Original Article

Role of Liquid-based Cytology and Cell Block in the Diagnosis of Endometrial Lesions

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Abstract

Background: Liquid-based cytology (LBC) offers an alternative method to biopsy in screening endometrial cancer. Cell block (CB), prepared by collecting residual cytological specimen, represents a novel method to supplement the diagnosis of endometrial cytology. This study aimed to compare the specimen adequacy and diagnostic accuracy of LBC and CB in the diagnosis of endometrial lesions.

Methods: A total of 198 women with high risks of endometrial carcinoma (EC) from May 2014 to April 2015 were enrolled in this study. The cytological specimens were collected by the endometrial sampler (SAP-1) followed by histopathologic evaluation of dilatation and curettage or biopsy guided by hysteroscopy. The residual cytological specimens were processed into paraffin-embedded CB after LBC preparation. Diagnostic accuracies of LBC and CB for detecting endometrial lesions were correlated with histological diagnoses. Chi-square test was used to compare the specimen adequacies of LBC and CB.

Results: The specimen inadequate rate of CB was significantly higher than that of LBC (22.2% versus 7.1%, $P < 0.01$). There were 144 cases with adequate specimens for LBC and CB preparation. Among them, 29 cases were atypical endometrial hyperplasia (11 cases) or carcinoma (18 cases) confirmed by histology evaluation. Taking atypical hyperplasia and carcinoma as positive, the diagnostic accuracy of CB was 95.1% while it was 93.8% in LBC. When combined LBC with CB, the diagnostic accuracy was improved to 95.8%, with a sensitivity of 89.7% and specificity of 97.4%.

Conclusions: CB is a feasible and reproducible adjuvant method for screening endometrial lesions. A combination of CB and LBC can improve the diagnostic accuracy of endometrial lesions.

Key words: Atypical Endometrial Hyperplasia; Cytological Technique; Endometrial Carcinoma; Paraffin Embedding; Sensitivity; Specificity

Introduction

Endometrial cancer (EC) is a common gynecological cancer worldwide, accounting for about half of all gynecological cancers.[1-3] The etiology and pathogenesis of EC remain unclear. Risk factors for EC include age ≥40 years, obesity, diabetes, hypertension, estrogen using, tamoxifen treatment, and family history of malignant tumor. An effective screening strategy for women with high-risk factors may contribute to early detection and management of EC. Direct endometrial sampling procedures, including dilatation and curettage (D&C) and biopsy, are traditional and efficacious diagnostic methods in EC since they can obtain endometrial specimens for histopathological analysis.[4] However, endometrial curetting is painful and costly which requires dilatation and anesthesia. Therefore, D&C is a less practical screening tool and usually performed in hospital settings.[5] Cytology-based Papanicolaou smears have steadily decreased the incidence and mortality of cervical cancer in countries with successful national screening programs.[6] However, no mass screening programs are available for the early detection of EC. Therefore, the incidence and mortality of patients with EC remain high.[2] Endometrial cytology is a relatively painless and simple

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method to detect endometrial lesions.[7,8] Liquid-based cytology (LBC) is a method approved by the Food and Drug Administration for cervical cancer screening in America in 1996.[9] LBC prepares samples for cytology examination by depositing the collected sample into a bottle of preservative liquid.[10,11] Through removing obscuring factors such as mucus or blood, LBC can reduce obscuring factors and provide thin-layer specimens for cytology examination.[12] Cell block (CB) is prepared from the residual cytological specimens. Both the morphology of endometrial cells and glandular architectures are critical to EC diagnosis. LBC is helpful for studying cell details, whereas the evaluation of glandular architecture relies indirectly on the morphology of cell clumps.[13] CB can maintain cell morphology and tissue architecture and is thus a useful complement to liquid-based smears for definitive diagnosis.[14] CB preparation is used as a complementary diagnostic tool in gynecocytology, fine-needle aspiration, and effusion cytology.[15]

In the current study, we compared the specimen adequacy of LBC and CB preparation. In addition, we investigated the diagnostic accuracy of LBC and CB in detecting atypical endometrial hyperplasia and carcinoma.

