Cervical Pap Smears - Cytomorphological Features
A Comparative Study In South Indian Population

Author
Dr. B. Rajsekhar
Associate Professor, Dept. of pathology
P.K. Das Medical College, Ottapalayam, Kerala

Abstract
The present study was undertaken to evaluate the cytomorphological pattern of cervical PAP smears in symptomatic women attending the Rajah Muthiah Medical College and Hospital for a period of 3 years (2003 to 2005) between 20 yrs to 80 years. Study was conducted to evaluate the suffering of women who had symptoms white discharge, irregular menstrual bleeding, postmenopausal bleeding and post coital bleeding. In each case after recording relevant clinical data, Pap smear obtained by using Ayre’s spatula was stained with pap stain. The study was conducted to screen, evaluate and detect the prevalence of various gynecological problems.

Aims and Objectives: The present study was undertaken to evaluate the cytomorphological pattern of cervical PAP smears in symptomatic women attending the Rajah Muthiah Medical College and Hospital for a period of 3 years (2003 to 2005) and between 20 yrs to 80 years.

Introduction
Material for study of cells can be obtained either by aspiration cytology or exfoliated cells.

Exfoliative Cytopathology
Exfoliative Cytopathology is the study of the normal and the disease altered desquamated cells from various sites. There are two types of cellular exfoliation

1. Natural Spontaneous Exfoliation
The physiologically desquamated cells will often show, besides pathologic changes and the normal changes of natural ageing the results of their separation from confinement in the organized structures

2. Artificial Exfoliation (surface microbiopsy)
Surface of the mucosa in scraped or tissue is aspirated with a needle and viable cells are traumatically exfoliated before their natural time of shedding.

Cytology of Normal Female Genital Tract
Anatomy and Histology
The vagina and portio vaginalis or ectocervix are normally covered by a smooth, white nonkeratinized stratified squamous epithelium. Squamocolumnar junction is seen in 60% of adult patients.

Endocervical Glands
Endocervical glands are seen mainly in the portio supravaginalis of the cervical canal.

Histology of Stratified Squamous Epithelium
Section of the nonkeratinized vagino-cervical stratified squamous epithelium has 4 distinct zones:
   1. Basal layer or stratum cylindricum
2. Parabasal layer or stratum spinosum
3. Intermediate layer
4. Superficial layer or cornified layer.

**Cytology of Stratified Squamous Epithelium**

**Deep Basal-Germinial Cells**
Originate from single-layered lining of the basal membrane. Deep basal cells are smallest epithelial cells found in vaginal smear.

**Parabasal Cells**
Originate from deep layer of the stratified squamous epithelium. Physiologically exfoliated from atrophic epithelium.

**Intermediate Cells**
Originate from precornified or superficial spinous layer. Most commonly seen during pregnancy, postovulatory time, menopause or as the result of hormones (progesterone or adrenocortical hormones).

**Superficial Cells**
Originate from stratum corneum. These cells are physiologically exfoliated. Commonly seen in the vaginal smear at the preovulatory time of cycle, after estrogentic therapy or in a case of functioning ovarian tumor.

**Squamous Pearls**
Originate from the bottom of an epithelial pit or crevasse. The component cells can be either superficial, intermediate or parabasal in type according to the depth of the pit.

**Anucleated Cells**
Rare in normal smear except when contaminated by perineal cells or hands of the doctor. Originate from lower 3rd of vagina.

**Cytology of the Columnar Epithelium**

**Endocervical Reserve Cells**
These are young endocervical, parabasal cells with a multipotential differentiation.

**ciliated endocervical cells**
Seen commonly in cervical scrapings or endocervical aspirations than in the vaginal pool smear.

**Nonciliated Endocervical Cells**
Occurs single, in clusters or in palisade form. Size varies from 20-30μ, elongated in shape.

**Hyper Secretory Endocervical Cells**
These cells are seen in chronic irritation, in pregnancy, in the presence of glandular endocervical polyps, or during the use of various contraceptive pills.

**Endocervical Stripped Nuclei**
Commonly seen in smears from postmenopausal or pregnant women or women with an endocervical entropion or ectopy.

