A STUDY OF PAPANICOLAOU [PAP] STAIN AND CERVICAL ACID PHOSPHATASE - PAPANICOLAOU [CAP-PAP] STAIN IN THE DETECTION OF CERVICAL INTRA-EPITHELIAL NEOPLASM - A COMPARATIVE STUDY

By

DR. RAJINI. T

Dissertation submitted to the
SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH, KOLAR, KARNATAKA

DOCTOR OF MEDICINE

in

PATHOLOGY

Under the guidance of

Dr. SUBHASHIS DAS
Professor of Pathology

Department of Pathology
SRI DEVARAJ URS MEDICAL COLLEGE
Tamaka, Kolar.

2018

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Dr. SHEELA S.R.
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Tamaka, Kolar.

2018

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DECLARATION BY THE CANDIDATE

I, Dr. RAJINI. T, hereby declare that this dissertation entitled, **"A Study of Papanicolaou [Pap] Stain and Cervical Acid Phosphatase - Papanicolaou [Cap-Pap] Stain in The Detection of Cervical Intra-Epithelial Neoplasm - A Comparative Study",** is a bonafide and genuine research work carried out by me under the guidance and direct supervision of Dr. SUBHASHIS DAS, Professor, Department of Pathology, Sri Devaraj Urs Medical College Tamaka, Kolar.

Date:

Place: Kolar

Signature of the candidate

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CERTIFICATE BY THE GUIDE

This is to certify that the study entitled **"A Study of Papanicolaou [Pap] Stain and Cervical Acid Phosphatase - Papanicolaou [Cap-Pap] Stain in The Detection of Cervical Intra-Epithelial Neoplasm - A Comparative Study",** is a bonafide research work done by Dr RAJINI. T, in partial fulfilment of the requirement for the degree of Doctor of Medicine in Pathology.

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CERTIFICATE BY THE CO-GUIDE

This is to certify that the study entitled **"A Study of Papanicolaou [Pap] Stain and Cervical Acid Phosphatase - Papanicolaou [Cap-Pap] Stain in The Detection of Cervical Intra-Epithelial Neoplasm - A Comparative Study"** is a bonafide research work done by Dr. RAJINI. T in partial fulfilment of the requirement for the degree of Doctor of Medicine in Pathology.

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ENDORSEMENT BY THE HOD/PRINCIPAL OF THE INSTITUTION

This is to certify that the study entitled **"A Study of Papanicolaou [Pap] Stain and Cervical Acid Phosphatase - Papanicolaou [Cap-Pap] Stain in The Detection of Cervical Intra-Epithelial Neoplasm - A Comparative Study",** is a bonafide research work done by Dr Rajini. T, under the guidance and supervision of Dr. Subhashis Das, Professor, Department of Pathology, Sri Devaraj Urs Medical College, Kolar, in partial fulfilment of the requirement for the degree of Doctor of Medicine in Pathology.

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ETHICAL COMMITTEE CERTIFICATE

This is to certify that the ethical committee of Sri Devaraj Urs Medical College, Tamaka, Kolar has unanimously approved the work of Dr Rajini T, a postgraduate student in the department of Pathology of Sri Devaraj Urs Medical College, entitled **A Study of Papanicolaou [Pap] Stain and Cervical Acid Phosphatase - Papanicolaou [Cap-Pap] Stain in The Detection of Cervical Intra-Epithelial Neoplasm - A Comparative Study",** to be submitted to the Sri Devraj URS Academy of Higher Education and Research, Tamaka, Kolar, Karnataka**.**

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Signature:

Principal,

Sri Devaraj Urs Medical College,

Kolar.

ACKNOWLEDGEMENT

While I was working on this thesis, I would often dream of the moment when I would get to this part: when the manuscript would be written, the final stress would be gone, and I would look back at this period of my life with pleasure, nostalgia and gratitude. Finally, the moment has come. The process has been enjoyable. So, I am glad to complete it by remembering many wonderful people who have contributed to it in various ways.

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Thanks to one and all.

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Kolar.

LIST OF ABBREVIATIONS

AGUS	-	Atypical glandular cells of unknown significance
ASC-H	-	Atypical squamous cells cannot exclude an HSIL
ASCUS	-	Atypical squamous cells cannot exclude an HSIL
CAP	-	Cervical acid phosphatase
CAP-PAP	-	Cervical acid phosphatase – Papanicolaou stain
CIN	-	Cervical intraepithelial neoplasia
DNA	-	Deoxy ribose nucleic acid
FIGO	-	International Federation of Gynaecology and Obstetrics
HCG	-	Human chorionic gonadotropin
HIV	-	Human immunodeficiency virus
HLA	-	Human leukocyte antigen
HPV	-	Human papilloma virus
HSIL	-	High grade squamous intraepithelial lesion
HSV	-	Herpes Simplex Virus
IARC	-	International Agency for Research on Cancer
IUD	-	Intrauterine device
LSIL	-	Low grade squamous intraepithelial lesion
NIH	-	National Institutes of Health
No	-	Number
NPV	-	Negative Predictive value
PAP	-	Papanicolaou

LIST OF ABBREVIATIONS

PBCR	-	Population Based Cancer Registry
PMN	-	Polymorphonuclear leukocytes
PPV	-	Positive Predictive Value
TRAP	-	Telomeric Repeat Amplification Protocol
WDPV	-	White Discharge per vagina
WHO	-	World Health Organisation

ABSTRACT

BACKGROUND :

CAP Pap test is a new test developed as an adjunct to routine PAP to improve its sensitivity. This test combines a simple biochemical test of enzyme labelling with conventional Pap Test in which abnormal squamous cells of the cervix are labelled for the presence of the lysosomal enzyme, Cervical Acid Phosphatase (CAP). Visualization of CAP inside abnormal cervical cells on smears makes this enzyme a biomarker for cervical dysplasia, and hence a possible surrogate for PAP smear in detection of CIN.

OBJECTIVES :

1.To perform CAP-PAP and PAP staining on cervical smears.

2.To compare CAP-PAP results with PAP results.

3.To correlate CAP-PAP and PAP results with cervical histopathology reports.

METHODS :

The study comprised of 150 females who underwent cervical cancer screening at Obstetric and Gynaecological Department R L Jalappa Hospital and Research Centre during Jan 2016 to December 2017. Two cervical smears were collected from all patients and subjected to PAP and CAP-PAP staining. Histopathology was the gold standard test against which PAP and CAP-PAP results were compared and correlated.

RESULTS :

75 PAP positive cases out of which 34 were LSIL, 17 were HSIL, 13 were ASCUS, 9 were ASC-H, and 2 were SCC cases. In the remaining 75 PAP negative smears, 38 cases were inflammatory smears and 37 cases were of NLIM smears. Among the PAP positive cases, CAP-PAP results were similar to PAP results in all the LSIL, HSIL, ASCUS, ASC-H and SCC cases. Among the PAP negative cases, CAP-PAP was negative in all the 37 NILM cases, but of the 38 inflammatory cases, CAP-PAP was negative in 30 cases but was positive in 8 cases. CAP-PAP results had good agreement with PAP results and correlated well with histopathology results. CAP PAP showed greater sensitivity(100%) as compared to Pap test(93.67%), while specificity(89.33%) was little lesser than Pap(98.59%).

CONCLUSION :

CAP positivity helps in easy and early detection of abnormal cells because of red colored granules which are easily identified and thus speeds up the screening process. CAP-PAP fulfils the criteria of screening test and serve as a quick, economical and efficient method for large scale screening of cervical cancer. The test promises a great future at health centers where trained persons are not available as technicians can be easily trained for identification of abnormal smears. A larger trial is required before the widespread use of CAP-PAP staining can be recommended for clinical use.

Key Words: Cervical cancer; cytology; cervical acid phosphatase; screening.

TABLE OF CONTENTS

.

LIST OF TABLES

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES

LIST OF CHARTS

INTRODUCTION

INTRODUCTION

Cervical cancer ranks as the most common cancer among women in India, and most frequent cancer among women between 15 and 44 years of age.[1] Cervical smear cytology screening by Papanicolaou (PAP) - stained smears is the most efficacious and cost-effective method of cancer screening in developed countries, decreasing the incidence and mortality from cervical cancer.[2] However, cervical smear screening has significant rates of false-positive and false-negative results.[3,4]

To improve the detection of precancerous cervical lesions using Pap smear screening, a number of adjunctive tests have been developed including thin layer cytology;[5] use of magnified chemiluminescent screening examination (speculoscopy) combined with Pap smear (Papsure);[6] combined cytology and cervicogram[7] automated rescreening methods; detection of Human Papilloma Virus DNA (HPV DNA) by the Hybrid Capture 2 or polymerase chain reaction tests; evaluation of telomerase repeat activity by the Telomeric Repeat Amplification Protocol (TRAP), Fourier-Transform Infrared (FTIR) Spectroscopy and immunocytochemical detection of p16INK4a protein.[8] However, limited by their prohibitive cost factor and unavailability beyond few tertiary care referral centres, these newer technologies have no role in large scale screening programs of developing countries.

Recently, an inexpensive modification of the conventional Pap test, viz., cervical acid phosphatase-Papanicolaou test (CAP-PAP Test; MarkPap® Test) has been described. In this test, abnormal squamous cells of the cervix are labelled for the presence of the lysosomal enzyme, cervical acid phosphatase (CAP).[3,9-18] Cytochemistry for CAP results in red, granular deposits against a modified Papanicolaou background. CAP is not

present in the squamous cells of the normal female genital tract. Endocervical cells and monocytes however, contain CAP.[12] Preneoplastic lesions of the cervix arise in the transformation zone, which contains CAP. Hence, abnormal cells of both Low- and High-Grade Squamous Intraepithelial Lesions (LSIL and HSIL, respectively) are positive for CAP. Other lesions like ASC-H (Atypical Squamous Cells cannot rule out High-Grade SIL) and other Cervical Intraepithelial Neoplasia (CIN) lesions also express CAP.

Studies conducted decades ago have described acid phosphatase in cervical cancer, preneoplastic lesions of the cervix and in vaginal secretions of patients suffering from cervical and uterine cancer.[8] Hence it has become possible to explore this enzyme as a biomarker for cervical dysplasia, and as an adjuvant for PAP smear in detection of cervical intraepithelial neoplasia. Thus, this study was undertaken to assess the utility of CAP-PAP in addition to routine Pap smears as an aid for visualization of abnormal squamous cells by its correlation with the gold standard test, cervical histopathology.

AIMS and OBJECTIVES

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1. To perform Cervical Acid Phosphatase - Papanicolaou (CAP-PAP) and Papanicolaou (PAP) staining on cervical smears.
2. To compare CAP-PAP results with PAP results.
3. To correlate CAP-PAP and PAP results with histopathology reports.

REVIEW of

LITERATURE

REVIEW OF LITERATURE

EMBRYOLOGY

The paramesonephric ducts appear in human embryo at about 40 days. Each duct develops as thickening and invagination of the coelomic epithelium on the lateral aspect of the intermediate mesoderm.[19]

The site of invagination later becomes the abdominal ostium of the uterine tube. In the female fetus, the absence of testicular hormones allows regression of the mesonephric ducts and development of the paramesonephric ducts. The upper segment of each paramesonephric duct develops fimbriae at its cephalic end and subsequently forms the uterine tube. Uterus develops from the fused part of paramesonephric ducts.[20]

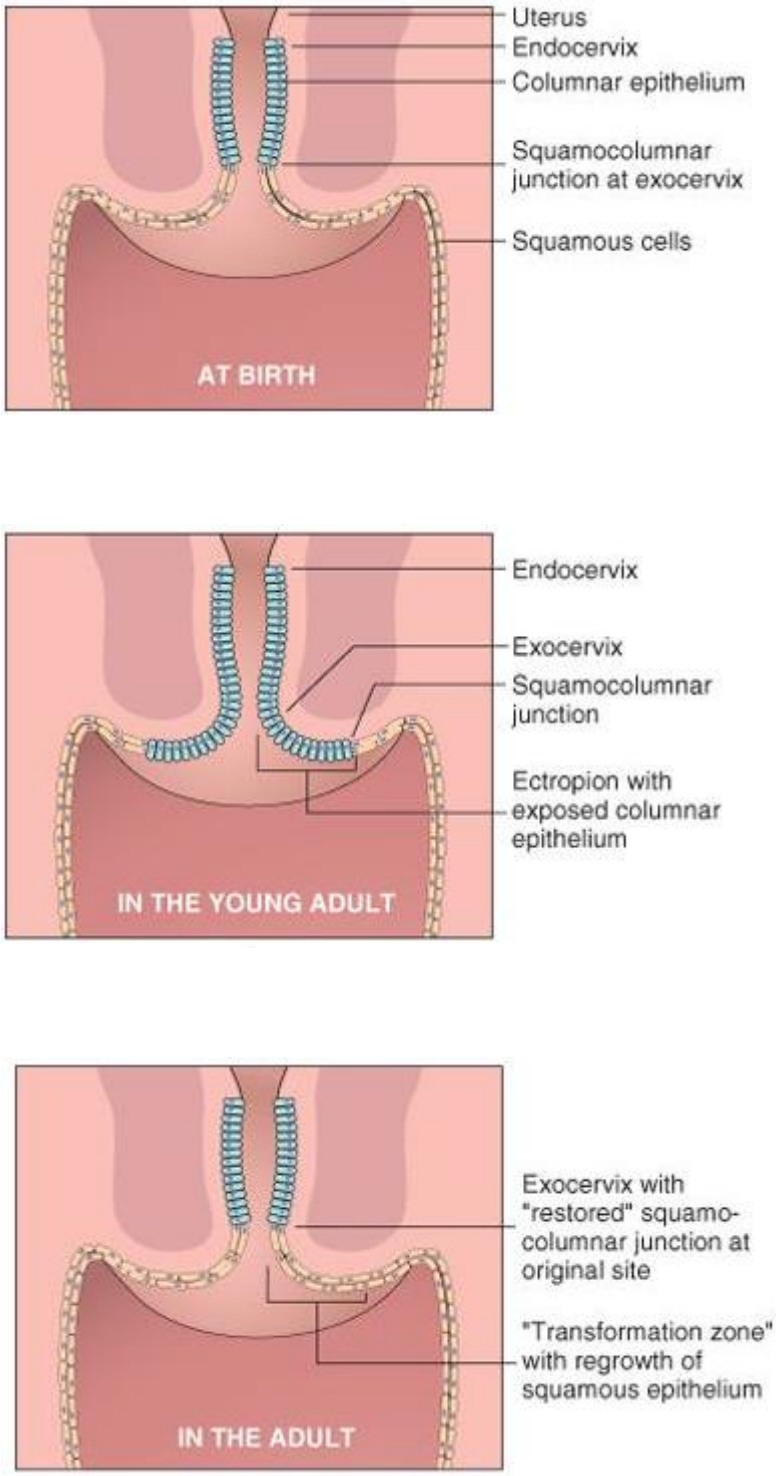
The cervix which forms the distal two-thirds of the fetal uterus is of paramesonephric origin.[21] Its mucous membrane is derived from the urogenital sinus[22]. About the 17th week, cervical glands appear. At 22 weeks, the cervical canal is lined by stratified squamous epithelium and at term, the squamo-columnar junction is situated external to the os.[23]

ANATOMY[20]

Uterus is a hollow, thick walled organ situated between the bladder and rectum. It is divided into Body or Corpus and Cervix. The body of the uterus receives the fallopian tubes and the portion of corpus uteri above this level is the fundus.

The uterine cervix is about 2.5 cm in length. Inferiorly, the cervix projects into the vault of the vagina and is enclosed by the vaginal fornices. This anatomical arrangement divides the cervix into supravaginal and vaginal segments. The cervix has a covering epithelium with an underlying stroma that is composed predominantly of elastic tissue with a small amount of smooth muscle as shown in figure 1.

Fig 1 : Diagrammatic representation of cervical epithelium in different age groups



HISTOLOGY AND CYTOLOGY OF THE NORMAL CERVIX[24,25]

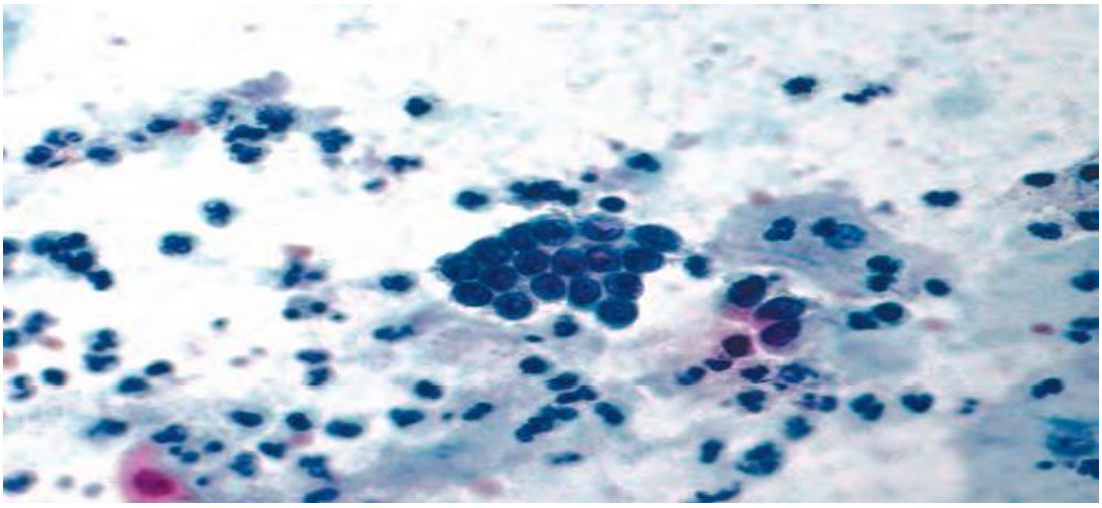
EPITHELIUM OF THE CERVIX

The squamous epithelium has three zones.

1. The Basal or Germinative layer: is composed of one row of small elliptical cells measuring approximately 10μm in diameter with vesicular nuclei. The nuclei display nucleoli and occasional mitoses. The process of epithelial regeneration is confined to the basal layer.

CYTOLOGY : The basal cells are practically never seen in smears. These cells are very small, round to oval cells with very scant basophilic cytoplasm as shown in figure 2. The nuclei appear to be larger and have fine chromatin with occasional nucleoli. These cells have high nucleo-cytoplasmic ratio.

Fig 2 : Basal cells of cervical epithelium. [40X]



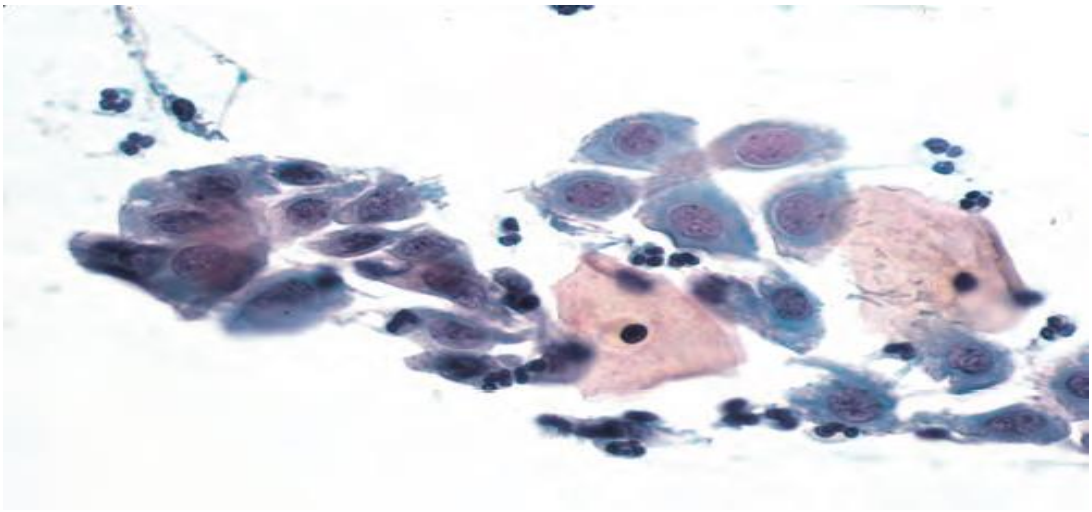
2. The Midzone: is composed of maturing squamous cells and comprises the parabasal and intermediate layers. The two or three layers of smaller cells of the deeper portion of the midzone are designated as parabasal cells. The larger cells adjacent to the superficial

zone, form the intermediate cell layers. As the maturation of the epithelium progresses towards the surface, the amount of cytoplasm per cell increases whereas, the sizes of the vesicular nuclei remain fairly constant.

CYTOLOGY : Parabasal cells

The parabasal cells measure from 12 to 30 μm in diameter. The cytoplasm is basophilic, and the nuclei are vesicular. Few cells of this type are seen in normal smears from women in their 20s and early 30s, but their number increases in women more than 35 years of age. Such cells may become the dominant cell type in postmenopausal women. In the presence of inflammatory processes, the proportion of parabasal cells in the smears may increase substantially. Immature parabasal cells generally lie in sheets, while the more mature cells are usually dissociated as shown in figure 3.

Fig 3: Parabasal cells of cervical epithelium [40X]



Intermediate squamous cells[25]

It is particularly prominent in pregnancy and with the use of progestational agents. The nucleus is larger than that of the superficial cell, with a cross-sectional nuclear area of 35 μm^2 and a finely granular chromatin pattern. The nucleus is often elongate with a longitudinal nuclear groove. The intermediate cell nucleus serves as the basic size reference for other cells in cervical cytology.

3. The Superficial zone: is composed of several layers of loosely attached cells that are still larger than intermediate cells. The nuclei of these cells are smaller and pyknotic.

CYTOLOGY : Superficial squamous cells vary from 35 to 45μm in size. They are large and polygonal and have eosinophilic cytoplasm. The staining properties of cytoplasm depend on the state of maturation of these cells. Their nuclei are pyknotic. Nuclear pyknosis represents the last stage in the maturation process of the squamous epithelium.

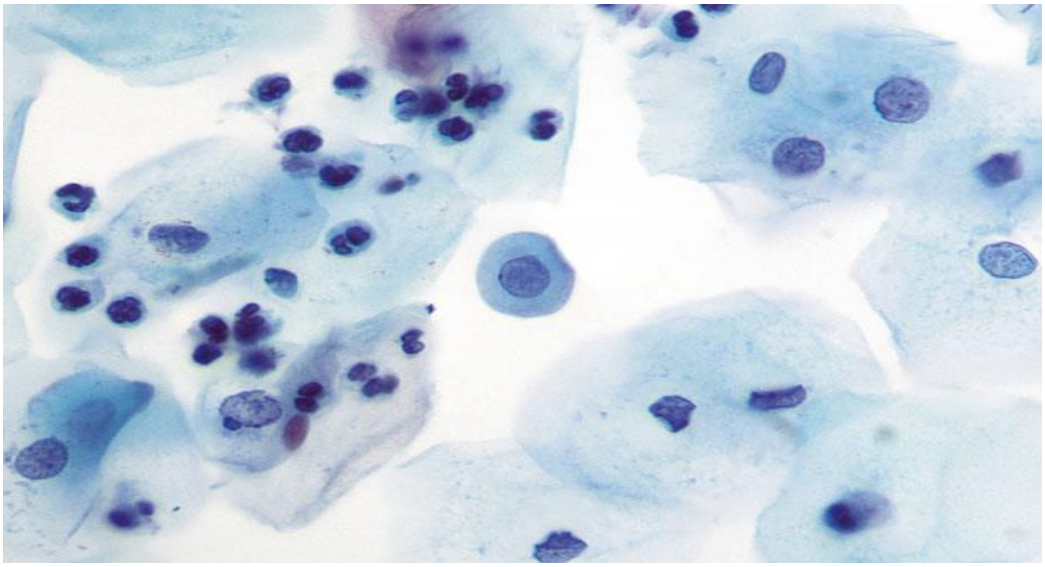
The Squamo-columnar junction (Transformation zone)

In a normal quiescent cervix, the transition is often a sharp one and is designated as squamo-columnar junction. The anatomic location may vary and may be found at the external os, inside the endocervical canal or on the surface of the portio. In many women, the squamo-columnar junction is not static and frequently transformation of the endocervical epithelium into squamous epithelium takes place in this area of the cervix. Hence the name transformation zone has been given to this area.

CYTOLOGY : Squamous metaplastic cells which show a range of cytoplasmic differentiation from immature parabasal-like cells to those that approximate the

appearance of differentiated intermediate/superficial cells. The mean nuclear area is larger than that of the intermediate cell and similar to the parabasal cell as shown in figure 4.

Fig 4 : Squamo-columnar junction cells of cervical epithelium [40X]



The Endocervical epithelium

The epithelial lining of the endocervical canal and of the endocervical glands are formed by a single layer of mucin producing tall columnar cells with oval nuclei which are basally located. The endocervical glands are of the simple tubular branching type.

CYTOLOGY : Endocervical cells appear in tight clusters and resemble a honeycomb because of the clear cytoplasm surrounding the nuclei. The cytoplasm is usually very finely vacuolated and faintly basophilic. The nuclei are finely granular and are of approximately the same size as the nuclei of intermediate and parabasal squamous cells, 1-2 small nucleoli are frequently observed as represented in figure 5.

