COMPARATIVE STUDY OF CONVENTIONAL PAPANICOLAOU SMEAR AND CYTOSPIN SMEAR IN CERVICAL CANCER SCREENING

By

Dr. AMRUTHA.M.R. **MBBS**

Dissertation submitted to the
BLDE University, Vijayapur, Karnataka

In partial fulfillment of the requirements for the award of the degree of

DOCTOR OF MEDICINE
IN
PATHOLOGY

UNDER THE GUIDANCE OF

Dr. MAHESH.H.KARIGOUDAR **M.D., DNB**

PROFESSOR,

DEPARTMENT OF PATHOLOGY

BLDE UNIVERSITY'S, SHRI B.M. PATIL MEDICAL COLLEGE,
HOSPITAL & RESEARCH CENTRE, VIJAYAPUR, KARNATAKA.

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Date: **Dr. AMRUTHA.M.R**

Place: Vijayapur

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& RESEARCH CENTRE, VIJAYAPUR

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Date:

Place: Vijayapur

Dr. MAHESH.H.KARIGOUDAR

Professor

Department of Pathology,

BLDEU Shri B.M.Patil Medical College,

Hospital & Research Centre, Vijayapur,

Karnataka

B.L.D.E UNIVERSITY'S

SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL

& RESEARCH CENTRE, VIJAYAPUR

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Date:

Place: Vijayapur

Dr. NEELAMMA PATIL
Associate Professor
Department Of OBG,
BLDEU Shri B.M.Patil Medical College, Hospital & Research Centre, Vijayapur, Karnataka

B.L.D.E UNIVERSITY'S

SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL

& RESEARCH CENTRE, VIJAYAPUR

ENDORSEMENT BY HEAD OF DEPARTMENT

This is to certify that the dissertation entitled **"COMPARATIVE STUDY OF CONVENTIONAL PAPANICOLAOU SMEAR AND CYTOSPIN SMEAR IN CERVICAL CANCER SCREENING"** is a bonafide research work done by **Dr. AMRUTHA.M.R** in partial fulfillment of the requirements for the degree of **Doctor of Medicine (Pathology).**

Date:

Place: Vijayapur

DR. B. R. YELIKAR

Professor and H.O.D,

Department of Pathology,

BLDEU Shri B.M. Patil Medical College, Hospital & Research Centre, Vijayapur, Karnataka.

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SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL

& RESEARCH CENTRE, VIJAYAPUR

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Date:

Place: Vijayapur

DR.S P GUGGARIGOUDAR

Principal,
BLDEU Shri B.M. Patil
Medical College, Hospital &
Research Centre, Vijayapur,
Karnataka.

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Date: **Dr. AMRUTHA.M.R**

Place: Vijayapur

ACKNOWLEDGEMENT

This study has been accomplished with the grace of almighty God. It gives me immense pleasure to express my heartfelt gratitude to all. I dedicate this page to each and everyone who have helped me to explore the expanses of knowledge.

A line from Sanskrit Shloka says "Guru r brahma guru r Vishnu gurudevo maheshwaraha, guru ssakshaat parabrahma tasmay shri gurave namaha" – meaning a teacher is next to god and without him knowledge is always incomplete.

Firstly I would like to express my sincere and deepest gratitude to my teacher and guide ***Dr.Mahesh.H.Karigoudar,*** *Professor, Department of Pathology, for his encouragement and invaluable guidance throughout the course of my study. His guidance helped me in all the time of research and writing of this thesis. I could not have imagined having a better advisor and mentor for my study.*

I am equally grateful to ***Dr. B.R. Yelikar,*** *Professor and H.O.D, Department of Pathology for his valuable suggestions, indispensable guidance given at all the steps of the study. He has a profound influence on both my personal growth and professional pursuits.*

I am thankful to ***Dr.Neelamma Patil****, Asso.Professor, Department of OBG for her valuable suggestions and guidance in all the time of research and thesis writing.*

My sincere thanks to ***Dr Padmaja Kulkarni,*** *Asso.Professor for guiding me, suggesting this research topic and being a well-wisher.*

I am also extremely fortunate to have a caring, approachable and supportive department, who have advised and mentored me and made it possible for me to expedite this dissertation. I am thankful to ***Dr. S.U. Arakeri*** *Prof****, Dr. S.B. Hippargi*** *Prof,* ***Dr.R.M.Potekar*** *Prof,* ***Dr. Girija Patil*** *Assoc Prof,* ***Dr. Prakash M. Patil*** *Assoc Prof,* ***Dr. Vijayalaxmi S Patil*** *Asst. Prof****, Dr. Savitri M. Nerune*** *Asst prof,* ***Dr. Anita P Javalgi*** *Asst prof,* ***Dr. Mamatha K.*** *Asst Prof and* ***Dr Sneha Jawalkar*** *Asst Prof****.*** *for their supervision, assiduous concern and positive feedback at all steps of this work.*

My heartful thanks to ***Dr.Teena D Murthy, Dr. Himanshu D Mulay, Dr.Abhigna V, Dr.Sumith S Deep****, statistician* ***Mohd. Shannawaz****, my seniors, batchmates and juniors who have helped and encouraged me during my work.*

I am very grateful to all non-teaching staff of Department of Pathology ***Ashok, Jessy, Praveen, Ningappa Mathad*** *and others who have helped me during this work.*

I am deeply indebted to my parents ***Mrs.Lathamani.G*** *,* ***Mr. Ranganatha.G****, my husband* ***Dr.Lohith.B.M,*** *my brother* ***Dr.Sathish Chandra.M.R*** *and in-laws* ***Mrs.Puttamma****,* ***Mr.Marulaiah.B*** *for their help, constant encouragement and moral support that led me to successfully complete this dissertation work.*

*Last but not the least****,*** *my sincere gratitude to all my study subjects whose cooperation has contributed to this study.*

Date: ***DR. AMRUTHA. M. R***

Place: Vijayapur

LIST OF ABBREVIATIONS USED

AGC-NOS	-	Atypical Glandular cells- Not Otherwise Specified
AJCC	-	American Joint Committee on Cancer
ASCUS	-	Atypical Squamous Cells of Undetermined Significance
ASC-H	-	Atypical Squamous Cells cannot exclude HSIL
CIS	-	Carcinoma in-situ
CIN	-	Cervical Intraepithelial Neoplasia
CPS	-	Conventional Papanicolaou Smear
DNA	-	Deoxyribonucleic acid
ELISA	-	Enzyme- linked immunosorbent assay
FIGO	-	Federation of International of Gynecologists and Obstetricians
FISH	-	Fluorescence In-Situ Hybridization
FDA	-	Food and Drugs Administration
H and E	-	Hematoxylin and Eosin
HIV	-	Human Immunodeficiency Virus
HPV	-	Human Papilloma Virus
HSIL	-	High-grade Squamous Intraepithelial Lesion
IS	-	Inflammatory smear
LBC	-	Liquid- Based Cytology
LBP	-	Liquid- Based Preparations
LSIL	-	Low-grade Squamous Intraepithelial Lesion
MLBC	-	Manual Liquid- Based Cytology

NILM	-	Negative for Intraepithelial Lesion/ Malignancy
OBG	-	Obstetrics and Gynecology
PAP	-	Papanicolaou
PCR	-	Polymerase Chain Reaction
PV	-	Per Vaginal
SCC	-	Squamous Cell Carcinoma
SIL	-	Squamous Intraepithelial Lesions
TBS	-	The Bethesda System
TNM	-	Tumor, Nodes, Metastasis
TZ	-	Transformation Zone
WHO	-	World Health Organization

ABSTRACT

Background:

Cervical carcinoma is the second most common malignancy in women worldwide after breast cancer, but is the most common among women in developing countries. Cytological screening leads to a reduction in the rate of invasive cancer of uterine cervix. The present study highlights the use of cytospin i.e, manual method of liquid based cytology in preparing cervical smears which is a cost effective alternative in low resource settings to improve the efficacy of Pap smears.

Objectives:

1. To study the conventional Papanicolaou smears according to the Bethesda system of classification, 2014.
2. To study the cytospin Papanicolaou smears according to the Bethesda system of classification, 2014.
3. To compare efficacy of cytospin Papanicolaou smears with conventional Papanicolaou Smears in cervical cancer screening based on Bethesda system of classification, 2014.

Materials and methods:

A prospective study of 134 samples was carried out in the Department of Pathology, B.L.D.E.U'S Shri B M Patil Medical College, Hospital & Research Centre, Vijayapur by split smear technique for conventional Pap smear and liquid-based cytology during 1st December 2014 to 30th June 2016. Cervical cytology samples from women from 18-65 years attending the Obstetrics and gynaecology Out Patient Department were

taken by using Ayre’s spatula and one slide was prepared and immediately fixed in fixative and the residual material was rinsed in fixative then spun in cytospin to obtain direct smear. Both smears were stained by Pap stain.

Results:

Of the 134 cervical cytospin smears studied, 3 were unsatisfactory, 118 were non-neoplastic and 13 were neoplastic lesions. The most common neoplastic lesion was LSIL and HSIL accounting for 4 cases each followed by ASCUS, squamous cell carcinoma and adenocarcinoma. Cytospin showed significant difference in the morphological features compared to conventional.

Interpretation and Conclusion:

Cytospin method of manual liquid based cytology is strongly recommended in the best interest of public health as it improves the sample quality, reduces the likelihood of false negative results and better morphology. It over comes the limitation of conventional smear as it significantly reduces unsatisfactory smears, improves specimen adequacy, detects more intraepithelial lesion. It is of value as an alternative more effective screening strategy in low resource settings, like developing countries including India where women are at high risk for developing cervical cancer.

Keywords:

Cervical carcinoma, conventional Pap smear, cytospin Pap smear.

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INTRODUCTION

Cervical cancer is the second most common malignancy among women worldwide after breast cancer, but is the most common among women in developing countries. Globally, cervical cancer constitutes about 12% of all cancers in women. India bears one fifth of the world's burden, with an incidence of 1,22,844 cases (22.9%) and mortality of 20.7%. The incidence in developed countries is as low as 2.9%. The age standardized incidence rate for cervical cancer in India is 22/1,00,000 and age standardized mortality rate of 12.4/1,00,000 which are the highest in South Central Asia.[1]

Cytological screening leads to a reduction in the rate of invasive cancer of uterine cervix. The sensitivity of the conventional Pap smears for the detection of cervical cancer is less due to several limitations including inadequate transfer of cells to slide, inhomogenous distribution of abnormal cells, presence of obscuring blood, inflammation or thick areas of overlapping epithelial cells. To overcome these limitations liquid-based cytology (LBC) came into existence.[2] The screening Pap test remained unchanged for over half a century, until recently, when several new advances were developed. The SurePath® & ThinPrep® LBC systems are now well established for cervical cancer screening. Ample research on ThinPrep® and SurePath® techniques has evaluated the efficacy of these cytological methods and their technical and economic impact on cytology laboratories and cervical cancer screening. However, there is a lack of studies evaluating the screening efficacy of direct-to-vial systems based on cytocentrifugation. Cytocentrifugation LBC techniques consist of the PapSpin system (ThermoShandon Inc, Pittsburgh, USA), Turbitec (Labonord, Templemars, France) and CytoSCREEN (Seroa, Monaco, Monaco).[3]

In the last 15 years new cytological techniques have been developed to improve the sensitivity of Pap smear. Liquid-based cytology is the most accepted method, in which obscuring cells, mucus and blood are removed. It allows for a better morphological assessment and improves the sensitivity of Pap smear. Other advantages include reduction of unsatisfactory/ inadequate smears, provision for detection of Human Papilloma Virus (HPV) DNA by PCR or in situ DNA hybridization and other ancillary techniques like immunocytochemistry and cell blocks which can be performed on the residual sample.[4]

ThinPrep® and SurePath® are the two techniques approved by Food and Drug Administration (FDA), USA, which are liquid-based preparations and are used to obtain cervical smears, which have significantly improved the sensitivity of the screening program.[5]

LBC accounts for more than 90% of the Pap tests performed in the United States.[6] However this is not same in India as well in many developing countries where conventional Pap test is still commonly followed. Cytocentrifugation (cytospin) LBC techniques could be cheaper alternatives to SurePath® & ThinPrep® LBC systems.

Studies by NM Nandini et al[7], Kavatkar AN et al [5] and Lee JM et al [8] have found manual liquid-based cytology to be comparable with the conventional Pap smears. This low cost screening technique can be a potential adjuvant for the conventional Pap screening technique.

The present study aimed at preparing cervical cytology smears using the cytospin method of liquid-based cytology and comparing its results with that of conventional Pap smears.

AIMS AND OBJECTIVES

1. To study the conventional Papanicolaou smears according to the Bethesda system of classification, 2014.
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REVIEW OF LITERATURE

FEMALE GENITAL TRACT

The female genital tract is composed of the vulva, the vagina, the uterus, the fallopian tubes, and the ovaries etc.

Ovaries - Produce female gametes (oocytes) and female sex hormones.

Uterine tubes - Convey oocytes toward uterus; site of fertilization.

Uterus - Site of implantation; protects and sustains embryo and fetus during pregnancy, plays active role in parturition (childbirth).

Vagina - Conveys uterine secretions to outside of body and serves as passage way for fetus during partition.

Labia majora - Form margins of pudendal cleft; enclose and protect labia minora.

Labia minora - Form margins of vaginal vestibule; protect openings of vagina and urethra.

Clitoris - Glans of the clitoris is richly supplied with sensory nerve endings.

Pudendal cleft - Cleft between labia majora within which labia minora and clitoris are located.

Vaginal vestibule- Cleft between labia minora within which vaginal and urethral openings are located.

Vestibular glands - Secrete fluid that moistens and lubricates the vaginal vestibule.[9]

ANATOMY OF NORMAL CERVIX

The cervix is a cylindrical fibromuscular structure measuring 2.5 to 3cm in length in the lower portion of uterus. The endocervical canal connects the body of the

uterus through the internal os and with the vagina exteriorly through the external os. The protruding lower portion of the cervix forms the fornices in the upper vagina where pooling of secretions and exfoliated cells occurs. The outer aspect of the cervix is known as the ectocervix or portio vaginalis. [9]

NORMAL HISTOLOGY

The cervix consists of relatively little smooth muscle and predominantly fibroelastic connective tissue. The ectocervix is covered by non-keratinizing stratified squamous epithelium in continuity with vaginal epithelium distally. The lining of the endocervix is mucin-secreting tall columnar epithelium and it is not exposed to the vaginal pH. An inconspicuous layer of reserve cells lies beneath the endocervical lining epithelium. The glandular mucosa extends into the stroma of the cervix in a racemose pattern forming branching crypts. Squamocolumar junction is the junction of endocervical mucosa with ectocervical squamous epithelium.[10]

TRANSFORMATION ZONE:

Mature (pale) squamous and immature (dark pink) squamous metaplastic cells along with endocervical columnar epithelium constitute the cervical squamocolumnar junction. The original squamocolumnar junction lies at the junction of native ectocervical epithelium and endocervical columnar epithelium. Whereas the functional squamocolumnar junction lies at the junction of metaplastic squamous cells with the endocervical columnar cells, and the transformation zone is the area between the two squamocolumnar junctions and recedes into the endocervix in post-menopausal women.[11]

EMBRYOLOGY

The paired paramesonephric ducts form the uterine tubes and fuse caudally forming the epithelium of the uterine canal including the corpus, cervix uteri and upper part of vagina. The surrounding mesoderm forms the myometrium. The thickness of myometrium increases and the unfused part forms the fundus of the uterus. The fold which extends from the lateral sides of the fused paramesonephric ducts toward the pelvic wall forms the broad ligament of the uterus.[12]

PHYSIOLOGY

The epithelium of the ectocervix is subject to cycles of remodeling by proliferation, maturation, and desquamation during the reproductive period. The epithelium is completely replaced by a new population of cells every 4–5 days by the action of oestrogen. As the serum oestrogen levels fall, maturation ceases and glycogen disappears rapidly. The endocervix undergoes cyclic changes in the cervical mucus- Oestrogen makes the mucus profuse, watery and alkaline while progesterone makes it thick, scanty and acidic. During ovulation, the mucus is the thinnest forming spinnbarkeit and increasing the elasticity. It also dries in a fern-like pattern when spread on a slide.[11]

Table 3.1: Differential characteristics of normal squamous genital cells [13]

Criteria	Basal	Parabasal	Intermediate	Superficial
Size (µ)	8-10	15-25	30-60	40-60
Shape				
Polygonal (%)	0	5	85	75
Oval (%)	5	40	10	20
Round (%)	95	55	5	5
Occurrence	Sheets 90%	Single 60% Sheets 40%	Single 80%	Single 90%
Amount of cytoplasm	Scanty	Adequate	Abundant	Abundant
Cytoplasmic border curling	Rare	Rare	Common	Rare
Cytoplasmic stain	Deep blue	Blue	Pink or blue	Orange
Cytoplasmic vacuolization	None	Occasional	Occasional	None
Nuclei- cytoplasmic ratio	8:10	5:10	2:10	1:10
Nuclear size (µ)	7-9	8-13	10-12	5-7
Nuclear shape	Round	Round to oval	Round to oval	Round to oval
Chromatin pattern	Coarse	Granular	Finely granular	Pyknotic
Multinucleation	Rare	Few	Few	Rare
Nucleoli	None	Occasional and prominent	Small	None

CYTOLOGICAL INDICES:

The degree of proliferation, maturation and desquamation of cells is influenced by various hormones.

Maturation index (MI), Karyopyknotic (KPI) or Cornification index (CI), Eosinophilic index (EI) Folded cell index (FCI), Superficial cell index (SCI), Crowded cell index (CCI), Maturation value (MV).

Maturation index(MI)- The percentage of the basal, intermediate and superficial cells are presented as a three-part ratio with the basal cells stated first, the intermediate cells second and the superficial cells third.

NORMAL CYTOHORMONAL AVERAGES

Newborn (upto 8 weeks) MI= 0/90/10 +/- 10

The increased number of intermediate cells, often with glycogen in their cytoplasm, in the vaginal smear of the newborn is the result of the persisting effect of maternal hormones in the infant's blood.

Infancy and childhood (from 3weeks to puberty) MI= 80/20/0 +/- 20

They contain mainly parabasal cells. This cellular pattern persists until 1 to 2 years.

Menstrual age (Reproductive period) MI= 0/60/40 +/- 20

Superficial and intermediate cells are always present, but few parabasal cells are found if the mucosa is intact.

Menopause MI= 100/0/0 +/- 10

Maturation index varies greatly. The exfoliated superficial and intermediate squamous cells become progressively smaller.[13]

Dr. George N. Papanicolaou, in early 1940s, for the first time described that the vaginal smears could be prepared to screen for cervical cancers and introduced the Papanicolaou (Pap) stain. Papanicolaou and Traut also published their famous monograph "Diagnosis of Uterine Cancer by the Vaginal Smears". Dr. J Ernest Ayre introduced the wooden spatula to scrape the cervix at the transformation zone in 1947 now referred to as the Ayre's spatula.[14,15]

Since Papanicolaou's introduction of Pap smear, a variety of terms have been used to describe accompanying cytological diagnoses. The Papanicolaou classification introduced in 1954 graded dysplastic cells into 5 classes depending of the presence/ absence of atypia and whether suggestive of or not or conclusive of malignancy. The WHO scheme graded the precancerous squamous lesions of the cervix into dysplasias (mild/moderate/severe depending on the extent of morphological changes) and carcinoma in situ. The CIN system introduced by Richart in 1969 followed next which emphasized on dysplasias and carcinoma in situ as a continuum. Cervical intraepithelial neoplasia was subdivided into grades 1 to 3 according to the degree of abnormality encountered. [16]

The first Bethesda workshop in 1988 at Bethesda, Maryland , chaired by Robert Kurman, focused on addressing the issues related to the wide variability in reporting results of cervical cytology when cytologists used either the numeric 'Pap Class' system or the 'dysplasia' terminology. The objective was to establish terminology that would provide clear-cut thresholds for management and decrease interobserver variability.

The 3 fundamental principles emerged and have guided The Bethesda System (TBS) to this day:

1. Terminology must communicate clinically relevant information from the laboratory to the patient's health care provider.

2. Terminology should be uniform and reasonably reproducible across different pathologists and laboratories and also flexible enough to be adapted in a wide variety of laboratory settings and geographic locations.

