

Research Article

Application of Bethesda system for conventional cervical cytology: a study of 340 cases

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ABSTRACT

Background: Cervical carcinoma is an important women's health problem worldwide. The Bethesda system is a complete diagnostic system that recognizes the cytopathology report as a medical consultation. The aim of this study is to evaluate the abnormal cytological entities detected by cervical Pap smear, to classify the cytological findings according to the recently recommended Bethesda system and to discuss the significance of Pap smears screening programs.

Methods: A prospective study on conventional cervical cytology was performed including 340 women, 111 postmenopausal and 229 reproductive age group, attending the Gynaecologic out-patient department at a tertiary care hospital in Bhavnagar district of Gujarat state. Cytological findings including incidence of unsatisfactory smears, inflammatory smears and epithelial abnormalities were classified according to the Bethesda system 2001.

Results: Overall incidence of unsatisfactory smears was 9.4%, of squamous cell abnormality was 4.1% and of glandular cell abnormality was 1.2%. Highest frequency of epithelial cell abnormalities (squamous as well as glandular) was seen in 61-70 years age-group women (35.71%) and in women presented with complaints of post-menopausal bleeding (22.5%).

Conclusions: The study had shown a relatively low prevalence of epithelial abnormalities in cervical smears. The 2001 Bethesda system seems to reduce the number of cervical smear diagnoses of Atypical Squamous Cells (ASC), without causing any impairment in the diagnosis of High grade Squamous Intraepithelial Lesion (HSIL) thus decreasing the number of unnecessary interventions.

Keywords: Screening, Pap smear, Cervical cancer, Squamous intraepithelial lesion

INTRODUCTION

Cancer of the uterine cervix is the second most common cancer among women in the world after breast cancer. Cervical cancer accounted for 493000 new cases and 273000 deaths in 2002 worldwide. More than 80% of the cervical cancer cases occurred in developing countries.¹ Taking the lives of 75000 women each year, cervical cancer is the leading cause of death for women in India. This number accounts for a third of all cancers that affect women in India.²

In 1939 Papanicolaou in association with Herbert Trant, found that vaginal pool smears showed cancer cells in a number of patients with malignant tumors of uterine cervix and endometrium, some that had not been suspected clinically. To avoid difficulties with tissue pathologist, Papanicolaou proposed a series of classes from I to V, to describe the smears.³ Before 1988, laboratories generally utilized a numeric "Pap class" system of reporting results. Though the Papanicolaou classification has a historical association with the development of cytology, it does not reliably

communicate clinically relevant information. Four major deficiencies were pointed out:

1. Papanicolaou classification does not reflect current understanding of cervical/vaginal neoplasia.
2. Papanicolaou classes do not have equivalent in diagnostic histopathology terminology.
3. Papanicolaou classes do not provide diagnosis for non-cancerous entities.
4. As a result of numerous idiosyncratic modifications, the specific Papanicolaou classes no longer reflect diagnostic interpretations uniformly.⁴

In December 1988, a small group of individuals participated in a National Cancer Institute-sponsored meeting in Bethesda, Maryland, with the goal of developing a system for reporting Pap smears that would communicate the cytology interpretation to the clinician in a clear and relevant fashion. The result of this first meeting was The 1988 Bethesda System (TBS). This new terminology reflected three fundamental principles:

1. The terminology must communicate clinically relevant information from the laboratory to the patient's health-care provider.
2. The terminology should be uniform and reasonably reproducible across different pathologists and laboratories, and also flexible enough to be adapted in a wide variety of laboratory settings and geographic locations.
3. The terminology must reflect the most current understanding of cervical neoplasia.⁵

The National Cancer Institute sponsored a second workshop in April 1991 and a third workshop in May 2001, to critically assess TBS and suggest further emendations. Currently the 2001 Bethesda system is in use. The cytological diagnosis of cervico-vaginal inflammatory, reactive and reparative changes is important for minimizing false positives, for minimizing false negatives and for diagnosis of some specific nonneoplastic conditions. The 2001 Bethesda system of reporting requires the reporting of all other abnormal changes apart from neoplasia.⁶ The 2001 Bethesda system includes specific statements about specimen adequacy, general categorization, and interpretation and results.⁷ The 2001 Bethesda system seems to reduce the number of cervical smear diagnoses of ASC, without causing any impairment in the diagnosis of HSIL thus decreasing the number of unnecessary interventions like cervical biopsy and decreasing the cost, inconvenience, anxiety and discomfort.⁸

