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Research Article

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Recognizing Histological Patterns in the Endometrial Scrape Cytology of Hysterectomy Specimens

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ABSTRACT

Background: There is an absence of standardized diagnostic criteria for endometrial cytology. **Aims**: This study aims at recognizing various patterns seen in endometrial scrape cytology from hysterectomy specimens against histopathological features. The dependability of these patterns for accurate cytological diagnosis of endometrial pathology was assessed. **Materials and methods**: 106 uteri in two separate batches of 50 and 56 received consecutively in surgical pathology laboratory were grossed and their features documented. Each endometrium was scraped by a scalpel, subsequently two H&E and two PAP smears were prepared. Inadequate smears and specimens which were received cut-opened were excluded. The cytological findings were broadly categorized into benign endometrium (including phases of menstrual cycle, endometritis and atrophic endometrium), decidual change, pill endometrium, endometrial hyperplasia, suspicious of malignancy and malignancy. Sensitivity, specificity, likelihood ratio, pre-test odds, post-test odds and diagnostic odds ratio were calculated for hyperplasia/malignancy and only malignancy. **Results**: The cytological evaluation for endometrial malignancy showed sensitivity, specificity of 66.7% and 99% respectively. The pre-test odds were 3%. For positive results, the likelihood ratio and the post-test odds were 69 and 67% respectively. The likelihood ratio was 0.34 with a post-test odds of 1% for negative results. The diagnostic odds ratio was 204. **Conclusions**: Endometrial hyperplasia was missed in cytology because the cellularity in comparison to benign endometrium was not different. Enlarged vesicular nuclei with prominent nucleoli were seen on review. One cytologic misdiagnosis of adenocarcinoma as benign endometrium was owing to low cellularity wherein the patterns were not seen.

Key Words: *cytological, patterns, endometrial, histopathology*

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INTRODUCTION

Hysteroscopy with directed biopsy is the 'gold standard' endometrial diagnostic procedure. Dilatation and Curettage or Examination Under General Anesthesia are cumbersome. Papanicolaou and Traut had reported that carcinoma of the uterine corpus is detectable by cytologically examining cervical and vaginal secretions. Various studies thereafter have supported this statement but also expressed markedly poorer accuracy in diagnosing endometrial adenocarcinoma than cervical squamous cell carcinoma[1]. It is difficult to distinguish between benign and malignant cells in the endometrium than in the cervix. Attaining well-preserved endometrial cells becomes necessary. There's no established diagnostic criteria but endometrial aspiration is uncomplicated with potential utility.

Materials and methods

This was a prospective study over a period of two years. 106 uteri in two separate batches of 50 and 56 received consecutively in the Surgical Pathology Laboratory under the Department of Pathology of a Tertiary Care Center were studied.

Inclusion Criteria: Consecutive uncut hysterectomy specimens sent to the Surgical Pathology Laboratory.

Exclusion Criteria: Hysterectomy uteri of women which have been cut and then sent for histopathological study and smears with inadequate cellularity.

The Co-investigators (Co-I and Co-II) documented all the clinical details like age, last menstrual period, duration and/or severity of the presenting complaints and USG pelvis findings wherever available.

All consecutive uteri (n=106) were cut; uterine size and endometrial thickness were measured by the Co-I and/or Co-II under the guidance of the Principal investigator (P-I). Other gross findings like thin slit-like or dilated endometrial cavity, any polyp or endometrial growth were documented by the P-I.

Endometrium of each specimen was scraped by scalpel and four smears were prepared from the material on the scalpel. The smears were fixed immediately in 95% ethyl alcohol followed by staining with Haematoxylin and Eosin (H&E), and Papanicolaou (PAP) stain. The staining procedure was done by lab technicians. Reading, evaluation and reporting was done by the P-I with simultaneous teaching of the Co-I and Co-II.

The smears were evaluated and reported as benign (proliferative/ secretory phase/ atrophic) endometrium, inflamed endometrium, endometrial hyperplasia or adenocarcinoma. The cytomorphological criteria used in various conditions of the endometrium were as given by Shu et al[2] and Meisels et al[3]. These findings were correlated with the subsequent histopathological results. Final clinicopathologic and histopathologic correlation were performed.

