DNA methylation detection is a significant biomarker for screening endometrial cancer in premenopausal women with abnormal uterine bleeding

Xingping Zhao,[1,2,3] Yanfei Yang,[1] Yinqin Fu,[1] Weigang Lv,[1] Dabao Xu [1]

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[1]Gynecology, the Third Xiangya Hospital, Central South University, Changsha, Hunan, China
[2]Jiangwan Research Institute, Central South University, Changsha, Hunan, China
[3]Postdoctoral Station of Clinical Medicine, the Third Xiangya Hospital, Central South University, Changsha, China

Correspondence to
Dr Dabao Xu and Dr Weigang Lv, Gynecology, the Third Xiangya Hospital, Central South University, Changsha, Hunan, 410013, China; dabaoxu2022@163.com, lvweigang@sklmg.edu.cn

XZ and YY contributed equally.

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ABSTRACT

Objective The aim of our study was to explore the value of DNA (CDO1m/CELF4m) methylation detection in exfoliated cervical cells collected for screening endometrial cancer in premenopausal women with abnormal uterine bleeding.

Methods A total of 296 premenopausal women with abnormal uterine bleeding admitted to the Department of Obstetrics and Gynecology at the Third Xiangya Hospital of Central South University from November 2021 to October 2022 were selected. Clinical characteristics, endometrial thickness measured by transvaginal ultrasound and serum CA125 were collected. Exfoliated cervical cells from the thinPrep cytogic test were collected for DNA (CDO1m/CELF4m) methylation testing. Endometrial tissue was collected under hysteroscopy for pathological diagnosis as the gold standard. A univariate logistic regression model was used to analyze risk factors for endometrial cancer. The receiver operating characteristic (ROC) curve and area under the curve (AUC) were used to measure the diagnostic efficacy of DNA methylation detection in endometrial cancer screening of women with abnormal uterine bleeding.

Results Univariate logistic regression analysis showed that age, body mass index (BMI) ≥25 kg/m^2, endometrial thickness ≥11 mm, CDO1 methylation (CDO1mΔCt≤8.4), CELF4 methylation (CELF4mΔCt≤8.8), and dual gene methylation (CDO1mΔCt≤8.4 or CELF4mΔCt≤8.8) were independent risk factors for endometrial cancer in women with abnormal uterine bleeding. The odds ratio (OR) values (95% confidence interval (CI) were 0.87 (0.80–0.95), 4.76 (1.89–11.96), 8.41 (3.13–22.59), 64.49 (20.46–203.33), 12.79 (4.91–33.30), and 42.53 (11.90–152.04), respectively. Among these indicators, dual gene methylation had the higher sensitivity and specificity for endometrial cancer screening (85.7% and 87.6%). Moreover, dual gene methylation combined with BMI or endometrial thickness could further improve the screening efficiency of endometrial cancer in women with abnormal uterine bleeding.

Conclusions In premenopausal women with abnormal uterine bleeding, the clinical efficacy of DNA (CDO1m/CELF4m) methylation detection in exfoliated cervical cells for endometrial cancer screening was better than that of other noninvasive clinical indicators. In addition, dual gene methylation combined with BMI or endometrial thickness was a good predictor of endometrial cancer screening.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ At present, there are still many shortcomings in the early screening of endometrial cancer, including transvaginal ultrasound and serum tumor markers etc. There is a need to develop a simple, more sensitive, non-invasive and easy-to-administer test to diagnose and deliver personalized treatment. CDO1 and CELF4 methylation test kits have been developed, but their predictive effect on endometrial cancer has not been fully validated clinically.

WHAT THIS STUDY ADDS

⇒ This study found cytological DNA methylation provides a simple, non-invasive and highly sensitive detection method for endometrial cancer detection in premenopausal women with abnormal uterine bleeding, which effectively reduces the invasive procedure in endometrial cancer screening, and reduces the patient's psychological burden.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This study highlights the value of DNA methylation detection in the screening of endometrial cancer in premenopausal women with abnormal uterine bleeding, and this technology deserves to be promoted.

INTRODUCTION

Endometrial cancer has become the most common female genital tract malignancy in developed countries, with increasing morbidity and mortality rates.[1–3] The increasing incidence of endometrial cancer is related to higher body mass index (BMI), increased age, early menarche, late menopause, family history and endogenous or exogenous estrogen exposure.[3] Although abnormal uterine bleeding is the most common and important clinical manifestation symptom of endometrial cancer, it is not typical since many other diseases have the same symptoms.[3 4] Only a minority of women with abnormal uterine bleeding are eventually diagnosed with endometrial cancer, and some endometrial carcinoma patients have no clinical manifestations of abnormal uterine bleeding.[4]

Therefore, it is often difficult to detect and intervene early for patients with potentially at-risk endometrial cancer, which delays the treatment time and affects the prognosis.

