COMPARISON OF CONVENTIONAL PAP SMEAR AND LIQUID BASED CYTOLOGY AS A SCREENING METHOD FOR CERVICAL CANCER AND ITS CORRELATION WITH BIOPSY

DISSERTATION

SUBMITTED FOR M.D. (PATHOLOGY)

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DECLARATION

I Dr.B.Saranya solemnly declare that this Dissertation **" COMPARISON OF CONVENTIONAL PAP SMEAR AND LIQUID BASED CYTOLOGY AS A SCREENING METHOD FOR CERVICAL CANCER AND ITS CORRELATION WITH BIOPSY"** is a bonafide record of work done by me in the Department of Pathology, Thanjavur Medical College and Hospital, Thanjavur under the Guidance and Supervision of my Professor **Dr.AL.SANTHI, M.D.,D.G.O**, The Head of the Department, Department of Pathology, Thanjavur Medical College, Thanjavur between 2013 and 2016.

This Dissertation is submitted to The Tamilnadu Dr. M.G.R Medical University , Chennai in partial fulfilment of University regulations for the award of M.D Degree (Branch – III) in Pathology to be held in April 2016.

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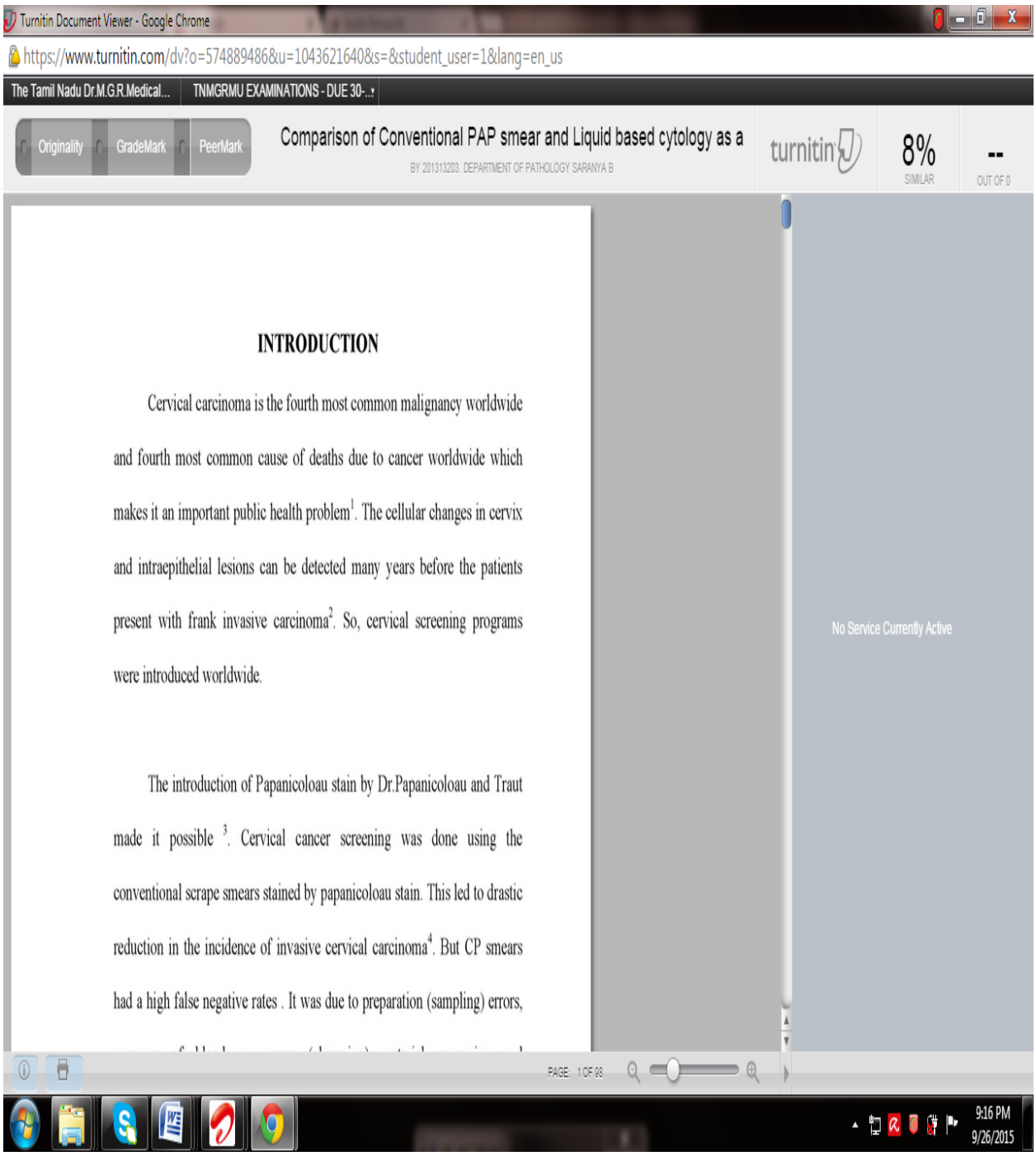
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INTRODUCTION
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The introduction of Papanicoloau stain by Dr.Papanicoloau and Traut made it possible [3]. Cervical cancer screening was done using the conventional scrape smears stained by papanicoloau stain. This led to drastic reduction in the incidence of invasive cervical carcinoma[4]. But CP smears had a high false negative rates . It was due to preparation (sampling) errors,
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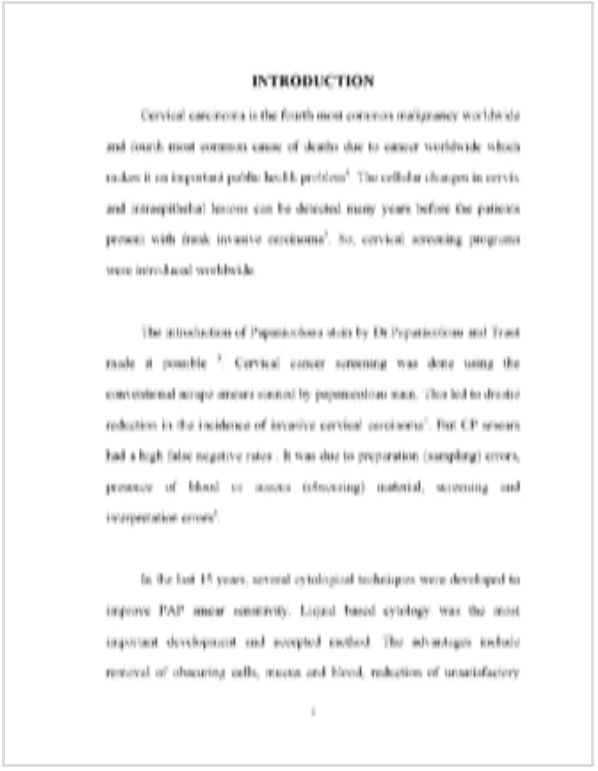