3. Terminology must reflect the most current understanding of cervical neoplasia.[17]

Subsequently, The Bethesda System (TBS) was revised in 1991, 2001 and 2014 and classifies the squamous cells as atypical squamous cells (atypical squamous cells of undetermined significance (ASCUS), atypical squamous cells-cannot exclude HSIL (ASC-H), low-grade squamous intraepithelial lesions (LSIL), high-grade squamous intraepithelial lesions (HSIL) and squamous cell carcinomas. The glandular lesions have been classified as atypical glandular cells- not otherwise specified (AGC-NOS), atypical glandular cells- favor neoplastic, adenocarcinoma in-situ and adenocarcinoma.[18]

Table 3.2: Different reporting systems for cervical cytological squamous epithelial abnormalities.[16]

Papanicolaou	WHO	CIN	Bethesda (2014)
Class I			Negative for intraepithelial lesion/malignancy
Class II	Mild dysplasia	CIN 1	Atypical squamous cells Mild dysplasia
			Low grade SIL
Class III	Moderate dysplasia	CIN 2	High-grade SIL
	Severe dysplasia	CIN 3	High-grade SIL
Class IV	Carcinoma in situ	CIN 3	High-grade SIL
Class V	Carcinoma	Carcinoma	Carcinoma

The occasional failure of conventional cervical smear led to the development of liquid-based preparations in the early 70s in Germany, which also favored the development of computer assisted cervical screening. This helped in achieving a monolayer of cells and a high contrast between nuclei and cytoplasm with a clear background. There are many liquid-based systems available. Some of them are ThinPrep® (Hologic), SurePath® (Becton Dickinson), PapSpin (ThermoShandon), LiquiPrep (LGM International), Turbitek (Labonord), NovaPrep (Novacyt), GluCyte (BestPrep, CellSolutions). The standardized preparations, omission of cell damage, random distribution of cells on the slide and possibility of other ancillary test performance on the residual sample has made the technique favorable. However the

disadvantages are high cost of equipment and consumables, training of cytotechnicians, expertise of cytopathologists and no standardization of number of cells in the smear. The manual liquid-based preparations are cost-effective compared to automated methods.[19]

Muskett JM et al[20] in 1966 conducted a study on 906 women comparing two methods- the scrape smears using Ayre spatula introduced by Papanicolaou and irrigation smears introduced by Davis using a cytopipette. Davis pipette smears were more time consuming and the spectrum of cells seen with Ayre smear was not seen with it. Hence they concluded that the Ayre smear was more effective than the Davis cytopipette in detecting malignancy and that it was the method of choice for population screening of cervical cancers.

ThinPrep® was the first liquid-based system to obtain USA FDA approval in the year 1996. For the ThinPrep® Pap test, the specimen collected from the patient with a cervical sampling device is rinsed into ThinPrep® vial containing PreservCyt transport medium which is then placed in the ThinPrep® Processor. Monodispersion is achieved through rotation and monolayer through a membrane filter. The representative sample is transferred to the slide by randomization and a 20mm diameter smear is produced.[19]

The second LBC system approved by FDA was SurePath®, in the year 1999. In this system, the sampling device used is a combination brush/spatula with detachable head which is placed in SurePath® preservative fluid. Using the principle of density gradient centrifugation, the BD PrepStain slide processor generates a thin-layer smear of 13mm diameter which is also stained. Thus the granulocytes, erythrocytes and debris are discarded due to lower density.[19]

Hutchinson ML et al[21] in a population-based study conducted in Costa Rica in 1999 evaluated 8000 women as the province had a high incidence of cervical cancer. The sample was collected using Cervex brush and conventional smears were prepared following which residual cells were rinsed into vials containing PreservCyt preservative. ThinPrep® slides were prepared by the processor using the membrane filter method and a 20mm circular smear was obtained. They compared the conventional method with the ThinPrep® method of LBC in all cases and correlated with histopathological diagnoses and HPV DNA in some cases. The diagnosis of ASCUS by ThinPrep® was 12.7% while it was 6.7% by conventional smears. The diagnosis of HSIL and SCC by ThinPrep® was significantly higher (92.9% and 100%) when compared to conventional smears. Therefore the ThinPrep® LBC method increased the colposcopy referrals and sensitivity of Pap smears.

Richard K et al[22] in 1999 studied 100 cases of cervicovaginal fluid-based ThinPrep® Pap specimens and subjected them to cell block preparation based on the morphologic findings on the slide. Cell block aided the diagnoses in 20% cases and was immensely useful in situations where architecture and morphology were critical to the diagnosis like differentiating immature squamous metaplasia from HSIL. Therefore they concluded that cell block preparation can be a valuable adjunct to LBC in the diagnosis of cervical lesions.

A retrospective cohort study by Schorge JO et al[23] in 2002 on accuracy of detection of cervical and endometrial adenocarcinoma by ThinPrep® was carried out. Sample was collected with cytobrush/plastic spatula and slides were prepared using ThinPrep® 2000 automated slide processor (Cytyc). Over a 24 month period, smears

diagnosed as adenocarcinoma or AGC were identified and correlated with biopsy findings. By ThinPrep®, 186 cases were reported and confirmed by histology whereas 77 cases were detected by conventional smears. Thus the ThinPrep® test was observed to have significantly inproved the detection of cervical and endometrial adenocarcinomas.

Chacho MS et al[24] in their study in 2003 studied the cytohistologic correlation rates between conventional Pap smears and ThinPrep® in a retrospective analysis of 1544 cytology and histology slides. The ThinPrep® specimens were processed after all their laboratory personnel completed appropriate training. They found no statistically significant difference between the correlation rates of conventional and ThinPrep® smear results. They also observed that the ThinPrep® was less consistent in predicting invasive carcinoma compared to conventional Pap smears.

Abulafia O et al[25] in 2003 reviewed 24 articles published in the English literature wherein ThinPrep® method of LBC was compared with conventional cytology and histology. The ThinPrep® method by which the obscuring material was decreased by means of dispersion, centrifugation and membrane filtration was studied in comparison with conventional Pap smears. They found that the two methods agreed in 92% of dichotomous classifications and 89% of five-level classifications. They concluded that ThinPrep® tends to be more sensitive and specific in detecting cervical dysplasia than conventional smears.

Nam JH et al[26] in 2004 published their study wherein modified MonoPrep2 (Monogen) method was compared with ThinPrep® method of LBC. MonoPrep2 was a new method using manual filtration system to provide monolayers of cells. A nylon mesh was used in front of a filter to eliminate mucus. The results of the two methods were

compared and histopathological correlation was done on samples from 1218 patients. Obscuring factors were slightly more frequent with MonoPrep2 method and 13 specimens were excluded due to poor specimen quality. However the MonoPrep method was comparable with the ThinPrep® method and provided a cost-effective alternative to the ThinPrep® method.

Alves VAF et al[27] in their study in 2004 compared three different methods of liquid-based cytology- ThinPrep® (automated), Autocyte (manual) (TriPath Imaging) and DNACITOLIQ (manual) (Digene, Brazil). They evaluated for 16 morphologic parameters and found that in spite of different methodologies all the three systems provided good cellular morphology preservation for evaluation although more cellular overlapping and inflammatory infiltrate was found with the manual methods compared with the ThinPrep® method. Hence, the choice of method therefore depends on the price, procedure and availability of the methods.

Fremont-Smith M et al[28] in a study in 2004 conducted a study comparing the SurePath® LBC smears with conventional smears among 58580 SurePath® slides. They used direct-to-vial sampling device and the obtained sample was subjected to density gradient centrifugation to remove debris and excess inflammatory cells. The results of the study showed higher detection rates of HSIL and LSIL when compared with the conventional smears. Also, a decrease in the unsatisfactory smears and higher rate of ASCUS detection was noted although ASCUS/SIL ratio was reduced overall. Thus they concluded that SurePath® outperformed the conventional slides.

Another study by Hussein T et al[29] in 2005 studied the results of the two methods – conventional smear and ThinPrep® and compared it with histological

diagnoses in all the 563 cases. The split-sample technique was used to obtain the material. Using the T2000 processor (Cytyc) to prepare LBC slides, the rate of inadequate smears was significantly reduced. LBC had better sensitivity with higher detection rates of low and high grade lesions although conventional smears had better specificity. They however indicated that larger studies are required to verify the findings.

In a study by Garbar C et al[30] in 2005, inexpensive LBC techniques were studied using a liquid fixative Easyfix (Labonord corp) and cytocentrifuge like Papspin (ThermoShandon) and Turbitec (Labonord) and the diagnoses compared with those obtained by histolology. The sample obtained was first vortexed to obtain a homogenous solution. For Turbitec centrifugation with alcoholic fixative liquid diluted with polyethylene glycol was done and polylysined slide was used. For the Papspin technique ThermoShandon Megafunnel was used and centrifuged at 1250 rpm for 5 mins. Papspin and Turbitec had sensitivities of 82.6% and 75% and specificities of 92.6% and 96.2% respectively. They also evaluated the efficiency of the liquid fixative by testing for HPV DNA in the collected samples by Hybrid Capture II assays and compared it with those of HPV PCR and found good correlation between them with a kappa value of 0.89. They concluded that LBC performed by cytocentrifugations showed excellent efficiency and also allowed HPV detection by molecular methods.

Lee JM et al[8] conducted a retrospective study in 2006 of 300 samples cases including 150 HIV positive and 150 low-risk cases. They used 2ml residual sample from SurePath® collection vials to make MLBC slide by centrifuging it for 10 minutes at 800g and resuspending it in an alcoholic-agar solution consisting of polyvinyl alcohol, polyethylene glycol, agar, glycerin, polyanionic alcohol soap and gelatin glue. They

found a good overall agreement (76.3% agreement) between the manual membrane method of liquid-based cytology and conventional cytology diagnoses. They concluded that the low-cost manual membrane method of liquid-based cytology method is comparable with the standard commercial method and may be used as an alternative screening strategy in limited resource settings.

The study conducted by Sherwani RK et al[2] in 2007 on 160 cases observed that PapSpin is for the best interest of public health in low resource settings as it reduces false negative results and improves sample quality. They used the residual material from Ayre's spatula and endocervical brush by rinsing it in LiquiPrep fluid and spun it in Shandon cytospin for 10 min at 1500rpm. They found higher detection rates of abnormal smears by liquid-based cytology (26.2%) than by conventional Pap smears (15%) which were histologically proven.

Zhu J et al[31] in their study conducted in Sweden in 2007, used the split-sample technique for ThinPrep® method of LBC and compared it with conventional Pap smears. They concluded that ThinPrep® liquid-based preparation had 66% sensitivity when compared to conventional Pap having 47% sensitivity in detecting HSIL. There was a significant decrease in reporting of ASCUS by LBC. The ThinPrep® technique used here had lesser false negative rates and better concordance with histological diagnoses. But the ThinPrep® method increases the laboratory costs due to disposables which necessitates the economical evaluation before including it for screening programs.

In a study by Kavatkar AN et al[5] in 2008, sample from 105 patients was collected by Cervex-brush (Rovers medical devices) and rinsed in SurePath® preservative fluid in half the cases and fixative prepared with water, sodium chloride, sodium citrate,

10% formalin and alcohol in the remaining for manual method of liquid-based cytology. Following centrifugation, 1-2ml of polymer solution was applied and vortex mixed to obtain smear on to glass slide. The results of the manual method were found to be comparable with the conventional Pap smears and showed a good concordance and a good overall agreement. Thus, they concluded that the MLBC favored better morphology visualization and hence can be considered as a cost effective alternative to liquid-based cytology.

In a study by Kitchener HC et al[31], liquid-based cytology in combination with HPV DNA testing was done among 24510 women in primary screening for CIN and compared with LBC screening alone by randomized controlled trials. LBC samples were prepared using ThinPrep® T3000 processor and HPV DNA testing by Digene Hybrid Capture 2 test. They found that combined HPV DNA and LBC testing showed lower detection rates of lesions in comparison to LBC testing alone.

A study by Kim JH et al[33] in 2010 in Korea investigated the feasibility of detection of methylated DNA in cervical LBC samples as a screening tool for SIL and SCC. They observed an increasing methylation of HIN-1, MGMT, RAR-β, RASSF1A and SHP-1 genes with increasing severity of cervical SILs. They concluded that although aberrant DNA methylation can be a potential biomarker for SIL and SCC in LBC samples, additional genes need to be studied for better clinical performance.

Lopez-Cuervo JE et al[34] in their study stated that there was a gain in sensitivity by 4.52% and drop in the number of unsatisfactory smears to 0.5% using the NovaPrep Processor system when compared to conventional Pap smears, with an additional benefit of safety from viral/ bacterial particle contamination as the vial is never

opened again once the sample is assessed. LBC samples were processed by NPS50 instrument using a fixative medium with direct-to vial sampling.

A prospective study of 100 patients was conducted by Nandini NM et al[7] in 2012 on manual liquid-based cytology in primary screening for cervical cancer. They used a liquid fixative for sample preservation composed of sodium chloride, sodium citrate, 10% formalin and isopropyl alcohol and centrifuged it with a polymer solution containing agarose, polyeythlene glycol, poly-l-lysine and alcohol. They concluded that MLBC was better than conventional Pap in the diagnosis of precursor lesions with better sensitivity in diagnosis of Low-grade Squamous Intraepithelial Lesions. The percentage agreement by the two methods was found to be 68%. Thus it was concluded that MLBC can be used as an alternative strategy for cervical cancer prevention when resources are limited.

A study by Verma K[35] in 2014 compared 200 cases of liquid-based cytology using cytospin (ThermoShandon) with conventional Pap smears in the screening of unhealthy cervix. The sample collected was centrifuged for 5 minutes at 2000rpm in cytospin and slides were fixed with alcohol. The study showed better sensitivity and specificity and detection of more abnormal smears with liquid-based cytology compared to the conventional Pap smear. In view of these findings, they have strongly recommended the advocation of LBC systems for the best interest of public health as it improves the quality of the sample and reduces the false negative results.

In a study by Singh VB et al[36] in 2015, split sampling was conducted for 1000 samples and the results of liquid-based cytology were compared with the conventional Pap smear results. Multiple parameters were considered as per the Bethesda

system 2001. In the prospective study using SurePath® technique, the number of unsatisfactory samples was significantly reduced and the LBC technique offered better morphology, uniformity of cellular distribution. The sensitivity and specificity of LBC and conventional Pap smears were found to be equivalent.

Cervical Neoplasia-

WHO histological classification for tumors of uterine cervix [37]

Epithelial Tumors-

Squamous tumors and precursors- Squamous cell carcinoma NOS and variants

Glandular tumors and precursors- Adenocarcinoma

Other epithelial tumors- Adenosquamous carcinoma

Adenoid cystic carcinoma

Adenoid basal carcinoma

Neuroendocrine tumors

Undifferentiated carcinoma

Mesenchymal tumors and tumor-like conditions

Mixed epithelial and mesenchymal tumors

Melanocytic tumors

SQUAMOUS CELL CARCINOMA

Introduction:

Cervical carcinoma accounts for 7.9% of all malignancies in women worldwide. It is also the second most common malignancy in women in India, bearing one fifth of the world's burden, with an incidence of 1,22,844 cases (20.2%).[1]

Definition:

An invasive carcinoma composed of squamous cells of varying degrees of differentiation.

Risk Factors:

Increased number of sexual partners

Early sexual activity (especially less than 16 years of age)

Sexually transmitted diseases

Human Papillomavirus (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 & 66)

Herpes simplex virus

Chlamydia trachomatis infection

Early age of first pregnancy

Multiparity

Low socioeconomic class

Cigarette smoking

Human immunodeficiency virus

Immunosuppression from any cause

Oral contraceptive use [11]

Natural history of cervical carcinoma:

The understanding of natural history of cervical cancer is of paramount importance in planning and implementing a rational and cost-effective cervical cancer prevention program. Exposure to risk factors like HPV have been noted both in patients with SIL/ dysplasia and invasive cancer suggesting strong evidence of links between SIL and invasive cancers. The cytogenetic studies further confirm the theory as both SIL and cancers have been found to have similar chromosomal abnormalities. Thus an observation was made that the disease progressed from mild dysplasia/ Low-grade SIL to severe dysplasia and carcinoma-in situ/ High grade SIL to cancer. Several studies have suggested that the high grade SILs progress to invasive cancer in a period of up to 10 years.[38]

Table 3.3 : The natural history of cytologic pre-invasive squamous lesions (Follow-up at 24 months) [39]

	Regress (%)	**Progress to HSIL (%)**	**Progress to invasive cancer (%)**
ASCUS	68	7	0.25
LSIL	47	21	0.15
HSIL	35	_	1.4

Cervicitis:

Inflammation of the cervix may be acute or chronic based on the accompanying inflammatory cells. Acute cervicitis may be associated with focal necrosis and pus formation. Chronic cervicitis is associated with activation of fibroblasts and capillary

vessels in the affected area with formation of granulation tissue. It is termed specific when the nature of invading microorganisms is known. For example, mycobacterium tuberculosis infection resulting in granulomatous inflammation is considered specific. It is termed nonspecific when no causative organism is identified.[11,16]

CIN I:

There is increased thickness of the epithelium with a slight disturbance in the regular arrangement of cells. The upper two-thirds of the epithelium shows regular arrangement of cells with preserved stratification. Aberrations of nuclear morphology are confined to the basal layers of the epithelium.[11,16]

CIN II:

There is a moderate disturbance in the stratification. The nuclear abnormalities are more prominent in the lower two-thirds of the thickness of the epithelium with immature basaloid-type cells. Mitoses are found in the lower two-thirds of the epithelium. The upper third shows evidence of stratification and flat squamous cells usually.[11,16]

CIN III:

The cells of all the three layers of the epithelium are disturbed with cells showing less maturation and increase in nuclear size and loss of cytoplasmic volume. The cells fail to differentiate and show hyperchromatic nuclei with irregularly distributed, coarsely granular chromatin. Presence of mitoses throughout all epithelial layers is characteristic.[11,16]

Clinical features:

Late stage carcinoma presents with post-coital or abnormal vaginal bleeding. Intermittent spotting, serosanguinous discharge, and frank hemorrhage are other frequent

complaints. Weakness, pallor, weight loss, edema of the lower extremities, rectal pain and hematuria are symptoms and signs of either locally advanced or metastatic disease.

Colposcopic features:

Colposcopic examination usually reveals atypical, tortuous vessels varying widely in size and configuration. Early carcinomas are most commonly localized within the transformation zone, with variable degrees of encroachment onto the neighboring native portio. Most advanced tumors are endophytic or exophytic. Endophytic carcinomas are ulcerated or nodular. The exophytic varieties of cervical carcinoma have a polypoid or papillary appearance.[11]

Gross findings- SCC presents as an exophytic friable polypoid or Papillary excrescence (frequently in the ectocervix) and as a nodular, ulcerated, endophytic mass (more frequently involving the endocervix) with extensive infiltration of the cervical wall resulting in a barrel shaped configuration or as an ulcerative lesion.

Microscopic findings- Invasive squamous cell carcinoma is characterized by anastomosing cords or nests or irregular and ragged islands of neoplastic epithelium infiltrating the stroma. Cells in the center of the invading nests frequently become necrotic or undergo extensive keratinization. The cells are oval to polygonal, often with eosinophilic cytoplasm and prominent cellular membranes. The nuclei are relatively uniform but may display pleomorphism with coarse and granular chromatin. Mitoses are common with frequent atypical forms.[11]

Morphological variants:

Subtypes-

1. Large cell nonkeratinizing (most common, lacks keratin pearls but can have keratinization of individual cells).
2. Large cell keratinizing (marked keratin pearl formation).
3. Small cell nonkeratinizing (composed of small cells with scant cytoplasm and small nuclei).

