Interpreting epithelial cell abnormalities detected during cervical smear screening – a cytohistologic approach

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Summary

Purpose: To determine the underlying pathology and clinical significance of epithelial cell abnormalities (ECA) identified during cervical Papanicolaou (Pap) smear screening.

Material and Methods: A total of 19,215 Pap smears stained by Papanicolaou stain were screened during a 36-month period. They were classified according to The Bethesda System (TBS) for cervical cytology screening and the results of ECA were compared with histology. The chi square test was applied to determine the significance and validity of high-grade lesions on cytology diagnosis.

Result: 360 cases of ECA were identified. Mean age at presentation was 50.58 years. Cytohistologic correlation of ECA was possible in n = 249 (69.17%) of cases. Out of these, 18 cases were negative, six were inflammatory, three were benign, and 222 cases showed pathology ranging from mild dysplasia to invasive carcinoma. The chi square test showed a highly significant predictive value ($p < 0.001$) for high-grade lesions detected on Pap smears.

Conclusion: Pap smear results of high-grade squamous intraepithelial lesions (HSIL) or invasive malignancy are highly reliable and the patient may be considered directly for therapeutic intervention. Of cytologically detected ASCUS cases 38.89% were diagnosed as CIN (1-3) on histology. Thus the management of these patients needs to be re-evaluated. Atypical glandular cells of undetermined significance (AGUS) detected in postmenopausal women signify an underlying pathology. These patients should be further investigated and followed to biopsy if necessary.

Key words: Epithelial cell abnormalities (ECA); Histology; Pap smear; TBS.

Introduction

The idea of screening for the early detection of cancer has gained wide-spread acceptance after the development of techniques of exfoliative cytology initiated by the pioneer work of Dr. George Papanicolaou in the year 1941 [1]. The main goal of any screening programme is to detect disease at the preinvasive stage to reduce the incidence and the mortality resulting from it. At present, the only cytological screening test that has been generally accepted as fulfilling these criteria is the cervical smear used for detecting preneoplastic lesions of the cervix [2].

Detection of preneoplastic lesions of the cervix and vagina by the use of Pap smears catapulted the obscure field of cytology into clinical practice. For effective communication between pathologists and clinicians, and to convey the diagnostic interpretation of the morphologic findings of the Pap smears, a uniform descriptive terminology for cervico-vaginal cytopathology was introduced in the form of The Bethesda System (TBS) [3]. TBS was developed in 1988, with minor modifications in 1991, which were based on actual laboratory and clinical experience after its implementation [4]. With utilization of new technologies and recent findings from research studies the Bethesda 2001 workshop was convened to evaluate and update the 1991 Bethesda System terminology [5]. The format of TBS report includes an evaluation of specimen adequacy and a descriptive diagnosis of the cellular changes detected on Pap smears.

Descriptive diagnoses specify the categories of benign cellular changes, epithelial cell abnormalities (ECA) and other malignant neoplasms. ECA that can be detected on Pap smears range from atypical squamous cells of undetermined significance (ASCUS) and atypical glandular cells of undetermined significance (AGUS) to invasive malignancy like squamous cell carcinoma (SCC), adenocarcinoma and other malignant neoplasm.

The correlation of the ECA detected in Pap smears with histological findings helps in achieving better diagnostic accuracy between cytopathologists, surgical pathologists and clinicians for optimal patient management. It also serves as an internal quality control [6]. The purpose of the current study was to determine the underlying pathology and clinical significance of ECA identified by cervical Pap smear screening.

Material and Methods

Pap smears from a total of 19,215 patients were evaluated over a 36-month period. The study was conducted at the cytology department of The Gujarat Cancer and Research Institute, a regional cancer referral center in West India. We received smears from patients referred to our hospital, from the cervical screening camps held by the hospital at different district levels and health check-ups conducted in the hospital itself.

All 19,215 smears were stained by Papanicolaou stain and were screened by a single group of cytotechnologists and

Revised manuscript accepted for publication July 1, 2004

pathologists by the criteria mentioned in TBS [7]. The pathologist would be responsible for the result signed out by the cytotechnologists. Any discrepancy in the cytological diagnosis between pathologists was reviewed and re-evaluated by a senior pathologist before a final diagnosis was given.

