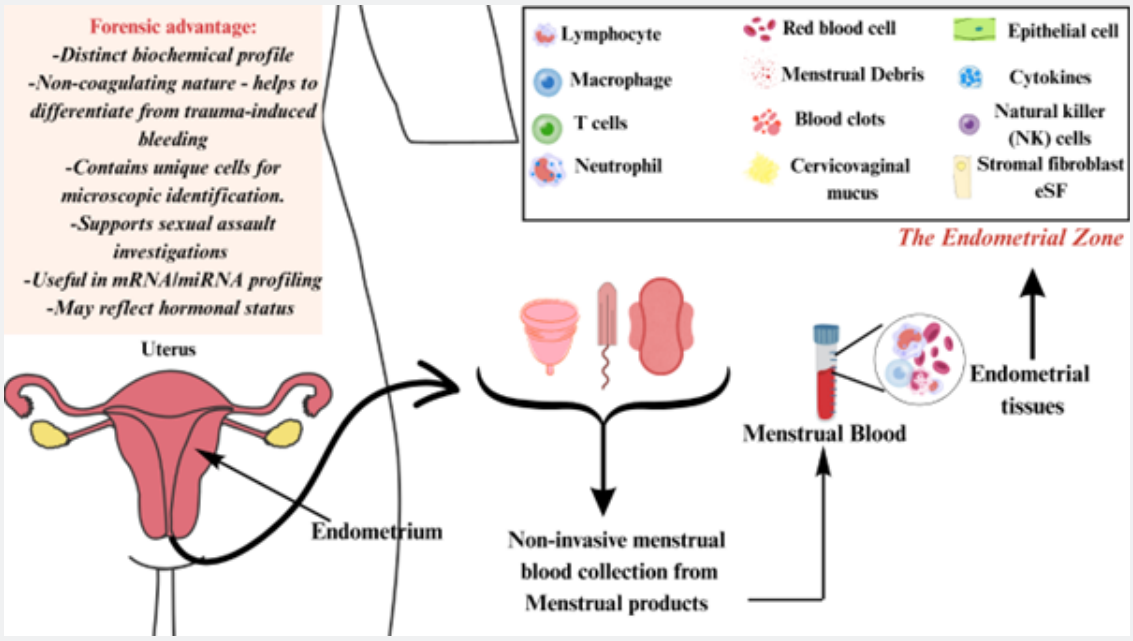

Figure 1: Menstrual blood collection methods and composition.