Variants-

1. Basaloid – composed of nests of small oval-shaped immature basal type squamous cells with scant cytoplasm. Cells have hyperchromatic nuclei and are associated with brisk mitotic activity.
2. Verrucous- typically has a warty growth with an undulating, hyperkeratotic surface and prominent acanthosis. It invades the underlying stroma in the form of bulbous pegs with a pushing border. The cells have abundant eosinophilic cytoplasm, minimal cytologic atypia and no koilocytosis.
3. Warty (condylomata)- invasive SCC with morphological features of HPV infection.
4. Papillary-characterized by broad or thin Papillae with fibrovascular cores covered by squamous epithelium.
5. Lymphoepithelioma- like: characterized by ill-defined islands of undifferentiated cells associated with a marked lymphocytic background within the stroma.
6. Squamotransitional- rare entity and indistinguishable from Papillary transitional cell carcinoma of urinary bladder.[37]

Cytology:

Cytological preparations of keratinizing squamous cell carcinoma show bizarreshaped dyskeratotic cells including —fibre cells‖ and —tad-pole cells‖ with eosinophilic cytoplasm and large irregular hyperchromatic nuclei. In non-keratinizing carcinoma, anisokaryosis is seen in cells arranged singly or in syncytia. The nuclei are large with coarsely clumped chromatin and macronucleoli. A dirty background (tumor diathesis) including necrotic debris, fibrin, blood and inflammation is seen more commonly in nonkeratinizing SCC but can also be seen in keratinizing carcinoma.[18]

Immunohistochemistry:

IHC helps to identify precursor lesions of cervix and to differentiate SCC from other tumors. p16 a cell cycle protein is diffusely positive in precancerous and cancerous lesions of the cervix and measures the carcinogenic activity of HPV. Cyclin B1 positivity in the basal and parabasal cells indicates the presence of HPV. Staining of the middle and upper thirds of the squamous epithelium by Ki-67 antigen indicates the presence of intraepithelial lesion. Cytokeratin 8,18 and 19 are positive in both SCC and adenocarcinomas, while CK 5,10 and 13 are more consistently positive in cervical SCC exclusively. p63 is an excellent marker of SCC and its expression highly correlates with HPV 16. Synaptophysin and chromogranin help to exclude small cell neuroendocrine carcinoma.[16,40,41]

In situ hybridization:

Fluorescent in situ hybridization (FISH) technology has been recognized as a valuable tool to evaluate cervical dysplasia. Gain of chromosome arm 3q has been consistently identified in about 70% cases of cervical carcinoma. Studies have found higher percentages of cells with 3q26 gain in patients with HSIL or squamous cell carcinoma.[42]

TNM classification of carcinomas of uterine cervix-

Tx- Primary Tumor cannot be assessed.

T0- No evidence of primary tumor

Tis- Carcinoma in-situ / Pre-invasive carcinoma

T1- Cervical carcinoma confined to uterus

T1a- Invasive carcinoma diagnosed only by microscopy

T1b- Clinically visible lesion confined to the cervix or microscopic lesion > 5 mm

T2- Tumor invades beyond uterus but not to pelvic wall or to lower third of vagina

T2a- without parametrial invasion

T2b-with parametrial invasion

T3-Tumor extends to pelvic wall, involves lower third of vagina or causes hydronephrosis or non-functioning of kidney.

T3a-Tumor invades lower third of vagina, no extension to pelvic wall hydronephrosis or non-functioning of kidney.

T3b-Tumor extends to pelvic wall or causes hydronephrosis or non-functioning of kidney.

T4- Tumor invades mucosa of bladder or rectum or extends beyond true pelvis.[37]

FIGO staging:

Stage I (T1, N0, M0): Cervical carcinoma confined to the uterus.

- **Stage IA (T1a, N0, M0):** Invasive carcinoma diagnosed only by microscopy.

  -Stage IA1 (T1a1, N0, M0): Stromal invasion no greater than 3.0 mm in depth and 7.0 mm or less in horizontal spread.

  -Stage IA2 (T1a2, N0, M0): Stromal invasion more than 3.0 mm and not more than 5.0 mm with a horizontal spread 7.0 mm or less.

- **Stage IB (T1b, N0, M0):** Clinically visible lesion confined to the cervix or microscopic lesion greater than T1a2/IA2.

  -Stage IB1 (T1b1, N0, M0): Clinically visible lesion 4.0 cm or less in greatest dimension.

  -Stage IB2 (T1b2, N0, M0): Clinically visible lesion more than 4 cm in greatest dimension.

Stage II (T2, N0, M0): Tumor invades beyond uterus but not to pelvic wall or to lower third of the vagina.

- Stage IIA (T2a, N0, M0): Without parametrial invasion
- Stage IIB (T2b, N0, M0): With parametrial invasion

Stage III (T3, N0, M0): Tumor extends to pelvic wall, involves lower third of vagina, or causes hydronephrosis or non-functioning kidney

- Stage IIIA (T3a, N0, M0): Tumor involves lower third of vagina, no extension to the pelvic wall.
- Stage IIIB (T3b, N0, M0; OR T1-T3, N1, M0): Tumor extends to pelvic wall or causes hydronephrosis or non-functioning kidney.

Stage IV: Tumor spread to nearby organs or other parts of the body

- Stage IVA (T4, N0, M0): Tumor invades mucosa of bladder or rectum or extends beyond true pelvis.
- Stage IVB (any T, any N, M1): Distant metastasis.[43]

Prognosis and Treatment:

Cervical dysplasias regress to normal limits in most cases with progression to invasive SCC in 24 months being 0.15% for LSIL and 1.44% for HSIL. The primary modality for abnormal Pap smears is colposcopy guided biopsy of acetowhite areas. Factors that indicate poor prognosis in SCC are depth of stromal invasion, presence of lymphovascular invasion, tumor volume and involvement of resection margins. Also, the single most important factor for prognosis is staging. The 5-year survival rate for stage 1A tumors is 93%, 80% for stage 1B tumors, 63% for stage II tumors and drops to 35% for stage III tumors and 16% for stage IV tumors. Early invasive carcinoma is treated with conservative surgery or radiotherapy. Radiotherapy and radical hysterectomy with bilateral pelvic lymphadenectomy have similar results for stages 1B and IIA cancer. In advanced carcinomas- stage IIB-IV, combined external and intracavitary radiation with chemotherapy is recommended.[11]

Screening & Prevention:

Screening techniques for cervical cancer include –

Speculoscopy- Visual inspection (VI)

Visual inspection of cervix with application of acetic acid(VIA)

Visual inspection of cervix with application of lugols iodine(VILI)

Conventional exfoliative cytology

Liquid-based cytology

Automated cervical screening techniques

HPV testing

HPV vaccines

p16INK4a and Ki-67 dual staining kits for Pap smears

Cervicography

Biopsy.[11]

Exfoliative cytology (conventional Pap smear):

Since the introduction of exfoliative cytology by Papanicolaou, it has been regarded as the gold standard for cervical cancer screening programs. The Pap smears have created a benchmark in the screening for preinvasive lesions and have been a success story for decades. The technique for Pap smear collection is to sample the ectocervix and the endocervical canal using a Ayre spatula and endocervical brush. The sample thus collected is smeared on a slide and then fixed immediately with cytology fixative. Although the conventional cytology is highly effective, there are various problems observed to be associated with it like incorrect and inadequate sampling as only up to 20% of harvested cells are being transferred on the slide, with a mean sensitivity of only 55-60%. Also the interobserver variability and the likelihood of preventing only less than 60% of cervical cancer cases according to epidemiological data led to the development of several new techniques. They were developed in an attempt to automate the various steps of Pap smear preparation and to improve the sensitivity and specificity of conventional cytology.[44,45]

Liquid-based cytology:

Liquid-based cytology has gained popularity because of a marked improvement in the adequacy of the specimen attributable to an even distribution of cells and reduction in cellular debris and RBCs. A decrease in the incidence of false positive diagnosis of cytological atypia and an excellent correlation with the detection of squamous abnormalities has been observed. Two USFDA approved techniques that have been tested and widely accepted are ThinPrep® (Cytyc Corp, Booxborough, MA) and SurePath® (TriPath Imaging, Burlington, NC) in which a special sampling device is used for sampling the cervix which is then placed in a vial containing a special preservative solution. A well preserved sample is thus obtained that is automatically transferred to a slide as a small sized thin layer smear by the principle of membrane filtration (ThinPrep®) or density gradient centrifugation (SurePath®).[19]

CERVICAL CYTOLOGY

Superficial cells are large, polygonal with pink-orange, translucent cytoplasm, sharply defined boundaries and a small pyknotic central nucleus. Superficial cell diameter is approximately 40µm with a nuclear diameter of 3-5µm. Cells from granular cell layer display small dark blue keratohyaline granules evenly distributed in cytoplasm; known as Polka-Dot sign.

Intermediate cells are polygonal in shape with cyanophilic cytoplasm and folded edges. The cytoplasm stores glycogen and secretory products. The nuclear diameter is 8- 10µm and is round to oval, vesicular with fine chromatin. The cellular diameter is approximately 30µm, giving a low N/C ratio.

Parabasal cells are round to oval cells with small dense green cytoplasm with a diameter of 10μm. The cytoplasm is dense with distinct borders and may contain vacuoles. The nuclear diameter is about 8μm and occupies about one-half of the cells and has a fine chromatin pattern with occasional nucleoli.

Metaplastic cells are usually the size of parabasal cells or early intermediate cells and appear in small sheets. The cells have variable nuclear sizes with vesicular chromatin and high N:C ratio. The cytoplasm appears densely cyanophilic which may be prematurely keratinized. The cells have a spidery contour due to the presence of cytoplasmic projections due to loosened intercellular bridges. As these cells mature, they resemble intermediate and superficial squamous cells.

Endocervical cells are mucus-secreting tall columnar cells with oval nuclei having fine chromatin and 1-2 small nucleoli. The cytoplasm appears clear, cyanophilic or vacuolated. They are usually seen in small sheets or groups having a honey-comb appearance when viewed from above and picket-fence pattern when seen from the sides.[16,46]

Endometrial cells are seen normally in smears for the first 12 days of the cycle following menstruation. The presence of these cells reflects endometrial pathology or exogenous hormonal manipulation at other times of the cycle. During different stages of the menstrual cycle the appearance of endometrial cells varies. During the menstrual phase, they are grouped in well-formed 3-dimensional clusters with a peripheral rim of epithelial cells and a central core of stromal cells. Following which degenerative changes appear with crumpling of the nuclei and disorganization of the cells. Nuclei are small round with inconspicuous nucleoli and scant, basophilic cytoplasm.[48] The presence of

these benign-appearing endometrial cells should be reported in women aged >45 years with a note suggesting endometrial evaluation to be performed only in post menopausal women.[17]

Conventional Pap smears:

The conventional Pap smear devised by Papanicolaou has successfully been used for cervical cancer screening for more than 50 years and continues to perform well provided the preventable causes of suboptimal smear preparation are addressed. However the conventional Pap smears are reported to have low sensitivity which can be attributed to large field for screening, more time consumption, cellular overlap and obscuring factors like inflammatory cells and hemorrhage. This led to the advent of Liquid-based preparations with an objective of minimizing cellular overlap, improving smear quality and performance of adjunctive HPV testing.[47]

Liquid-based preparations:

LBPs were originally developed by the Germans in the early 70s to minimize cell overlap for better performance of automated screeners in the identification of abnormal cells. Eventually they have almost replaced the use of conventional cervicovaginal smears in the developed world after successful clinical trials. ThinPrep® and SurePath® are the two FDA approved liquid-based methods that are used for the preparation of such smears. Most developed countries have employed these systems for routine cervical screening.

SurePath® works on the principle of density gradient centrifugation and ThinPrep® on membrane filtration. Liquid-based cytology is a technique achieved by rinsing the collection device in a preservative/fixative fluid to generate a suspension of

cells that is processed to deposit a monolayer of cells on a microscope slide. Both these systems result in the formation of a small circular smear on the slide after the sample is placed in a fixative solution and processed by the machine. The advantages being: relative absence of blood and debris, monolayer formation, better quality of smears and smaller field for screening thus accelerating the screening process. The cells show a high contrast between the nucleus and cytoplasm with a clear background and thus requiring special training in interpretation of material.[47]

Cytocentrifugation

A cytocentrifuge is a device that spins cells in a fluid suspension directly onto a glass slide. Since the introduction of the Cytospin I by Thermo Electron Corporation, other instruments have been developed with slightly different features. Following the guidelines and procedures recommended by the manufacturer of the instrument usually results in excellent cytologic material.

Cytocentrifuges Shandon Cytospin II and III

Newer Cytospin models (Thermo Electron Corporation) have features that increase cell recovery. The Cytospin II and III form an air bubble between the sample and the slide which increased cell recovery rates when compared to the Cytospin I. Also available is a Megafunnel for use with the Cytospin II or III, which allows the processing of up to 12 times the sample volume (6 ml) and deposits the cells over an area 10 times larger than the cell deposition area of Cytofunnel. The Megafunnel is designed for highly cellular samples such as effusions, bronchial washings and sputums.[47]

The use of LBP in cervicovaginal screening has been widely accepted as it has shown an increased percentage of specimens reported as —satisfactory, better detection

rates of LSILs and HSILs over conventional Pap smears and a decrease in the reporting rate of ASCUS. LBP has also been found to be comparable to conventional Pap smears in diagnosing adenocarcinomas and other glandular lesions.[16,18,19,47]

Table 3.4: Advantages and Disadvantages of Liquid-based cytology[19]

Advantages	**Disadvantages**
➢ Preparations are standardized. ➢ Cells are treated gently to avoid damage. ➢ Clear background. ➢ Better fixation of cells and preservation of nuclear details. ➢ Possibility to carry out other tests on the rest of the liquid (cytochemistry, immunocytochemistry, molecular pathology, HPV testing, cell block method). ➢ Reduced number of inadequate smears. ➢ Cells are distributed randomly on the slide. ➢ Lesser time consuming	➢ Greater demands on cost of equipment and logistics. ➢ Needs special training for interpretation. ➢ The number of cells used are not standardized. ➢ Monolayer is not always formed. ➢ Cells appear shrunken and are ➢ more circular.

Aims of liquid-based cytology-

- To recruit all exfoliated material for the cytological examination.
- To document the material for further morphological and non-morphological examinations.
- Monodispersion and monolayer preparation to avoid cell clumps.
- Enable computer-based screening.
- Elimination of disruptive elements (e.g. inflammatory cells, mucous, fibrin).[19]

Papanicolaou's Classes

In the year 1954, the initial classification of cervicovaginal smears was proposed by Papanicolaou who formulated a series of guidelines of smear interpretation in five classes:

Class I. Absence of atypical or abnormal cells.

Class II. Atypical cytology but no evidence of malignancy.

Class III. Cytology suggestive of, but not conclusive for, malignancy.

Class IV. Cytology strongly suggestive of malignancy.

Class V. Cytology conclusive for malignancy.[46]

THE 2014 BETHESDA SYSTEM

Specimen type :

Conventional smear (Pap smear) vs. liquid-based preparation vs. other

Specimen adequacy:

Satisfactory for evaluation

Unsatisfactory for evaluation

Specimen rejected/not processed

Specimen processed and examined, but unsatisfactory for evaluation of epithelial abnormality

General categorization (optional):

Negative for intraepithelial lesion or malignancy

Other

Epithelial cell abnormality

Interpretation/ Result:

Negative for intraepithelial lesion or malignancy:

Non-Neoplastic Findings (optional):

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

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 aged >45 years)

Epithelial Cell Abnormalities:

Squamous Cell

Atypical squamous cells

- Of undetermined significance (ASC-US)

- Cannot exclude HSIL (ASC-H)

Low-grade squamous intraepithelial lesion (LSIL)

High-grade squamous intraepithelial lesion (HSIL)

- With features suspicious for invasion

Squamous cell carcinoma

Glandular Cell

Atypical

- Endocervical cells (NOS)

- Endometrial cells (NOS)

- Glandular cells (NOS)

Atypical

- Endocervical cells, favor neoplastic
- Glandular cells, favor neoplastic

Endocervical adenocarcinoma in situ

Adenocarcinoma

- Endocervical
- Endometrial
- Extrauterine
- Not otherwise specified (NOS)

Other malignant neoplasms:

Adjunctive testing:

Computer-assisted interpretation of cervical cytology:

Educational notes and comments appended to cytology reports :[17]

Updates of The Bethesda system 2014 from 2001 includes-

1. Adequacy- Inclusion of additional guidance for special situations, such as assessing cellularity in specimens obtained from post-radiation patients, interfering substances (eg, lubricant, blood), and the effects of adequacy on HPV testing.
2. Non-neoplastic findings- An expanded variety of “normal” findings as well as non-neoplastic mimics of classic epithelial abnormalities such as squamous metaplasia are included.
3. Endometrial cells- The age for reporting of “cytologically benign appearing” endometrial cells has been increased to women aged 45 years

4. Atypical squamous cells - ASC remains the general category; subcategorization as ASC-US / ASC-H. Guidance on use of ASC with HPV test results to monitor quality and consistency among practitioners and laboratories.
5. Squamous epithelial cell abnormalities- This include problematic patterns and mimics that may lead to locator and/or interpretation errors of non-neoplastic changes as HSIL/ASC-H and vice versa.
6. Glandular epithelial cell abnormalities- This includes many differential diagnostic considerations of glandular lesions.
7. Other malignant neoplasms- Special variants of cervical carcinoma/ uterine or adnexal tumors and metastasis from other primaries are included.
8. Anal cytology- Anal cytology was first included in the 2001 Bethesda atlas and has gained acceptance as a tool for anal cancer screening in conjunction with high-resolution anoscopy and biopsy—in a role similar to that of the Pap test. TBS 2014 includes sampling devices used to collect anal cytology specimens, criteria for adequacy and the role of cytohistologic/high-resolution anoscopy correlation.
9. Adjunctive testing- Data concerning use and reporting for the current HPV testing schemes and adjunctive immunocytochemistry procedures (eg, p16/Ki67) are included.
10. Computer-assisted interpretation - If a cervical cytology case is examined by an automated device, the report should specify the following: 1. Device utilized 2. Type of review 3. Result of the automated review process 4. The individual(s) involved in the process and their role stipulated.

11. Educational notes and comments- standardization of reports to facilitate widespread electronic health record implementation has been encouraged. These changes may have further implications for the use of recommendations in pathology reports, and a relevant discussion is now included.
12. Risk assessment in cervical cancer- the results of various screening and triage test combinations relate to the patient's risk for cervical cancer.[17]

Adequate smears:

On conventional smears, a minimum of approximately 8000-12000 well preserved and well visualized squamous epithelial cells with exclusion of endocervical cells and completely obscured cells.

On liquid-based preparations, a minimum of approximately 5000 well preserved and well visualized squamous epithelial cells.

The presence of an endocervical or transformation zone component is noted and not necessary for smear adequacy [Satisfactory but limited by presence or absence of endocervical or transformation zone component].

It is considered that the endocervical or transformation zone has been adequately sampled when atleast 10 well preserved endocervical or squamous metaplastic cells are seen singly or in clusters.

Specimens with more than 75% of squamous cells obscured should be termed unsatisfactory. When 50% to 75% of the cells are obscured, a statement describing the specimen as partially obscured should follow the satisfactory term. Any smear containing abnormal cells and requiring further action is by definition satisfactory for evaluation.[18]

Infections:

Trichomonas Vaginalis- Pear shaped, oval or round cyanophilic organism ranging in size from 15 to 30µm. Nucleus is pale, vesicular and eccentrically located. Eosinophilic cytoplasmic granules are evident. In LBPs organisms tend to be smaller due to rounding. Nuclei and cytoplasmic eosinophilic granules are often better visualized.

Candida- Budding yeasts (3-7 µm); pseudohyphae are eosinophilic to gray-brown. Fragmented leukocyte nuclei and rouleaux formation of squamous epithelial cells speared by hyphae may be seen. Spearing of epithelial cells is more common in LBPs called —shish kebab effect.

Shift in Flora Suggestive of Bacterial Vaginosis- Individual squamous cells are covered by a layer of bacteria that obscures the cell membrane, forming so called —clue cells. Filmy background of small coccobacilli is evident. There is a conspicuous absence of lactobacilli. LBPs have a clean background.

Herpes virus- Nuclei have a —ground-glass‖ appearance due to intranuclear viral particles and enhancement of the nuclear envelope caused by peripheral margination of chromatin. Dense eosinophilic intranuclear inclusions surrounded by a halo or clear zone are present. Large multi-nucleated epithelial cells with molded nuclei are characteristic.

Leptothrix- Long, curving, filamentous organisms, most commonly observed in conjunction with vaginal trichomoniasis.

Epithelial cell abnormalities:

ASCUS- Nuclei are approximately two and half to three times the area of the nucleus of a normal intermediate squamous cell with slightly increased ratio of nuclear to cytoplasmic area (N/C). Minimal nuclear hyperchromasia and irregularity in chromatin distribution or nuclear shape and atypical parakeratosis are characteristic.

LSIL- Nuclear enlargement more than three times the area of normal intermediate nuclei results in a slightly increased N/C ratio. Variable degrees of nuclear hyperchromasia are accompanied by variations in nuclear size, number and shape. Chromatin is uniformly distributed and coarsely granular. Nucleoli are absent or inconspicuous. Perinuclear cavitation (—koilocytosis‖) consisting of a sharply delineated clear perinuclear zone and a peripheral rim of densely stained cytoplasm is a characteristic feature. Alternatively, the cytoplasm may appear dense and orangeophilic (keratinized). In LBPs angulated clusters of atypical/dysplastic cells are more clearly visualized.

ASC-H- Cells usually occur singly or in small fragments of less than 10 cells. Cells are the size of metaplastic cells with nuclei that are about 1½ to 2½ times larger than normal. Ratio of nuclear to cytoplasmic (N/C) area may approximate that of HSIL.