Historical study has found a positive correlation between the decrease in mortality from carcinoma cervix and the rate of cytological screening.⁹ The overall results of various studies reaffirm that cervico-vaginal cytology (Pap smear) remains the most inexpensive and effective tool for the elimination of cervical cancer.¹⁰

Since data from Gujarat is not well documented and since no other previous study of Pap smears was ever conducted in Bhavnagar district, the present study was conducted on the patients having various symptoms related to lower genital tract and/or having unhealthy looking cervix, attending the outpatients department of gynecology at Sir T. Hospital, Bhavnagar, to evaluate the abnormal cytological entities detected by cervical Pap smear, to classify the cytological findings according to the recently recommended Bethesda system and to discuss significance of Pap smears screening programs.

METHODS

The study was conducted over a span of 16 months at a tertiary care hospital in Bhavnagar on women attending the Gynaecologic OPD having various symptoms related to lower genital tract and/or having unhealthy looking cervix. Three patients of physical fitness with no complaints were also included in this study. As antimicrobial therapy was given according to clinical suspicion and followed up if symptoms and/or signs persisted, such patients were also taken up for this study.

Complete history including complaints, personal and marital history was noted. Material was collected from the ecto cervix by cervical scrapping with the help of an Ayre's wooden spatula and from endocervix with cytobrush and immediately smeared on a clean glass slide that was premarked as 'ecto' or 'endo' with patient's identity. Smears were stained by rapid Pap method and reported according to The 2001 Bethesda system⁵ by the consultant pathologist as follows:

(A) *Specimen type*: Indicate conventional smear vs. liquid-based preparation vs. other.

(B) *Specimen adequacy*:

- Satisfactory for evaluation (describe presence or absence of endocervical/transformation zone components and any other quality indicators such as partially obscuring blood, inflammation etc.)
- Unsatisfactory for evaluation... (specify reason)
- Specimen rejected or not processed (specify reason)
- Specimen processed and examined, but unsatisfactory for evaluation of epithelial abnormality because of (specify reason)

(C) *Interpretation/result*:

- Negative for intraepithelial lesion or malignancy (NILM):

Organisms:

- Trichomonas vaginalis
- Fungal organisms morphologically consistent with Candida species
- Shift in flora suggestive of bacterial vaginosis

- Bacteria morphologically consistent with Actinomyces species
- Cellular changes consistent with herpes simplex virus

• *Other non-neoplastic findings (optional to report; list not inclusive):*

- Reactive cellular changes associated with: Inflammation (includes typical repair), radiation, intrauterine contraceptive device (IUD)
- Glandular cells status post hysterectomy
- Atrophy

Other: Endometrial cells (in a women ≥ 40 years of age)

- Epithelial cell abnormalities:

Squamous cell:

- Atypical squamous cells (ASC)
 - ASC of undetermined significance (ASC-US)
 - ASC, cannot exclude high-grade squamous intraepithelial lesion (ASC-H)
- Low-grade squamous intraepithelial lesion (LSIL) (Encompassing: HPV, mild dysplasia, and CIN 1)
- High-grade squamous intraepithelial lesion (HSIL) (Encompassing: moderate and severe dysplasia, carcinoma in situ, CIN 2, and CIN 3)
- Squamous cell carcinoma

Glandular cell:

- Atypical
 - Endo-cervical cells (NOS or specify in comments)
 - Endometrial cells (NOS or specify in comments)
 - Glandular cells (NOS or specify in comments)
- Atypical
 - Endo-cervical cells, favour neoplastic
 - Glandular cells, favour neoplastic
- Endo-cervical adenocarcinoma in situ (AIS)
- Adenocarcinoma
- Other malignant neoplasm: (specify)

(D) Ancillary testing and automated review (Include as appropriate)

(E) Educational notes and suggestions (optional)

Statistical analysis: The P value was calculated using the Chi-square test.

