Review

ANNALS OF MEDICINE & SURGERY

OPEN

Potential for and challenges of menstrual blood as a non-invasive diagnostic specimen: current status and future directions

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Abstract

Menstrual blood, which is often discarded as a waste product, has emerged as a valuable source of health information. The components of menstrual blood, such as endometrial cells, immune cells, proteins, and microbial signatures, provide insights into health. Studies have shown encouraging results for using menstrual blood to diagnose a variety of conditions, including hormonal imbalances, cervical cancer, endometriosis, chlamydia, diabetes, and other endocrine disorders. This review examines the potential of menstrual blood as a non-invasive diagnostic specimen, exploring its composition, promising applications, and recent advances. This review also discusses challenges to utilizing menstrual blood testing, including ethical considerations, the lack of standardized collection protocols, extensive validation studies, and the societal stigma around menstruation. Overcoming these challenges will open new avenues for personalized medicine and revolutionize healthcare for individuals who menstruate.

Keywords: cervical cancer, chlamydia, diabetes, endometriosis, HbA1c, menstrual blood, STI

Introduction

Diagnostic tools, both non-invasive and invasive, have played a key role in the assessment, diagnosis, and management of patients. From the stethoscope to complete blood counts (CBC) to bodily fluid assessment assessments, diagnostic tools all contribute to the ultimate diagnosis of various diseases. Changes in bodily fluids have been related to disease since the time of the ancient Greek's[1]. With the development of the modern clinical laboratory in the early 20th century, phlebotomy and blood testing became a crucial diagnostic tool[1]. Though invasive diagnostic procedures, including phlebotomy, have become an integral part in the management of patients, non-invasive diagnostic tests are equally important. Non-invasive diagnostic tests are often rapid, safe, less technically complex, less painful for patients, and less costly compared to invasive diagnostic tests[2].

Menstrual blood has garnered increased attention in recent years for its potential as one of the non-invasive diagnostic specimens and health indicators[3]. While traditionally viewed as a

HIGHLIGHTS

- Menstrual blood is a valuable source of health information, containing endometrial cells, immune cells, proteins, and microbial signatures.
- Studies have shown promising results for diagnosing hormonal imbalances, cervical cancer, endometriosis, chlamydia, diabetes, and other endocrine disorders using menstrual blood.
- Challenges to utilizing menstrual blood testing include ethical considerations, lack of standardized collection protocols, extensive validation studies, and societal stigma.
- The integration of advanced technologies like mass spectrometry and molecular biology has enhanced the diagnostic potential of menstrual blood.
- Food and Drug Administration (FDA) approval for using menstrual blood in diabetes diagnosis and ongoing research signify its promising future as a non-invasive diagnostic method.
- Limitations include its inapplicability in postmenopausal women, men, and young children, as well as the need to address the stigma surrounding menstrual health awareness.

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Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

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Annals of Medicine & Surgery (2024) 86:4591–4600

Received 19 February 2024; Accepted 29 May 2024

Published online 10 June 2024

http://dx.doi.org/10.1097/MS9.0000000000002261

discardable waste product, recent research has highlighted the dynamic nature of menstrual blood and its potential utility in health diagnostics[4,5]. Menstrual blood comprises shed endometrial tissue, blood, cervical mucus, and a myriad of cellular components, offering an insight into the reproductive and overall health of individuals[6].

By using menstrual blood composition and looking at patterns of menstrual cycle, many diseases—including those that predominately affect non-reproductive organs—can now be better understood. Menstrual blood is highly accessible for individuals who menstruate, as It is estimated that individuals without heavy menstrual bleeding use up to 10 000 pads in their lifetime[7]. In terms of menstrual cycle patterns, a study found that ~53% of reproductive-age women reported heavy menstrual bleeding, which was a sign for an underlying reproductive disease[8]. An irregular menstrual cycle is one whose length is less than 21 days or more than 35 days. The irregularity of menstruation has been associated with medical conditions affecting the heart such as coronary heart disease, as well as disease that affecting the endocrine system including type 2 diabetes mellitus and metabolic syndrome[9]. In one study, woman with irregular menstrual cycle patterns were found to have a 28% increased risk for coronary heart disease along with an increased risk for type 2 diabetes compared to women with normal cycle lengths[10]. Metabolic syndrome also has a significant association with menstrual irregularity in reproductive-age women[11]. Furthermore, in recent years, numerous studies have investigated the potential of menstrual blood as a diagnostic specimen. These studies have analyzed various components of menstrual blood to assess their diagnostic value. In this review, we embark on an exploration of the composition of menstrual blood, its emerging role as a potential diagnostic specimen, and the hurdles that accompany its integration into the broader healthcare landscape. By navigating these intricacies, we aim to unravel the potential of menstrual blood as a valuable source of health information and pave the way for innovative approaches in medical diagnostics.

The inspiration for this project arises from the noteworthy development of the Food and Drug Administration's (FDA's) approval for using menstrual blood in testing hemoglobin A1c (HbA1c), a crucial marker for diabetes[12]. This regulatory acknowledgement signifies the potential of menstrual blood beyond its traditional reproductive health scope[5]. Furthermore, emerging research not only supports the feasibility of utilizing menstrual blood in testing for HbA1C but also indicates its potential in detecting common biomarkers associated with various other diseases[6,13].

