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ORIGINAL RESEARCH

Innovative Hematology Analysis Using Menstrual Blood

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Purpose: The main aim of this study was to compare and analyze hematological profiles using menstrual blood, as an alternative to peripheral blood.

Patients and Methods: This study used menstrual and peripheral blood samples from women who were menstruating. The design of this research is analytical observational.

Results: Menstrual blood can show an overall hematological profile similar to peripheral blood. Data shows the detection of blood component parameters, white blood cells and reticulocytes in MB with a range within and outside normal blood. Data on MB that show higher values (WBC, MCH, MCHC, PLT, RDW-CV, PDW, MPV, P-LCR, PCT, neutrophils, lymphocytes, monocytes, basophils, reticulocytes, LFR, Ret-He) and lower values lower (RBC, HGB, HCT, MVC, RDW-SD, Eosinophils, IRF, MFR, HFR) when compared with peripheral blood controls. The hematological profiles of Menstrual and peripheral blood showed significant differences ($p < 0.01$) for several parameters, while several other parameters did not show significant differences ($p > 0.05$) according to the Wilcoxon test.

Conclusion: All hematological profile parameters were detected in menstrual blood. The new concept that menstrual blood can be used as a supporting medium for hematological examinations opens up opportunities for developing independent hematological detection tools in productive women.

Keywords: hematological, menstrual blood, peripheral blood, WBC, RBC, reticulocyte

Introduction

Menstrual Blood Flows Through Arterial and Venous Blood

Menstrual blood (MB) is the result of the process of shedding the endometrial lining of the uterus due to the fall of estrogen and progesterone concentrations. The expulsion of MB occurs due to bleeding from the uterus approximately fourteen days after ovulation in a cyclical pattern. On the third day of menstruation, there is a significant release of MB.[1] Menstruation is regulated by hormonal signals that trigger local, ischemic, and immunological reactions in the uterus, ultimately leading to the shedding of the endometrium. The hormonal process of MB release is associated with the role of immune cells, endothelial cells, and stromal cells, which release various cytokines through autocrine, paracrine, or endocrine mechanisms.[2] Menstrual blood consists mostly of arterial blood and 25% venous blood. It contains prostaglandins, tissue debris, and substantial amounts of fibrinolysis products originating from endometrial tissue. Fibrinolysis prevents clot formation, ensuring that MB does not clot. The duration of menstruation typically ranges from 3 to 5 days in most women, with MB volume ranging from 30 to 80 mL.[3]

Menstrual Blood Can Detect Cytokine Proteins

Previous research has shown a relationship between the amount of MB and nutritional intake with hemoglobin (Hb) levels in girls, using a menstrual pictogram questionnaire. There is a significant relationship between the amount of MB,

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vitamin C intake, and hemoglobin levels in adolescent girls.[1] No studies have described the hematological profile of MB in relation to health and disease. Hematological profile examination includes hemoglobin, erythrocytes, hematocrit, MCV, MCH, MCHC, RDW-CV, platelets, leukocytes, eosinophils, basophils, neutrophils, lymphocytes in the blood, and reticulocytes. However, previous research has successfully described the cytokine pattern (IL-6, IL-1β, and TNF) in MB plasma, which differs significantly from peripheral blood, using ELISA measurements. Interindividual MB cytokine profiles in healthy donors are limited and remain stable over time. Minor injury from endometrial biopsy does not alter the cytokine profile throughout the menstrual cycle.[2] This information suggests that there are various cell types present in MB which secretes these cytokines.

