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THE USE OF CYTOLOGY FOR THE DETECTION OF ENDOMETRIAL PATHOLOGIES IN VAGINAL AND URINE SAMPLES IN COMPARISON WITH HISTOPATHOLOGY

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ABSTRACT

Background: Endometrial cancer is the most common gynecological malignancy with increasing incidence. The most common symptom of endometrial cancer is the abnormal uterine bleeding that causes women with this malignancy to be early investigated and treated increasing the rate of successful management because the prognosis of endometrial cancer depends on the stage; it is favorable in those diagnosed at an early stage. Proofing the accuracy of cytology in the screening and diagnosis of endometrial cancer helps to provide a triage method that refers those at high risk for more invasive investigations while avoiding unnecessary interventions in the majority of women who are at low risk. **Aim of the study:** To evaluate the accuracy of vaginal and urine cytology for the detection of endometrial cancer and other pathologies in comparison with histopathology. **Patients & methods:** This is a prospective study which enrolled 80 women with postmenopausal bleeding who attended the outpatient clinics of gynecology and oncology in Baghdad Teaching Hospital. Vaginal and urine samples were collected and sent for cytology. Hysteroscopy guided endometrial biopsy was done and the histopathological results were used as standard diagnosis. **Results:** Both the mean age and BMI were higher in the endometrial cancer group while parity was lower in this group. Diabetes was more prevalent in women with EC. Endometrial thickness was more than five in all women with EC. The sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio and negative likelihood ratio of vaginal cytology were 94%, 97%, 88.2%, 98.4%, 31 and 0.06 respectively. All urine samples were negative for malignant cells. **Conclusion:** This study concluded that vaginal cytology can be used as a simple easy noninvasive method to test those with postmenopausal bleeding, allowing reassurance of the majority of those with PMB while referring those with positive test for invasive investigations. Urine cytology is not useful for the detection of endometrial cancer and other pathologies.

KEYWORDS: Hysteroscopy guided endometrial biopsy was done and the histopathological results were used as standard diagnosis.

INTRODUCTION

Endometrial cancer is the most common gynecological malignancy with increasing incidence. It is the malignancy that originates from the uterine epithelial lining. Histologically, it is classified into two types; type 1 which is the most common and it's of adenocarcinoma type. Type 2 endometrial cancer includes serous and clear cell carcinoma. Risk factors of endometrial cancer are numerous, the most important of these factors cause excessive and long-term exposure of endometrium to the estrogen (like obesity and diabetes).[1] Grading is based on the percentage of solid non squamous growth and it is an important predictor of the prognosis.[2] The most common symptom of endometrial cancer is the abnormal uterine bleeding that causes women with this malignancy to be early investigated and treated increasing the rate of successful management.[3][4]

The most prevalent gynecological cancer in developed countries is endometrial cancer.[5] It is fourth in terms of

cancer incidence but only sixth in terms of cancer-related deaths in women since the majority of patients receive an early diagnosis and recovery.[6] In Arabic countries, age-standardized incidence rate of endometrial cancer in UAE, Kuwait, Saudi Arabia and Levant countries is higher than Northern Africa Arab countries at all ages and higher than the world rate at age 55 years and older.[7] In Iraq, uterine cancer was the second most common cancer in Middle Euphrates Region[8] and the fourth most common cancer in females in Basra.[9] The incidence of endometrial cancer was 16.54% among patients from Baghdad.[10]

Postmenopausal bleeding (PMB) is of high sensitivity in the detection of endometrial cancer[11], therefore; women with this symptom are targeted by series of investigations for the diagnosis of endometrial cancer. These investigations include transvaginal sonography (TVS), outpatient hysteroscopy and endometrial biopsy.[12]

Management is based on the surgical staging. In general surgery alone with total hysterectomy, bilateral salpingo-oophorectomy, lymphadenectomy and peritoneal washing is sufficient and no need for further treatment in patient with cancer confined to the uterus. However; in patients with advanced disease, radiotherapy, hormonal therapy or chemotherapy are often needed in addition to the surgical treatment. The prognosis of endometrial cancer depends on the stage; it is favorable in those diagnosed at an early stage.[1]