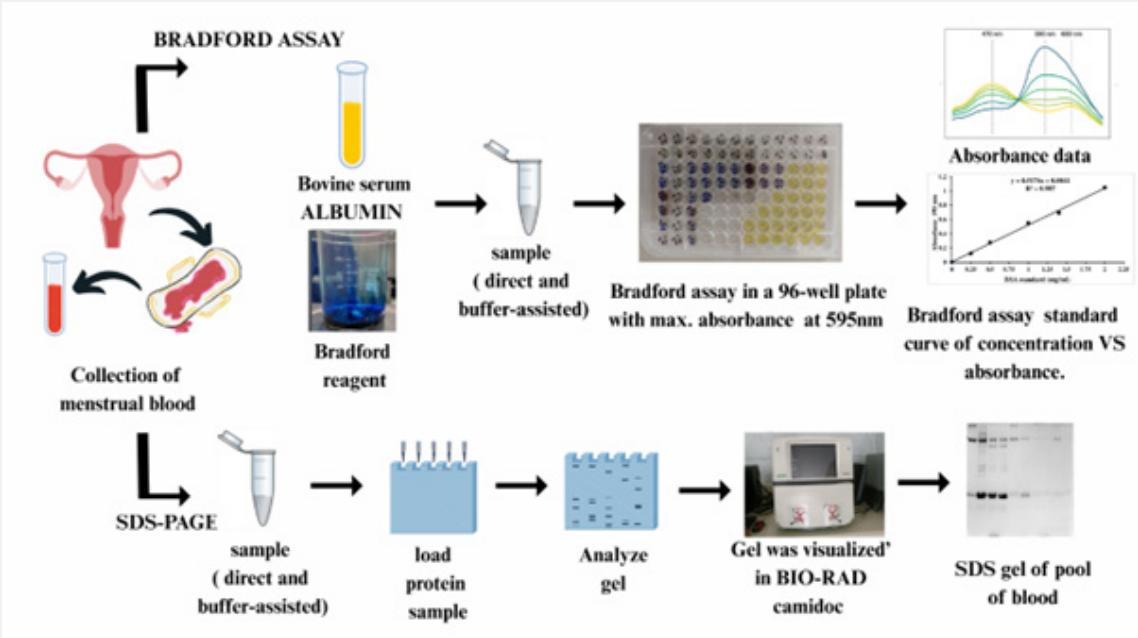

Figure 2: Overview of methodology for analyzing menstrual blood samples using Bradford assay for quantification and SDS-PAGE for protein separation.

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The Endometrium

The endometrium is an extremely regenerative tissue that sheds in pieces and regenerates quickly without leaving wounds. The human endometrium has two layers: functionalis and basalis [7]. Functionalis includes a columnar epithelium that borders the uterine canal and endometrial glands that pierce the vascularised stroma. The stroma is made up of fibroblasts and immunological cells (mostly leukocytes) [8].

During the menstrual cycle, functionalis cells respond to oestrogen and multiply. The basalis consists of horizontal glands that produce vertical endometrial glands, as well as a dense stroma containing stem/progenitor cells. These cells heal the luminal epithelium through menstruation and renew the functionalis in the cycle that follows.

Common proteins in Menstrual blood

The most plentiful proteins frequently known include those with roles in proliferation or growth(fatty acid binding protein5, ninjurin-2), cell repair (macrophage migration inhibitory factor, lactotransferrin, galectins1and3, matrix metalloproteinase [MMP]-9), cell migration (neutrophil gelatinase-associated lipocalin, AHNAK nucleoprotein, osteopontin, secretory leukocyte protease inhibitor), angio genesis (vascular endothelial growth factor A [VEGF-A], stanniocalcin), inflammation (interleukins-6,-8, and 1b[IL-6, IL-8,andIL-1b], ,C-X-C motif chemokine ligand 8, tumor necrosis factor-alpha[TNF-a]) [9].

Menstrual blood is compositionally distinct from peripheral blood, comprising a mixture of endometrial lining, vaginal secretions, and elevated protein concentrations [10]. These unique biochemical properties are a consequence of physiological processes linked to the menstrual cycle, including endometrial shedding and ovulation [11]. A key distinction lies in the absence of coagulating factors in menstrual blood, leading to degradation pathways markedly different from those observed in peripheral blood [12]. This distinction underlines the forensic relevance of studying menstrual blood as a separate biological fluid.

The Bradford Assay, developed by [13], is a robust and widely adopted analytical technique for protein quantification. Its mechanism is based on the colorimetric interaction between Coomassie Brilliant Blue G-250 dye and protein molecules, resulting in a quantifiable spectral shift. The assay is highly sensitive, efficient, and cost-effective, rendering it a valuable tool for forensic applications involving protein analysis.

This study investigates protein quantification in menstrual blood using the Bradford Assay, with a specific focus on degradation dynamics. Moreover, the analysis of menstrual blood proteins extends to their separation and characterization via Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE). SDS-PAGE operates by denaturing proteins and separating them based on their molecular weight, a principle initially elucidated by [14]. This methodology is well-established for providing qualitative and visual evidence of protein stability and degradation. The unique proteomic profiles observed in menstrual blood samples via SDS-PAGE confirm its differentiation from other fluids, further strengthening its forensic applicability.