Results of MB analysis have revealed subpopulations of T cells and natural killer cells immunophenotyped by flow cytometry. The frequency of T cells, such as CD4 + Foxp3+, CD4 + Foxp3 + CD25-, CD4 + Foxp3 + CD25+, IL17+, and TCR αβ+, CD45RO+, CD16-, IFNγ + and IL17+ NKT (CD56 + CD3+) cells, was significantly higher in MB compared to peripheral blood. This indicates that MB contains both inflammatory and anti-inflammatory cells, suggesting good cell homeostasis during menstruation. MB can be seen as an easily accessible specimen for monitoring endometrial immune cells, especially those with preferential endometrial localization.[4] Additionally MB, 1061 proteins have been identified compared to circulating blood (1774 proteins) and vaginal fluid (823 proteins), with 385 unique proteins found in MB. These unique proteins in MB suggest that extramedullary uterine hematopoiesis or parenchymal hemoglobin synthesis can occur in late endometrial tissue.[5]

Independent Hematology Examination Using Menstrual Blood

The prevalence of heavy menstrual bleeding (HMB) in women of childbearing age is 37.9%. Ferritin levels and physical function were found to be significantly reduced, with increasing menstrual duration. The negative effects on anemia, fatigue, and quality of life caused by this issue can be prevented, and treatment plans can be identified through laboratory examinations and regular follow-ups.[6] The use of MB is easier, non-invasive, and does not require injections or surgery. Examination of the hematological profile in this study is crucial to understand the differences in composition between MB and peripheral blood. This hematological profile is necessary for various diagnostics related to different diseases. This research could assist in the diagnosis and monitoring of health problems associated with changes in MB composition. For instance, it could be relevant in the management of menstrual disorders, endometriosis, or bleeding disorders. It is hoped that sophisticated and effective identification tools or health technology for blood analysis will be developed in the future, allowing women to conduct independent examinations using MB. This could be a step toward preventing health issues, especially for those located far from healthcare facilities.

Materials and Methods

Hematological Profile Parameters

This study used an analytical objective design that compared the hematology profile of menstrual blood samples with peripheral blood as a control. Hematology profile parameters measured were: blood components including WBC, RBC, HGB, HCT, MVC, MCH, MCHC, PLT, RDW-SD, RDW-CV, PDW, MPV, P-LCR, PCT; leukocyte cell types including neutrophils, lymphocytes, monocytes, eosinophils, basophils; and reticulocyte measurement types including percentage and number of reticulocytes, IRF, LFR, MFR, HFR, Ret-He.

How to Take and Measure Hematological Profiles

Menstrual blood was obtained from women of reproductive age between 18–55 years. All of them were volunteer donors who provided signed informed consent forms in accordance with ethical guidelines. This research had previously passed the Ethics Commission of Faculty of Medicine, Islamic State University Syarif Hidayatullah Jakarta which complies with the Declaration of Helsinki (ethical approval No: B-023/F12/KEPK/TL.00/04/2023). Respondents were asked to collect their MB using a sterile plastic pot that did not use EDTA. The pot does not contain anticoagulants and can hold 10 mL of fluid. Respondents were guided on how to collect MB without mixing it with urine. The number of MB collected is not limited, but should not exceed the capacity of the pot. Menstrual blood was taken on the third day and peripheral blood

was taken on the same day, approximately 3 mL each was put into a tube containing EDTA. There were 25 respondents who were willing to have menstrual and peripheral blood taken at the same time on the third day of menstruation. Respondents were obtained through consecutive sampling. An automated hematology analyzer was performed using an automated hematology analyzer, from the Sysmex XP-300™ series, was used to measure the concentration of menstrual and peripheral blood hematological profiles. Menstrual and peripheral blood were processed similarly without centrifugation. Samples were taken directly through the automatic pipette on the device as much as 20 µL. The reagent used was Cellpack™ DCL (DCL-300A). The data results were analyzed using the Wilcoxon test due to the non-normality of the distribution via IBM SPSS version 22.