The survival time of patients with endometrial cancer is closely dependent on the stage at diagnosis.[3] Transvaginal ultrasound is the most common diagnostic tool for endometrial cancer since it measures the thickness of the endometrium with the characteristic of being less invasive.[5] However, transvaginal ultrasound does not effectively distinguish between benign and malignant lesions, and the dividing line for endometrial thickness is controversial.[6] Diagnostic curettage and hysteroscopy combined with endometrial biopsy are the most reliable means to diagnose endometrial cancer and can also be combined with genetic testing to assist diagnosis.[7] But the acceptance rate of this invasive examination is low, and some patients who undergo hysteroscopy do not have indications, resulting in excessive medical treatment, which is not conducive to the protection of female reproductive ability.[8] Serum carbohydrate antigen 125 (CA125) is a classic serum tumor marker with wide clinical application and is non-invasive, but with low accuracy.[9–11] Therefore, there is an urgent need for a simple, more sensitive, non-invasive and easy-to-administer test to diagnose and deliver personalized treatment.

DNA methylation is the most well-researched and best characterized epigenetic alteration in humans,[12] which can produce different biological effects on gene activity by methylating different gene regions[13] and can regulate gene expression without changing the DNA sequence.[14] Thus, DNA methylation detection can be used as a new early screening method for cancer with broad prospects. It has been reported that CDO1, CELF4 and BHLHE22 in cervical scrapings are effective for the detection of endometrial cancer.[4] CDO1 encodes a protein that reduces cytotoxicity caused by overproduction of cysteine and is also related to drug sensitization due to reactive oxygen species (ROS) elevation.[4] CELF4 encodes a protein-binding ribonucleic acid (RNA) recognition motif with three domains and may participate in RNA alternative splicing.[4] BHLHE22 is associated with cell differentiation.[4] Currently, CDO1 and CELF4 methylation detection kits have been developed, while BHLHE22 methylation kits have not. However, its predictive effect on endometrial cancer has not been fully validated clinically.

In our study, CDO1 and CELF4 methylation test kits were used to screen endometrial cancer for premenopausal women with abnormal uterine bleeding and to evaluate their effectiveness. In addition, we explored the effectiveness of CDO1 and CELF4 methylation test kits in combination with BMI or endometrial thickness measured by transvaginal ultrasound for screening endometrial cancer women with abnormal uterine bleeding.

METHODS

Clinical Samples

Our study included 296 women with abnormal uterine bleeding with hysteroscopic indications who were admitted to the Department of Obstetrics and Gynecology of the Third Xiangya Hospital of Central South University from November 2021 to October 2022. According to the inclusion criteria, study participants had to be premenopausal, between the ages of 18 and 55 and free of other cancers and precancerous lesions. Clinical characteristics such as age, past history, BMI and detection indicator data such as endometrial thickness measured by transvaginal ultrasound, serum CA125 were collected. Endometrial thickness ≥11 mm was a high risk of endometrial cancer for premenopausal women.[15] Hysteroscopy was performed by qualified senior practitioners in the Department of Obstetrics and Gynecology of the Third Xiangya Hospital of Central South University. Subsequently, the histological examination of endometrial tissue was performed by two senior pathologists. Informed consent was acquired from all participants. The study was approved by the Ethics Committee of the Third Xiangya Hospital of Central South University (no.22246), and all participants gave informed written informed consent.

DNA Methylation Analysis

Exfoliated cervical cells were obtained from the thinPrep cytogic test. Through DNA extraction, bisulfite conversion, and quantitative methylation-specific PCR, the difference in the cycle threshold (Ct) of CDO1, CELF4 and GAPDH was recorded and analyzed. ΔCt CDO1=Ct CDO1 – Ct GAPDH and ΔCt CELF4=Ct CELF4 – Ct GAPDH. CDO1 methylation (CDO1mΔCt≤8.4) or CELF4 methylation (CELF4mΔCt≤8.8) indicated that dual gene methylation was positive.

