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

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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) aed between 20 yrs to 80 years.*

Introduction

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

Exfoliative Cytopathology[1,]

Exfoliative Cytopathology is the study of the normal and the disease altered desquamated cells from various sitesThere 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[2]

The vagina and portiovaginalis 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[3]

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-Germinal 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 eostrogenic 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[4]

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/ cytoplasmic 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 μ m 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 [5]

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

Nonepithelial Cells [6]

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[7].

Buckley et al and Ferenczy (1982) graded Cervical Intraepithelial Neoplasia(CIN)[8].

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]

PAPANICOLAOU SYSTEM	WHO SYSTEM (1973)	BETHESDA SYSTEM (1988)
Class I	Normal	Within normal limits
Class II	Atypical	Reactive or reparative changes
ClassIII	Dysplasia	Atypical or abnormal squamous cells
	Mild	Low grade includes HPV- SIL
	Moderate	Low grade SIL
	Severe	High grade SIL
Class IV	Ca in situ	Squamous cell carcinoma
Class V	Adenocarcinoma	Adeno-carcinoma and glandular cell abnormalities

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]

Annamma.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 Caterorization (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 dyplasia, 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 pap 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 1week 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 [14]

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

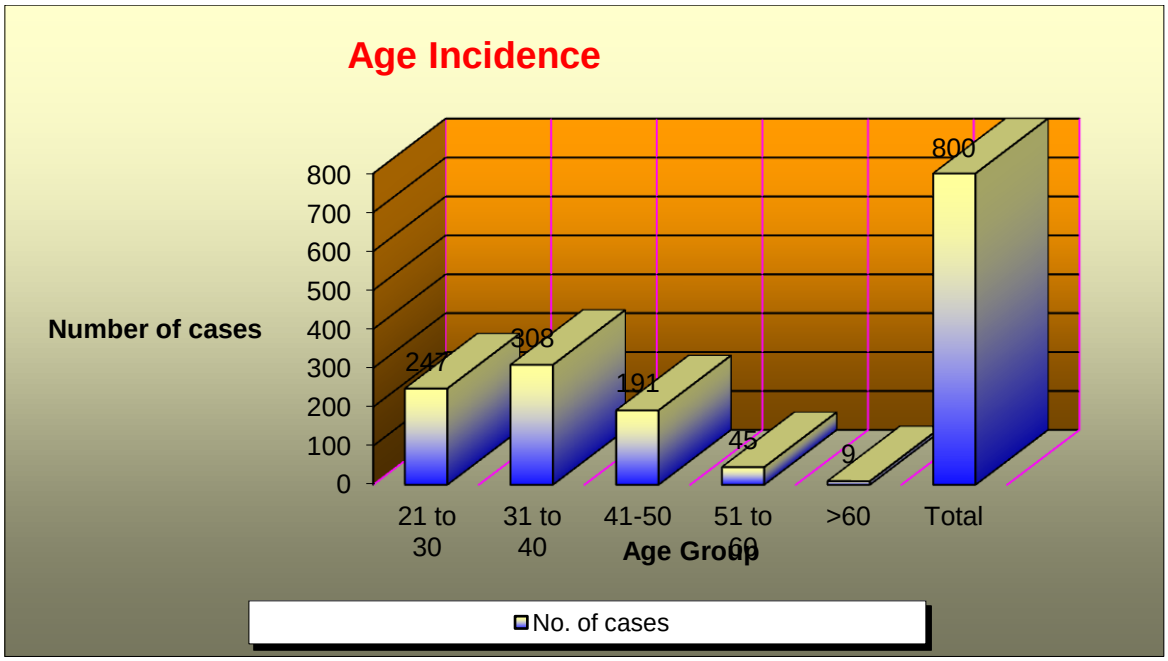
In the present study, high incidence of 397 cases (49.62%) presented with complaint of white discharge followed by 284 cases (35.50%) with irregular bleeding and low incidence of 13 cases (1.62%) with complaint of post coital bleeding.