Composition of menstrual blood

If egg fertilization does not occur following ovulation, hormonal changes trigger menstruation—the shedding of the endometrial lining, leading to the expulsion of blood and other materials from the uterus through the vagina[14]. This cyclical process is central to the reproductive health of individuals assigned female at birth[15]. Understanding the composition of menstrual blood is crucial for several reasons. First and foremost, it provides insights into the overall health and functioning of the reproductive system. The menstrual cycle serves as a valuable diagnostic specimen, offering indicators of hormonal balance, potential underlying health issues, and the regularity of the menstrual cycle[16]. Moreover, a thorough comprehension of menstrual blood composition contributes to demystifying common misconceptions and dispelling cultural taboos surrounding menstruation. Knowledge about menstrual blood compositions can empower individuals to make informed decisions about their reproductive health, seek appropriate medical care when needed, and challenge societal norms that may perpetuate stigma and misinformation[17].

Menstrual blood is a unique mixture of blood, endometrial tissue, cervical mucus, and vaginal secretions. Menstrual blood contains live endometrial and immune cells, proteins, nucleic acids, and even vaginal microbes like Lactobacillus species. An estimated 300 distinct proteins are found in menstrual blood, which are not present in peripheral blood[18]. The menstrual cell proteome consists of proteins originating from immune cell types such as cytokines, proteolytic enzymes, and proteins that contribute to apoptotic pathways[6]. Unlike normal blood, menstrual blood has a higher proportion of tissue, mucus, and water, but a lower level of proteins, cholesterol, bilirubin, iron, oxygen, red blood cells (RBCs), white blood cells (WBCs), and hemoglobin than venous blood[19]. Despite its thick viscosity, menstrual blood has decreased coagulation factors and lacks fibrinogen, found in venous blood[20]. Essentially, menstrual blood is a waste product that contains dead or non-functional tissue and yields negative findings on the Teichmann blood test, a confirmatory blood test[21].

A recent study found that the menstrual blood plasma cytokine profile is distinctly different from peripheral blood plasma. High concentrations of C5/C5a, interleukin-6 (IL-6), IL-1β, and CXCL8 were detected in menstrual plasma, while IL-2, IL-12p70, XCL1/Lymphotactin, and interferon-γ were found in low concentrations. The most noticeable differences between menstrual and peripheral blood plasma were observed for IL-6, IL-1β, and CXCL8[22]. Mononuclear cells obtained from menstrual samples, known as menstrual blood mononuclear cells (MMCs), exhibit clear distinctions from peripheral blood mononuclear cells (PBMC) and show a strong resemblance with biopsy-derived endometrial mononuclear cells[18]. A study also suggested the levels of granulocyte-colony stimulating factor (G-CSF) and macrophage-colony stimulating factor (M-CSF) positively correlate with the abundance of Lactobacillus spp. in the microbiota of menstrual samples. This suggests a potential association between the presence of Lactobacillus species and the concentrations of these immune mediators in the analyzed menstrual blood samples[23].

The endometrial cells found in menstrual fluid are the result of a dynamic, cyclical process that centers around conception and procreation. As such, these cells express a large number of proteins, such as progesterone receptor (PR), estrogen receptor (ER), insulin-like growth factor (IGF), vascular endothelial growth factor (VEGF), leukemia inhibitory factor (LIF), mucin proteins and cytokines and chemokines, in order to prepare for embryo implantation and development[6]. While the specific identity of non-endometrial cells in menstrual blood remains insufficiently explored, subjecting menstrual blood cells to adherent culture conditions reveals the presence of a cell type termed menstrual blood-derived stromal cell (MenSC). MenSCs exhibit a multipotent phenotype, showcasing mesenchymal stem cell properties, and are considered promising candidates for cell-based therapies in regenerative medicine and immune-related diseases[24]. MenSCs are a recently discovered type of adult stem cells (ASC)

that exhibit several benefits over other types of stem cells, including a non-invasive collection process, a high rate of proliferation, multipotency, low immunogenicity, and a reduced risk of karyotypic abnormalities[25].

Menstrual blood as a health indicator

The unique proteins of menstrual blood have the potential to serve as biomarkers for various disorders, including endometriosis and chlamydia (Fig. 1). According to recent research, menstrual blood has the potential to estimate serum levels of eight biomarkers, including HbA1c, cholesterol, creatinine, high sensitive C-reactive protein (hsCRP), low-density lipoprotein (LDL), triglycerides, high-density lipoprotein (HDL), and FSH[6].

Diagnosis of diabetes mellitus (DM)

HbA1c serves as the established diagnostic and prognostic benchmark in the clinical oversight of DM. However, suboptimal patient compliance with the recommended periodic testing is a challenge, and notable gender-related discrepancies exist in the management and outcomes of DM. Emerging research indicates that menstrual blood might offer a viable alternative to systemic blood for tracking biomarkers, including HbA1c. This introduces a potential avenue for more accessible, less invasive, and equitable monitoring methods, addressing some of the current limitations (requires skin poking for peripheral venous blood collection and collection of blood only at the lab) in routine DM management and contributing to a more comprehensive understanding of gender-specific influences on disease outcomes. A study comparing HbA1c levels in menstrual and systematic blood in women with either type 1 or type 2 diabetes showed no significant difference in HbA1c levels between blood types. This indicates that menstrual blood, facilitated by self-collection technologies, may offer a reliable and non-invasive alternative for monitoring HbA1c levels in reproductive-age women with diabetes[12]. A menstrual blood-based diagnosis has many benefits. Firstly, it is non-invasive and easily collected compared to traditional peripheral venous blood collection. Secondly, it offers the potential for home-based testing[26].