RESULTS

Three hundred and forty patients between the ages of 20-103 years were examined. All except one were married, with mean age at marriage being 19 years. 6 patients were of total hysterectomy in which vaginal vault smears were taken. 111 patients were post-menopausal.

Follow up Pap smear examination was done in 6 patients and the follow up Pap smear findings were same as the first diagnosis in all cases except in one in whom previous unsatisfactory result became satisfactory-NILM with atrophy on follow up.

In our study 308 (90.6%) smears were satisfactory according to the Bethesda system. 32 (9.4%) smears were Unsatisfactory for evaluation - 10 were obscured by blood, 8 were obscured by inflammation, 6 were obscured by blood and inflammation, 6 due to low squamous cellularity and 2 due to poor fixation of smear. The epithelial cell abnormalities (squamous cell abnormalities: 4.1% and glandular cell abnormalities: 1.2%) constituted 5.3% of all cases and rest of 209 cases (85.3%) fell in the category of NILM (Table 1). Within the category NILM, 36 (10.6%) smears were of NILM with specific organism - 33 were of shift in flora suggestive of bacterial vaginosis and 3 were of trichomonas vaginalis.

Maximum numbers of cases (34.11%) were in age group 31-40 years, followed by 26.17% in age group 41-50 years. A progressive rise was seen in the frequency of cytopathological abnormalities with increasing age. It increased from 1.72% in the 31-40 year age group to as high as 35.71% in the 61-70 year age group. Although it appears low in >70 year age group, it can't be concluded; it could be due to the reason that total number of patient in >70 year age-group were only 7. Highest frequency of epithelial cell abnormalities (squamous as well as glandular) was seen in 61-70 year age-group (Table 2). Representative pictomicrographs of smears classified as Negative for Intraepithelial Lesion or Malignancy (NILM), atrophy with inflammation, High grade Squamous Intraepithelial Lesion (HSIL) and Squamous Cell Carcinoma (SCC) are shown in Figure 1, 2, 3 and 4 respectively.

Common presenting complaints of the subjects were excessive white discharge (45.3%), menorrhagia (15.3%) and uterine prolapsed (10.9%). Among various presenting complaints highest frequency of epithelial cell abnormalities were seen in post-menopausal bleeding (22.5%) followed by 10.5% in intermenstrual/post coital bleeding (Table 3).

Table 1: Distribution of women according to the 2001 Bethesda system Pap smear reports.

Cytological examination finding		No. of subjects (N=340)	Percentage
Main category	Sub-category		
Unsatisfactory for evaluation		32	9.4
Satisfactory for evaluation: 308 (90.6%)			
NILM: 290 (85.3%)	Only NILM	102	30.0
	NILM-Acute inflammation	115	33.82
	NILM-Reactive due to inflammation	24	7.05
	NILM-with specific organism	36	10.6
	NILM-with atrophy	2	0.6
	NILM-with atrophy and inflammation	11	3.23
Epithelial cell abnormalities: 18 (5.3%)			
Squamous cell abnormalities: 14 (4.1%)	ASCUS	8	2.35
	ASC-H	3	0.88
	LSIL	1	0.29
	HSIL	1	0.29
	SCC	1	0.29
Glandular cell abnormalities: 4 (1.2%)	Atypical endocervical cell-NOS	2	0.6
	Atypical endometrial cell-NOS	1	0.3
	Atypical glandular cell-NOS	1	0.3

NILM: Negative for intraepithelial lesion or malignancy, ASCUS: Atypical squamous cell of undetermined significance, ASC-H: Atypical squamous cell can't exclude HSIL, LSIL: Low grade squamous intraepithelial lesion, HSIL: High grade squamous intraepithelial lesion, SCC: Squamous cell carcinoma

Table 2: Age-wise distribution of smears and prevalence of various cytological findings.