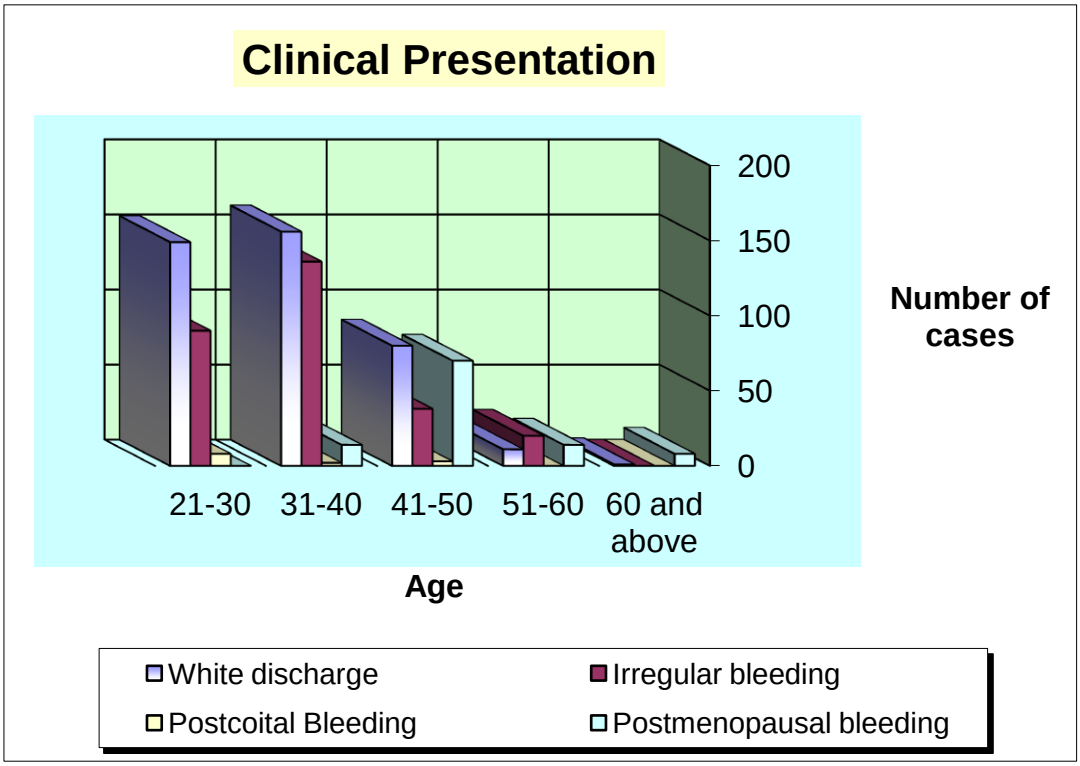
Cytology Vs Age

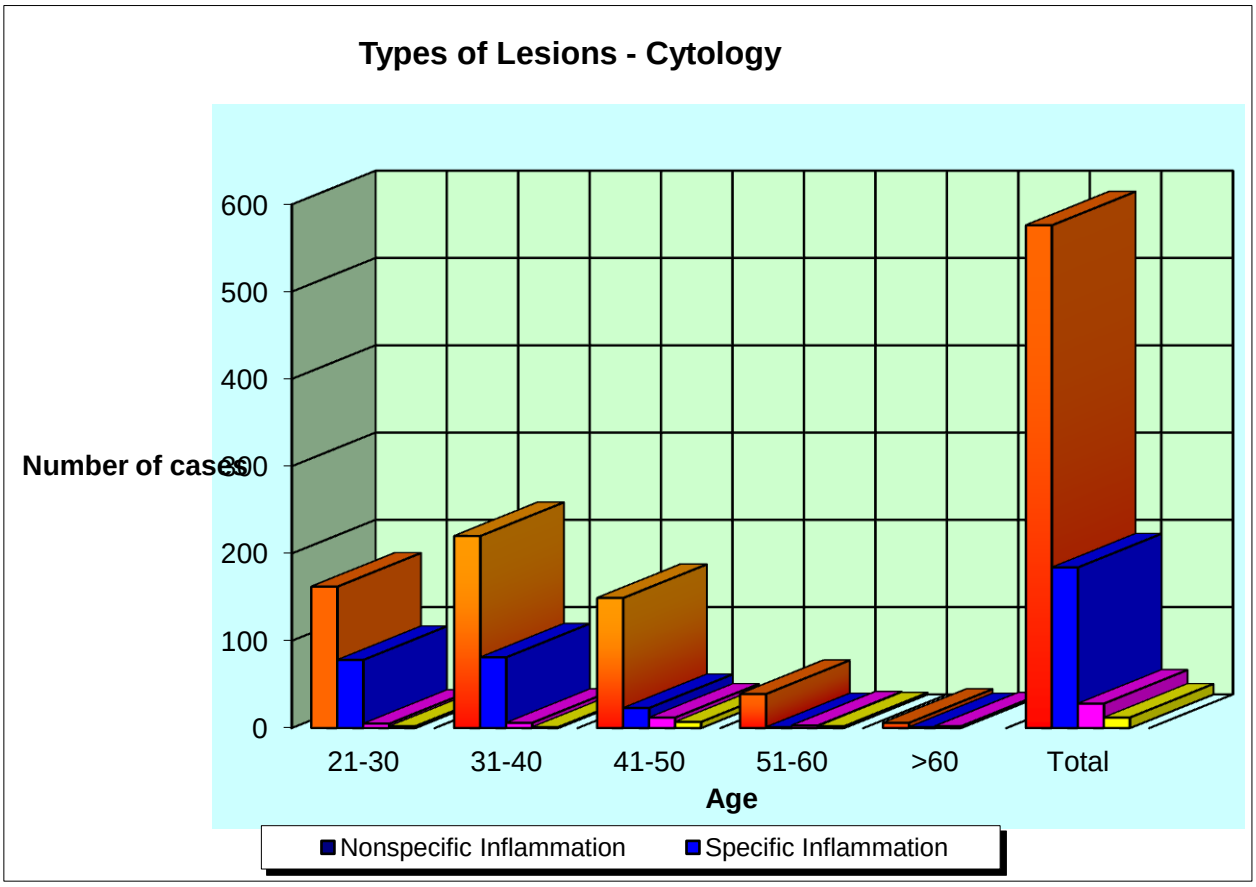
In the present study, high incidence of chronic inflammation was observed in the 3rd decade followed by 2nd decade.