Recently, Qvin received FDA clearance for its groundbreaking Q-Pad and HbA1c test, introducing the first healthcare service capable of collecting menstrual blood samples as an alternative to conventional venous blood draws for HbA1c testing. This regulatory approval signifies a significant milestone, enabling millions of women living with diabetes to undergo HbA1c monitoring through tests conducted on the Q-Pad. Beyond diabetes management, the clearance opens opportunities for testing other biomarkers for over 80 million menstruating individuals in the U.S. Menstrual blood testing is not only beneficial for individuals with diabetes, but this test also provides a means for non-diabetics to monitor their blood sugar levels and assess associated health impacts.

Diagnosis of cervical cancer

Globally, cervical cancer ranks among the fourth most prevalent cancers in women, with an estimated 660 000 new cases of cervical cancer were reported in 2022, resulting in around 350 000 fatalities[27]. Approximately 99.7% of cases of cervical cancer are attributable to persistent genital high-risk human papillomavirus (HPV) infection.

In a study conducted by Naseri and colleagues evaluated the agreement and acceptability of utilizing a modified menstrual pad

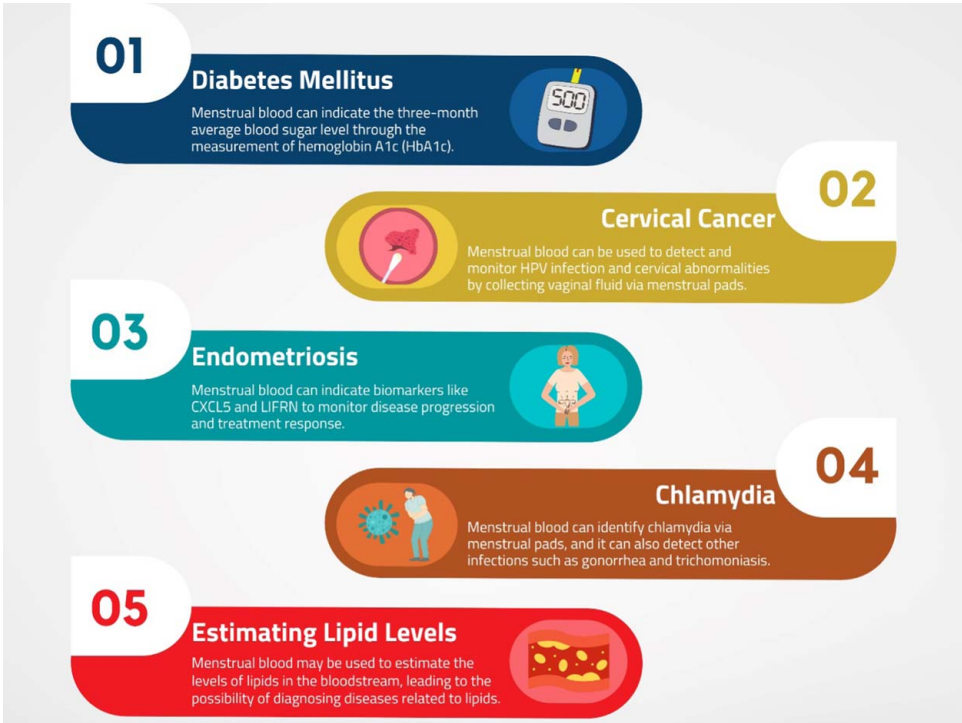

Figure 1. Menstrual blood as a health indictor (created with canva.com).

for high-risk HPV screening in comparison to samples collected by clinicians. Each participant provided three types of samples: clinician-collected cervical specimens, self-collected vaginal swabs, and samples collected using the modified menstrual pad. The study found a high level of acceptability, with 94% of participants expressing a preference for the menstrual pad over clinician-collected samples, highlighting the method's user-friendliness. Furthermore, the strong concordance of 94% between menstrual pad samples and traditional clinician-collected specimens underscores the reliability and consistency of results, supporting the potential integration of menstrual blood-based HPV screening into cervical cancer prevention programs as an alternative and more widely accepted screening method. One key benefit is the convenience of this method, as it allows for non-invasive and easily accessible collection of menstrual blood at home during menstruation. This eliminates the need for a clinic visit and simplifies the screening process for women.

Other studies indicate that testing for HPV in menstrual blood has the potential to achieve sensitivity and specificity rates exceeding 80%[13,28]. A study by Lee and colleagues, demonstrated a 87.5% sensitivity and 45.5% specificity for detecting cervical intraepithelial neoplasia (CIN) 3 or worse on first and second menstrual cycle days for women with high-grade squamous intraepithelial lesions or HR-HPV infections via MB HR-HPV test. The agreement rate between positive cervical and MB HR-HPV test results was 87.5% on day 1 and 62.5% on day 2 for CIN 3 or worse. While indicating promise as a cervical cancer screening modality, the study stressed the need for large prospective randomized trials to confirm efficacy and establish a standardized method[29].

