Original Article

A Study to Determine the Diagnostic Accuracy of Endometrial Aspiration Cytology with Histopathological Correlation at Rural Tertiary Health Care Hospital

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Abstract

Background: Endometrial cytopathology is a powerful tool for the detection of a wide variety of benign lesions, atypias, inflammatory changes and infectious organisms. It is also helpful for the cyto-hormonal evaluation of patients and the detection of endometrial malignancies. Aim: To assess the adequacy of the aspirated endometrial sample and identify the morphological parameters with regard to making a definite diagnosis. The aim of this study is to analyse the accuracy of endometrial aspiration cytology diagnosis in comparison with histopathological correlation.

Methods: Endometrial aspiration using a Karman’s cannula was done in 58 patients who presented with abnormal uterine bleeding to the department of Obstetrics and Gynecology during the period of July 2013 to Jan 2015. The cytology smears were stained with Papanicolaou stain and May-Grünwald-Giemsa stain. Following aspiration Dilatation and Curettage was done for all cases. The formalin fixed tissue samples of curettage were stained with Hematoxylin and eosin staining for obtaining the histopathological diagnosis. The statistical analysis was performed using EPI info version 3.5.3. Chi square test was used to correlate the parameters.

Results: In the present study endometrial aspiration cytology and histopathological correlation were performed on 58 cases. The patterns identified on EAC were 27 cases of benign endometrium (46.6%), 24 cases of simple hyperplasia (41.4%), 4 cases of endometritis (6.8%), 1 atypical hyperplasia (1.7%) and 2 cases of malignancies (3.4%). Secretory phase endometrium was the most common benign lesion amounting to 36.2%.

Conclusion: Endometrial aspiration cytology was found to be a safe outpatient procedure in the diagnosis of cyclic changes and endometrial lesions. The accuracy of identifying benign, inflammatory and malignant lesions was 98.28% to 100% making EAC an effective procedure.

Keywords: Abnormal uterine bleeding (AUB), Endometrial malignancy, Endometrial aspiration cytology (EAC), Dilatation and curettage, Cyclic endometrium, Karman’s cannula

Introduction

Endometrium is subjected to changes due to hormonal influence and so it is an important area in gynecologic practice for identification of proliferative changes, malignancy and other hormonal changes. [1] Until recently the standard method of endometrial assessment was by Dilatation and Curettage (D&C) which is an invasive and time-consuming procedure. It has to be done under paracervical block or general anesthesia, which requires hospitalization. It is also associated with complications such as uterine perforation, vaso vagal shock, hemorrhage and infection. [2,3,4,5]

Endometrial aspiration cytology (EAC) is a valuable asset in gynecologic practice for determining the lesions in women with abnormal bleeding, both in premenopausal and postmenopausal age group. [2] Endometrial cytology is a powerful test for the detection of a wide variety of lesions ranging from atypias and inflammatory changes to infectious conditions. [2] Further this technique has a role to play in monitoring the status of the endometrium in women at high risk of endometrial malignancy particularly in patients receiving Hormone Replacement Therapy (HRT). [2,6] This technique was first described by Papanicolaou and Marchetil. They employed sample aspiration method in the diagnosis of cancer and other uterine lesions, using a metal cannula, which was developed by Cary in 1943. [3] Subsequent modifications of the endometrial aspiration technique included the Endometrial brush, Gravlee jet washer, Isaac endometrial sampler, Endocyte, Endo pap sampler and disposable plastic cannula. [2,7]

Endometrial aspiration was found to be a safe outpatient procedure when done with Karman’s plastic cannula in diagnosis of endometrial lesions of all ages, where anesthesia and hospital admission can be avoided. [2,3,4,5] Endometrial sample represent the functional endometrium, which includes stromal and glandular elements as well as cells from surface epithelium. [2] Moreover endometrial aspiration is as accurate as D&C in diagnosis of endometrial
atyria and carcinoma. The cells of endometrial aspiration show better morphology than endometrial cells found in cervico-vaginal smear. Hence the sensitivity and specificity of the endometrial aspiration cytology in comparison with the endometrial biopsy is documented with the hope of replacing the standard invasive procedure with less complicated minimally invasive procedure as a routine screening tool. The aim of this study is to analyse the accuracy of endometrial aspiration cytology diagnosis in comparison with histopathological correlation.