Results

The identification of respondents in this study is presented in Table 1. There was no intervention when collecting MB and peripheral blood samples, because it was carried out according to the donor's natural condition, respectively on the third day of menstruation. From the results of respondent identification, data was obtained regarding the patient's age, natural conditions when blood was drawn, and medical history. In this study, Table 2 displays the comparison results of various blood component parameters in MB and peripheral blood. Table 3 presents the comparison results of various white blood cell parameters in MB and peripheral blood. Meanwhile, Table 4 shows the comparison results of various reticulocyte parameters of MB and peripheral blood. The results from Tables 2–4 indicate that MB can be used for hematological profile analysis, similar to that performed on peripheral blood. The p values in all tables varied between

Table 1 Respondent Identification (N=25)

Age (years)	
12–25	16%
26–45	50%
> 46	34%
Blood Collection Time	Menstruation on the third day
Duration of menstruation	7–10 days
Menstrual disorders	None
Operation for pregnancy	12%
Contraceptive use	4%
Respondent's condition at the time of blood collection	
Dengue Fever Recovery	8%
Dizziness/weakness	25%
Normal	67%
Disease History	
Tuberculosis	8%
Hypertension	17%
Hypercholesterolemia	8%
Obesity	17%
Diabetes mellitus	8%
Hyperuricemia	8%

Table 2 The Comparison of Blood Component Parameters in Menstrual and Peripheral Blood

Blood Components	Normal	Peripheral Blood	Menstrual Blood	Analysis			*p value*
				High	Low	Difference High/ Low	
WBC (10^3/uL)	5–10	6.0	11.6	√		5.65	0.000**
RBC (10^6/uL)	4–5	3.81	1.71		√	2.11	0.006**
Hb (g/dL)	12–16	12.2	11.7		√	0.49	0.570
HCT (%)	36–48	33.0	15.0		√	18.07	0.003**
MCV (fL)	80–100	86.3	72.0		√	14.24	0.100
MCH (pg)	27–31	32.4	83.7	√		51.29	0.003**
MCHC (g/dL)	32–36	37.7	131.2	√		93.52	0.003**
PLT & O (10^3/uL)	177–401	376	511	√		134.73	0.131
RDW-SD (fL)	40–55	87.6	80.0		√	7.65	0.021*
RDW-CV (%)	11–15	28.4	31.2	√		2.75	0.041*
PDW (fL)	9.2–16.7	14.7	17.0	√		2.32	0.073
MPV (fL)	8.4–12	11.48	11.71	√		0.23	0.421
P-LCR (%)	15–35	36.69	37.71	√		1.02	0.475
PCT (%)	0.22–0.24	0.41	1.19	√		0.77	0.060*

Notes: *Significantly different at the 95% confidence level. **Very significantly different at the 99% confidence level.
Abbreviations: WBC, white blood cell; RBC, red blood cell; Hb, hemoglobin; HCT, hematocrit; MCV. mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, Mean corpuscular hemoglobin concentration; PLT, platelet; RDW-SD, Red cell distribution width-standard deviation; RDW-CV, red cell distribution width-corpuscular volume; PDW, platelet distribution width; MPV, Mean Platelet Volume; P-LCR, Platelet-large cell ratio; PCT, platelet count.

Table 3 The Comparison of Leukocyte Cells in Peripheral Blood and Menstrual Blood

Leukocyte Cells	Normal	Peripheral Blood	Menstrual Blood	Analysis			*p value*
				High	Low	Difference High/ Low	
NEUT (10^3/uL)	1.5–8.0	5.14	8.32	√		3.18	0.033*
LYMPH (10^3/uL)	1.4–4.8	0.456	1.993	√		1.54	0.016*
MONO (10^3/uL)	0.2–0.6	0.013	0.713	√		0.70	0.003**
EO(10^3/uL)	0.03–0.35	0.172	0.121		√	0.05	0.050
BASO (10^3/uL)	<0.2	0.176	0.442	√		0.27	0.016*
NEUT %	50–70	85.65	74.98		√	10.66	0.026*
LYMPH %	20–40	7.85	13.51	√		5.66	0.056
MONO %	1–10	0.24	5.21	√		4.97	0.003*
EO %	0–6.0	3.04	2.61		√	0.43	0.656
BASO %	0–1	3.24	3.69	√		0.45	0.328

Notes: *Significantly different at the 95% confidence level. **Very significantly different at the 99% confidence level.
Abbreviations: NEUT, neutrophils; LYMPH, lymphocytes; MONO, Monocytes; EO, Eosinophils; BASO, Basophils.