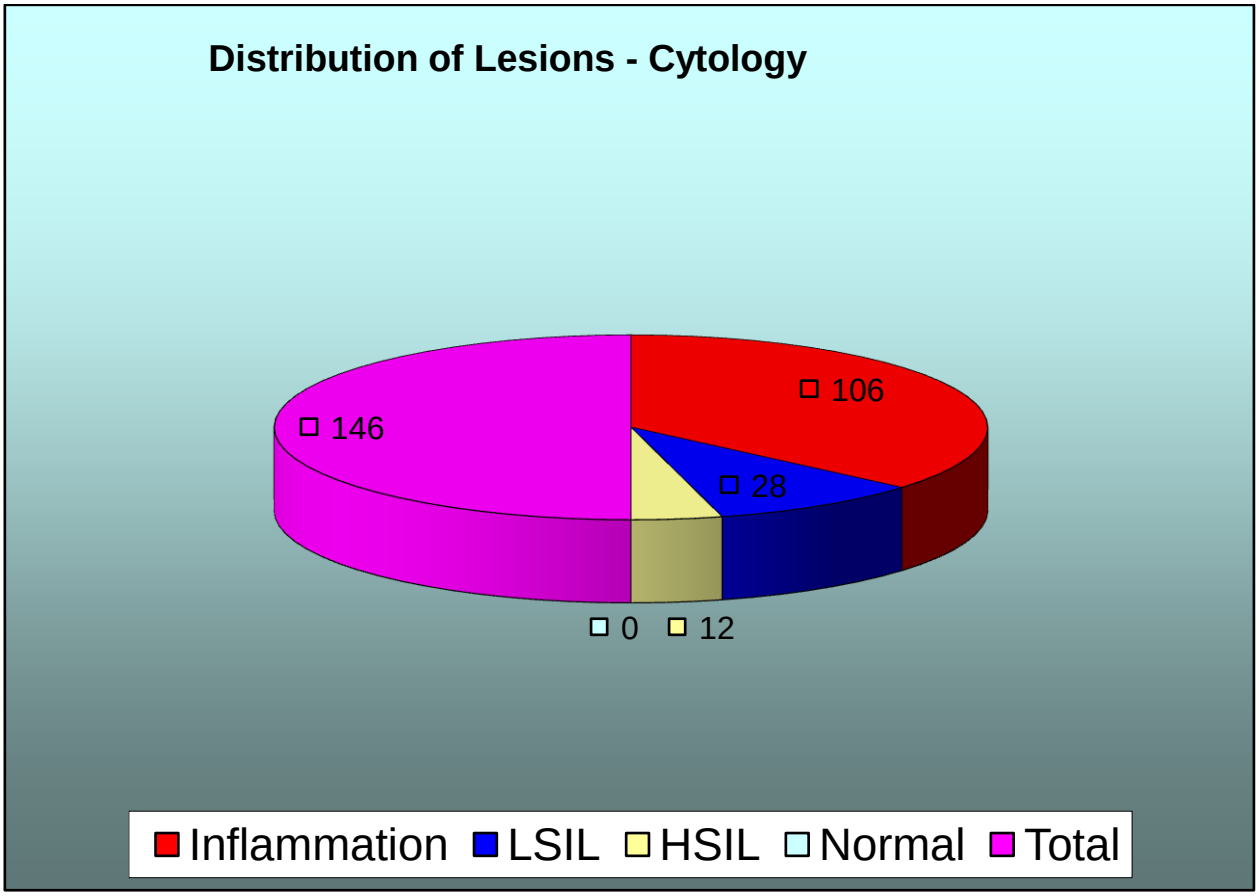
Adequacy of Specimen

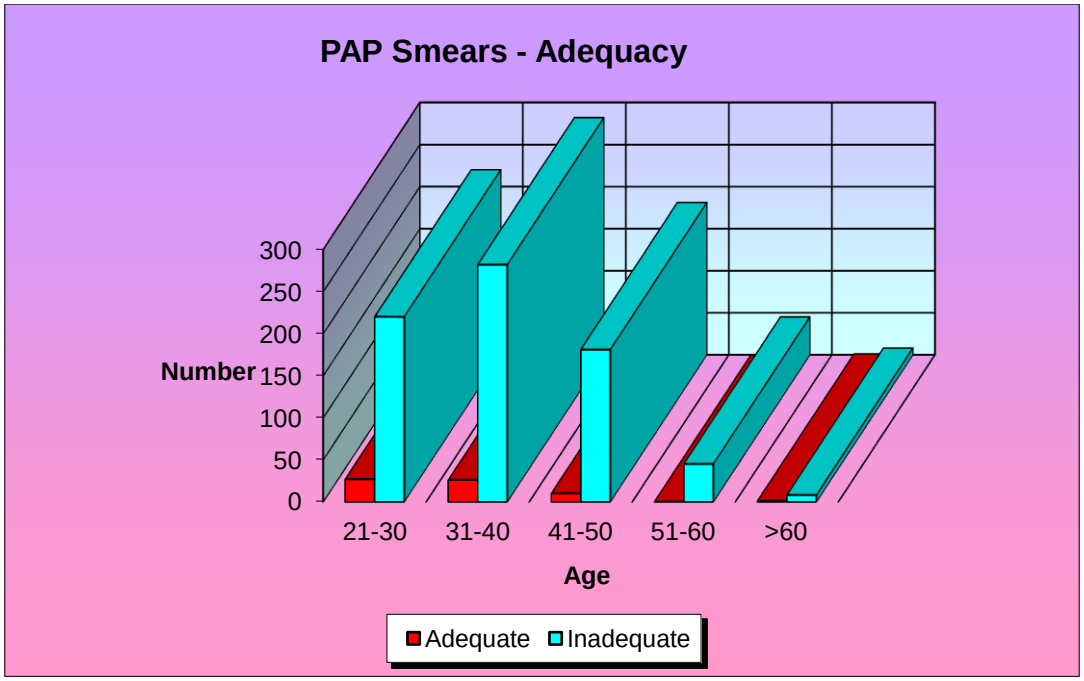
In the present study, 736 cases (92%) were inadequate-No endocervical cells were observed in the smcar and the remaining 64 cases (8%) had adequacy-presence