**Materials and Methods:**

The present study was a cross sectional prospective study conducted in the Departments of Pathology and Obstetrics and Gynaecology in Sri Manakula Vinayagar Medical College and Hospital, Puducherry, over a period of two years with a clinical diagnosis of AUB, after obtaining the ethical committee approval. Patients having acute inflammatory disorders of genital tract, cervical malignancy and pregnancy were excluded from the study and the study included patients above 40 years of age with menorrhagia, polymenorrhoea and post menopausal bleeding who required D&C as recommended by the Gynaecologist. After obtaining a detailed history, thorough general, systemic and local examination and all relevant investigations. The procedure was properly explained to the patients, and their consent was obtained for endometrial aspiration cytology, followed by diagnostic curettage. Under aseptic precautions the endometrial aspiration was carried out in the OPD or in the operation theater prior to Dilatation & curettage (D&C). A plastic disposable Karman’s cannula measuring 4mm was inserted into the endometrial cavity with an 20cc disposable syringe for aspiration. The aspirated material obtained was expelled onto clean glass slides, smears were prepared, air-dried, followed by fixation in methanol. These were stained with May-Grünwald-Giemsa stain. Some of the smears were immediately fixed in 95% ethyl alcohol and then stained with Papanicolaou stain. The cytomorphological features were analyzed to diagnose the lesion, the cytology smears were screened for adequacy using criteria suggested by Bistoletti and Hjerpe. They suggested that the yield of 10-20 endometrial fragments to be considered as adequate. Subsequently, D and C were done for all the patients, The formalin fixed tissue samples of curettage were routinely processed and paraffin blocks were made. Sections of 5micron thickness were cut using microtome and stained with Hematoxylin and eosin (H and E) staining. Finally the histopathological diagnosis of endometrial curettages were compared with cytological diagnosis. The morphological patterns were tabulated and statistical analysis was performed using EPI info version 3.5.3. Chi square test was used to correlate the parameters. The sensitivity, specificity, positive predictive value, negative predictive value and accuracy of the EAC diagnosis in comparison to gold standard histopathological diagnosis were calculated.

**Result**

The present study was carried out on 58 female patients above 40 years of age with the complaints of AUB, the endometrial aspirates were performed following which the endometrial biopsies were taken. The result of 58 cytological aspirations in the present study was broadly categorized as benign endometrium including normal phases of endometrium, (secretory phase, proliferative phase, menstrual phase) as well as decidual reaction, Inflammatory smear, Hyperplasia, Atypical hyperplasia and malignancy (adenocarcinoma, adenosquamous carcinoma).

Most of our patients were in the age group of 40-45 years (60.3%) followed by 46-50 years (25.7%). Hence, the age group ranging between 41 and 50 constitutes most of our patients (86.0%). Perimenopausal age group 41-50 years was the largest group in our study (Table 1). Majority of the patients were presented with menorrhagia 37 (64%), followed by menorrhagia with dysmenorrhea 16 (27.6%). Out of two cases of malignancies, one patient presented with postmenopausal bleeding and other with menorrhagia which was depicted in (Table 2). Out of 58 cases studied a clinical diagnosis of DUB was obtained in 30 (51.7%) cases, followed by fibroid in 20 (34.5%) cases shown in (Table 3). In the 30 patients with clinical diagnosis of DUB, simple hyperplasia was the commonest finding on EAC (53.3%). Other patterns observed were secretory phase endometrium (20%), endometritis (13.3%), and menstrual phase (6.8%). Atypical hyperplasia and malignancy were found in one patient each. Among the 20 patients with clinically suspected fibroid 10 patients showed secretory phase endometrium, 6 were showing simple hyperplasia and remaining 4 EAC findings were one each of endometritis, Menstrual phase, Atypical hyperplasia and carcinoma (Table 4). In simple hyperplasia endometrial changes in EAC of 23 cases correlated with the histopathological diagnosis, out of 24 cases. One case which did not correlate was reported as Endometrial polyp on histopathology. Endometrium in secretory phase correlated in 20 cases out of 21 cases, with the exception of a case reported as Secretory hyperplasia on histopathology. One case of Endometritis was underdiagnosed as menstrual endometrium. Among all patterns simple hyperplasia was found to be the commonest lesion 41.4%, followed by secretory phase 36.2% (Table 5). 60.3% of the smears were hypercellular, 34.4% of the hypercellular smears...
showed hyperplasia. Both cases of carcinomas were hypercellular. Most of the menstrual endometrium showed low cellularity [Table 6]. In the present study 37 cases (63.7%) of the cellular patterns were in sheets.