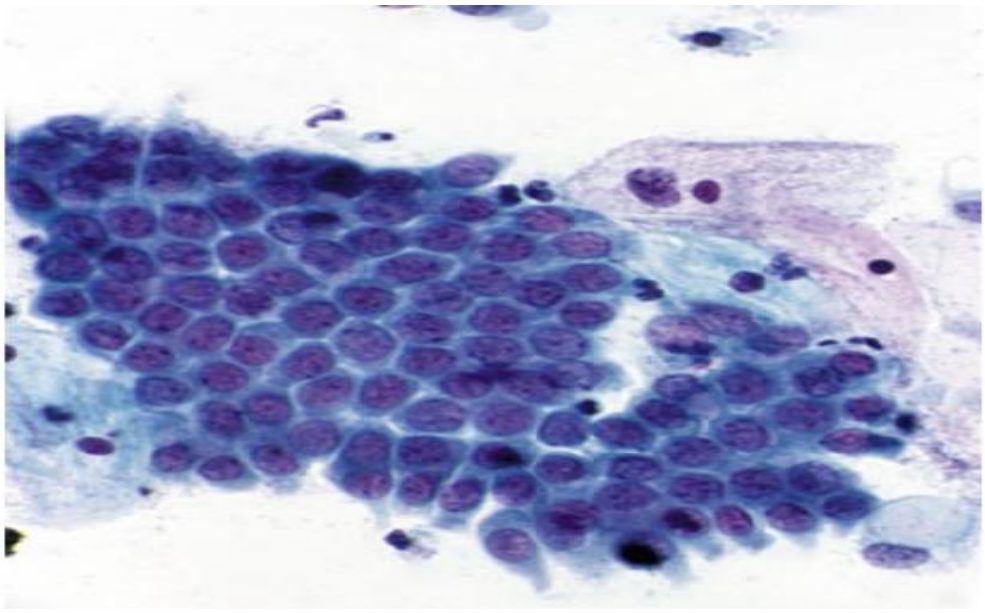


Fig 5 : Endocevical cells of cervical epithelium [40X]

Other types of cells seen in normal smears:

1. Endometrial cells:

   Normally seen in cervical smears from the time of beginning of menstrual flow until the 10th or 12th day of the cycle. The finding of endometrial cells in vaginal smears after the 12th day is abnormal. In smears, endometrial cells most often appear in the form of round or oval cell clusters with a central core made of small, elongated tightly packed stromal cells and at the periphery, larger glandular cells with vacuolated cytoplasm and round to oval nuclei.

2. Leucocytes:

   Lymphocytes and neutrophils are frequently present even in the absence of inflammation. Plasma cells are uncommon and signify chronic inflammation.

3. Macrophages:

   Smaller mononuclear macrophages are seen during the late part of the menstrual bleeding. Larger size and multinucleation are associated with menopause or inflammatory processes.

PHYSIOLOGY[26]

Physiological changes in cervix:

- Birth to seven days: Cervical epithelium of the new born is fully mature showing mature superficial squamous cells due to maternal hormones.
- From the first week to puberty: Cervical smears normally manifest atrophy with many parabasal cells.
- Puberty: The cervical epithelium slowly reaches maturity. At first many intermediate epithelial cells are seen and when menstrual cycles begin, many superficial cells are also present.

Menstrual cycle

1. Menstrual phase (1st - 5th day): The smear contains erythrocytes, leucocytes, endometrial cells, superficial and intermediate squamous cells.
2. Proliferative phase (6th - 10th day): A large number of histiocytes in loose aggregates are seen. Intermediate squamous cells, polymorphs and endometrial cells are present.
3. Ovulation (11th - 13th day): There are numerous superficial epithelial cells which are discrete.

4. Secretory phase (14th - 28th day): Intermediate squamous cells predominate. Polymorphs and Doderlein's bacilli are seen.

Pregnancy:

The cytological pattern of pregnancy is that of progesterone activity, with clustering and folding of navicular cells. These cells are distended with glycogen, becoming boat shaped with a rim of folded cytoplasm and the nucleus pushed to periphery.

Post-partum and lactation :

Smear pattern is chiefly composed of parabasal epithelial cells, which are somewhat angular and contain glycogen or may keratinize. Such cells are called lactational or postpartum cells.

Postmenopausal smear :

In the early postmenopausal phase there are many flat dispersed intermediate epithelial cells. With advancing age, parabasal cells predominate and they are arranged singly or in variable sized sheets of undifferentiated cells with scant cytoplasm.

CERVICAL CANCER

Cancer is an uncontrolled division of abnormal cells in a part of the body. Cancer is derived from the Latin word for crab, i.e., they adhere to any part that they seize in an obstinate manner. Cancer can affect all living cells in the body, at all ages and in both genders. The causation may be multi-factorial and the disease processes may differ for cancers at different sites.

Although cancer of the cervix can develop in women of all ages, it usually develops in women aged 35-55 years, with the peak age for incidence varying with populations. In India, the peak age for cervical cancer incidence is 45-54 years, which is similar to rest of South Asia [WHO/ICO Information Centre on HPV and Cervical Cancer].[27] In terms of incidence rates, Cervical cancer is the leading cancer among women in 2 out of the 12 Population Based Cancer Registries (PBCRs) in India and has the second highest incidence rate after breast cancer in the rest of the PBCRs. The age adjusted incidence is highest in Chennai, a metropolitan city in the south, and lowest in Thiruvananthapuram, the capital of Kerala. There is a high incidence belt in the north eastern districts of Tamil Nadu, as well as in two districts in the North-Eastern region of the country.

CERVICAL CANCER BURDEN

As per the Global Cancer Burden of 2012[27], cervical cancer was the 4th most common cause of cancer death among women in the world, and had:

- 528,000 new cases
- An age-standardized incidence rate (global) of 16 per 100,000 women in 2002
- 1-year prevalence of 528,000 and 5-year prevalence of 1.55 million in 2012

- 2,66,000 deaths (7.5% of all cancer deaths)

CERVICAL CANCER IN INDIA[27]

India has a disproportionately higher burden of cervical cancer accounting for nearly one-third of global cervical cancer deaths. There is considerable excess mortality from cervical cancer in India compared to the rest of the world and the South Asia region.

According to IARC estimates, mortality from cervical cancer is expected to witness a 79% increase from 74,118 deaths in 2012 to 1,32,745 deaths by 2025.

- Age-standardized incidence rate of 16.7 per 1,00,000 women in 2012
- 1-year prevalence of 1,23,000, and 5-year prevalence of 3,09,000 in 2012
- 67,000 deaths (nearly 10% of all female cancer deaths)

AETIOLOGY OF CERVICAL NEOPLASM[28]

1. Infectious agents
   i) Human Papilloma Virus: HPV types 7, 11, 42, 44 are associated with low grade CIN and only rarely with invasive tumours. Low-grade CIN which is progressive, high-grade CIN and invasive carcinomas are associated mainly with HPV types 16, 18 & 33 (But also 31, 35, 39, 45, 51, 52, 56 and 58). [29,30]
   ii) Herpes Simplex Virus : The epidemiological evidence for involvement of Herpes simplex virus in cervical carcinogenesis is suggestive. Two hypotheses have been formulated to explain it are : The 'hit and run' hypothesis and synergism between HSV and HPV.[29]

2. Sexual factors: More common in women who have had multiple sexual partners, who are promiscuous. It is absent in virgins.[31]
3. Smoking: Overall two-fold increase in risk for the development of cervical intra-epithelial neoplasia and invasive cervical cancer.[32]
4. Oral contraceptives: There is a significantly increased risk of cervical cancer in patients who have used oral contraceptives, the incidence increasing with the duration of use.[33]
5. Socio-economic condition and parity: There is increased incidence of cervical cancer in women of low socio-economic status and in multiparous women.[34]
6. Immunosuppression and cervical cancer: An increased incidence of CIN has been described in patients who have received renal transplants and in patients who are infected with HIV.[35]
7. Diet and cervical cancer: Some studies show an inverse relationship between the intake of vitamin A and C and the risk of cervical cancer.[36]
8. Host Factors: Patients who are HLA DQW3 positive are at a greater risk of cervical neoplasia because of a reduced ability to clear HPV infection.[37]

Cytology And Histopathology Of Pre-Malignant And Malignant Lesions Of Cervix[24,28]

1. Low-grade Squamous Intra-Epithelial Lesion (LSIL) (CIN I and HPV changes)

Cytology:

- Cells occur singly and in sheets.
- Cytologic changes are usually confined to cells with "mature" or superficial type of cytoplasm.
- Overall cell size is large, with fairly abundant well-defined cytoplasm.
- Nuclear enlargement with slightly increased nucleo-cytoplasmic ratio.
- Nuclear hyperchromasia.
- Uneven distribution of chromatin.
- Irregularity of the nuclear membrane.
- Nucleoli are generally absent or inconspicuous.
- Binucleation and multinucleation are common.
- Koilocytosis, consisting of sharply delineated clear perinuclear zone and a peripheral rim of densely stained cytoplasm is a characteristic feature but not required for the interpretation of LSIL.[38]

Histopathology:

Maturation present in the upper two-thirds of epithelium. Loss of polarity of cells in the basal third of the epithelium due to the presence of abnormal immature cells replacing the normal basal and parabasal layers.[39] The immature cells are neoplastic and have nuclear abnormalities like dyskaryosis. Mitoses are not

infrequent. Normal or abnormal mitotic figures can be seen in the basal third of the epithelium in up to 50% of cases of CIN I.[40]

2. High- grade Squamous Intraepithelial Lesion (HSIL)(CIN II and CIN III)

Cytology:

- Cytologic changes affect cells that are smaller and less "mature".
- Cells occur singly, in sheets or in syncytium.
- HSIL is characterized by smaller cells of basal or parabasal type with rounded borders showing dense cytoplasm and pronounced nuclear abnormalities like markedly increased nucleo-cytoplasmic ratio, hyperchromasia, nuclear irregularity and coarse chromatin clumping.

Histopathology:

CIN II - Maturation is present in the upper third of the epithelium. Nuclear abnormalities are more marked than in CIN I and extend further through the epithelium. Mitotic figures are present and are confined to the basal two-thirds of the epithelium. Abnormal forms may be seen.

CIN III - Maturation may be absent or confined to the superficial third of the epithelium. Nuclear abnormalities are marked throughout most of the thickness of the epithelium. Mitotic figures are numerous and are found at all the levels of the epithelium. Abnormal mitoses are frequent.

3. Micro-invasive carcinoma

Cytology:

Micro-invasive carcinoma cannot be diagnosed reliably from a smear because the severe dysplasia of CIN III may be morphologically indistinguishable from severe dysplasia of invasive carcinoma.[41]

Histopathology:

Approximately 4% to 7% of CIN lesions have been associated with superficial invasion and is a subset of invasive squamous cell carcinoma of the cervix, which is termed as micro invasive carcinoma.[42] The term is restricted to cervical carcinoma that infiltrates the cervical stroma by not more than 3 mm to 5 mm in depth and is defined as FIGO stage Ia1 when the depth is less than 3 mm.[43]

4. Invasive squamous cell carcinoma and its variants

Invasive squamous cell carcinoma is defined as a malignant neoplasm that invades the underlying stroma of the cervical epithelium by infiltration.

Reagan's sub-classification of squamous cell carcinoma[44]

1. Large cell keratinizing carcinoma or well-differentiated squamous cell carcinoma.
2. Large cell non-keratinizing carcinoma or moderately differentiated squamous cell carcinoma.
3. Small cell non-keratinizing carcinoma or poorly differentiated carcinoma.

Cytology[45]

Non-Keratinizing carcinoma

Cells are present in syncytial aggregates and are round to oval with moderate variations in size and shape. The cells have abundant cyanophilic cytoplasm, large, round to oval nuclei. The nuclear chromatin is hyperchromatic, irregularly distributed and coarsely granular. Nucleoli are found, with macro nucleoli in less differentiated tumors. The presence of extensive necrosis, cellular debris and blood cells in the background should raise the suspicion of a malignant process.

Keratinizing carcinoma

The tumor cells may be elongated, caudate or bizarre which frequently lie singly. The cells have large amounts of cytoplasm, which is eosinophilic. Large irregular cells and cytoplasmic eosinophilia are characteristic of keratinizing cancer. Nuclei are elongated and irregular. Bizarre nuclear forms may be present. Nuclear chromatin is hyperchromatic and coarsely granular.

Small cell carcinoma

The tumor cells are relatively small with large nuclei and scanty cyanophilic cytoplasm causing a high nucleo-cytoplasmic ratio. The nuclei may be round to oval but are more often irregular and have a hyperchromatic, coarsely granular chromatin. Nucleoli are usually present.

Histopathology[45,46]

Non-keratinizing carcinoma

They contain cells which are generally recognizable as squamous from their polygonal shape and there may be little keratin formation and individual cell keratinization, but keratin pearls are not seen. Cellular and nuclear pleomorphism is more obvious than in the well- differentiated tumors and mitotic figures are usually quite numerous.

Keratinizing carcinoma

These carcinomas are composed of characteristic epidermoid cells and the most striking features are the circular whorls of cells with central nests of keratin. Intercellular bridges contain kerato-hyaline granules. The nuclei are usually large and hyperchromatic with coarse chromatin. Mitotic figures are not frequent and are usually seen in the less well-differentiated cells at the periphery of the invasive masses.

Small cell carcinoma:

It is characterized by small, round cells with high nucleo-cytoplasmic ratio. Epithelial pearls and individual cell keratinization are not observed. A high mitotic index is seen. There is frequently an inflammatory cell infiltrate composed mainly of lymphocytes and plasma cells in the stromal tissue between the invasive islands of the tumor. These cells represent the hosts immune response against the tumor antigens.

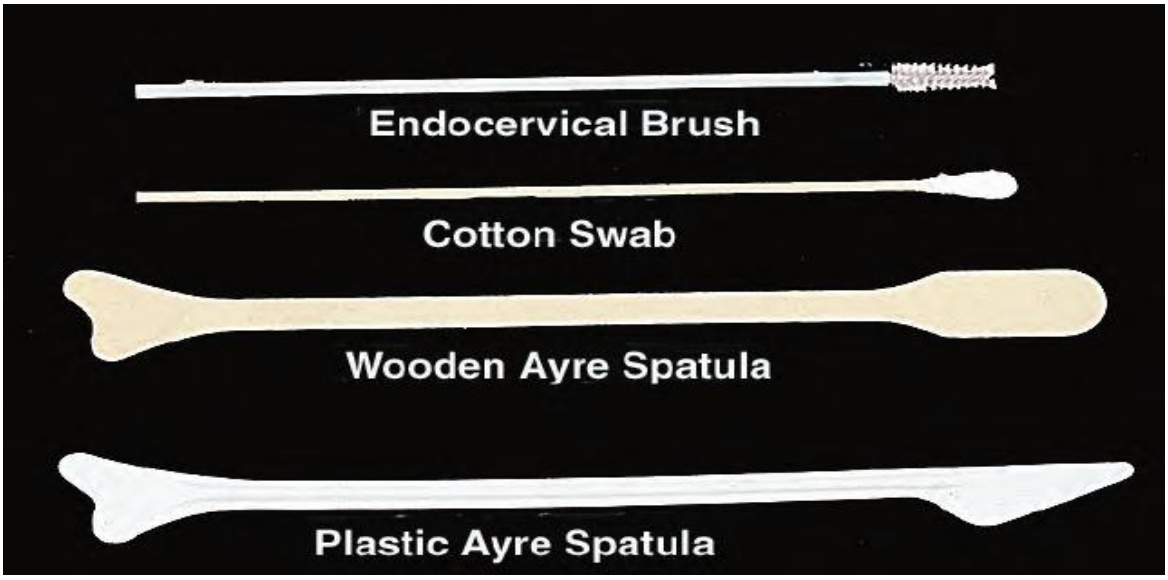
SCREENING OF CERVICAL CANCER

Pap test is the most used and probably the most successful and economical cancer prevention measure currently available. It is recommended for prophylaxis of healthy women.[47] The staining procedure was introduced by George Papanicolaou in the years 1940.[48]

This procedure dramatically improved detection of cervical dysplasia leading to aggressive treatment including surgery. As a result, many lives were saved.[49] Before systematic administration of Pap test, cervical cancer was among the leading causes of death in women with malignant disease. Pap test screening of healthy or oligo-symptomatic women resulted in sharp reduction of cervical cancer incidence and mortality rates. Reported are reductions of 80% (Iceland), 70% (U.S.), 50% (Finland) and 34% (Sweden). Recent WHO reports cite that about 4 of every 5 cases of cervical cancer occur in those countries without screening programs.[50]

Cervical smears and its variants are utilized for diagnosis of precancerous lesions and cancer of the uterine cervix. Regardless of the instrument used, the cervical smear must be obtained under direct vision after introduction of the unlubricated speculum. If there are difficulties in introducing the speculum, a few drops of normal saline solution may be used to moisten it. Several methods and instruments for securing cytologic material from the uterine cervix are available (Fig. 6). Although many are no longer used, they are generally much less expensive and more affordable in developing countries.

Fig 6: Instruments for sampling of the uterine cervix[24]



Pap test is based on cytological examination of excoriated (abraded), not exfoliative cells. Physicians, using a spatula and a brush scrape mucosal cells from the cervix, at the neck of the uterus, and smear them on a microscopic slide. Smears are sprayed with a fixative and sent to laboratories, where slides are stained by Papanicolaou staining.

Cytopathologists examine Pap smear under the microscope and make a cytological diagnosis (table 1). There are varying degrees of dysplasia defined by the degree of cellular atypia. All types of dysplasia must be observed and treated as precancerous.

Table 1: Classification Systems for cervical smears[51]

Sl. No	Dysplasia (Cytological)	CIN	The Bethesda System
1	Benign	Benign	Normal
2	Benign with inflammation	Benign with inflammation	Normal-Benign-Infection-Reactive-ASCUS/AGCUS
3	Mild dysplasia		Low grade SIL
	Moderate Dysplasia	CIN I	ASCUS/AGCUS Low grade SIL
	Severe Dysplasia	CIN II	High grade SIL
4	Carcinoma in Situ	CIN III	High grade SIL
5	Invasive Cancer	Invasive cancer	High grade SIL

Cyto-pathologists consider PAP smear as a medical consultation and will recommend further diagnostic procedures, treatment for infection and comment on factors that prevent adequate evaluation of the specimen.

The Pap test is purely a screening procedure. The presumptive diagnosis is made by cytologic screening of an asymptomatic population with no grossly visible cervical changes. All visibly abnormal cervical lesions should be examined by biopsy.

Histological examination of cervical tissue obtained by biopsy/hysterectomy is the "golden standard" for detection of cervical dysplasia. This standard is best defined by Cervical Intraepithelial Neoplasia (CIN) criteria for cervical cancer and the recent Bethesda System classification. The Pap negative women are referred for next screening test after 6 to 12 months. Pap smear positive and non-negative women are kept on

observation with a next exam after three months or they are referred to gynecological interventions - Colposcopy and Biopsy. This practice has produced dramatic reduction of cervical cancer mortality and morbidity in screened populations.[52]

These positive trends encouraged some of leading workers in the field of cancer prevention to believe that cervical cancer is a curable disease, and that we already have tools for its eradication. The major obstacle for reaching this ultimate goal of every disease prevention is the high rate of false negative readings of the Pap test, during the primary screening. False negative rates ranging from 1.1 to 69% have been reported in various literatures. The reason for false negatives is inherent to the Pap test itself, Sampling and Technical errors are under thorough investigation, and much effort has been given to improve both techniques.[47] Also, false positive cases are reported, with false positive rates ranging from 10.3% to 14.8%.[53]

In 1996, an NIH Consensus Conference on Cervical Cancer revealed that 20% of women with a single negative Pap test, developed cervical cancer in the next five years.[47] The Conference recommended increasing the frequency of screening (annually), to improve sampling techniques, and to improve staining and interpretation.

The interpretation of PAP test is done by The Bethesda System for reporting cervical cytology as described in the following section.[54]

The 2014 BETHESDA SYSTEM FOR REPORTING CERVICAL CYTOLOGY[54,55]

SPECIMEN TYPE:

Indicate conventional smear (Pap smear) vs. liquid-based preparation vs. other

SPECIMEN ADEQUACY

- Satisfactory for evaluation (*describe presence or absence of endocervical/transformation zone component and any other quality indicators, e.g., partially obscuring blood, inflammation, etc.*)

- Unsatisfactory for evaluation . . . (*specify reason*)
  - Specimen rejected/not processed (*specify reason*)
  - Specimen processed and examined, but unsatisfactory for evaluation of epithelial abnormality because of (*specify reason*)

GENERAL CATEGORIZATION (optional)

- Negative for Intraepithelial Lesion or Malignancy
- Other: See Interpretation/Result (*e.g., endometrial cells in a woman ≥45 years of age)*
- Epithelial Cell Abnormality: See Interpretation/Result (*specify* 'squamous' *or* 'glandular' *as appropriate)*

INTERPRETATION/RESULT

NEGATIVE FOR INTRAEPITHELIAL LESION OR MALIGNANCY

(W*hen there is no cellular evidence of neoplasia, state this in the General Categorization above and/or in the Interpretation/Result section of the report -- whether or not there are organisms or other non-neoplastic findings*)

NON-NEOPLASTIC FINDINGS (*optional to report optional to report; list not inclusive*)

- Non-neoplastic cellular variations
  - Squamous metaplasia
  - Keratotic changes
  - Tubal metaplasia
  - Atrophy
  - Pregnancy-associated changes
- Reactive cellular changes associated with:
  - Inflammation (includes typical repair)
- Lymphocytic (follicular) cervicitis
  - Radiation
  - Intrauterine contraceptive device (IUD)
- Glandular cells status post hysterectomy

ORGANISMS

- *Trichomonas vaginalis*

- Fungal organisms morphologically consistent with *Candida* spp.
- Shift in flora suggestive of bacterial vaginosis
- Bacteria morphologically consistent with *Actinomyces* spp.
- Cellular changes consistent with herpes simplex virus
- Cellular changes consistent with cytomegalovirus

OTHER

- Endometrial cells (in *a woman ≥45 years of age)*

(Specify *if "negative for squamous intraepithelial lesion")*

EPITHELIAL CELL ABNORMALITIES

SQUAMOUS CELL

- Atypical squamous cells
  - of undetermined significance (ASC-US)
  - cannot exclude HSIL (ASC-H)
- Low-grade squamous intraepithelial lesion (LSIL) (*encompassing: HPV/mild dysplasia/CIN 1)*
- High-grade squamous intraepithelial lesion (HSIL) (*encompassing: moderate and severe dysplasia, CIS; CIN 2 and CIN 3)*
  - with features suspicious for invasion (*if invasion is suspected)*
- Squamous cell carcinoma

GLANDULAR CELL

- Atypical

– endocervical cells (NOS *or specify in comments)*

– endometrial cells (NOS *or specify in comments)*

– glandular cells (NOS *or specify in comments)*

• Atypical

– endocervical cells, favor neoplastic

– glandular cells favor neoplastic

• Endocervical adenocarcinoma in situ

• Adenocarcinoma

– endocervical

– endometrial

– extrauterine

– not otherwise specified (NOS)

OTHER MALIGNANT NEOPLASMS: *(specify)*

ADJUNCTIVE TESTING

Provide a brief description of the test method(s) and report the result so that it is easily understood by the clinician.

COMPUTER-ASSISTED INTERPRETATION OF CERVICAL CYTOLOGY

If case examined by an automated device, specify device and result

Atypical Squamous Cells – Undetermined Significance (ASC-US)

- **Definition:** ASC refers to cytologic changes suggestive of SIL, but which are qualitatively or quantitatively insufficient for a definitive interpretation as such [41,42.]
- **Criteria :**
  - Nuclei are approximately two and one half to three times the area of the nucleus of a normal intermediate squamous cell (approx. 35 mm^2) or twice the size of a squamous metaplastic cell nucleus (approximately 50 μm^2).[43]
  - Slightly increased ratio of nuclear to cytoplasmic area (N/C).
  - Minimal nuclear hyperchromasia and irregularity in chromatin distribution or nuclear shape.
  - Nuclear abnormalities associated with dense orangeophilic cytoplasm ("atypical parakeratosis"), cytoplasmic changes that suggest HPV cytopathic effect (incomplete koilocytosis) – including poorly defined cytoplasmic halos or cytoplasmic vacuoles resembling koilocytes but with absent or minimal concurrent nuclear changes.

Common ASC-H Patterns[55]

- **Small Cells with High N/C Ratios ("Atypical Immature Metaplasia")**
- **Criteria**
  - Cells usually occur singly or in small groups of less than ten cells; occasionally, in conventional preparations, cells may "stream" in strands of mucus.

- Cells are the size of metaplastic cells with nuclei that are about 1.5–2.5 times larger than normal.
- Nuclear to cytoplasmic ratio may approximate that of HSIL
- In considering a possible interpretation of ASC-H or HSIL, nuclear abnormalities such as hyperchromasia, chromatin irregularity, and abnormal nuclear shapes with focal irregularity favor an interpretation of HSIL.

Low-Grade Squamous Intraepithelial Lesion (LSIL)[55-60]

- Squamous cell changes associated with HPV infection encompass "mild dysplasia" and "CIN 1." Several studies have demonstrated that the morphologic criteria for distinguishing "koilocytosis" from mild dysplasia or CIN I vary among investigators and lack clinical significance. In addition, both lesions share similar HPV types, and their biologic behavior and clinical management are similar, thus supporting a common designation of LSIL.[44,45,46]
- **Criteria**
  - Cells occur singly, in clusters, and in sheets.
  - Cytologic changes are usually confined to squamous cells with "mature" intermediate or superficial squamous cell-type cytoplasm.
  - Overall cell size is large, with fairly abundant "mature" well-defined cytoplasm.