Out of these, 8,022 smears were within normal limits, 9,996 showed benign cellular changes, 837 were labeled as being unsatisfactory due to various reasons and 360 smears fell into the category of ECA consisting of ASCUS, AGUS, low-grade squamous intraepithelial lesions (LSIL), high-grade SIL (HSIL), squamous cell carcinoma (SCC), adenocarcinoma and other types of malignancies not otherwise specified (NOS) according to the criteria specified in TBS [7]. Among these 360 smears, 249 cases had colposcopic biopsies performed within three months after the cervical cytological diagnosis (Figure 1). A three-month interval was specified in order to obtain the most valid correlation between the cytological and histological diagnoses. Usually at our institute a Pap smear is taken following the first gynecology check-up. Further follow-up depends on the cytology report – either a regular follow-up or to be followed to colposcopy or colposcopic-guided biopsy or hormonal therapy. Colposcopic-guided biopsies were sent to the histopathology department and sections were stained with haematoxylin and eosin (three deeper sections as and when needed). An experienced gynecological histopathologist reviewed all the slides and the interpretation was considered as the final diagnosis. The histopathologist was not aware of the original cytological diagnosis. Histological diagnoses were classified as negative for malignancy, reactive or inflammatory change, cervical intraepithelial neoplasia (CIN) of grade I to III, squamous cell carcinoma (SCC), adenocarcinoma and other types of malignancy.

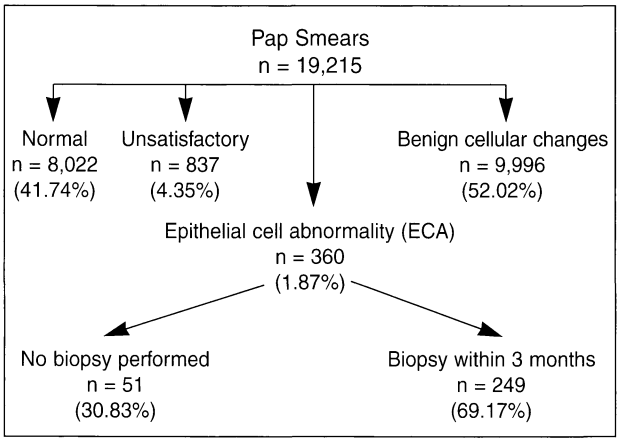

Figure 1. — Results of the Pap smears screened during the three-year period.

Statistical analysis:

All the smears were reported according to the criteria specified in TBS. However, for the purpose of application of the chi square test only, we divided ECA into two major categories as follows: the first category of high-grade lesions included HSIL, SCC and adenocarcinoma and the other category included ASCUS, AGUS and LSIL. The histological diagnoses were correlated with these two categories. The chi square test was applied and it showed a highly significant value ($p < 0.001$) for detecting high-grade lesions, a p value of 0.05 was considered significant.

Results

During the study period, 360 (1.87%) of the 19,215 smears were identified in the category of ECA, among which 279 (69.17%) women underwent colposcopic-guided biopsy, endocervical curettage and/ or diagnostic dilatation and curettage.

Patient age ranged from 25 years to 85 years with a median age of 50.58 years; 79% of patients were above 40 years of age (Table 1). Of the 360 smears with ECA, 30.83% showed ASCUS, 8.33% had AGUS, 4.17% had LSIL, 19.17% showed HSIL, 25% were labeled as SCC, 11.94% had adenocarcinoma and 0.56% had malignancy not otherwise specified (NOS) (Table 2). The ratio of SIL versus ASCUS in our study was 1:1.3 which does not fall into the expected range of 1:3 as suggested in TBS [7]. The reason for this difference is that our study was from a regional cancer referral center, thus the bulk of patients were those who had already been screened and referred to us on suspicion of malignancy.

