Histopathology-like categories based on endometrial imprint cytology in dysfunctional uterine bleeding

ABSTRACT

Background: Cytology of the endometrium is an underused technique in diagnostic pathology. It has been in use since 1943 when it was used by Papanicolau and Marchetti and also by Cary. Its advantage has been shown in the past for endometrial hyperplasia and endometrial carcinoma by Segadal and Iversen in 1980, Grimes in 1982, Polson et al. in 1984 and Skaarland in 1986. All these studies had used a sampler for obtaining endometrial aspiration-like metal cannula attached to syringe, Isaacs sampler, Gynoscan (Endoscann) and insemination cannula. These studies have shown that cytology is a useful test and gives comparable and sometimes even better results than histopathology of curettage in endometrial hyperplasia and carcinoma. The literature search showed that endometrial imprint has been used only occasionally in immunocytochemistry for the diagnosis of endometrial carcinoma. Only a few studies have used endometrial cytology for the study of dysfunctional uterine bleeding (DUB). Some authors have reservations about endometrial cytology and have drawn the line for usage of endometrial cytology in condition of low levels of clinical suspicion or with direct guidance. The present study was hence conducted to evaluate whether it is possible to assign histopathology-like diagnosis by imprint cytology and also to evaluate its usefulness in the assessment of patients of dysfunctional uterine bleeding of low clinical suspicion.

Aim: The present study was conducted to evaluate whether it is possible to assign histopathology-like diagnosis by imprint cytology and also to evaluate its usefulness in the assessment of patients of dysfunctional uterine bleeding of low clinical suspicion.

Materials and Methods: Imprint smears were made from 93 curettage materials during a study of DUB. Blinded analysis of imprint smears was performed by using McKenzie’s criteria and some criteria devised for the requirements of this study. Results of cytology were correlated with histopathology. Statistical analysis was carried out by GraphpadInStat Demo.

Results: Majority of the patterns classifiable in histopathology could also be classified in this study on imprint cytology. The overall sensitivity and specificity of cytology in the detection of endometrial patterns in DUB patients were 91.23% and 83.87%, respectively, although the sensitivities and specificities differ according to the phase of endometrium.

Conclusion: Histopathology-like categories can be assigned on imprint smears in the diagnosis of DUB. Endometrial imprint cytology can be helpful in centers where histopathology laboratories are not available and even in well-established institutes. It is possible to improve the sensitivity and specificity with better imprinting techniques.

Key words: Cytology in dysfunctional uterine bleeding; direct endometrial smears; endometrial cytology; endometrial imprint cytology

Introduction

Cytology of the endometrium is an underused technique in diagnostic pathology, although it has been in use since 1943 when it was used by Papanicolau and Marchetti and also by Cary. Its advantage has been shown in the past for endometrial hyperplasia and endometrial carcinoma by Segadal and Iversen in 1980, Grimes in 1982, Polson et al. in 1984 and Skaarland in 1986. All these studies had used a sampler for obtaining endometrial aspiration-like metal cannula attached to syringe, Isaacs sampler, Gynoscan (Endoscann) and insemination cannula. These studies have shown that cytology is a useful test and gives comparable and sometimes even better results than histopathology of curettage in endometrial hyperplasia and carcinoma. The literature search showed that endometrial imprint has been used only occasionally in immunocytochemistry for the diagnosis of endometrial carcinoma. Only a few studies have used endometrial cytology for the study of dysfunctional uterine bleeding (DUB). Some authors have reservations about endometrial cytology and have drawn the line for usage of endometrial cytology in condition of low levels of clinical suspicion or with direct guidance. The present study was hence conducted to evaluate whether it is possible to
assign histopathology-like diagnosis by cytology and also to evaluate the usefulness of endometrial imprint cytology in the assessment of patients of DUB of low clinical suspicion.

**Materials and Methods**

A prospective study of imprint smears of curettage material was carried out over a 12-month period. Imprint smears were made randomly from 93 curettage materials during a histopathology study of 237 patients of DUB between July 2012 and July 2013 at a tertiary medical care hospital. Patients included in the study were those with history of DUB of all age groups having a low suspicion of malignancy. Patients excluded from the study were those who were known to have systemic bleeding disorders, acute pelvic inflammatory disease or those with in situ intrauterine contraceptive device.