- Nuclear enlargement more than three times the area of normal intermediate nuclei results in a low but slightly increased nuclear to cytoplasmic ratio.
- Nuclei are generally hyperchromatic but may be normochromatic.
- Nuclei show variable size (anisonucleosis).
- Chromatin is uniformly distributed and ranges from coarsely granular to smudgy or densely opaque.
- Contour of nuclear membranes is variable ranging from smooth to very irregular with notches.
- Binucleation and multinucleation are common.
- Nucleoli are generally absent or inconspicuous if present.
- Koilocytosis or perinuclear cavitation consisting of a broad, sharply delineated clear perinuclear zone and a peripheral rim of densely stained cytoplasm is a characteristic viral cytopathic feature but is not required for the interpretation of LSIL.
- Cells may show increased keratinization with dense, eosinophilic cytoplasm with little or no evidence of koilocytosis.
- Cells with koilocytosis or dense orangeophilia must also show nuclear abnormalities to be diagnostic of LSIL; perinuclear halos or clearing in the absence of nuclear abnormalities does not qualify for the interpretation of LSIL.

High-Grade Squamous Intraepithelial Lesion (HSIL) [55-60]

- **Criteria**
    - The cells of HSIL are smaller and show less cytoplasmic maturity than cells of LSIL.
    - Cells occur singly, in sheets, or in syncytial-like aggregates.
    - Syncytial aggregates of dysplastic cells may result in hyperchromatic crowded groups of immature cells which should always be carefully assessed for nuclear abnormalities.
    - While overall cell size is variable, in general, the cells of HSIL are smaller than those of LSIL. Higher-grade lesions often contain quite small basal-type cells.
    - Degree of nuclear enlargement is more variable than that seen in LSIL. Some HSIL cells have the same degree of nuclear enlargement as in LSIL, but the cytoplasmic area is decreased, leading to a marked increase in the nuclear to cytoplasmic ratio.
    - Other cells have very high nuclear/ cytoplasmic ratios, but the actual size of the nuclei may be considerably smaller than that of LSIL, at times even as small as a normal intermediate cell nucleus.
    - Nuclei are generally hyperchromatic but may be normochromatic or even hypo chromatic.
    - Chromatin may be fine or coarsely granular and is evenly distributed.
    - Contour of the nuclear membrane is quite irregular and frequently demonstrates prominent indentations or grooves.

- Nucleoli are generally absent, but may occasionally be seen, particularly when HSIL extends into endocervical gland spaces or in the background of reactive or reparative change.
- Appearance of the cytoplasm is variable; it can appear "immature," lacy, and delicate or densely metaplastic; occasionally, the cytoplasm is "mature" and densely keratinized (keratinizing HSIL).

Keratinizing Squamous Cell Carcinoma[55-60]

- **Criteria**
  - Presents predominantly as isolated, single cells and less commonly in cellular aggregates.
  - Marked variation in cellular size and shape is typical, with caudate and spindle cells that frequently contain dense orangeophilic cytoplasm.
  - Nuclei vary markedly in area, nuclear membranes may be irregular, and numerous dense opaque nuclei are often present.
  - Chromatin pattern, when discernible, is coarsely granular and irregularly distributed with chromatin clearing.
  - Macro nucleoli may be seen but are less common than in nonkeratinizing squamous cell carcinoma.
  - Associated keratotic changes (hyperkeratosis or parakeratosis) may be present but are not sufficient for the interpretation of carcinoma in the absence of nuclear abnormalities.

- A tumor diathesis may be present but is usually less than that seen in nonkeratinizing squamous cell carcinomas.

The current screening protocols call for every woman to repeat the pap test at least once every year for the next five years. It is estimated, if healthy women comply with this schedule, they would have very low probability of developing cervical cancer within this period. However, the majority of women do not comply.[47] The false negatives in the PAP test are Sampling error (omission to bring to the microscopic slides with the abnormal cells otherwise present in vaginal fluids or cervical mucosa), and technical error (omission to detect abnormal cells present on smears).

Recent Improvements of PAP Test:

The NIH Consensus Conferences call for PAP test improvement was followed by major development in medical devices industry. Many new patents and instruments have been developed to improve the accuracy of the conventional Pap test. The new technologies are roughly classified into three categories:

Liquid based smear preparation[61]--to remove unwanted elements and drying artefacts. This technology provides excellent smears for image analysis assessment. However, it removes not only debris but also rare cells that may be diagnostic. (Early detection of cancer is based upon detection of a few malignant cells). Inflammatory cells are also removed. This may affect correct diagnosis.

Microscope tracking systems--cytologist's workstation that integrates specimen automated scanning stage. Human participation in decision making is an integral part of this technology.

Computer-assisted image analysis---to improve detection and interpretation of abnormal cells, helps human observer during the decision-making processes. It does not replace human assessment.

1) Thin Prep Pap Test

This is a liquid sampling technique. This method is intended to replace the Pap test (only in the Sample preparation phase). In this technology, a physician collects cervical sample in the usual manner, but rather than smearing a small portion of the cervical cells into a slide, a collection device is rinsed in a vial of preservative solution, capturing virtually the entire cell sample. Thin Prep is an instrument that disperses and filters the specimen to reduce blood, mucus and inflammation layer of cells to a microscopic slide. Thin Prep 2000 Processor is offered for diagnosis of cervical cancer, lung, bladder, gastrointestinal cancer, and for fine needle biopsy of thyroid and breast cancer.[61]

Disadvantage : The final sample does not represent the original sample obtained from a patient. Many “inflammatory” and other cells removed by this cleaning technology could contribute for diagnosis of the body condition. Many small cancer cells may also be lost during the procedure. Implementation needs additional training of Pap test providers and cytotechnologists and also requires buying new equipment, the Thin Prep 2000 Processor. In borderline cases, other techniques are needed to improve sensitivity. The use of this technology adds to the cost of the conventional Pap test.

2) PapNet.RTM./ AutoPap.RTM[62]

All "negative" slides are screened by an automated microscope with digital video output. Each cell and cell cluster are analyzed using neural network processing (NNP). NNP is a form of artificial intelligence that has the capacity to "learn," "recognize" and "generalize." Instrument selects 128 cells/clusters for senior cytotechnologist to review "atypical" images. Pap Net is particularly good for detection of small atypical cells of high grade lesions that mimic inflammatory cells. The cost is very high over the conventional Pap test cost.

Disadvantage of this system is a need for human (high qualified cytologist) interaction after the instrument selected 128 "characteristic" fields. Problems arise during assessment process. This instrument takes images at a single focus and magnification. This is an oversimplification of a microscope as an input device. When a human operator investigates cervical smears using microscopy, he/she always has option to increase magnification and change focus in order to clarify what he/she is seeing and to add more certainty into classification of findings. This is a 3D observation providing more information than any of 2D images or prints that are available to a cytologist interacting with images of microscopic fields already selected by a computer. Computer created images do not have the advantage of human selection.

3) Hybrid Capture.RTM.HPV Test.

The only approved system for detection of HPV in cervical smears needs women's DNA to identify HPV (infection that may contribute to cervical cancer). It is always

performed together with the Pap test for morphological determination. It can use Thin Prep solution for specimen collection.[63]

All devices described above qualify for category III medical devices, meaning they need to be at least equivalent (safety and efficacy) to an approved device (method) already on the market. However, the "gold standard" to measure safety and efficacy of Pap test related devices is the conventional Pap test itself. And this test has never been standardized or approved as a single in vitro diagnostic device.[50]

In view of the above problems, a new simple cytochemical stain was launched which utilized acid phosphatase detection in cervical epithelium as a marker for dysplasia.

Cervical Acid Phosphatase[CAP] Staining.

Introduction

Uterine cervix has been evaluated for a number of histo-chemical reactions including ribonucleic acid, glycogen, acid phosphatase, nonspecific esterase, glucuronidase, and phosphamidase for evaluation of benign and malignant lesions. Of these, acid phosphatase evoked considerable interest due to its easy intracellular localization using cytochemical techniques. High acid phosphatase activity was seen in normal basal cells, 'atypical' basal cells, histiocytes, and endocervical cells as well as in malignant squamous

cells. It was first proposed for its role in detecting atypical squamous cells on routine Pap smears by Markovic N et al .[3]

Acid phosphatases are very common enzymes. They are found in plants and animals. In humans they have been intensely investigated in prostate, liver, kidney and connective tissue, particularly blood cells.[64,65] As enzymes, they all release phosphate from organophosphate. About substrate preference, they have shown species and tissue specificity.

Demonstration of acid phosphatase in tissues and cells is based on enzyme catalysis of organophosphate substrate, capture of phosphate by a metallic ion (i.e. lead), or an organic radical (aromatic ring) by a diazonium salt, formation of a product which is insoluble at acid pH range (pH<5.0), and precipitation of a colorful, granular deposit at sites of enzyme activity (FRP). FRP is available for microscopic examination. The amount is measurable and is proportional to acid phosphatase activity.

Many human cell types and tissues contain acid phosphatase. In humans, acid phosphatase is confined inside lysosome.

Lysosomes are cytoplasmic bodies, 200 to 800 nm in diameter, containing acid phosphatase and other acid hydrolyses. They originate from Golgi membrane or from endoplasmic reticulum. The enzyme is lipoprotein bound and inactive. Lysosomes play important role in cell defense mechanism due to abundance of hydrolytic enzymes, lysosomes are involved in both physiologic processes as atrophy and involution, and pathologic processes as cytolysis, necrosis, metaplasia (cytolysosomes). If hydrolytic enzymes are released outside cell, they affect surrounding cells and membranes producing hydrolysis of their structures. It is believed, release of these enzymes from

malignant cells substantiate local spread (invasion) of cancer tissue.[12,64,65] Acid phosphatase is abundant in metabolically active cells in inflammation and malignancy.[64]

Medical literature contains much data related to acid phosphatase activity in different human cells and tissues.[64,65] Acquired knowledge is helpful as guidance for understanding the mechanisms involved in the cervical acid phosphatase testing. The alterations of synthesis, processing and trafficking of lysosomal enzymes in malignancy have been demonstrated. A consistent increase of lysosomal enzymes (i.e., prostatic acid phosphatase) has been found in tumor cells in comparison with their normal counterparts. This property contributes to "aggressiveness" of malignant cells.

There are many studies in forensic medicine that are all related to demonstration of semen acid phosphatase in vaginal fluid as evidence for sexual intercourse. Information of acid phosphatase activity in cervical epithelial cells has been scarcely studied.[66]

In healthy women, normal looking cervical epithelial cells contain alkaline phosphatase. Acid phosphatase has been described rarely if it has been described at all. However, acid phosphatase in vaginal fluid/smears has been suited intensely in forensic medicine as indicator of rape.

Medical literature contains only a few articles related to cervical acid phosphatase. In 1960, Gross and Kinzie found the gradient of acid phosphatase activity in malignant epithelium to be similar to the normal cervical epithelium; however poorly differentiated, malignant cells had a higher degree of activity. They used a Gomori's method for visualization of cervical acid phosphatase. In 1961 Berger showed semi-quantitative difference between acid phosphatase activity in basal and malignant cells. Mature cervical epithelial cells do not present that type of activity.[12]

In I974, Malvi et al described acid phosphatase in carcinoma of the cervix uteri. Using a staining technique according to Gomori, they found increased enzyme activity in malignant cells as opposing to "normal" activity in basal cells.[12,17] By using azo dye diazonium salt technique, it was easy for the demonstration of acid phosphatase activity inside abnormal cervical cells.[66,67,12]

Principle of the CAP Test

CAP reaction occurs because of the following:

CAP catalyses the liberation of phosphate from a substrate alpha-naphthyl AS-BI phosphate. The remaining aromatic moiety of the molecule simultaneously couples with Fast Garnet GBC producing an insoluble brown-red diazonium salt on the sites of the enzyme activity.[56] Counterstaining of nuclei is done by haematoxylin .CAP activity appears as a distinct red-brown granular deposit.[68]

This technique is used for evaluating the acid phosphatase activity on cervical smears to easily detect atypical cervical epithelial cells, a condition which may lead to diagnosis of cervical dysplasia or cancer in asymptomatic women. Sigma procedure for staining leukocyte acid phosphatase [Cat. No. 387A][69] was used for staining cervical smears.

Sigma-Aldrich procedure: Cervical swabs are fixed to a microscope slide. The resulting film is incubated in a solution of naphthol AS-BI phosphoric acid and freshly diazotized fast garnet GBC. The following reaction occurs

Naphthol AS-BI Phosphate >> Acid Phosphatase >> Naphthol AS-BI

Naphthol AS-BI + Fast Garnet GBC >> Insoluble Maroon Pigment.

The intracellular chemical reaction involving acid phosphatase splitting a naphthol substrate and donating the aromatic ring to a diazonium salt--producing an insoluble colorful deposit that precipitates inside cytoplasm at sites of enzyme activity.

Clinical Significance

Cervical Dysplasia

Cervical dysplasia is a term describing changes of normal composition of cervical epithelium. In healthy women, this epithelium is composed of several layers of cells, beginning with basal cells attached to a basal membrane, through intermediate cell layers to the superficial layers of squamous epithelial cells [47].

Both scrubbing and brushing excoriation techniques use force to damage cervical mucosal tissue, and to obtain a specimen of this tissue composed of cells from the superficial layers. These cells are smeared on microscopic slide, stained by Papanicolaou technique and evaluated by the Bethesda system.

In healthy women this specimen consists of superficial, large squamous cells with relatively small nucleus with condensed chromatin. Below are several layers of intermediate cells and, attached to the epithelial basal membrane are the cuboid, small cells with relatively larger nuclei. Local pathological processes and risk factors (i.e., unprotected sexual activity, human papilloma virus [HPV] infection, smoking),[70] may lead to cellular abnormalities, which over a period can result in the development of epithelial cell changes like increase in the thickness of mucosal layers, increasing the probability for cells from deeper layers to appear on cervical smears.

The most important change could be found when cervical intraepithelial neoplasia (CIN) dysplasia or cancer is present. Depending on the size of the underlying tumor, upper layers are reduced, and intermediate and basal cells could be found on cervical smears. This diagnostic condition is called cervical dysplasia. Relation between superficial, intermediate and basal cells present on a cervical smear determines a degree of dysplasia. Sometimes a few cancer cells could be present defining diagnosis of cervical cancer.[71]

Basal cells are biochemically most active and contain more acid phosphatase activity than intermediate and particularly more than superficial cells which are almost without lysosomal activity. Atypical cells usually contain more acid phosphatase activity than "typical cells" at all layers.[18]

All degrees of cervical dysplasia are considered as precancerous and must be confirmed by clinical procedures (Colposcopy, biopsy, surgery) and treated with anti-infective and/or antitumor therapy.[47,48,50]

Inflammation

All inflammatory cells contain acid phosphatase. Lysosomal activity is increased during inflammation. However, inflammatory cells (PMN, monocytes) are smaller than cervical epithelial cells, and possess many other morphological characteristics for easy identification by cytotechnologists. Difficulty may appear if basal epithelial cells are involved in the inflammatory process.[50] However, their Lysosomal content is significantly smaller than in inflammatory cells of similar size (i.e., monocytes).

Follow-up of therapy

In equivocal morphology (ASCUS), when Cyto-pathologists cannot decide between Pap positive and Pap negative result, he/she usually recommends a repetition of the test within next three months following intensive anti-inflammatory therapy. The CAP-PAP test could provide valuable information of the effect of this therapy on reduction of inflammatory cells, and reparation of cervical epithelium (reduction of acid phosphatase activity).

A common practice is to use a meta-analysis of data from several Cytopathologists laboratories using the unstandardized, conventional Pap tend to create a historic group to be a comparator for the new device. Such a historic group is a very weak comparator for statistical analyses of equivalence. On the other side, all devices described above, have been designed rather as adjunct than a substitute for the conventional Pap test. Therefore, it is very unlikely, they could be able to sustain a scrutiny of statistical analyses in clinical trials (unless with a very large sample size).

Cost Effectiveness of CAP/PAP.

The CAP-PAP test, as presented, is a simple, low cost and rapid assay. It does not require special training for Pap test screeners or purchasing of additional instruments. Only a microscope is sufficient. Minimal additional cost for reagents per test was noted in the study. Our estimate is that CAP and PAP test could be performed for the same price as the Pap test today .Technical time for marker processing is 3 min plus 60 min incubation time. Duration of counterstaining is about 30 min as for conventional Papanicolaou staining. With 10-20 min for test preparation and post-test cleaning, the

entire CAP-PAP procedure should be completed for less than two hours. The technician is free for other activities during the incubation period. Multiple tests can be run simultaneously. If one uses large basket containers for transferring slides between staining dishes, theoretically, an unlimited number of slides can be stained within this time frame.[51]

CAP Evaluation

The "CAP-PAP" smear is evaluated for presence and degree of acid phosphatase activity among cervical epithelial cells. Cytochemical criteria are used for assessment of enzyme activity. The CAP Score is used for comparison with other criteria of cervical dysplasia. Haematoxylin used to stain the cellular background, allows cytological classification of cells. Because the CAP-PAP Test will be a standardized procedure with internal controls, it could be used reciprocally to test the PAP test practices.[51]

The criteria for screening of CAP activity are taken as the presence of red granules in the cytoplasm of the squamous cells. Each cell is assessed for the presence of the granular deposits as 0 (Negative) if no visible granules are seen, 1 (Low) if few granules are seen which are barely visible, 2 (Moderate) if several to many granules clearly visible and scattered throughout the cytoplasm, 3 (High) if abundant, large aggregates of granules are seen. Also, the entire slide is seen for the presence of granules and assessed as 0 (Negative) if majority of cells are negative or only some cells showing low activity, and as +(nonnegative) if all degree of positivity is seen in majority of cells. One or two clusters of cells with high activity or majority of cells with moderate or high activity and atypical cells with any degree of activity are noted.[51]

Roughly 100 cells are screened and the degree of each is added and cells/smear classified according to the activity. The internal control for all the slides are noted (monocytes, macrophages, histiocytes) and only if the internal control is positive the slides are considered for evaluation.[51]

METHODOLOGY

METHODOLOGY

MATERIALS: SOURCES OF DATA

The sources of data were patients undergoing cervical cancer screening at Obstetric and Gynecological Department of R L Jalappa Hospital and Research Centre during January 2016 to December 2017. This was an opportunistic type of screening method where patients presented with complaints. Informed consent was obtained from the patients after which two smears were collected simultaneously for PAP and CAP-PAP staining.

Cervical biopsy / hysterectomy specimen of these patients received in Department of Pathology, R L Jalappa Hospital and Research Centre for histopathological examination were included for correlation. Histopathology was the Gold standard test for the present study.

Inclusion criteria:

- All patients undergoing cervical cancer screening from whom two cervical smears would be collected

Exclusion criteria:

- Smears showing Atypical glandular cells and adenocarcinoma
- Cases who have undergone radiation changes.

SAMPLE SIZE :

Sample size was estimated by using based on the sensitivity and specificity of PAP at 75% and 100% with respect to histopathology findings obtained from the study Neha Batra et al.[18] Based on these values at 80% power and 5% alpha error, and at arbitrary 0.25 prevalence, sample size is 136 subjects undergoing histopathology will be included in the study. Considering 10% non-response rate, sample size of 136 ± 13.6 ≈ 150 cases of Cervical histopathology will be included in the study.[72,73] Refer figure 7.

Fig 7: Sample size estimation formula of the study

FORMULA

$$n = \frac{Z^2 * P(1-P)}{\Delta^2} \qquad (1)$$

n will be $(a+c)$ if we use Sensitivity as P, and n will be $(b+d)$ if we use Specificity as P in formula (1).

$$N = \frac{(a+c)}{\text{Prevalence}} \qquad (2)$$

$$N = \frac{(b+d)}{(1-\text{Prevalence})} \qquad (3)$$

METHODS :

All the patients who underwent cervical cancer screening at Obstetric and Gynecological Department R L Jalappa Hospital and Research Centre during Jan 2016 to December 2017 participated in this study after obtaining an informed consent. Demographic details and Clinical history was obtained from all the patients. All patients underwent two cervical smear collections.

The first smear for routine cervical PAP stain was fixed by alcohol and second smear for CAP PAP stain, was fixed by fixative made by 25 ml Citrate Solution ,65 ml acetone and 8 ml 37% formaldehyde and stored at 2 to 8^0C. Later, all smears were stained by PAP stain and interpreted as per 2014 BETHESDA system.[46] Among them, 75 PAP positive and 75 negative cases were included in the study. CAP-PAP staining was performed on all the included cases and interpreted as mentioned in the following section.

CAP-PAP and PAP results were compared. Histopathological study was done on the biopsy/hysterectomy specimens of all the cases included in the study. Histopathology was the gold standard test against which PAP and CAP-PAP results were compared and correlated with their respective histopathology reports.

STAINING and INTERPRETATION

RAPID PAP STAIN [71]

First smear was stained as per the routine Pap staining procedure.

- The fixed smear was dipped for a minute in tap water and excess water was blotted out
- It was dipped for 45 seconds in RAPID-PAP (nuclear stain)
- It was washed in Scotte's tap water buffer for 30 seconds and excess water was blown out.
- It was dipped for 30 seconds in RAPID-PAP.(dehydrant)
- It was dipped for 45 seconds in working cytoplasm stain.
- It was washed in Scotte's tap water for 20 seconds and excess water was blown out.
- Dehydration was repeated in a second bath of RAPID PAP (dehydrant) for 30 seconds and air-dried
- It was dipped in xylene, for 20 seconds, dried and mounted with cover glass using a drop of D.P.X.

INTERPRETATION OF PAP CASES[54]

NEGATIVE FOR INTRAEPITHELIAL LESION OR MALIGNANCY

(When there is no cellular evidence of neoplasia, state this in the General Categorization above and/or in the Interpretation/Result section of the report -- whether or not there are organisms or other non-neoplastic findings)

NON-NEOPLASTIC FINDINGS (optional to report optional to report; list not inclusive)

- Non-neoplastic cellular variations
  - Squamous metaplasia
  - Keratotic changes
  - Tubal metaplasia
  - Atrophy
  - Pregnancy-associated changes
- Reactive cellular changes associated with:
  - Inflammation (includes typical repair)
- Lymphocytic (follicular) cervicitis
  - Radiation
  - Intrauterine contraceptive device (IUD)
- Glandular cells status post hysterectomy

ORGANISMS

- Trichomonas vaginalis
- Fungal organisms morphologically consistent with Candida spp.
- Shift in flora suggestive of bacterial vaginosis
- Bacteria morphologically consistent with Actinomyces spp.
- Cellular changes consistent with herpes simplex virus
- Cellular changes consistent with cytomegalovirus

OTHER

• Endometrial cells (in a woman ≥45 years of age)

(Specify if "negative for squamous intraepithelial lesion")

EPITHELIAL CELL ABNORMALITIES

SQUAMOUS CELL

• Atypical squamous cells

– of undetermined significance (ASC-US)

– cannot exclude HSIL (ASC-H)

• Low-grade squamous intraepithelial lesion (LSIL) (encompassing: HPV/mild dysplasia/CIN 1)

• High-grade squamous intraepithelial lesion (HSIL) (encompassing: moderate and severe dysplasia, CIS; CIN 2 and CIN 3)

– with features suspicious for invasion (if invasion is suspected)

• Squamous cell carcinoma

GLANDULAR CELL

• Atypical

– endocervical cells (NOS or specify in comments)

– endometrial cells (NOS or specify in comments)

– glandular cells (NOS or specify in comments)

• Atypical

– endocervical cells, favor neoplastic

– glandular cells favor neoplastic

• Endocervical adenocarcinoma in situ

• Adenocarcinoma

– endocervical – endometrial

– extrauterine – not otherwise specified (NOS)

OTHER MALIGNANT NEOPLASMS: (specify)

CAP-PAP STAIN

Second smear labelled as CAP PAP was stained by following technique.

Staining procedure[11] was same as Pap staining but also included following additional steps for staining of enzyme - Marker visualization(CAP) and counterstaining.(by Rapid Pap stain)

- Slides would be fixed at room temp for 50 sec, followed by rinsing in two changes of distilled water.
- Slides are then air dried and stored in dust free container at 4-6^{0}C.
- At the time of staining, incubation solution would be freshly prepared by mixing

  a.10 drops sodium nitrate

  b. 10 drops of Fast Garnet GBC (incubated at room temp for three minutes) in an Erlenmeyer flask containing 46ml pre-warmed distilled water and 2.5ml acetate solution.

  c. 10 drops of substrate solution (containing alpha naphthyl ASBI phosphate) finally added to this mixture.

- Slides were rehydrated and incubated for 45min at 37^0C in the dark , followed by serial rinsing in running tap water, distilled water ,and finally in phosphate buffered saline for 5min each .
- Slides were counterstained using a modified pap smear with different incubation time in Gill hematoxylin (4min), OG-6(3min), EA -65 solution (5 sec).
- Slides were mounted and evaluated at magnification between 100x and 400x

Interpretation of CAP-PAP smears[18]

CAP appears as brown-red deposit scattered throughout the cytoplasm of "abnormal" cervical cells. PAP staining produces a color that is combination of original Papanicolaou recommendation:

- Hematoxylin stains cervical cells nuclei blue and adds a bluish coloration to the color of cytoplasm.
- Orange G (OG-6) stains cervical cell cytoplasm orange (red + yellow).
- Eosin Alcohol (EA-65) stains cervical cell cytoplasm red.