**Glandular Endometrial Cells**
Seen in routine vaginal smear, only during the menstrual flow, early in pregnancy, during abortion or postpartum period.

**Endometrial Stromal Cells**
Originate from the spongiosa layer (deep) or the compact layer (superficial) of the endometrium.

**Cytology of the Normal Menstrual Cycle**

**Menstrual Phase**
Occupies the first 5 days of the cycle, is characterized by the presence of large numbers of red blood cells, and groups of endometrial cells, together with large number of histiocytes and polymorphs.

**Postmenstrual Phase**
Occupies 5th to 8th day of the cycle. Histiocytes and polymorphs are present in large numbers. Predominantly intermediate squamous cell is seen.

**Proliferative Phase**
Occupies 9th to 14th day of the cycle. There is decrease number of histiocytes and polymorphs. Superficial cells increase to 60%.

**Ovulatory Phase**
The ovulatory phase smear on the 15th and 16th day consists almost entirely of superficial cells with their characteristic pyknotic nuclei and eosinophilic transparent cytoplasm.

**Secretory Phase**
From the 17th to 24th day, the secretory phase is under the influence of progesterone secretion from the corpus luteum.

**Pre-Menstrual Phase**
From the 25th day to the onset of menstruation and the start of a new cycle there is an increase in polymorphs. Predominantly intermediate cells are seen.
Anovulatory Pattern
This pattern is the result of a defect in the maturation of the follicle. As ovulation does not occur the pattern is influenced by a variable degree of ovarian oestrogen secretion.

Pregnancy Pattern
Presence of large clumps of navicular cells. They are intermediate in origin, but modified to a boat shaped morphology with curled cell edges

Postmenopausal Pattern
The cytological patterns are diverse, ranging from the typical proliferative patterns found in the ovulatory cycle, to the atrophic pattern composed of mainly of parabasal cells

Patterns of Specific Atypical Conditions
Cervical Metaplasia
Atypical epithelial cells will occasionally be found in cervical and vaginal smears, which suggests a metaplastic in origin.

Dyskaryosis
Dyskaryosis is a term applied to cells that exhibit nuclear changes consistent with malignancy whilst maintaining a normal cytoplasm and nuclear/cyttoplasmic ratio.

Carcinoma of the Cervix
Carcinoma-in-situ of the cervix is a cytologically malignant lesion, arising in the squamous epithelium, which by definition shows no invasion across the basement membrane. Smears in carcinoma-in-situ will show large number of dyskaryotic cells together with frankly malignant cells.

Adenocarcinoma
These cells are most commonly detected in specimens from the posterior fornix pool

Identification of Vaginal Infections
Doderlein Bacilli
Most common commensals inhabiting the vagina which aperies as small plump rod to a longer bacilli form. Found mainly in the secretory phase of the cycle.

Trichomonas Vaginalis
Small, unicellular, flagellate organism which is pear shaped in wet preparations

Candida Albicans
Candida albicans appears as long, brightly eosinophilic filaments with adjacent small, ovoid, unicellular and eosinophilic yeast forms.

Chlamydia
Chlamydia is an essential obligate intracellular Gram-negative, non-motile organism between 0.2 and 1.5 μm in size.

Herpes Simplex
Presence of typical giant multinucleate squamous epithelial cells in cervical smears.

Human Papilloma Virus (HPV)
The characteristic feature of HPV infection is the “koilocyte” an epithelial cell with a well clear perinuclear halo, surrounded by dense peripheral staining of the cytoplasm.

Actinomyces
In Papanicolaou stained preparations the organism appears as discrete, dense blue-stained aggregates in which parallel filaments radiate from a central core.

Leptothrix Vaginitis
Filamentous organism, similar to Actinomyces, is often mistaken for a fungus. They are elongated and very thin with rare branching

Non-epithelial Cells
Mesodermal cells, Red blood cells (erythrocytes), Polymorphonuclear leukocytes (Neutrophils, eosinophils and Basophils), Lymphocytes, Plasmacytes and histiocytes.

Non-Epithelial Contaminants
Sperm, Pollen, Trichomes, Lubricant, Talcum and Yeast.