Dyscohesive clusters and single cells were seen in 100% of the malignancies. In 5 cases of simple hyperplasia inflammatory cells and cyst macrophages were seen. All the 4 cases of endometritis showed plasma cells and lymphocytes. Stromal and vascular predominance were seen in simple hyperplasia. Bare nuclei were seen in almost all the cases except in carcinoma[Table 7]. There was 100% sensitivity for benign, hyperplastic and malignant lesions. The specificity was 100% for endometritis and malignancy. The accuracy was ranging from 98.28% to 100%. Accuracy of EAC was 96.55% in the diagnosis of benign lesions which included secretory phase endometrium, menstrual phase and decidual reaction. Accuracy was 98.28% in the diagnosis of simple hyperplasia. Accuracy was 100% in the diagnosis of carcinoma[Table 8].

Table 1: Age incidence of the patients.

| Age incidence | No of cases | Percentage (%) |
|---------------|-------------|----------------|
| 40-45yrs      | 35          | 60.3%          |
| 46-50yrs      | 15          | 25.7%          |
| 51-55yrs      | 8           | 14%            |
| TOTAL         | 58          | 100%           |

Table 2: Clinical presentation of the patients

| Clinical presentation                          | No of cases | Percentage (%) |
|------------------------------------------------|-------------|----------------|
| Menorrhagia                                    | 37          | 64.0%          |
| Menorrhagia with dysmenorrhea                  | 16          | 27.6%          |
| Amenorrhea followed by menorrhagia             | 3           | 5.2%           |
| Post-menopausal bleeding(POM)                  | 2           | 3.2%           |
| TOTAL                                          | 58          | 100%           |

Table 3: Clinical diagnosis in the patients aspirated

| Clinical diagnosis                      | No of cases | Percentage (%) |
|-----------------------------------------|-------------|----------------|
| Dub                                     | 30          | 51.7%          |
| Fibroid                                 | 20          | 34.5%          |
| Adenomyosis                             | 6           | 10.4%          |
| Endometrial Hyperplasia(Eh)             | 1           | 0.2%           |
| Endometrial Polyp                       | 1           | 0.2%           |
| TOTAL                                   | 58          | 100%           |

Table 4: EAC patterns in clinically suspected DUB and fibroid cases

| EAC Patterns               | No of cases in DUB | Percentage (%) | No of cases in Fibroid | Percentage (%) |
|----------------------------|---------------------|----------------|------------------------|----------------|
| Simple hyperplasia        | 16                  | 53.3%          | 6                      | 30%            |
| Secretory phase           | 6                   | 20.0%          | 11                     | 55%            |
| Endometritis              | 4                   | 13.3%          | 1                      | 5%             |
| Menstrual phase           | 2                   | 6.8%           | 1                      | 5%             |
| Atypical hyperplasia      | 1                   | 3.3%           | --                     | --             |
| Malignancy                | 1                   | 3.3%           | 1                      | 5%             |
| TOTAL                     | 30                  | 100%           | 20                     | 100%           |
Table 5: Endometrial patterns obtained by endometrial aspiration cytology.

| Endometrial patterns [EAC] | No of cases | Percentage (%) |
|----------------------------|-------------|----------------|
| Simple Hyperplasia (SH)    | 24          | 41.4%          |
| Secretory Phase endometrium (SP) | 21          | 36.2%          |
| Endometritis (ETS)         | 4           | 6.9%           |
| Menstrual phase endometrium (ME) | 6           | 10.4%          |
| Atypical Hyperplasia (SHA) | 1           | 1.7%           |
| Carcinoma (CA)             | 2           | 3.4%           |
| **Total**                  | **58**      | **100.0%**     |

Table 6: Distribution of cellularity of various lesions in EAC-

| Cellularity | SH | SP | ME | ETS | DE | SHA | CA | TOTAL |
|-------------|----|----|----|-----|----|-----|----|-------|
| High        | 20 | 10 | 0  | 1   | 1  | 1   | 2  | 35    |
| Moderate    | 3  | 11 | 1  | 3   | 0  | 0   | 0  | 18    |
| Low         | 1  | 0  | 4  | 0   | 0  | 0   | 0  | 5     |
| **Total**   | 24 | 21 | 5  | 4   | 1  | 1   | 2  | 58    |

