RESEARCH ARTICLE

Pap Smears in Endometrial Adenocarcinoma: Does It Have a Role?

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Abstract

Background: Historically the conventional Pap smear has been an effective screening tool for Carcinoma cervix. However the reporting of benign and malignant endometrial cells on Pap smear has remained controversial. There are very few studies addressing the utility of Pap smears in diagnosing endometrial carcinomas. **Objective:** To rescreen the Pap smears of patients with endometrial adenocarcinoma for the detection of normal/ atypical endometrial cells and to correlate with clinico-pathological parameters. **Methods:** The present study was carried out at SDM College of Medical Sciences, Dharwad, Karnataka, India for a period of 7 years. Of the 89 endometrial cancers diagnosed, Pap smear slides were available in 32 patients, which were reviewed and classified as normal cells/ atypical cells. Corresponding biopsy slides were reviewed for tumour type, nuclear grade, myometrial invasion and stage. Statistical tests of independence were applied for selected clinico-pathological parameters. **Results:** Cervical cytology was normal in nine patients (28.1%) and atypical in 23 patients (71.2%). The most common histological type was endometrioid carcinoma in 27 cases (84%). 13 cases(40.5%) had nuclear grade 1 whereas 9 cases(28%) and 10 cases(31.2%) had grades 2 and 3, respectively. Of the 22 cases assessed for invasion and stage, 12 cases had <1/2 of invasion (41.2%) and remaining 10 cases had >1/2 of invasion (58.8%). Early stages (I and II) had 17 cases (77.27%) and advanced stage (III and IV)had 5 cases (23.5 %). Significant correlation was found between post-menopausal status and higher nuclear grades (p<0.05).**Conclusion:** Pap smear is primarily a screening test for squamous cell carcinoma cervix. If atypical glandular cells are seen, further investigations are required to rule out neoplasia. All women with atypical endometrial cells on Pap tests need endometrial sampling irrespective of age/menstrual status.

Keywords: Pap smear- endometrial cancer- histological type- nuclear grade

Introduction

The incidence of endometrial cancers varies throughout the world with the rates being higher in developed countries while the developing countries share the lower burden of disease (Kurman et al., 2011). According to WHO, endometrial cancers are the most common female genital tract malignancy in the developed countries accounting 12.9/100,000 women (Tangjitgamol et al., 2010). However, this frequency is comparatively lower in India with approximately 4.3/100,000 women (Balsubramaniam et al., 2013).

The mortality rates of endometrial cancer appear to be quite high which demands its early detection (Kurman et al., 2011). However, till date no standard screening tests are available for the early detection of endometrial cancers. Pap tests are used since ages for the screening of premalignant and malignant lesions of the cervix (Thrall et al., 2005). The Bethesda system recommends to report the benign appearing endometrial cells in all women above 40 years of age irrespective of menopausal status

and in 2014, Bethesda increased the age of reporting to 45 years (Browne et al., 2005; Nair and Wilbur., 2015). Exfoliated endometrial cells can be normally seen during menstruation and proliferative phases but the presence of these cells in postmenopausal women requires further evaluation (Simsir et al., 2005). Implementation of these guidelines of reporting endometrial cells helped in increase of the detection rate of endometrial cancer. However, the protective effects of Pap smears to detect endometrial carcinoma is significantly less as compared to cervical cancer as atypical endometrial cells can be detected only in a subset of patients with endometrial carcinomas (Lai et al., 2015).

Very few studies are available addressing Pap smears in the early detection of endometrial carcinomas. Hence, the present retrospective study was undertaken at the Department of Pathology of SDM College of Medical Sciences and Hospital, Dharwad, Karnataka, India with the following objectives - i. To rescreen the Pap smears of patients already diagnosed with endometrial adenocarcinoma for the detection of normal/ atypical

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endometrial cells and ii. To correlate atypical endometrial cells with clinical parameters, histopathological grade and stage, wherever possible.