Statistical Analysis

Statistical analysis was performed using SPSS 27.0 and R 4.2.2. The statistical data were described by the number of cases and percentage, and the Chi-squared (χ^2) test was used for comparison between groups. The risk factors for endometrial cancer were analyzed by a univariate logistic regression model. The receiver operating characteristic (ROC) curve was plotted to evaluate the value of each indicator in screening endometrial cancer, and the area under the curve (AUC), sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), odds ratio (OR) and 95% confidence interval (CI) were calculated. Net reclassification improvement (NRI) and integrated discrimination improvement (IDI) were used to compare the enhancement value of dual gene methylation combined with different indicators for endometrial cancer screening. A two-tailed p-value < 0.05 was considered statistically significant.

RESULTS

Clinical Features and Differential Expression of CDO1 and CELF4 Methylation in the Control Group and Endometrial Cancer Group

Between 296 patients with abnormal uterine bleeding, there were 21 patients with endometrial cancer, 10 patients with endometrial intraepithelial neoplasia, and 265 patients with non-diseased endometrium (including normal endometrium, simple hyperplasia, and complexity). As shown in Table 1, there were significant differences in age, BMI, and endometrial thickness measured by transvaginal ultrasound, CDO1mΔCt, CELF4mΔCt, and dual gene methylation among the three groups ($P_1<0.05$), while there were no significant differences in serum CA125 ($P_1>0.05$). There was no difference in the levels of CDO1mΔCt and CELF4mΔCt between endometrial intraepithelial neoplasia and non-diseased endometrium groups, which were significantly higher than those in endometrial cancer groups. Therefore, endometrial intraepithelial neoplasia and non-diseased patients were combined as the control group and the

Table 1 The clinical and DNA methylation characteristics of patients with abnormal uterine bleeding

	Cases (n=296)	Non-diseased endometrium group (n=265)	Endometrial intraepithelial neoplasia group (n=10)	Control group (n=275)	Endometrial cancer group (n=21)	X_1^2	P_1	X_2^2	P_2
Age, years, mean (medium, range)	41.95 (43,22-55)	41.50 (43,22-55)	42.8 (46,24–54)	41.55 (43,20-55)	47.29 (49,31-55)	13.55	0.001	13.07	<0.001
BMI, No. (%)						12.4	0.002	11.1	<0.001
<25 kg/m^2	213 (71.96)	199 (75.09)	6 (60.00)	205 (74.55)	8 (38.10)				
≥25 kg/m^2	83 (28.04)	66 (24.91)	4 (40.00)	70 (25.45)	13 (61.90)				
Endometrial thickness, No. (%)						20.33	<0.001	21.23	<0.001
<11 mm	218 (73.65)	204 (76.98)	8 (80.00)	212 (77.09)	6 (28.57)				
≥11 mm	78 (26.35)	61 (23.02)	2 (20.00)	63 (22.91)	15 (71.43)				
CA 125, No. (%)						0.3	0.86	0.01	0.918
<35 U/mL	249 (84.12)	224 (84.53)	8 (80.00)	232 (84.36)	17 (80.95)				
≥35 U/mL	47 (15.88)	41 (15.47)	2 (20.00)	43 (15.64)	4 (19.05)				
CDO1mΔCt, mean (medium, range)	15.04 (16.74, 1.88–19.91)	15.61 (16.95, 1.88–19.91)	14.43 (15.18, 8.35–18.24)	15.57 (16.93, 1.88–19.91)	8.22 (7.37, 2.31,18.46)	38.91	<0.001	37.32	<0.001
CELF4mΔCt, mean (medium, range)	14.29 (15.86, 3.68–19.47)	14.69 (16.42, 4.06–19.47)	13.34 (14.07, 8.23–17.79)	14.65 (6.37, 4.06–19.47)	9.60 (8.59, 3.68–18.46)	24.4	<0.001	22.77	<0.001
Dual gene methylation, No. (%)						57.28	<0.001	67.51	<0.001
positive	244 (82.43)	235 (88.68)	6 (60.00)	241 (87.64)	18 (85.71)				
negative	52 (17.57)	30 (11.32)	4 (40.00)	34 (12.36)	3 (14.29)				

P_1: the comparison of Non-diseased endometrium group, Endometrial intraepithelial neoplasia group and Endometrial cancer group; P_2: the comparison of Control group and Endometrial cancer group.
BMI, body mass index.

endometrial cancer group served as the study group. After further analysis and comparison, the results of other indicators were the same as before in the control group and endometrial cancer group (Table 1). The levels of CDO1mΔCt and CELF4mΔCt in the control group and the endometrial cancer group are shown in online supplemental figure S1.