The collective findings from the studies suggest that menstrual blood holds substantial promise as a breakthrough in diagnosing cervical cancer. The findings collectively suggest that menstrual blood could revolutionize cervical cancer diagnosis, offering a more accessible, user-friendly, and effective approach for early detection and monitoring. Furthermore, menstrual blood can also be used for the screening of biomarkers associated with conditions like ovarian and endometrial cancers, as well as other types of cancers[30]. However, further large-scale prospective randomized trials are needed to validate its efficacy and establish standardized protocols for clinical use[13,28–30].

Diagnosis of endometriosis

Endometriosis is a condition where the growth of uterine tissue occurs outside of the uterus, leading to infertility and severe pain for women of reproductive age[31]. More than 190 million women globally experience endometriosis, with many cases going undiagnosed[32]. Traditional diagnostic methods, like laparoscopy, are invasive and may cause discomfort, making it difficult for patients. However, recent research has found that CXCL5 and IL1RN proteins in menstrual blood are significantly elevated in endometriosis patients as opposed to healthy subjects. While elevated levels of IL-1 have been identified in peritoneal fluid and serum and were correlated with the severity of endometriosis, these methods are not non-invasive compared to analyzing IL-1 in menstrual blood. This discovery suggests that these proteins in menstrual blood could serve as non-invasive biomarkers to detect and monitor the disease's progression[33]. It's worth noting that menstrual blood may not be the only non-invasive diagnostic specimen for testing endometriosis biomarkers, as previous research has also investigated the presence of specific biomarkers in urine[34]. Both menstrual blood and urine samples offer non-invasive alternatives to laparoscopy for diagnosing endometriosis. However, menstrual blood contains endometrial tissue itself and can provide a reliable diagnosis of endometriosis compared to other non-invasive specimen[35].

Diagnosis of chlamydia

Chlamydia is a common sexually transmitted disease (STD) that can be identified in menstrual blood through the collection of vaginal fluid in menstrual pads. This method has demonstrated a sensitivity (92–100%) and specificity (99–100%) that are equivalent to, or even exceeds, existing approaches that involve vaginal swabs or urine samples. The non-invasive nature of this approach offers women greater convenience and privacy[36].

Estimation of serum lipid levels

A study has shown a statistically significant correlation between certain biomarker values in systemic and menstrual blood samples, specifically cholesterol, LDL, triglycerides, and HDL[33]. Since menstrual blood can predict the levels of cholesterol, LDL, triglycerides, and HDL, can aid in diagnosing diseases like atherosclerosis, coronary artery disease, and dyslipidemia via non-invasive approach.

The advantages and disadvantages of menstrual blood as a diagnostic medium are shown in Figure 2.

Role of technology and other challenges in utilizing menstrual blood as a diagnostic specimen

The field of menstrual blood diagnosis technologies has come a long way from using traditional diagnostic methods to now using modern and innovative approaches. For instance, in the past, the diagnosis of heavy menstrual bleeding relied on self-reported bleeding histories, which may be influenced by recall bias, personal beliefs regarding "normal" flow volume, and other physical symptoms or disruptions to daily life. However, in today's evolving landscape of healthcare, advanced technologies have become instrumental in redefining diagnostic and therapeutic approaches. Within this context, the narrative explores the transformative role of technology in the detection and diagnosis of diseases using menstrual blood. The types of technologies utilized for using menstrual blood for diagnosis can be broadly classified into (1) mass spectrometry (MS) and chromatography (2) dried blood spot (DBS) technology (3) molecular biological techniques (4) culture-based method.

MS and chromatography

Research has identified proteins found in menstrual blood, such as CXCL5 and IL1RN, as potential biomarkers for diagnosing endometriosis. This discovery was made through data independent acquisition (DIA)-based MS and bioinformatic analysis[35,37]. MS is an analytical laboratory technique that separates the components of a sample by their mass and electrical charge[38]. It can be used to identify, quantify, and analyze differentially expressed protein samples from menstrual phase plasma, which can be used to diagnose certain medical conditions such as endometriosis that could be even undetected by ultrasonography due to its high sensitivity (98%) and specificity (81%)[35,37].