Materials and Methods

It is a retrospective study of 7 years duration carried out between 2008 and 2015. All the Pap smear slides of patients diagnosed with endometrial carcinoma on biopsy were retrieved from the archives of Pathology. The relevant clinical data such as menopausal status and the presenting complaints were collected from the medical records section.

All the Pap smear slides of 32 cases diagnosed with endometrial cancer were reviewed thoroughly by two pathologists for the presence of endometrial cells and classified into two categories as normal cells and atypical cells. Simultaneously, the haematoxylin and eosin slides of the endometrial biopsies and radical specimens were retrieved. Biopsy slides were reviewed for tumour type, nuclear grade, myometrial invasion and stage, wherever possible.

Statistical methods

Statistical tests for independence of association were carried-out between the normal/atypical cytology on Pap smears with the clinico-pathological parameters such as menopausal status, histological type, nuclear grade, myometrial invasion and stage wherever possible. The data was entered in Microsoft Office 2007 Excel worksheet and statistical analysis was calculated. Descriptive statistics were applied and results were expressed in percentage. Social science statistics website was used (www. socscistatistics.com). Significance test was calculated using Chi Square calculator 2x2 contingency table. The Chi Square statistic and p value was calculated. The statistical classes having zero values were subjected for tests of significance applying Yates correction and then the p value was calculated. p value <0.05 was considered significant.

Results

A total of 89 cases of endometrial carcinomas were diagnosed during the study period. Of the 89 cases, Pap smears were available in 32 cases. Radical surgery was performed in 22 cases. The patients were in the age group of 55 and 70 years (mean age-40 years). With respect to menopausal status, 10 patients (31.2%) were premenopausal who presented with heavy menstrual bleed and 22 patients (68.8%) were post-menopausal who presented with postmenopausal bleed.

The cervical cytology was normal in 9 patients (28.1%) and atypical in 23 patients (71.2%) (Table 1). The evaluation of nuclear grade indicated that as many as 13 cases (40.5%) had low nuclear grade whereas 19 cases (59.2%) had high nuclear grade (Table 1). The most common histological type was endometrioid which was found in 27 cases (84%). Other 5 cases (2 papillary serous, 2 clear cell and 1 malignant mixed mullerian tumour) were of different histologic type (13.6%). Myometrial

invasion and staging were assessed in radical hysterectomy cases only. Of the 22 cases assessed, 12 cases had <1/2 of invasion to the extent of 41.2% and remaining 10 cases had >1/2 of invasion (58.8%). Surgical staging was divided into early and advanced stage. Early stage which included Stages I and II had 17 cases (77.27%) and advanced stage that included Stages III and IV had 5 cases (23.5 %) (Table 1). Further correlation was done between the presence of endometrial cells (normal and atypical) and
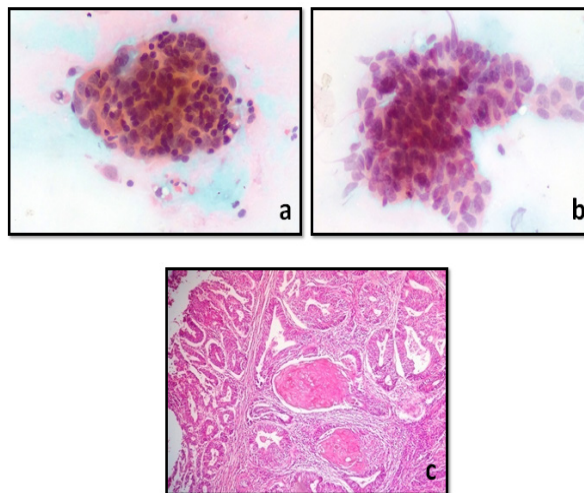

Figure 1. (a-c) Pap Smears Showing Tumour Cells Arranged in Clusters and Glandular Pattern Having Round to Oval Nucleus, Fine Granular Chromatin, Inconspicuous Nucleoli and Moderate Cytoplasm (a and b); Histopathology Shows Well Differentiated Endometrioid Adenocarcinoma with Squamous Morules (Low Grade) (c)