of endocervical cells in the smear.

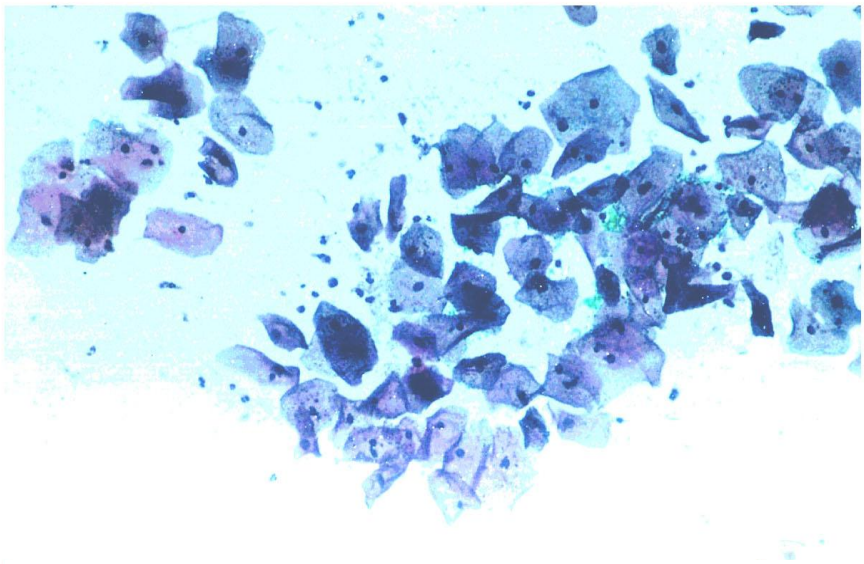



Clinical Presentation
200
150
100
50
0
Number of cases
21-30
31-40
41-50
51-60
60 and above
Age
White discharge
Irregular bleeding
Postcoital Bleeding
Postmenopausal bleeding