However, Light Green added to the solution of EA, causes green color of cytoplasm.

The "ideal" staining will produce the following results: CAP -red-brown individual granules scattered through cytoplasm. Other cytological features (nucleus, nuclei, vacuoles, another granulation) distinguished by conventional PAP stain. CAP staining produces a red-brown granular precipitate at intracellular sites of enzyme activity. Counterstaining assists presenting cell morphology, cell identification and classification as per the Bethesda 2014 criteria.

Statistical Methods:

Data was entered Microsoft excel data sheet and was analyzed using SPSS 22 version software. Categorical data was represented in the form of Frequencies and proportions. Chi-square test was used as test of significance for qualitative data. Continuous data was represented as mean and standard deviation.

Screening of Disease:

Screening test results	Diagnosis		Total
	Diseased	Healthy	
Positive	a (True postive)	b (False Postive)	a+b
Negative	c (False Negative)	d (True Negative)	c+d
Total	a + c	b + d	a+b+c+d

- Sensitivity = a/(a+c) x 100 = True positive / True positive + False Negative
- Specificity = d/(b+d) x 100 = True Negative / True Negative + False Postive
- Positive predictive value = a/ (a+b) x 100 = True Postive / True positive + False Postive
- Negative predictive value = d/ (c+d) x 100 = True Negative / True Negative + False Negative
- Diagnostic accuracy = a + d / a + b + c + d = True postive + True Negative / Total

Sensitivity: Defined as ability of a test to identify correctly all those who have the disease i.e. true positive

Specificity: It is the ability of test to identify correctly those who do not have the disease i.e. true negative.

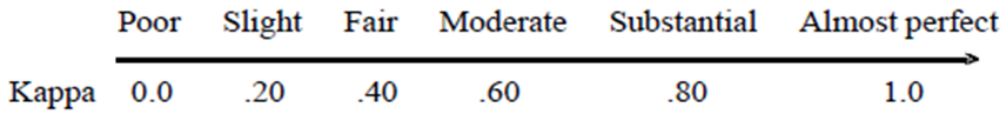
Positive predictive value (PPV): The proportion of patients who test positive and have the disease.

Negative predictive value (NPV): The proportion of patients who test negative who are actually free of the disease.

Diagnostic accuracy: Is the ability of screening test to detect true positives and true negatives in the total population studied.

Kappa Statistics: Agreement between two or more observers/ between two or more methods or instruments and equipments was assessed by using Kappa statistics.[74] Refer figure 8

Fig 8 : Interpretation of Kappa



Kappa	Agreement
< 0	Less than chance agreement
0.01–0.20	Slight agreement
0.21– 0.40	Fair agreement
0.41–0.60	Moderate agreement
0.61–0.80	Substantial agreement
0.81–0.99	Almost perfect agreement

Graphical representation of data: MS Excel and MS word were used to obtain various types of graphs such as bar diagram, Pie diagram.

p value (Probability that the result is true) of <0.05 was considered as statistically significant after assuming all the rules of statistical tests.

Statistical software: MS Excel**,** SPSS version 22 **(**IBM SPSS Statistics, Somers NY, USA) was used to analyze data.

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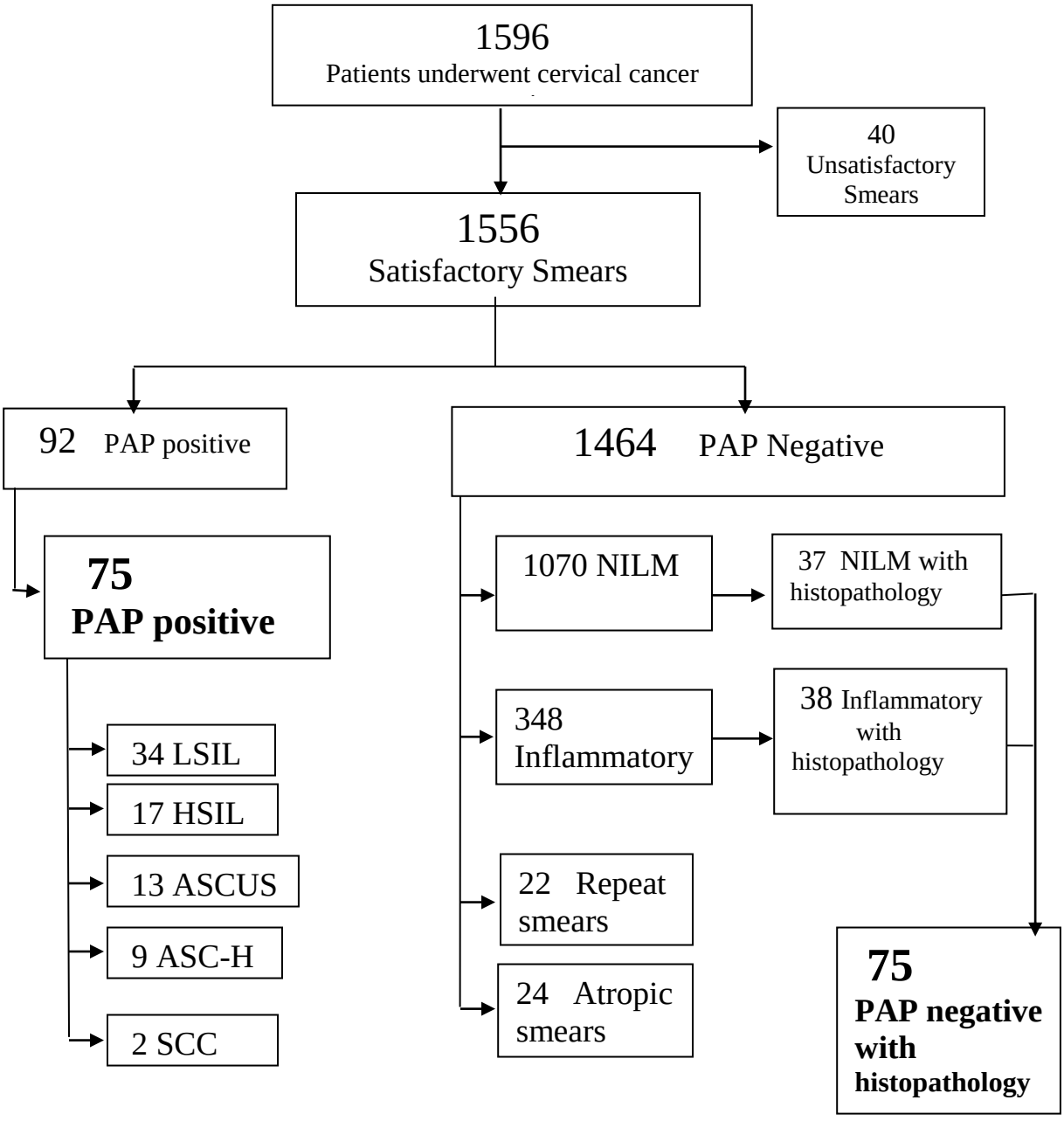
RESULTS

Results

A total of 1596 patients underwent cervical cancer screening at Obstetric and Gynecological Out Patient Department of R L Jalappa Hospital and Research Centre during Jan 2016 to December 2017. The present study included 75 PAP Positive and 75 PAP Negative patients, a total of 150 patients who were selected as represented chart 1.

Chart 1 : Flowchart of selection of study subjects



AGE AT PRESENTATION

Age of patients ranged from 20-88 years in our study. The mean age of presentation in our study was 42.96 ± 13.73 yrs. Age distribution of all the subjects in the study is shown in table 2 and chart 2. 65% of the cases of PAP negative were below 40 years of age group. Among PAP positive cases, 58.7% were above 40 years as shown in table 3 and chart 3.

Table 2: Age distribution of all subjects in the study

		Count	%
Age	<30 years	30	20.0%
	31 to 40 years	50	33.3%
	41 to 50 years	41	27.3%
	51 to 60 years	12	8.0%
	61 to 70 years	11	7.3%
	>70 years	6	4.0%
	Total	150	100.0%

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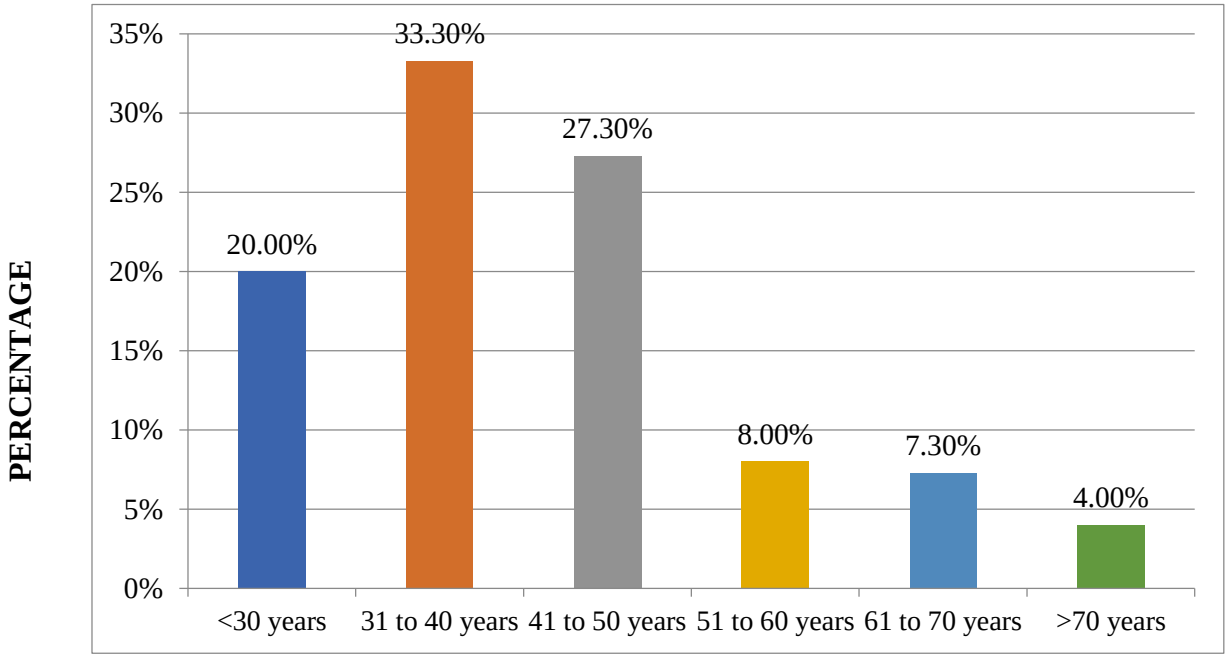


Chart 2: Bar diagram showing Age distribution of subjects in the study

Table 3: Comparison of Age distribution with respect to PAP results

		Group			
		PAP Negative		PAP Positive	
		Count	%	Count	%
Age	<30 years	23	30.7%	7	9.3%
	31 to 40 years	26	34.7%	24	32.0%
	41 to 50 years	21	28.0%	20	26.7%
	51 to 60 years	2	2.7%	10	13.3%
	61 to 70 years	2	2.7%	9	12.0%
	>70 years	1	1.3%	5	6.7%
	Total	75	100.0%	75	100.0%

$\chi 2 = 21.09$, df = 5, p = 0.001*

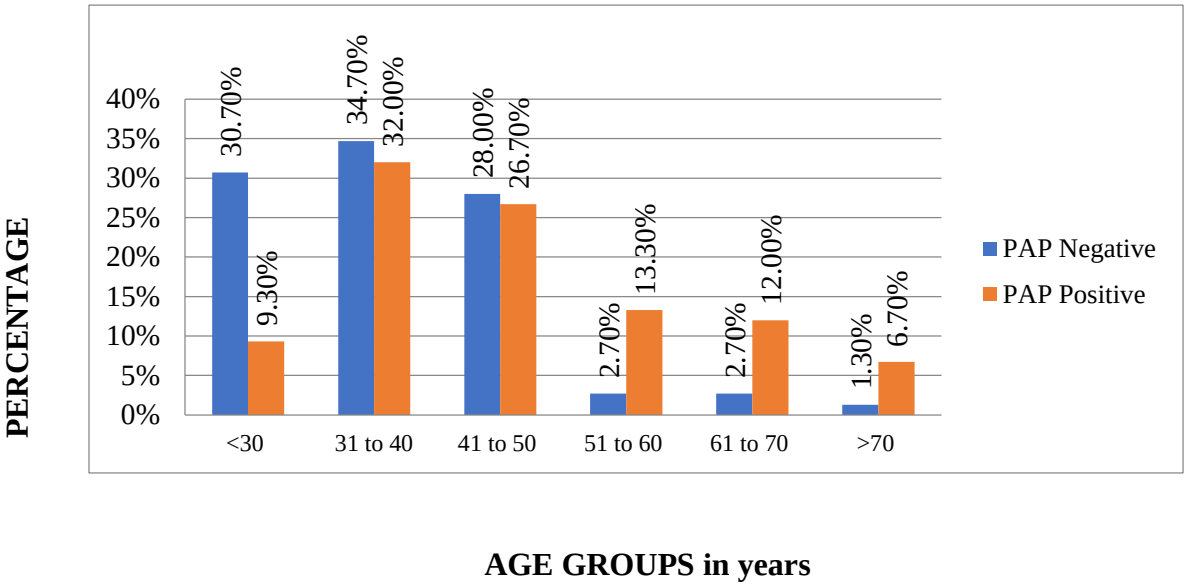


Chart 3: Bar diagram showing Comparison of Age distribution with respect to PAP results

PRESENTING COMPLAINTS

Among the 150 patients studied, 49.3% had WDPV, 18% had Mass PV, 19.3% underwent routine screening, 6% had pain abdomen, 6% had bleeding PV and 1.3% had burning Micturition. Of those with PAP negative, 52% had WDPV, 25.3% had Mass PV, 9.3% had routine screening, 6.7% had pain abdomen, 4% had bleeding PV and 2.7% had burning Micturition. Among those with PAP positive, 46.7% had WDPV, 10.7% had Mass PV, 29.3% had routine screening, 5.3% had pain abdomen, 8% had bleeding PV.as shown in table 4 and chart 4.

Table 4: Comparison of Presentation with respect to PAP results

		Group			
		PAP Negative		PAP Positive	
		Count	%	Count	%
Presenting Complaint	Bleeding PV	3	4.0%	6	8.0%
	Burning Micturition	2	2.7%	0	0.0%
	Mass PV	19	25.3%	8	10.7%
	Pain Abdomen	5	6.7%	4	5.3%
	Routine Screening	7	9.3%	22	29.3%
	WDPV	39	52.0%	35	46.7%

$\chi 2 = 15.56$, df = 5, p = 0.008*

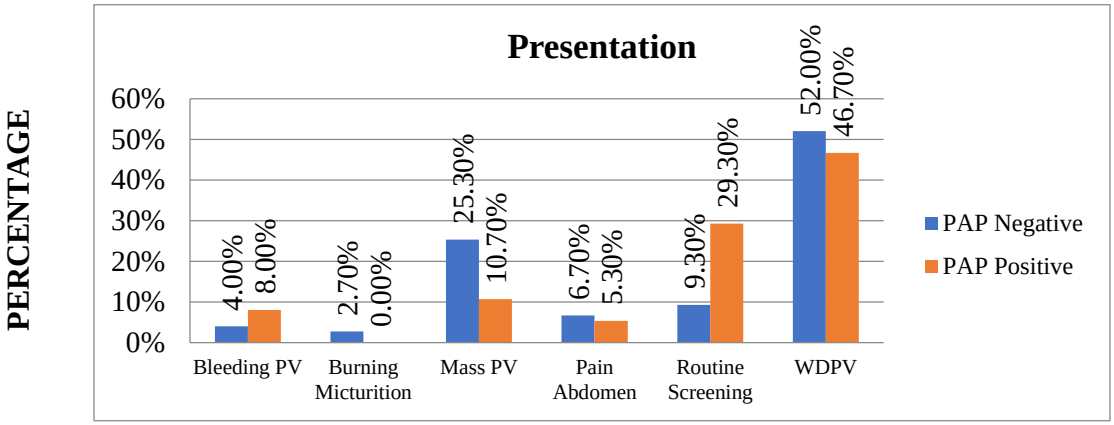


Chart 4: Bar diagram showing Comparison of Presentation with respect to PAP results

CAP-PAP Staining Results

All smears stained by CAP PAP, showed positivity in the form of red granular deposits at the site of enzyme activity inside the cell, while other cellular structures and cells devoid of CAP enzyme were stained by modified Pap method. Endocervical cells, metaplastic cells and inflammatory cells showed marked cytoplasmic red granular deposits as shown in figure 11,12. There was no extra-cellular diffusion but, in some cases, staining was intense which obscured the nuclear details. Inflammatory cells contain acid phosphatase are smaller than cervical epithelial cells and possess many other morphological characteristics for easy identification. Metaplastic cells were always positive for the red granular deposit which could be identified in low power, however for nuclear details high power was used as shown in figure 16. For analysis, smears showing CAP positive squamous cells with nuclear atypia or enlargement were considered positive by CAP PAP as shown in figures 9,10,13-24 in comparison with PAP.

Figure 9: PAP staining of NILM smear [40X]

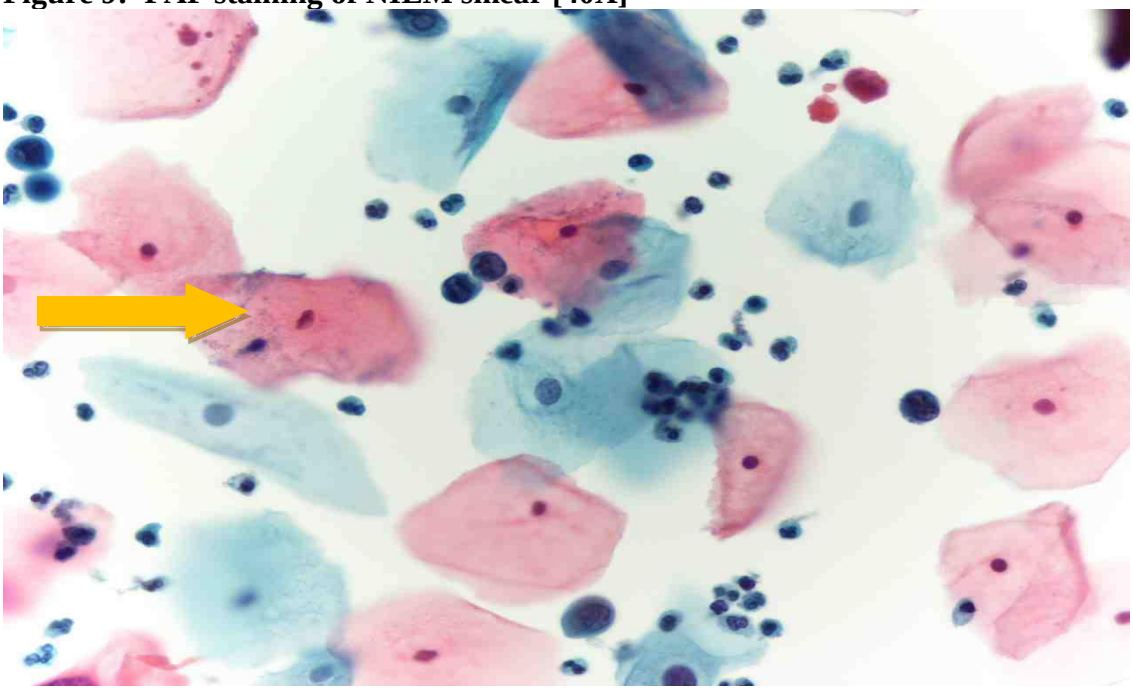


Figure 10: CAP-PAP staining of NILM smear [40X]

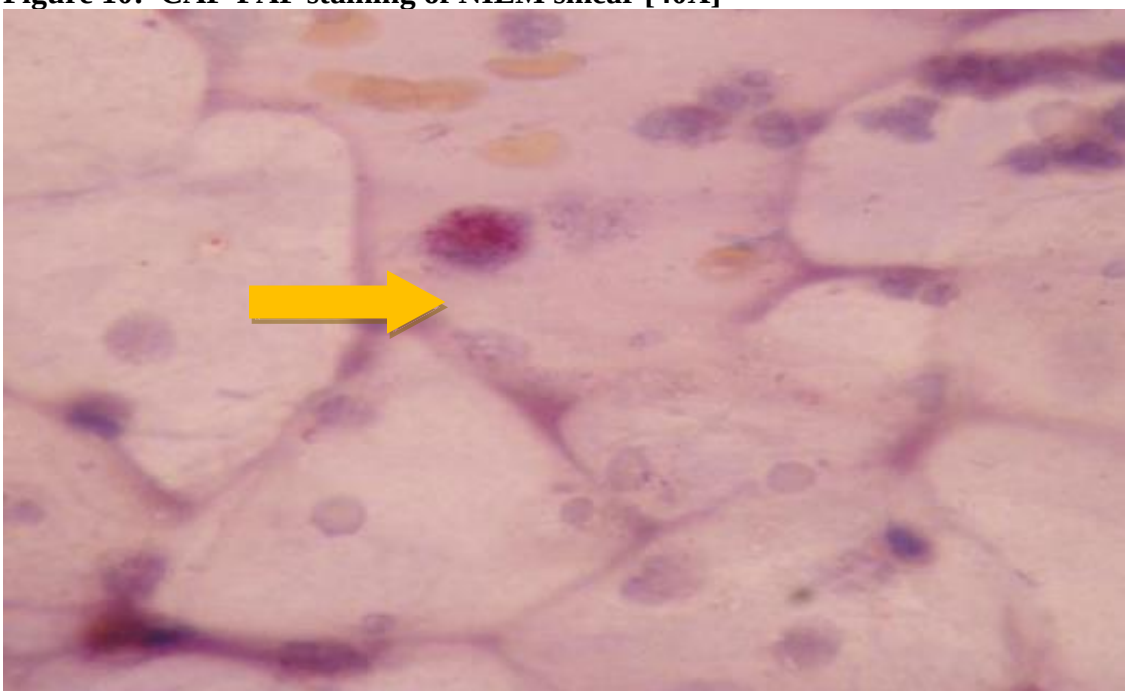


Figure 11: - Endocervical cells showing CAP positivity which act as an internal control.(CAP PAP, Oil immersion)(100X)

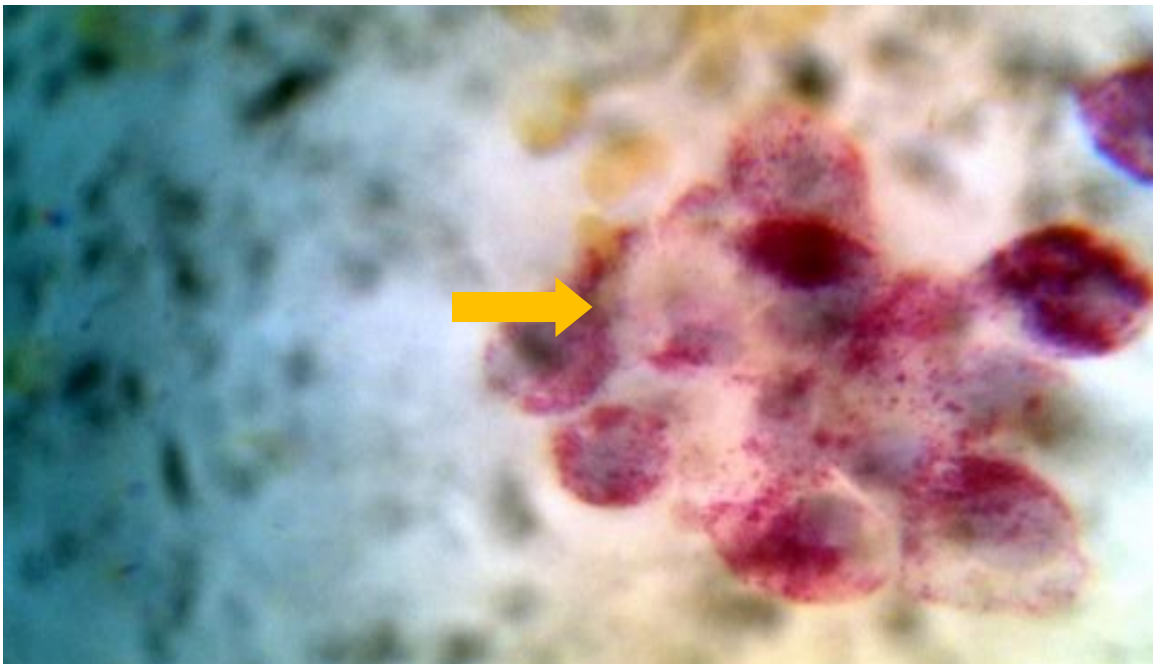


Figure 12: - Metaplastic Squamous cells CAP positivity which act as an internal control. CAP stains (40X)