Methods

Patients and samples

A total of 198 consecutive women were enrolled in this cross-sectional study. The cytological specimens were obtained from May 2014 to April 2015 at Department of Obstetrics and Gynecology, Xuanwu Hospital, Capital Medical University, Beijing. The inclusion criteria included: women aged ≥40 years, abnormal uterine bleeding (AUB), or thickened endometrium (postmenopausal women ≥4 mm or menopausal women ≥20 mm). The exclusion criteria included malformation of the genital tract, adhesion of endometrial cavity or cervical canal, and intrauterine contraceptive device. After signing informed consent, all women were submitted first to endometrial cytological test (ECT), then to D&C or biopsy guided by hysteroscopy. The Ethics Committee of Xuanwu Hospital approved the study.

Cytology preparation

Cytological sampling of endometrium was performed using a SAP-1 sampler (Saipujiuzhou Co., Beijing, China) without cervical dilatation. The device was made of soft plastic, which measured 3 mm diameter and 25 cm length, consisting a scalable latex ring with some fine teeth inside a 16-cm outer protective sheath to prevent contamination from endocervical and vaginal cells. After collection of the endometrial sample, the ring was then immersed in SurePath™ cell preservative container (SurePath™ Preservative Fluid; BD Diagnostic, Burlington, NC, USA), where it was shaken to allow cells to release. The 5 ml specimens were transferred into centrifuge tubes with some density reagent (BD Diagnostic, Burlington, NC, USA) to remove blood and mucus. The centrifuge tube was inserted into the SurePath™ semi-automated slide processor and stained using Papanicolaou stain [Figure 1a and 1b].

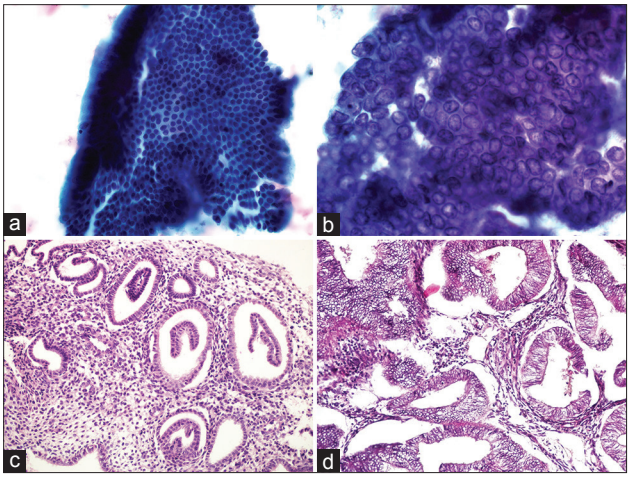

Figure 1: (a) Secretory phase endometrium in liquid-based cytology preparation (Papanicolaou, Original magnification ×40). (b) Endometrial carcinoma in liquid-based cytology preparation (Papanicolaou, Original magnification ×100). (c) Secretory phase endometrium in cell block preparation (HE, Original magnification ×40). (d) Endometrial carcinoma in cell block preparation (HE, Original magnification ×40).

Cell block and histology preparation

The residual fluid was collected in a 10-ml disposable centrifuge tube and then centrifuged at 1000 round/min for 2 min and 15 s and 2000 round/min for 10 min and 15 s (Rotina 46S, Hettich Co., Germany). The cell pellets with the endometrial cells were wrapped up in a cassette and fixed in formalin, embedded in paraffin, and stained with hematoxylin and eosin (HE) [Figure 1c and 1d].

Histological samples were collected from D&C or biopsy guided by hysteroscopy. Histological samples were routinely fixed in neutral buffered formalin, embedded in paraffin, and stained with HE.

Evaluation of cytology, cell block, and histology

Liquid-based smears were executed blindly by two gynecocytologists. Histological sections from CB and histology were executed blindly by two clinical pathologists according to the WHO classification scheme.[16] The cytological diagnosis was made according to the criteria of Buccoliero *et al*.[17] The histological results were considered as the gold diagnostic standard. The findings of LBC and CB were correlated with the histological results. In cases of discordant diagnosis, both pathologists reviewed the case together and reached an agreement on the diagnosis. We classified the endometrial lesions into four categories: normal endometrium, nonatypical hyperplasia, atypical hyperplasia, and carcinoma. Normal endometrium and nonatypical hyperplasia were considered as negative while atypical hyperplasia and carcinoma as positive.