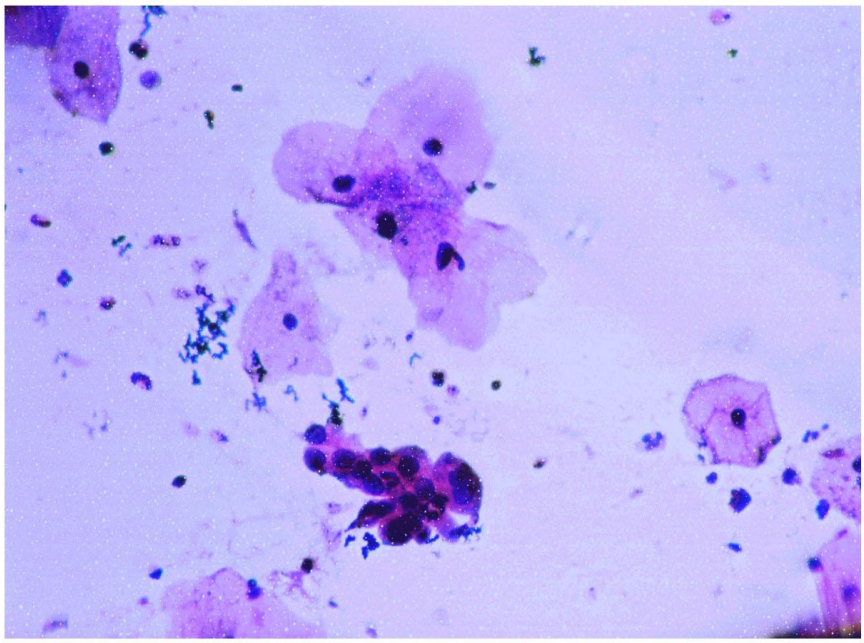

Types of Lesions - Cytology
600
500
400
300
200
100
0
Number of cases
21-30
31-40
41-50
51-60
>60
Total
Age
Nonspecific Inflammation
Specific Inflammation


Distribution of Lesions - Cytology
106
28
0
12
146
Inflammation
LSIL
HSIL
Normal
Total

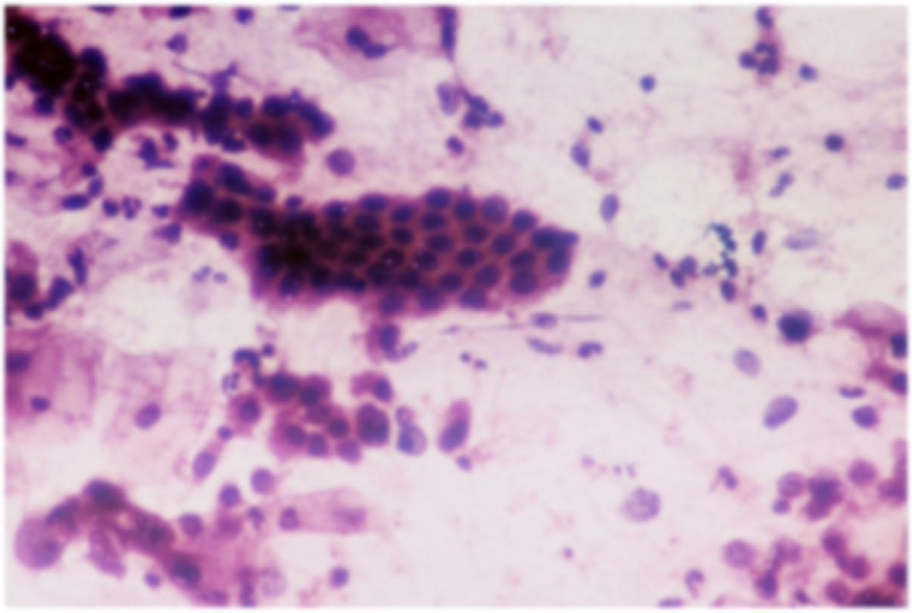

PAP Smears - Adequacy
Number
300
250
200
150
100
50
0
21-30
31-40
41-50
51-60
>60
Age
Adequate
Inadequate



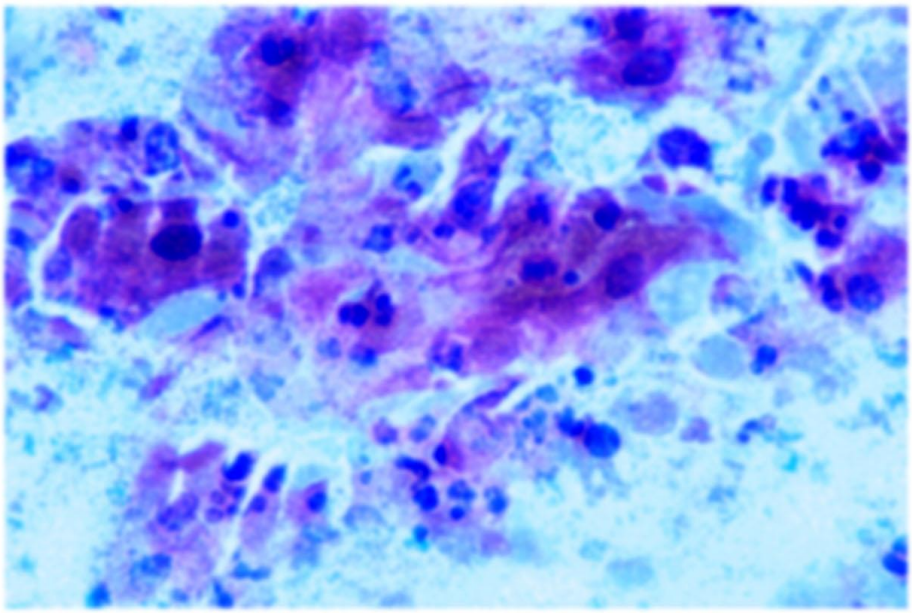
Intermediate cells, superficial cells and Neutrophils X 20 PAP