The Independent Risk Factors for Endometrial Cancer Analyzed by Univariate Logistic Regression

To confirm the risk factors associated with endometrial cancer, univariate logistic regression analysis was performed. The results showed that age, BMI ≥25 kg/m^2, endometrial thickness ≥11 mm, CDO1mΔCt≤8.4, CELF-4mΔCt≤8.8, and dual gene methylation were independent risk factors for endometrial cancer. The OR values (95% CI) were 0.87 (0.80–0.95), 4.76 (1.89–11.96), 8.41 (3.13–22.59), 64.49 (20.46–203.33), 12.79 (4.91–33.30) and 42.53 (11.90–152.04), respectively (Table 2).

Clinical efficacy of each indicator in endometrial cancer screening

Then the clinical efficacy of each indicator was measured in the screening of endometrial cancer among women with abnormal uterine bleeding. By comparing sensitivity and specificity, dual gene methylation had the higher sensitivity and specificity compared with other indicators (sensitivity 85.7% and specificity 87.6%) (Table 3). And dual gene methylation with BMI or endometrial thickness did not change the value, but all combinations reduced sensitivity (66.7%) and increased specificity (94.5%). The ROC curves of CDO1 methylation and CELF4 methylation are shown in online supplemental figure S2A. The AUCs (95% CIs) of BMI ≥25 kg/m^2, endometrial thickness ≥11 mm, CDO1 methylation, CELF4 methylation and dual gene methylation were 0.682 (0.573–0.792), 0.743 (0.641–0.845), 0.857 (0.763–0.951) and 0.753 (0.645–0.861), respectively. Moreover, the AUCs (95% CIs) of dual gene methylation combined with BMI, endometrial thickness, BMI and endometrial thickness were 0.929 (0.892 to 0.966), 0.920 (0.855–0.985) and 0.942 (0.900–0.985), respectively (Table 3, online supplemental figure S2B).

In addition, the differences in AUC and NRI between dual gene methylation alone and combined with BMI were 0.062 and 0.444, respectively. Similarly, the differences in AUC and NRI between dual gene methylation alone and combined endometrial thickness were 0.053 and 0.590, respectively. The differences in AUC and NRI between dual gene methylation alone and combined BMI and endometrial thickness were 0.075 and 0.590, respectively. In addition, combined endometrial thickness or combined BMI and endometrial thickness showed significant differences in IDI from dual gene methylation alone (online supplemental table S1). The above results indicated that combining dual gene methylation with

Table 2 Univariate logistic regression analysis

	Control group (n=275)	Endometrial cancer group (n=21)	χ^2	P- value	OR (95% CI)
Age	43.0 (35.0, 48.0)	49.0 (44.0, 52.0)	13.75	0.001	0.87 (0.80 to 0.95)
BMI					
<25 kg/m^2	205 (74.55)	8 (38.1)	11.34	0.001	4.76 (1.89 to 11.96)
≥25 kg/m^2	70 (25.45)	13 (61.9)			
Endometrial thickness					
<11 mm	212 (77.09)	6 (28.57)	20.28	<0.001	8.41 (3.13 to 22.59)
≥11 mm	63 (22.91)	15 (71.43)			
CA125					
<35 U/mL	232 (84.36)	17 (80.95)	0.16	0.681	1.27 (0.41 to 3.96)
≥35 U/mL	43 (15.64)	4 (19.05)			
CDO1mΔCt					
ΔCt>8.4	262 (95.27)	5 (23.81)	62.02	<0.001	64.49 (20.46 to 203.33)
ΔCt≤8.4	13 (4.73)	16 (76.19)			
CELF4mΔCt					
ΔCt>8.8	244 (88.73)	8 (38.10)	27.24	<0.001	12.79 (4.91 to 33.30)
ΔCt≤8.8	31 (11.27)	13 (61.90)			
Dual gene methylation					
Negative	241 (87.64)	3 (14.29)	52.16	<0.001	42.53 (11.90 to 152.04)
Positive	34 (12.36)	18 (85.71)			

BMI, body mass index; CI, confidence interval; OR, odds ratio - PLEASE CONFIRM.

BMI, endometrial thickness or BMI and endometrial thickness could improve the screening efficiency.