HSIL- Degree of nuclear enlargement is more variable than that seen in LSIL. Cytoplasmic area is decreased, leading to a marked increase in N/C ratio. Contour of nuclear membrane is irregular and frequently shows prominent indentations. Nucleoli are absent. Cytoplasm is immature, lacy and delicate.[18]

Automated screening technology:

The recently developed automated screening techniques can perform quality control rescreening and also can be used for primary screening of cervical smears. They rely on neural network technology and are based on the computerized imaging and identification of abnormal cervical cells by utilizing a specialized high speed video microscope, image interpretation software, and specially designed field of view computers to image, analyze and classify abnormal cervical cells.

1. AutoPap300 (TriPath Imaging, Burlington NC) -approved by the USFDA for primary and secondary cervical screening.
2. PAPNET (Neuromedical systems) -approved for secondary screening.[49-51]

METHODOLOGY

In the present study, cervical smears were prepared using the cytospin method of liquid-based cervical cytology and compared with the conventional Pap smears.

A prospective study of 134 samples (sample size) was carried out in the Department of Pathology, B.L.D.E.U'S Shri B M Patil Medical College, Hospital & Research Centre, Vijayapur by split smear technique for conventional Pap smear and liquid-based cytology during 1st December 2014 to 30th June 2016 (one year and seven months). Institutional Ethical Committee clearance was obtained.

Cervical cytology samples from all women from 18-65 years attending the OBG (Obstetrics and Gynaecology) Out Patient Department with presenting complaints of white discharge per vagina, post-coital bleeding, mass per vagina, pain abdomen, irregular menstruation, infertility and for routine cervical cancer screening were evaluated and their details examined according to the proforma.

Inclusion criteria:

Female patients attending Outpatient and Inpatient Department of OBG in the age group of 18-65 years were included in the study.

High risk patients who were included in the study are-

1.Coitus before the age of 18yrs.

2.Multiple sexual partners.

3.Delivery of 1st baby before the age of 20years.

4.Multiparity with poor birth spacing between pregnancies.

5.Poor socioeconomic status.

6.Women with STD, HIV infection, herpes simplex virus 2 infection, human Papilloma virus infection(16,18,31,33) or condylomata.

Exclusion criteria:

1. Non co-operative patients/ patients who do not give consent.

2. Technically defective slides like broken slides and slides having drying artefacts.

Sample collection and processing: In all these cases after a detailed history and thorough clinical examination, conventional Pap smears were prepared from cervix with an Ayre's spatula and one slide was prepared and immediately fixed in 100% methanol. Then residual material on spatula were rinsed in 5ml of 100% methanol. It is allowed to sediment for 1 hour, supernatant was decanted and around 100 microlitre of the sediment material was transferred into cytofunnel with filter Paper placed between slide and funnel and spun in Cytospin [MedSpin4] at 800rpm for 5 minute. Both conventional and Cytospin smears were stained by Papanicolaou stain.

The smears prepared by the conventional method and cytospin method were observed for the following parameters-

1. Cellularity

2 .Cellular overlapping

3. Morphological changes

4. Clear background,

5. Nuclear featues

6. Endocervical cells.

The Bethesda system of classification, 2014 was used to report the smears.

Fig 4.1: The cytospin(MedSpin4) instrument used to prepare direct Pap smears on slides.

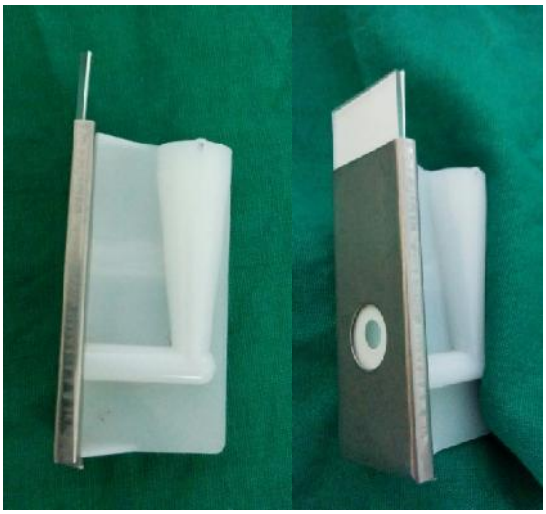


Fig 4..2: MedSpin4 cytofunnel used in preparing direct smears, filter card placed between glass slide and cytofunnel.

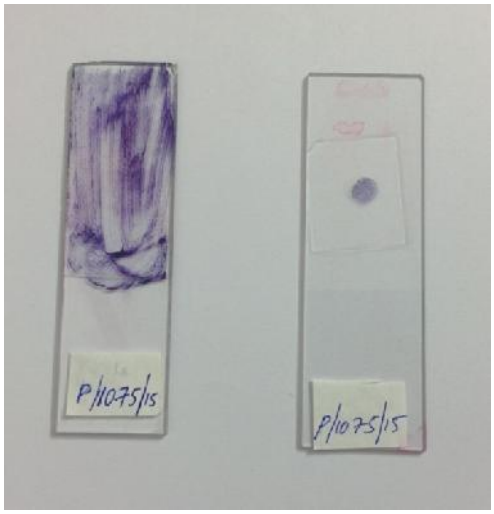


Fig 4.3: Comparison of Pap smear size prepared by conventional (left) & cytospin methods (right).

The Bethesda System – 2014 Revised for reporting cervical cytology smears-

Specimen type :

Conventional smear (Pap smear) vs. liquid-based preparation vs. other

Specimen adequacy:

Satisfactory for evaluation

Unsatisfactory for evaluation

Specimen rejected/not processed

Specimen processed and examined, but unsatisfactory for evaluation of epithelial abnormality

General categorization (optional):

Negative for intraepithelial lesion or malignancy

Other

Epithelial cell abnormality

Interpretation/ Result:

Negative for intraepithelial lesion or malignancy:

Non-Neoplastic Findings (optional):

- Non-neoplastic cellular variations
    - Squamous metaplasia
    - Keratotic changes
    - Tubal metaplasia
    - Atrophy
    - Pregnancy-associated changes
- Reactive cellular changes associated with:

- Inflammation - Lymphocytic (follicular) cervicitis
- Radiation
- Intrauterine contraceptive device (IUD)

- Glandular cells status posthysterectomy

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 aged >45 years)

Epithelial Cell Abnormalities:

Squamous Cell

Atypical squamous cells

- Of undetermined significance (ASC-US)

- Cannot exclude HSIL (ASC-H)

Low-grade squamous intraepithelial lesion (LSIL)

High-grade squamous intraepithelial lesion (HSIL)

- With features suspicious for invasion

Squamous cell carcinoma

Glandular Cell

Atypical

- Endocervical cells (NOS)
- Endometrial cells (NOS)
- Glandular cells (NOS)

Atypical

- Endocervical cells, favor neoplastic
- Glandular cells, favor neoplastic

Endocervical adenocarcinoma in situ

Adenocarcinoma

- Endocervical
- Endometrial
- Extrauterine
- Not otherwise specified (NOS)

Other malignant neoplasms:

Adjunctive testing:

Computer-assisted interpretation of cervical cytology:

Educational notes and comments appended to cytology reports :[17]

STATISTICAL METHODS

Study design:

A prospective cross sectional study.

Sample size:

Using the cervical cancer incidence rate in India as 20.2%,[1] the expected sensitivity as 88.9%, specificity as 92.3%,[7] the minimum sample size is 134 at 5% level of significance (95% confidence limit).

This sample size will give precision of 12 % or less.

The formula used here is

$$n = \frac{Z^2 P(1-P)}{D^2}$$

n- sample size

Z= 1.96 at α= 5%

α- level of significance

P- Prevalence

D- Desired precision

The calculated sample size is 134.

Hence 134 cases were included in the study.

Statistical analysis:

All characteristics were summarized descriptively. For continuous variables, the summary statistics of N, mean, standard deviation (SD) were used. For categorical data, the number and percentage were used in the data summaries. Chi-square (χ^2)/Fisher exact test was employed to determine the significance of differences between groups for categorical data. The difference of the means/proportion of analysis variables was tested with the t-test/z test and ANOVA. If the p-value was < 0.05, then the results will be considered to be significant. Data were analyzed using SPSS software v.23.0.

Statistical software:

Data were analyzed using SPSS software v.20.0.

RESULTS AND ANALYSIS

The present study was undertaken to prepare cervical cytology smears using the cytospin method of liquid-based cervical cytology (LBC) and to compare it with the conventional Pap smears and observe morphological features.

During the period of this study from 1st December 2014- 30th June 2016, comparative study of 134 cases of conventional Pap smear and cytospin Pap smear were made.

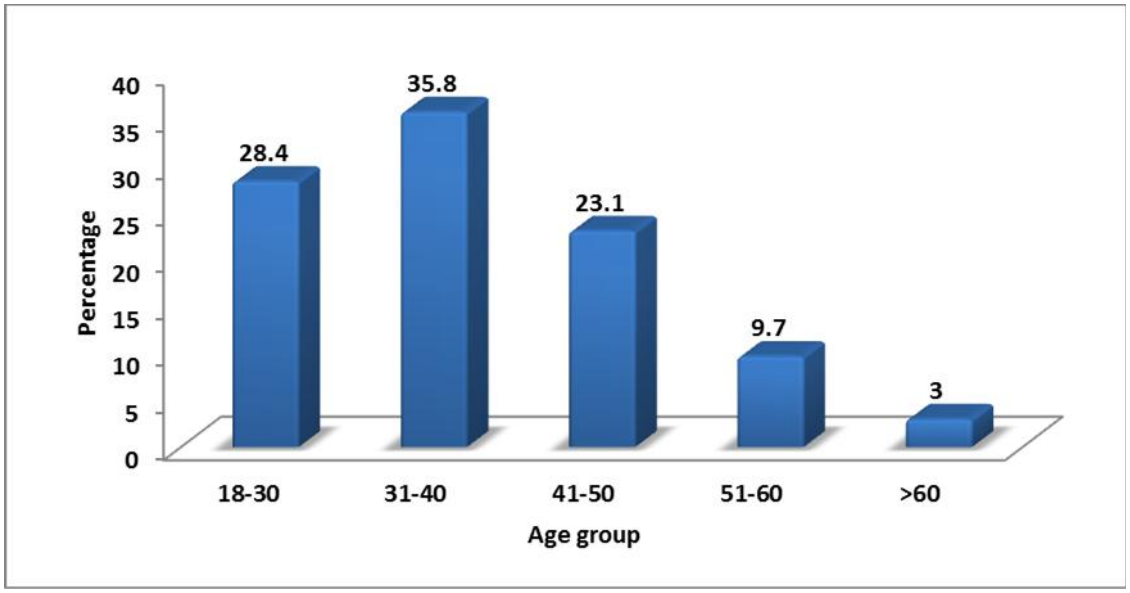
Age

Age group of patients ranged from 18 to 65 years with the youngest patient aged 18 years and the oldest 65 years with a mean age of 38.3 years. Majority of the patients were in the age group of 31-40 years. (Table 5.1, Graph 1)

Table 5.1: Age wise distribution of cases

Age group (Years)	Number of cases (N)	Percent (%)
18-30	38	28.4
31-40	**48**	**35.8**
41-50	31	23.1
51-60	13	9.7
>60	4	3
Total	**134**	**100**

Graph 1: Age wise distribution of cases.



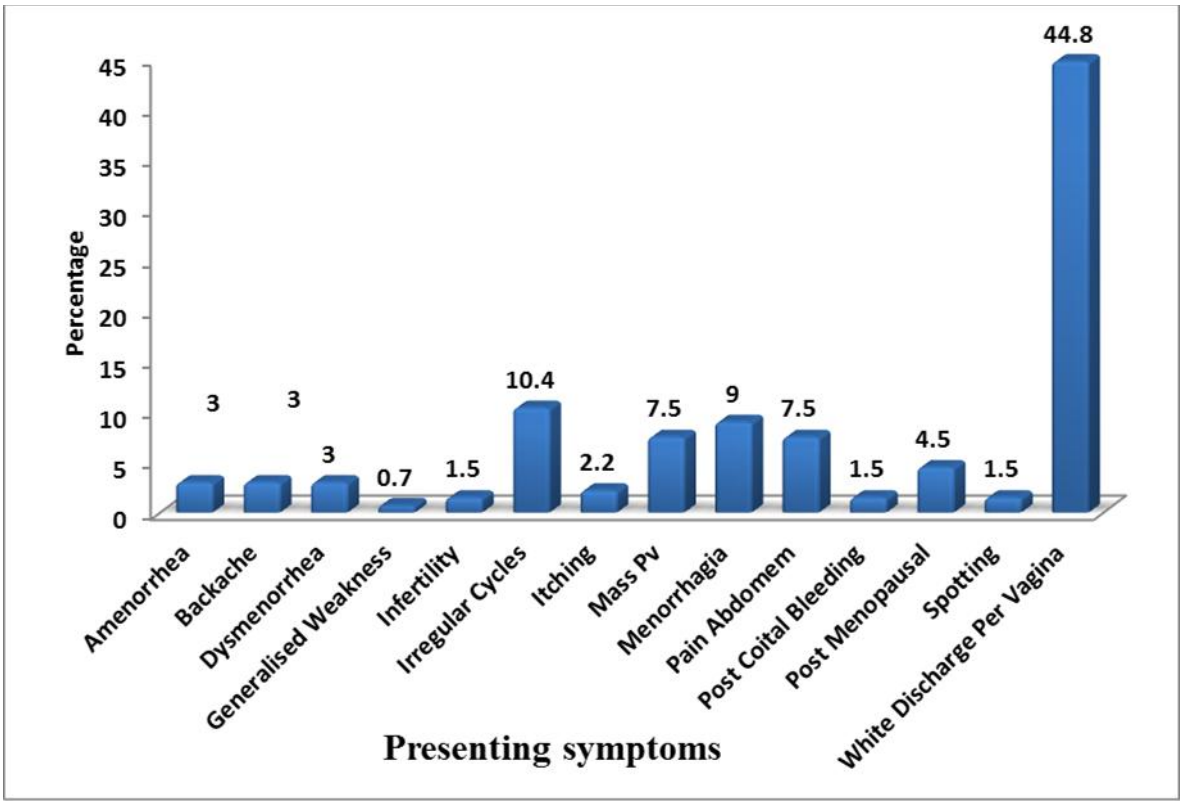
Clinical Presentation

The most common clinical presentation was white discharge per vagina followed by irregular cycles and menorrhagia. (Table 5.2, Graph 2)

Table 5.2: Distribution of Presenting Symptoms of cases

Presenting Symptoms	Number of cases (N)	Percent(%)
Amenorrhea	4	3
Backache	4	3
Dysmenorrhea	4	3
Generalized Weakness	1	0.7
Infertility	2	1.5
Irregular Cycles	14	10.4
Itching	3	2.2
Mass Per vagina	10	7.5
Menorrhagia	12	9
Pain Abdomen	10	7.5
Post Coital Bleeding	2	1.5
Post Menopausal	6	4.5
Spotting	2	1.5
White Discharge Per Vagina	60	44.8
Total	**134**	**100**

Graph 2: Distribution of Presenting symptoms of cases



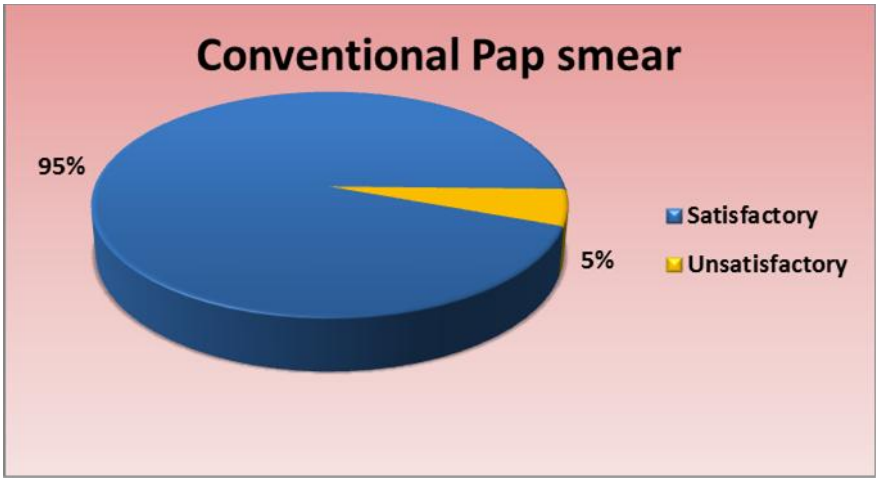
Sample Adequacy

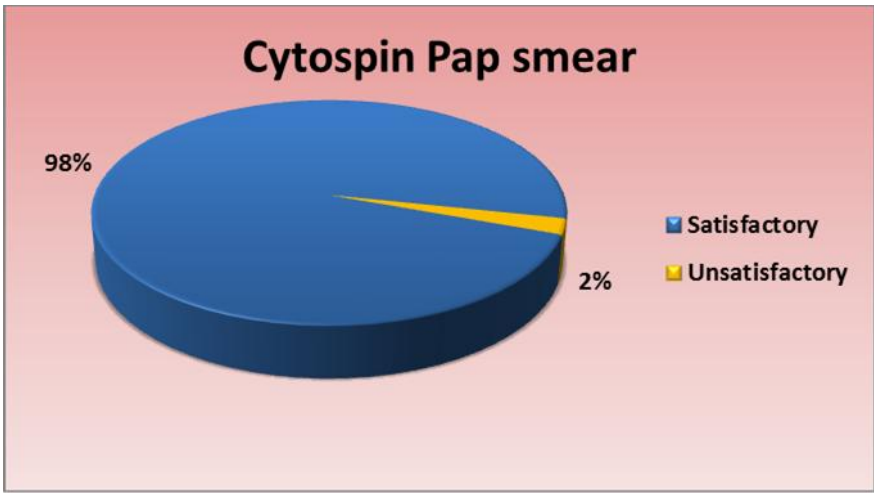
Satisfactory cell samples were obtained in 127 out of the 134 cases of conventional Pap smear and 131 cases of cytospin Pap smear. (Table 5.3, Graph 3)

Table 5.3: Comparison of adequacy of samples in Conventional and Cytospin Pap smears

Smear	Conventional pap smear		Cytospin pap smear		p value
	N	Percent	N	Percent	
Satisfactory	127	94.8	131	97.8	0.197
Unsatisfactory	7	5.2	3	2.2	0.197
Total	**134**	**100.0**	**134**	**100.0**	

Graph 3: Comparison of adequacy of samples in Conventional and Cytospin Pap smears





Cytology

In the present study 134 cervical smears prepared by conventional were studied, out of which 116 (91.3%) were reported as non-neoplastic lesions and 11(8.7%) cases were reported as neoplastic lesions.

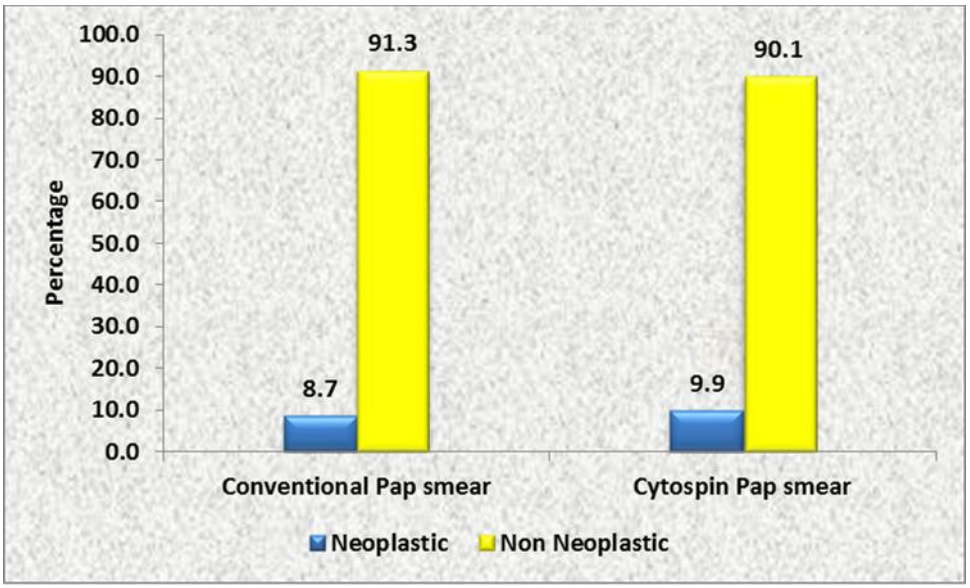
Out of 134 cervical smears prepared by cytospin studied, 118 (90.1%) were reported as non-neoplastic lesions and 13(9.9%) cases were reported as neoplastic lesions.