Table 4 Comparison of Reticulocyte Parameters in Peripheral Blood and Menstrual Blood

Reticulocyte Parameters	Normal	Peripheral Blood	Menstrual Blood	Analysis			*p value*
				High	Low	Difference High/ Low	
RET% (%)	28–36	11.49	36.71	√		25.22	0.013*
RET(10^6/uL)	0.5–2.5	0.05	0.29	√		0.24	0.003**
IRF (%)	2.3–15.9	46.33	17.39		√	28.94	0.013*
LFR (%)	87.9–98.4	53.67	82.61	√		28.94	0.013*
MFR (%)	1.6–11.0	33.04	8.72		√	24.32	0.062
HFR (%)	0.0–1.7	13.29	8.62		√	4.67	0.507
RET-He (pg)	28–36	9.90	11.69	√		1.79	0.003*

Notes: *Significantly different at the 95% confidence level. **Very significantly different at the 99% confidence level.
Abbreviations: Ret, reticulocyte; IRF, Immature Reticulocyte Fraction; LFR, low fluorescence ratio; MFR, medium fluorescence ratio; HFR, high fluorescence ratio; Ret He, reticulocyte Hb.

menstrual and peripheral blood (non-significant and significant). This analysis uses third day MB from the same individual/respondent. The data results show that the blood component parameters, white blood cell parameters, and reticulocyte parameters in MB are mostly outside the normal blood range, although some are still within the normal range. The data also highlight differences in high and low values compared with peripheral blood controls. These differences can be correction factors when used as a basis for diagnosis and tool development. In addition, some data regarding blood component parameters, white blood cell parameters, and reticulocyte parameters in MB and peripheral blood as controls showed very significant differences ($p < 0.01$), some were considered significant ($p < 0.05$), while others are insignificant. In addition, some data on blood component parameters, white blood cell parameters, and reticulocyte parameters in MB blood showed significant differences compared to peripheral blood.

Discussion

This study presents the novelty that hematological profile analysis of MB can be used As a media for supporting hematology examinations in confirming the diagnosis of diseases, as evidenced by the consistency of all hematological profile parameters with those found in peripheral blood. Although there are aspects that may require correction when used as a foundation for diagnostic tools and tool development, including the establishment of correction factors for the normal blood range and alignment with peripheral blood analysis controls, which are the current standards for diagnostic purposes. Moreover, the potential benefits of utilizing MB as a diagnostic tool should also be considered. For instance, MB can be collected non-invasively, offering a more convenient option for some individuals. However, it is important to recognize that this initial step in developing diagnostic media and validating new diagnostic tools using MB requires rigorous research and testing before they can be widely implemented in medical practice.

In our bodies, blood consists of three main types of cells: erythrocytes, leukocytes, and platelets. Red blood cells contain hemoglobin which functions as an oxygen transporter, while hematocrit is the percentage of red blood cell count and leukocytes are part of the immune system in fighting infection. Leukocytes can be grouped into granulocytes and agranulocytes based on their origin, and they can be further classified into myeloid and lymphoid cells, including neutrophils, eosinophils, lymphocytes, basophils, and monocytes. The main function of leukocytes is to protect the body from infection and aid in wound healing.[7] In this study, we examined various parameters in the blood, including erythrocyte count, leukocyte count, platelet count, Hb (hemoglobin) levels, hematocrit, and others. We found that erythrocyte levels in MB were lower compared to peripheral blood. This difference may be related to inflammation when the uterine wall sheds during menstruation.[4] However, when we measured Hb (hemoglobin) levels, we did not observe any significant difference between MB and peripheral blood. This suggests that menstruation does not

significantly affect Hb levels. Upon analyzing erythrocytes, we observed that the erythrocyte count in peripheral blood (venous blood) was higher compared to MB. This difference is likely because only about 25% of venous blood is mixed with MB.[3] We acknowledge that further analysis of other parameters is needed to fully understand the distinctions between peripheral blood and MB. Additionally, we must consider the physiological conditions during measurements to develop diagnostic standards that are more valid, free from bias, and accountable.