Clinical efficacy of endometrial cancer screening in different BMI subgroups and different endometrial thickness subgroups

Later, the patients were divided into two subgroups with BMI ≥25 kg/m^2 and BMI <25 kg/m^2. The ROC curves of endometrial thickness, CDO1 methylation, CELF4 methylation, dual gene methylation, and endometrial thickness combined with dual gene methylation for endometrial cancer screening are shown in Figure 1A,B. In different BMI subgroups, endometrial thickness combined with dual gene methylation had the highest AUC value (BMI ≥25 kg/m^2, AUC=0.898; BMI <25 kg/m^2, AUC=0.955). Moreover, the patients were divided into two subgroups with endometrial thickness ≥11 mm and endometrial thickness <11 mm. And BMI combined with dual gene methylation also had the highest AUC

Table 3 The efficacy of each indicator in endometrial cancer screening

	Sensibility (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	AUC (95% CI)
BMI (≥25 kg/m^2)	0.619 (0.411 to 0.827)	0.745 (0.694 to 0.797)	0.157 (0.078 to 0.235)	0.962 (0.937 to 0.988)	0.682 (0.573 to 0.792)
Endometrial thickness (≥11 mm)	0.714 (0.521 to 0.908)	0.771 (0.721 to 0.821)	0.192 (0.105 to 0.280)	0.972 (0.951 to 0.994)	0.743 (0.641 to 0.845)
CDO1^m (+)	0.762 (0.580 to 0.944)	0.953 (0.928 to 0.978)	0.552 (0.371 to 0.733)	0.981 (0.965 to 0.998)	0.857 (0.763 to 0.951)
CELF4^m (+)	0.619 (0.411 to 0.827)	0.887 (0.850 to 0.925)	0.295 (0.161 to 0.430)	0.968 (0.947 to 0.990)	0.753 (0.645 to 0.861)
Dual gene methylation	0.857 (0.707 to 1.000)	0.876 (0.837 to 0.915)	0.346 (0.217 to 0.475)	0.988 (0.974 to 1.002)	0.867 (0.788 to 0.946)
BMI+dual gene methylation	0.857 (0.707 to 1.0)	0.876 (0.837 to 0.915)	0.346 (0.217 to 0.475)	0.988 (0.974 to 1.0)	0.929 (0.892 to 0.966)
Endometrial thickness+dual gene methylation	0.857 (0.707 to 1.0)	0.8763 (0.837 to 0.915)	0.346 (0.217 to 0.475)	0.988 (0.974 to 1.0)	0.92 (0.855 to 0.985)
BMI+endometrial thickness+dual gene methylation	0.667 (0.465 to 0.868)	0.945 (0.919 to 0.972)	0.483 (0.301 to 0.665)	0.974 (0.955 to 0.993)	0.942 (0.900, 0.985)

AUC, area under the curve; BMI, body mass index; CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value.