Age groups (years)	Total cases (340)		NILM (290)		Squamous cell abnormalities (14)		Glandular cell abnormalities (4)		Unsatisfactory (32)	
	No.	%	No.	%	No.	%	No.	%	No.	%
20-30	67	19.7	63	94.03	0	0	0	0	4	5.97
31-40	116	34.11	108	93.10	2	1.72	0	0	6	5.17
41-50	89	26.17	73	82.02	2	2.24	1	1.12	13	14.61
51-60	33	9.7	28	84.85	2	6.06	0	0	3	9.09
61-70	28	8.23	15	53.57	7	25.0	3	10.71	3	10.71
>70	7	2.05	3	42.86	1	14.28	0	0	3	42.86

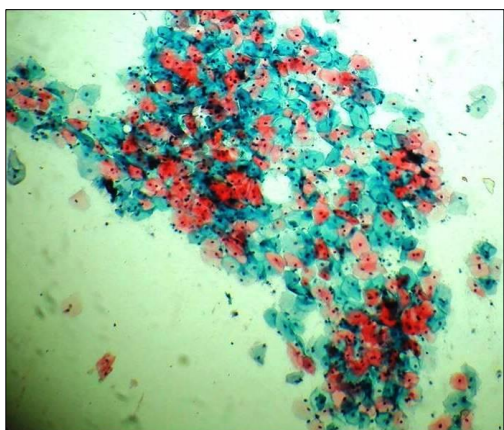


Figure 1: Negative for intraepithelial lesion or malignancy (NILM); Pap stain, 100x.

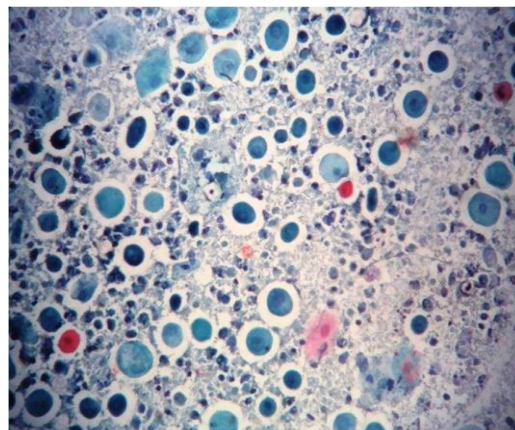


Figure 2: Atrophy with inflammation; Pap stain, 400x.

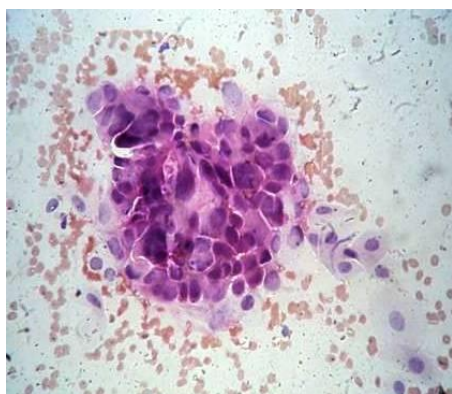


Figure 3: High grade squamous intraepithelial lesion (HSIL); Pap stain, 400x.

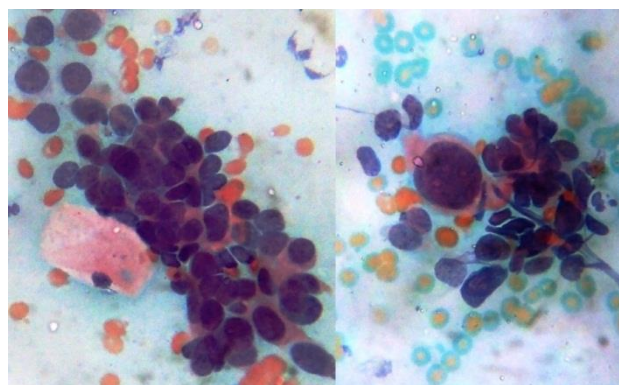


Figure 4: Squamous cell carcinoma (SCC); Pap stain, 400x.

Table 3: Association of various cytological findings with presenting complaint.