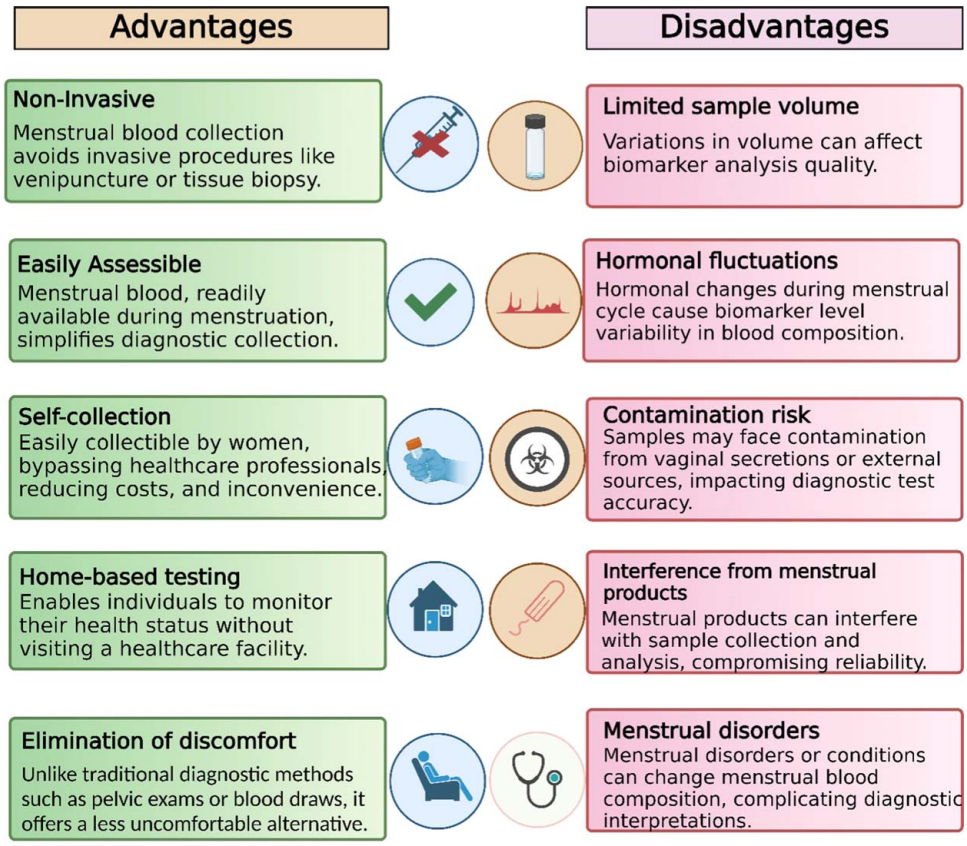

Figure 2. Summary of advantages and disadvantages of menstrual blood as a diagnostic specimen (created with biorender.com).

Serving as a powerful method for separating, identifying, and quantifying complex mixtures of compounds, another pivotal advancement in disease detection through menstrual blood analysis is liquid chromatography (LC)[39]. When coupled with MS, LC becomes a powerful analytical technique that enables the detection of the desired molecule according to the mass/charge ratio (m/z) after chromatographic separation. Ovarian cancer is one example that uses menstrual blood samples and liquid chromatography-tandem mass spectrometry (LC-MS/MS). Researchers measured the levels of 11 ovarian cancer biomarkers in menstrual blood samples from 20 women with ovarian cancer and 20 healthy controls. They found that the levels of the 11 biomarkers were significantly different between the two groups and that a combination of these biomarkers could accurately discriminate ovarian cancer from healthy controls with 93% accuracy. They concluded that menstrual blood could be a useful source for ovarian cancer detection, and that LC-MS/MS could be a reliable and sensitive technique for measuring ovarian cancer biomarkers[40]. LC-MS/MS also stands out as a potent tool in the screening and diagnosis of various endocrine disorders, as it is a straightforward and effective approach for monitoring the concentrations and fluctuations of endogenous steroids in disorders related to steroid metabolism[41]. Additionally, this method has been employed for the quantification of gamma-aminobutyric acid (GABA) levels in plasma, revealing a noteworthy positive correlation between GABA levels and symptoms of post-traumatic stress disorder (PTSD) in women[42].

DBS technology

DBS is characterized by simplicity and convenience, as they require minimal blood volumes collected on filter paper and can be efficiently employed in biobanks for blood sample storage[43]. The samples collected as DBS have shown to provide good diagnostic performance in diagnosing HCV infections, HIV infections, metabolic diseases, lysosomal storage disorders, and several other diseases[44–47]. Analyte loss resulting from partial and ineffective extraction and/or analyte degradation poses a difficulty for DBS quantification, especially for unstable analytes, and this is one of the major technical challenges in regulatory acceptance[48]. In January 2024, Qvin unveiled a pioneering healthcare service that revolutionizes blood testing by collecting menstrual blood samples in a DBS strip (Q-Pad). The Q-Pad eliminates the need for traditional venous blood draws and is currently FDA for HbA1c testing. The Q-Pad marks a significant milestone in healthcare monitoring, which is not only a non-invasive and more accessible method for collecting samples but also proves to be a cost-effective and time-efficient solution, particularly beneficial for the millions of women in America managing diabetes[49].

Molecular biological techniques

Molecular methods play a pivotal role in modern medical diagnostics. By analyzing genetic and molecular components, molecular methods enable the accurate detection of pathogens, genetic mutations, and disease biomarkers. Their ability to identify even

trace amounts of genetic material facilitates early disease diagnosis and personalized treatment strategies, ultimately improving patient outcomes[50].

Polymerase chain reaction (PCR)

The PCR is a method used to amplify DNA or cDNA derived from RNA, generating numerous copies of a particular genomic segment. In this process, various genomic changes, including single nucleotide variants (SNVs or somatic point mutations), deletions, insertions, copy number variants, and gene fusions, can be identified, depending on the assay design. One such example is the identification of the presence of matrix metalloproteinases (MMPs) which are involved in tissue remodeling and the cleavage of extracellular matrix proteins and can serve as biomarkers for menstrual blood identification and endometriosis diagnosis[51].