While prior research [15] has highlighted the efficacy of SDS-PAGE in forensic proteomics, its specific application to menstrual blood analysis remains limited [16]. This study addresses this gap by employing both Bradford Assay and SDS-PAGE to explore the stability and degradation dynamics of menstrual blood proteins. The results emphasize the potential of these proteomic techniques to advance forensic methodologies, particularly in differentiating menstrual blood from peripheral blood and estimating the post-depositional age of biological samples.

With the focus on protein quantification, degrading kinetics, and evidential importance, the present study highlights the present state of the art in menstrual blood proteomics. The study evaluates the methodological progress of the Bradford procedure and SDS-PAGE in forensic contexts, bring out problems with sample integrity and analysis, and discuss potential directions of research and operational integration based on current experimental findings and comparative literature. This work aims to transform menstrual blood from a peripheral fluid to a main evidential substrate through combining biochemical characterisation with forensic application, which leads to more complex, inclusive, and scientifically valid crime scene recreation.

Forensic applications of menstrual blood:

Distinct biochemical profile: Menstrual blood has unique composition of biochemicals like endometrial tissues fragments, lysed erythrocytes and elevated levels of fibrinolytic enzymes. This distinguishes it from peripheral blood and other body fluids, aiding forensic investigation

Non-coagulating nature: Presence of plasmin and other fibrinolytic agents, menstrual blood typically does not clot which helps to differentiate from trauma-induced bleeding, which usually exhibits coagulation unless disrupted by anticoagulants.

Microscopic identification: Presence decidual and endometrial cells make them morphologically distinct under microscope. These cells serve as markers for body fluid identification.

Supports sexual assault investigations: The detection of menstrual blood in sexual assault cases can corroborate victim testimony and even could define specific timeline and assist in reconstructing chain of events. It can also support mixed DNA profiling when present alongside semen or epithelial cells

Useful in mRNA/miRNA profiling: Menstrual blood is a viable substrate for molecular assays targeting mRNA and MIRNA markers and these profiles enable tissue specific differentiation and enhance the accuracy of body fluid identification.

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May reflect hormonal status: Hormonal fluctuation during menstruation influences the composition of menstrual blood and analysis of hormone -regulated transcripts could provide insights into behavioral or temporal and reproductive aspects for forensic coursework.

Materials and Methods

Sample Collection

For analysis purpose menstrual blood can be collected from sanitary pad sterile or even polypropylene containers should be used to collect menstrual blood samples non-invasively in order to increase protein output and reduce degradation [16]. Proteomic integrity has been preserved by processing the samples within the first two hours of collecting and storing them at 4°C [17]. For proteomic research, this method is in line with established biorepository protocols, which prioritise rapid cooling and little exposure to light or outside temperatures to prevent oxidative damage and protein denaturation [18]. Figure 2 provides a visual representation of the processes discussed in the paper.

At first, the supernatant has been separated from cellular debris after centrifugation [19]. While the pellet was kept for buffer-assisted extraction, the supernatant—which included soluble proteins—was aspirated and reserved for direct analysis. Direct stabilisation, dosing, and analysis of menstrual blood on collecting platforms such as sanitary pads or microfluidic devices is accomplished by the use of specialised buffers and fluid-control systems and or reliable diagnostics, buffers are essential for maintaining pH, preventing clotting, and enabling consistent biomarker detection [20].

Cold centrifugation is essential for maintaining native protein conformations and reducing proteolytic activity, especially in complex biological fluids such as menstrual blood [21]. This buffer method was chosen because proteome profiling studies of menstrual blood showed that it was effective in solubilising cytoplasmic proteins while preserving structural integrity.

A colorimetric technique based on the binding of Coomassie Brilliant Blue G-250 dye to basic and aromatic amino acid residues, the Bradford test, was used to quantify proteins (Burokerkilgore & Wang, 1993). Following the dissolution of 100 mg of Coomassie G-250 in 50 mL of 95% ethanol, 100 mL of 85% phosphoric acid was added, and the mixture was diluted to 1 L with distilled water to create the Bradford reagent [22]. Using standards of bovine serum albumin (BSA) ranging from 10 to 100 µg/mL, to create a standard curve [23]. Comparative comparison of extraction techniques and degradation time points was carried out, and protein quantities were interpolated from the standard curve.