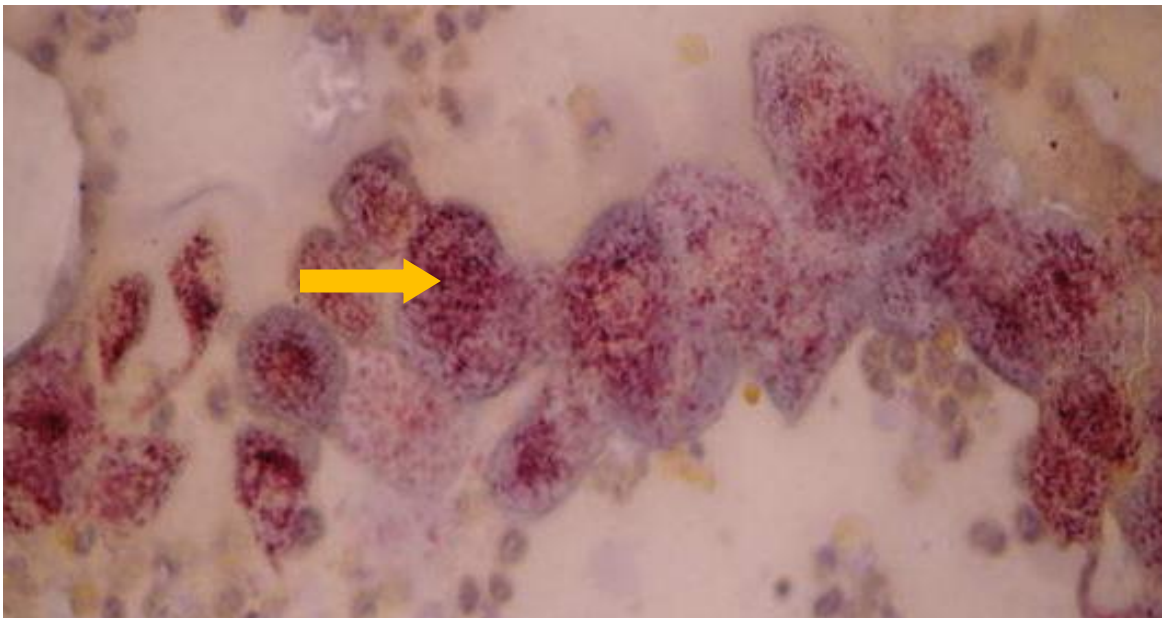


Figure 13 : PAP staining of INFLAMMATORY smear [40X]

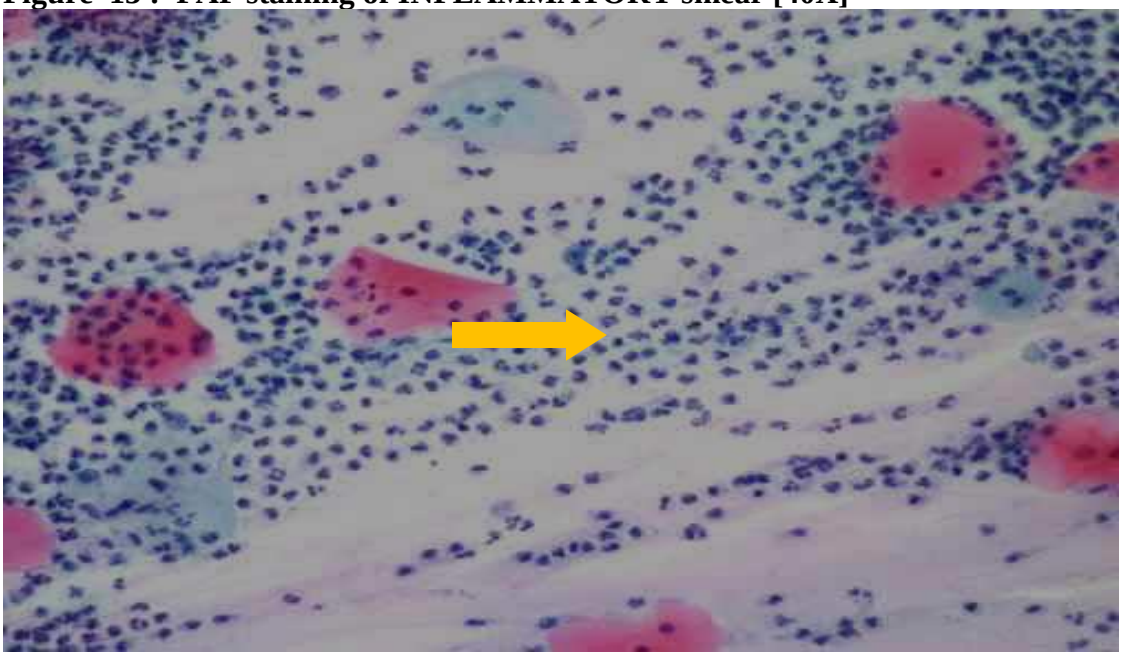


Figure 14 : CAP-PAP staining of INFLAMMATORY smear [100X]

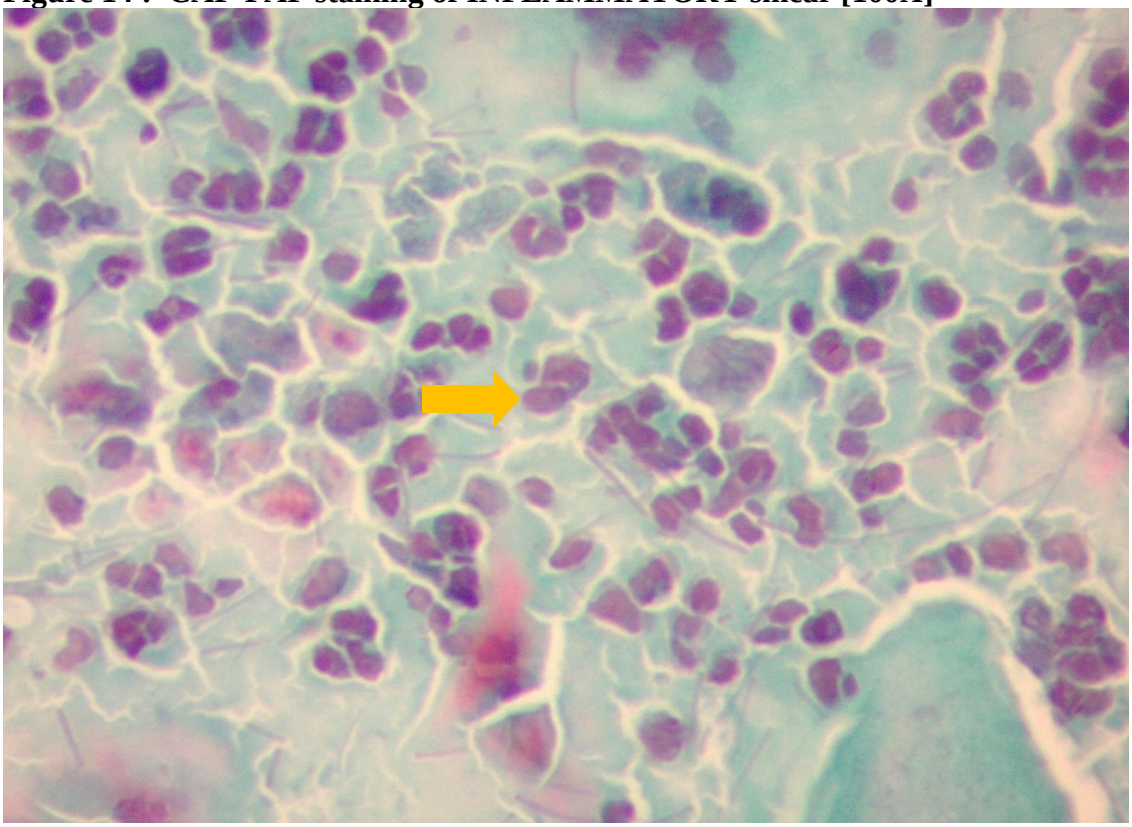


Figure 15 : PAP staining of ASCUS smear [40X]

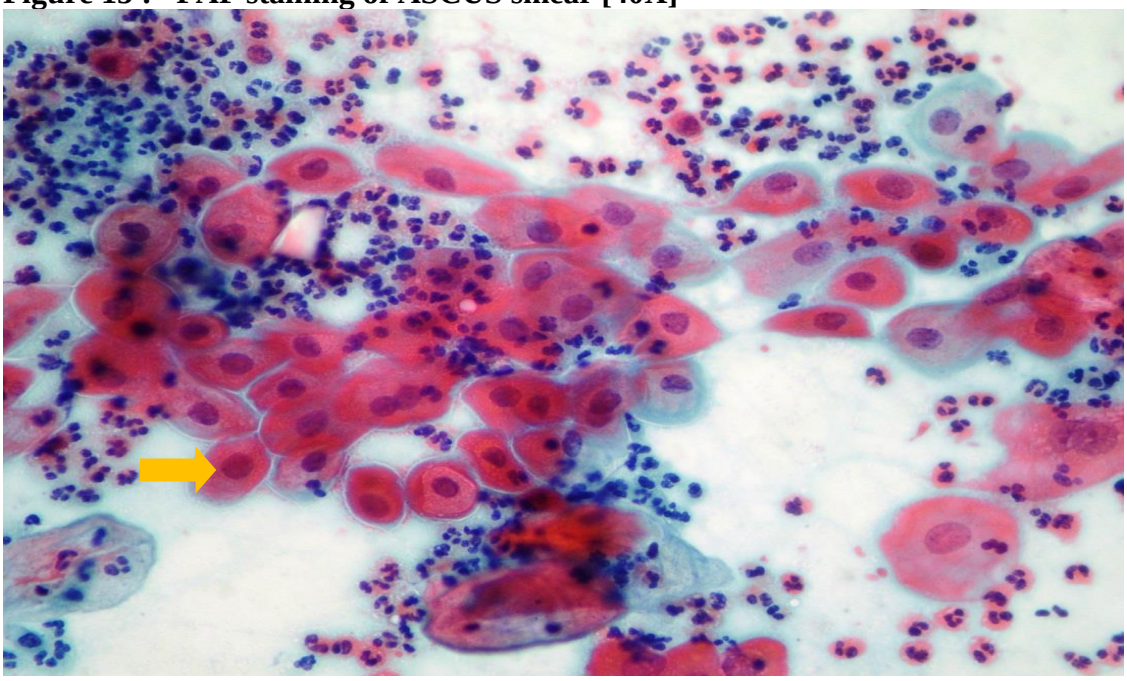


Figure 16: CAP-PAP staining of ASCUS smear [100X]

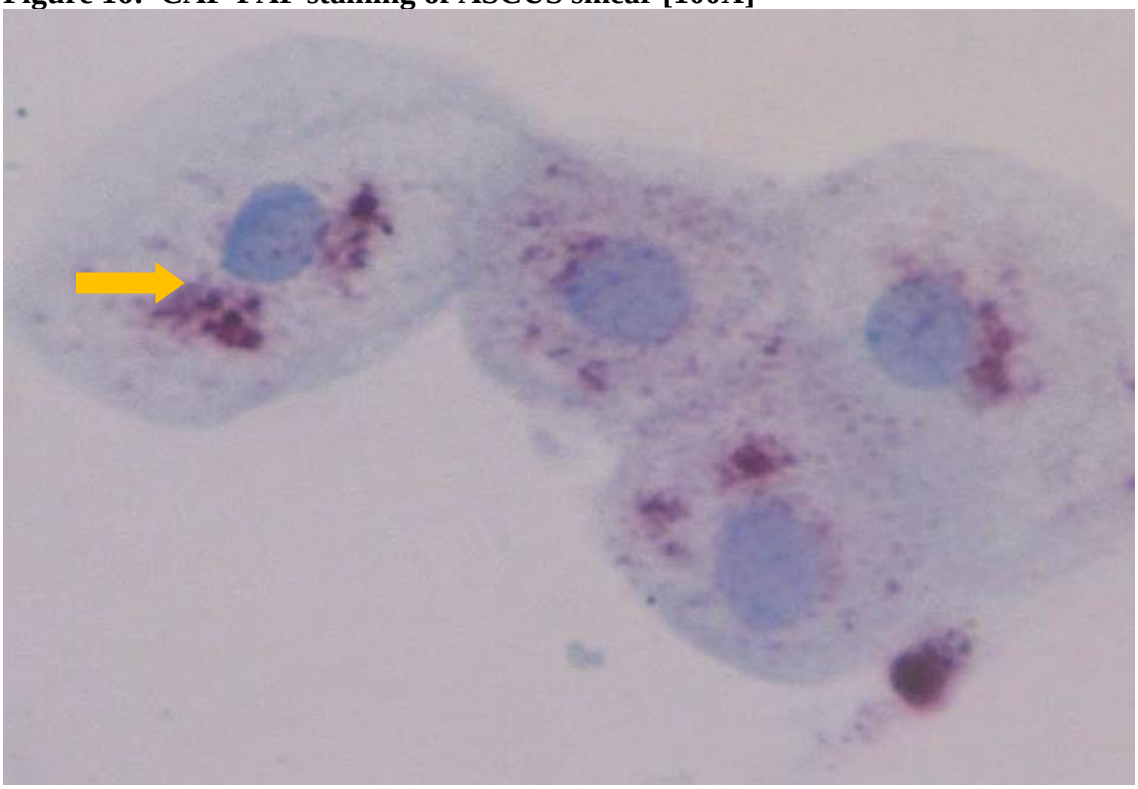


Figure 17: PAP staining of LSIL smear [40X]

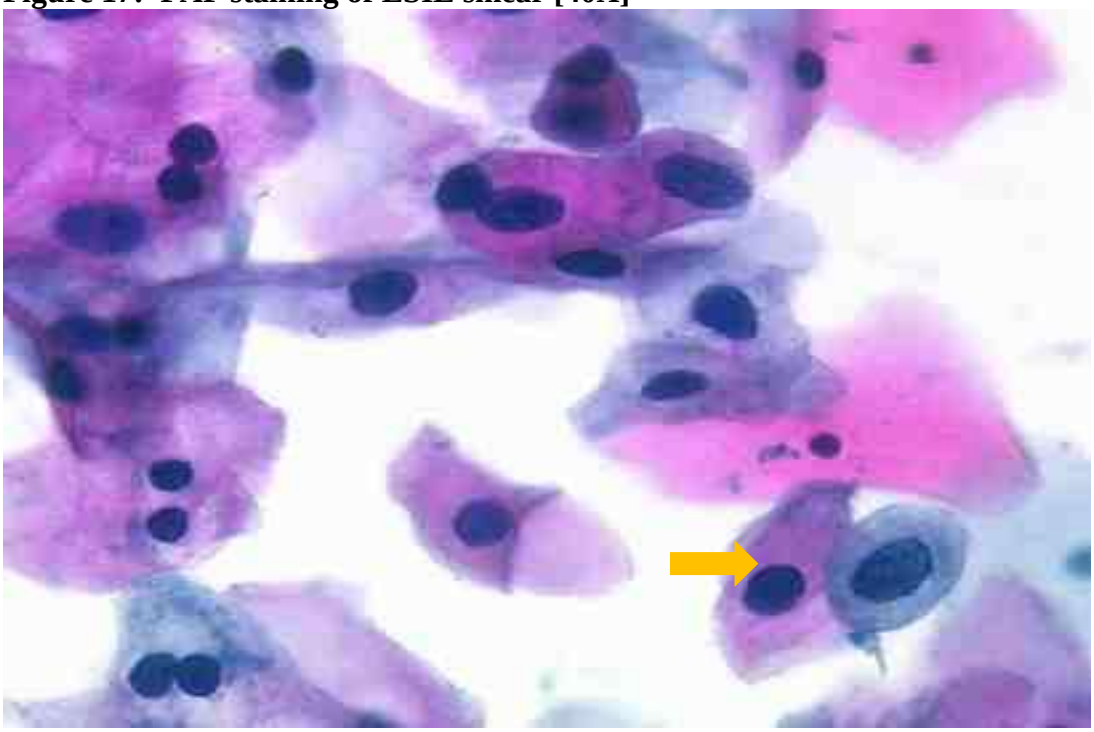


Figure 18: CAP-PAP staining of LSIL smear [100X]

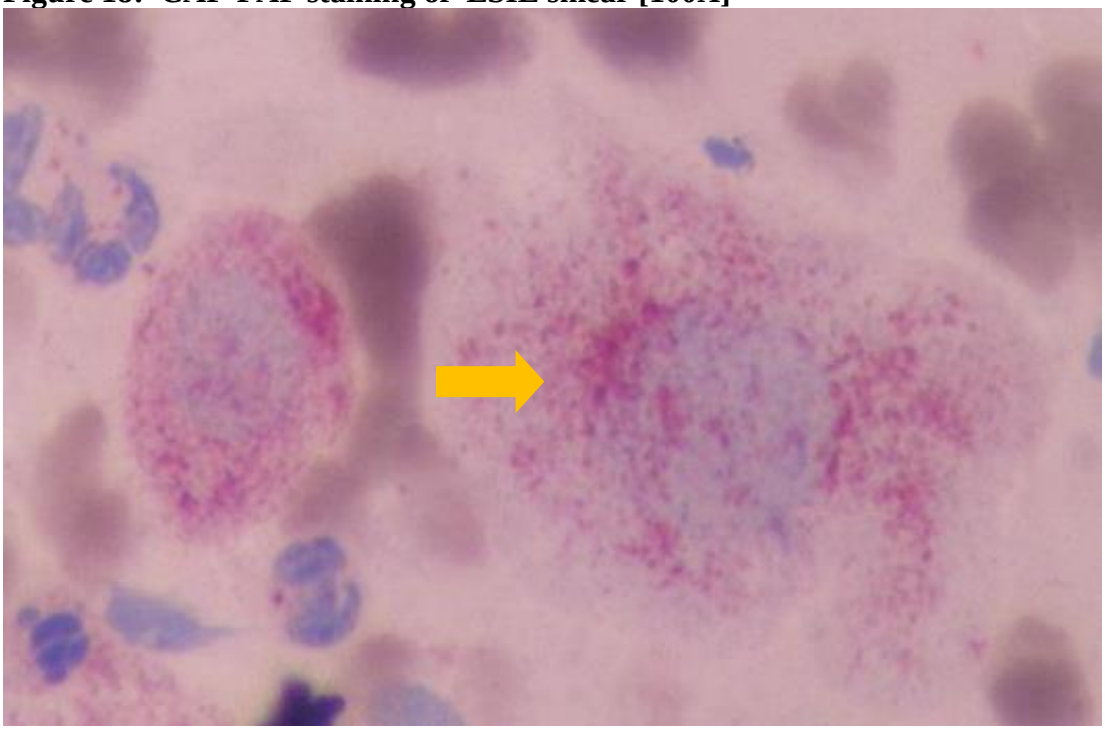


Figure 19: PAP staining of ASC-H smear [40X]

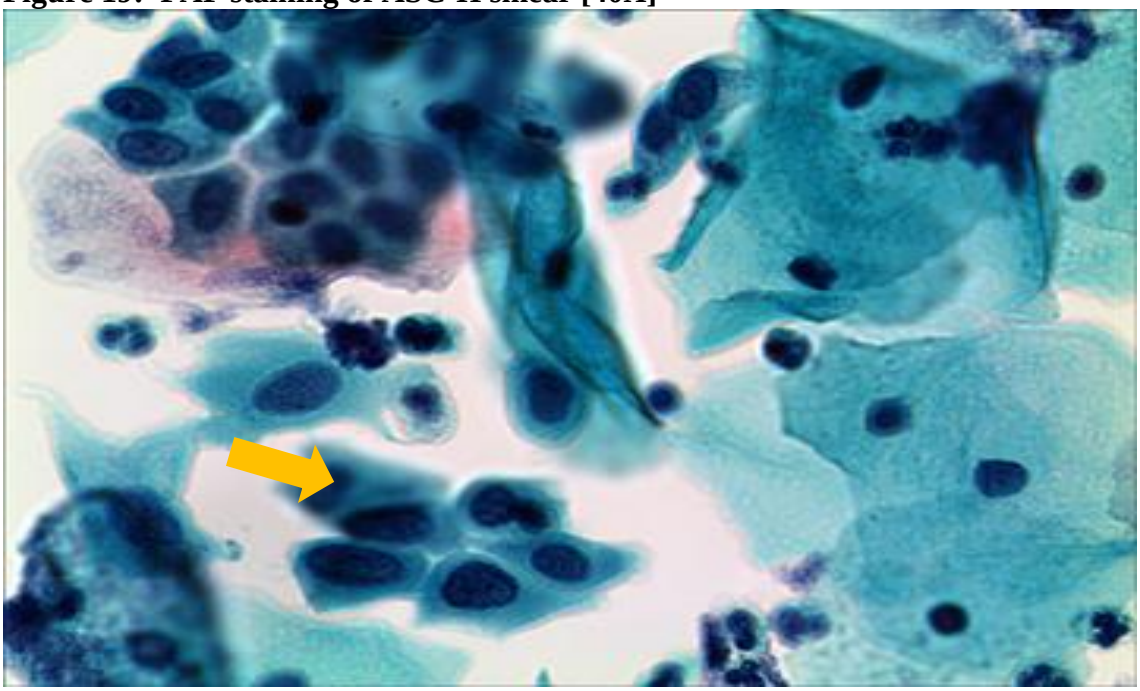


Figure 20: CAP-PAP staining of ASC-H smear [100X]

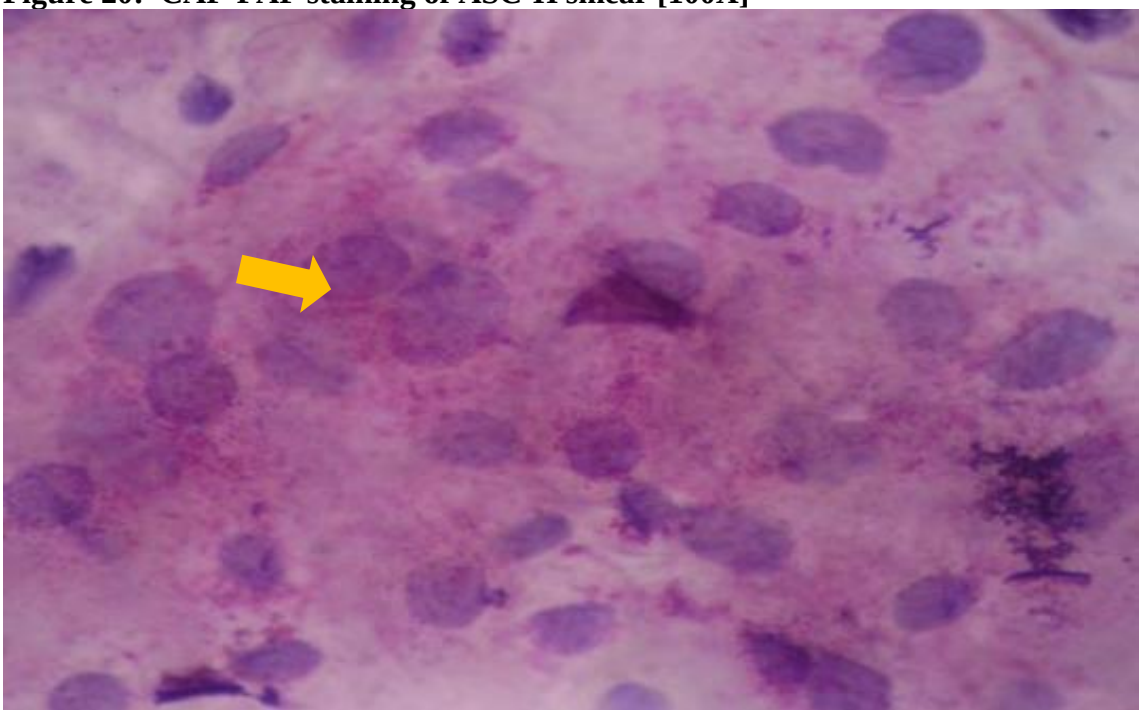


Figure 21: PAP staining of HSIL smear [40X]

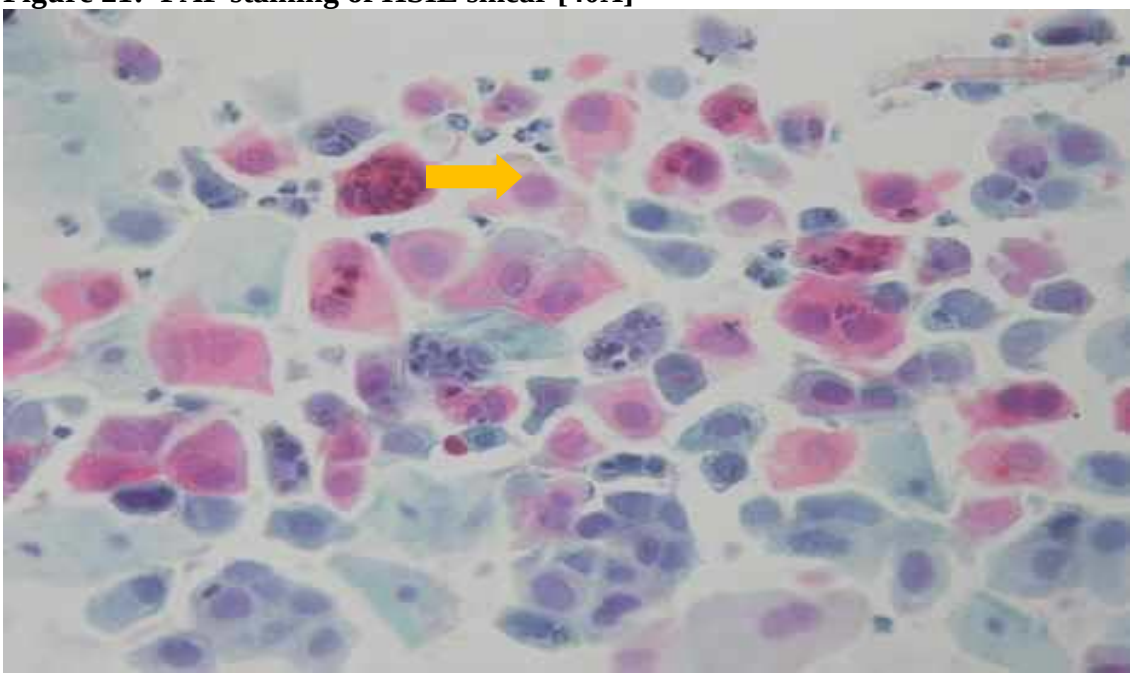


Figure 22: CAP-PAP staining of HSIL smear [100X]