Statistical analysis

Statistical analyses were performed using the Statistical Program/SPSS for Windows (version 10.0, SPSS Inc., Chicago, IL, USA). The adequacy of specimens was compared using Chi-squared test. A $P < 0.05$ was considered statistically significant. A double access table was created to evaluate the accuracy, sensitivity, specificity, the positive

predictive value (PPV), and negative predictive value (NPV) of LBC smears and CBs. The accuracy was defined as dividing the true positive and true negative cases by all the cases studied. The sensitivity was defined as dividing the number of true positive cases by all the positive cases confirmed by histology. The specificity was defined as dividing the number of true negative cases by all the negative cases. The PPV was defined as dividing the true positive cases by the overall true positive and false positive cases. The NPV was defined as dividing the true negative cases by the overall true negative and false negative cases.

Results

Clinical characteristics

Direct endometrial specimens were collected from 198 perimenopausal and postmenopausal women. Among them, 89 (44.9%) were postmenopausal. The median age was 52.5 years (range: 40–78 years). The 198 cases consisted of one hundred cases with thickened endometrium, 13 cases with AUB, and 85 cases with both thickened endometrium and AUB.

Specimen adequacy of liquid-based cytology and cell block

Of the 198 cases, 44 (22.2%) CB specimens were inadequate, whereas 14 (7.1%) LBC specimens were inadequate. The specimen inadequate rate of CB was significantly higher than LBC ($\chi^2 = 18.18$, $P < 0.01$). Among 184 adequate LBC smears, 144 (78.3%) residual specimens were processed into CBs. However, among 14 inadequate LBC smears, 10 residual specimens were successfully processed into CBs. In addition, postmenopausal women accounted for 63.6% in 44 inadequate CB cases and 35.7% in 14 inadequate LBC specimens. A total of 144 specimens were adequate for both CB and LBC preparation [Figure 2].

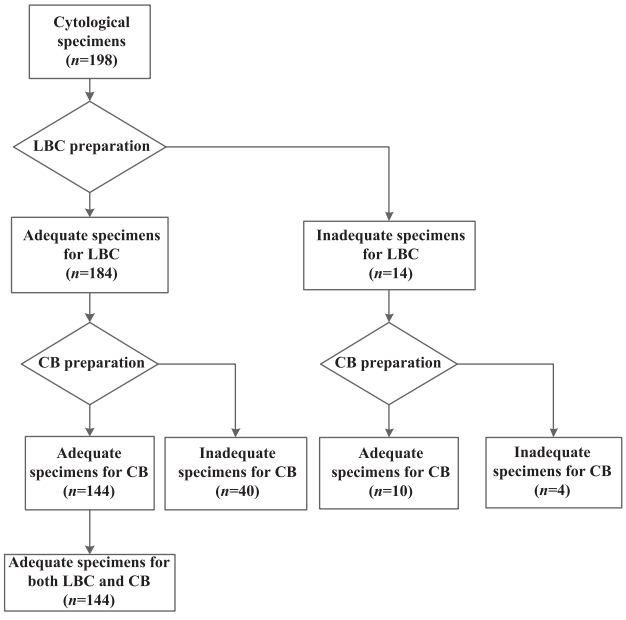

Figure 2: Flow chart of specimen preparation. LBC: Liquid-based cytology; CB: Cell block.

Diagnostic accuracy of liquid-based cytology and cell block

Among 144 specimens adequate for both CB and LBC, histological results demonstrated that there were 115 negative cases (37 normal endometria and 78 nonatypical hyperplasia endometria) and 29 positive cases (11 atypical hyperplasia endometria and 18 endometrial carcinomas). LBC correctly recognized 23 positive cases and 112 negative cases, whereas six positive cases were underrated and three negative cases were misdiagnosed as positive. CB correctly diagnosed 24 positive and 113 negative cases, whereas five positive cases were underrated and two negative cases were misdiagnosed. A combination of LBC and CB correctly diagnosed 26 positive cases and 112 negative cases. There were three positive cases (atypical hyperplasia) underrated as negative and three negative cases (nonatypical hyperplasia endometria) misdiagnosed as positive (atypical hyperplasia).

The diagnostic accuracy of LBC was 93.8%, with a sensitivity of 79.3%, specificity of 97.4%, PPV of 88.5%, and NPV of 94.9%. The diagnostic accuracy of CB was 95.1%, with a sensitivity of 82.8%, specificity of 98.3%, PPV of 92.3%, and NPV of 95.8%. When combined LBC with CB, the diagnostic accuracy was improved to 95.8%, with a sensitivity of 89.7%, specificity of 97.4%, PPV of 89.7%, and NPV of 97.4% [Table 1].