The gynecology residents were asked to make imprints from the curettage material. The tissue was to be blotted lightly on a gauze piece to remove excess blood and imprints were to be prepared by pressing the material firmly for 3-5 seconds. Two slides were prepared from each case. No adhesive material was used. The slides were immediately fixed in methanol. The curetted material was then put in formalin and sent for histopathology.

The imprints were stained by hematoxylin and eosin and reported by a single experienced observer. Because the histological diagnosis was made by the same observer, to facilitate blinding, the histopathology slides were diagnosed first and cytology smears were assessed later without the knowledge of the histopathology diagnosis. Finally, the cytological diagnosis was compared with the histopathological diagnosis. The direct endometrial adequacy criteria used was that mentioned in Koss (presence of at least six epithelial cell clusters of at least five to 10 cells each). The criteria used for assessing cytology were those of McKenzie et al. We designed few points to suit the requirements for our study of DUB as most studies and textbooks have concentrated on the diagnosis of hyperplasia and carcinoma.

In general, we used the following parameters to assess each case.

**Overall cellularity**

**Glands**

- **Size**
  - Architecture: Packing of endometrial cells
  - Cell: Size, type
  - Cytoplasm: Amount, color, vacuolization
  - Nucleus: Irregularity in membrane, chromatin and nucleoli.

**Stroma**

- Amount
- Morphology of cell
- Nucleus

**Blood vessels**

**Inflammatory cells**

Each parameter was graded either from 1 to 4 or small/medium/large size or present/absent. The cytological characteristics we found most useful are mentioned in Table 1.

**Statistical analysis**

Statistical analysis was performed by GraphpadInStat Demo-[DATASET1.ISD] software.

**Results**

Imprint smears from 93 random curettage materials were analyzed. Twenty-six cases had no material on the slide, four cases showed only blood, two cases showed cervical/endocervical tissue and four cases had less than optimal cellularity. These 36 cases were excluded from further calculations.

The remaining 57 cases were further evaluated and rendered categories similar to the histopathological DUB categories based on the assessment of their glandular, stromal, blood vessel and inflammatory cell characteristics as mentioned earlier. The endometrial patterns reported are shown percentage-wise in Table 2.

None of the smears showed morphology of carcinoma and none of the histopathology slides showed carcinoma.

Proliferative pattern: Thirty-four of 38 cases diagnosed cytologically matched with their corresponding histopathology. The remaining four cases showed a different endometrial pattern on histopathology (two showed secretory endometrium, one showed pill effect and one showed hyperplasia). One case that showed proliferative endometrium on histology was missed on cytology and diagnosed as late secretory endometrium. These 38 cases were further categorized into early, mid, late [Figure1a, b and c, respectively] and weak proliferative phase (12, 12, three and 11 cases each).

Secretory pattern: Six of seven cases diagnosed cytologically were also secretory phase on histology. One case showed histology of proliferative endometrium. These seven cases were further categorized into early and late secretory phase (three and four cases each and Figure 2a and b, respectively).
Shedding endometrium: All the five cases reported as shedding endometrium in cytology showed similar features on histology [Figure 2c].

Atrophic pattern: Both cases of atrophic endometrium diagnosed on cytology also had histology of atrophic endometrium histology.

Hyperplasia: Of two cases of hyperplasia reported on cytology [Figure 3], one showed hyperplasia on histology while another showed inactive endometrium in the initial sections and hyperplastic endometrium on the deeper sections.

Overall, the anovulatory and ovulatory patterns detected were 68.4% and 31.6%, respectively.
Hence, sensitivity and specificity for competency of cytology to diagnose these endometrial patterns are as shown in Table 3. Sensitivity and specificity for pill effect and hyperplasia may not reflect true values because of the lesser number of cases in these categories. Sensitivity and specificity of interval phase, atrophied endometrium and DPE could not be calculated because of the absence of true-negative and false-positive cases.