Presenting complaint	Total cases (340)		NILM (290)		Squamous cell abnormalities (14)		Glandular cell abnormalities (4)		Unsatisfactory (32)	
	No.	%	No.	%	No.	%	No.	%	No.	%
Excessive white discharge	154	45.3	144	93.5	3	1.9	1	0.6	7	4.5
Blood stained vaginal discharge	14	4.1	11	78.6	0	0	0	0	3	21.4
Intermenstrual/post coital bleeding	19	5.6	16	84.2	2	10.5	0	0	1	5.3
Postmenopausal bleeding	31	9.1	20	64.5	6	19.3	1	3.2	4	12.9
Uterine prolapse	37	10.9	27	73.0	2	5.4	0	0	8	21.6
Menorrhagia	52	15.3	45	86.5	0	0	1	1.9	6	11.5
Post hysterectomy vaginal discharge	8	2.4	6	75.0	0	0	0	0	2	25.0
Others*	25	7.3	22	88.0	1	4.0	1	4.0	1	4.0

*Includes - Burning micturition, burning/itching over perineum, lower abdominal pain/mass, uterine fibroid, physical fitness purpose

Squamous cell abnormalities were higher among postmenopausal women (10.0%) in comparison to reproductive age group women (1.31%) and the difference in squamous cell abnormalities between the two groups was statistically significant with P value 0.0001 (Table 4).

Table 4: Comparison of various cytological findings in postmenopausal and reproductive age group women.

Cytological finding	Post-menopausal women (N=111)		Reproductive age-group women (N=229)		P value
	No.	%	No.	%	
NILM	82	73.8	208	90.83	<0.05
Squamous cell abnormalities	11	10.0	3	1.31	0.0001
Glandular cell abnormalities	3	2.7	1	0.44	0.069
Unsatisfactory	15	13.5	17	7.42	0.07

When same 340 cases were reported by both TBS 1991 and TBS 2001 reporting system, incidence of ASC-US and ASC-US/SIL ratio was higher with TBS 1991 than TBS 2001. The 2001 Bethesda system seems to reduce the number of cervical smear diagnoses of ASC, without causing any impairment in diagnosis of HSIL (Table 5).

Table 5: Comparison of result of same Pap smears reported by TBS 1991 and 2001 reporting system.

Category	TBS-1991	TBS-2001
Unsatisfactory	8.0%	9.4%
ASCUS-favour reactive	1.17%	-
ASCUS-NOS/ASC-US	1.76%	2.35%
ASCUS-favour SIL/ASC-H	0.88%	0.88%
ASCUS/SIL ratio	6.57	4.05%
LSIL	0.29%	0.29%
HSIL	0.29%	0.29%

TBS: The Bethesda System

DISCUSSION

Cancer of the cervix is a preventable disorder as the different screening, diagnostic and therapeutic procedures are effective. Uterine cervix is ideal for screening due to easy accessibility of cervix for inspection, palpation, exfoliative cytology, and screening. Cervical cancer screening represents one of the great success stories in cancer prevention.¹¹ The goal of The Bethesda System (TBS) is to provide a uniform system of cytopathological reporting. Effective communication between the pathologist and the clinician is essential for patient care. The Bethesda system is a complete diagnostic system that recognizes the cytopathology report as a medical consultation.¹²

From the inception of TBS in 1988; it has been well received by the pathology and clinical communities. Although explanations for such a quick acceptance of TBS are many, one of the leading reasons is, most certainly, the sound and thoughtful basis for all of the elements of TBS. These include: (1) nomenclature that provides uniform diagnostic terminology to facilitate unambiguous communication between the laboratory and the clinician; (2) diagnostic categories that incorporate the latest scientific information on the pathogenesis and prognosis of cervical lesions; and (3) incorporation of the evaluation of specimen adequacy as an integral part of the report.¹³

In present study 340 female patients with symptoms related to lower genital tract and/or having unhealthy looking cervix were taken for Pap smear examination and reported by The Bethesda System 2001; the age group was between 20-103 years, of which 111 were postmenopausal and 229 were of reproductive age group. 9.4% Pap smear results were Unsatisfactory for evaluation, majority of which were due to squamous cells obscured by excessive blood. Incidence of unsatisfactory smears in postmenopausal women was 13.5% compared to 7.42% in reproductive age group women, however the difference was statistically non-significant ($P=0.07$).

Clinical diagnosis of specific organisms was accurate and many patients were treated by a course of specific antimicrobial therapy before the Pap smear examination, hence these were not detected in large number on Pap smear.