(Table 5.4, Graph 4)

Table 5.4: Comparison of distribution of cases into neoplastic and non-neoplastic lesions by conventional Pap smear and cytospin Pap smear

Lesions	Conventional Pap smear		Cytospin Pap smear		p value
	N	%	N	%	
Neoplastic	11	8.7	13	9.9	0.727
Non-neoplastic	116	91.3	118	90.1	0.727
Total	**127**	**100**	**131**	**100**	

Graph 4: Comparison of distribution of cases into neoplastic and non-neoplastic lesions by conventional Pap smear and cytospin Pap smear

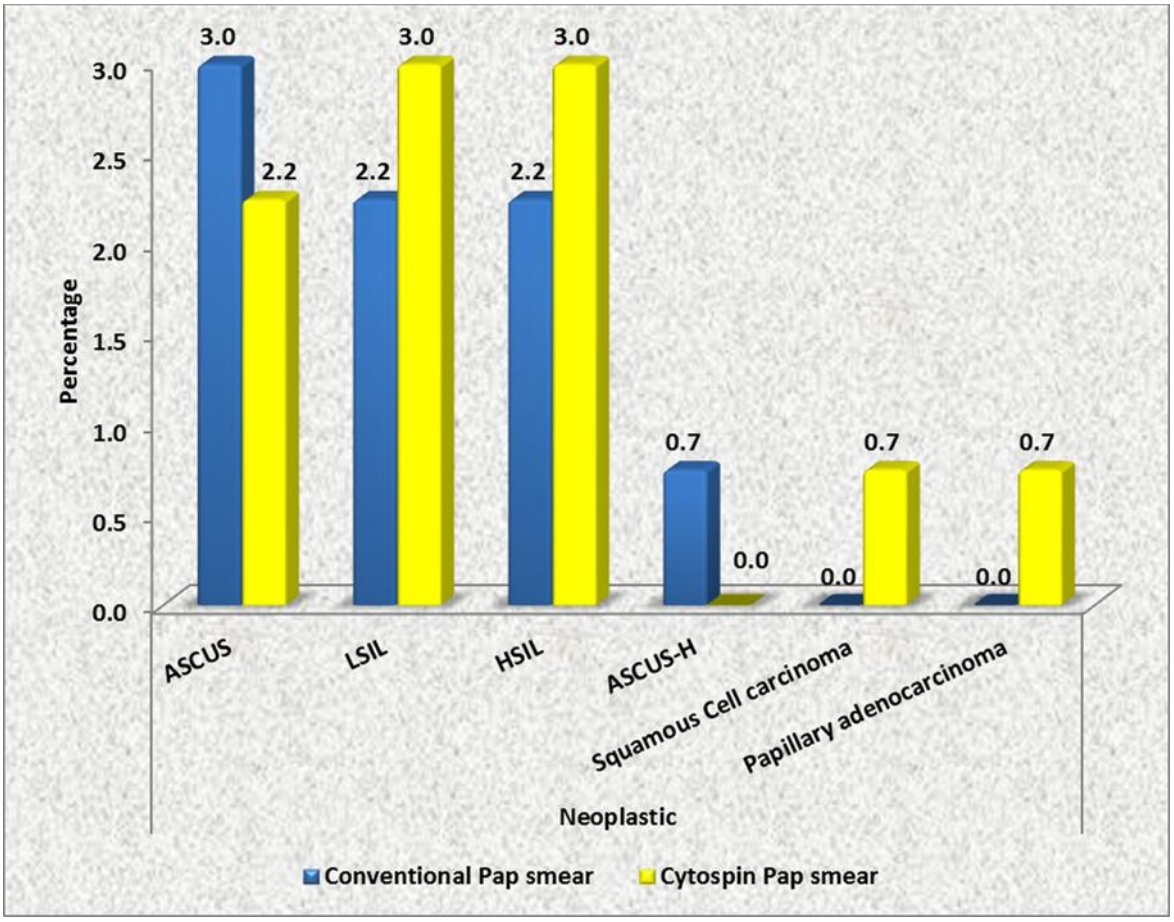


By conventional technique, the most common neoplastic lesion was found to be ASCUS accounting for 4 cases, followed by LSIL (3 cases), HSIL (3cases) and ASCUS-H (1case). By the cytospin technique, the most common neoplastic lesion was found to be LSIL, HSIL accounting for 4 cases each, followed by ASCUS (3 cases), squamous cell carcinoma (1case) and papillary adenocarcinoma (1 case).(Table 5.5, Graph 5)

Table 5.5: Comparison of morphological distribution of neoplastic cases diagnosed on conventional Pap smear and cytospin Pap smear

Morphological Distribution		**Conventional Pap smear**		**Cytospin Pap smear**	
		N	**%**	**N**	**%**
Neoplastic	**ASCUS**	4	3.0	3	2.2
	LSIL	3	2.2	4	3.0
	HSIL	3	2.2	4	3.0
	ASCUS-H	1	0.7	0	0.0
	Squamous Cell carcinoma	0	0.0	1	0.7
	Papillary adenocarcinoma	0	0.0	1	0.7
Total neoplastic		**11**	**8.7**	**13**	**9.9**
Total number of cases		**134**	**100**	**134**	**100**

Graph 5: Comparison of morphological distribution of neoplastic cases diagnosed on conventional Pap smear and cytospin Pap smear.



By conventional technique, the most common non-neoplastic lesion was inflammatory smear (65 cases) followed by normal study (26 cases), bacterial vaginosis (16 cases), candidal infestation (5 cases), trichomonas vaginalis (2 cases), atrophic smear (2 cases). By the cytospin technique, the most common non-neoplastic lesion was inflammatory smear (63 cases) followed by normal study (27 cases), bacterial vaginosis (17 cases), candidal infestation (5 cases), trichomonas vaginalis (3 cases), atrophic smear (2 cases), leptothrix infestation (1 case). (Table 5.6, Graph6)

Table 5.6:Comparison of morphological distribution of non-neoplastic cases diagnosed on conventional Pap smear and cytospin Pap smear

Morphological Distribution		**Conventional Pap smear**		**Cytospin Pap smear**	
		N	**%**	**N**	**%**
Non-neoplastic	**Inflammatory Smear**	65	48.5	63	47.0
	IS-Leptothrix	0	0.0	1	0.7
	Atrophic smear	2	1.5	2	1.5
	Trichomonas Vaginalis	2	1.5	3	2.2
	Candida	5	3.7	5	3.7
	Bacterial vaginosis	16	11.9	17	12.7
	Normal study	26	19.4	27	20.1
Total non-neoplastic		**116**	**91.3**	**118**	**90.1**
Total number of cases		**134**	**100**	**134**	**100**

Graph 6: Comparison of morphological distribution of non- neoplastic cases diagnosed on conventional Pap smear and cytospin Pap smear .

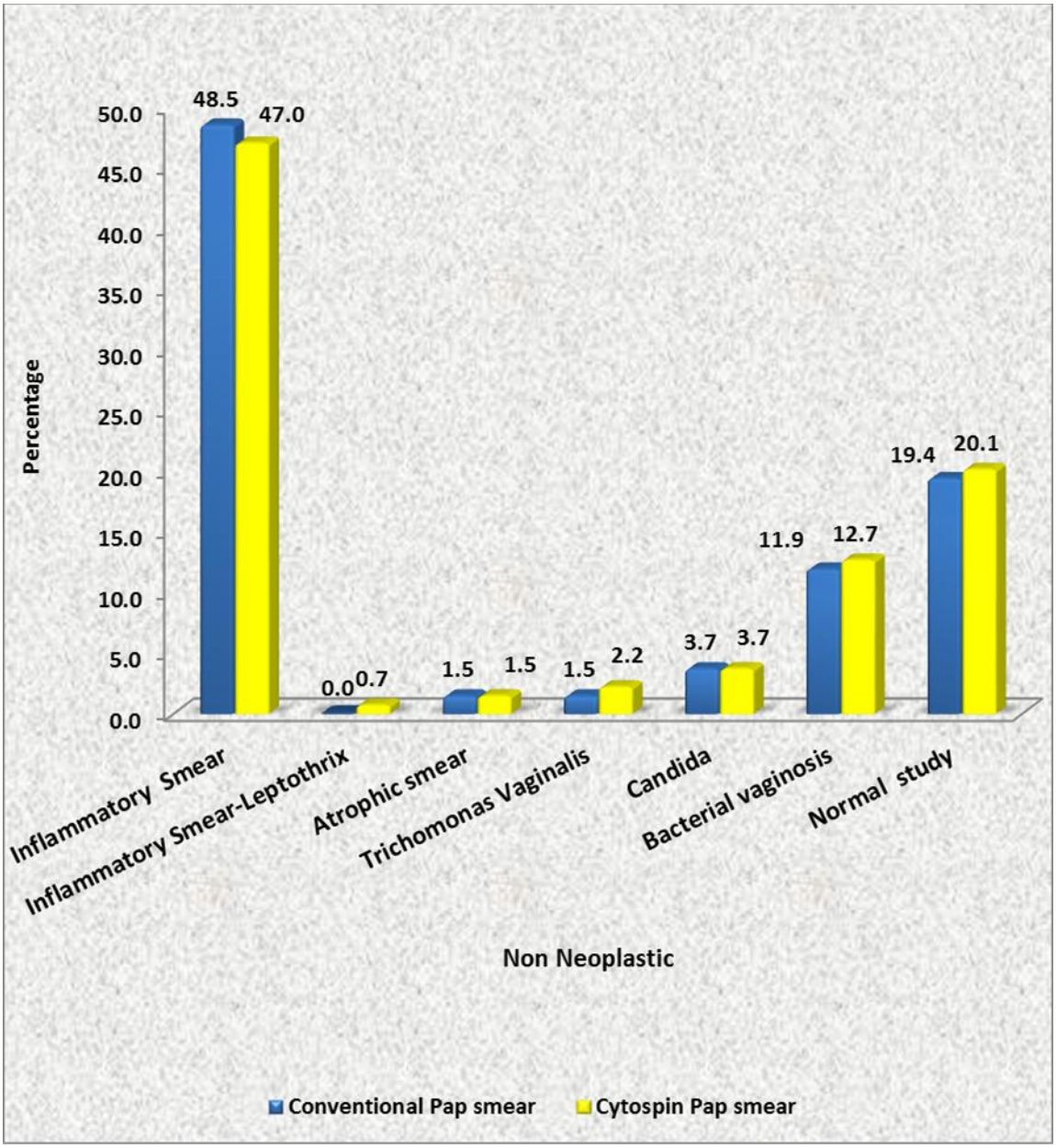


Table 5.7:Comparison of morphological distribution of total number of cases studied on conventional Pap smear and cytospin Pap smear

Morphological Distribution		Conventional Pap smear		Cytospin Pap smear		p value
		N	%	N	%	
Neoplastic	ASCUS	4	3.0	3	2.2	0.702
	LSIL	3	2.2	4	3.0	0.702
	HSIL	3	2.2	4	3.0	0.702
	ASCUS-H	1	0.7	0	0.0	0.316
	Squamous Cell carcinoma	0	0.0	1	0.7	0.316
	Papillary adenocarcinoma	0	0.0	1	0.7	0.316
Non Neoplastic	Inflammatory Smear	65	48.5	63	47.0	0.807
	Inflammatory Smear-Leptothrix	0	0.0	1	0.7	0.316
	Atrophic smear	2	1.5	2	1.5	--
	Trichomonas Vaginalis	2	1.5	3	2.2	0.652
	Candida	5	3.7	5	3.7	--
	Bacterial vaginosis	16	11.9	17	12.7	0.853
	Normal study	26	19.4	27	20.1	0.878
Unsatisfactory		7	5.2	3	2.2	0.197
Total		**134**	**100.0**	**134**	**100.0**	

Agreement of morphological distribution between Conventional Pap smear and Cytospin Pap smear is 94.03%

In present study, histopathology correlation was not done. Taking cytospin as standard sensitivity and specificity of conventional Pap smear was calculated {Sensitivity-84.6%, Specificity-100%, Positive Predictive Value (PPV)-100%, Negative Predictive Value (NPV)- 98.4% and accuracy (ACC)- 98.5% [True positive (TP)=11, True negative (TN) = 121, False positive(FP) =2, False negative(FN) = 0]}.

Cellularity was adequate in most of the cytospin smears while the number of unsatisfactory smears were more in CPS. Cellular morphologic change was present in most of CPS samples. Cellular overlapping and inflammatory infiltrate were prominently present in CPS but decreased in cytospin smears. Clean background was observed in most cases of cytospin smears which was not seen in majority of CPS. Nuclear changes were very clear by cytospin smears, but not very clear by CPS. Endocervical cells were more in CPS than in cytospin.

Table 5.8: Comparison of morphological features in CPS and Cytospin Pap smear

Morphological features	**Conventional Pap smear**		**Cytospin Pap smear**		**P value**
	N	**Percent(%)**	**N**	**Percent(%)**	
Cellularity present	131	97.8	133	99.3	**0.315**
Absence of cellular overlapping	99	73.9	119	88.8	<0.001*
Absence of morphologic changes	123	91.8	131	97.8	<0.004*
Clean background	109	81.3	130	97	<0.001*
Better nuclear features	126	94	128	95.5	0.585
Presence of Endocervical cells	50	37.3	47	35.1	0.704
Total	**134**	**100**	**134**	**100**	

Note: * - Difference is statistically significant at 5% level of significance.

Graph 7: Comparison of morphological features in CPS and Cytospin Pap smear

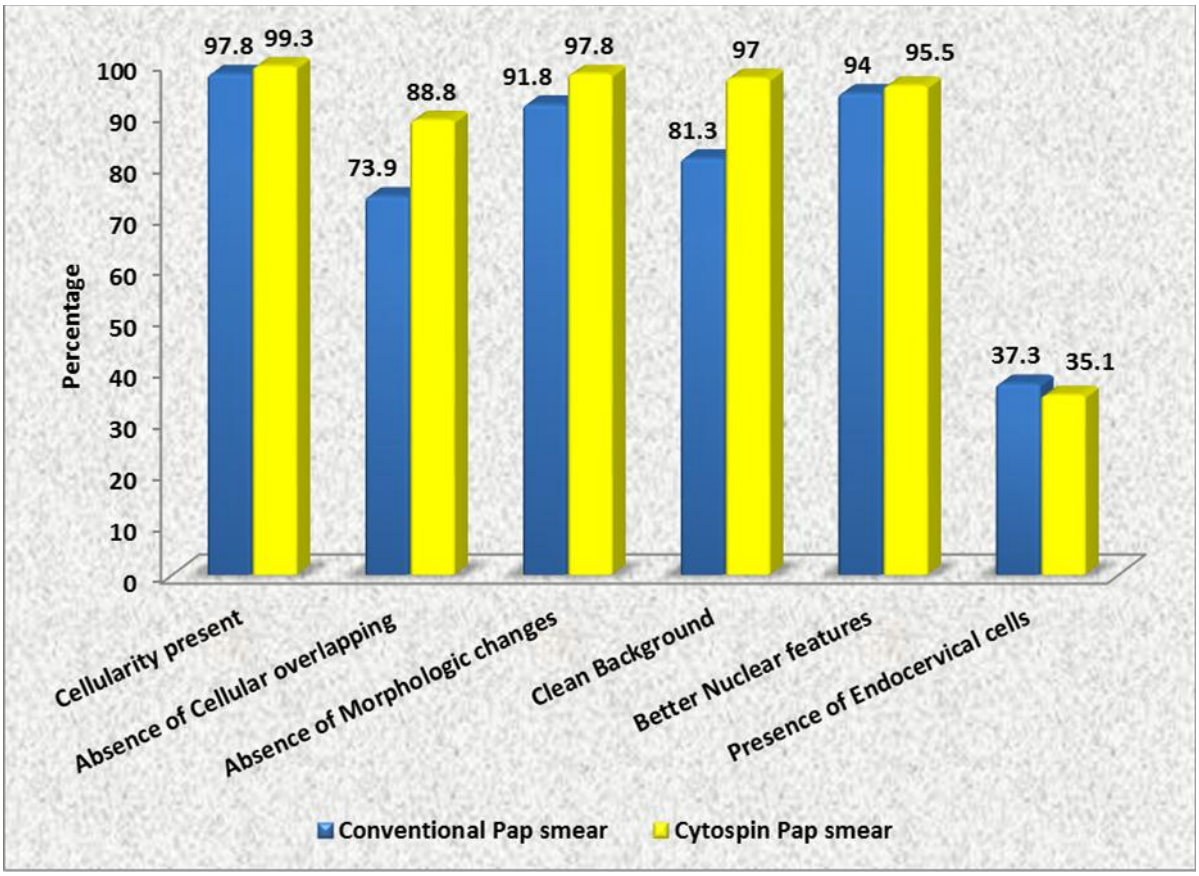


Table 5.9: Comparison of morphological parameters between the 134 Conventional Pap smears and Cytospin Pap smears

Morphological features	**Conventional Pap smear**	**Cytospin Pap smear**
Cellularity	Satisfactory in 127 cases	Satisfactory in 131 cases
Cellular overlapping	Present	Absent/Mild
Morphologic changes	Present	Absent
Clean background	Absent and obscuring factors such as mucus and blood seen	Present in most
Nuclear features	Clear	Clear
Endocervical cells present	More	Less

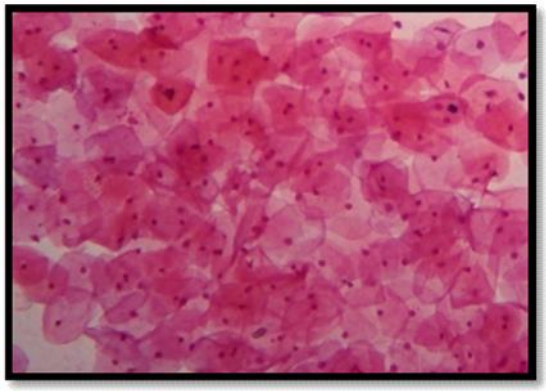


Fig.5.1: CPS (Pap 400X): Normal study- Smear is satisfactory and shows superficial and intermediate squamous cells of normal morphology. There is overlapping of cells.

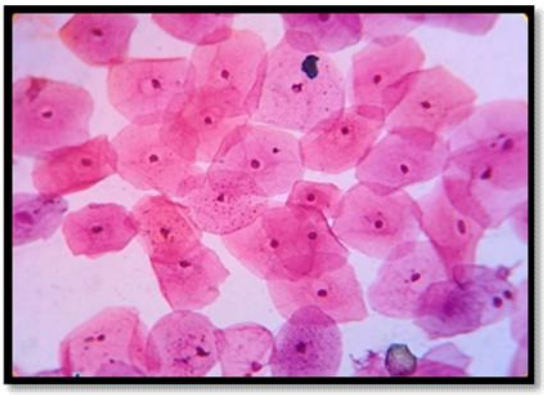


Fig.5.2:Cytospin smear (Pap 400X): Normal study- Superficial and intermediate squamous cells of normal morphology in a clean background.

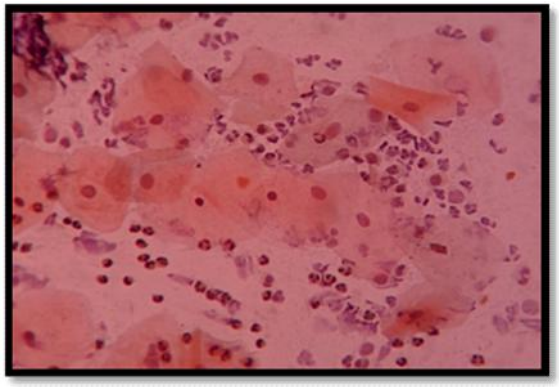


Fig.5.3: CPS (Pap400X): Inflammatory Smear- Superficial and intermediate squamous cells of normal morphology with inflammatory cells in the background.

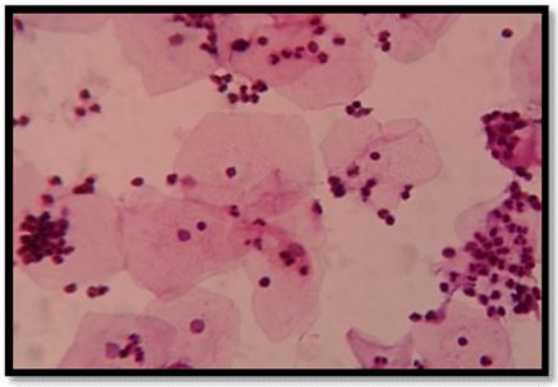


Fig.5.4: Cytospin smear (Pap 400X): Inflammatory Smear- Superficial and intermediate squamous cells of normal morphology with inflammatory cells in a clean background.