Next-generation sequencing (NGS):

NGS is a cutting-edge DNA sequencing method that rapidly and simultaneously sequences millions of DNA fragments. It offers high-throughput, cost-effective analysis, revolutionizing genomics in tasks such as whole-genome sequencing, targeted sequencing, and RNA sequencing[52]. It is related to analyzing menstrual blood for the diagnosis of diseases because menstrual blood contains endometrial tissue with viable cells, immune cells, nucleic acids, proteins, and microorganisms from the vaginal microbiome. These components can provide valuable information about the health status of the person who menstruates, such as their genetic variations, gene expression, epigenetic modifications, and microbial diversity[53].

Culture-based method

Culture-based methods for menstrual blood analysis are techniques that involve growing microbes from a sample of menstrual blood on different media and identifying them based on their characteristics. These methods can detect bacteria, fungi, and parasites that may cause infections or inflammation in the reproductive tract[54]. Such techniques enable the isolation of pure cultures, facilitating further in-depth analysis or long-term storage. Moreover, they possess the capability to detect novel or uncultured microbes that may go unnoticed when employing molecular methods[54].

Challenges in utilizing menstrual blood as a diagnostic specimen

Despite the potential of menstrual blood-based diagnosis, it is still not commonly used. The lack of standardized protocols for the collection, processing, and analysis of menstrual blood samples is one reason. Additionally, more research is required to validate the use of menstrual blood biomarkers for various conditions[33].

While advancements in analyzing menstrual blood for the diagnosis and detection of diseases show promise, other challenges persist. Stigma is one of the biggest taboos in promoting open discussions about menstrual health and hygiene[55]. This, coupled with the fact that it may be perceived as an unconventional diagnostic method due to its novelty, limits the widespread acceptance and exploration of menstrual blood as a promising source for diagnosing diverse diseases. Another challenge is that many healthcare providers and their patients aren't aware of the benefits of using menstrual blood for diagnosis despite it being a non-invasive test. More awareness campaigns are needed for both doctors and women in general so that more people recognize it as a useful diagnostic specimen. Furthermore, certain blood clotting factors, like von Willebrand factor and factor VIII, undergo fluctuations throughout the menstrual cycle. Consequently, the accuracy of diagnoses for conditions involving these factors may be influenced by the timing of menstrual blood collection. For instance, women with von Willebrand disease might exhibit lower levels of von Willebrand factor during menstrual and early follicular phases, potentially causing false-negative results[56]. Studies have also identified some challenges in using menstrual blood for high-risk HPV detection. One of the challenges is the variability of menstrual blood composition, which can affect the sensitivity and specificity of the diagnostic results[28].

Comparison of menstrual blood with other body fluids as a diagnostic specimen

Body fluid analysis plays a key role in providing valuable insights for diagnosis in clinical medicine. Available tests vary in specificity and sensitivity, and few provide a comprehensive evaluation of the clinical picture. It is essential to combine lab findings with clinical assessment of the patient through history and examination in order to reach a successful diagnosis[57]. Routine blood tests offer insights on the different aspects of homeostasis in the human body and can be used to make diagnoses for a variety of conditions including dehydration, infections, pregnancy, anemia, and cancer[58]. Urine samples are regularly used to test for pregnancy, diabetes, and urinary tract infections[59]. Saliva is diagnostically valuable in identifying certain neuropsychiatric disorders as well as certain malignancies[60,61]. Cerebrospinal fluid (CSF) is commonly employed for diagnosing conditions such as meningitis, encephalitis, and other neurological disorders[62].

Comparison with normal blood

The amount of RBC, WBC and Hb are lower in menstrual blood compared to venous blood. RBC in menstrual blood range between 2 and 3 million/cm, while in normal blood it is found to be 4–5 million/cm. WBC in menstrual blood range between 4 and 6 cu/mm while in normal blood they range between 5 and 8 cu/mm. Hb levels fall between the range of 2–5 gm/dl in menstrual blood compared to normal blood. Therefore, menstrual blood is not an appropriate fluid to test for RBC, WBC, and Hb levels as the consistently lower counts in comparison to venous blood may lead to inaccurate assessments and misinterpretations of health status. However, menstrual blood can be used to determine the blood group precisely. Additionally, performing a forensic analysis using Teichmann test (confirm presence of hemoglobin) yields a negative analysis using menstrual blood. It is positive however in normal blood. This is due to the low levels of Hb (2–5 gm/dl) in the menstrual blood[63]. Creatinine, LDL, glucose, and triglyceride levels are found to be consistently higher in normal blood compared to menstrual blood. The mean difference between the menstrual blood and systematic blood sample's LDL, creatinine, glucose, triglyceride was 12.2, −0.24, 80.7 and 12.49 mg/dl, respectively. This carries significant implications for diagnostic testing as variations among normal and menstrual blood underscore the importance of considering these differences