INTRODUCTION

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In the last 15 years, several cytological techniques were developed to improve PAP smear sensitivity. Liquid based cytology was the most important development and accepted method. The advantages include removal of obscuring cells, mucus and blood, reduction of unsatisfactory

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I would like to dedicate my work to my dear son and my husband who was with me throughout helping and extending their moral support.

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ABBREVIATIONS

CP – CONVENTIONAL PAP SMEAR

PAP – PAPANIOLOAU

HPV – HUMAN PAPILLOMA VIRUS

LBC – LIQUID BASED CYTOLOGY

VIA – VISUAL INSPECTION OF CERVIX WITH ACETIC ACID

VILI – VISUAL INSPECTION OF CERVIX WITH LUGOLS IODINE

WHO – WORLD HEALTH ORGANISATION

ICMR – INDIAN COUNCIL OF MEDICAL RESEARCH

CIN – CERVICAL INTRAEPITHELIAL NEOPLASIA

LSIL – LOW GRADE SQUAMOUS INTRAEPITHELIAL LESION

HSIL – HIGH GRADE SQUAMOUS INTRAEPITHELIAL LESION

LP – LIQUIPREP

N/C – NUCLEAR : CYTOPLASMIC

SIL – SQUAMOUS INTRAEPITHELIAL LESION

ASC – ATYPICAL SQUAMOUS CELLS

ASC – US – ATYPICAL SQUAMOUS CELLS OF UNDETERMINED SIGNIFICANCE

ASC – H – ATYPICAL SQUAMOUS CELLS - CANNOT EXCLUDE HSIL

SCC – SQUAMOUS CELL CARCINOMA

IUD – INTRAUTERINE DEVICE

HSV – HERPES SIMPLEX VIRUS

STD – SEXUALLY TRANSMITTED DISEASE

CIS – CARCINOMA IN SITU

AIS – ADENOCARCINOMA IN SITU

NOS – NOT OTHERWISE SPECIFIED

DES – DIETHYL SILBESTEROL

FIGO – FEDERATION INTERNATIONAL OF GYNAECOLOGY AND OBSTETRICS

SGO – SOCIETY OF GYNAEOLOGIC ONCOLOGISTS

CEA – CARCINO EMBRYONIC ANTIGEN

ER – ESTROGEN RECEPTOR

PAS – PERIODIC ACID SCHIFF

HPE – HISTOPATHOLOGICAL EXAMINATION

TP – TRUE POSITIVE

FP – FALSE POSITIVE

TN – TRUE NEGATIVE

FN – FALSE NEGATIVE

+ VE – POSITIVE

-VE – NEGATIVE

SCJ – SQUAMOCOLUMNAR JUNCTION

PSP – PILOT SCREENING PROJECT

OP NO – OUT PATIENT NUMBER

S NO – SERIAL NUMBER

CC – CHRONIC CERVICITIS

N - NORMAL

US – UNSATISFACTORY

ASC – US – ATYPICAL SQUAMOUS CELLS OF UNDETERMINED SIGNIFICANCE

ASC – H – ATYPICAL SQUAMOUS CELLS CANNOT EXCLUDE HIGH GRADE SQUAMOUS INTRAEPTHELIAL LESION

EMA – EPITHELIAL MEMBRANE ANTIGEN

ABSTRACT

Cervical carcinoma is the fourth most common malignancy worldwide and fourth most common cause of deaths due to cancer worldwide which makes it an important public health problem. The cellular changes in cervix and intraepithelial lesions can be detected many years before the patients present with frank invasive carcinoma. So, cervical screening programs were introduced worldwide. For many years , Conventional PAP smears were used for screening. Though it led to drastic reduction in number of cervical carcinoma cases, it had high false negativity. So, newer methods like Liquid based cytology were introduced. This study was undertaken to compare Liquid based cytology with Conventional PAP smear and to correlate the results with biopsy obtained from the same patient. This study was done on randomly selected 100 patients attending the Pilot screening project at Department of Obstetrics and Gynaecology , Thanjavur medical college , Thanjavur and their personal details like age , puberty age , number of children and their presenting complaints were obtained. The sample for Conventional PAP smear was taken using Ayre's spatula and slide prepared. Sample for Liquid based Cytology was taken using the Cytobrush and the sample was rinsed in the fixative provided by the manufacturer. The sample was then centrifuged and slide prepared. Both the slides were then stained using the Rapid PAP stain. Colposcopy was done

and biopsy was taken from the suspicious area which was then processed and stained by Haematoxylin and Eosin. The slides were analysed and the following results were obtained. Most of the patients who attended the screening program were in the fourth decade of life. Dysplasia was diagnosed in 26% of cases and most were in the age group of 21 – 40 years. Most of the cases were in the Socio economic Class II of the Modified Prasad's classification. Dysplasia was found more in the Socio economic class III(12% of cases). 90% of cases started sexual activity before 25 years of age and out of these 90 patients,92.3% had dysplasia. Dysplasia was more in patients with parity 3(14% of cases). 46% of cases presented with white discharge per vaginum. Cytological abnormality was found in 28 cases (28%) by LBC, whereas conventional Pap smear detected abnormality in only 22 cases (22%). 96 cases (96 %) were satisfactory for evaluation on LBC and 92 cases (92%) on conventional Pap smear. ASC was found in 12% of cases in Conventional PAP whereas it was detected in only 6% of cases in LBC. LSIL and HSIL was found in 8% and 2% of cases in conventional PAP smear whereas it was found in 12% and 8% of cases in LBC. No carcinoma was found in Conventional PAP smear whereas 2% of cases had carcinoma features in LBC. Sensitivity and specificity of PAP smear in detecting LSIL was 40% and 93% whereas for HSIL it is 50% and 100%. Sensitivity and specificity of LBC in detecting LSIL is 66% and 94% whereas for HSIL it was 100% and 96%. Overall sensitivity and specificity