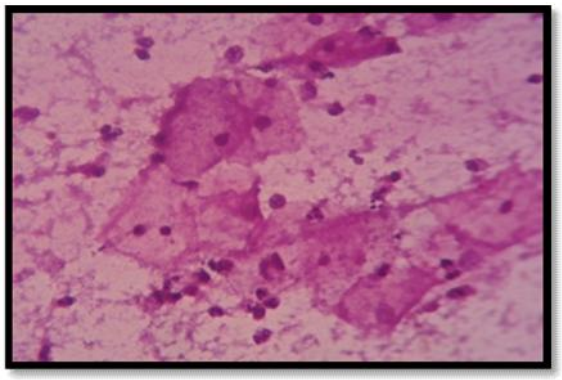


Fig.5.5: CPS (Pap400X): Bacterial vaginosis – Superficial and intermediate squamous epithelial cells in a background of plenty of coccobacilli which are present intracellularly suggestive of clue cells.

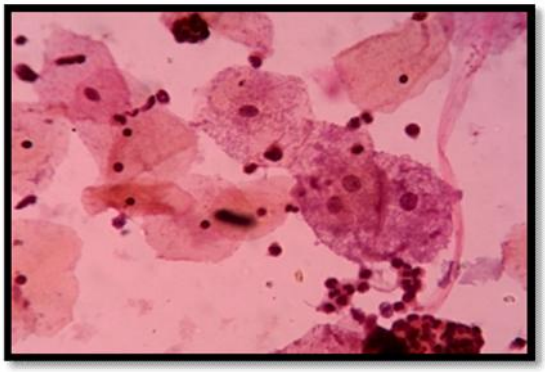


Fig.5.6: Cytospin smear (Pap400X): Bacterial vaginosis – Superficial and intermediate squamous epithelial cells in a background of plenty of coccobacilli which are present intracellularly suggestive of clue cells in a clean background.

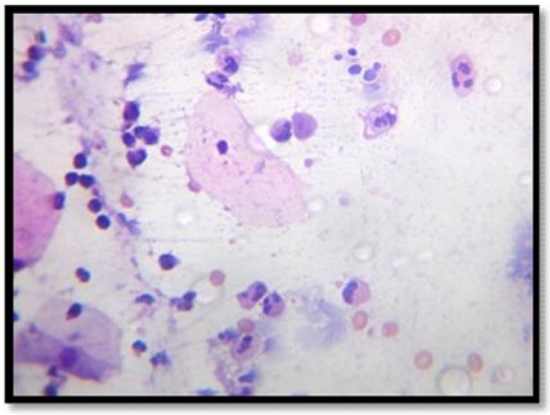


Fig.5.7: CPS (Pap400X): Trichomonas vaginalis organisms. Superficial squamous cells of normal morphology with pear-shaped cyanophilic organisms with eccentric vesicular nuclei.

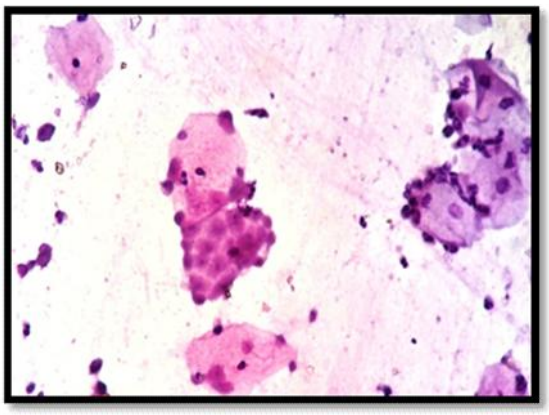


Fig.5.8: Cytospin smear (Pap400X): Trichomonas vaginalis organisms. Superficial squamous cells of normal morphology with pear-shaped cyanophilic organisms with eccentric vesicular nuclei.

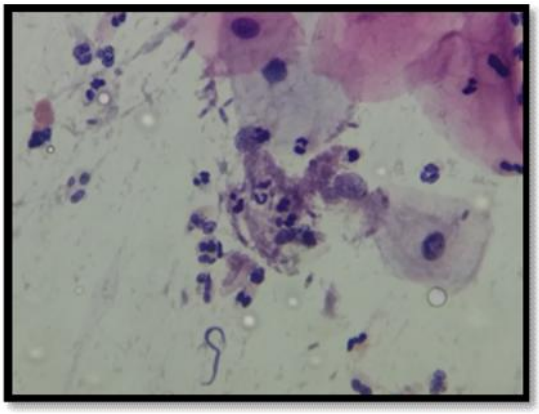


Fig.5.9: CPS (Pap400X): Candidiasis-Superficial squamous cells of normal morphology with budding yeasts forms of candida with inflammatory cells.

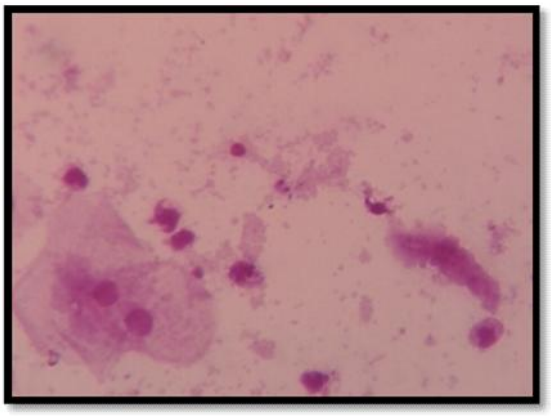


Fig.5.10: Cytospin smear(Pap400X): Candidiasis-Superficial squamous cells of normal morphology with budding yeasts forms of candida with inflammatory cells.

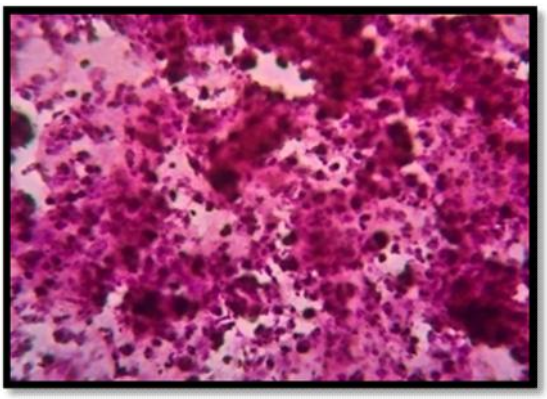


Fig.5.11: CPS (Pap400X): Unsatisfactory - Smear shows severe degree of inflammatory cells obscuring the morphology of squamous cells.

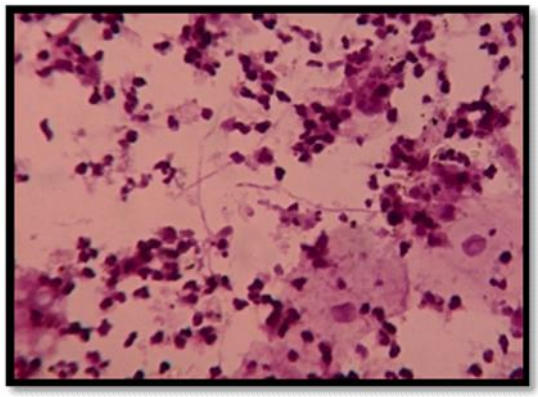


Fig.5.12: Cytospin Smear (Pap400X): Inflammatory smear- Leptothrix infestation- Long curving filamentous organisms and superficial squamous cells seen reducing the inflammatory cells. But was unsatisfactory on CPS(Fig-5.11)

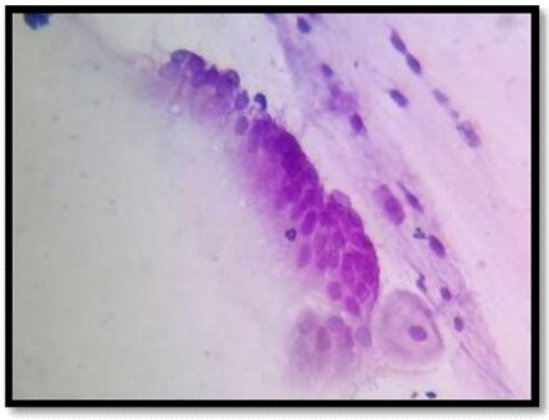


Fig.5.13: CPS (Pap 400X): Endocervical cells arranged in picket fence pattern.

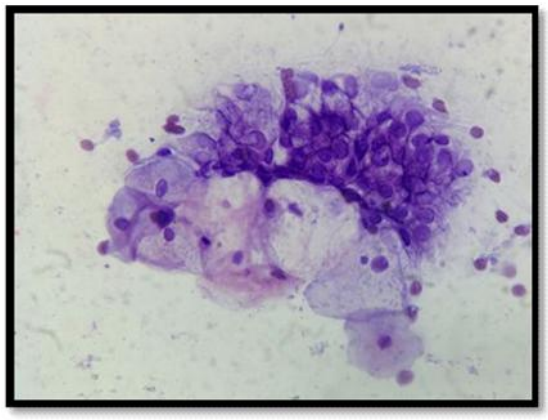


Fig.5.14: CPS (Pap 400X): Endocervical cells arranged in honeycomb pattern.

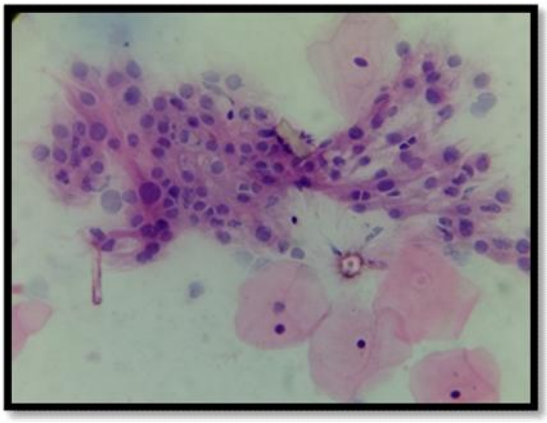


Fig.5.15:CPS (Pap 400X): ASCUS- Atypical squamous cells arranged in a 2-dimensional sheet with abundant cytoplasm showing a "pulled-out" or streaming effect. Nuclei exhibit pleomorphism of size and shape.

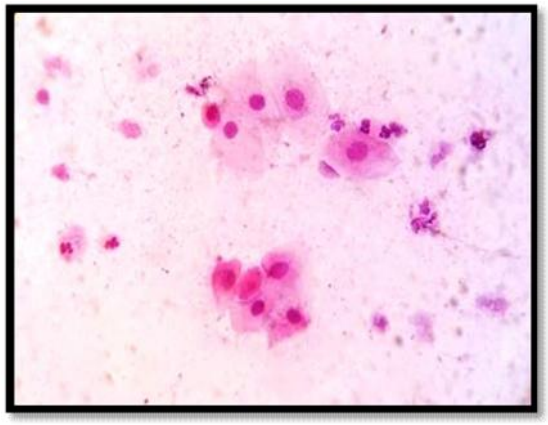


Fig.5.16: Cytospin Smear (Pap400X): ASCUS- Atypical squamous cells with increased N:C ratio, cyanophilic to basophilic cytoplasm with few dyskeratotic cells.

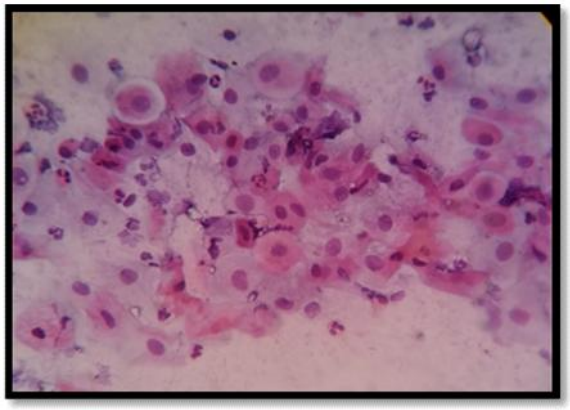


Fig.5.17: CPS (Pap 400X):
LSIL- Superficial and intermediate squamous cells showing nucleomegaly and hyperchromasia. The nucleus occupies less than half the area of the cell. Cellular overlapping is seen.

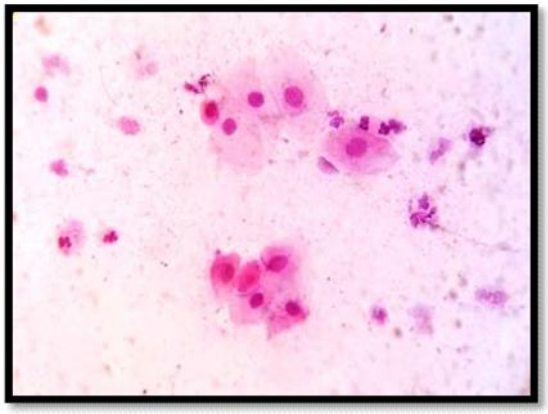


Fig.5.18: Cytospin smear (Pap 400X):
LSIL- Smear shows superficial and intermediate squamous cells, many showing nucleomegaly and hyperchromasia. The nucleus occupies less than half the area of the cell.

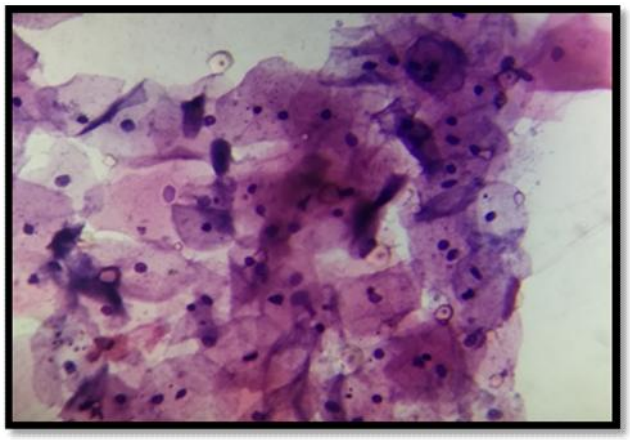


Fig.5.19: CPS (Pap 400X):
ASC-H - Smear show metaplastic cells showing nucleomegaly, hyperchromasia and coarse nuclear chromatin.

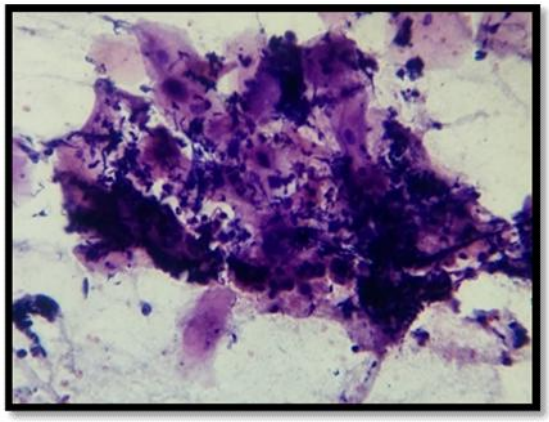


Fig.5.20: CPS (Pap 400X):
HSIL- Smear shows sheets of parabasal and metaplastic cells showing nucleomegaly and hyperchromasia in a background of inflammatory cells obscuring the morphology.

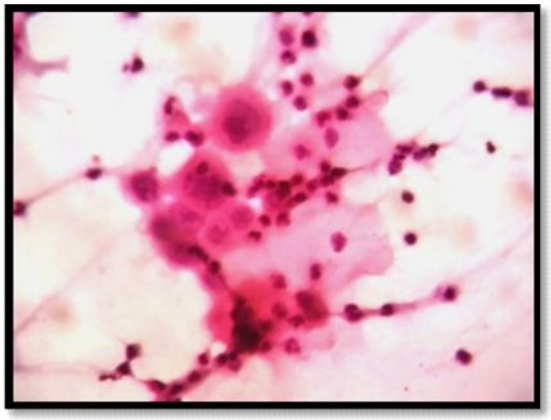


Fig.5.21: Cytospin smear (Pap 400X):
HSIL- Smear shows sheets of parabasal and metaplastic cells showing nucleomegaly and hyperchromasia in a background of inflammatory cells obscuring the morphology.

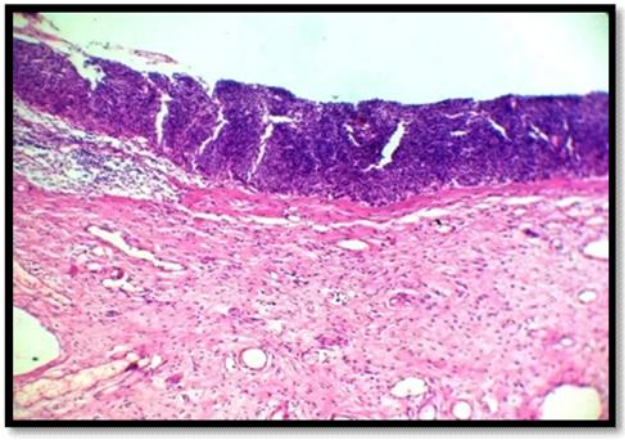


Fig.5.22: HPE (H&E-100X):
HSIL- Section shows crowding of cells in the basal two third of the epithelium. Nuclei are enlarged and hyperchromatic.

A case of HSIL diagnosed on conventional and cytospin Pap smears and was confirmed on histopathology.

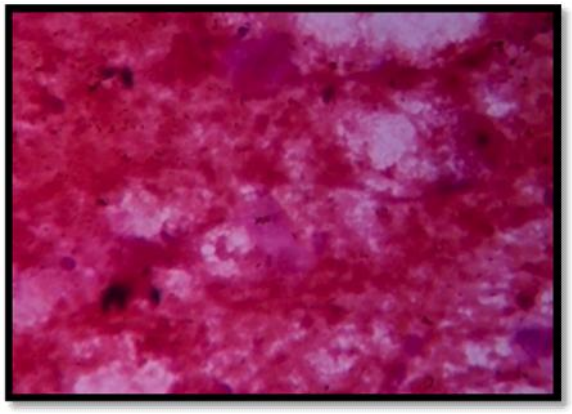


Fig.5.23: CPS (Pap400X): Unsatisfactory smear- Smear shows only hemorrhage.

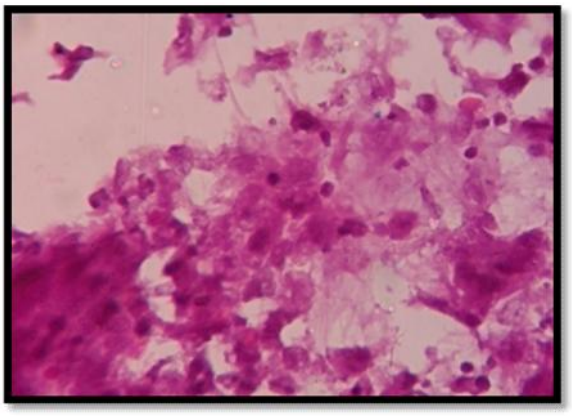


Fig.5.24: Cytospin smear (Pap 400X): Squamous cell carcinoma- Smear shows pleomorphic cells with high N/C ratio, in singles and small clusters having scant cytoplasm, large round hyperchromatic nucleus with irregular nuclear membranes.

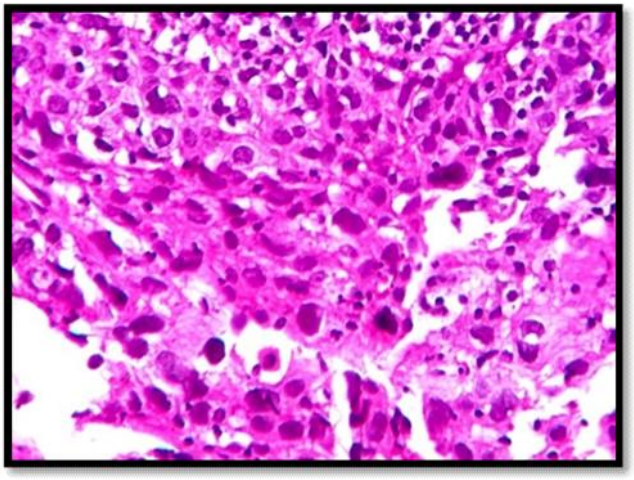


Fig.5.25: HPE (H& E-400X): Squamous cell carcinoma- Tumor cells showing nucleomegally and individual cell keratinization.

A case of Squamous cell carcinoma diagnosed on cytospin Pap smears and was confirmed on histopathology. But conventional Pap smear was unsatisfactory because of hemorrhage.