Table 1. — *Distribution of epithelial cell abnormalities (ECA) according to age group (n = 360).*

Age (years)	No. of patients	Percentage
Below 30	12	3.33
31-40	66	18.33
41-50	144	40.00
51-60	66	18.33
61-70	51	14.17
71-80	15	4.17
Above 80	06	1.67

Table 2. — *Distribution of patients according to types of epithelial cell abnormalities (ECA) in line with TBS (n = 360).*

Types	No. of patients	Percentage
ASCUS	111	30.83
AGUS	30	8.33
LSIL	15	4.17
HSIL	69	19.17
SCC	90	25.00
Adenoca.	45	11.94
Malignant (NOS)	02	0.56

TBS: The Bethesda System (terminology). ASCUS: Atypical squamous cells of undetermined significance. AGUS: Atypical glandular cells of undetermined significance. LSIL: Low-grade squamous intraepithelial lesions. HSIL: High-grade squamous intraepithelial lesions. SCC: Squamous cell carcinoma. Adenoca.: Adenocarcinoma. Malignant (NOS): Malignancy not otherwise specified.

Histopathology of the followed-up cases (n = 249) revealed that 7.23% of these cases were negative for malignancy, 2.41% showed inflammation, 1.20% had benign endometrial hyperplasia, 3.61% showed CIN1 and CIN2 each, 6.02% had CIN3, 53.01% were labelled as SCC, 22.08% showed adenocarcinoma and 0.83% were small cell carcinoma (Table 3). The histopathology and cytology correlation of cases with ECA is shown in Table 4.

Table 3. — *Histological diagnosis of cases with epithelial cell abnormalities (ECA) on cytology (n = 249).*

Histological diagnosis	No. of patients	Percentage
Negative	18	07.23
Inflammatory	06	02.41
Benign	03	01.20
CIN1	09	03.61
CIN2	09	03.61
CIN3	15	06.02
SCC	132	53.01
Adenoca.	55	22.08
Small cell carcinoma	02	0.83

CIN: Cervical intraepithelial neoplasia. SCC: Squamous cell carcinoma. Adenoca.: Adenocarcinoma.

Table 4. — *Histocytologic correlation of cases with epithelial cell abnormalities (ECA) (n = 249).*

Cytology	Histology								
Types	Negative	Infla.	Benign	CIN1	CIN2 .	CIN3	SCC	Adeno.	Small.
ASCUS (54)	12	03	–	09	06	06	18	–	–
AGUS (21)	06	–	03	–	–	–	–	12	12
LSIL (09)	–	03	–	–	–	03	–	–	–
HSIL (51)	–	–	–	–	–	06	45	–	–
SCC (69)	–	–	–	–	–	–	69	–	–
Adenoca. (43)	–	–	–	–	–	–	–	45	45
Malig. (NOS) (02)	–	–	–	–	–	–	–	–	02

ASCUS: Atypical squamous cells of undetermined significance. AGUS: Atypical glandular cells of undetermined significance. LSIL: Low-grade squamous intraepithelial lesion. HSIL: High-grade squamous intraepithelial lesion. SCC: Squamous cell carcinoma. Adenoca.: Adenocarcinoma. CIN: Cervical intraepithelial neoplasia. Malig. (NOS): Malignancy not otherwise specified. Inflamm.: Inflammatory. Small.: small cell carcinoma.

Table 5. — *Age distribution of patients with atypical squamous cells of undetermined significance (ASCUS) on Pap smears (n = 111).*

Age (years)	No. of patients
21-30	6
31-40	24
41-50	42
51-60	18
61-70	15
71-80	6

Discussion

Carcinoma of cervix is the second most common malignancy among females in our country accounting for 19% of all malignancies [8]. The screening programme for carcinoma of the cervix has a significant advantage in terms of its anticipated outcome, because the natural history of the disease allows it to be detected at the preinvasive stage of dysplasia or carcinoma in situ. With appropriate treatment, progression to invasive cancer is prevented. This being the case, screening programmes can be expected to result in reduced incidence and mortality from cancer of the cervix.

The Bethesda system for reporting on cervical cytology was developed as a uniform system of terminology that would provide clear guidance for clinical management [3]. To validate the significance of cytological diagnosis and for quality control of the screening results, the biopsy report is considered to be the gold standard.