Following the Laemmli procedure, SDS-PAGE was used to separate the proteins, ammonium persulfate (APS, 10%), acrylamide/bis-acrylamide (30%), Tris-HCl buffers (pH 8.8 and 6.8 respectively), SDS (10%), and TEMED were used to create a 15% resolving gel and a 5% stacking gel [24]. Laemmli buffer, which contains SDS, β-mercaptoethanol, glycerol, and bromophenol blue, was combined with protein samples and heated to 95°C for five minutes to denature the proteins [25]. The electrophoresis conducted at 180 V using Tris-Glycine-SDS running buffer [26]. After electrophoresis, gels were stained for an hour with Coomassie Brilliant Blue R-250 and then destained with a 4:1:5 methanol, acetic acid, and water solution until the background disappeared [27]. Molecular weight distribution and degradation markers, such as fragmentation and smear formation, were examined in band patterns. The high-resolution separation of menstrual blood proteins using this methodology has been verified for use in clinical and forensic proteomics [28].

Discussion

The current study emphasises how menstrual blood is an unique biological fluid with forensic value, especially in situations when conventional blood evidence could be unclear or deteriorated. Menstrual blood, in contrast to peripheral blood, has distinct proteomic properties because it is composite, containing endometrial tissue, vaginal secretions, and higher protein concentrations [29]. It degrades quickly because to the lack of coagulating components, which provides a biochemical foundation for estimating post-depositional age [12].

The application of the Bradford Assay enabled precise quantification of protein content across varying degradation time points, revealing a consistent decline in protein concentration with increased exposure duration [30]. This trend aligns with established proteolytic kinetics and supports the assay's utility in forensic timelines. SDS-PAGE analysis provided complementary insights, with band fragmentation and smear formation serving as visual markers of protein breakdown [28]. These electrophoretic patterns not only confirmed the presence of menstrual blood but also differentiated it from peripheral blood and other biological fluids [31]. Operationally, the study highlights the importance of rapid sample processing and cold-chain preservation to maintain proteomic integrity. The use of sanitary pads and polypropylene containers for non-invasive collection proved effective, suggesting practical avenues for field deployment. Future research should explore integration with microfluidic platforms and AI-based pattern recognition to enhance diagnostic precision. Collectively, these findings advocate for the inclusion of menstrual blood proteomics in forensic workflows, expanding the evidentiary landscape and enabling more nuanced crime scene reconstruction.

Conclusion

This study establishes menstrual blood as a biochemically distinct and forensically valuable substrate, particularly in cases involving sexual assault, homicide, and ambiguous biological evidence. Through the combined application of Bradford Assay and SDS-PAGE, the study demonstrates reliable quantification and separation of degraded proteins, revealing unique proteomic

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signatures that differentiate menstrual blood from peripheral blood and other fluids. The use of non-invasive collection platforms such as sanitary pads and polypropylene containers further supports field applicability. The use of menstrual blood as a primary evidential fluid in this study improves forensic proteomics. Future initiatives should concentrate on standardising procedures for menstrual blood analysis, including AI-based pattern recognition, and creating portable diagnostic platforms in order to improve forensic resolution and aid in the administration of justice.

In conclusion, this study contributes significantly to the field of forensic proteomics by providing a comprehensive analysis of menstrual blood proteins and their degradation patterns. By addressing the challenges associated with menstrual blood identification and preservation, this study lays the groundwork for future advancements in forensic methodologies. The development of cost-effective and portable tools for menstrual blood analysis could revolutionize forensic science, enabling investigators to collect, preserve, and analyze biological evidence with greater efficiency and reliability. These innovations have the potential to strengthen forensic casework, ensuring justice and enhancing the scientific resolution of crimes.

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