Cytology is used as a screening test in Japan, but it has not been utilized internationally for screening. It is a useful diagnostic method for endometrial cancer because it can be done in an outpatient clinic without the need for anesthesia, is inexpensive, and is well tolerated. Cytological sampling includes urine, vaginal, cervical and uterine sampling and uses different techniques like brushings, suction devices and washings.[13] Pelvic and peritoneal wash cytology was reviewed for the diagnosis of endometrial cancer. Positive cytology is associated with adverse outcome when accompanied by extrauterine spread of the disease but it indicates adverse prognostic factors such as high grade, non-endometroid histology, deep myometrial invasion, lymph node metastasis and lymphovascular space invasion.[14]

Urogenital cytology can provide a simple method for reassuring most women presenting with postmenopausal bleeding with high sensitivity and specificity (sensitivity 91.7% and specificity 88.8%). The vaginal fluid collected contains the malignant cells that shed from the uterine cavity to the lower genital tract and the urine sample is contaminated with shed tumor cells.[15] However, the use of cytology in the diagnosis and as a screening method in the high risk group is not well studied. Proofing its accuracy helps to provide a triage method that refers those at high risk for more invasive investigations while avoids unnecessary interventions in the majority of women who are at low risk.

The aim of our study is to evaluate the accuracy of vaginal and urine cytology for the detection of endometrial cancer and other pathologies in comparison with histopathology.

PATIENTS AND METHODS

Study design and setting

A prospective study was conducted in Iraq, Baghdad, Medical City, Baghdad Teaching Hospital during the period from 10th of February to the 1st of November 2024. 100 women with postmenopausal bleeding were selected for this study. They attended outpatient clinics of gynecology and oncology. 20 women were lost, either by losing contact with them or they refused to complete work up.

Approval and official permission

This study has been approved by the Scientific Council of Gynecology and Obstetrics of the Iraqi Board of Medical Specializations. An official permission has been taken from the National Center for Educational Laboratories.

Ethical consideration

A verbal consent has been taken from all women participating in this study after adequate explanation of the aim of the study and the sampling technique used. They were assured that their information are anonymous and confidential.

Exclusion criteria

Women with previous hysterectomy (total and subtotal hysterectomy) were excluded from this study. All women were assessed by history and examination to exclude other causes of vaginal bleeding like cervical pathology, vaginal pathology, endometrial pathology like polyp, bleeding disorders and drugs like warfarin. Also, those with confirmed endometrial cancer were excluded from the study.

Data collection

After explanation of the study and the sampling technique and taking verbal consent, history was taken and examination was done. Age, parity, date of menopause, past medical and surgical history, drug history and BMI were recorded. General examination was done looking for features of anemia, BMI was calculated by the following equation: BMI= Weight in kg / (Height in meter)^2. Abdominal examination and bimanual examination to assess the uterine size, direction and mobility were done. Speculum examination was done to look for any cervical or vaginal pathology.

Vaginal and urine samples were collected prior to any clinical procedures that can cause contaminations of the samples by endometrial cells shed to lower genital tract; these procedures include transvaginal ultrasound,

hysteroscopy and endometrial sampling. Urine sample was collected first to avoid mixing with debris removed during vaginal sampling. Urine samples were self-collected in a sterile container. For vaginal sample collection, patient was placed in lithotomy position, a sterile cuscus speculum was used and a spatula was used to collect the vaginal secretions from the posterior fornix. The collected material was spread on a slide and put in a preservative material (95% alcohol). Both samples were transferred to the National Center for Educational Laboratories-cytology department in less than one hour. These samples were processed and stained at the same day and examined by cytologist. Cytology was positive when malignant cells with hyperchromatic cytoplasm and atypical large nuclei were detected. Transvaginal ultrasound was done for all women to measure endometrial thickness and exclude other uterine pathologies.

Endometrial sampling was done for all the cases by hysteroscopy, either in outpatient clinic or under general anesthesia. The results of cytology were compared to the histopathology results of endometrial biopsy that was considered as standard diagnosis. All women were sent for further workup like tumor markers and imaging studies.

Data entry and statistical analysis

Data entering and analysis was done by the software program IBM-SPSS version 26. Descriptive statistics were presented as mean ± standard, the groups were compared using Chi-Square test and a P value <0.05 was considered statistically significant. The accuracy of cytology in the diagnosis of endometrial cancer was assessed by sensitivity, specificity, positive and negative predictive values, and positive and negative likelihood ratios.

RESULTS

Patient characteristics

All women were from Baghdad. They were all menopause and presented with postmenopausal bleeding. Based on the histopathological results of endometrial biopsy, the 80 cases were divided into two groups; those with endometrial cancer and those with PMB due to other causes.