Era of Development And Expansion
In 1928-Aurel A. Babes from University of Bucharest published an article “Diagnosis of cancer of uterine cervix by smears” in French journal ‘La Presse Medicale’. He used platinum loop to collect the material, methyl alcohol for fixation and Giemsa stain for cytology.


Workshop was held at National Cancer Institute in Bethesda, Maryland (1988) where reporting system by Papanicolaou (1940) was replaced by...
The Bethesda system for reporting cervical/vaginal cytologic diagnoses.9

<table>
<thead>
<tr>
<th>PAPANICO LAOU SYSTEM</th>
<th>WHO SYSTEM (1973)</th>
<th>BETHESDA SYSTEM (1988)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class I</td>
<td>Normal</td>
<td>Within normal limits</td>
</tr>
<tr>
<td>Class II</td>
<td>Atypical</td>
<td>Reactive or reparative changes</td>
</tr>
<tr>
<td>Class III</td>
<td>Dysplasia</td>
<td>Atypical or abnormal squamous cells</td>
</tr>
<tr>
<td></td>
<td>Mild</td>
<td>Low grade includes HPV- SIL</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>Low grade SIL</td>
</tr>
<tr>
<td></td>
<td>Severe</td>
<td>High grade SIL</td>
</tr>
<tr>
<td>Class IV</td>
<td>Ca in situ</td>
<td>Squamous cell carcinoma</td>
</tr>
<tr>
<td>Class V</td>
<td>Adenocarcinom a</td>
<td>Adeno-carcinoma and glandular cell abnormalities</td>
</tr>
</tbody>
</table>

Krane J.F et al (2001) observed that erosion cervix was commonly associated with inflammatory changes (46.6%) Associated with gynaecological symptoms White discharge-1152 (13.7%) Menorrhagia-4.2%. Contact bleeding 46 (0.5%) Postmenopausal bleeding-21 (0.2%)10 Annamama. T et al (2003) – In their study of clinical profile and cervical cytomorphology in symptomatic postmenopausal women showed high incidence of candidiasis (12%). Cytology in postmenopausal can be used to screen for malignancy, pathogens in inflammation and to monitor hormone replacement therapy.11
JS Misra et al (2003)-In their study revealed a higher incidence of LSIL and of frank cancer in menopausal women than in women in the reproductive age group (9.1% and 3.3% as against 2.1% and 0.9% respectively).12

**The 2001 Bethesda System for Reporting Cervical Cytologic Diagnoses**13

**Specimen Adequacy**
Satisfactory for evaluation
Presence or absence of endocervical or transformation zone components or other quality indicators such as partially obscuring blood or inflammation
Unsatisfactory for evaluation (specify reason)
Specimen rejected or not processed (specify reason)
Specimen processed and examined, but unsatisfactory for evaluation of epithelial abnormalities (specify reason)

**General Categorization (Optional)**
Negative for intraepithelial lesion (IEL) or malignancy
Epithelial cell abnormality
Others

**Interpretation/Result**
Negative for intraepithelial lesion or malignancy

**ORGANISMS**
Trichomonas vaginalis
Fungal organisms morphologically consistent with Candida species
Shift in flora suggestive of bacterial vaginosis
Bacterial morphologically consistent with Actinomyces species
Cellular changes consistent with Herpes Simplex Virus
Other non-neoplastic findings (optional to report)

**Reactive Cellular Changes Associated With**
Inflammation (includes typical repair)
Radiation
Intrauterine contraceptive device
Glandular cells status post hysterectomy
Atrophy
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: Human Papillomavirus (HPV), mild dysplasia, and Cervical Intraepithelial Neoplasia (CIN) 1
High-grade Squamous Intraepithelial Lesion (HSIL)
Encompassing: moderate and severe dysplasia, Carcinoma-in-situ, CIN2, and CIN3
Squamous cell carcinoma
Glandular cell
Atypical glandular cells (AGC)
Specify endocervical, endometrial, or glandular cells not otherwise specified
Atypical glandular cells, favor neoplastic
Specify end cervical or not otherwise specified
Endocervical adenocarcinoma in situ (AIS) Adenocarcinoma Other (list not comprehensive) Endometrial cells in a woman 40 years or older

Materials and Methods
Women aged 21yrs and above attending the gynecology OPD of RMMCH with complaints of white discharge, irregular menstrual bleeding, postmenopausal bleeding and post coital bleeding for a period of 3 years (Dec 2003 to June 2005) were included in this study. In each case after recording relevant clinical data, Pap smear obtained by using Ayre’s spatula was stained with papanicolaou’s stain.