Documentation of the relevant details was done by the Co-I and/or Co-II. Data analysis was done by the Co-I and conclusions drawn by the P-I.

Histological patterns of endometrium expected on cytology: [2,3]

1. Benign endometrium- proliferative phase: Uniform sized endometrial cells are present in sheets or clusters with regular, round, darkly stained nuclei showing uniform chromatin pattern and a thin rim of cyanophilic cytoplasm.
2. Benign endometrium- secretory phase: The cells are present in sheets with vacuolated cytoplasm and slightly vesicular nuclei forming a honey-comb pattern.
3. Atrophic endometrium: Here, cellular sheets are rarely seen. The nuclei are small and darkly stained with scanty cytoplasm.
4. Decidual changes: The decidual cells are round to polygonal with abundant eosinophilic cytoplasm and sharp distinct borders. The centrally placed nucleus is enlarged with a round to oval shape and has vesicular chromatin with a prominent nucleolus.
5. Endometrial hyperplasia: There is overlapping of cells in glandular clusters or sheets, presence of nucleoli, anisokaryosis, granularity of chromatin and presence of stromal cells.
6. Malignancy: Smears show increased cellularity with crowding, disorganization and variation of cellular and nuclear sizes in sheets of cells, extreme variation of shape and size of cells, increased nuclear chromatic granularity, increased size, altered shape and increased number of nucleoli, abnormal mitotic figures, loss of cellular cohesiveness and cancer diathesis. Cells may be arranged in a papillary pattern.
7. Endometritis: There are clusters of cells and presence of neutrophils, lymphocytes and plasma cells.

The various cytological features were noted down in each case. The cytological findings were broadly categorized into benign endometrium (including the phases of menstrual cycle, endometritis and atrophic endometrium), decidual change, pill endometrium, endometrial hyperplasia, suspicious of malignancy and malignancy. The cytological results were compared with the histopathological diagnosis. Sensitivity, specificity, likelihood ratio, pre-test odds, post-test odds and diagnostic odds ratio were calculated for hyperplasia/malignancy and also for malignancy separately.

Results

Differentiating proliferative from secretory endometrium was difficult, so calling them as benign endometrium was sufficient. These smears showed benign tall columnar epithelial cells arranged in either clusters, monolayered sheets, as glands or isolated. Mild anisonucleosis was seen in a few smears, of which most were in the proliferative phase, but this feature was non-specific and not indicative of any frank pathology.

No specific features indicated toward atrophy and like other benign endometrium, cellularity was variable. Therefore, these cases were also included under benign endometrium.

Consequently, a total of 86 cases were designated as benign endometrium after cytological evaluation. Two cases were false positive for endometrial hyperplasia and one false positive as highly suspicious of malignancy. Four cases were misdiagnosed and included under benign endometrium.

There were three cases of endometrium undergoing decidual change, of which the first case encountered was false negative and cytological impression was that of benign endometrium, but on review decidual cells were identified. The next two cases were accurately identified on cytology and correlated with histopathology and clinical history of obstetric hysterectomy done for intractable postpartum bleeding. Smears in these cases were blood-mixed. The microscopy showed plenty of RBCs with scant to moderate cellularity. There were few isolated to tiny clusters of characteristic large decidual cells with abundant cytoplasm and large vesicular nucleus. Few columnar epithelial cells were also seen.

Four out of five pill endometrium were true positives. After the first case was misdiagnosed as simple endometrial hyperplasia and then reviewed, a similar dilemma was not faced again. Pseudodecidual cells seen were intermediate to

large sized cells, arranged in monolayered sheets or isolated, containing moderate cytoplasm with a large nucleus and well-defined cell border. Benign tall columnar cells were also seen. The smears also showed lymphocytes and neutrophils in the background.