The mean age of the population was 57.59 (±7.4) years, for those with endometrial cancer (mean age 60.4 years) and for those without EC (mean age 56.8 years). Mean BMI for those with endometrial cancer was 32.5 kg/m2 and for those without EC 29.5kg/m2. There was statistically significant difference between the two groups regarding BMI and having diabetes. (**Table 1**).

Table 1: Basic characteristics of the studied sample.

Parameter	Groups		Total	P value
	EC	PMB due to other causes		
Age group (years)				
45-54	4	31	35	0.2
	25%	48.4%	43.8%	
55-64	8	22	30	
	50%	34.4%	37.5%	
65-75	4	11	15	
	25%	17.2%	18.8%	
Total	16	64	80	
	100.0%	100.0%	100.0%	
BMI (Kg/m^2)				
Normal weight (18.5 – 24.9)	0	2	2	0.03
	0.0%	3.1%	2.5%	
Overweight (25.0 – 29.9)	1	32	33	
	6.3%	50.0%	41.3%	
Obese ≥30.0	15	30	45	
	93.8%	46.9%	56.3%	
Total	16	64	80	
	100.0%	100.0%	100.0%	
Parity				
Nulliparous	1	2	3	0.3
	6.3%	3.1%	3.8%	
Primiparous	2	3	5	
	12.5%	4.7%	6.3%	
Multiparous	7	21	28	
	43.8%	32.8%	35.0%	

Grand multiparous	6	38	44	
	37.5%	59.4%	55.0%	
Total	16	64	80	
	100.0%	100.0%	100.0%	
Diabetes				
Not diabetic	3	33	36	0.01
	18.8%	51.6%	45.0%	
Diabetic	13	31	44	
	81.3%	48.4%	55.0%	
Total	16	64	80	
	100.0%	100.0%	100.0%	

Ultrasound and endometrial thickness

Transvaginal ultrasound was done for all the patients. All women with endometrial cancer had endometrial thickness (ET) more than 5 mm.16/64 (25%) of those without endometrial cancer had ET less than 5 mm and 48/64 (75%) had ET more than 5mm, P<0.03. (**Table 2**)

Table 2: ET in both study groups.

Endometrial thickness	**Groups**		**Total**	**P value**
	Endometrial Cancer	**PMB due to other causes**		
Less than 5	0	16	16	0.03
	0.0%	25.0%	20.0%	
5 and more	16	48	64	
	100.0%	75.0%	80.0%	
Total	16	64	80	
	100.0%	100.0%	100.0%	

Definitive diagnosis

Endometrial sampling was done for all the 80 women participating in the study by hysteroscopy guided biopsy; in 15/80 (19%) the sampling was done by office hysteroscopy and by hysteroscopy under general anesthesia in 65/80 (81%).

The cause of PMB was found to be endometrial cancer in 16/80 (20%), endometrial hyperplasia without atypia in 22/80 (28%), endometrial hyperplasia with atypia in 15/80 (19%), polyp in 12/80 (15%), vaginal atrophy in 10/80 (12%) and no specific pathology was found in 5 cases (6%). Of the endometrial cancer cases, 3 cases were of serous type, and all the rest were of endometroid type. (**Table 3**)

Table (3): Causes of PMB in the studied sample.

Diagnosis	**Percentage**
Endometrial cancer	16
	20%
Endometrial hyperplasia without atypia	22
	28%
Endometrial hyperplasia with atypia	15
	19%
Polyp	12
	15%
Atrophic vaginitis	10
	12%
No significant pathology	5
	6%

Vaginal and urine cytology

Both vaginal and urine samples were obtained for all women. (**Figure 3.1**)