Method of Collection of Pap Smear
For preparation of smears the following reagents and equipments were needed.

1) Clean glass slides.
2) Diamond marking pencil
3) Cover slip
4) Absolute methanol in Coplin jar as fixative
5) Wooden Ayre spatula
6) Sterile vaginal speculum
7) A pair of sterile gloves

Patient Preparation
1) Abstinence from coitus for 24hrs prior to the procedure
2) No intravaginal medication for 1 weekend prior to the test.
3) No lubricants should be used during the procedure.
4) 12th day of cycle is ideal for taking the smear.

Collection of Pap Smear
For collecting the material patient was put in the lithotomy position. Vulva was inspected for the lesions after inspecting and stabilizing the cervix. Ayre’s spatula was inserted into the external os and gently and firmly rotated at 360° degrees. By doing this procedure material was obtained from squamocolumnar junction.

After obtaining, material was transferred on clean glass slide and was uniformly spread using spatula and immediately kept in Coplin jar containing absolute alcohol for fixation. Slide was stained by modified Papanicolaou’s stain.

Preparation of Pap Stain
1. Preparation of Harris Haematoxylin
   - Haematoxylin powder 2.0gm
   - Ethylene glycol 250ml
   - Aluminium sulphate 17.6gm
   - Distilled water 750ml
   - Sodium iodate 0.2gm
   - Glacial acetic acid 20.0ml

   The ingredients were added in order as written above in a dry clean bottle and the mixture was vigorously shaken for 1 hour at room temperature. The staining solution could be stored over one year.

2. Preparation of Orange G Stain
   - Orange G stock solution
     - Orange G stain 9.05gm
     - Distilled water 100ml

   - Orange G working solution
     - Orange G stock solution 20ml
     - Phosphotungstic acid 0.15gm
     - 95% ethyl alcohol 980ml

   Orange G working solution was changed every fortnight. The stain was filtered before use and stored in a dark bottle.

3. Preparation of E.A Stain (Modified)
   - Light green stock solution
     - Light green SF yellowish 3.17gm
     - Distilled water 100ml

   - Eosin Y Stock Solution
     - Eosin Y 20.8gm
     - Distilled water 100ml

   - E.A Working Solution
     - 95% ethyl alcohol 700ml
     - Stock eosin y 20ml
     - Stock light green 10ml
     - Absolute methanol 250ml
     - Phosphotungstic acid 2gm
     - Glacial acetic acid 20ml

   This working solution was changed every week.

Staining Of Pap Smear

The fixed slides are transferred directly from the fixative into the following solutions.
1. 80% ethyl alcohol 10dips
2. 70%ethyl alcohol 10dips
3. 50%ethyl alcohol 10dips
4. Distilled water 10dips
5. Harris haematoxylin 3min
6. Running tapwater 1min
7. 0.5%HCL 5dips
8. Running tapwater 1min
9. Dilute solution of lithium carbonate 1min
10. Running tapwater 1min
11. 50%ethyl alcohol 10dips
12. 70%ethyl alcohol 10dips
13. 80%ethyl alcohol 10dips
14. 95%ethyl alcohol 1min
15. Orange G-6 1min
16. 95% ethyl alcohol 10dips
17. 95%ethyl alcohol 10dips
18. EA-36 4min
19. 95%ethyl alcohol 10dips
20. 95%ethyl alcohol 10dips
21. Absolute alcohol 4min
22. Xylene 5min

Slides are then mounted in DPX.

Results
Nuclei-Blue
Superficial cell cytoplasm-Pink
Intermediate and Parabasal cell cytoplasm-Blue-green
Red Blood cells-Orange

Observations
Age Incidence
In the present study, a high incidence of 308 cases (38.5%) was observed in the 3rd decade followed by 241 cases (30.8%) in the 2nd decade.