Table 7: Pattern of cells in various lesions by EAC-

| Pattern         | SH  | SP  | ME | ETS | DE | SHA | CA |
|-----------------|-----|-----|----|-----|----|-----|----|
| Sheets          | 16  | 15  | 2  | 3   | 1  | 0   | 0  |
| Papillaroid     | 8   | 6   | 0  | 1   | 0  | 1   | 0  |
| Small clusters  | 0   | 0   | 3  | 0   | 0  | 0   | 0  |
| Dyscohesive clusters | 0 | 0   | 0  | 0   | 0  | 0   | 0  |
| Singles         | 0   | 0   | 3  | 0   | 0  | 0   | 0  |
| Stroma          | 12  | 4   | 0  | 0   | 1  | 0   | 0  |
| **predominant** |     |     |    |     |    |     |    |
| Vascular        | 23  | 6   | 0  | 0   | 0  | 0   | 2  |
| **predominant** |     |     |    |     |    |     |    |
| Inflammatory    | 5   | 2   | 3  | 4   | 0  | 1   | 2  |
| Bare nuclei     | 22  | 21  | 4  | 1   | 1  | 1   | 0  |

Table 8: Level of accuracy with statistical analysis of EAC in comparison with histopathology

| Parameter         | Number of cases (cytology) EAC | Number of cases (HPE) | Sensitivity (%) | Specificity (%) | Positive predictive value (%) | Negative predictive value (%) | Accuracy (%) |
|-------------------|--------------------------------|-----------------------|-----------------|-----------------|-------------------------------|-------------------------------|--------------|
| Simple Hyperplasia [SH] | 24                              | 23                    | 100             | 97.14           | 95.83                         | 100                           | 98.28        |
| Secretary Endometrium [SP] | 21                              | 20                    | 100             | 97.37           | 95.24                         | 100                           | 98.28        |
| Endometritis [ETS] | 4                               | 5                     | 80              | 100             | 98.15                         | 80                            | 98.28        |
| Menstrual Endometrium [ME] | 5                               | 4                     | 100             | 98.15           | 80                            | 100                           | 98.28        |
| Decidual Reaction [DE] | 1                               | 1                     | 100             | 100             | 100                           | 100                           | 100          |
| Atypical Hyperplasia [SHA] | 1                               | 1                     | 100             | 100             | 100                           | 100                           | 100          |
| Carcinoma [CA] | 2                               | 2                     | 100             | 100             | 100                           | 100                           | 100          |
Discussion
In the present study 58 patients above 40 years of age with menorrhagia, polymenorrhoea or postmenopausal bleeding who required D&C were taken for endometrial aspiration and histopathological correlation was done for all the cases. Among 58 cases majority were simple hyperplasia and secretory phase endometrium and only 2 cases of malignancy were identified. Adequacy of endometrial aspirates was studied on the basis of presence of endometrial cells or tissue, Bistoletti and Hjerpe et al. [9] suggested the yield of 10-20 endometrial cell fragments to be taken as adequate. [9] Using the above criteria, all 58 cases were found to be adequate in the present study. In the present study there was 94.8% correlation in cytological and histological findings and this was comparable with Hemalatha et al. [14] this study which also compared histopathological and cytological findings in cases of dysfunctional uterine bleeding using 4mm Karman cannula and found 95% correlation. [4] According to our study the commonest presenting age group among the patients was peri menopausal age group with 86.3% of cases recorded, which was similar to the study done by Farah MJ et al., with 80% of cases in perimenopausal age group. [10] Among the clinical presentations DUB was the commonest presentation seen in 52% of cases which correlated with studies by Kaur N et al. [11] The cytological diagnoses of patients presenting with DUB in the present study were comparable with previous literature. [11, 22]