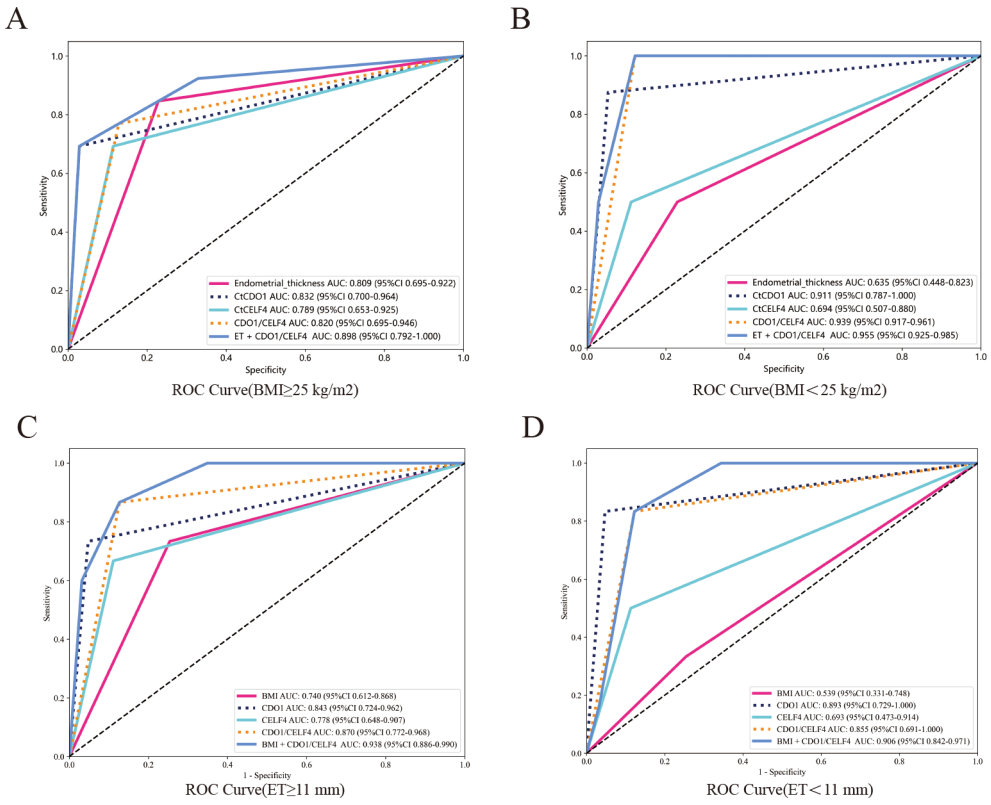

Figure 1 The ROC curves of each indicator on screening endometrial cancer in different BMI (A–B) and endometrial thickness (C–D) subgroups.

value in endometrial thickness subgroups (endometrial thickness ≥11 mm, AUC=0.938; endometrial thickness <11 mm, AUC=0.906) (Figure 1C,D). These results further suggested that BMI and endometrial thickness combined with dual gene methylation was a good predictor of endometrial cancer screening.

DISCUSSION

Summary of Main Results

In this study, DNA methylation testing was used as a screening test for endometrial cancer in patients with abnormal uterine bleeding, presenting a good predictive result compared with BMI, endometrial thickness and CA125. DNA methylation combined with BMI or endometrial thickness can further improve its prediction efficiency.

Results in the Context of Published Literature

Abnormal uterine bleeding is one of the high-risk factors for endometrial cancer.[16] Due to nonovulation, the endometrium is chronically affected by estrogen, leading to hyperplasia, endometrial intraepithelial neoplasia or even endometrial cancer.[17] Though diagnostic curettage of endometrial tissue for pathological examination is the gold standard for the diagnosis and treatment of endometrial cancer,[18] patients have poor acceptance and cooperation due to the invasive and painful nature of pathological examination. Thus, in this study, we used exfoliated cervical cells for DNA methylation testing to screen endometrial cancer noninvasively and with less pain. Several studies have used exfoliated cervical cells for cytopathological analysis, target gene detection, exosome analysis, diagnosis of Lynch syndrome and multiomics detection.[19–23] However, there has been little research on the epigenetic analysis of exfoliated cervical cells. There is no reliable, uniform and standardized endometrial cancer screening program that can be widely used in the clinic.

The use of DNA methylation in cancer screening has attracted the attention of many researchers. Park et al., find that using DNA methylation of PCDHGA12 and CDO1 genes in bronchial flushes to predict the diagnosis of lung cancer can reduce invasive procedures for lung cancer diagnosis.[24] What's more, the epigenetic inheritance of abnormal methylation of CDO1 may play a crucial role in the clinical treatment and prognosis of breast cancer.[25] And a prospective, continuous observational cohort study in the United Kingdom has shown that the novel WID-qEC DNA methylation test performs better than imaging tests in detecting uterine cancer in women with abnormal uterine bleeding.[26] Interestingly, our study also found that DNA (CDO1m/CELF4m) methylation detection had excellent predictive performance for endometrial cancer screening in patients with abnormal uterine bleeding. The difference was that compared with the EPI-SURE study, our study was limited to premenopausal women with abnormal uterine bleeding. In a word, DNA methylation detection to predict the screening of cancer will hopefully be further promoted in clinical applications.

Obesity is one of the high-risk factors for endometrial cancer, especially in women of reproductive age.[18] The 2015 American College of Obstetricians and Gynecologists (ACOG) indicated that people with BMI ≥25 kg/m^2 have a two to fourfold increased risk of endometrial cancer.[27] And it has been proved that endometrial thickness ≥11 mm is strongly associated with atypical endometrial hyperplasia/endometrial cancer in premenopausal women.[15] Additionally, we also analyzed the screening efficacy of DNA (CDO1m/

CELF4m) methylation detection in combination with BMI or endometrial thickness. The data showed that the sensitivity of dual gene methylation screening for endometrial cancer was 85.7%, and the specificity was 87.6%. Dual gene methylation combined with BMI or endometrial thickness can further improve the sensitivity and specificity as its AUC value increased. Furthermore, we divided the patients into different subgroups according to BMI and endometrial thickness. The results indicated dual gene methylation combined with endometrial thickness had the best prediction performance in BMI subgroups. Similarly, dual gene methylation combined with BMI was a good predictor in endometrial thickness subgroups. Therefore, the use of BMI and endometrial thickness in combination with DNA methylation tests to assess endometrial cancer risk in patients with abnormal uterine bleeding had greater accuracy, which could be of great value in clinical applications.