CLINICAL PRESENTATION
In the present study, high incidence of 397 cases (49.62%) presented with complaint of white discharge followed by 284 cases (35.50%) with irregular bleeding and low incidence of 13 cases (1.62%) with complaint of post coital bleeding.

Cytology Vs Age
In the present study, high incidence of chronic inflammation was observed in the 3rd decade followed by 2nd decade.

Adequacy of Specimen
In the present study, 736 cases (92%) were inadequate-No endocervical cells were observed in the smear and the remaining 64 cases (8%) had adequacy-presence of endocervical cells in the smear.

![Age Incidence Chart](chart.png)
**Clinical Presentation**

![Bar chart showing clinical presentations by age and type of discharge/bleeding.](chart1)

**Types of Lesions - Cytology**

![Bar chart showing types of lesions by age and inflammation type.](chart2)
Distribution of Lesions - Cytology

- Inflammation
- LSIL
- HSIL
- Normal
- Total

PAP Smears - Adequacy

Adequate
Inadequate

Age:
- 21-30
- 31-40
- 41-50
- 51-60
- >60

Number:
- 0
- 12
- 28
- 106
- 146
Intermediate cells, superficial cells and Neutrophils  X 20 PAP

Intermediate cells, endocervical cells and neutrophils X 20 PAP
Endocervical (columnar and reserve) cells and intermediate cells X 40 PAP

Endocervical (columnar) cells and intermediate cells
Parabasal cells, Intermediate cells and neutrophils X 40 PAP

Intermediate cells with perinuclear halo (arrow), Parabasal cells and neutrophils X 40 PAP
Tight collections of neutrophils overlying the superficial cells (BB shot), Intermediate cells and RBCs X40 PAP
Candida hyphae showing branching

Leptothrix and Intermediate cells
Low grade Squamous Intraepithelial Lesion (LSIL)

High Grade Squamous Intraepithelial Lesion (HSIL) X100 PAP

**Discussion**

The different systems were employed to categorize the cytological features—viz. PAP system, WHO system and BETHESDA system. The Bethesda System (TBS) 2001 is currently used so as to ensure uniformity in reporting and allow comparison of results from different centers.

**Clinical Presentation**

Misra JS et al (2004) conducted study on 19,449 women, of which 1574 presented with white discharge 1152 (13.7%), menorrhagia 355(4.2%), contact bleeding 46(0.5%) and postmenopausal bleeding 21(0.2%).

In the present study (2006), out of 800 women, 397(49.62%) presented with white discharge, 284(35.5%) with irregular bleeding, 13 (1.62%) with postcoital bleeding and 106 (13.25%) postmenopausal bleeding.

**Age Incidence**

Misra JS et al (2004) reported in their study, a high incidence (64.7%) of inflammatory smears in women between 31-40 years of age followed by
women between 21-30 years of age (28.9%). 201 cases (8.3%) of LSIL and 7 cases (0.2%) of HSIL were observed in the 2nd decade. 

In the present study (2006), high incidence of inflammatory smears was observed in women between 31-40 years of age [81 cases (44.02%)] followed by women between 21-30 years of age [78 cases (42.39%)]. A high incidence of LSIL – 12 cases (42.85%) and HSIL – 7 cases (58.33%) was observed in the 4th decade.

Cytology
Misra JS et al (2004) conducted study on 19,449 women, which revealed inflammatory smears in 8354 (42.9%). Out of the inflammatory smears LSIL accounted for 403 (4.8%) and HSIL 14 (0.1%). HPV accounted for 108 cases (1.2%), Trichomonas 433 (5.1%) and Candida albicans 88 (1.1%).

In the present study, 800 women were cytologically screened. Of these, 576 (72%) showed non-specific inflammation, 184 (23%) specific inflammation, 28 (3.5%) LSIL and 12 (1.5%) HSIL.

Summary
800 women with gynecological symptoms were subjected to screening by PAP smear. Patients above 20 years of age were included in the study. The youngest patient was 21 years of age and the oldest patient 80 years. The mean age of the patients was found to be 41.8 years. An overall accuracy 130/146 (89.04%) is found in the present series.

Interest of conflict: None

Bibliography