Two out of two cases of endometrial hyperplasia were missed in cytology because there was no difference in cellularity in cases of benign or hyperplastic endometrium. Cellularity was moderate in these cases. The smears showed benign tall columnar epithelial cells in clusters, sheets or isolated. There was mild anisonucleosis with some cells showing conspicuous nucleoli. Also seen were few apoptotic bodies in the cell clusters. A diagnosis of endometrial hyperplasia was not made on the basis of these findings but could possibly be considered cytological features seen in cases of endometrial hyperplasia.

Of the three cases of endometrial adenocarcinoma, two were true positives, including one case which was categorized as highly suspicious of malignancy. One of these cases, which was definitively adenocarcinoma on cytological examination, showed tumor cells arranged in clusters, glands, papillary pattern and isolated. Individual cells were columnar with high nuclear-cytoplasmic ratio, enlarged vesicula to hyperchromatic nuclei and moderate amount of cytoplasm. Background showed RBCs and tumor necrosis in the form of granular material and cells with pyknotic nuclei. Cellular features were similar when highly suspicious for malignancy, with an exception of lack of architectural pattern such as papillary pattern with doubtful tumor necrosis in the background. One case reported as benign endometrium on cytology turned out to be well-differentiated adenocarcinoma on histopathology. The reason for cytologic mis-diagnosis being scant cellularity (most probably due to inappropriate scraping) wherein the patterns were not seen.

Most of the patients were in the age group of 40 to 49 years (52.7%) followed by 30-39 years (24.5%). Majority of the patients had presented with the complaints of heavy menstrual bleeding, followed by abdominal pain and dysmenorrhoea. All the 5 cases of endometrial hyperplasia and adenocarcinoma were examined for AUB and were within the age group of 40-50 years.

The statistical calculations were carried out to measure how reliable specific cytological features could be in drawing inferences and assigning diagnoses for endometrial pathologies. It was not the method, of course, which was being tested. However, this method of sampling provided us with direct access to the endometrium. The cytological evaluation for endometrial hyperplasia/malignancy showed sensitivity and specificity of 40% and 97% respectively. The pre-test odds were at 5%. For positive results, the likelihood ratio and the post-test odds were 13 (95% CI: 2.87, 63) and 39% (95% CI: 12%, 76%) respectively. Meanwhile, the likelihood ratio was 0.62 (95% CI: 0.30, 1.27) with a post-test odds of 3% (95% CI: 1%, 6%) for negative results. The diagnostic odds ratio was 21.7 (95% CI: 2.5977 TO 182.5767) with a significance level (P) of 0.0045.

The sensitivity and specificity improved to 66.7% and 99% when only malignant cases were considered. Pre-test odds were calculated to be 3% with positive likelihood ratio and post-test odds as 69 (95% CI: 8.34, 565) and 67% (95% CI: 20, 94) respectively. When negative for malignancy, the likelihood ratio was 0.34 (95% CI: 0.07, 1.67) and post-test odds was 1% (95% CI: 0%, 5%). Hence, the diagnostic odds ratio for malignancy was 204 (95% CI: 9.1433, 4551.5481) with a significance level (P) of 0.0008.

DISCUSSION

In this study, we evaluated if specific cytologic features were associated with certain endometrial pathologies and if they can be useful diagnostic guidance, especially for endometrial hyperplasia and malignancy.

Endometrial malignancies are accountable for nearly 50% of all new diagnoses of gynecological cancers making it the most prevalent cancer of the female genital tract, especially in developed countries[4,5]. Unfortunately, for endometrial cytology, there is an absence of an internationally accepted system for reporting. Endometrial cytology examination could be an unavoidable method for endometrial cancer screening or an adjuvant in the diagnostic workup. Follow-up with cytological studies can help monitor the status of the endometrium in women who receive hormone replacement therapy with higher risk of developing endometrial cancer[6,7,8]. Patient survival can also be enhanced by earlier detection of this tumor[9].