Out of total 8 cases (2.35%) of ASC-US, 5 patients were in the age group 61-70 year and 3 were in the age group 40-50 year. 3 had complaint of excessive white discharge, 2 had uterine prolapse, 1 had perimenopausal bleeding, 1 had post-menopausal bleeding and 1 had post coital bleeding. Out of total 3 cases (0.88%) of ASC-H, 2 were in the age group 60-70 year and 1 was in the age group 40-50 year. 2 had complaint of post-menopausal bleeding, 1 had intermenstrual bleeding. Total 2 cases (0.6%) of Atypical Endocervical Cells (NOS) detected, one 41 year aged and another 70 year aged with complaints of menorrhagia and excessive white discharge respectively. Single case (0.3%) was detected in each of atypical endometrial cells (NOS) and atypical glandular cells (NOS), both were 70 year aged presented with post-menopausal bleeding. Incidence of SIL & invasive carcinoma was 0.87% and all cases were seen in patients older than 50 years of age presented with complaints of post-menopausal bleeding.

Majority of the patients with excessive white discharge, menorrhagia, and uterine prolapse were NILM on Pap smear. However in comparison to postmenopausal and post coital bleeding, patients of uterine prolapse & excessive white discharge show epithelial cell abnormalities whereas patients with blood stained vaginal discharge showed no epithelial cell abnormality indicating that not all conditions traditionally linked with abnormal Pap smear give abnormal results and even conditions not associated with traditional symptoms related to epithelial cell abnormality gave abnormal Pap results.

There was wide range of variations in squamous cell abnormalities in different studies from India and abroad (Table 6). The difference in the incidence of epithelial cell abnormalities were mostly due to difference in geographic area of study and difference in the socioeconomic standards, racial, religious and other ethnic factors peculiar to the population studied. Low incidence of different epithelial cell abnormalities in present hospital based study correlate well with other hospital based studies done in India by Kuastubh Mulay¹⁴ and Anita N. Kavatkar.¹⁵ Incidence of epithelial cell abnormalities were also lower in studies done in developed world by Vassilakos¹⁶ and T. P. Thamboo¹⁷ than the studies done in under-developed world by Dall' Agnol M¹⁸ and Elhakeem HA.¹⁹

Table 6: Comparison of squamous cell abnormalities in Pap smears using the Bethesda system 2001 in studies from India and abroad.

Category	Kuastubh Mulay ¹⁴	Anita N. Kavatkar ¹⁵	Vassil-akos ¹⁶	Thamboo TP ¹⁷	Dall' Agnol M ¹⁸	Elhakeem HA ¹⁹	Present study
ASCUS	0.64%	1.23%	3.72%	0.92%	3.5%	2.76%	2.35%
LSIL	0.22%	1.23%	0.82%	0.53%	2.18%	1.3%	0.29%
HSIL	0.16%	1.23%	0.29%	0.38%	1.18%	0.66%	0.29%
ASCUS/SIL ratio	1.68	0.50	3.37	1.01	1.04	1.40	4.05
SCC	0.06%	-	-	0.06%	0.06%	0.33%	0.29%

In present study although overall incidence of CIN or invasive carcinoma was very low, incidence of ASC-US and ASC-US/SIL ratio were high. The ASC-US/SIL ratio is a quality indicator of reporting quality of Pap smears and should be less than 1.5:1 for satisfactory reporting quality. In present study the ASC-US/SIL ratio was high, it indicates that the reporting quality was suboptimal and it should be improved in future.

The study had shown a relatively low prevalence of epithelial abnormalities in cervical smears and conventional Pap smear is a good tool to find cervical dysplasia in population. The Bethesda system used for cervical cytology is not only a uniform and standard method but also gives descriptive diagnosis that helps the gynaecologist in individualized patient management. The 2001 Bethesda System seems to reduce the number of cervical smear diagnoses of ASC, without causing any impairment in the diagnosis of HSIL thus decreasing the number of unnecessary interventions like cervical biopsy and decreasing the cost, inconvenience, anxiety and discomfort. Communication between gynaecologist and cytopathologist is required for best results of the cervical cytology reporting.

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