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Comparison of DNA Extraction Feasibility from Menstrual Blood and Endometrial Tissue in Reproductive-Aged Women

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ABSTRACT

The endometrium is a highly vascularized tissue composed of numerous blood vessels, with cells that are formed and destroyed during each menstrual cycle. Menstrual blood, which is shed cyclically from the endometrium in women of reproductive age, contains endometrial tissue or cells. Both deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) are the primary nucleic acids present in cells with nucleic. The aim of this study was to compare the feasibility of DNA molecule extraction results from menstrual blood specimens and endometrial tissue in women of reproductive age.In this study, 20 samples of menstrual blood and endometrial tissue were collected from women of reproductive age. Menstrual blood was gathered using specially designed filter paper, and DNA was extracted using the QIAamp DNA Mini Kit (Qiagen, Cat No.: 51304). Endometrial tissue was obtained via biopsy, and DNA was extracted using the Geneaid DNA Isolation Kit (Geneaid, New Taipei, Taiwan). DNA purity and concentration were measured using a Thermo Scientific Nano-Drop microvolume Spectrophotometer. The results indicated an average DNA purity of 1.88 ± 0.09 and a mean concentration of 116.9 ng/µL for menstrual blood, while endometrial tissue had a median DNA purity of 1.92 and a concentration of 192 ng/µL. Statistical analysis revealed no significant difference in DNA purity between menstrual blood and endometrial tissue ($p = 0.083$), but a significant difference in DNA concentration was observed ($p = 0.002$). Although there was a slight difference in DNA concentration, both menstrual blood and endometrial tissue samples were equally effective in preserving optimal purity and concentration of DNA molecules, proven by the absence of any difference in purity between menstrual blood and endometrial tissue.

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INTRODUCTION

The endometrial tissue, the innermost layer of the uterus, underwent significant changes and hormonal fluctuations during the menstrual cycle.[1] This tissue, predominantly composed of highly vascularized structures formed by numerous blood vessels, was periodically built up and shed with each cycle.[2] Menstrual blood, comprising shed endometrial tissue, cyclically occurred in women of reproductive age.[3] It contained blood and uterine wall fluid, making it a potential source of nucleic acids (DNA and RNA) derived from endometrial cells, which could aid in identifying biomarkers of reproductive diseases.[4,5] Genetic material was located within cells that possessed nuclei, and nucleic acids regulated the biological development of all cellular life. The two primary nucleic acids, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), were extracted from cell nuclei using appropriate isolation methods.[6,7],

The physiological retrograde implantation of endometrial cells into the pelvic cavity through the fallopian tubes, where these cells infiltrated and proliferated in surrounding tissues, was known as retrograde menstruation and was a characteristic feature of endometriosis.[8,9] This phenomenon necessitated consideration when menstrual blood was used as a sample,[10] for example in endometriosis, laparoscopy is the standard diagnostic method. This procedure is invasive, expensive, and can only be performed by experienced professionals. Therefore, there is a need to develop non-invasive diagnostic methods, such as evaluating pathological biomarkers from menstrual blood.[3]

Menstrual blood collection had been previously utilized in imaging, proteomics, and genomics research.[11] Among the methods for collection, menstrual cups, which are cup-shaped devices inserted into the vagina during menstruation, were commonly used. Additionally, earlier studies documented the use of filter paper for collecting menstrual blood for proteomic analysis.[6,4,12]

The DNA isolation process involved three fundamental steps: lysing cells to analyze DNA, separating DNA from other molecules such as RNA, lipids, proteins, and carbohydrates, and recovering purified DNA.[13] When this technique produced pure and intact nucleic acids, it was considered highly effective.[14,15] In this study, a modified DNA isolation technique was employed to enhance the purity of menstrual blood DNA extracts obtained using filter paper during the lysis and purification processes. Therefore, this research aimed to compare the feasibility of extracting deoxyribonucleic acid (DNA) molecules from menstrual blood specimens and endometrial tissue in women of reproductive age.