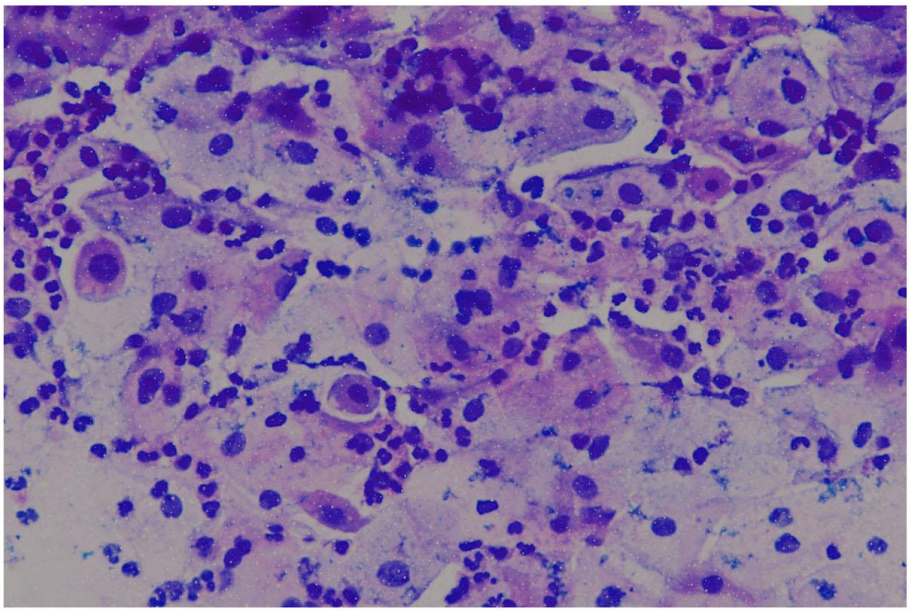
Intermediate cells, endocervical cells and neutrophils X 20 PAP



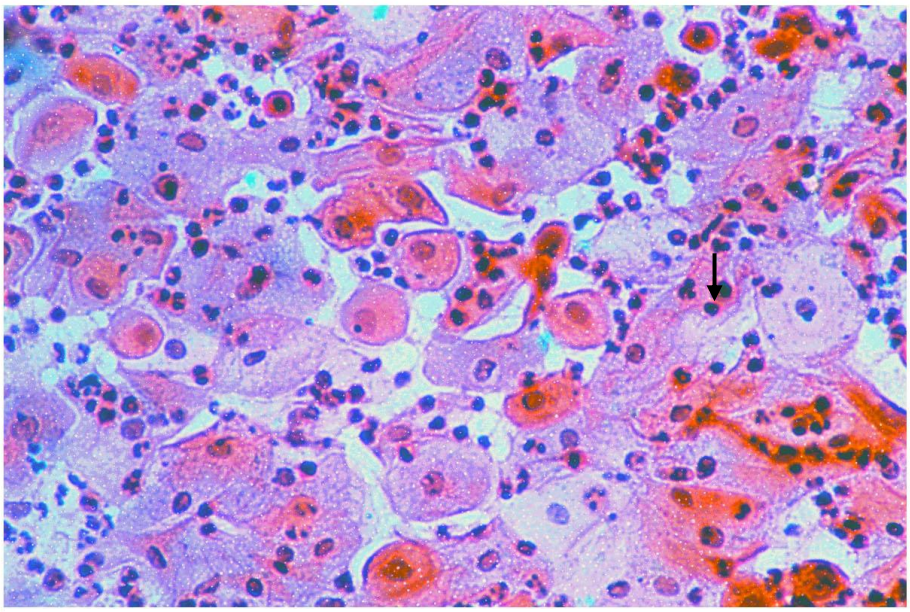
Endocervical (columnar and reserve) cells and intermediate cells X 40 PAP



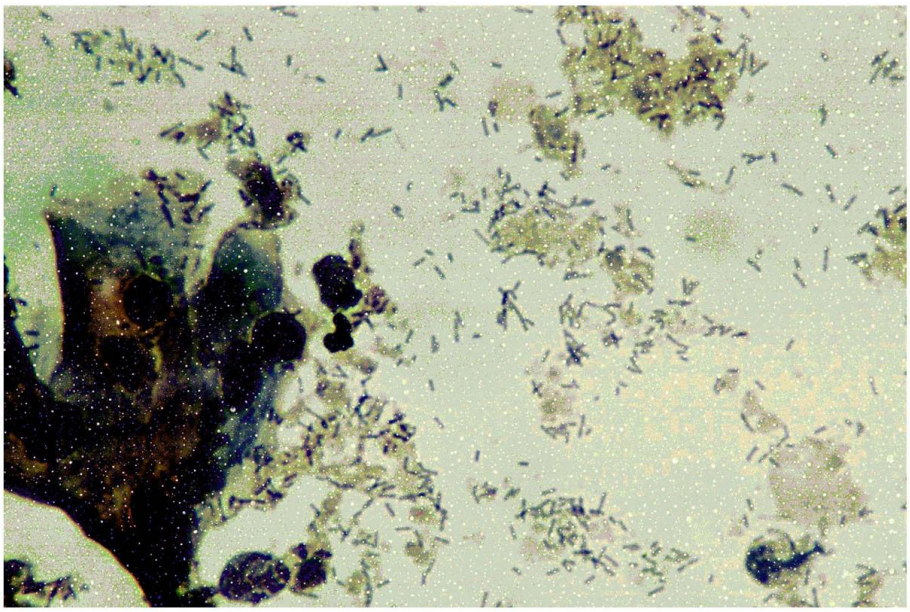
Endocervical (columnar) cells and intermediate cells