Strengths and Weaknesses

Our study used DNA methylation detection of exfoliated cervical cells to screen endometrial cancer in premenopausal women with abnormal uterine bleeding, which can reduce invasive operations in the uterine cavity and has positive significance for protecting female fertility.[28] However, there are some limitations in this study. First, the incidence of endometrial cancer among premenopausal women with abnormal uterine bleeding reached 7% in this study, a higher rate than in other studies, which may be related to hospital technology rankings. There is specificity, which requires scaling the cohort in conjunction with other multicenter platforms to eliminate differences. Second, endometrial intraepithelial neoplasia screening is as important as endometrial cancer and should be treated as study groups. However, there was no significant difference between the endometrial intraepithelial neoplasia and nondiseased groups in our study, which may be due to the small number of cases. The overall methylation characteristics and the differences between groups were not obvious. Finally, the study included 71% of the population with a BMI $<25\,kg/m^2$, and there may be racial differences in the findings. Further analysis needs to include ethnic subgroup analysis as well as endometrial cancer related genetic susceptibility analysis.

Implications for Practice and Further Research

Although our results showed that DNA methylation had a high sensitivity and specificity for predicting endometrial cancer screening in premenopausal patients with abnormal uterine bleeding, the population analyzed in this study was limited to premenopausal patients with abnormal uterine bleeding and other high-risk groups associated with endometrial cancer development were not included. It is necessary to expand the population sample. However, the development and use of DNA methylation kits also provide a new option for women with abnormal uterine bleeding to predict endometrial cancer screening, which will be more convenient and compliant for patients if applied in clinical practice on a larger scale.

CONCLUSION

In conclusion, the DNA methylation detection of cervical exfoliated cytology provides a new option for endometrial cancer screening in premenopausal women with abnormal uterine bleeding. This option is noninvasive, convenient to operate, and highly sensitive.

Contributors Conception of study: XZ, YY, WL and DX. Guarantor: WL and DX. Design and development: XZ and YY. Data collection: XZ, YY and YF. Data analysis: XZ and YY. Preparation of tables: XZ, YY and YF. Initial draft of manuscript: XZ, YY, WL and DX. Manuscript writing, review, and approval: all authors. WL and DX are joint guarantors.

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Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by the Ethics Committee of the Third Xiangya Hospital of Central South University (no.22246). Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request.

ORCID iD

Dabao Xu http://orcid.org/0009-0006-5486-763X

REFERENCES

1 Siegel RL, Miller KD, Wagle NS, *et al*. Cancer statistics. *CA Cancer J Clin* 2023;73:17–48.
2 Liew P-L, Huang R-L, Wu T-I, *et al*. Combined genetic mutations and DNA-Methylated genes as biomarkers for endometrial cancer detection from cervical scrapings. *Clin Epigenetics* 2019;11:170.
3 Cancer Genome Atlas Research Network, Kandoth C, Schultz N, *et al*. Integrated genomic characterization of endometrial carcinoma. *Nature New Biol* 2013;497:67–73.
4 Huang R-L, Su P-H, Liao Y-P, *et al*. Integrated epigenomics analysis reveals a DNA methylation panel for endometrial cancer detection using cervical scrapings. *Clin Cancer Res* 2017;23:263–72.
5 Herzog C, Marín F, Jones A, *et al*. A simple cervicovaginal epigenetic test for screening and rapid triage of women with suspected endometrial cancer: validation in several cohort and case/control SETS. *J Clin Oncol* 2022;40:3828–38.
6 Wong AS-W, Lao TT-H, Cheung CW, *et al*. Reappraisal of endometrial thickness for the detection of endometrial cancer in postmenopausal bleeding: a retrospective cohort study. *BJOG* 2016;123:439–46.
7 Clark TJ, Voit D, Gupta JK, *et al*. Accuracy of hysteroscopy in the diagnosis of endometrial cancer and hyperplasia: a systematic quantitative review. *JAMA* 2002;288:1610–21.
8 Costas L, Frias-Gomez J, Guardiola M, *et al*. New perspectives on screening and early detection of endometrial cancer. *Int J Cancer* 2019;145:3194–206.
9 Calis P, Yuce K, Basaran D, *et al*. Assessment of cervicovaginal cancer antigen 125 levels: a preliminary study for endometrial cancer screening. *Gynecol Obstet Invest* 2016;81:518–22.
10 Kil K, Chung J-E, Pak HJ, *et al*. Usefulness of CA125 in the differential diagnosis of uterine adenomyosis and Myoma. *Eur J Obstet Gynecol Reprod Biol* 2015;185:131–5.
11 Zhou J, Tomashefski JF, Khiyami A. Thinprep PAP tests in patients with endometrial cancer: a histo-cytological correlation. *Diagn Cytopathol* 2007;35:448–53.
12 Pan Y, Liu G, Zhou F, *et al*. DNA methylation profiles in cancer diagnosis and therapeutics. *Clin Exp Med* 2018;18:1–14.
13 Pennings S, Allan J, Davey CS. DNA methylation, Nucleosome formation and positioning. *Brief Funct Genom Proteom* 2005;3:351–61.
14 Ghavifekr Fakhr M, Farshdousti Hagh M, Shanehbandi D, *et al*. DNA methylation pattern as important epigenetic criterion in cancer. *Genet Res Int* 2013;2013:317569.