METHOD

Material

Sanitary napkins are made from filter paper sewn onto cloth layers, with the absorbent or blotting paper commonly being of the Whatman type. Menstrual blood collected on sanitary napkins is typically obtained on the second day of the menstrual phase. Equipment used in the DNA extraction process included 1.5 ml collection tubes, micropipettes, pipette tips, centrifuges, tissue crushers, and other necessary tools. In contrast, endometrial tissue was obtained through an endometrial biopsy procedure.

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Sampel Collection

Menstrual blood samples were collected using Whatman filter paper sewn onto a cloth pad on the second or third day of menstruation. The blood samples were promptly sent to the laboratory for analysis. The filter paper was first dried, separated from the cloth pad, cut into small pieces, and stored. DNA was extracted from both menstrual blood and endometrial tissue and subsequently preserved in 1.5 mL microcentrifuge tubes at -20°C.

The DNA extraction

DNA extraction from menstrual blood was conducted using a Qiagen DNA Extraction Kit (Qiagen, Venlo, Netherlands). The process began by adding 360 µL of ATL buffer to a 1.5 mL tube containing 50 mg of the specimens, followed by incubation at 85°C for 30 minutes. After incubation, the samples were vortexed and centrifuged, and 20 µL of proteinase K was added. The samples underwent further incubation at 56°C for two hours, after which they were centrifuged again. Subsequently, 400 µL of AL buffer was added, vortexed, and incubated at 70°C for 10 minutes. After the incubation, 400 µL of 100% ethanol was added to the sample, and the mixture was transferred to a spin column, which was placed in a fresh collection tube. Next, 500 µL of AW1 buffer was added, and the mixture was centrifuged once. This was followed by the addition of 500 µL of AW2 buffer, with the centrifugation step repeated twice. To elute the DNA, 50 µL of AE buffer was added to the spin column, incubated at room temperature for three minutes, and then centrifuged. This step was performed once. The concentration and purity of the eluted DNA were measured using a Nanodrop spectrophotometer, and the DNA was subsequently stored at -20°C for preservation. DNA extraction from endometrial tissue was conducted using the Geneaid DNA Isolation Kit (Geneaid, New Taipei, Taiwan). The process began by mixing 200 µL of GST buffer, 20 µL of Proteinase K, and 10 mg of the endometrial tissue sample. The mixture was incubated overnight at 60°C. Following incubation, 200 µL of 100% ethanol and 200 µL of GSB buffer were added to the sample. The mixture was then transferred into a GS column fitted into a 2 mL collection tube. Subsequently, 400 µL of W1 buffer was added to the column. Afterward, the GS column was treated with 600 µL of wash buffer and centrifuged. The filtrate was discarded, and additional centrifugation was performed to dry the column matrix. The spin column was then transferred to a fresh microtube, and 100 µL of preheated elution buffer was added to the center of the column matrix. After standing for three minutes, a final centrifugation step was carried out. The purity and concentration of the eluted DNA were measured using a Nanodrop spectrophotometer.

Purity measurement

The purity and concentration of deoxyribonucleic acid (DNA) samples were assessed using spectrophotometry with absorbance fluorescence labeling. A Thermo Scientific NanoDrop Microvolume Spectrophotometer was utilized for these measurements. The NanoDrop instrument operates based on the principles of spectrophotometry. The ratio of absorbance at 260 nm (λ260) to 280 nm (λ280) was used to evaluate the purity of nucleic acids. For DNA extracts, purity values in a 1 µL solution typically ranged between 1.8 and 2.0, indicating high-quality DNA suitable for downstream applications.

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Statistical analysis

DNA purity and concentration were analyzed using univariate statistical methods. The results were presented as tabulated averages of concentration and purity scores. The results of the examination in the form of numerical data with a normal distribution are presented in the mean ± standard deviation, while those that are not normally distributed are presented in the median and minimum maximum values. Statistical comparisons were conducted using the Student's *t*-test or the Mann-Whitney *U* test, depending on the data distribution. Proportions were analyzed using the chi-square test or Fisher's exact test, as appropriate.

Ethical approval

The protocol of this study has been approved by the Ethics Commission of the Faculty of Medicine, Universitas Indonesia (Protocol No. 23-12-1961).

RESULTS AND DISCUSSION

Table 1. Extraction outcome : Concentration and purity value of DNA in menstrual blood specimen.