Endometrial sampling in this study was done by taking scrapings from hysterectomy specimens received in an uncut state. The endometrium hence was not exposed to formalin or other fixatives and the cytological features were preserved. Many investigators have tested the accuracy of cytological techniques using various instruments. Adequacy rates were found to be ranging from 59-100%, depending on the technique/device used for endometrial sample collection[6,10,11,12]. The sensitivity and specificity of the cytological results were independent of the method used, as inadequate samples were excluded from such studies[6].

The age of the patients ranged from 24 to 62 years in this study, of which 52.7% were in the 4th decade. All the cases of endometrial hyperplasia and adenocarcinoma were aged within 40-50 years with AUB as presentation. In 2010,

a retrospective study was carried out by Bhosale and Fonseca[13], wherein 112 perimenopausal women with abnormal uterine bleeding were in the age group of 40-52 years, and reported that 78.6% belonged to the age group of 40-49 years. In our study, 73% of women belonged to the age group of 40-49 years amongst perimenopausal women.

The sensitivity in our study was found to be 40% and the specificity was 97%. One of the limitations of this study was the small number of patients in the carcinoma/hyperplasia group. Hence, as the two cases of hyperplasia were missed, the resultant sensitivity was low. Wang et al[14], performed a meta-analysis of 4179 patients, wherein the pooled sensitivity and specificity of the cytological method in detecting endometrial atypical hyperplasia or cancer was 91% and 96% respectively. In a study by Meissels et al[3], five of the criteria described to provide an increased probability of correctly diagnosing endometrial hyperplasias on the cytologic sample were: the overlapping of cells in the glandular clusters or sheets, the presence of nucleoli, anisokaryosis, granularity of chromatin and the presence of sheets of stromal cells. The chances of identifying endometrial hyperplasia in the Endopap sample increases as more of these criteria are met. The two cases of simple endometrial hyperplasia which were diagnosed as benign endometrium owe the discrepancy to there being no difference in cellularity with benign endometrium and smears consisting majorly of benign tall columnar epithelial cells without any obvious overlapping. Anisonucleosis was mild and nucleoli was also seen in a few cells which did not seem remarkable enough until a retrospective review was done. But these features were also seen in a few cases of proliferative endometrium. As stated by Kashimura et al[15], these features varied in the different types of endometrial hyperplasia and that it is not clear that these cytologic findings can be used to cytologically diagnose endometrial hyperplasia because some of the findings involve subjective judgments by different cytologists.

For endometrial adenocarcinoma, smears showed tumor cells with high nuclear-cytoplasmic ratio, enlarged hyperchromatic nuclei and moderate amount of cytoplasm. When the papillary pattern and necrotic background were seen, it was diagnosed as endometrial adenocarcinoma (Figure 1a and b); but in their absence, it was considered as highly suspicious of malignancy. One case misdiagnosed as benign endometrium was owing to probable sampling error whereby cells were few and did not show features of malignancy. When sensitivity and specificity were calculated for only endometrial adenocarcinoma, the values were 66.7% and 100% respectively. So, though the results for endometrial hyperplasia was unsatisfactory, it is beneficial to utilize cytological methods to screen for malignancy. However, as there were only 3 cases of carcinoma, these results should be confirmed from studies with larger samples.

Endometrial dating was not deemed mandatory and hence both proliferative and secretory endometrium were simply diagnosed as benign endometrium in cytology. The cytological features such as vacuolated cytoplasm and vesicular nuclei were not exclusively seen in cases of secretory endometrium, nor was there any feature specific to proliferative endometrium which were seen. However, on review, few of the smears of proliferative endometrium showed epithelial cells with mild anisonucleosis and presence of nucleoli. One of the smears also showed the finger-in-glove pattern (Figure 2a) which is considered characteristic of proliferative endometrium[16] with glandular cells arranged in straight or twisted tubular structures, resembling glove fingers, with irregular shearing at the ends. But Norimatsu et al[17], described finger-in-glove patterns in endometrial hyperplasia. Only occasional cases of secretory endometrium showed stromal cells in streaming pattern (Figure 3a) and hence could not be relied upon for endometrial dating.