for Conventional PAP smear is 55.5% and 83.7% whereas for LBC it is 83% and 86.5% respectively. Statistically, LBC and histopathology was highly correlated (r = 0.617) whereas only medium level of correlation was found for Conventional PAP smear (r = 0.4651).

So, Liquid based cytology is strongly advocated in the best interest of public health especially in countries like India where more number of people are in the lower socioeconomic status category, it improves the sample quality and reduces the likelihood of false negative results and hence improving the efficacy of the screening programs and thereby reducing the incidence of cervical cancer.

Key Words: Liquid based cytology, conventional Pap smear, cytology.

INTRODUCTION

Cervical carcinoma is the fourth most common malignancy worldwide and fourth most common cause of deaths due to cancer worldwide which makes it an important public health problem[1]. The cellular changes in cervix and intraepithelial lesions can be detected many years before the patients present with frank invasive carcinoma[2]. So, cervical screening programs were introduced worldwide.

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In the last 15 years, several cytological techniques were developed to improve PAP smear sensitivity. Liquid based cytology was the most important development and accepted method. The advantages include removal of obscuring cells, mucus and blood, reduction of unsatisfactory smears and inadequate smears, provision of cells for detection of HPV, presence of residual sample for performing ancillary techniques such as immunocytochemistry. LBC gives standardized slides

containing a monolayer of well stained well preserved cells which is easier to interpret than the conventional smears[7].

So , the aim of this study is to compare the results of conventional PAP smear and liquid based preparation and to correlate the results with the histopathological findings obtained from the biopsy.

AIM OF THE STUDY

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1. To study the Conventional PAP smears according to the Bethesda system of classification 2001

2. To study the Liquid based preparations according to the Bethesda system of classification 2001

3. To study the histopathological findings from the cervix biopsy of the same patient

4. To compare the results of Conventional pap smears and Liquid based preparations and to correlate with the histopathological findings of the cervix biopsy

MATERIALS AND METHODS

MATERIALS AND METHODS

This prospective study was conducted at the Department of Pathology,Thanjavur medical college,Thanjavur. In our study, we proposed to compare Conventional PAP with the new method Liquid based cytology and to compare the results with the histopathological examination of the biopsy from the same patient.

The study was conducted on 100 patients selected randomly from patients coming for pilot screening project to the department of Obstetrics and Gynaecology,Thanjavur medical college.

Exclusion criteria:

1. Non co-operative patients.
2. Patients who do not give consent.
3. Patients with massive bleeding per vaginum.
4. Pregnant women.
5. Treated cervical carcinoma cases.

After obtaining proper consent, proforma (APPENDIX I) was given to each patient and detailed history was obtained. After that, physical examination was done and the patient was put in lithotomy position for specimen collection.

For obtaining the specimens, first for Conventional PAP, Ayer’s spatula was inserted into the cervix and gently rotated at 360degree. Then, sample was smeared

onto a grease free slide and fixed in alcohol. After fixation, smear was stained with the PAP stain.

For Liquid based cytology, endocervical brush issued by the manufacturer was similarly inserted into the endocervical canal and rotated 360 degrees 3-4 times. Then, the brush is detached and placed into a vial containing fixative issued by the manufacturer for transport. The vial is closed and shaken to obtain a homogenous mixing. The vial is taken to the lab where it is again shaken with the vortex to obtain a homogenous mixture. After agitation, centrifugal chambers are prepared by placing the slide onto the support; the chamber is then placed onto the slide and tightened. Into the centrifugal chamber, 2ml of the separator solution given by the manufacturer and 5ml of the sample is placed and fixed into a rotor and then centrifuged at the rate of 2100rpm/min for 10minutes. After centrifugation, liquid is thrown into a container containing disinfectant. Some drops of alcohol (100%)are poured along the inner side of cytochamber. The chamber is then turned onto a absorbant paper and drained. Then all the parts are disassembled and slides are dried before staining.