15 Zhang J, Zhang Q, Wang T, *et al*. Multimodal MRI-based radiomics-clinical model for preoperatively differentiating concurrent endometrial carcinoma from atypical endometrial hyperplasia. *Front Oncol* 2022;12:887546.
16 Clarke MA, Long BJ, Sherman ME, *et al*. Risk assessment of endometrial cancer and endometrial intraepithelial Neoplasia in women with abnormal bleeding and implications for clinical management algorithms. *Am J Obstet Gynecol* 2020;223:549.
17 Ring KL, Mills AM, Modesitt SC. Endometrial hyperplasia. *Obstet Gynecol* 2022;140:1061–75.
18 Makker V, MacKay H, Ray-Coquard I, *et al*. Endometrial cancer. *Nat Rev Dis Primers* 2021;7:88.
19 Odashima H, Yoshioka H, Ota K, *et al*. Morphological differences between liquid-based cytology and conventional preparation in endometrial endometrioid carcinoma grade 1 and grade 3, and the differentiation of grades in each method. *Acta Cytol* 2021;65:227–34.
20 Akahane T, Kitazono I, Yanazume S, *et al*. Next-generation sequencing analysis of endometrial screening liquid-based cytology specimens: a comparative study to tissue specimens. *BMC Med Genomics* 2020;13:101.
21 Srivastava A, Moxley K, Ruskin R, *et al*. A non-invasive liquid biopsy screening of urine-derived Exosomes for miRNAs as biomarkers in endometrial cancer patients. *AAPS J* 2018;20:82.
22 Silveira AB, Bidard F-C, Kasperek A, *et al*. High-accuracy determination of microsatellite instability compatible with liquid biopsies. *Clin Chem* 2020;66:606–13.
23 Buglyó G, Styk J, Pös O, *et al*. Liquid biopsy as a source of nucleic acid biomarkers in the diagnosis and management of Lynch syndrome. *Int J Mol Sci* 2022;23:4284.
24 Park SJ, Kang D, Lee M, *et al*. Combination analysis of PCDHGA12 and CDO1 DNA methylation in bronchial washing fluid for lung cancer diagnosis. *J Korean Med Sci* 2024;39:e28.
25 Yang J, Sun L, Liu X-Y, *et al*. Targeted demethylation of the CDO1 promoter based on CRISPR system inhibits the malignant potential of breast cancer cells. *Clin Transl Med* 2023;13:e1423.
26 Evans I, Reisel D, Jones A, *et al*. Performance of the WID-qEC test versus Sonography to detect uterine cancers in women with abnormal uterine bleeding (EPI-SURE): a prospective, consecutive observational cohort study in the UK. *Lancet Oncol* 2023;24:1375–86.
27 Management of endometrial intraepithelial Neoplasia or atypical endometrial hyperplasia: ACOG clinical consensus No.5. *Obstet Gynecol* 2023;142:735–44.
28 Rodríguez-Eguren A, Bueno-Fernandez C, Gómez-Álvarez M, *et al*. Evolution of biotechnological advances and regenerative therapies for endometrial disorders: a systematic review. *Hum Reprod Update* 2024:dmae013.