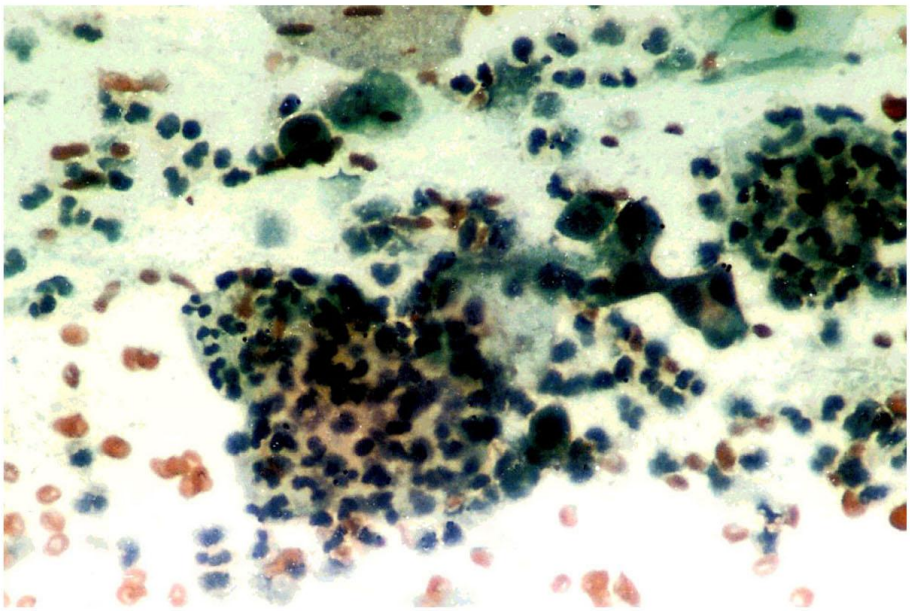
Parabasal cells, Intermediate cells and neutrophils X 40 PAP



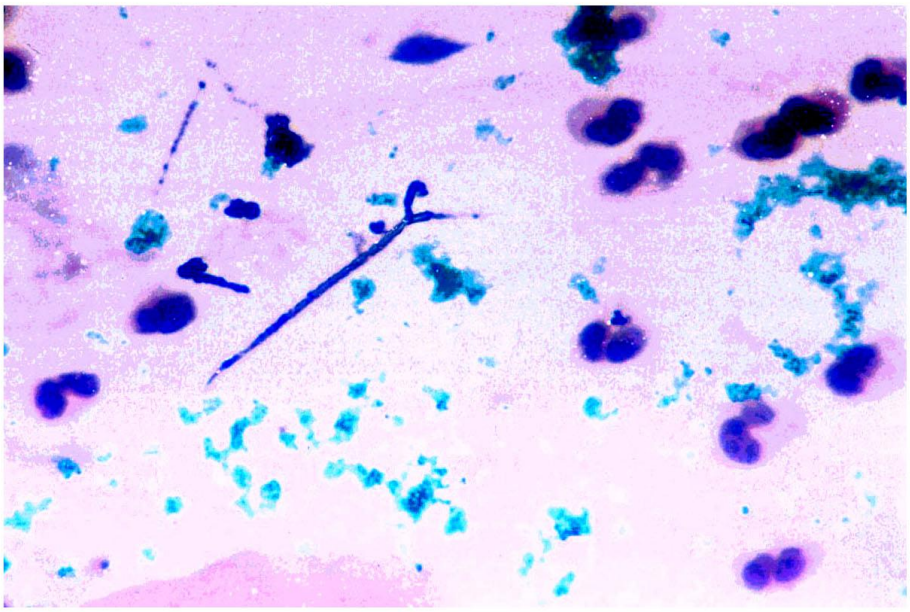
Intermediate cells with perinuclear halo (arrow), Parabasal cells and neutrophils X 40 PAP