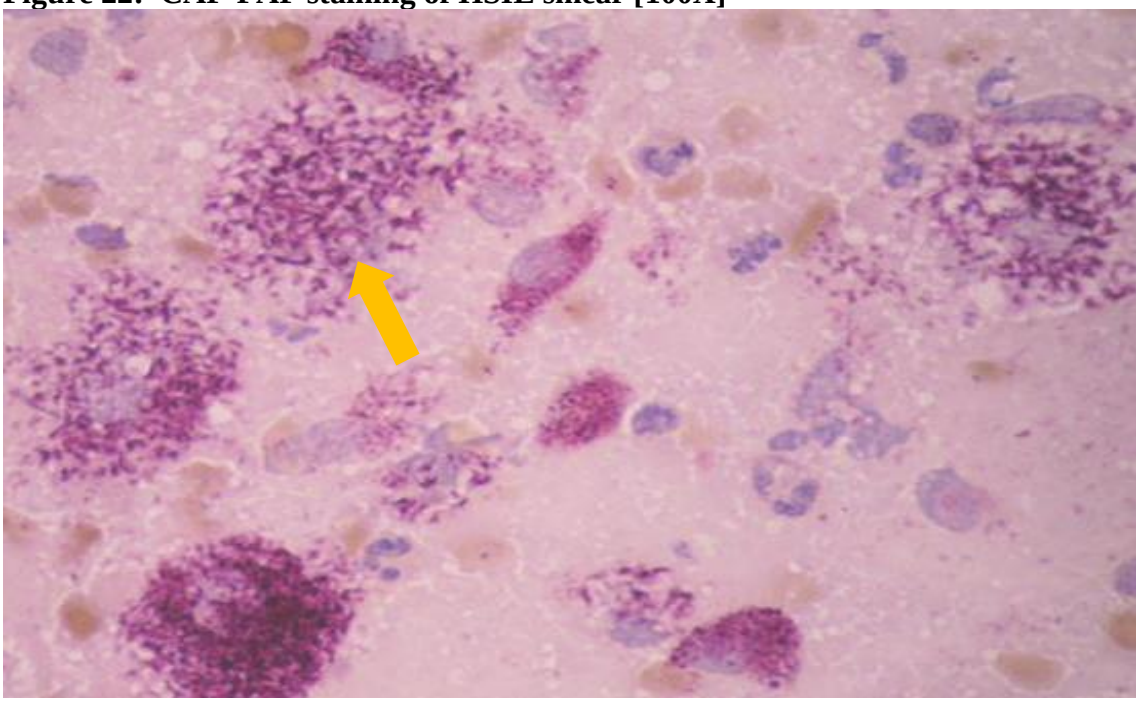


Figure 23: PAP staining of SCC smear [40X]

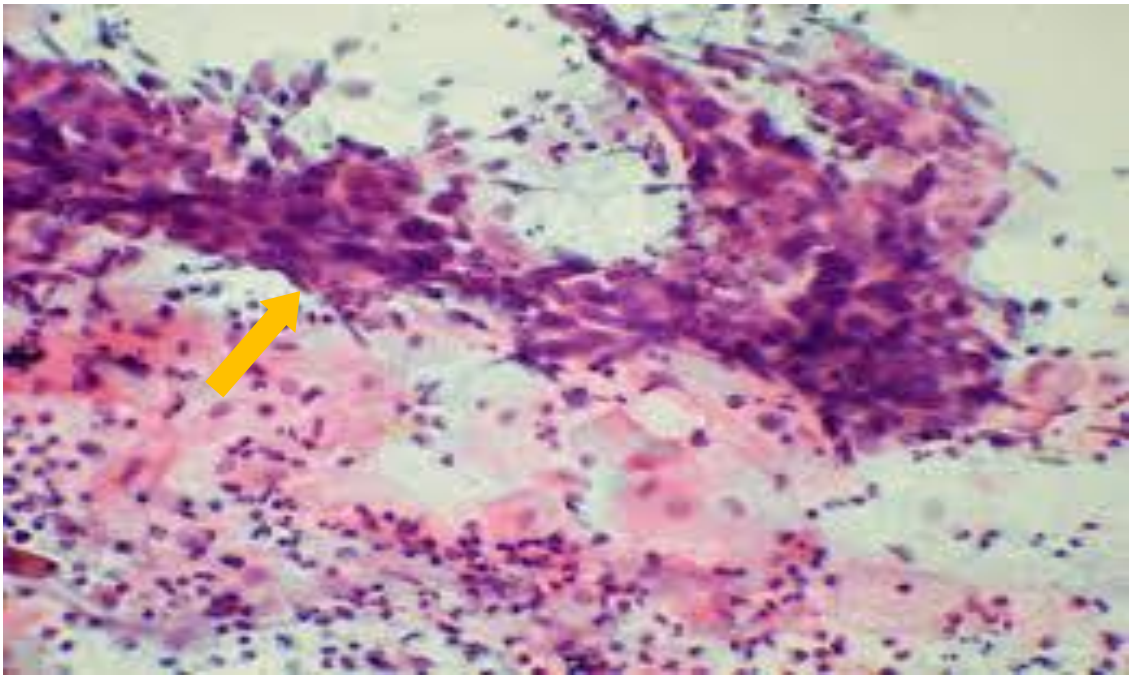
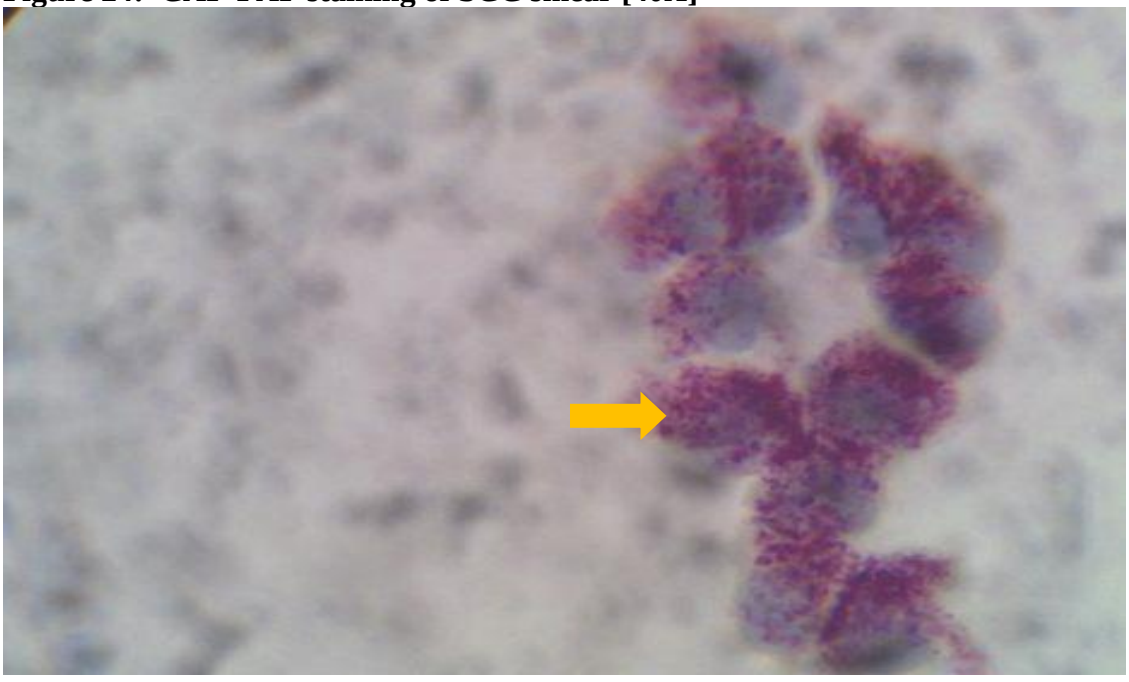


Figure 24: CAP-PAP staining of SCC smear [40X]



Distribution of PAP and CAP-PAP results

In our study, there were 75 PAP positive cases out of which 34 were LSIL, 17 were HSIL, 13 were ASCUS, 9 were ASC-H, and 2 were SCC cases. In the remaining 75 PAP negative smears, 38 cases were inflammatory smears and 37 cases were of NLIM smears. Among the PAP positive cases, CAP-PAP result was similar in all LSIL, HSIL, ASCUS ASC-H and SCC cases. Among the PAP negative cases, CAP-PAP was negative in all the 37 NILM cases, but of the 38 inflammatory cases, CAP-PAP was negative in 30 but was positive in 8 cases as shown in table 5.

Table 5: Distribution of PAP and CAP-PAP results

GROUP	LESIONS	No of PAP cases	No of CAP – PAP Positive cases	No of CAP – PAP Negative cases
PAP Positive – 75	ASCUS	13	13	0
	LSIL	34	34	0
	ASC-H	9	9	0
	HSIL	17	17	0
	SCC	2	2	0
PAP Negative – 75	NILM	37	0	37
	INFLAMMATORY SMEARS	38	8	30

Comparison of CAP-PAP and PAP results

Among the 75 PAP positive cases, all 75 [100%] were CAP PAP positive and of the 75 PAP negative, 8[10.7%] were CAP PAP positive and remaining 67 [89.3%] were CAP PAP negative as shown in tables 6 and chart 5. Statistical analysis showed that CAP PAP had sensitivity of 100%, Specificity of 89.33%, PPV of 90.36%, NPV of 100% and

Diagnostic Accuracy of 94.67% as shown in table 7 and chart 6. As per the Kappa statistic, agreement between CAP PAP and PAP was 0.893 (Almost perfect agreement) which shows that CAP-PAP results were similar to PAP result

Table 6: Comparison between CAP PAP and PAP results

		PAP			
		Positive		Negative	
		Count	%	Count	%
CAP-PAP	Positive	75	100.0%	8	10.7%
	Negative	0	0.0%	67	89.3%

χ 2 = 121.084, df = 1, p < 0.001*

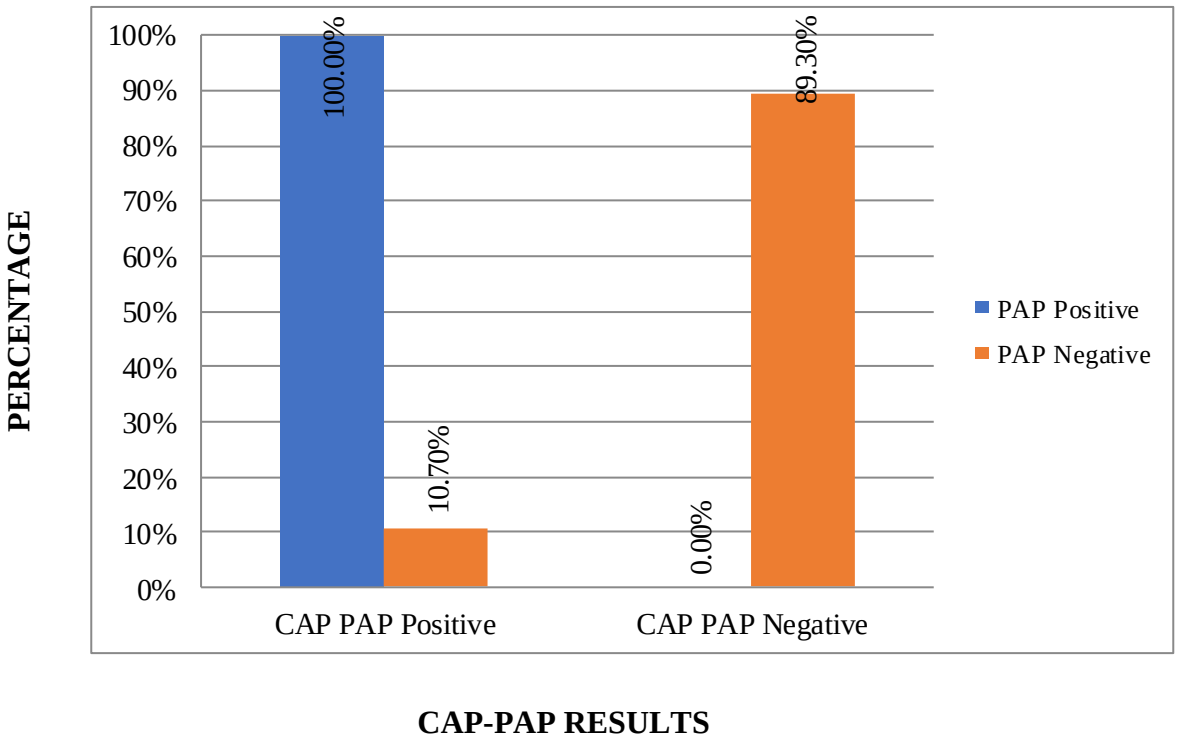


Chart 5: Bar diagram showing Comparison between CAP - PAP and PAP results

Table 7: Validity of CAP PAP results with respect to PAP results

Parameter	Estimate	Lower - Upper 95% Cis
Sensitivity	100%	95.13, 100
Specificity	89.33%	80.34, 94.5
Positive Predictive Value	90.36%	82.12, 95.03
Negative Predictive Value	100%	94.58, 100
Diagnostic Accuracy	94.67%	89.83, 97.27
Cohen's kappa (Unweighted)	0.8933	0.7342 - 1.052

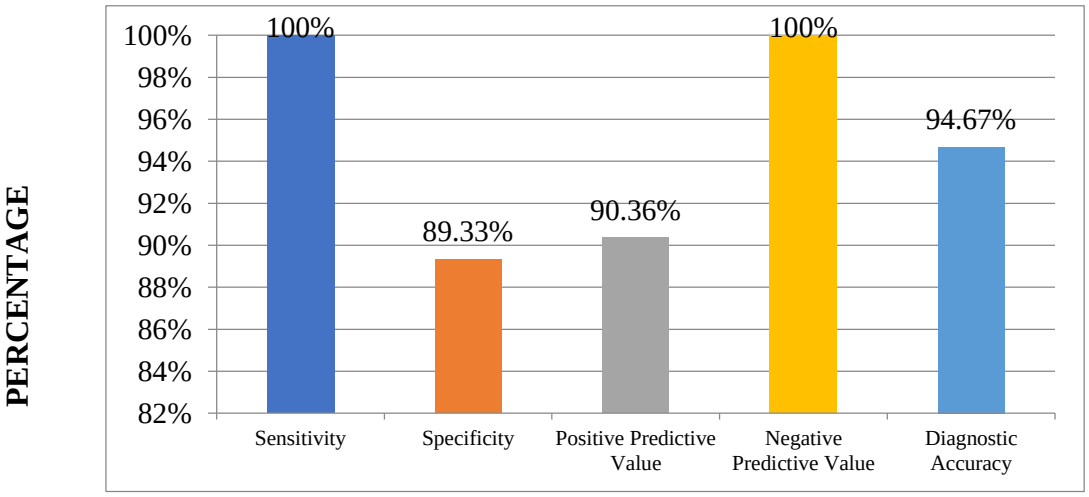


Chart 6: Bar diagram showing Validity of PAP results with respect to CAP PAP results

HISTOPATHOLOGY RESULTS

Histopathology was the Gold standard test for the present study and was obtained for all the 150 patients. Histopathology results showed 71[47.3%] negative and 79 positive for dysplasia among which 39[26%] had CIN1, 18[12%] had CIN2, 20[13.3%] had CIN3 and 2[1.3%] had SCC as shown in table 8 and chart 7. The histopathology of various lesions is depicted in figures 25-28.

Table 8: Histopathology results among study subjects

		Count	%
Histopathology Report	Negative	71	47.3%
	CIN1	39	26.0%
	CIN2	18	12.0%
	CIN3	20	13.3%
	SCC	2	1.3%

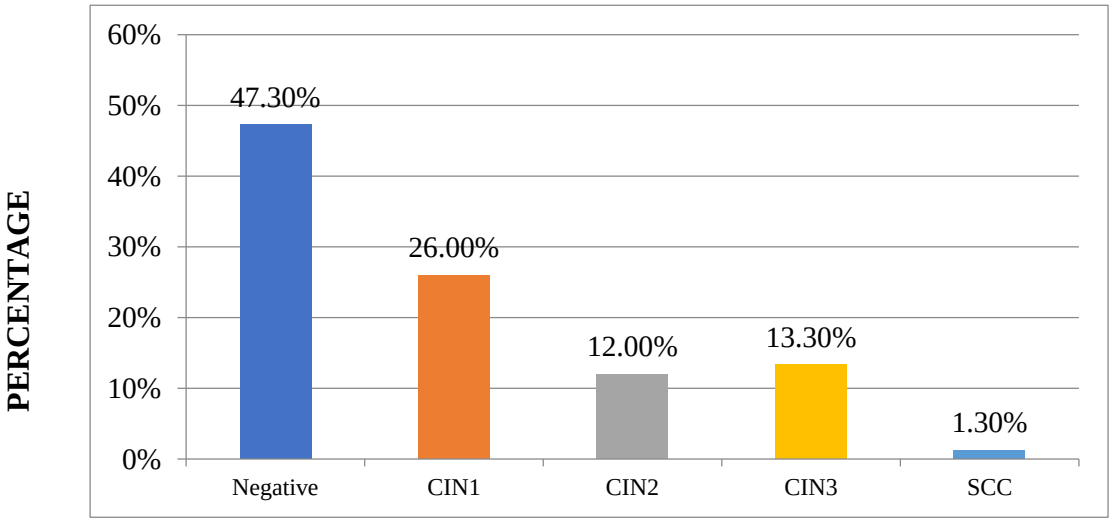


Chart 7: Bar diagram showing Histopathology results among study subjects

Figure 25: CIN 1 involving lower 1/3rd of the cervical epithelium [H&E,40X]

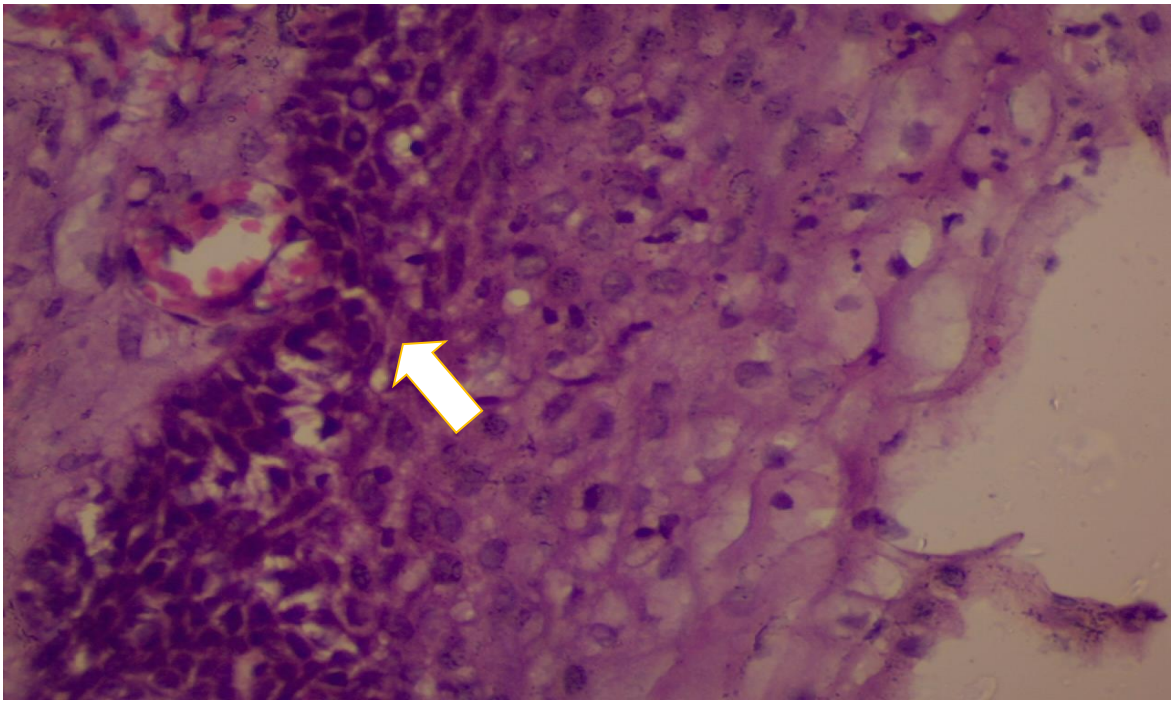


Figure 26: CIN 2 involving lower 2/3rd of the cervical epithelium [H&E,10x]

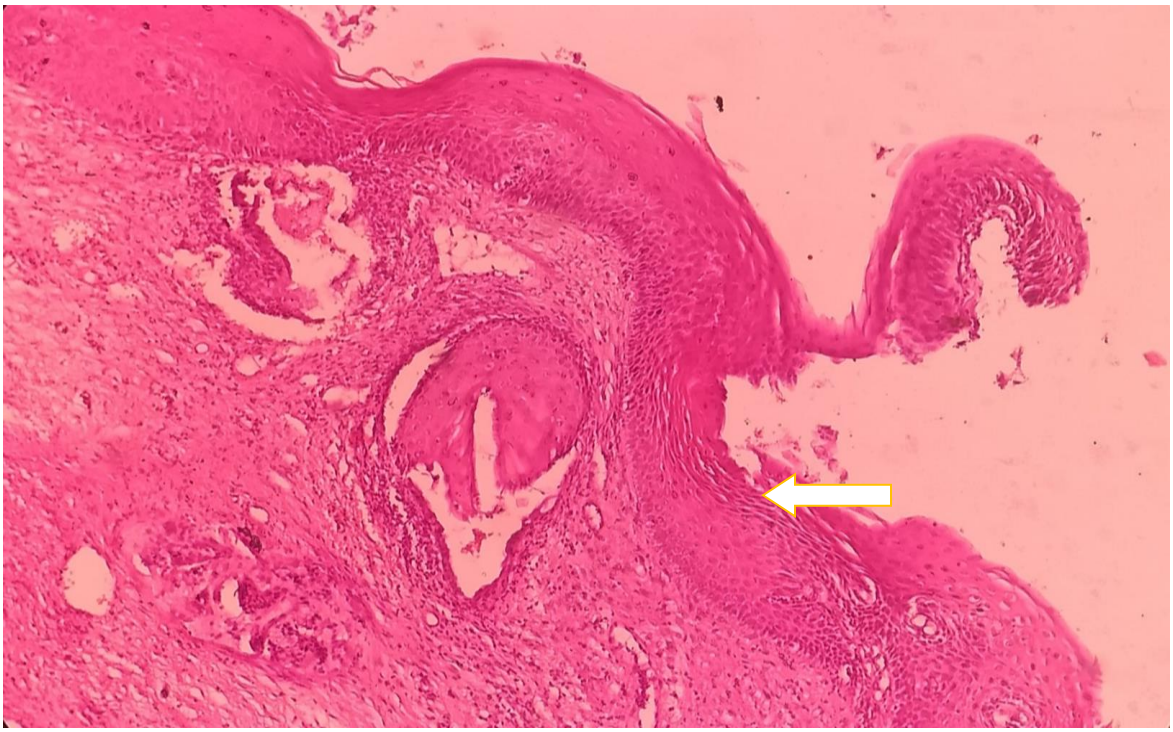


Figure 27 : CIN 3 involving entire thickness of cervical epithelium [H&E, 40x]