In this study ASCUS accounted for almost one-third (30.83%) of all patients of ECA. They were further classified according to TBS. The results were as follows. ASCUS favours reactive in 26.99% cases, ASCUS favours neoplastic in 5.45% cases, ASCUS not otherwise specified (NOS) in 67.56% cases. ASCUS was seen in patients with ages ranging from 25 to 75 years (mean age 48.1 years). Age distribution of patients with ASCUS is shown in Table 5. In our hospital, patients with ASCUS on Pap smears are reassessed clinically and some are given a course of either antibiotic or hormone therapies depending on the inflammatory or atrophic changes that are judged by the cytopathologist and gynecologist. After completion of therapy, a repeat Pap smear is done, and if necessary colposcopic and guided biopsy is carried out on subsequent follow-up. Thus in our patients with ASCUS, biopsies were done in only 48.64% of cases. Out of these 20.22% were normal, 5.56% were inflammatory, 16.67% had CIN1, 11.11% showed CIN2 and CIN3 each and SCC was seen in 33.3% of cases on histopathology (Table 4). In a study by Losuebsakul *et al.* [9] of 421 ASCUS cytological diagnoses, 13% were normal, 34% were reactive, 4.8% were atypical, 43% were LSIL and 4% were HSIL. The results of our study are congruent with those of Losuebsakul *et al.* and others, [9-11] where the percentage of squamous intraepithelial lesions (SILS) found in ASCUS cases ranged from 13.5% to 83.1%. In our study 38.89% (n = 21) of cytologically detected ASCUS cases were diagnosed as CIN, ranging from 1 to 3 on histology. We underdiagnosed 33.33% cases of invasive malignancy as ASCUS on Pap smears. We re-reviewed all these smears and possible causes of false negative diagnoses in these cases were scarcity of abnormal cells, with exuberant inflammation, which precluded optimal visualization of the cellular material. The diagnosis was attempted in these paucicellular smears following TBS suggestion that any epithelial abnormality must be reported regardless of specimen adequacy [7]. The percentage of SILs in ASCUS smears observed from the present and previous studies indicate that a significant proportion of ASCUS smears represent preneoplastic lesions [9-11].

Of our patients, 4.17% (n = 15) were categorized as having LSIL. Cervical biopsies were done in 60% of cases; 33.33% showed inflammation, 33.33% showed CIN2 and remaining had CIN3.

In our knowledge, this is the first study to provide biopsy follow-up of high grade and invasive lesions using the Bethesda System. Histological follow-up was available in 85.29% of our patients. Diagnostic accuracy of Pap smears for high-grade lesions was 100%.

In our study AGUS, using the Bethesda system, accounts for 8.33% (n = 30) of the total in which more than 70% of cases were in the postmenopausal age group. Cytohistologic correlations were possible in 70% of cases. Cervical smears with AGUS favouring endometrial cells were seen in six postmenopausal females of which

two had endometrial hyperplasia, one had complex endometrial hyperplasia with atypia and the rest showed endometrioid carcinoma of the uterus on histology. This states that biopsies should be performed in postmenopausal women diagnosed with AGUS favouring endometrial cells. Obenson *et al.* [12] drew a similar conclusion in a series of 30 cases of AGUS in postmenopausal women. Eighteen of our cases showed AGUS favours reactive changes. Six of them were negative for malignancy on histologic follow-up. Nine cases were diagnosed as AGUS, probably neoplastic, and turned out to be adenocarcinoma on biopsy. The study by Da Gloria *et al.* [13] concluded that the chance of finding SIL involving glands in AGUS smears was 5.32 times higher than in those with no AGUS. A larger series of cases with AGUS should be studied in this regard to give any definite conclusion.

Conclusion

The presence of high-grade lesions and invasive malignancies in cervical smears has a high predictive value ($p < 0.001$) suggesting that cervical smear cytology is a sufficient diagnostic modality for deciding the further management and treatment of these patients. The finding of ASCUS in this study supports the finding of other series – that a large proportion of smears diagnosed as ASCUS are really SIL [9-11]. Thus, the management of patients having ASCUS needs to be re-evaluated; they should be followed-up adequately to prevent the development to advanced lesions. Of cases diagnosed as having AGUS 71.43% showed abnormal histopathology. Hence it is advisable that all patients with AGUS should be followed-up by biopsy, especially postmenopausal women.

Acknowledgement

We are thankful to our Medical Registry Department for providing the data, to the Gynaecological Oncology Department for their therapy-related guidance and the staff members of the Cytology Department for their assistance.

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