Samples	Concentration (ng/ul)	Purity
1	158.8	1.905
2	63.53	1.805
3	59.57	1.837
4	149.6	1.787
5	107.4	2.044
6	64.84	1.99
7	115.3	1.869
8	148.2	1.948
9	83.9	1.786
10	75.54	1.813
11	99.15	1.983
12	264.1	1.839
13	198.9	1.972
14	151.2	1.783
15	77.02	1.728
Samples	**Concentration (ng/ul)**	**Purity**
16	77.18	1.709
17	114.8	1.882

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18	83.68	1.772
19	116.2	1.867
20	129.2	1.797
mean ± SD	**116.9 ± 51.22**	**1.88± 0.09**
Median (Min-Max)	**111.1 (59.57-264,1)**	**1.83 (1.71-2.04)**

Table 2. Extraction outcome: Concentration and purity value of DNA in Endometrial tissue specimen.

Samples	**Concentration (ng/ul)**	**Purity**
1	209.6	1.83
2	189.6	1.948
3	218.2	1.953
4	253.2	1.956
5	127.3	1.888
6	200	1.949
7	194.9	1.748
8	183.5	1.936
9	80.26	2.014
10	294.1	1.846
11	133.5	1.775
12	97.63	1.776
13	414.4	2.008
14	168.4	1.91
15	345	1.91
16	145	1.795
17	91.52	2.097
18	287.1	2.03
19	62	1.838
20	325.9	1.968
mean + SD	**201.0 ± 95.22**	**1.90 ± 0.09**
Median (Min-Max)	**192.22 (62 – 414.4)**	**1.92 (1.75 – 2.10)**

p=0.083

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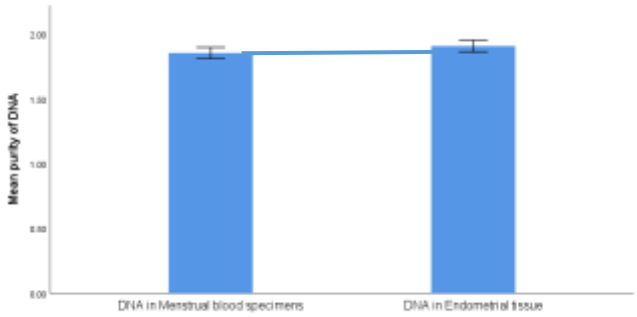

Figure 1. DNA Purity between Menstrual Blood and Endometrial Tissue

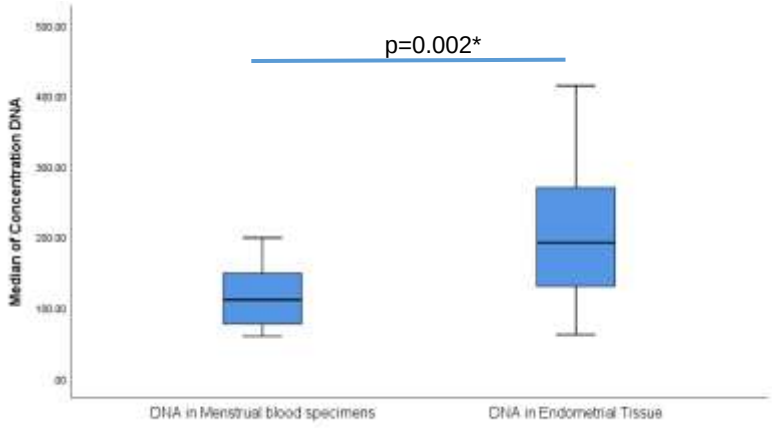

Figure 1. Differences in DNA Concentration between Menstrual Blood and Endometrial Tissue

This study assessed the concentration and purity of DNA extracted from menstrual blood samples collected on filter paper, with results summarized in Table 1. The average DNA purity obtained was 1.88. DNA purity is categorized as follows: contamination with proteins when purity is below 1.7, pure DNA when between 1.7 and 2.0, and contamination with RNA when above 2.0.[16] Understanding DNA purity and concentration is essential for exploring biological processes and diseases. Filter paper was used to stabilize the specimens and ensure their condition remained intact until laboratory analysis.[17,18] The filter paper used is Whatman which can filter particles with high efficiency, can separate particles and rapid filtration and can achieve the same level of precision and reproducibility as expected from clinical methods that usually use blood collected in tubes or pipettes.[19]