Discussion

Endometrial cell morphology may be influenced by certain hormonal situations which makes the diagnosis more confusing.[18] Abnormal cell clumps in cytological smears complicate the differentiation between metaplastic endometrium and carcinoma.[19] Therefore, it is challenging to detect EC by cytological screening and the incidence and mortality increase annually in most developed countries. However, the overall death rate in Japan was reduced from 20/100,000 in 1950 to 8/100,000 in 1999. This reduction was mainly attributed to the endometrial cytological screening, a widely accepted method in Japan.[20] CB slides can preserve both cellular (nuclear and cytoplasm) characteristics and architectural patterns of endometrial glands and stroma, thus increasing the diagnosis accuracy. Moreover, CB preparation also allows for long-term preservation and provides more materials for immune stains in later studies.

In the current study, we compared the specimen adequacy of LBC and CB preparation. Our result demonstrated that the inadequate rate of CB (22.2%) preparation was significantly higher than that of LBC (7.1%) ($P < 0.01$). Garcia *et al.*[21] showed that the inadequate rate of LBC was 15%, lower than that in endometrial biopsies (26%). A validation study of 1514 ECT showed that the inadequate rate of cytology was lower than that of histology collected from D&C or biopsy. In particular, the inadequate rate was markedly lower in LBC than in endometrial biopsies in postmenopausal women.[22] The high inadequate rate of CB in our study might be due to several reasons. CB was collected from the

Table 1: Diagnostic accuracy of LBC and CB in endometrial lesions, % (*n*/*N*)

Method	Accuracy	Sensitivity	Specificity	PPV	NPV
LBC	93.8 (135/144)	79.3 (23/29)	97.4 (112/115)	88.5 (23/26)	94.9 (112/118)
CB	95.1 (137/144)	82.8 (24/29)	98.3 (113/115)	92.3 (24/26)	95.8 (113/118)
LBC and CB	95.8 (138/144)	89.7 (26/29)	97.4 (112/115)	89.7 (26/29)	97.4 (112/115)

LBC: Liquid-based cytology; CB: Cell block; PPV: Positive predictive value; NPV: Negative predictive value.

residual specimens of LBC and CB preparation processes result in cell losses.

Recent studies have emphasized the diagnostic potential of LBC and CB in endometrial lesions. Garcia *et al*.[21] performed a prospective study of 103 symptomatic women and reported a sensitivity of 78% and specificity of 96% with PPV of 78% and NPV of 96% in the detection of endometrial abnormalities. A review by Fambrini *et al*.[20] showed that the overall sensitivity of LBC in diagnosing EC was from 78% to 100% in different literatures, with a specificity of 95–100%. Our results showed that the diagnostic accuracy of LBC was 93.8%, with a sensitivity of 79.3% and specificity of 97.4%. In addition, the diagnostic accuracy of CB in our study was 95.1%, with a sensitivity of 82.8% and specificity of 98.3%.

In the present study, the combination of CB and LBC improved the diagnostic accuracy of EC to 95.8%, with a sensitivity of 89.7% and specificity of 97.4%. Importantly, no endometrial carcinoma was missed or misdiagnosed when CB and LBC were combined. Another study by Kyroudi *et al*.[23] reported that CB preparation dramatically increased the overall diagnostic accuracy in endometrial lesions. They showed that the diagnostic accuracy was improved significantly in hyperplasia with atypia, from 55% to 95.3% and in adenocarcinoma, from 98.6% to 100%. Dharan[24] also reported the utility of CB in unclear cytological smears in four cases. They concluded that CB preparation was an excellent adjunctive tool in the evaluation of endometrial lesions because of its advantages to preserve the quality for immunohistochemistry. However, few studies investigated the combination of CB and LBC in the diagnosis of endometrial lesions.

In conclusion, CB is a feasible and reproducible adjuvant method in diagnosing endometrial lesions. However, CB demonstrates a higher specimen inadequate rate than LBC smears. In addition, not all cytological specimens are cost-effective for CB preparation and it may be used in confusing cases as an auxiliary diagnosis tool. Used together, LBC and CB can improve the diagnostic accuracy of EC.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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