when interpreting test results and making clinical decisions, enhancing diagnostic accuracy. However, levels of FSH, cholesterol and high sensitivity c-reactive protein (CRP) levels were found to be the same in menstrual blood and in normal blood, suggesting the possibility that menstrual blood can be used to detect the levels of these health indicators effectively while providing a non-invasive approach[33]. If specific biomarkers consistently exhibit similar levels in both systemic and menstrual blood, it opens the door to the feasibility of utilizing menstrual blood in diagnostic testing. This potential alternative could offer greater convenience and accessibility, particularly for certain populations, thereby reshaping approaches to diagnostic procedures. In a study conducted for comparative analysis of natural killer (NK) cells of women with Recurrent Spontaneous Abortion (RSA) and fertile women, menstrual blood and normal blood samples were compared. Samples were taken from both groups on the second day of the menstrual cycle. In both groups, the proportions of CD16+ and CD45RO− cells were lower in menstrual blood than in normal blood. However, levels of CD56+CD16−CCR7− and CCR7+ cells were greater in menstrual blood compared to normal blood[64]. Though it was not mentioned how much lower or greater these cells were present in menstrual blood compared to normal blood, knowledge of these cells may still have implications for potential diagnostic and therapeutic strategies for patients experiencing RSA. Additionally, matrix metalloproteinases such as MMP7 and MMP11 are detected in menstrual blood samples. These proteins are involved in tissue remodeling and extracellular matrix protein cleavage and are absent in normal blood[51].

Comparison with urine

Urine is another commonly used body fluid for the diagnosis of diseases. It is important to properly collect, store, and analyze the urine sample for an accurate diagnosis. Collection methods for urinalysis include non-invasive and invasive techniques. Non-invasive technique involves a spontaneous voiding method and is preferred for adults and children that can control voiding. It is important to clean the genital area to avoid contamination, and a sample of 15–40 ml is collected in a sterile container. Invasive techniques include urethral catheterization for patients who are unable to control voiding. A small French urinary catheter is passed through the urethral meatus, and urine is collected. Another invasive technique used for urine collection is suprapubic needle aspiration. This involves inserting a needle into the bladder through the abdominal wall. For the purpose of analysis, menstrual blood collection is a non-invasive procedure, where women are asked to collect the effluent by Tampon or pads[6]. However, to date, no studies have investigated the presence of biomarkers of chronic kidney disease (CKD), such as uromodulin, neutrophil gelatinase-associated lipocalin (NGAL), and kidney injury molecule-1 (KIM-1), in menstrual blood[65]. These biomarkers are primarily found in urine. Therefore, the potential of menstrual blood to replace urine for the diagnosis of CKD remains unknown. Exploring this would be beneficial for women with severe CKD who are unable to urinate. In such cases, menstrual blood could provide an alternative non-invasive approach for the diagnosis of CKD.

Comparison with saliva

Saliva analysis offers a valuable specimen for assessing endocrine function, particularly in measuring steroid hormone levels. Salivary hormone measurements hold clinical significance when they accurately mirror serum hormone levels or maintain a consistent correlation with them. Also, saliva offers a convenient, non-invasive sampling method compared to traditional blood draws, potentially enhancing patient compliance and reducing discomfort[66]. Analyzing hormonal markers in saliva offers a non-invasive method for predicting pubertal onset and understanding hormonal changes in pre-pubertal girls. Hormones like estradiol, LH and FSH provide insights into hormonal balance and developmental stages even before menstruation starts. These markers serve as indicators of pubertal progression, offering valuable information on hormonal dynamics in girls before menstruation begins. Endocrine markers in saliva may thus serve as an objective and sensitive specimen for assessing development, providing a non-invasive alternative to traditional methods[67].

Saliva has also shown promise as an alternative to serum for assessing hscRP levels in myocardial infarction (MI) patients, with studies indicating a direct correlation between saliva and serum levels in both control subjects and MI patients[68]. However, CRP levels in menstrual blood during the early follicular phase are significantly higher than during the luteal phase of the menstrual cycle. Thus, using CRP levels for cardiovascular risk assessment during menstruation in reproductive-age women may not be appropriate due to the fluctuation in levels[69].

Menstrual blood analysis for HbA1c and saliva-based measurement of alpha-2-macroglobulin (A2MG) levels offer promising avenues for assessing glycemic index and preventing diabetes-related complications. A cross-sectional study suggests salivary A2MG's high specificity in diagnosing diabetes[70]. While menstrual blood analysis allows for convenient self-administration at home, saliva-based A2MG measurement offers a non-invasive and cost-effective alternative for glycemic status assessment. However, further research and validation in clinical settings are crucial to determine the optimal utilization and integration of these techniques in diabetes management[12].

Recent research suggests that there is a potential association between salivary lipids and hyperlipidemia. Studies have found increased levels of salivary lipids in patients indicating a possible link between overall health and lipid metabolism. Saliva, being easy to collect and non-invasive, presents an opportunity to assess lipid status as a diagnostic specimen for monitoring hyperlipidemia[71]. Moreover, menstrual blood has emerged as a promising medium for estimating serum lipid levels, including cholesterol, LDL, triglycerides, and HDL. This indicates that menstrual blood could potentially serve as a surrogate to serum lipid profiles, offering a non-invasive and accessible method for lipid assessment[33]. However, further research is necessary to validate the specificity and reliability of menstrual blood analysis in estimating serum lipid levels accurately. In summary, both saliva and menstrual blood show promise as an alternative means for lipid assessment, with saliva particularly suited for overall health monitoring and menstrual blood potentially offering a non-invasive method for lipid profile estimation. Further research is necessary to determine the specificity for using it as a replacement for serum lipid profiles. Ongoing studies will contribute to establishing their clinical usefulness and precision in lipid evaluation[71].