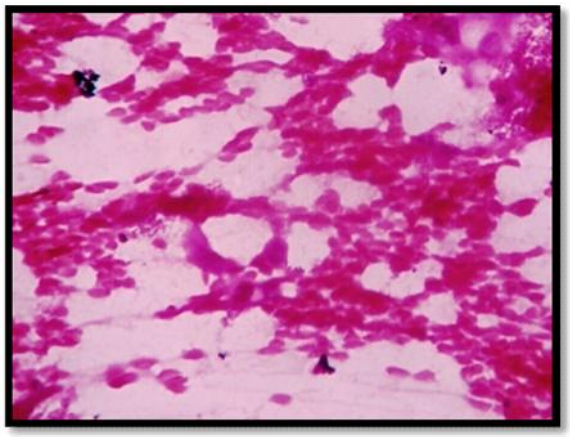


Fig.5.26: CPS (Pap400X): Unsatisfactory smear- Show hemorrhagic background

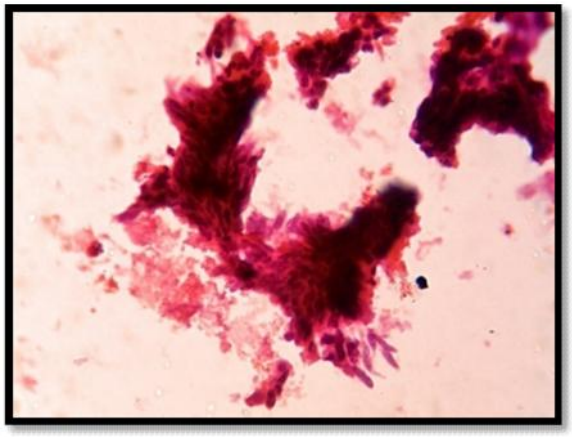


Fig.5.27: Cytospin smear (Pap 400X): Papillary Adenocarcinoma- Smear shows pleomorphic cells in three dimensional clusters and papillary pattern having nucleomegaly, hyperchromasia with scant cytoplasm.

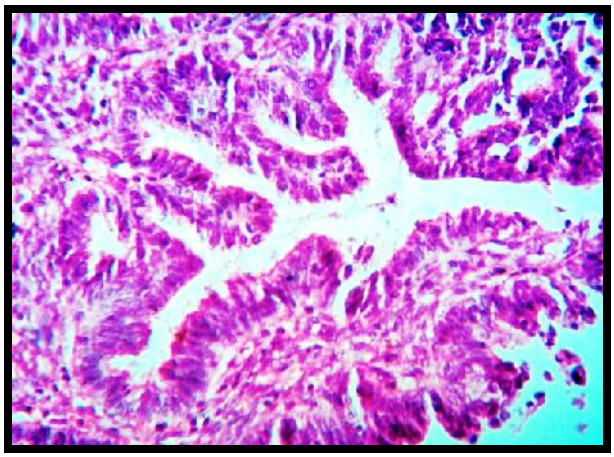


Fig.5.28: HPE (H& E-100X): Papillary Adenocarcinoma-Section shows a tumor displaying features of invasive endocervical adenocarcinoma with papillary pattern

A case of papillary cell carcinoma diagnosed on cytospin Pap smears and was confirmed on histopathology. But conventional Pap smear was unsatisfactory because of obscuring blood.

DISCUSSION

The conventional Papanicolaou smear (CPS) has been very successful in detecting pre-cancerous and cancerous lesions of the cervix through the development of quality assured and comprehensive, cytology-based population screening programs. It has remarkably reduced the mortality and morbidity due to cervical cancer. However, the CPS has many limitations owing to high number of unsatisfactory and suboptimal smear because of drying artefacts, obscuring factors like inflammatory cells and hemmorhage.[44,46]

Inadequate sampling most frequently results from discarding of most of the sample taken from the patient with the sampling device in CPS. This is a major cause for false negatives. The CPS was found to have a false-negative rate of about 14% to 33%. The CPS was also reported to have a broad range of sensitivity (30%-87%) for the detection of high-grade lesions.[6]

To address these shortcomings, new technologies were introduced, the most successful one being liquid-based cytology (LBC). It was introduced with an objective to improve the overall cervical cancer detection. It reduces the sampling error due to loss of cellular material and also avoids the heterogeneity problems by presumably transferring all the cellular material.[52]

LBC were originally developed by the Germans in the early 70s to minimize cell overlap for better performance of automated screeners in the identification of abnormal cells. Eventually they have almost replaced the use of conventional cervicovaginal smears in the developed world after successful clinical trials. ThinPrep® and SurePath® are the two FDA approved liquid-based methods that are used for the

preparation of such smears. Most developed countries have employed these systems for routine cervical screening. SurePath® works on the principle of density gradient centrifugation and ThinPrep® on membrane filtration. Liquid-based cytology is a technique achieved by rinsing the collection device in a preservative/fixative fluid to generate a suspension of cells that is processed to deposit a monolayer of cells on a microscope slide. Both these systems result in the formation of a small circular smear on the slide after the sample is placed in a fixative solution and processed by the machine. The advantages being: relative absence of blood and debris, monolayer formation, better quality of smears and smaller field for screening thus accelerating the screening process. The cells show a high contrast between the nucleus and cytoplasm with a clear background and thus requiring special training in interpretation of material.[16,18,19,46]

The disadvantages of closed systems like SurePath® and ThinPrep® are huge capital investment for equipment and logistics. The proprietary reagents adds to the maintenance and per test kit cost further.

Cytospin method of LBC is a relatively inexpensive and cost-effective method and has shown to be better than conventional Pap smears (CPS). Hence we wanted to test this technique to assess its adequacy and utility in routine screening.

In present study, we have used the cytospin which is a manual method of Liquid-based Cytology.[30,2,35] The cervical smears were taken using wooden ayre spatula. Using the split sample technique, the cells were smeared onto a glass slide for CPS and remaining cells were rinsed into a liquid fixative vial i.e, 100% methanol. The sample was left for 1hour for sedimentation.[7,53]

The sample was centrifuged with cytospin to obtain a direct smear. The cells were seen uniformly dispersed onto the glass slide with a cell deposition area of 6X6 mm with increased cell yield.[53-55] The slide was fixed with 100% methanol and stained with the Papanicolaou stain. In this way, 134 cases were sampled and smears obtained were compared with the corresponding conventional Pap smears.

In present study, the youngest patient was 18 and the oldest 65 years. The mean age of the patients was 38.3 years similar to studies by authors like Jena et al[56] and Verma K et al.[35]

Table 6.1: Comparison of Age Range and Mean age of different studies and present study

Authors	**Age Range (yr)**	**Mean Age (yr)**
Present Study	31-40	38.3
Jena et al 73 (2012)	20-55	41.3
Bukhari et al 74 (2012)	30-39	44.7
Saha K et al 75 (2013)	31-40	38.6
Verma K et al 37 (2014)	31-40	38.2

The most common presenting symptom of the patients in present study was white discharge per vagina which was similar to other studies by authors like Sherwani et al[2], Bukhari et al[57]. Other common symptoms were irregular cycles and menorrhagia.

Table 6.2: Comparison of Presenting symptoms in various studies and present study

Authors	Presenting symptoms	Percentage (%)
Present study	White discharge per vagina	44.8
Sherwani et al 29 (2007)	Discharge per vaginum	42.5
Bukhari et al 74 (2012)	Abnormal vaginal discharge	91.2
Bal et al 76 (2012)	Discharge per vaginum	59
Al Eyd GJ 77 (2012)	Vaginal discharge	27
Saha K et al 75 (2013)	Lower abdominal pain	53.8

Specimen adequacy was significantly increased in present study as also observed by Lee KR et al [58], Ronco G et al [59] and Hutchinson et al [21].

Table 6.3: Comparison of Sample Adequacy on MLBC and CPS

Authors	LBC (%)	CPS (%)
Present study	97.8	94.8
Sherwani et al 29 (2007)	83.1	31.9
Behtash N et al 80 (2008)	94.7	92.1
Kavatkar et al 3 (2008)	88.8	90.1
Nandini NM et al 7 (2012)	99	91
Singh VB et al 38 (2015)	98.3	95.7

Many authors have reported decreased rate of unsatisfactory smears by liquid based cytology owing to optimal fixation and easy monitoring of preparation quality. Also, another advantage is that the unsatisfactory samples can be reprocessed.[3] In the present study reprocessing was done in one case of adenocarcinoma and adequate material was obtained. Studies by Bishop JW et al[60], Fremont-Smith M et al[28], Marino

JF et al[61] and Utagawa ML et al[62] have reported an decrease in the unsatisfactory rates while studies by Davey E[63] et al have found no reduction in proportion of unsatisfactory smears. Weynand et al[54], however found that endocervical cells were absent in 5.3% of the cases which was attributed to a bias in methodology.

In present study, we found that 3 cases (2.2%) of cytospin were unsatisfactory compared to 7 cases (5.2%) on CPS. Hence there was a significant decrease in the number of unsatisfactory samples agreeing with studies by Sherwani RK et al[2], Deshou H et al[4], Rimiene J et al[3] and Nandini NM et al[7].

However, keeping collecting device in LBC vial ensures optimal sampling and increased number of satisfactory smears as loss of cellular material especially the endocervical clusters can be prevented. Intense rinsing process decreases the loss in cellularity.[52] The cellular material obtained can be used to prepare at least five satisfactory smears when the sampling device is discarded with the vial.[64] In present study three satisfactory smears were obtained from the residual sample. However, split sampling technique may not demonstrate full benefit due to the sampling bias as LBC sample is prepared only after the conventional smears.[28]

Conventional Pap smears have been replaced by Liquid-based cytology in many western countries as it offers further testing of HPV with the same sample besides offering better results.[65-67] Liquid-based cytology has been reported to have a significant rise in sensitivity without a significant decrease in specificity.[60]

In a study by Park et al[68], split sampling technique showed percentage agreement of 91.4% between the two methods. LBC and conventional smears had

equivalent sensitivity and LBC was more specific as there was a reduction in the detection of ASCUS/ false positive cases.

A study by Hutchinson et al[21], documented that the ThinPrep® method had heightened the sensitivity significantly when compared with CPS. They also reported a percentage agreement of 85.8% between ThinPrep® and conventional smear diagnoses.

The sensitivity of CPS as found by Hussein et al[29] was 82% versus 92% by LBC while the specificity was 76% versus 83% by LBC. Hence the sensitivity and specificity was better with LBC when compared to conventional smears. However, they recommended larger studies to verify the findings. They observed 73% agreement between conventional and LBC preparations.

In present study cytospin Pap smears showed high detection of carcinomas hence keeping that as standard sensitivity and specificity of CPS were calculated. The sensitivity and specificity of CPS was 84.6% and 100% respectively. The percentage agreement between cytospin Pap smear and conventional Pap smear was 94.03%.

In present study, we found an increased detection rate for LSIL, HSIL, squamous cell carcinoma and adenocarcinoma with cytospin smears when compared to conventional smears. The overall sensitivity was improved owing to better sampling technique, absence of obscuring factors, decreased reading time , better preservation of cells and also prevents drying artefacts due to direct fixation of cells in the liquid medium. Similar increase in sensitivity was reported by authors like Sherwani et al[2] and Bolick et al[66]. Most studies including present showed an overall increased detection rate of epithelial cell abnormalities by the LBC method. The percentage agreement between LBC and conventional smears in present study was 94.03% which was similar to studies

by Hussein et al[29] and Park et al[68]. Another advantage of cytospin Pap smear includes decreased reading time because of smaller dimension of 6X6 mm in the present study. Deshou H etal[4] prepared slide manually with dimension with a dimension of 15-17mm. Alves VAF etal[27] in their study on automated method of LBC showed the dimensions of smears being 20mm by ThinPrep® , 13mm by Autocyte® Prep and 25mm by DNACITOLIQ®.

In present study, we found a good correlation between cytospin and CPS in the detection of infectious agents. This could be due to removal of obscuring factors like blood and inflammatory cells and smaller field of smear for observation. A total of 26 cases (19.3%) of infectious diseases by cytospin smear comprising of 17 cases (12.7%) of bacterial vaginosis , 5 cases (3.7%) of candida, 3 cases (2.2%) of trichomonas and 1 case (0.7%) of leptothrix. CPS detected 23 cases (17.1%). One case of bacterial vaginosis, one case of trichomonas and one case leptothrix was missed on CPS. Similarly Sherwani RK etal[2] also reported enhancement of microscopic details of infectious agents like candida, coccobacilli and trichomonas.

In present study, morphological features between CPS and cytospin Pap smears were compared. Cytospin showed better cellularity in 133 cases (99.3%), absence of cellular overlapping in 119 cases (88.8%), absence of morphological changes in 131 cases (97.8%), clean background in 130 cases (97%), better nuclear features in 128 cases (35.1%) compared to conventional Pap smears having 131 cases (97.8%), 99 cases (73.9%), 123 cases (91.8%), 109 cases (81.3%) and 126 cases (94%) respectively. Morphological features like absence of cellular overlapping , absence of morphological changes and clean background showed p value less than 0.005 which was statistically

significant at 5% level of significance. Similar study done by Siebers AG et al[72] showed significant difference between the morphological features between LBC and conventional cervical cytology.

Some studies have reported a higher percentage of endocervical component in conventional cases which has been attributed to the split-sample collection protocols and use of the residual sample for the liquid-based preparations.[7,34,52,54,70,71] This can be prevented by direct-vial sampling which allows the entire cervical sample to be rinsed into the liquid-preservative fluid, allow an equal percentage of thin-layer slides to have the endocervical component when compared to CPS.[28,52] The technique used in present study was split-sample technique and hence there was no increase in rates of endocervical component on cytospin smears. In contrast, endocervical cell component was decreased in the cytospin Pap smears in the present study. Similar limitations were seen in a study by Nandini NM etal[7].

The following are the advantages of liquid-based cytology- the provision for long term storage of the liquid sample, thin monolayer of cells within a clean background attributed to fixative used.[19,73,74] Thus liquid-based cytology improves the quality of screening of slides by giving a clear background and removal of obscuring factors and also by reducing reading time.[70,75,76]

Besides, cytospin Pap smear method of MLBC has other advantages as the residual specimens can be used for ancillary testing like detection of HPV-DNA by PCR or In-situ DNAHybridization[3,30,32,49,77,78] and immunocytochemistry by cell block preparations.[7,19,79]

Numerous automated computer assisted systems have been developed for screening of slides for abnormal cells like the PAPNET system and Focal point analyser which reduces the screening time and increases the detection rate but is an expensive method to be employed for routine screening in a country like ours.[45,49-51,80]

Limitations of the study were that split sample method for sample collection was used which could lead to ineffective sampling as we found endocervical component were less in cytospin smears when compared with CPS which can be attributed to the split sample collection protocol. This can be overcome by direct sampling method and sampling by experienced gynecologists.[7,28,70,81]

Another limitation of the study is that histopathological correlation. Sensitivity and specificity could have been better achieved when there is histopathological correlation.

CONCLUSION

Cervical cancer is the second most common malignancy among women worldwide. Cytological screening leads to a reduction in a rate of invasive cancer of uterine cervix. Conventional Pap smear screening for detection of cervical cancer is less sensitive due to several limitations. Cytospin type of liquid based cytology is strongly recommended in the best interest of public health as it improves the sample quality, reduces the likelihood of false negative results, better morphology and cost effective. It over comes the limitation of conventional smear as it significantly reduces unsatisfactory smears, improves specimen adequacy, detects more intraepithelial lesion.It is of value as an alternative more effective screening strategy in low resource settings, like developing contries including India where women are at high risk for developing cervical cancer, as it is an inexpensive, cost effective method compared to ThinPrep® and SurePath® method of liquid based cytology. Also, further ancillary testing like HPV DNA by PCR or insitu DNA hybridization and immunocytochemistry can be performed with the remaining sample.Thus it will significantly improve early detection and treatment of cervical lesions.

SUMMARY

A prospective study of cytospin method of liquid-based cytology for screening cervical smears and comparing the same with conventional Pap smears was undertaken to evaluate its diagnostic utility, during 1st December 2014 to 30th June 2016 in the Department of Pathology, B.L.D.E.U'S Shri B M Patil Medical College, Hospital & Research Centre, Vijayapur.

The salient features observed in this study are –

A comparative study for 134 cases of conventional Pap smears and cytospin Pap smears were prepared. The mean age of the patients was 38.3 years with majority of the patients in the age group of 18-65 years. The most frequent presenting complaint was white discharge per vagina seen in 44.8% of the women.

Smears were adequate in 131 cases (97.8%) of cytospin smear and 127 cases (94.8%) of CPS. Of the 131 cases on cytospin method of Pap smear, 118 cases (90.1%) were reported as non-neoplastic lesions and 13 cases (9.9%) as neoplastic. The most common non-neoplastic lesion was inflammatory smear 63 cases (47%) followed by normal study 27 cases (20.1%), bacterial vaginosis 17 cases (12.7%), candidal infestation 5 cases (3.7%), trichomonas vaginalis infestation 3 cases (2.2%), menopausal/atrophic smear 2 cases (1.5%) and leptothrix infestation 1 case (0.7%). The most common neoplastic lesion was LSIL and HSIL each accounting for 4 cases (3%) followed by ASCUS 3 cases (2.2%), squamous cell carcinoma 1 case (0.7%) and 1 case (0.7%) of adenocarcinoma.

Comparison of morphological features between CPS and cytospin Pap smears such as cellularity, absence of cellular overlapping, absence of morphological changes, clean background, nuclear features and presence of endocervical cells were done and difference was statistically significant at 5% level of significance.

Cytospin detected more number of neoplastic lesions, proving it as a better screening test in diagnosis of precancerous and cancerous lesions, in view of better morphology, sensitivity, low cost per test and easy technical training with available resources.

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ETHICAL CLEARANCE CERTIFICATE

B.L.D.E.UNIVERSITY'S
SHRI.B.M.PATIL MEDICAL COLLEGE, BIJAPUR – 586103
INSTITUTIONAL ETHICAL COMMITTEE

Institutional Ethical Committee BIJAPUR-586 103. OUTWARD No. 55/2015 Date. 10/10/15 BLDE U's Shri B.M. Patil Medical College

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this college met on 10/10/2015 *at* 03-30pm *scrutinize the Synopsis of Postgraduate Students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected and revised version synopsis of the Thesis has accorded Ethical Clearance.*

Title "Comparative Study of conventional papanico-laou Smear and cytospin smear in cervical cancer Screening"

Name of P.G. Student : Dr Amrutha. M.R.
Dept of Pathology.

Name of Guide/Co-investigator : Dr. Mahesh. H. Karigoudar.
Professor of Pathology.

DR.TEJASWINI VALLABHA
CHAIRMAN
Institutional Ethical Committee
BLDEU's Shri B.M. Patil
Medical College,BIJAPUR-586103.

Following documents were placed before E.C. for Scrutinization
1)Copy of Synopsis/Research Project
2)Copy of informed consent form.
3)Any other relevant documents.

BLDE University's
Shri B M Patil Medical College, Hospital & R.C
Vijayapur, Karnataka

INFORMED CONSENT FOR PARTICIPATION IN DISSERTATION/RESEARCH

I, the undersigned, _____, D/O W/O, _____ aged ___years, ordinarily resident of _____do hereby state/declare that Dr. AMRUTHA.M.R of _____Hospital Shri B M Patil Medical College, Hospital & R.C Vijayapur, Karnataka has examined me thoroughly on_____ at _____ (place) and it has been explained to me in my own language _____that I am suffering from _____disease (condition) and this disease/condition mimic following diseases _____. Further Dr. AMRUTHA.M.R informed me that she is conducting dissertation/research titled **"Comparative Study of Conventional Papanicolaou Smear and Cytospin Smear In Cervical Cancer Screening"** under the guidance of Dr. MAHESH H KARIGOUDAR requesting my participation in the study. Apart from routine treatment procedure the pre-operative, operative, post-operative and follow-up observations will be utilized for the study as reference data.

Further doctor has informed me that my participation in this study help in evaluation of the results of the study which is useful reference to treatment of other similar cases in near future, and also I may be benefited in getting relieved of suffering or cure of the disease I am suffering.

The Doctor has also informed me that information given by me, observations made/ photographs/ video graphs taken upon me by the investigator will be kept secret and not assessed by the person other than me or my legal hirer except for academic purposes.

The Doctor did inform me that though my participation is purely voluntary, based on information given by me, I can ask any clarification during the course of treatment / study related to diagnosis, procedure of treatment, result of treatment or prognosis. At the same time I have been informed that I can withdraw from my participation in this study at any time if I want or the investigator can terminate me from the study at any time from the study but not the procedure of treatment and follow-up unless I request to be discharged.