After VIA/VILI,biopsy was taken from the doubtful areas.

FIG 1: Ayre's spatula for Conventional PAP smear.

FIG 2: Specimen brush with detachable head, prefilled vial with fixative liquid and slide for Liquid based cytology.

Method of staining:

PAP smear after fixation in alcohol and LBC smears are taken for staining with PAP stain(rapid) (APPENDIX II)

The biopsy specimen obtained was submitted intoto for routine histopathologic processing. The tissue sections were stained with Haematoxylin and Eosin (APPENDIX III).

The PAP smears and the LBC slides were examined and recent 2001 Bethesda system of classification (APPENDIX IV) were used for reporting.

Both the reports were correlated with the histopahological report of the biopsy which is considered the gold standard.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Normal anatomy: [8]

It is located in the uterus lower portion. It is cylindrical in shape and the length is 2.5 to 3cm and diameter is 2.5 to 3 cm . It consists of two portions:1. portio vaginalis 2. portio supravaginalis which is divided according to the vaginal reflection. Portio vaginalis outer portion is covered by ectocervix which is lined by stratified squamous epithelium and the endocervical canal is lined by mucin producing columnar epithelium. It has two openings external and internal os. Squamocolumnar junction is the point at which the ectocervical squamous epithelium and endocervical columnar epithelium joins. Transformation zone is the zone between the SCJ at puberty and SCJ after squamous metaplasia that occurs as the age advances.

Epithelium of the ectocervix:[8]

The lining epithelium is nonkeratinising stratified squamous epithelium. It consists of superficial, intermediate, parabasal and basal layers. The cells of the basal layer has a vertically oriented oval nuclei with a dense chromatin and scanty cytoplasm. The cells of this layer are inactive mitotically. Above this layer is the parabasal layer .They are larger with large amount of cytoplasm. Mitosis is seen in this layer and they express Ki-67. The next layer is the intermediate cell layer. These cells have vesicular nuclei and these have abundant and clear cytoplasm due

to glycogen accumulation. The superficial cells have round and small nuclei with a clear cytoplasm. There is abundant glycogen in these cells. During menstrual cycle, there are changes in the epithelium due to the influence of hormones. Superficial cells predominate when there is estrogen in the preovulation stage. In the post ovulatory phase, progesterone increases and hence there is predominance of intermediate cells.

Endocervical epithelium and endocervical glands:[8]

It is covered by a layer of columnar cells (mucin producing) .The nucleus is basally situated oval small nuclei with dense chromatin.

Epithelium of the transformation zone:[8]

Squamous epithelialisation and metaplasia are responsible for the endocervical epithelium transformation to squamous epithelium. In epithelialisation, mature squamous cells move below the endocervical epithelium and push the endocervical cells off the basement membrane and there is extension of these process to the clefts. Squamous metaplasia is the proliferation and differentiation of the endocervical reserve cells to the squamous cells. The reserve cells initially look like parabasal and basal cells. When these cells acquire cytoplasm,they are called as immature squamous metaplasia. When these cells acquire glycogen, they are called as mature

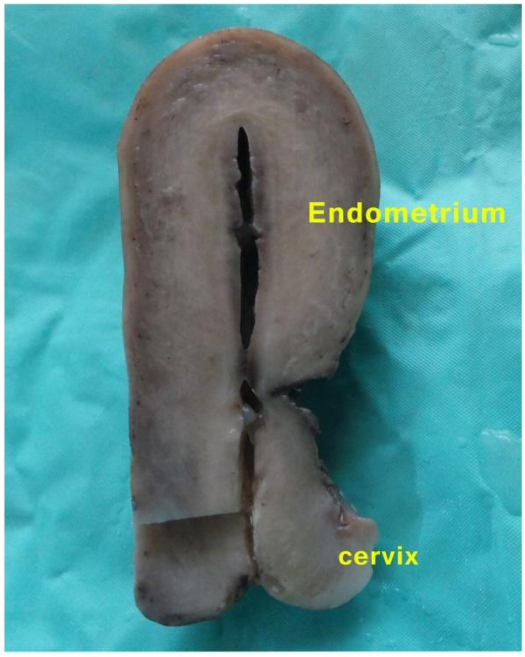


FIG 3: Cut surface of uterus showing endometrium and cervix.

squamous metaplasia. The nuclei of these cells are uniform, smooth contours and nuclear abnormalities are minimal.