Table 1. Percentages of Pap Smear Results with Respect of Some Clinico-Pathological Parameters and Independence of Association among Them as Observed in Pap Smear Results

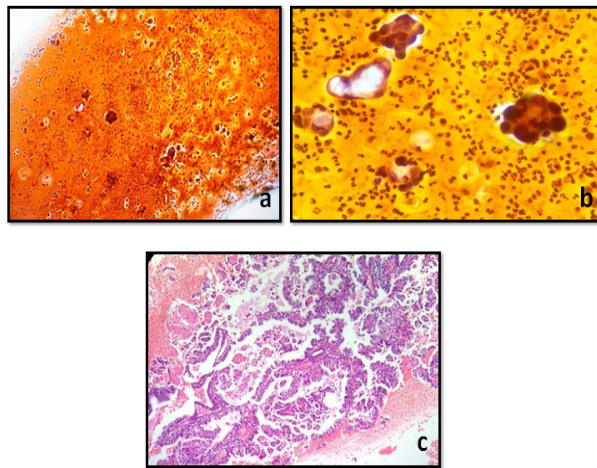| Clinico-pathological parameters	Pap smear results		
n=32	Normal	Atypical	p value
Menopausal status			
Pre-Menopausal	6(18.75%)	4(12.5%)	0.006
Post-Menopausal	3(9.37%)	19(59.37%)	
Histologic Type			
Endometrioid	9(33.3%)	18(66%)	0.17
Non-Endometrioid	0	5(100%)	
Nuclear Grade			
Low Grade	9(69.2%)	4(30.7%)	0.001
High Grade	0	19(100%)	
n=22			
Myometrial Invasion			
<1/2	3(25.1%)	9(74.9%)	0.78
>1/2	2(20%)	8(80%)	
Surgical Stage			
Early	5(29.4%)	12(70.6%)	>0.05
Advanced	0	5(99.9%)	

Figure 2. (a-c) Pap Smears Showing Tumour Cells Arranged in Tight 3D Clusters, Papillae and Singles Having Vesicular Nucleus, Prominent Nucleoli and Moderate amount of Cytoplasm with Cytoplasmic Vacuoles. Background Showed Haemorrhagic Diathesis (a and b); Histopathology – Serous Carcinoma (High Grade)(c)
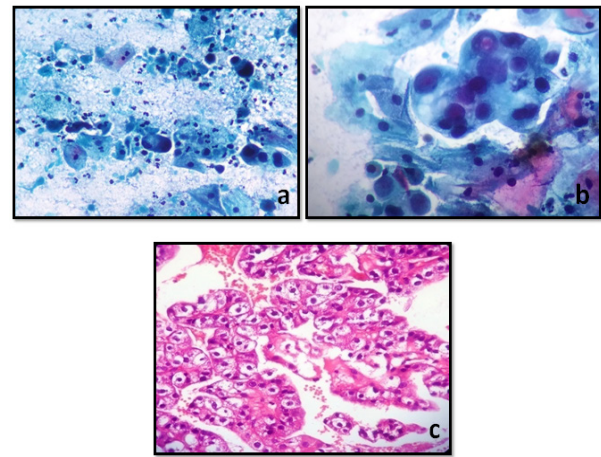

Figure 3. (a-c) Pap Smears Showing Tumour Cells Arranged in Singles and Clusters Having Pleomorphic Vesicular Nucleus, Prominent Nucleoli and Abundant Clear Cytoplasm (a and b); Histopathology – Clear Cell Carcinoma (High Grade) (c)

the histological type, nuclear grade, myometrial invasion & surgical stage with the calculation of p value for each of them (Table 1).

With respect of independence of association between different classes of clinico-pathological parameters, correlation was found significant for postmenopausal status and higher nuclear grades (p<0.05).

Discussion

Cervical cancer shares the major cancer burden in the world and more prevalent in developing countries like India. It is also a commonest cause of death among women in developing countries like in India with the death rates recorded to be as high as 67477 in 122844 i.e. approximately 1 in 2 women diagnosed with cervical cancer (Sreedevi et al., 2015). The morbidity and mortality due to cervical cancer has come down drastically in countries with well established screening programs at national level (Kulkarni et al., 2013).