Cytological Findings: Cytodiagnosis of secretory endometrium correlated in 20 cases out of 21 cases, with the exception of a case reported as secretory hyperplasia on HPE. In secretory endometrium, there was presence of sheets of single layered cells with round or oval nuclei showing evenly distributed fine granular chromatin and presence of vacuolation in cytoplasm. [2, 11, 13] Present study observed same findings but in addition to it bare nuclei, moderate vascularity and neutrophils were also seen in background which denoted increased cytolysis of cells in secretory phase. The EAC findings of simple hyperplasia correlated with HPE in 23 cases out of 24 cases. One case which did not correlate was reported as endometrial polyp on HPE. Features of simple hyperplasia on EAC were increase in cellularity, cells in small and large groups, nuclei of the cells showed mild to moderate anisonucleosis, finely granular even chromatin and scant cytoplasm. [2, 14, 15] Similar findings were observed in present study but in addition, bare nuclei, high vascularity and cyst macrophages were also seen in the background. Presence of cyst macrophages in EAC was often associated with cystically dilated hyperplastic glands in HPE.

In decidual reaction, the cells were round to polygonal in shape with abundant eosinophilic cytoplasm and centrally placed nucleus. [16, 17, 18] In the present study also similar findings were observed in the single case of decidual reaction encountered.

Menstrual phase endometrial cells appeared exhausted with scanty cytoplasm. Single normal cells were seen with hemorrhage, necrotic cellular debris and inflammatory cells in the background. [19] In addition to these findings bare nuclei, low cellularity and inflammation were also seen in the background in the present study.

Features of endometritis in our study were minimal increase in cellularity, dense inflammation obscuring cellular details and presence of plasma cells. Atypical endometrial hyperplasia was observed by presence of hypercellularity of smears, three dimensional structures, sheets of glandular and papillary formation, increased N:C ratio, presence of nucleoli, anisonucleosis and nuclear crowding. [9, 17, 20] Present study observed a single case of atypical hyperplasia.

In terms of malignancy, sensitivity was 100% and specificity was 100%. One case of adenocarcinoma was diagnosed in the present study on the basis of cytological features of irregular glands and dyscohesive clusters of cells with nuclear pleomorphism, atypical mitosis and individual atypical cells in a necrotic background. [2, 11, 13] There was one case of adenosquamous carcinoma of endometrium diagnosed on EAC, based on large numbers of dyscohesive single cells and spindle shaped keratinised cells, hyperchromatic nuclei with variation in size and shape admixed with malignant glandular pattern in a granular necrotic background. Both cases of malignancies showed absence of bare nuclei in contrast to the benign lesions. The lesions were identified by aspiration cytology and were correlated with histopathological examination done on endometrial samples obtained by D&C. The diagnoses were correlated and sensitivity of the EAC was analysed. The values were correlated with other studies done by Kaur N et al. [11] and Malik et al. [2]

An accuracy of 98.28% was observed for secretory phase, in the present study, which is comparable to Malik et al. [2] In the present study, accuracy of 98.28 % was observed in diagnosis of hyperplasia, similar to that of Kaur N et al. [11] and Malik et al. [2]

The percentage accuracies in diagnosing benign conditions of endometrium, hyperplasia and malignancy on aspiration
cytology in our study were 96.55%, 98.28%, 100% respectively. These findings were comparable to that of Kaur N et al.\cite{29} (benign 93.88%, and hyperplasia 96.94%) and Kyroud et al.\cite{30} (benign 93.88% and hyperplasia 92.5%). Comparison of accuracy in the diagnosis of endometrial adenocarcinoma was 100%, similar to the study of done by Malik et al.\cite{19,20} and Liza et al.\cite{21,22,23}

Pitfalls in the diagnosis on EAC are Endometrial polyp could not be identified by EAC since it also showed high cellularity vascularity, bare nuclei and absence of secretory activity as seen in simple hyperplasia. Secretory hyperplasia was misdiagnosed as secretory phase due to the presence of the secretory activity in the cells.Endometritis was misinterpreted as menstrual endometrium since the plasma cells were over looked on the cytology smear.

**Conclusion**

We conclude from our study the endometrial cytology is an excellent initial diagnostic evaluation tool and it aids a useful, effective, and a minimally invasive procedure. Karman endometrial aspiration cannula attached to a disposable syringe is a cheap and easily available device that yields good cellular material. A very important criteria is the cytologist's ability in reporting the findings, it can be used routinely for the primary investigation of women with AUB, Endometrial aspiration is of special value in the investigation of women in whom there are much greater risks associated with general anesthesia and in whom no curettings are obtained. And also, we can avoid unnecessary hospital admissions and requirement of anesthesia for D and C. Hence, it can be used as an initial screening procedure in low resource settings.

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