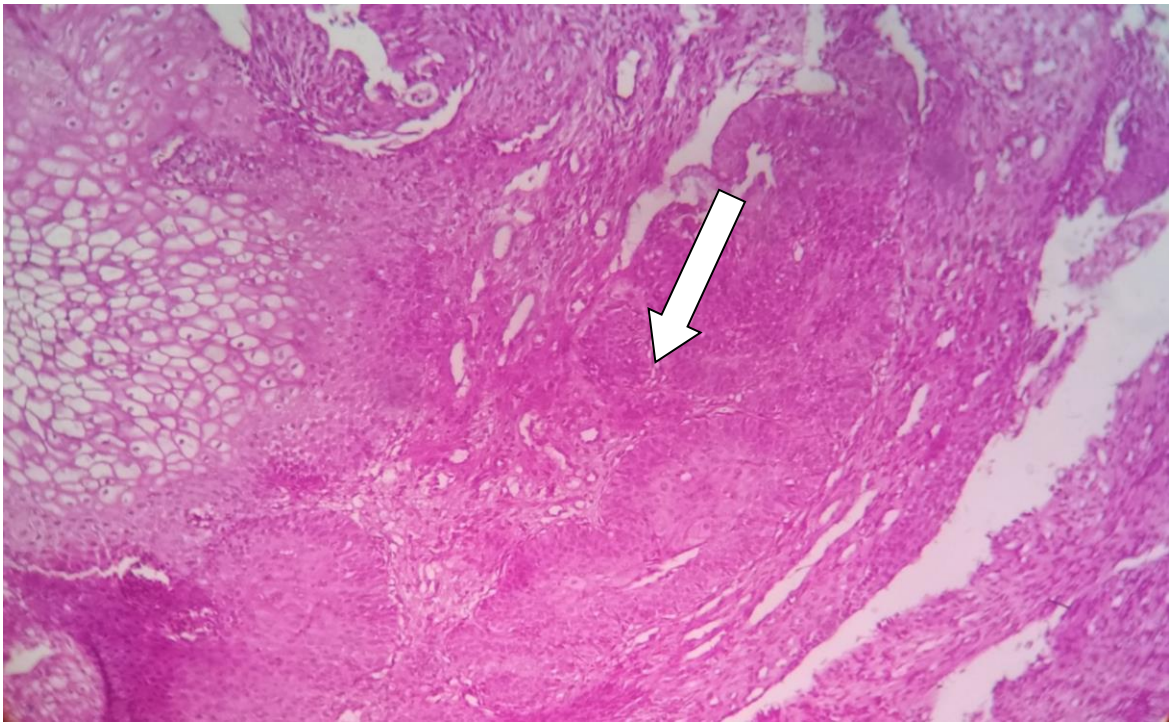
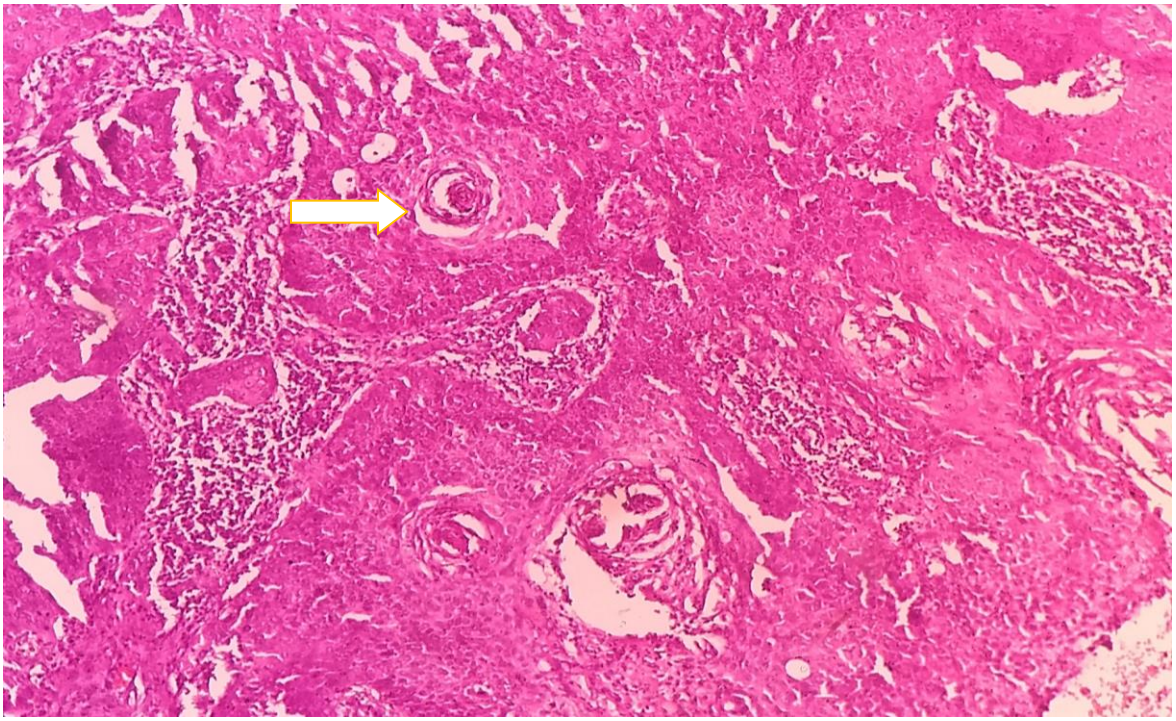


Figure 28 : Histopathology of SCC cervical epithelium [H&E, 40x]



Comparison between PAP results and Histopathology results

Among 75 subjects who were PAP positive, 74 were positive on histopathology (true positive) but 1 was negative (false positive). Among 75 PAP negative, 70 were negative on histopathology (true negative) but 5 were positive (false negative) as shown in table 9 and chart 8 . The association between PAP and histopathology results was significant but the single false positive case had reactive inflammatory changes and was reported as ASCUS on PAP , while the 5 false negative cases had very few abnormal cells which were unevenly distributed in the smear. Thus, PAP showed lower sensitivity of 93.67%, better specificity of 98.59%, PPV of 98.67%, but lower NPV of 93.3% and Diagnostic Accuracy of 96% as shown in table 10 and chart 9.

Table 9: Comparison between PAP and histopathology results

		Histopathology Results			
		Positive		Negative	
		Count	%	Count	%
PAP Results	Positive	74	98.6%	1	1.4%
	Negative	5	6.7%	70	93.3%

$\chi 2 = 127.322$, df = 1, $p < 0.001$*

.

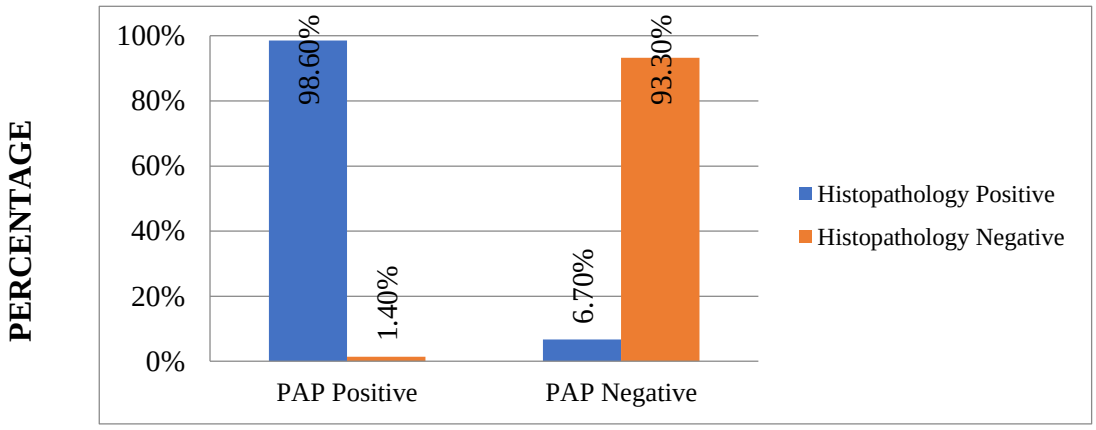


Chart 8: Bar diagram showing Comparison between PAP & Histopathology results

Table 10: Validity of PAP results with respect to Histopathology results

Parameter	Estimate	Lower - Upper 95% Cis
Sensitivity	93.67%	86.02, 97.27
Specificity	98.59%	92.44, 99.75
Positive Predictive Value	98.67%	92.83, 99.76
Negative Predictive Value	93.33%	85.32, 97.12
Diagnostic Accuracy	96%	91.55, 98.15
Cohen's kappa (Unweighted)	0.92	0.7602 - 1.08

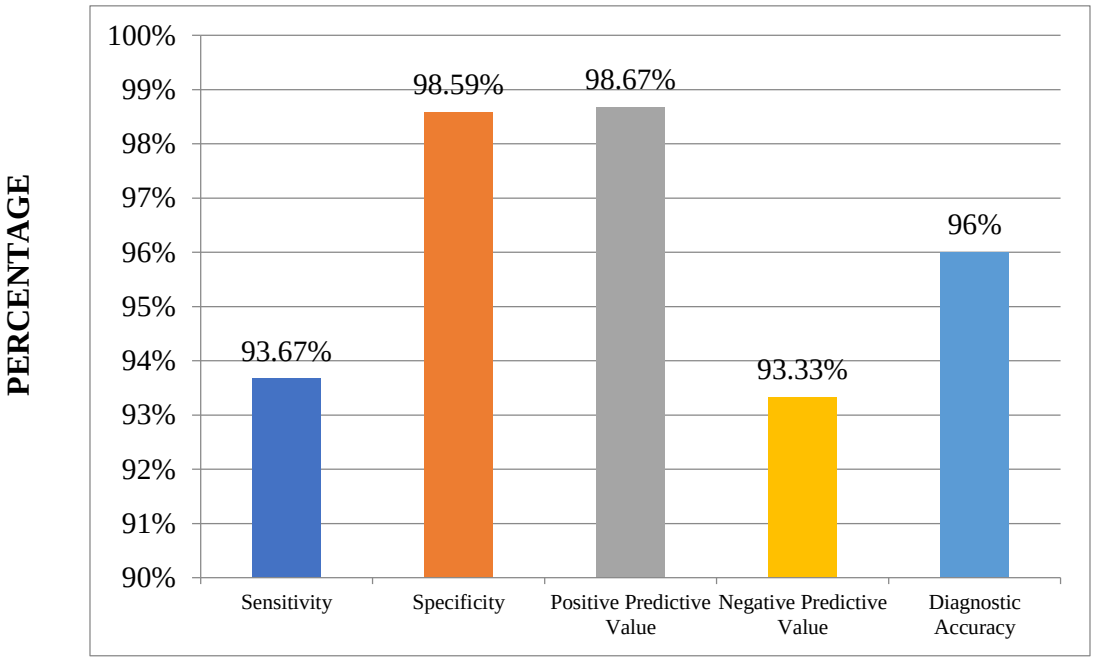


Chart 9: Bar diagram showing Validity of PAP results with respect to Histopathology results

Comparison between CAP PAP results and histopathology results

Among 83 CAP-PAP positive subjects, 79 [94.4%] were positive on histopathology (True positive) and 4 [5.6%] negative (false positive). Of the remaining 67 CAP-PAP negative, 100% were histopathology negative (True negative) and none were positive[no false negative] as shown in table 11 and chart 10. There were four cases reported falsely positive on CAP PAP of which.

- Three cases had intense inflammation where marker positivity in inflammatory cell was so intense, that morphology of squamous epithelial cells was obscured.
- In one case, many endocervical cells were mis-interpreted as ASCUS because of their inherent positivity.

There was significant association between CAP PAP and histopathology reports. CAP-PAP had better sensitivity of 100%, Specificity of 94.37%, Positive Predictive Value of 95.18%, Negative Predictive Value of 100% and Diagnostic Accuracy of 97.33%. Agreement between CAP PAP result and histopathology results was 0.946 which was almost perfect agreement as shown in table 12 and chart 11.

Table 11: Comparison between CAP PAP results and Histopathology results

		Histopathology results			
		Positive		Negative	
		Count	%	Count	%
CAP PAP results	Positive	79	94.4%	4	5.6%
	Negative	0	0.0%	67	100.0%

$\chi 2 = 134.72$, df = 1, $p < 0.001$*

.

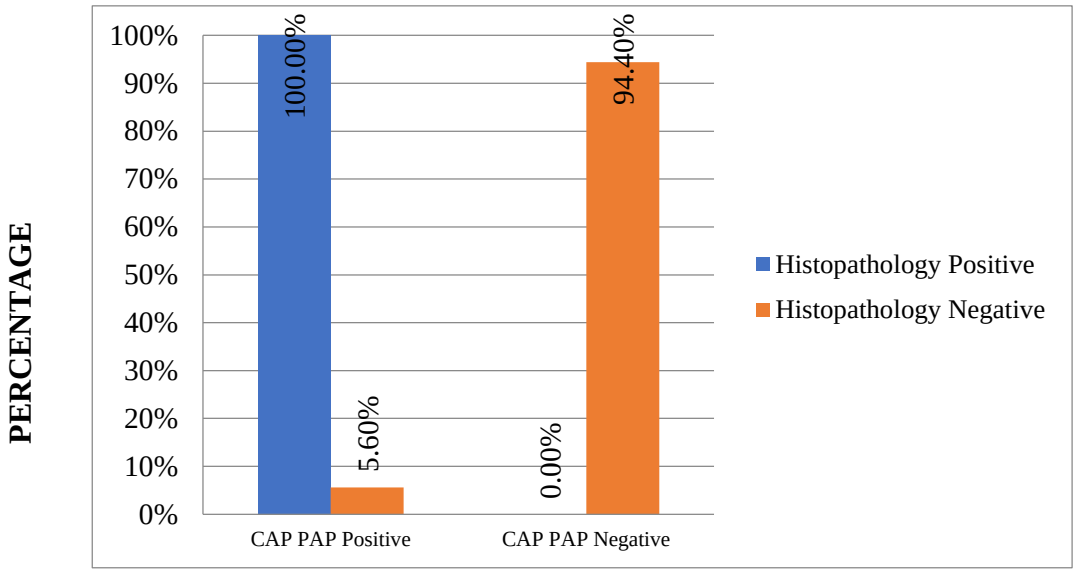


Chart 10: Bar diagram showing Comparison between CAP PAP results and Histopathology results

Table 12: Validity of CAP PAP results with respect to Histopathology results

Parameter	Estimate	Lower - Upper 95% Cis
Sensitivity	100%	95.36, 100
Specificity	94.37%	86.39, 97.79
Positive Predictive Value	95.18%	88.25, 98.11
Negative Predictive Value	100%	94.58, 100
Diagnostic Accuracy	97.33%	93.34, 98.96
Cohen's kappa (Unweighted)	0.9464	0.7866 - 1.106

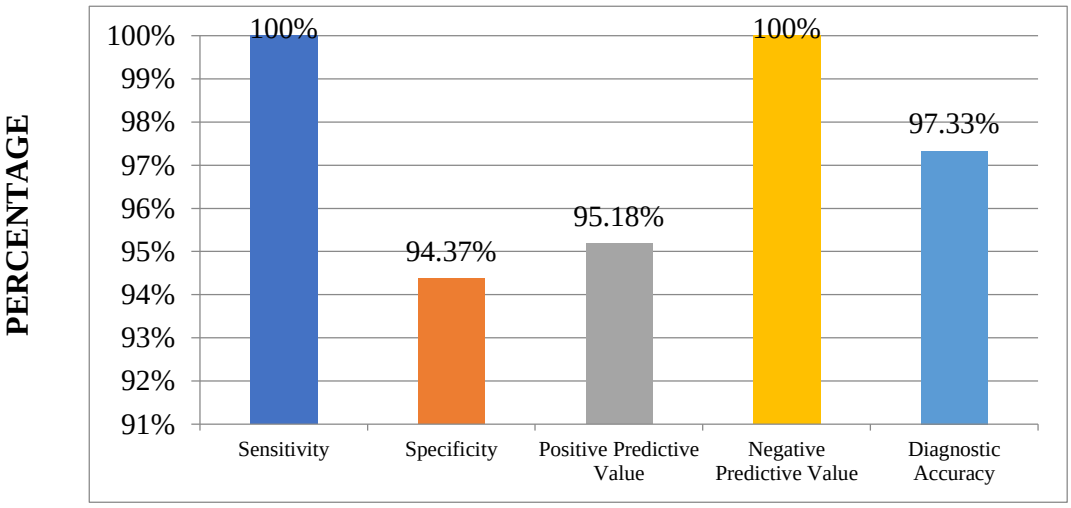


Chart 11: Bar diagram showing Validity of CAP PAP results with respect to histopathology results

DISCUSSION

DISCUSSION

The CAP-PAP Test is a new double-staining, single-slide microscopic method for the diagnosis of cervical dysplasia.[18] In this technique acid phosphatase enzyme would be labelled in abnormal cervical epithelial cells on Pap smears stained by conventional Papanicolaou technique and, thus improving visibility of abnormal cells and interpretation of Pap smears. Due to its increased accuracy (better sensitivity and equivalent specificity as Pap test) the CAP-PAP test could reduce false negative readings of the conventional Pap test.

Normal cervical epithelium contains acid phosphatase, but the enzyme activity gradually reduces subsequently to the maturation from basal to intermediate cells. Superficial cells are always negative. However, abnormal intraepithelial growth such as hyperplasia, dysplasia (mild and severe) and cancer are always positive for the enzyme. This discrepancy between enzyme activity inside normal and abnormal cells, makes cervical acid phosphatase a natural biomarker for detecting abnormal growth. CAP activity is not detected in cervical smears containing normal squamous cells excoriated from superficial layer of cervical epithelium. In the same time, CAP activity is detected in cervical smears containing squamous cells showing morphological signs of cell abnormality.[10,75,76]

A total of 150 patients were included in our study. There were 75 PAP negative smears of which 38 cases were inflammatory smears and 37 cases were of atrophic smears. In the remaining 75 PAP positive smears, 34 were LSIL, 13 were ASCUS, 17 were HSIL, 9 were ASC-H, and 2 were SCC cases

AGE AT PRESENTATION

Age of patients ranged from 20-88 years in our study. Majority of the cases of inflammation was seen in the reproductive age group. Cervical intraepithelial lesions/dysplasia was observed mostly in the age group of 31-50 years and malignancy was present after the age of 60 years. The mean age of presentation in our study was 42.96 yrs as compared to 40.4 yrs in a study done by Niranjan et al[51] and 35.4 yrs in another study done by Prabal Deb et al[8] the comparison of different age groups among various other studies is shown in table 13. This supports that cervical screening is delayed by many years in majority of females in India. There is a need for educating the people and increasing awareness about the benefits of early pap smear testing by conducting regular education programs. Moreover, the physicians and healthcare professionals should also motivate and educate the general public regarding pap testing.

Table 13 : Comparison of different age groups among various studies.

AGE GROUP	< 30 years	31 to 40 years	41 to 50 years	51 to 60 years	61 to 70 years	> 70 years
Present study	20.00%	33.30%	27.30%	8.00%	7.30%	4.00%
Ranabhat SK et al 2011[77]	23.52%	40.23%	24.55%	8.41%	2.38%	0.91%
Bhagya Lakshmi A et al 2014[78]	16.67%	26.67%	30%	23.33%	3.33%	-
Niranjan et al 2015[51]	19.86%	30.82%	31.51%	17.81%	-	-
Malpani G et al 2016[79]	33.40%	38.10%	20.34%	5.4%	2.20%	0.56%
Akinfolarin AC et al 2017[80]	8.00%	27.50%	35.90%	23.10%	5.60%	-

PRESENTING COMPLAINT

Among the 150 patients, 49.3% had WDPV, 18% had Mass PV, 19.3% underwent routine screening, 6% had pain abdomen, 6% had bleeding PV and 1.3% had burning Micturition. This was in comparison with a study done by Bhagya Lakshmi A et al[70] in which 43.3% were asymptomatic, 30% had menstrual abnormalities, 20% had WDPV, 6.7% had bleeding on touch.

DISTRIBUTION OF PAP RESULTS

In our study, there were 75 PAP positive smears out of which 34 were LSIL, 17 were HSIL, 13 were ASCUS, 9 were ASC-H, and 2 were SCC cases. In the remaining 75 PAP negative smears, 38 cases were inflammatory smears and 37 cases were of NILM smears. Of all epithelial cell abnormalities, LSIL was found to be most common followed by HSIL, ASCUS, ASC-H and then squamous cell carcinoma. This was similar to study by Gupta et al[81] who also reported LSIL to be most common epithelial cell abnormality. However, in a study by Ranabhat et al[77] HSIL was the most common lesion.

COMPARISON OF CAP-PAP AND PAP RESULTS

In our study, out of 75 subjects who were PAP positive, all 75 were CAP PAP positive and among those with PAP negative, 8 [10.7%] were CAP PAP positive and 67 [89.3%] were CAP PAP negative. Hence , CAP-PAP was positive in 83 cases and negative in 67 cases. To find out if there was difference in CAP and PAP smears examined, we tested the agreement between the two. We found that the observed agreement was 97%. Statistical significance was assessed by applying Kappa test and was found to be 89%, which is more than 80%. This proved that there was considerably good agreement between CAP-PAP and PAP. Similar agreement was observed in study done by Niranjan et al[51].

CORRELATION OF PAP RESULTS WITH HISTOPATHOLOGY

Among 75 subjects who were PAP positive, 74 were positive on histopathology and one was negative. Of the 75 PAP negative subjects, 70 were negative on histopathology but 5 were positive. The overall concordance rate of the study was 92% which is comparable with other studies like Nawaz et al[82], Yeoh et al[83] and Rasbridge et al[84] who have reported concordance rate of 74%, 52%, 81.2% respectively.[66,67,68] PAP had overall sensitivity of 93.67%, Specificity of 98.59%, Positive Predictive Value of 98.67%, Negative Predictive Value of 93.3% and Diagnostic Accuracy of 96%. This shows a good correlation between pap smear and histopathology and was compared with other studies[Table 14]

Table 14: Comparison of the PAP statistics with of various studies

Study	Sensitivity %	Specificity %	PPV %	NPV %	Diagnostic Accuracy %
Present study	93.67	98.59	98.67	93.33	96
Mallur et al[85]	41.66	81.2	86.21	78.26	40
Jain et al[86]	78	26.9	91	11.3	73.2
Ashmita et al[87]	19.51	83.3	80	86.5	23.26
Chaudary et al[88]	25.4	99.27	94.12	74.3	76
Naik et al[89]	79.4	58.3	86.1	46.6	74.5
Saha et al[90]	76	83.3	86.4	71.4	79.1

CORRELATION OF CAP-PAP RESULTS WITH HISTOPATHOLOGY

Among 67 CAP PAP negative cases, all of them were negative on histopathology and among the 83 CAP PAP positive cases, 79 were positive on histopathology but 4 were negative. Hence, these 4 cases were reported falsely positive on CAP PAP. In 3 of them, marker positivity in inflammatory cell was so intense, that morphology of squamous epithelial cells was obscured. In other case, many endocervical cells were mis-interpreted as malignant squamous cells because of their inherent positivity.

There was significant association between CAP PAP and histopathology. CAP PAP had sensitivity of 100%, Specificity of 94.37%, Positive Predictive Value of 95.18%, Negative Predictive Value of 100% and Diagnostic Accuracy of 97.33%. Agreement between CAP PAP and histopathology was 0.946 (Almost perfect agreement). This is similar to study done by Batra eta al[18] and Deb et al[8] [Table 15]

Table 15 : Comparison of the CAP-PAP results of various studies

STUDY	Sensitivity %	Specificity %	PPV %	NPV %	Diagnostic Accuracy %
Present study	100	94.37	95.18	100	97.33
Deb et al	100	89	50	100	-
Batra et al	100	97.2	93.33	100	-

Our study revealed that CAP-PAP detected 8 cases which PAP could not detect out of which 5 cases were asymptomatic[routine screening] and 3 had symptoms[WDPV]. The sensitivity of PAP was only 93.67% in detecting dysplastic lesions whereas CAP-PAP was 100% sensitive. Similar results were obtained by Markovic N et al.[75] This signifies the importance of CAP-PAP screening over PAP in apparently healthy females. Also, patients with inflammatory PAP smears can transform into cervical dysplastic lesion because of repeated HPV infection and hence these patients will need further evaluation .The CAP PAP will be able to detect these dysplastic changes at early stages.[91]

Limitations:

The technical simplicity and ability to detect intra epithelial lesions are the advantages of CAP test, this technique had many interpretation problems. In comparison to the conventional Pap stain in evaluating nuclear features, certain nuclear features were lost by air drying step. Nuclear details are the most essential criteria for detecting atypical squamous cells.

In some instances, endocervical cells with very high CAP activity, the red granules masked the nucleus causing difficulty in evaluation. The presence of enzyme activity in squamous metaplastic cells implies that only a person well-versed with routine cytopathology can evaluate a CAP smear. Otherwise, all smears with metaplastic squamous cells would be considered positive, which in the absence of clear nuclear details, can mimic an atypical cell and lead to an incorrect diagnosis.

All CAP-positive cell with nuclear enlargement or atypia, regardless of its size or presumed nature, needs to be considered as being a positive result with CAP. Metaplastic squamous cells can show nuclear enlargement, such cells would also be CAP-positive and would be considered positive with the CAP test. In all these instances, a conventional Pap smear is essential for proper evaluation of nuclear features.

Advantages of CAP-PAP staining

The CAP-PAP stain was easy to perform following the manufacturer's instructions and gave uniform staining results.[69] Endocervical cells always showed marked cytoplasmic granular red deposits. There was no extra cellular diffusion although in many instances the positivity was intense and obscured nuclear details. Squamous metaplastic cells were always CAP-positive. Red granules were seen mostly as small foci diffusely distributed in the cytoplasm of metaplastic cells, although many cells showed quite intense staining of most of the cytoplasm.

Parabasal, intermediate and superficial cells were uniformly negative with some cells showing faint granules, except in smears considered positive with CAP-PAP. Of the inflammatory cells monocytes and histiocytes showed positivity and showed most intense cellular reaction with CAP-PAP.

Smears having CAP-positive mature squamous cells or CAP-positive squamous cells with nuclear enlargement or nuclear atypia were considered positive in the CAP test. In such smears CAP-positive cells were observed focally, either as isolated cells or cell groups. Staining intensity was variable but usually quite intense. There was no

deposition on the nucleus, in most of the instances which permitted evaluation of nuclear details.

The most important aspect of the present study is the re-evaluation prompted by CAP positivity. Of all cases found to be positive by CAP-PAP, 8 were initially negative in routine Pap smears. After the CAP test, 5 more smears were upgraded to being positive with CIN 1 type of abnormality on histopathology.

CONCLUSION

CONCLUSION

This study was undertaken to evaluate the utility of CAP PAP staining in detection of cervical epithelial abnormalities in comparison with routine PAP staining. The study included both PAP positive and negative patients with histopathology reports as gold standard. It was found that, CAP-PAP had better sensitivity and specificity in detecting cervical epithelial abnormalities when compared to PAP staining. Also, CAP-PAP had better correlation with histopathology reports. Overall CAP-PAP staining performance was better in early detection of cervical epithelial abnormalities.