**Discussion**

Different studies have used different adequacy criteria. Malik et al. have used the criteria of Bistoletti and Hjerpe for adequacy (10-20 endometrial fragments). We used the direct endometrial cytology adequacy criteria mentioned in Koss and found it useful in diagnosis, especially of the weak proliferative phase. Criteria of 10-20 fragments would have rendered many of the smears inadequate and would have required a repeat examination if only cytology had been used for the diagnosis of these patients.

Cytological criteria for diagnosis of hyperplasia have been used by Rascoe in 1963, Anforaker in 1978 and Morse in 1981. These are described in detail by Hemalatha et al. Because we wanted to assess cytology for DUB, we used the criteria of McKenzie and, with the specially devised additional criteria mentioned in Table 1, we were able to assign specific proliferative, secretory and weak proliferative endometrial patterns in our study.

### Table 2: Percentage distribution of endometrial patterns seen in cytology smear

| Imprint diagnosis                  | No. of cases | Percentage |
|-----------------------------------|--------------|------------|
| Proliferative pattern             | 38           | 66.6       |
| Interval pattern                  | 01           | 1.8        |
| Secretory pattern                 | 07           | 12.2       |
| Shedding endometrium              | 05           | 8.8        |
| Pill effect                       | 01           | 1.8        |
| Atrophic pattern                  | 02           | 3.5        |
| Hyperplasia                       | 02           | 3.5        |
| Disordered proliferative endometrium | 01         | 1.8        |

### Table 3: Sensitivity and specificity of cytology compared with histopathology (57 cases)

| Imprint diagnosis                  | Total (cases) | Non-match (cases) | Matching (in no.) | Sensitivity (%) | Specificity (%) |
|-----------------------------------|---------------|-------------------|-------------------|-----------------|-----------------|
| Proliferative pattern             | 38            | 04                | 34                | 97.14           | 82.16           |
| Interval pattern                  | 01            | 00                | 01                | —               | —               |
| Secretory pattern                 | 07            | 01                | 06                | 85.71           | 98              |
| Shedding endometrium              | 05            | 00                | 05                | 83.33           | 100             |
| Pill effect                       | 01            | 00                | 01                | 50              | 100             |
| Atrophic pattern                  | 02            | 00                | 02                | —               | —               |
| Hyperplasia                       | 02            | 00                | 02                | 66.67           | 100             |
| Disordered proliferative endometrium | 01          | 00                | 01                | —               | —               |
Inadequacy rates reported in the literature for aspiration varies from 1.6% to 27%, depending on the technique used. In our study, this pre-analytical error was very high (36.6%). The corresponding histopathological slides had adequate material, indicating that faulty imprinting technique was the chief reason. Because the imprints were prepared in the operation theater by gynecology residents, to make this additional procedure less cumbersome for them, an adhesive material was not applied. This could be an additional reason of no cellularity in such a high percentage of our cases and a lesser yield of cells in most of the smears studied.

Sensitivity and specificity of endometrial cytology in different studies range from 81% to 96% and 83% to 96%, respectively. These studies have used either Isaac’s sampler, insemination cannula or endopap sampler and hence cellularity achieved is higher. The overall sensitivity and specificity in our study were 91.23% and 83.87%, respectively, even though the cellularity was low. It can be seen from Table 3 that imprints were most sensitive in detecting proliferative phase (97%) and more specific in detecting secretory phase, pill effect and hyperplasia (98-100), although the sensitivity to detect these was not good.

Hemalatha et al. have compared cytological findings in cases of DUB with histology and found a 95% correlation. They found overall diagnostic accuracy higher with aspiration cytology than with D&C due to two cases of hyperplasia that were missed out by histopathology. Kawana et al. have revealed endometrial cancers on cytology even though histopathology was normal. Our study also showed one case of complex hyperplasia on cytology that showed inactive endometrium in the initial sections. Later, complex typical hyperplasia was detected on deeper sections, proving that cytology may have a better diagnostic accuracy.