Comparison with CSF

CSF analysis is crucial for diagnosing various central nervous system (CNS) diseases including multiple sclerosis (MS), encephalitis, meningitis, brain tumors, Creutzfeldt–Jakob disease, and Alzheimer's disease. Changes in CSF components like total protein, glucose, neurospecific proteins, and cell counts indicate disease presence or inflammation. Oligoclonal bands in CSF are diagnostic for MS, while Guillain–Barré syndrome (GBS) shows increased total protein concentration. CSF analysis aids in differentiating viral and bacterial meningitis and identifying atypical cells in CNS tumors like lymphoma, leukemia, and primary brain tumors. Alzheimer's disease exhibits increased Tau protein and decreased β-amyloid in CSF. Overall, CSF examination provides valuable insights for accurate diagnosis and treatment implementation in various CNS disorders[72]. Studies have yet to investigate the presence of Tau protein and β-amyloid in menstrual blood or its potential in diagnosing meningitis. Further research should determine whether menstrual blood shows promising results, potentially serving as a non-invasive alternative diagnostic option for some of the above-mentioned conditions, currently diagnosed via CSF analysis.

CSF CRP serves as a valuable diagnostic marker for meningitis owing to its link with CNS inflammation[73]. However, detecting CRP from menstrual blood in reproductive-age women might be less reliable due to hormonal fluctuations during the menstrual cycle[69]. These hormonal variations can influence CRP levels, potentially leading to misleading conclusions about inflammation markers in menstrual blood.

In nutshell, a comparison of menstrual blood with other common diagnostic specimens such as normal blood, urine, saliva, and CSF reveals various advantages and limitations. Menstrual blood offers the advantage of being non-invasive compared to normal blood and can be used for analyzing markers present at similar levels in normal blood, such as FSH and HbA1c. However, it cannot be used for measuring blood cell counts or for forensic analysis purposes, where normal blood is preferred. When compared to urine, which is also non-invasive, menstrual blood lacks specific biomarkers such as NGAL and KIM-1 that provide information on CKD. It remains unclear if menstrual blood contains these markers or others relevant for CKD diagnosis, potentially offering an alternative diagnostic approach for patients unable to provide urine samples. Saliva, another non-invasive diagnostic specimen, provides insight into various diseases, including diabetes, similar to menstrual blood. However, a comparative study is needed to determine which specimen, saliva or menstrual blood, offers greater reliability for diagnosing diseases such as diabetes. Finally, CSF, obtained through invasive lumbar puncture, offers information on several diseases. Future research should explore the possibility of markers present in CSF also being present in menstrual blood, potentially providing an alternate non-invasive diagnostic approach.

Limitations of utilizing menstrual blood as a diagnostic specimen

Several limitations need acknowledgement when considering the use of menstrual blood for diagnosis of diseases. Firstly, there is a lack of standardized protocols for both collecting and processing menstrual blood samples. Additionally, there have been no significant advancements in methodologies aimed at controlling the fluctuation of menstrual blood composition, which poses challenges for accurate diagnosis. Moreover, menstrual blood may not be applicable for diagnosing diseases in certain populations, such as postmenopausal women, men, and young children who do not undergo menstrual cycles. Furthermore, societal stigma surrounding menstrual health and awareness, particularly prevalent in certain regions, presents a significant barrier that may not be fully addressed within the scope of the study. This stigma could impact both the willingness of individuals to participate in research involving menstrual blood samples and the accessibility of menstrual health services.

Conclusion

Menstrual blood has an immense potential to be used as a diagnostic specimen in healthcare. Its composition offers insights into reproductive and overall health, aiding in the detection and monitoring of various diseases such as diabetes, endometriosis, cervical cancer, and cardiovascular diseases via non-invasive approach. Several factors contribute to the opportune timing for utilizing menstrual blood in disease diagnosis. Among them is the integration of advanced technologies such as mass spectrometry, chromatography, and molecular biological techniques. Additionally, recent studies investigating the diagnostic potential of menstrual blood, along with FDA approval for its use in diabetes diagnosis, have created opportunities for innovative diagnostic approaches using this biological specimen. Despite some limitations, as mentioned above, ongoing research and regulatory approvals signify the promising future of menstrual blood-based testing as a non-invasive, accessible, diagnostic method for various diseases. Further validation through large-scale prospective trials is needed to confirm the efficacy and establish standardized protocols for the clinical use of menstrual blood-based diagnostics. Additionally, investigation aimed at identifying an optimal approach for managing fluctuations in menstrual blood sampling may have the potential to improve its accuracy in diagnosing diseases.

Ethical approval

Ethics approval was not required for this review.

Consent

Informed consent was not required for this review.

Source of funding

Not applicable.

Author contribution

Concept and outline: A.A. Initial draft: A.Z., A.K., M.B.A.B., A.K.S., A.S.S., M.M.K., A.A., M.H.K, Figures: A.Z., M.B.A.B., M.M.K. Critical revision of draft: A.A. Revision of final draft: A.Z., A.K., M.B.A.B., A.K.S., A.S.S., M.M.K., A.A., M.H.K., A.A.

Conflicts of interest disclosure

Not applicable.

Research registration unique identifying number (UIN)

Not applicable.

Guarantor

Not applicable.

Data availability statement

Not applicable.

Provenance and peer review

Not applicable.

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