As indicated by the sensitivity of 100 %, CAP PAP fulfils the criteria of screening test, so this test could be very useful as a quick, economical and efficient method of large scale screening. CAP positivity helps in easy and early detection of abnormal cells because of red colored granules which are easily identified and thus speeds up the screening process. The test promises a great future at health centers where trained persons are not available, technicians can be easily trained for identification of abnormal smears.

SUMMARY

SUMMARY

Cervical cancer is the most common cancer among women in India. PAP staining is the most efficacious and cost-effective method of cancer screening. However, PAP staining has higher rates of false-positive and false-negative results. This study was conducted to evaluate the role of CAP-PAP staining in cervical cancer screening and compare these results with that of PAP staining and correlate them with histopathology results which is the gold standard test for detection of cervical cancer.

A total of 150 patients undergoing cervical cancer screening at SDUMC were included in the study. PAP staining and CAP-PAP staining were peformed and analysed on SPSS 17.0 and Microsoft excel. Chi-square test was used as test of significance for qualitative data. Kappa test was used to assess the agreement between various results.

On analysis, it was found that that CAP-PAP results had good agreement with PAP results. CAP-PAP staining had better correlation with histopathology result than PAP staining. With a sensitivity of 100%, CAP-PAP was the better test for screening of cervical cancers.

In conclusion, these results show that CAP-PAP staining can serve as an adjuvant to PAP smear for screening of cervical cancer. A larger trial is therefore needed before the widespread use of CAP-PAP staining can be recommended for clinical use.

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ANNEXURES

ANNEXURES

ANNEXURE – I : PATIENT INFORMATION AND CONSENT FORM

A Study of Papanicolaou [PAP] Stain and Cervical Acid Phosphatase - Papanicolaou [CAP-PAP] Stain in The Detection of Cervical Intra-Epithelial Neoplasm- A Comparative Study

Cervical cancer ranks as the most common cancer among women in India, and most frequent cancer among women between 15 and 44 years of age. Cervical carcinoma is the only cancer which documents the remarkable effects of screening, in early diagnosis and curative therapy on mortality rate. Application of Pap test has resulted in a dramatic reduction of both mortality and morbidity. Therefore, PAP test is extremely helpful in evaluating cervical cancer and prevents grave complications and mortality

You are requested to participate in a study conducted by the department of Pathology as a part of dissertation. This study will be done on the patients undergoing routine cervical cancer screening by pap smear.

This study has been approved by the institutional ethics committee. The information collected will be used only for dissertation and publication. There is no compulsion to agree to participate and the medical care you will get will not change. You are requested to sign or thumb impression only if you voluntarily agree to participate in this study.

All information collected from you will be kept confidential and will not be disclosed to any outsider. Your identity will not be revealed. You will have to pay for basic investigations and you will not receive any monetary benefits for participating in this research study.

For any further clarification you are free to contact the following investigator.

PRINCIPAL INVESTIGATOR: Dr Rajini. T

Contact no : 9916420738

E-mail : rajini11.gowda@gmail.com.

ರೋಗಿಯ ಮಾಹಿತಿ ಪತ್ರ

ಶೀರ್ಷಿಕೆ : A Study of Papanicolaou [PAP] Stain and Cervical Acid Phosphatase - Papanicolaou [CAP-PAP] Stain in The Detection of Cervical Intra-Epithelial Neoplasm- A Comparative Study

ಭಾರತದಲ್ಲಿ, ಗರ್ಭಕಂಠದ ಕ್ಯಾನ್ಸರ್ 15 ಮತ್ತು 44 ವರ್ಷ ವಯಸ್ಸಿನ ಮಹಿಳೆಯರಲ್ಲಿ ಅತ್ಯಂತ ಸಾಮಾನ್ಯ ಕ್ಯಾನ್ಸರ್ ಸ್ಥಾನ ಪಡೆದಿದೆ. ಸ್ಕ್ರೀನಿಂಗ್ ಕಾರಣದಿಂದ ಗರ್ಭಕಂಠದ ಕ್ಯಾನ್ಸರ್ ಸರಿಪಡಿಸುವ ಚಿಕಿತ್ಸೆ ಮತ್ತು ಮರಣ ಪ್ರಮಾಣ ಮೇಲೆ ಗಮನಾರ್ಹ ಸುಧಾರಣೆಗಳನ್ನು ಹೊಂದಿದೆ. ಪ್ಯಾಪ್ ಪರೀಕ್ಷೆ ಈ ರೋಗದ ಹರಡಿಕೆ ಮತ್ತು ಮರಣ ಸಂಖ್ಯೆಯನ್ನು ಗಣನೀಯ ಪ್ರಮಾಣದಲ ಇಳಿಕೆಗೆ ಕಾರಣವಾಗಿದೆ. ಆದ್ದರಿಂದ ಪ್ಯಾಪ್ ಪರೀಕ್ಷೆ ಗರ್ಭಕಂಠದ ಕ್ಯಾನ್ಸರ್ನ ಮಾಪಿಸುವ ಅತ್ಯಂತ ಸಹಾಯಕ ಪರೀಕ್ಷೆಯಾಗಿದೆ

ಈ ಅಧ್ಯಯನವು ಪ್ಯಾಪ್ ಸ್ಮೀಯರ್ ಮೂಲಕ ಗರ್ಭಕಂಠದ ಕ್ಯಾನ್ಸರ್ ಸ್ಕ್ರೀನಿಂಗ್ ಒಳಪಡುವ ರೋಗಿಗಳಲ್ಲಿ ಮಾಡಲಾಗುತ್ತದೆ. ನೀವು ಪ್ರೌಢಪ್ರಬಂಧದ ಅಂಗವಾಗಿ ಪೆಥಾಲಜಿ ವಿಭಾಗದಲ್ಲಿ ನಡೆಸಿರುವ ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಕೋರಲಾಗಿದೆ, ಸಂಗ್ರಹಿಸಿದ ಮಾಹಿತಿಯನ್ನು ಪ್ರೌಢಪ್ರಬಂಧದಲ್ಲಿ ಮತ್ತು ಪ್ರಕಟಣೆಗೆ ಮಾತ್ರ ಬಳಸಲಾಗುತ್ತದೆ.ನೀವು ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಮತ್ತು ಒಪ್ಪಿಕೊಳ್ಳಲು ಯಾವುದೇ ಕಡ್ಡಾಯ ಇರುವುದಿಲ್ಲ. ನೀವು ಸ್ವಯಂಪ್ರೇರಣೆಯಿಂದ ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಒಪ್ಪಿದಲ್ಲಿ ಮಾತ್ರ ಸೈನ್ ಅಥವಾ ಹೆಬ್ಬೆಟ್ಟಿನ ಗುರುತನ್ನು ಕೋರಲಾಗಿದೆ.

ಎಲ್ಲಾ ಮಾಹಿತಿಯನ್ನು ಗೌಪ್ಯವಾಗಿ ಇಡಲಾಗುತ್ತದೆ ಮತ್ತು ಯಾವುದೇ ಹೊರಗಿನವರ ಬಹಿರಂಗ ಮಾಡಲಾಗುವುದಿಲ್ಲ. ನಿಮ್ಮ ಗುರುತನ್ನು ಎಲ್ಲೂ ತೋರಿಸಲಾಗುವುದಿಲ್ಲ. ನೀವು ನಿಮ್ಮ ಮೂಲ ಪರೀಕ್ಷೆಗಳಿಗೆ ಹಣ ಕಟ್ಟಬೇಕು ಮತ್ತು ಈ ಸಂಶೋಧನಾ ಅಧ್ಯಯನದಲ್ಲಿ ಯಾವುದೇ ಪರಿಶೀಲನಾ ಲಾಭವನ್ನು ಸ್ವೀಕರಿಸುವುದಿಲ್ಲ.

ಯಾವುದೇ ಹೆಚ್ಚಿನ ಸ್ಪಷ್ಟೀಕರಣಗಳಿಗೆ ನೀವು ಕೆಳಗಿನ ಸಂಶೋಧಕರನ್ನು ಸಂಪರ್ಕಿಸಬಹುದು.

ಪ್ರಧಾನ ಸಂಶೋಧಕರು : **ಡಾ ರಜಿನಿ .T**

ಫೋನ್ : 9916420738

Email : rajini11.gowda@gmail.com

ANNEXURE 2 : PATIENT CONSENT FORM

Sl. NO:

Patient Name:

Mobile No:

Title: **A Study of Papanicolaou [PAP] Stain and Cervical Acid Phosphatase - Papanicolaou [CAP-PAP] Stain in The Detection of Cervical Intra-Epithelial Neoplasm- A Comparative Study**

I , the undersigned, agree to participate in this study and give consent for conducting study on PAP smear and disclosure of my personal information as outlined in this consent form.

I have been read out or explained in my local language i.e., in kannada and understand the purpose of this study and the confidential nature of the information that will be collected and disclosed during the study. I have had the opportunity to ask questions regarding the various aspects of this study and my questions have been answered to my satisfaction. The information collected will be used only for research.

I also understand there is no risk to my life and there is actually benefit from this study.

I understand that I remain free to withdraw from this study at any time. Participation in this study does not involve any extra cost to me.

Subject's name and signature/thumb impression

Name and signature of witness

1.

2.

Name and signature of Principal/Co-investigator

ರೋಗಿಯ ಸಮ್ಮತಿ ಪತ್ರ

ಕ್ರಮ ಸಂಖ್ಯೆ :

ರೋಗಿಯ ಹೆಸರು :

ಮೊಬೈಲ್ ನಂಬರ್ :

ಶೀರ್ಷಿಕೆ : **A Study of Papanicolaou [PAP] Stain And Cervical Acid Phosphatase - Papanicolaou [CAP-PAP] Stain In The Detection Of Cervical Intra-Epithelial Neoplasm- A Comparative Study**

ಈಕೆಳಗೆ ರುಜು ಮಾಡಿರುವ ನಾನು, ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು, ಪ್ಯಾಪ್ ಸ್ಮೀಯರ್ ಅಧ್ಯಯನ ನಡೆಸಲು ಮತ್ತು ಈ ಸಮ್ಮತಿ ನಮೂನೆಯ ಅಂಶಗಳಂತೆ ನನ್ನ ವೈಯಕ್ತಿಕ ಮಾಹಿತಿಯನ್ನು ಬಹಿರಂಗಪಡಿಸುವ ಒಪ್ಪಿಗೆ ನೀಡಿರುತ್ತೇನೆ.

ನನಗೆ ಈ ಅಧ್ಯಯನದ ಉದ್ದೇಶ ಹಾಗು ಗೋಪ್ಯತೆಯ ವಿಚಾರವನ್ನು ನನ್ನ ಭಾಷೆಯಾದ ಕನ್ನಡದಲ್ಲಿ ವಿವರಿಸಲಾಗಿದೆ. ಈ ಅಧ್ಯಯನದ ಕುರಿತಾದ ನನ್ನ ಎಲ್ಲ ಪ್ರಶ್ನೆಗಳಿಗೂ ಸಮಾಧಾನಕರ ಉತ್ತರ ನನಗೆ ದೊರಕಿರುತ್ತದೆ. ಎಲ್ಲ ಮಾಹಿತಿಗಳು ಸಂಶೋಧಗೆಗಾಗಿಯೇ ಬಳಸಲಾಗುವುದು.

ಈ ಅಧ್ಯಯನದಿಂದ ನನ್ನ ಜೀವಕ್ಕೆ ಯಾವುದೇ ಹಾನಿ ಇರುವುದಿಲ್ಲ ಮತ್ತು ಹೆಚ್ಚು ಅನುಕೂಲಕರವಾಗಿದೆ ಎಂದು ನನಗೆ ಅರ್ಥವಾಗಿರುತ್ತದೆ. ನಾನು ಯಾವಾಗ ಬೇಕಾದರೂ ಈ ಅಧ್ಯಯನದಿಂದ ಹೊರನಡೆಯಬಹುದು ಮತ್ತು ನನಗೆ ಯಾವುದೇ ರೀತಿಯ ಅಧಿಕ ಖರ್ಚಾಗಿರುವುದಿಲ್ಲವೆಂದು ನಾನು ಒಪ್ಪಿಕೊಂಡಿರುತ್ತೇನೆ.

ರೋಗಿಯ ಹೆಸರು ಮತ್ತು ರುಜು/ಬೆರಳುಗುರುತು

ಸಾಕ್ಷಿಗಳ **ಹೆಸರು ಮತ್ತು ರುಜು**

1.

2.

ಪ್ರಮುಖ ಸಂಶೋಧಕರ ಹೆಸರು ಮತ್ತು ರುಜು

ANNEXURE – II

PATIENT PROFORMA

NAME :

AGE/SEX:

HOSPITAL NUMBER:

CHIEF COMPLAINT :

BLEEDING PV ☐ DISCHARGE PV ☐ DYSPERUNIA ☐

AGE OF MENARCHE

MARITAL STATUS

FAMILY HISTORY

PREVIOUS PAP SMEAR REPORT (IF ANY)

ANNEXURE – III

MASTER CHART

*** To protect patients identity and to maintain confidentiality as stated in patient information and consent form (annexure 1), patients name and hospital registration number have not been mentioned in the master chart.

PAP Positive patients

SLNO	AGE [yrs]	Presenting Complaint	Pap Report	Cap Pap Report	Histopathology Report
1	54	ROUTINE SCREENING	HSIL	POSITIVE	CIN3
2	45	WDPV	ASC-H +	POSITIVE	CIN3
3	45	ROUTINE SCREENING	ASC-H	POSITIVE	CIN2
4	85	ROUTINE SCREENING	LSIL	POSITIVE	CIN1
5	38	ROUTINE SCREENING	ASCUS	POSITIVE	NEGATIVE
6	40	ROUTINE SCREENING	ASC-H	POSITIVE	CIN2
7	60	ROUTINE SCREENING	ATROPHIC	POSITIVE	CIN1
8	50	ROUTINE SCREENING	ASC-H	POSITIVE	CIN3
9	30	ROUTINE SCREENING	ASCUS	POSITIVE	CIN1
10	40	ROUTINE SCREENING	LSIL	POSITIVE	CIN1
11	48	WDPV	ASCUS	POSITIVE	CIN1
12	62	ROUTINE SCREENING	ASCUS	POSITIVE	CIN1
13	50	ROUTINE SCREENING	LSIL	POSITIVE	CIN1
14	45	ROUTINE SCREENING	LSIL	POSITIVE	CIN1
15	70	ROUTINE SCREENING	HSIL	POSITIVE	CIN3
16	75	PAIN ABDOMEN	HSIL	POSITIVE	CIN3
17	38	ROUTINE SCREENING	LSIL	POSITIVE	CIN1
18	65	WDPV	LSIL	POSITIVE	CIN1
19	55	ROUTINE SCREENING	LSIL	POSITIVE	CIN1
20	30	ROUTINE SCREENING	HSIL	POSITIVE	CIN3
21	45	ROUTINE SCREENING	LSIL	POSITIVE	CIN1
22	45	MASS PV	LSIL	POSITIVE	CIN1
23	75	MASS PV	LSIL	POSITIVE	CIN1
24	39	WDPV	LSIL	POSITIVE	CIN1
25	40	MASS PV	LSIL	POSITIVE	CIN1
26	60	MASS PV	LSIL	POSITIVE	CIN1
27	45	MASS PV	LSIL	POSITIVE	CIN1
28	35	ROUTINE SCREENING	LSIL	POSITIVE	CIN1
29	60	ROUTINE SCREENING	LSIL	POSITIVE	CIN1
30	50	WDPV	HSIL	POSITIVE	CIN3
31	23	MASS PV	LSIL	POSITIVE	CIN1
32	45	MASS PV	LSIL	POSITIVE	CIN1
33	60	MASS PV	LSIL	POSITIVE	CIN1

PAP Positive patients

SLNO	AGE [yrs]	Presenting Complaint	Pap Report	Cap Pap Report	Histopathology Report
34	70	BLEEDING PV	LSIL	POSITIVE	CIN1
35	32	BLEEDING PV	HSIL	POSITIVE	CIN3
36	65	BLEEDING PV	LSIL	POSITIVE	CIN1
37	50	PAIN ABDOMEN	LSIL	POSITIVE	CIN2
38	60	PAIN ABDOMEN	LSIL	POSITIVE	CIN2
39	35	WDPV	ASC-H	POSITIVE	CIN2
40	45	ROUTINE	ASC-H WITH	POSITIVE	CIN3
41	88	ROUTINE	ASCUS	POSITIVE	CIN1
42	37	WDPV	HSIL	POSITIVE	CIN3
43	35	WDPV	HSIL	POSITIVE	CIN3
44	60	WDPV	HSIL	POSITIVE	CIN3
45	50	WDPV	ASCUS	POSITIVE	CIN1
46	85	ROUTINE	LISL	POSITIVE	CIN1
47	40	BLEEDING PV	LSIL	POSITIVE	CIN2
48	45	BLEEDING PV	HSIL	POSITIVE	CIN3
49	40	PAIN ABDOMEN	HSIL	POSITIVE	CIN3
50	40	BLEEDING PV	HSIL	POSITIVE	CIN3
51	40	WDPV	ASCUS	POSITIVE	CIN1
52	54	WDPV	ASCUS	POSITIVE	CIN1
53	40	WDPV	LSIL	POSITIVE	CIN2
54	63	WDPV	LSIL	POSITIVE	CIN2
55	42	WDPV	HSIL	POSITIVE	CIN3
56	46	WDPV	HSIL	POSITIVE	CIN3
57	35	WDPV	LSIL	POSITIVE	CIN2
58	30	WDPV	LSIL	POSITIVE	CIN2
59	62	WDPV	SCC	POSITIVE	SCC
60	60	WDPV	HSIL	POSITIVE	CIN3
61	39	WDPV	ASCUS	POSITIVE	CIN1
62	63	WDPV	SCC	POSITIVE	SCC
63	31	WDPV	LSIL	POSITIVE	CIN2
64	30	WDPV	ASC-H	POSITIVE	CIN2
65	35	WDPV	LSIL	POSITIVE	CIN2
66	27	WDPV	LSIL	POSITIVE	CIN2
67	40	WDPV	HSIL	POSITIVE	CIN3
68	33	WDPV	ASC-H	POSITIVE	CIN2
69	48	WDPV	HSIL	POSITIVE	CIN3
70	47	WDPV	ASCUS	POSITIVE	CIN1
71	35	WDPV	ASC-H	POSITIVE	CIN2
72	64	WDPV	LSIL	POSITIVE	CIN2
73	23	WDPV	ASCUS	POSITIVE	CIN1
74	42	WDPV	ASCUS	POSITIVE	CIN1
75	36	WDPV	LSIL	POSITIVE	CIN2

PAP Negative patients

SLNO	AGE [yrs]	Presenting Complaint	Pap Report	Cap Pap Report	Histopathology Report
1	43	WDPV	NILM	NEGATIVE	NEGATIVE
2	44	WDPV	NILM	NEGATIVE	NEGATIVE
3	63	WDPV	INFLAMMATORY	POSITIVE	CIN1
4	44	WDPV	NILM	NEGATIVE	NEGATIVE
5	36	WDPV	NILM	NEGATIVE	NEGATIVE
6	40	WDPV	NILM	NEGATIVE	NEGATIVE
7	27	WDPV	INFLAMMATORY	NEGATIVE	NEGATIVE
8	29	WDPV	NILM	NEGATIVE	NEGATIVE
9	50	WDPV	NILM	NEGATIVE	NEGATIVE
10	32	WDPV	NILM	NEGATIVE	NEGATIVE
11	24	WDPV	NILM	NEGATIVE	NEGATIVE
12	40	WDPV	NILM	NEGATIVE	NEGATIVE
13	35	WDPV	NILM	NEGATIVE	NEGATIVE
14	24	WDPV	NILM	NEGATIVE	NEGATIVE
15	47	WDPV	NILM	NEGATIVE	NEGATIVE
16	35	WDPV	NILM	NEGATIVE	NEGATIVE
17	38	MASS PV	NILM + INFLAMMATORY	NEGATIVE	NEGATIVE
18	40	WDPV	NILM + INFLAMMATORY	NEGATIVE	NEGATIVE
19	37	WDPV	NILM + INFLAMMATORY	NEGATIVE	NEGATIVE
20	27	MASS PV	NILM + INFLAMMATORY	NEGATIVE	NEGATIVE
21	30	MASS PV	NILM + INFLAMMATORY	NEGATIVE	NEGATIVE
22	50	BURNING MICTURITION	ATROPIC VAGINITIS	NEGATIVE	NEGATIVE
23	75	WDPV	NILM	NEGATIVE	NEGATIVE
24	32	WDPV	NILM +	NEGATIVE	NEGATIVE
25	35	WDPV	NILM	NEGATIVE	NEGATIVE
26	50	WDPV	NILM +	POSITIVE	CIN1
27	26	WDPV	NILM +	NEGATIVE	NEGATIVE
28	28	WDPV	NILM	NEGATIVE	NEGATIVE
29	45	WDPV	NILM +	POSITIVE	CIN1
30	25	WDPV	NILM	NEGATIVE	NEGATIVE
31	36	WDPV	NILM + INFLAMMATORY	NEGATIVE	NEGATIVE
32	45	WDPV	NILM	NEGATIVE	NEGATIVE
33	50	WDPV	NILM	NEGATIVE	NEGATIVE
34	45	WDPV	NILM	NEGATIVE	NEGATIVE
35	70	WDPV	NILM	NEGATIVE	NEGATIVE
36	28	WDPV	NILM	NEGATIVE	NEGATIVE
37	30	WDPV	NILM	NEGATIVE	NEGATIVE
38	55	WDPV	NILM	NEGATIVE	NEGATIVE
39	40	MASS PV	NILM	NEGATIVE	NEGATIVE
40	20	MASS PV	NILM + INFLAMMATORY	NEGATIVE	NEGATIVE
41	50	MASS PV	NILM + INFLAMMATORY	POSITIVE	CIN1

PAP Negative patients

SLNO	AGE [yrs]	Presenting Complaint	Pap Report	Cap Pap Report	Histopathology Report
42	35	MASS PV	NILM + BACTERIAL	NEGATIVE	NEGATIVE
43	25	WDPV	NILM +	NEGATIVE	NEGATIVE
44	26	MASS PV	NILM	NEGATIVE	NEGATIVE
45	55	MASS PV	NILM	NEGATIVE	NEGATIVE
46	37	MASS PV	NILM +	NEGATIVE	NEGATIVE
47	32	MASS PV	NILM	NEGATIVE	NEGATIVE
48	30	MASS PV	NILM	NEGATIVE	NEGATIVE
49	28	WDPV	NILM	NEGATIVE	NEGATIVE
50	30	MASS PV	NILM	NEGATIVE	NEGATIVE
51	25	MASS PV	NILM + BACTERIAL	NEGATIVE	NEGATIVE
52	34	MASS PV	NILM	NEGATIVE	NEGATIVE
53	24	MASS PV	NILM	NEGATIVE	NEGATIVE
54	44	MASS PV	NILM +	POSITIVE	NEGATIVE
55	34	WDPV	NILM	NEGATIVE	NEGATIVE
56	37	MASS PV	NILM	NEGATIVE	NEGATIVE
57	49	MASS PV	NILM	NEGATIVE	NEGATIVE
58	38	ROUTINE	NILM +	NEGATIVE	NEGATIVE
59	45	ROUTINE	NILM +	POSITIVE	NEGATIVE
60	25	PAIN	NILM +	NEGATIVE	NEGATIVE
61	26	ROUTINE	INFLAMMATORY	NEGATIVE	NEGATIVE
62	38	ROUTINE	NILM WITH	NEGATIVE	NEGATIVE
63	30	PAIN	NILM + BACTERIAL	NEGATIVE	NEGATIVE
64	45	ROUTINE	NILM +	POSITIVE	NEGATIVE
65	50	ROUTINE	ATROPIC VAGINITIS	NEGATIVE	NEGATIVE
66	35	ROUTINE	NILM + REACTIVE	NEGATIVE	NEGATIVE
67	39	PAIN	NILM + BACTERIAL	NEGATIVE	NEGATIVE
68	46	PAIN	NILM + BACTERIAL	NEGATIVE	NEGATIVE
69	49	BLEEDING PV	NILM + BACTERIAL	NEGATIVE	NEGATIVE
70	23	PAIN	NILM + BACTERIAL	NEGATIVE	NEGATIVE
71	38	BLEEDING PV	NILM +	NEGATIVE	NEGATIVE
72	42	BURNING	NILM +	NEGATIVE	NEGATIVE
73	36	WDPV	NILM +	NEGATIVE	NEGATIVE
74	35	BLEEDING PV	NILM + BACTERIAL	NEGATIVE	NEGATIVE
75	50	WDPV	NILM +	POSITIVE	CIN1
70	23	PAIN	NILM + BACTERIAL	NEGATIVE	NEGATIVE
71	38	BLEEDING PV	NILM +	NEGATIVE	NEGATIVE
72	42	BURNING	NILM +	NEGATIVE	NEGATIVE
73	36	WDPV	NILM +	NEGATIVE	NEGATIVE
74	35	BLEEDING PV	NILM + BACTERIAL	NEGATIVE	NEGATIVE
75	50	WDPV	NILM +	POSITIVE	CIN1