The cases of atrophic endometrium were not segregated from those experiencing the menstrual cycle because in the vast majority of cases, the cellularity was not compromised and the epithelial cells showed similar features as those categorized under benign endometrium (Figure 2). While the expectation was to diagnose atrophic endometrium on the basis of features such as low-columnar to cuboidal cells with small and darkly stained nucleus and scant cytoplasm, these were seen only in two out of the 16 cases of atrophic endometrium.

Pseudodecidual cells were intermediate to large sized with large nucleus, abundant cytoplasm and well-defined cell border which was missed only in the first case we came across (Figure 3b and c). Presence of lymphocytes and neutrophils in the background was commonly seen in these smears. The cytological diagnosis correlated well with histopathology and history of being on oral contraceptive pills.

For obstetric hysterectomy, identifying the decidual cells was easy after reviewing the first encountered case. The cytological features of the cells correlated well with histopathology, showing large cells with abundant cytoplasm, large vesicular nuclei and abundant RBCs in the background (Figure 3c).

Failure of distinction of simple endometrial hyperplasia from benign endometrium was of concern. Even though the cases of simple endometrial hyperplasia showed epithelial cells with mild anisonucleosis and conspicuous nucleoli, similar features were also seen in a few proliferative endometrium. Atypical endometrial hyperplasia was not encountered during this study. Cases of malignancy, however, can be quite accurately detected on cytology, provided a good cellularity is attained. As per the calculations in our study, the odds of having malignancy after a positive test is increased by two times or about 1 in 1.5 with a positive test are likely to have the disease and almost each individual with a negative test can be ruled out for malignancy. Cytological screening is encouraged and is currently the commonest test for an initial evaluation of endometrial cancer in Japan, especially for women at high risk[18,19]. In a study by Tesuo

Kuroishi et al[20], the percent reduction in the average age-adjusted rate of PYLL (Potential Years of Life Lost) due to uterine cancer and the years of life saved per 100,000 females were found to be greater in the high coverage-rate areas than in the control areas. The results suggest that years of life have been saved by mass screening programmes for uterine cancer.

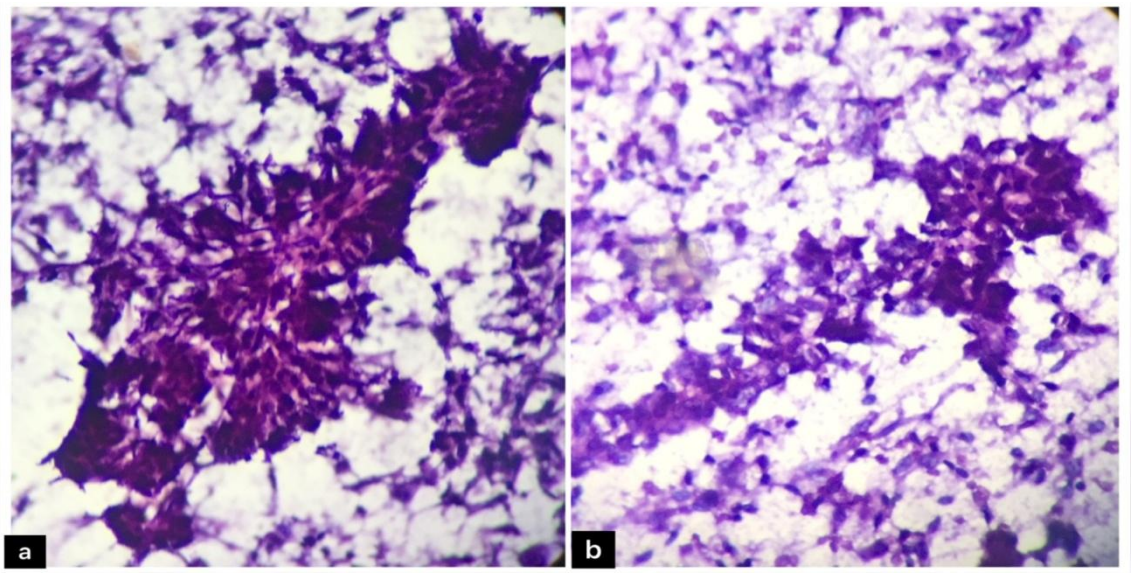

Figure 1 (a) and (b): Tumor cells arranged in papillary pattern with central fibrovascular core in a case of endometrioid adenocarcinoma [PAP 400x].