This research discusses the importance of blood profile analysis in diagnosing blood-related issues. In previous studies, a tool called Sysmex XN 9000 was used to identify problems with blood cells, such as high or low leukocyte counts, as well as other issues like large platelet aggregates and abnormal red blood cells.[8] In this study, we used a different tool, namely Sysmex XP-300™. Although this device differs from Sysmex XN 9000, both are generally capable of analyzing blood basics. We are confident that the results of the MB profile analysis we conducted with Sysmex XP-300™ are valid because we compared them with the results of hematological profile measurements from peripheral blood and found similarities. Despite some significant differences in certain parameters, which can be measured using the Wilcoxon test.

Hematocrit is a test that measures how many red blood cells are present in the blood, known as the packed cell volume (PCV). If the hematocrit result is low, it can indicate anemia, whereas if it's high, it may suggest polycythemia. The standard method for measuring hematocrit is by using a blood analysis instrument called micro-hematocrit. In the diagnosis of anemia, hemoglobin tests are typically used to assess how well blood carries oxygen. However, hematocrit measurements provide indirect information, as hematocrit results are converted to hemoglobin by multiplying them by 3 (Hemoglobin = Hematocrit X 3). This conversion may not be accurate in regions with malaria cases due to variations in red blood cell volume, and it requires consideration of context-dependent conversions.[9] For example, in a study on hemoglobin levels in MB, we found that hemoglobin levels in MB did not significantly differ from those in peripheral blood, but hematocrit levels in MB were approximately half as low as in peripheral blood. Analysis based on that formula and the Hb concentration we obtained in this study, in cases like this, we can use the formula where hemoglobin is six times hematocrit to measure the degree of anemia when measuring it with MB samples. However, caution is needed in specific cases involving MB samples, where different conversion approaches may be necessary for accurate evaluation and calculation.

Cardiovascular magnetic resonance (CMR) is used to measure the extracellular myocardial volume fraction (ECV) by connecting the longitudinal relaxation rates in blood and myocardium before and after contrast injection with hematocrit (HCT) in the blood. However, HCT can vary depending on body position, affecting ECV calculations. Standardizing the timing of HCT measurements in relation to CMR can reduce ECV calculation variability. Therefore, measuring HCT after CMR while the patient is in a supine position can enhance the accuracy of ECV measurements.[10] This supine position also provides an opportunity to collect MB samples for HCT or other hematological analyses if the patient is menstruating. However, innovative and hygienic handling of MB is required to ensure safety and convenience for patients undergoing treatment. It is important to note that this can only be done on patients who are currently menstruating and is not suitable for pregnant women, menopausal women, or premenstrual girls.

The measurement of platelet count in blood often yields results that are either too low or too high, especially when using automated counting instruments in various laboratories. To ensure accurate results with intact and undamaged cells, some laboratories also examine blood samples under a microscope or employ other methods (peripheral blood smears and counting in a Neubauer chamber). However, these methods are not always reliable, especially when platelet counts are very low.[11] To ensure more accurate results and gain a better understanding of blood cell conditions, further research can be conducted by obtaining fresher blood samples and directly performing automated hematological profile measurements. Additionally, this can be done using a technique called flow cytometry. This technique allows us to observe and measure the characteristics of blood cells in more detail. It is carried out by labeling blood cells with fluorescent markers and characterizing them using specialized instruments that utilize laser beams. This technique is highly valuable for identifying issues on the surface of blood cells and blood cell components.[12] A flow cytometer uses dyes to measure reticulocytes by observing the light or fluorescence they reflect. This instrument also provides additional information about reticulocytes, such as how young or mature they are, aiding in our understanding of erythrocyte formation activity.[13] Automated hematological measurements in blood are often imperfect. It is necessary to use additional methods (such as employing flow cytometry) to ensure more accurate results. The use of flow cytometry in measuring reticulocytes in MB and other methods is an area of research that could develop in the future.