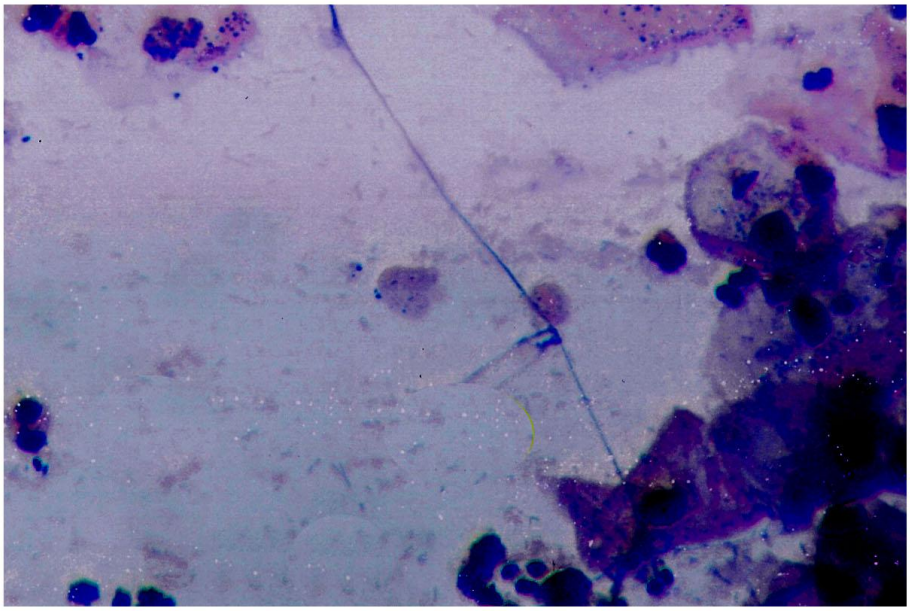
Doderlein Bacilli X100 PAP



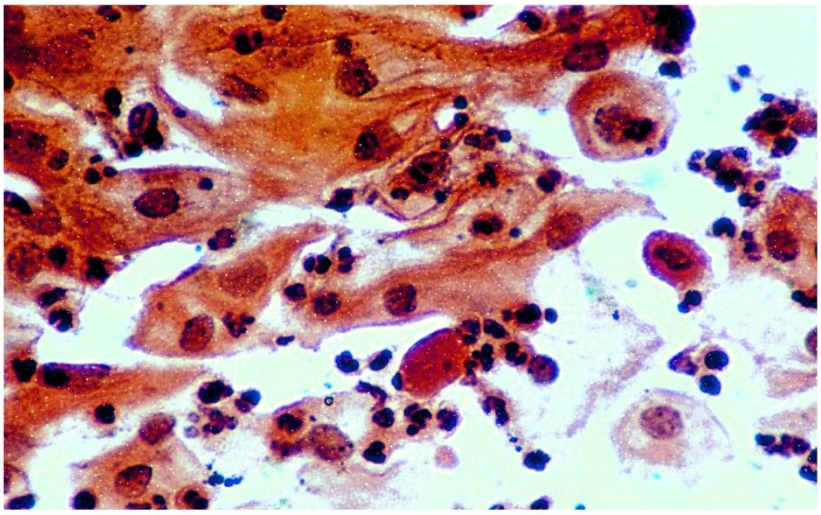
Tight collections of neutrophils overlying the superficial cells (BB shot), Intermediate cells and RBCs X40 PAP



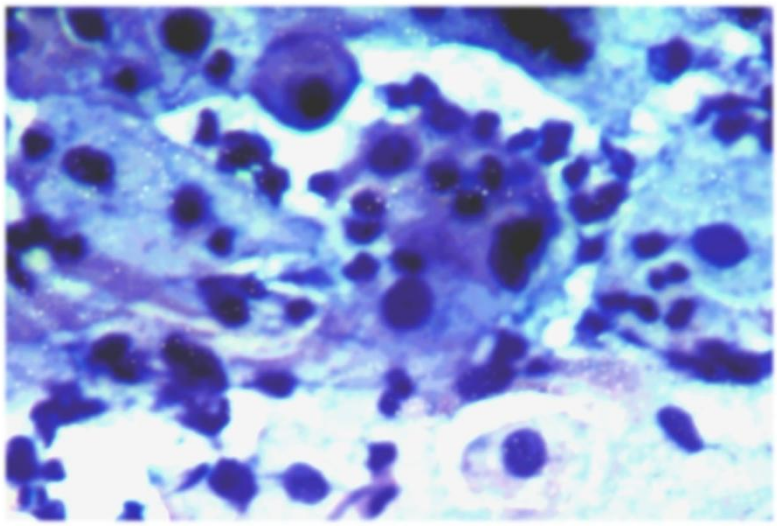
Candida hyphae showing branching



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[13] 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%). [10]

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%). 201cases (8.3%) of LSIL and 7cases (0.2%) of HSIL were observed in the 2nd decade.[10]

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%). [10]

In the present study, 800 women were cytology-cally 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

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