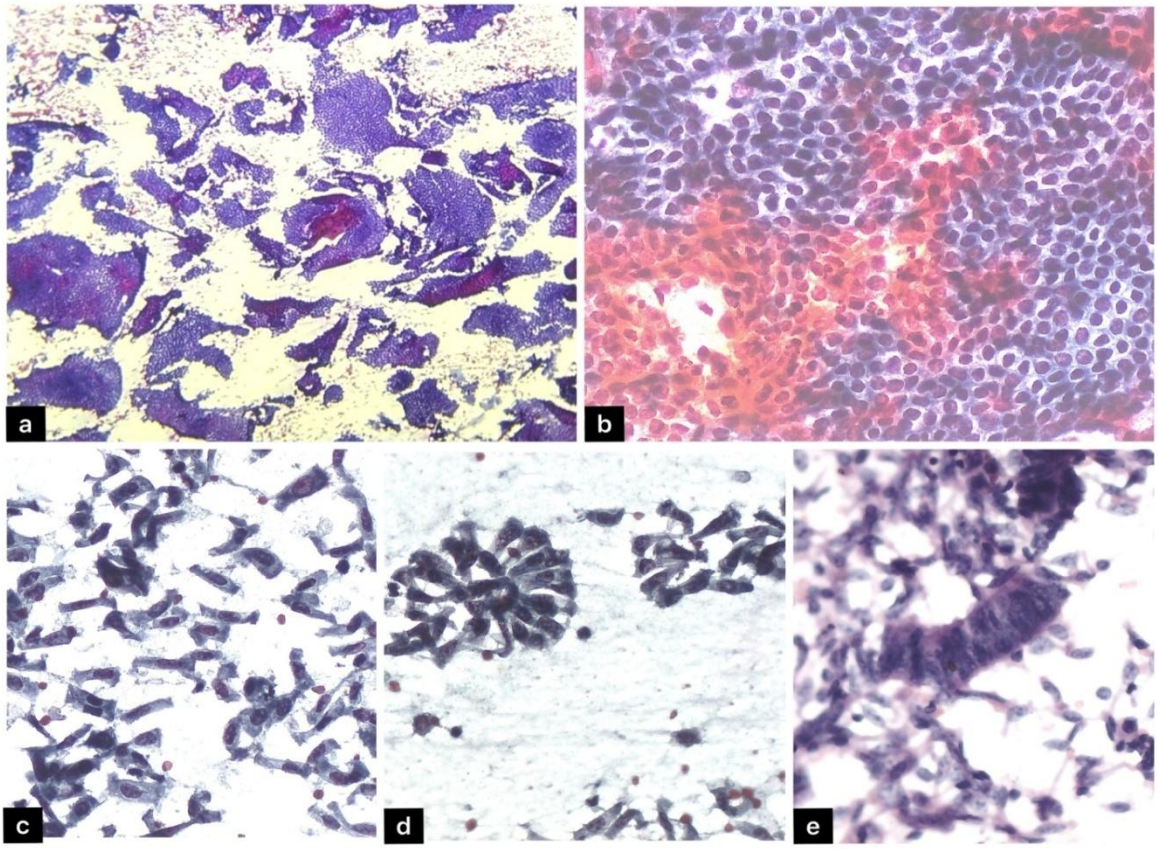

Figure 2: Benign endometrial epithelial cells. **(a)** Finger-in-glove pattern as seen in the proliferative phase [PAP 40x]. **(b)** Arrangement in monolayered sheet, **(c)** isolated tall columnar cells, **(d)** glandular pattern, and **(e)** picket fence pattern [PAP 400x].