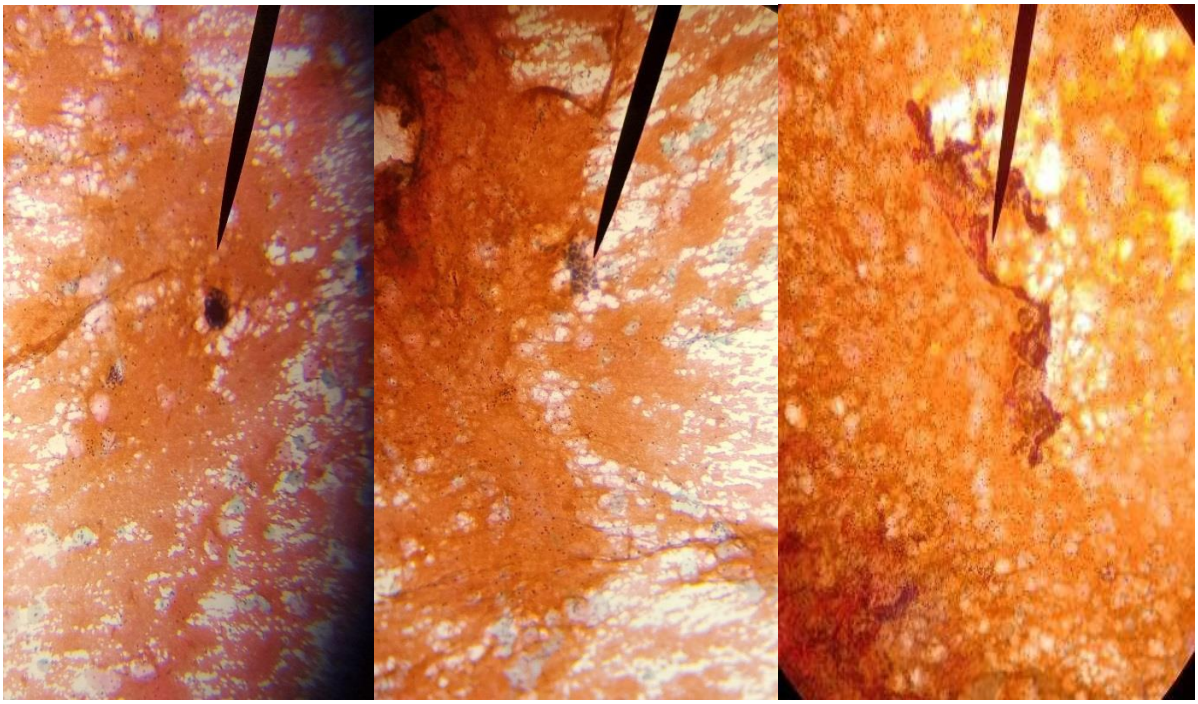

Figure 1: malignant cells in vaginal samples.

Vaginal cytology was positive in 17/80 (21%) of the cases with sensitivity 94% and specificity 97% for the detection of endometrial cancer. One case with endometrial cancer had negative cytology, with false negative rate of 6.3% and two cases with positive vaginal cytology had no endometrial cancer, giving false positive rate of 3.1%. The positive predictive value (PPV) of vaginal cytology in the detection of endometrial cancer was 88.2% and negative predictive value (NPV) was 98.4%. Positive likelihood ratio (LR+) was 31 and negative likelihood ratio (LR-) was 0.06. The detection rate by vaginal cytology was not affected by the type or the grade of the tumor. **(Table 4)**

Shedding of normal endometrial cells was seen in 11 vaginal samples, nine of them were with endometrial hyperplasia and atypia was seen in 7 cases (sensitivity 53.8% and specificity 94.0%) and P value was less than 0.05.

Table 4: Accuracy of vaginal cytology for the diagnosis of endometrial cancer.

Test measure	Vaginal cytology
Prevalence	21%
Sensitivity	94%
Specificity	97%
Positive predictive value	88.2%
Negative predictive value	98.4%.
Positive likelihood ratio	31
Negative likelihood ratio	0.06

Urine cytology was negative for malignant cells in all the cases; they showed benign urothelial cells, inflammatory cells or were acellular.

It had sensitivity 0%, specificity100%, negative predictive value 80% and negative likelihood ratio 1. The false positive rate was 0% and the false negative rate was 100%. (**Table 5**)

Table 5: Accuracy of urine cytology for the diagnosis of endometrial cancer.

Test measure	Urine cytology
Prevalence	0%
Sensitivity	0%
Specificity	100%
Negative predictive value	80%
Negative likelihood ratio	1

DISCUSSION

Comparing the results of our study to a study done by **O'Flynn** et al, the vaginal cytology had sensitivity 89.6 %, Specificity 88.7%, PPV 89.7, NPV 88.7, LR+ 7.90 and LR- 0.12. Prevalence of endometrial cancer by vaginal cytology in our study was 19%, while in a study done by O'Flynn et al, it was 52%[15], this can be explained by the relatively larger sample size, different population studied and because of targeting those with endometrial cancer while in our study, the cause of PMB was not confirmed as endometrial cancer before the study.