As shown in Table 1, the mean DNA concentration ± SD was 1.88 ± 0.09, with a purity value measured at a wavelength of Å260/280. The lowest DNA purity value was 1.709 and the highest was 2.044 in menstrual blood. The sample purity value was 1,709, the sample was 45 years old, had no history of smoking or endometriosis. Meanwhile, the sample with a DNA purity value of 2.044 was 43 years old, had no history of smoking or endometriosis. In this study there was no contamination, the DNA values obtained were within normal limits.

Similarly, DNA extracted from endometrial tissue displayed comparable results. Table 2 reports a median DNA purity of 1.92 (range: 1.75–2.10), also measured at Å260/280. The data normality test, performed using the Shapiro-Wilk test, confirmed normal distribution, allowing for analysis with an independent *t*-test. No significant difference in DNA purity values between menstrual blood and endometrial tissue was observed (p = 0.083), as illustrated in Figure 1.

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Menstrual blood is a complex biological fluid containing blood, vaginal secretions, immune cells, and endometrial cells shed from the uterine wall.[20,21] This study confirmed that menstrual blood is predominantly composed of endometrial tissue shed cyclically in women of reproductive age, making it a viable non-invasive source of nucleic acids, such as DNA and RNA.[22,7] This eliminates the need for invasive biopsy procedures and enables the identification of early diagnostic biomarkers for reproductive diseases.[23, 24]

Menstrual blood serves as a non-invasive source of endometrial tissue and identified 385 proteins in the menstrual blood proteome.[20] Additionally, research by Sabbaj et al. (2011) utilized menstrual blood to analyze the presence of T-cell types in endometrial cells, further demonstrating its potential in medical and diagnostic applications.[3]

The DNA concentration in menstrual blood showed a mean ± SD of 116.9 ± 51.22, while the DNA concentration in endometrial tissue had a median value of 192.22 (range: 62–414.4), as presented in Tables 1 and 2. Due to the non-normal distribution of the data, confirmed by the Shapiro-Wilk normality test ($p < 0.05$), the Mann-Whitney test was employed for analysis. The results indicated a significant difference in DNA concentration between menstrual blood and endometrial tissue ($p = 0.002$; Figure 2).

Endometrial tissue, the innermost layer of the uterus, undergoes dynamic changes across different phases of the menstrual cycle.[25] This variability may contribute to the observed differences in DNA concentration, as endometrial tissue samples could have been collected during varying phases, such as the proliferative or secretory (luteal) phases, where the tissue exhibits heterogeneous responses. In contrast, menstrual blood primarily consists of endometrial tissue shed cyclically during menstruation, resulting in more uniform composition.[26,27]

Another factor influencing the concentration difference is the method of sample collection. Menstrual blood is composed of uterine fluid and the inner layer of the uterus expelled through the vagina, whereas endometrial tissue samples are obtained directly through biopsy. This invasive method yields a more concentrated sample of endometrial tissue compared to the diluted nature of menstrual blood.

Conclusion

Both menstrual blood and endometrial tissue yield DNA with good purity and concentration, as menstrual blood consists of shed endometrial tissue which has the potential to be used for molecular screening without invasive procedures. However, endometrial tissue typically exhibits a slightly higher DNA concentration than menstrual blood. This difference is likely due to the direct biopsy method used to collect endometrial tissue, which provides a more concentrated sample. Various phases of the endometrial cycle also contribute to its heterogeneity, potentially affecting DNA concentration

Author Contributions

All authors were involved in research planning. FE collected subjects, took measurements, performed analysis, and wrote the manuscript. AS designed research idea, supervised the work, interpreting the results; AH supervised the work, interpreting the results; TT assisted in interpreting the results, revising and final approval of the manuscript; EH, ZD, BH, OT, CR and RA assisted in interpreting the results. All authors discussed the results and commented on the manuscript.

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