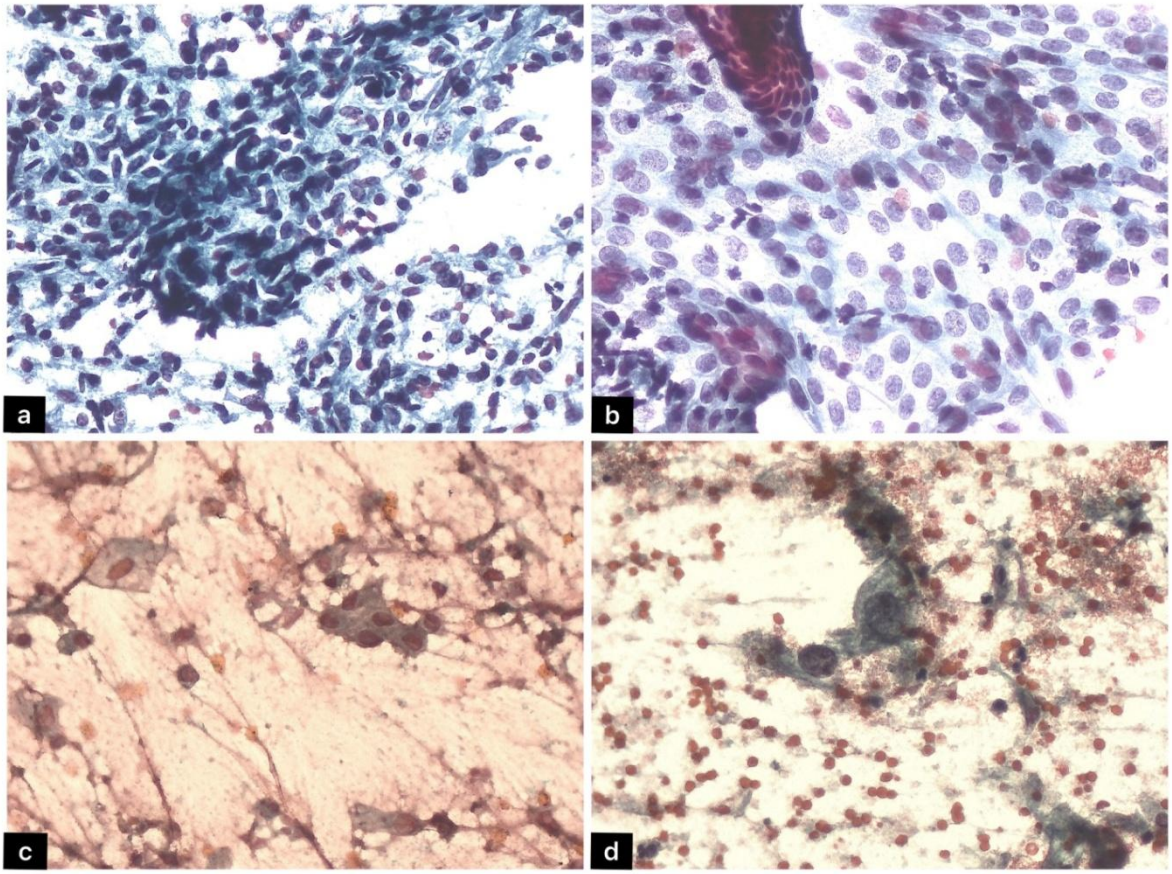

Figure 3: Benign endometrial stromal cells [PAP 400x]. **(a)** Streaming effect as seen in secretory endometrium, **(b)**monolayered sheet of pseudodecidual cells in a 'pill endometrium' showing many mitosis. **(c)** Pseudodecidual cells. **(d)** Decidual cells seen in obstetric hysterectomy.

CONCLUSION

Endometrial cytology is inexpensive and non-invasive which makes it a very good screening tool for endometrial malignancy on an out-patient basis. The diagnostic accuracy of endometrial carcinoma can be improved by the combination of histopathology, cytology and ultrasonography. Various studies have also supported its significant role in ruling out malignancy in women who are at high risk and improving survival in those detected early.

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