Monitoring the platelet count in the blood is important for assessing a patient's health, especially when they are undergoing specific treatments like chemotherapy or blood-thinning therapy. It is also highly advisable when following treatment procedures that may potentially trigger thrombocytopenia, such as anticancer chemotherapy and anticoagulants/heparin. The pre-analytical phase is critical, especially when using sodium citrate as an alternative to EDTA as an anticoagulant.[14] In this study, we used EDTA tubes for blood sample collection. This is not an issue when measuring the overall blood profile, but we need to consider different methods if we want to assess thrombocytopenia, as in the case mentioned above. As a side note, MB is generally challenging to separate into serum or plasma, as MB samples often contain clots, making collection with a micropipette difficult. However, in this study, we were able to use automated equipment that required only a small sample (20–50 µL).[13]

Iron Deficiency Anemia (IDA) is a condition in which the body lacks the iron necessary for the production of hemoglobin, which is vital for red blood cells. This can occur for various reasons, such as increased iron requirements, difficulties in absorbing iron, or chronic blood loss. The World Health Organization (WHO) defines hemoglobin values <130.0 g/L in men, <120.0 g/L in women, and <110.0 g/L in pregnant women as indicative of anemia. Typically, IDA is more common in women due to factors like menstruation and pregnancy. During adolescence, rapid growth and insufficient iron reserves can increase the risk of IDA if there is inadequate iron intake. Doctors can identify IDA by performing specific blood tests, such as measuring zinc protoporphyrin in erythrocytes, soluble transferrin receptor levels in the blood, or hemoglobin content in reticulocytes. These tests assist in diagnosing and monitoring IDA.[15] Reticulocyte Hemoglobin (RET-He) is one of the blood tests that helps us assess anemia due to iron deficiency. When the RET-He value is above 30 pg, it can be a good indicator that someone is not suffering from Iron Deficiency Anemia (IDA), but this is only valid if no other blood-affecting conditions are involved. In everyday medical practice, IDA is often mixed with other issues, so we still need to check ferritin levels for a more precise diagnosis.[16] This study has limitations because it did not examine the iron content and other molecules in the blood. This is essential information that can affect the results of hematological profiles in MB.

This study utilized MB on the third day, which is when the average blood volume is at its highest during the menstrual cycle. During menstruation on the third day, the average peripheral blood hemoglobin level was 12.3 g/dL (normal range 12–16 g/dL) (consistent with the findings of this study, which reported an average Hb level of 12.2 g/dL; Table 1). The third day of menstruation is a somewhat vulnerable condition as it can lead to iron loss, a decrease in high hemoglobin levels, and the development of anemia. Consequently, there is an increased risk of experiencing fatigue and disruptions in productivity. On the third day of menstruation, women undergo changes in erythrocyte morphology, including abnormalities in erythrocyte size (anisocytosis), erythrocyte shape (poikilocytosis), and erythrocyte color (normochromic, hypochromic). There is no relationship between hemoglobin levels and erythrocyte morphology on the third day of menstruation, such as erythrocyte size and shape. However, there is a correlation between hemoglobin levels and erythrocyte color on the third day.[17] In this study, using MB on this third day has been proven to be ideal for analysis using Sysmex equipment, but further observations and analyses of MB on other days are needed.

The use of MB for analyzing blood profiles opens up opportunities to discover early signs of diseases and develop more targeted treatments. It can also reduce the need for traditional blood draws or surgeries, which can be more comfortable for patients. Just like with MB-derived stem cells (MenSC), utilizing MB for these purposes requires a deeper understanding of how the body regulates the immune system or specific conditions, such as age, hormones, and medical history, which can impact measurement outcomes.[18] Diagnosis using MB has the potential to reduce the risk of serious illnesses and improve the quality of life for patients. This can indicate an effective therapeutic role in the prevention and control of various diseases more comprehensively. The relationship between hematological profiles and environmental conditions can lead to better-coordinated care.[19]