A meta-analysis by Wang et al reported that endometrial cytology using different sampling techniques like different types of brushes had combined sensitivity 0.84, combined specificity 0.98, combined LR+ 34.65, combined LR-0.21.[16] Another study by Wang Qing et al showed combined sensitivity, specificity, LR+ and LR- of endometrial cytology in the detection of endometrial cancer was 0.91, 0.96, 25.4 and 0.10 respectively.[17]

These results are comparable to our study results suggesting that vaginal cytology is as accurate as direct endometrial sampling in the detection of endometrial cancer.

Urine cytology was negative for all patients in our study with sensitivity 0%, specificity100%, negative predictive value 80% and negative likelihood ratio 1. There are limited studies about the role of urine cytology in the diagnosis of endometrial cancer. O'Flynn et al found that The sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio and negative likelihood ratio of urine cytology were 72%, 95%, 93.5%, 76.7%, 13.9 and 0.3 respectively.[15] This difference in the detection rate of urine cytology may be due to relatively smaller sample of our study.

A prospective multicenter study was done by Jones et al, it revealed that combined sensitivity and specificity of urine and vaginal cytology for detection of endometrial cancer were 80.8% and 92.6% respectively.[18]

The present study revealed that there is an association between shedding of endometrial cells without malignant features and endometrial hyperplasia with atypia with sensitivity 53.8% and specificity 94.0% and P value less than 0.05. Few studies have assessed the presence of endometrial cells in vaginal samples. In a study by Woolderink et al, endometrial cells were not detected in vaginal cytology in contrast to the ability of endometrial sampling in detecting endometrial tissue.[19]

In our study, the most common histologic type was endometroid adenocarcinoma (81%), the other type was serous type (19%). Rizescu et al reported that endometroid type was the most common type (85%), serous type was the second next type (9%), other types (mixed adenocarcinoma 4% and clear cell adenocarcinoma 2%) were also detected.[20]

The mean age of endometrial cancer group was 60.4 years; it is higher than that of the other group but the difference is not significant. This finding is different from other studies showing significantly higher age in the malignant group, Koual found that the mean age of patients with endometrial cancer was 63 years.[21] While in a study in India by Agarwal et al reported that the mean age of patient with endometrial cancer was 54 years.[22] This difference can be explained by the differences among populations regarding ethnicity, BMI, parity and other risk factors of endometrial cancer.

The present study revealed significantly higher BMI in the malignant group (32.5 kg/m2 vs 29.5kg/m2), this is in concordance with Kawachi et al who found that obesity is associated with elevated risk of endometrial cancer.[23] This ensures the association between obesity and endometrial cancer and enhances the impact of increasing obesity in increasing the incidence of endometrial cancer. Harvey et al found that life course obesity is associated with endometrial cancer; young adulthood obesity and weight gain or maintenance between young adulthood and adulthood were more in the endometrial cancer group.[24]

The parity was higher in the non- malignant group but the difference is not significant, this is probably due to the general higher parity of the population on which the study was performed. Harvey et al reported that nulliparity is higher in women with endometrial cancer than the control group.[24] A meta-analysis by Wu et al reported inverse association between parity and endometrial cancer.[25]

The present study revealed strong association between diabetes and endometrial cancer, 81% of patients with EC had diabetes vs 48% in those without EC. This association was also reported by Tamara et al showing that diabetes increases the risk of endometrial cancer.[26] A study by Agarwal et al found that 27% of the patients with EC had diabetes.[22]

The endometrial thickness in the present study was above the threshold of menopause (>5 mm) for all malignant cases and three quadrants of non-malignant cases, this finding was also reported by Tofiloska et al showing that endometrial thickness is an effective predictor of endometrial cancer with significant increase in the risk of EC with each millimeter increase in the endometrial thickness.[27]

Many limitations of this study exist, these include relatively small sample size and single center involved in the study. Also this study assessed the ability of the test to detect endometrial cancer in those with PMB while those who are asymptomatic or with atypical presentation were not assessed.

CONCLUSION

This study concluded that vaginal cytology can be used as a simple easy noninvasive method to test those with postmenopausal bleeding, allowing reassurance of the majority of those with PMB while referring those with positive test for invasive investigations. Urine cytology is not useful for the detection of endometrial cancer and other pathologies.

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