After understanding the nature of dissertation or research, diagnosis made, mode of treatment, I the undersigned Shri/Smt _____ under my full conscious state of mind agree to participate in the said research/dissertation.

Signature of patient:

Signature of doctor:

Witness: 1.

2.

Date:

Place:

PROFORMA

DEPARTMENT OF PATHOLOGY

Case No :

Particulars of the patient :

Name : Hospital :

Age : O.P.D/I.P No. :

Address : Socioeconomic status :

Occupation : Religion :

Presenting complaints and duration :

Menstrual history: Age of menarche -

Menstrual cycles -

Obstetric history: Age at 1st coitus -

Obstetric score -

Age at 1st delivery -

Spacing between 1st and 2nd child : _____

Spacing between 2nd and 3rd child : _____

Spacing between 3rd and 4th child : _____

Spacing between others : _____

Past history :

History suggestive of HIV : Yes ☐ No ☐ If yes, details:_____

History suggestive of HSV : Yes ☐ No ☐ If yes, details:_____

History suggestive of HPV : Yes ☐ No ☐ If yes, details:_____

Family history :

Personal history :

Multiple sexual partners : Yes ☐ No ☐

General physical examination :

Systemic examination :

Per speculum and per vaginal examination:

Clinical diagnosis:

Conventional Pap smear report:

Cytospin smear report:

KEY TO MASTER CHART

WDPV: White discharge per vagina

PV: Per vagina

Scoring

- Cellularity : 0- Inadequate
  1- Adequate
- Overlapping : 0- Presence of cellular overlapping
  1- Absence of cellular overlapping
- Morphological : 0- Presence of morphological changes
  1- Absence of morphological changes
- Background : 0- Obscured with mucus/blood
  1- Clean background
- Nuclear features : 0- Poor
  1- Better
- Endocervical cells : 0- Absent
  1- Present

MASTER CHART

Name	Age	c/o	Pap lab no.	conventional pap smear							cytospin pap smear						
				cellularity	overlapping	morphology	background	nuclear	endocervical	Impression	cellularity	overlapping	morphology	background	nuclear	endocervical cells	Impression
Tarabai	28	pain abdomem	9	1	1	0	0	1	1	Inflammatory smear	1	1	1	1	1	1	Inflammatory smear
Rajeshwari	45	irregular cycles	48	1	0	1	1	1	1	Normal study	1	1	1	1	1	0	Normal Study
Mahadevi	44	wdpv	57	1	1	1	1	1	0	Bacterial vaginosis	1	1	1	1	1	0	Bacterial vaginosis
Siddamma	32	wdpv	78	1	0	1	1	1	0	Inflammatory Smear	1	1	1	1	1	0	Inflammatory smear
Savatri	42	wdpv	79	1	0	1	0	1	0	Inflammatory Smear	1	1	1	1	1	0	Normal study
Radha	32	wdpv	90	1	0	1	1	1	0	Inflammatory Smear	1	0	1	1	1	0	Inflammatory smear
Parwati	52	pain abdomem	99	1	1	1	1	1	1	Inflammatory Smear	1	1	1	1	1	0	Inflammatory smear
Shainaj bi	28	dysmenorrhea	134	1	0	0	1	1	1	Inflammatory Smear	1	1	1	1	1	1	inflammatory smear
Neelamma	50	wdpv	135	1	1	1	0	1	0	Bacterial Vaginosis	1	0	1	1	1	0	Bacterial vaginosis
Sainaz	30	infertility	151	1	1	1	1	1	1	Inflammatory Smear	1	1	1	1	1	1	Inflammatory smear
Parvati	50	backache	152	1	0	1	1	1	0	Inflammatory Smear	1	0	1	1	1	0	Inflammatory smear
Savita	35	amenorrhea	153	1	0	1	1	1	0	Inflammatory Smear	1	0	1	1	1	0	Inflammatory smear
Devaki	40	irregular cycles	164	1	1	1	0	1	0	Inflammatory Smear	1	1	1	1	1	0	Inflammatory smear
Devakemma	35	dysmenorrhea	165	1	0	0	0	1	0	Inflammatory smear	1	0	1	1	1	0	Trichomonas Vaginalis
Shamshad	54	pain abdomem	182	1	1	1	1	1	1	Inflammatory Smear	1	1	1	1	1	0	Inflammatory smear
Sharadha	34	pain abdomem	206	1	0	1	0	1	0	Inflammatory Smear	1	1	1	0	1	0	Inflammatory smear
Indrabai	51	backache	255	1	1	1	1	1	1	Inflammatory Smear	1	1	1	1	1	1	Inflammatory smear

Anjana	26	wdpv	263	1	0	1	1	1	0	Inflammatory Smear	1	1	1	1	1	0	Inflammatory smear
Rajeshree	38	generalised weakness	374	1	1	1	1	1	1	Normal study	1	1	1	1	1	1	Normal study
Basamma	45	pain abdomem	381	1	1	1	1	1	0	Normal study	1	1	1	1	1	0	Normal study
Neelakka	38	wdpv	446	1	0	1	1	1	0	Inflammatory Smear	1	0	1	1	1	0	Inflammatory smear
Malawwa	40	wdpv	447	1	1	1	0	1	0	Inflammatory Smear	1	1	1	1	1	0	Inflammatory smear
Sharanamma	35	pain abdomem	5263	0	0	0	0	0	0	Unsatisfactory	1	1	1	1	0	0	Squamous Cell carcinoma
Lalita	50	wdpv	471	1	1	1	0	1	0	Inflammatory Smear	1	1	1	1	1	0	Inflammatory smear
Maya	48	wdpv	482	1	0	0	1	1	1	Inflammatory Smear	1	1	0	1	1	1	Inflammatory smear
Jagadevi	40	wdpv	513	1	1	1	1	1	1	Inflammatory Smear	1	1	1	1	1	1	Bacterial Vaginosis
Anasuya	32	wdpv	528	1	1	1	0	1	0	Inflammatory Smear	1	1	1	1	1	0	Inflammatory smear
Baginathi	44	wdpv	537	1	1	1	1	1	1	Inflammatory Smear	1	1	1	1	1	1	Inflammatory smear
Pramila m p	30	backache	4773	0	0	0	0	0	0	Unsatisfactory	1	1	1	1	0	0	Papillary adenocarcinoma
Yalamma	45	post coital bleeding	961	1	1	1	1	1	1	ASCUS-H	1	1	1	1	1	1	Hsil
Danamma	26	wdpv	539	1	1	1	1	1	1	Inflammatory Smear	1	1	1	1	1	1	Inflammatory smear
Shantabai	57	irregular cycles	973	1	0	0	0	0	0	Unsatisfactory	1	0	1	0	0	0	Inflammatory smear
Sudha	38	infertility	1026	1	1	1	1	1	1	Normal study	1	1	1	1	1	1	Normal study
Sujata	40	amenorrhea	1029	1	1	1	1	1	1	Normal study	1	1	1	1	1	1	Normal study
Jyoti	22	dysmenorrhea	1057	1	0	0	0	0	1	Unsatisfactory	1	0	0	1	0	1	Inflammatory Smear-Leptothrix
Umadevi	40	dysmenorrhea	1075	1	0	1	1	1	0	Inflammatory Smear	1	1	1	1	1	0	Inflammatory smear
Sunita kutur	24	irregular cycles	1102	1	1	1	1	1	1	Normal study	1	1	1	1	1	1	Normal study
Shila patil	25	wdpv	1140	1	1	1	0	1	1	Candida	1	1	1	1	1	1	Candida
Savita	36	wdpv	1150	1	0	1	0	1	0	Inflammatory Smear	1	1	1	1	1	0	Inflammatory smear
Nirmala	45	irregular cycles	1117	1	1	1	1	1	0	Inflammatory Smear	1	1	1	1	1	0	Inflammatory smear
Saraswati	44	wdpv	1126	1	1	1	0	1	0	Inflammatory Smear	1	1	1	1	1	0	Inflammatory smear
Mahadevi	20	wdpv	1127	1	0	1	1	1	1	Inflammatory Smear	1	1	1	1	1	1	inflammatory smear
Vijayalaxmi	55	post coital bleeding	1141	1	0	1	1	1	1	atrophic smear	1	1	1	1	1	1	atrophic smear

Shila patil	25	wdpv	1140	1	1	1	1	1	0	Candida	1	1	1	1	1	0	Candida
Bhagirati	45	irregular cycles	1142	1	1	1	1	1	0	Normal study	1	1	1	1	1	0	Normal study
Savita	36	wdpv	1150	1	0	1	0	1	0	Inflammatory Smear	1	1	1	1	1	0	Inflammatory Smear
Ambu	35	pain abdomem	1159	1	1	1	1	1	0	Inflammatory Smear	1	1	1	1	1	0	Inflammatory Smear
Neelawwa	41	pain abdomem	1170	1	1	1	1	1	0	Normal study	1	1	1	1	1	0	Normal study
Kalawati	33	menorrhagia	1178	1	1	0	0	1	0	ASCUS	1	1	1	1	1	0	ASCUS
Dodawwa	29	wdpv	1187	1	1	1	1	1	1	Inflammatory Smear	1	1	1	1	1	1	Inflammatory Smear
Channamma	50	mass pv	1220	1	1	1	1	1	1	Inflammatory Smear	1	1	1	1	1	1	Inflammatory Smear
Girajabai	60	post menopausal	1219	1	1	1	1	1	0	lsil	1	1	1	1	1	0	Lsil
Sujata	43	wdpv	1255	1	1	1	1	1	0	Candida	1	1	1	1	1	0	Candida
Indubai	45	mass pv	1282	1	0	1	1	1	0	Inflammatory Smear	1	1	1	1	1	0	Inflammatory Smear
Rachana	22	wdpv	1283	1	1	1	1	1	0	Inflammatory Smear	1	1	1	1	1	0	Inflammatory Smear
Kasturi	50	spotting	1285	1	1	1	1	1	1	lsil	1	1	1	1	1	1	Lsil
Mahadevi	60	mass pv	1286	1	1	1	1	1	0	Inflammatory Smear	1	1	1	1	1	0	Inflammatory Smear
Gurubai	35	menorrhagia	1308	1	1	1	1	1	1	hsil	1	1	1	1	1	1	Hsil
Vijayalaxmi	28	wdpv	1324	1	1	1	1	1	1	Inflammatory Smear	1	0	1	1	1	1	Inflammatory Smear
Savitri	32	wdpv	1344	1	1	1	1	1	1	Normal study	1	1	1	1	1	1	Normal study
Shridevi	27	wdpv	1343	1	0	1	1	1	0	Candida	1	1	1	1	1	0	Candida
Sunitha	50	post menopausal	1345	1	1	1	1	0	1	hsil	1	1	1	1	1	1	Hsil
Sharada	32	wdpv	1346	1	1	1	1	1	1	Inflammatory Smear	1	1	1	1	1	1	Inflammatory Smear
Shankuntala	60	post menopausal	1372	1	0	0	1	1	0	atrophic smear	1	1	1	1	1	0	atrophic smear
Sharada	49	wdpv	1373	1	1	1	1	1	1	Bacterial vaginosis	1	1	1	1	1	1	Bacterial vaginosis
Kasturibai	47	wdpv	1373	1	1	1	1	1	0	Bacterial vaginosis	1	1	1	1	1	0	Bacterial vaginosis
Kamala bai	65	mass pv	1395	1	1	1	1	1	0	Normal study	1	1	1	1	1	0	Normal study
Danamma	40	wdpv	1403	1	1	1	1	1	0	Normal study	1	1	1	1	1	0	Normal study
Laxmi	27	irregular cycles	1415	1	1	1	1	1	0	Inflammatory Smear	1	1	1	1	1	0	Inflammatory Smear
Laxmi	35	wdpv	1404	1	1	1	1	1	0	Inflammatory Smear	1	1	1	1	1	0	Inflammatory Smear
Sharada	55	mass pv	1416	1	1	1	1	1	0	Normal study	1	1	1	1	1	0	Normal study
Jayashree	35	wdpv	1406	1	1	1	0	1	1	Bacterial vaginosis	1	1	1	1	1	1	Bacterial vaginosis

Bisimilla	35	wdpv	1417	1	1	1	1	1	0	Normal study	1	1	1	1	1	0	Normal study
Yamanamma	32	wdpv	1424	1	1	1	1	1	0	Normal study	1	1	1	1	1	0	Normal study
Lalita	25	wdpv	1425	1	1	1	1	1	1	trichomonas vaginalis	1	1	1	1	1	1	Trichomonas Vaginalis
Neelabai	48	wdpv	1426	1	1	1	1	1	1	Bacterial vaginosis	1	1	1	1	1	1	Bacterial vaginosis
Shankremma	25	wdpv	1443	0	1	1	0	0	0	Unsatisfactory	0	1	1	0	0	0	Unsatisfactory
Savita	25	wdpv	1452	1	0	1	1	1	1	Bacterial vaginosis	1	1	1	1	1	1	Bacterial vaginosis
Laxmi	35	wdpv	1453	1	1	1	1	1	0	Inflammatory Smear	1	1	1	1	1	0	Inflammatory Smear
Rukuma bai	35	irregular cycles	1455	1	0	1	1	1	1	Inflammatory Smear	1	0	1	1	1	1	Inflammatory Smear
Bhimabai	50	mass pv	1456	1	1	1	1	1	0	Normal study	1	1	1	1	1	0	Normal study
Rajashree	19	menorrhagia	1475	1	1	1	1	1	0	Normal study	1	1	1	1	1	0	Normal study
Shahajanbai	35	menorrhagia	1494	1	0	0	0	0	0	Unsatisfactory	1	0	0	0	0	0	Unsatisfactory
Sangita	28	wdpv	1531	1	1	1	0	1	0	Inflammatory Smear	1	1	1	1	1	0	Inflammatory Smear
Mallawwa	35	menorrhagia	1529	1	1	1	1	1	0	Normal study	1	1	1	1	1	0	Normal study
Sunanda	35	wdpv	1530	1	0	1	0	1	0	Bacterial vaginosis	1	1	1	1	1	0	Bacterial vaginosis
Mahananda	40	wdpv	1532	1	1	1	1	1	0	normal study	1	1	1	1	1	0	normal study
Shail	40	wdpv	1535	1	0	1	1	1	1	Inflammatory Smear	1	1	1	1	1	1	Inflammatory Smear
Shabana	24	wdpv	1534	1	1	1	1	1	0	Bacterial vaginosis	1	1	1	1	1	0	Bacterial vaginosis
Kallawwa	50	wdpv	1549	1	1	1	1	1	0	Bacterial vaginosis	1	1	1	1	1	0	Bacterial vaginosis
Jayshree	45	mass pv	1548	1	0	1	1	1	1	Inflammatory Smear	1	1	1	1	1	1	Inflammatory Smear
Bhagirati	42	wdpv	1547	1	1	1	1	1	0	Bacterial vaginosis	1	1	1	1	1	0	Bacterial vaginosis
Kasturaibai	32	wdpv	1557	1	1	1	1	1	1	Bacterial vaginosis	1	1	1	1	1	1	Bacterial vaginosis
Lata chavan	25	wdpv	1550	1	1	1	1	1	0	Inflammatory Smear	1	1	1	1	1	0	Inflammatory Smear
Gangamma	29	menorrhagia	1570	1	0	1	1	1	0	Inflammatory Smear	1	0	1	1	1	0	Inflammatory Smear
Basamma	40	irregular cycles	1582	1	1	1	1	1	0	Inflammatory Smear	1	1	1	1	1	0	Inflammatory Smear
Mahadevi	30	menorrhagia	1583	1	1	1	1	1	0	Inflammatory Smear	1	1	1	1	1	0	Inflammatory Smear
Sheela	25	wdpv	1580	1	1	1	1	1	1	Bacterial vaginosis	1	1	1	1	1	1	Bacterial vaginosis
Mallawwa	30	menorrhagia	1579	1	1	1	1	1	0	Inflammatory Smear	1	1	1	1	1	0	Inflammatory Smear
Savitri	50	mass pv	1581	1	0	1	1	1	0	Inflammatory Smear	1	0	1	1	1	0	Inflammatory Smear
Neelamma	22	wdpv	1584	1	1	1	1	1	0	Inflammatory Smear	1	1	1	1	1	0	Inflammatory Smear

Sumitra	60	mass pv	1591	1	1	1	1	1	0	Unsatisfactory	1	1	1	1	1	0	Unsatisfactory
Sivanava	65	mass pv	1641	1	1	1	1	1	1	Normal study	1	1	1	1	1	1	Normal study
Mahananda	35	pain abdomem	1652	1	1	1	1	1	0	Inflammatory Smear	1	1	1	1	1	0	Inflammatory Smear
Suman	35	irregular cycles	1651	1	1	1	1	1	1	Inflammatory Smear	1	1	1	1	1	1	Inflammatory Smear
Kashibai	30	wdpv	1664	1	1	1	1	1	0	trichomonas vaginalis	1	1	1	1	1	0	Trichomonas Vaginalis
Mahadevi	30	wdpv	1666	1	1	1	1	1	0	Inflammatory Smear	1	1	1	1	1	0	Inflammatory Smear
Sarubai	35	wdpv	1663	1	1	1	1	1	0	Inflammatory Smear	1	1	1	1	1	0	Inflammatory Smear
Annapurana	25	wdpv	1665	1	1	1	1	1	1	Inflammatory Smear	1	1	1	1	1	1	Inflammatory Smear
Renuka	23	amenorrhea	658	1	1	1	1	0	0	ASCUS	1	1	1	1	1	0	ASCUS
Anushaya	35	wdpv	1668	1	1	1	1	1	0	Inflammatory Smear	1	1	1	1	1	0	Inflammatory Smear
Gourabai	40	irregular cycles	1737	1	1	1	1	1	1	Normal study	1	1	1	1	1	1	Normal study
Neelamma	40	wdpv	1738	1	1	1	1	1	0	Inflammatory Smear	1	1	1	1	1	0	Inflammatory Smear
Devamma	40	menorrhagia	1777	1	1	1	1	1	1	lsil	1	1	1	1	1	1	Lsil
Giraja	45	irregular cycles	1779	1	1	1	1	1	0	Normal study	1	1	1	1	1	0	Normal study
Sumitra	42	wdpv	1782	1	1	1	1	1	0	Bacterial vaginosis	1	1	1	1	1	0	Bacterial vaginosis
Bharati	60	spotting	2434	1	1	1	1	1	1	Inflammatory Smear	1	1	1	1	1	1	Inflammatory Smear
Prema	20	wdpv	2	1	1	1	1	1	0	Normal study	1	1	1	1	1	0	Normal study
Yamunawwa	33	itching	7	1	0	1	1	1	1	Candida	1	1	1	1	1	1	Candida
Sharubai	38	pain abdomem	35	1	1	1	1	1	0	Normal study	1	1	1	1	1	0	Normal study
Mala	40	itching	100	1	0	1	1	1	1	Bacterial vaginosis	1	0	1	1	1	1	Bacterial vaginosis
Balamma	25	irregular cycles	121	1	1	1	1	1	0	Normal study	1	1	1	1	1	0	Normal study
Sulochan	60	post menopausal	132	1	1	1	1	1	1	hsil	1	1	1	1	1	1	hsil
Sharanamma	64	menorrhagia	159	1	1	1	1	1	0	Normal study	1	1	1	1	1	0	Normal study
Chandbed	55	post menopausal	342	1	1	1	0	1	1	ASCUS	1	1	1	1	1	1	ASCUS
Neelabai	35	menorrhagia	1084	1	1	1	1	1	0	Inflammatory Smear	1	1	1	1	1	0	Inflammatory Smear
Sonali	25	irregular cycles	1099	1	1	1	1	1	1	Inflammatory Smear	1	1	1	1	1	1	Inflammatory Smear
Indrabai	32	menorrhagia	1108	1	1	1	1	1	1	Inflammatory Smear	1	1	1	1	1	1	Inflammatory Smear
Sunita	46	itching	1128	1	1	1	1	1	0	Bacterial vaginosis	1	1	1	1	1	0	Bacterial vaginosis
Mangla	22	wdpv	1158	1	1	1	1	1	0	Inflammatory Smear	1	1	1	1	1	0	Inflammatory Smear

Nagamma	18	wdpv	1159	1	1	1	1	1	0	Normal study	1	1	1	1	1	0	Normal study
Mala	30	amenorrhea	1287	1	0	1	1	1	0	Inflammatory Smear	1	0	1	1	1	0	Inflammatory Smear
Ambavva	65	post menopausal	427	1	1	1	0	1	0	ASCUS	1	1	1	1	1	0	lsil
Sukanya	42	backache	1298	1	1	1	1	1	0	Inflammatory Smear	1	1	1	1	1	0	Inflammatory Smear