It is an established fact that extensive use of routine screening by Pap smears has dramatically reduced the number of cervical cancers in the 20th century (Cibas ., 2009).

In the aftermath of the success of cervical cancer screening, many methods have been suggested for the early detection of endometrial cancers which are showing a rising trend. Till date, attempts are being made for the

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Table 2. Comparative Results of the Tests of Independence of Clinico-Pathological Parameters of Different Authors with the Results of Present Study

Authors	Histologic type (p value)	Nuclear grade (p value)	Myometrial invasion (p value)	Surgical stage (p value)
Fukuda et al (1999)	0.01	0.012	0.001	0.001
DuBeshter et al (1991)	-	0.006	0.011	0.024
Larson et al (1994)	0.017	0.001	-	0.035
Gu et al (2001)	-	0.01	0.27	0.01
Shin et al (2009)	0.029	0.006	0.026	0.029
Lai et al (2015)	0.19	0.095	-	0.29
Our study (2015)	0.17	0.00106	0.78	0.167

early diagnosis of endometrial carcinoma through Pap smears by detecting atypical endometrial cells. However, its role in detecting atypical endometrial cells is not yet well established. In our study we have re-screened the Pap smears for the detection of normal/atypical endometrial cells in the diagnosed cases of endometrial cancers and to check the utility of Pap smears in the diagnosis of endometrial cancer.

The constant growth and disintegration of the endometrium in the menstruating women does not favor the neoplastic process. Hence the most common age group for endometrial cancer is the post-menopausal period. This was supported by our observation with a significant p value of postmenopausal patients ($p<0.05$) for the detection of atypical cells on Pap smears and endometrial cancer. Few earlier studies also indicated positive correlation of postmenopausal advancing age with endometrial pathology (Zucker et al., 1985; DuBeshter et al.,1991; Koss, 2006)

Endometrial cells detected in the Pap smears can be normal/ atypical. Normal endometrial cells are shed and can be detected in the cervical smears up to day 14 of the cycle. The various benign conditions which are associated with the presence of endometrial cells even beyond 14 days are simple hyperplasia, endometrial polyps, IUCD and hormonal medications (Greenspan et al., 2006). It is important to differentiate typical from the atypical endometrial cells which are small rounded cells which occur in balls and moulded groups with pale chromatin, conspicuous nucleoli and vacuolar, basophilic cytoplasm (DeMay, 2012). Gu et al., (2000) reported that sensitivity of detecting atypical endometrial cells in Pap smears ranged from 40 to 70% whereas the detection of endometrial neoplasia was 70% in another study (Raab., 2000). The cervical smears of the fully developed carcinoma are characterized by presence of inflammation, necrotic material and the fresh/old blood along with leukocytes and macrophages having delicate vacuolated cytoplasm and eccentric kidney shaped nucleus. Malignant cells are likely to be missed unless carefully screened and hence it is very challenging to identify the cells in well differentiated tumors occurring in singles or clusters with small size and small visible nucleoli. Low grade tumours show mild atypia which is difficult to differentiate from benign endometrial cells. Also the cells in these tumours are not readily exfoliated from the endometrium.

The detection rate of atypical endometrial cells in Pap smears of patients of endometrial carcinoma was 31.3% in one of the studies (Fukuda et al., 1999). Similar detection rate of 29.9% was seen in the study done by Larson et al. (1994). However, a higher detection rate was found to the extent of 50% and 38% in the studies of DuBeshter et al. (1991) and Shin et al (2009), respectively. In our study, the detection rate of atypical endometrial cells in Pap smears was 70%. In the present study, 13 cases were of low grade with 9 of them having normal endometrial cells and only 4 cases (30.7%) having atypical endometrial cells in Pap smears (Figure 1a and b). High grade lesions on the contrary could be easily picked up because of larger cell size and more prominent nucleoli (Koss ., 2006). In this study, 19 cases were of high grade with all of them having atypical endometrial cells (100%) on Pap smears (Figure 2a&b and Figure3a &b). Identification of such high grade variants like papillary serous carcinoma is clinically valued for their aggressive course of disease (Park et al., 2005). In our study, 2 such cases of papillary serous carcinoma have been reported (Figure 2). In a study done by Li et al in 2012, who studied the utility of cervical cytology in detecting endometrial lesions, the background of degenerative endometrial debris and foamy histiocytes with the presence of atypical endometrial cells in Pap smears warranted a need for further evaluation by endometrial curettage (Li et al., 2012). In our study 70% of the cases had a hemorrhagic diathesis and 30% inflammatory background. We attribute the reason for this high detection rate in our study due to the following reasons:

i) Majority of the cases in our study were high grade (59%). High grade lesions are easily picked up because of larger cell size and more prominent nucleoli (Koss and Melamed, 2006).

ii) 100% of these high grade cases had endometrial cells shed in the Pap smears.

iii) Haemorrhagic diathesis was seen in the background of majority of the cases (70%), especially high grade lesions, which gives a clue to suspect and look for atypical cells.

The patients with atypical cervical cytology on Pap smears were found to have advanced FIGO stage, higher nuclear grade and deeper myometrial invasion (DuBeshter et al., 1991; Larson et al., 1994; Fukuda et al., 1999; Shin et al., 2009). Similar observations were seen in our study with the statistical correlation of atypical cervical cytology

with higher nuclear grade. However, because of the lower sample size the statistical test of independence was not significant for myometrial invasion and surgical stage. The Table 2 shows the comparative results of various studies in respect of statistical correlation of various histopathological characteristics.

Recent advances in this field of study and Scope for further study: In recent times, the traditional Pap smear has been replaced by a liquid based method with molecular assay. As an extension to the detection of atypical glandular cells in Pap smears, the detection of oncogenic DNA mutations characteristic of endometrial carcinoma from the DNA purified from routine liquid based Pap smears are more specific markers of neoplasia. In one study, presence of mutation was identified in all cases (100%) in Pap specimens from endometrial carcinoma patients thereby making this test (PAPgene test) to be more promising as a screening tool for endometrial cancer. The study observed that most commonly mutated genes included the phosphatidylinositol 3-kinase pathway genes PTEN and PIK3CA. It can also be used to monitor the response to hormonal therapy, monitor for local recurrence/progression of disease. Although molecular approaches are more promising, there is a need for validation of gene panel and standardization of technical procedures, for which large scale studies are required (Kinde et al., 2013).

Another study demonstrated the feasibility of detecting endometrial carcinoma via molecular analysis of DNA collected with vaginal tampons. They concluded that DNA hypermethylation in endometrial cancer tissues can also be identified in vaginal pool DNA collected via intravaginal tampon and proposed that refined collection methods are needed for early detection of endometrial cancers (Gamez et al., 2015).

Present Scenario in India, especially rural India: Although cervical cancer is preventable, there are no organized screening programs in any province or region of India. Resource constraint being the major hurdle in organizing them, thereby necessitating opportunistic screening. Similar cost considerations and lack of sophisticated laboratory infrastructures are the major setback for molecular testing in Indian setup (Senapathy et al., 2011; kulkarni et al., 2013).

Limitations of our study include smaller sample size, availability of only conventional Pap smear study without liquid based cytology and additional molecular studies, and lastly all the cases included in the study were biopsy proven cases of endometrial carcinoma.

Although the present study has shortcomings in terms of small sample size, the results are encouraging indicating application of Pap smears towards early detection of endometrial carcinoma which offers scope in handling large sample size through prospective study with follow up and biopsy correlation. Hence, endometrial curettage and biopsy are needed in elderly women with endometrial cells in cervical cytology.

It can thus be concluded that Pap smear is a primarily a screening test for Squamous cell carcinoma cervix. In the present Indian scenario, all women with atypical endometrial cells on Pap tests need further endometrial sampling irrespective of age/menstrual status for the early diagnosis and treatment.

Statement conflict of Interest
Authors declare that they have no conflict of interest.

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