Menstruation contains biological fluid that, in addition to blood, also includes vaginal secretions and endometrial cells from the uterine lining. It contains various proteins, including proteolytic enzymes, cytokines, components of the apoptosis pathway, and a variety of immune cell proteins, all of which are integral to menstruation.[5] A healthy vaginal microbiome is typically dominated by Lactobacillus, while Bacterial Vaginosis (BV) is a common vaginal microbiota disorder in reproductive-aged women. BV is known to be associated with adverse gynecological and obstetric outcomes.[20] The vaginal microbiome is a sensitive microenvironment susceptible to disruption by several factors, including the menstrual cycle. The use of vaginal hygiene products such as wet wipes, bath soaps, and feminine sprays is currently limited.[21] Contamination by microorganisms

from MB should also be considered as it can introduce bias that affects diagnostic results. Developing techniques to separate MB from potential contaminants should also be a future consideration.[20]

HPV screening using MB has been previously explored through a literature review. The basis for utilizing MB in HPV cases is the limited availability of cervical cancer screening tests due to a lack of organized screening facilities, resulting in low participation rates in screening programs, and many women succumb to cervical cancer. To reach a larger number of women, an easy, non-invasive, and time-saving screening method is required. There is research reporting that MB can be used as an alternative sample for HPV detection in cervical cancer screening. Studies have shown that MB's diagnostic accuracy, in terms of sensitivity, ranges from 82.8% to 97.7%, and specificity ranges from 50% to 98% in detecting cervical intraepithelial neoplasia or HPV infection. This suggests that the use of MB could be a viable alternative for cervical cancer screening, especially in developing countries where many women are reluctant to undergo screening due to reasons such as embarrassment, discomfort, or busy schedules.[22.] If MB is to be used as a diagnostic medium, consideration must also be given to societal acceptance and the influencing factors such as cultural, social, and psychological factors.

The menstrual cycle, based on the intensity of the menstrual flow is classified as light, medium, or heavy. The start and end dates of the menstrual cycle are determined based on the first day of menstruation, recorded by measuring the amount of blood discharged during menstruation. Ovulation occurs when there is a positive increase in luteinizing hormone (LH), indicating the end of the follicular phase, with estimated ovulation on the following day. An average cycle length of 28 days is not common in most women, with only 13.08% of cycles estimating ovulation on the 14th day. Age and stress become more significant factors than BMI in half of the cycles with long menstrual cycles and variability.[23] In this study, there were high and low concentrations of the MB hematologic profile compared to the peripheral blood control. This study reflects the conditions of the donors at the time of sample collection in their natural state, as indicated in Table 1 data. Variability in menstrual cycle length and in intensity, volume, composition of MB, and hormonal factors may potentially affect the ability of MB to provide consistent diagnostic results.

Information from donors in the study generally indicated difficulties in collecting MB, requiring extended time in the restroom to capture uncontaminated MB flow without mixing with urine, and avoiding skin contact around the vagina. Thus, there is a need to develop user-friendly and safe devices for MB collection that are conducive to reproductive health. Our vision as researchers in the future involves the creation of a device similar to a pregnancy test strip, capable of directly collecting MB and automatically detecting the tested parameters, including hematological profiles, with specific sensitivity and specificity levels. This would allow for the correction of fluctuations (as seen in Tables 2–4) in the hematological profile research, ensuring its accuracy compared to peripheral blood controls. In the context of screening tests, sensitivity and specificity are crucial to avoid misinterpretation of measurement data results. Sensitivity and specificity describe the relative accuracy of the examination compared to a reference standard. Sensitivity and specificity are often used to screen for highly individualized results. The ability to interpret metrics effectively is essential to provide maximum benefit to clients and the healthcare service system.[24] It's important to understand that the use of MB as a diagnostic tool is still an evolving research field and may require several years of investigation and understanding before valid and effective products become available for use in clinical diagnostics.

Conclusion

The novelty of this research provides a new concept that menstrual blood can be used as a supporting medium for hematological examination to diagnose a disease. The use of MB as a diagnostic support medium must consider: correction factors, validity, freedom from bias and accurate calculations according to normal peripheral blood standards; anticipation of contamination; and consideration of influencing factors (environmental, cultural, social and psychological of the patient).

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Disclosure

The authors report no conflicts of interest in this work.

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