Disordered endometrium cytologically showed more glandular and lesser stromal cellularity. Glands were closer in some areas but at normal distance to each other in other areas, and the endometrial cells had midproliferative morphology. Multinucleated giant cells were seen in one case of proliferative endometrium that had marked leukocytic infiltration; hence, the multinucleated cells may be multinucleated macrophages. Multinucleated giant cells are reported to be seen in atrophic endometrium.

Glandular or stromal mitoses were not seen in any of the proliferative smears. Nuclear features were not of much help in differentiating proliferative, secretory and hyperplastic patterns. Skaarland has also commented on the inability to diagnose hyperplasia based on nuclear characteristics.

Two studies of cytology on DUB found in the literature after extensive Internet search of Hemlatha et al. and Malik et al. (60 and 100 cases, respectively) have shown that it is possible to assign categories like secretory, non-secretory, proliferative endometrium and hyperplasia. They had correlation rates of 95% and sensitivity and specificity of 83% and 95%. We could also assign histopathology-like categories to most of our reportable cytology smears.

The advantage of endometrial imprint cytology is that because the diagnosis is available within 6 h, the patient can be relieved of anxiety on the same day and appropriate treatment can be started on the same day. In case different information like hyperplasia is thrown up by histopathology, the treatment may be modified in the patient’s next Outpatient Department visit. This is particularly helpful to decrease anxiety and morbidity by 3-7 days, especially if the patient is having continuous bleeding.

Conclusion

Histopathology-like categories can be assigned in the diagnosis of DUB by imprint cytology. It is possible to improve the sensitivity and specificity with better imprinting techniques. Thus, cytology can be used in the diagnosis of DUB in community health hospitals and smaller laboratories of India where fully established histopathology services are not available. It can also be used in well-established institutions to decrease the diagnostic time in DUB cases.

References

1. Polson DW, Morse A, Beard RW. An alternative to the diagnostic dilatation and curettage — endometrial cytology. Br Med J (Clin Res Ed) 1984;288:981-3.

2. Skaarland E. New concept in diagnostic endometrial cytology: Diagnostic criteria based on composition and architecture of large tissue fragments in smears. J Clin Pathol 1986;39:36-43.

3. An-Foraker SH, Kawada CY, McKinney D. Endometrial aspiration studies on Isaacs cell sampler with cytohistologic correlation. Acta Cytol 1979;23:303-8.

4. Liza Sister, Rameshkumar K, Lillian Sister. Value of endometrial aspiration cytology in assessing endometrial status in symptomatic peri and postmenopausal women. Indian J Cancer 1999;36:57-61.

5. Malik R, Agarwal R, Tandon P. Cytological assessment of endometrial washings obtained with an insemination cannula and its histological correlation. J Cytol 2008;25:128-32.

6. Konstantinos K, Marios S, Anna M, Nikolaos K, Efstratios P, Paulina A. Expression o6ki-67 as proliferation biomarker in imprint smears of endometrial carcinoma. Diagn Cytopathol 2013;41:212-7.

7. Hemalatha AN, Pai MR, Raghuveer CV. Endometrial aspiration cytology in dysfunctional uterine bleeding. Indian J Pathol Microbiol 2006;49:214-7.
8. Koss LG. Proliferative disorders and carcinoma of endometrium. In: Koss LG, Melamed MR, editors. Koss' Diagnostic Cytology and its Histopathologic Basis. 5th ed. Philadelphia: Lipincott Williams & Wilkins; 2006. p. 422-65.

9. McKenzie PR, Watson GF, Ng ABP. Cytology of body of uterus. In: Gray W, Mckee GT, editors. Diagnostic Cytopathology. 2nd ed. London: Churchill Livingstone; 2003. p. 821-46.

10. Bistoletti P, Hjerpe A. Routine use of endometrial cytology in clinical practice. Acta Cytol 1993;37:867-70.

11. Kawana K, Yamada M, Jimbo H, Shirai T, Takahasi M, Sano Y, et al. Diagnostic usefulness of endometrial aspiration cytology for endometrial cancer cases with normal curettage findings. Acta Cytol 2005;49:507-12.

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