Cervical stroma:[8]

It consists of a denser fibrous stroma admixed with 10 to 15% of the elastin and smooth muscle fibres. Many blood vessels and lymphocytes are seen.

Changes associated with pregnancy:[8]

Immature squamous metaplasia and decidual reaction can be seen in pregnancy.

Incidence:

Cervical cancer is the fourth most common cause of malignancy worldwide and it is also the fourth most common cause of cancer deaths according to latest cancer statistics issued by International agency for research on cancer (WHO). According to the report, 528000 new cases are identified every year and 266000 cancer deaths are due to cervical cancer. Every $1/5^{th}$ case of cancer is due to cervical cancer[1].

According to a review article on the magnitude of cancer cervix in India published by A.Nandakumar et. al, age associated incidence rates per 100000 women is 22.8% in 2004-05 and cancer cervix accounted for 16% of all cancer cases in the urban registries of ICMR. 90708 new cases are identified on an

average every year. In the hospital based registries cancer cervix is the leading cause of death in the urban and rural registries[9].

Etiology:

HPV continues to be the most common etiology for cervical cancers.HPV types most common are types 16,18,45,31,33,52,58 and 35[10]. High risk types are 16,18,31,33,35,39,45,51,52,56,58,59,68,73 and 82, low risk types are 6,11,40,42,43,44,54,61,70,72,81 and CP6108. HPV 16 and 18 are strongly associated with CIN III and invasive cervical cancer[11]. According to sherman et al, 93% of tumours expressed HPV DNA.HPV 16 was found in 50%,HPV 14 in 14%,HPV 45 in 8%,HPV 31 in 5% of specimens. HPV 16 predominated in squamous cell carcinomas(51% of such specimens),HPV 18 predominated in adenocarcinomas(56% of such specimens) and adenosquamous tumours(39% of such specimens)[12]. Multiparity and younger age of having first child is associated with cervical cancer. According to a study conducted by Louise et al,risk rose to 5.1 (95% confidence interval 2.7–9.7) for those with 14 or more pregnancies[13].Oral contraceptive usage had more incidence of invasive cancer than the the IUD's[14]. Cervical cancer is associated with long duration smoking[15]. According to latest WHO report, 70% of cases are in the areas of low developmental areas[1]. This difference is due to lack of access to effective screening and lack of facilities for

early detection and treatment. Genetic susceptibility for cervical cancer is related to HLA class II,HLA B7 and DQB1[16].

Pathogenesis:

Transformation zone recedes into the distal endocervical canal as the age advances[17].

SCC and adenocarcinoma of the cervix accounts for most common malignancies normally encountered in the cervix. In this, more than 70% of the cervical cancers are due to SCC which develops from the transformation zone [18,19].The second most common cause of cervical cancer is adenocarcinoma which arises from the endocervical cells[19].The other types of cancers arising in cervix are adenosquamous and other carcinomas or malignancies[19].

Precancerous lesion of cervix is known as CIN which is of 3 types.CIN 1(mild dysplasia), CIN 2(moderate dysplasia),CIN3(severe dysplasia),CIS and invasive carcinomas[20]. According to Bethesda system, CIN 1are LSIL and both CIN 2 and CIN 3 are combined and put into 1 category as HSIL[21,22].

The natural history of cervical cancer is that the precancerous lesions of cervix do not progress to invasive cervical cancers suddenly. According to Holowaty et al, risk of severe dysplasia developing from mild dysplasia was only 1% per year but moderate dysplasia progresses to severe dysplasia or worse was 16% within 2 years and 25% within 5 years. The risk of progression was more

within the first 2 years after a dysplastic smear[23].In another study conducted by Arends et al,approximate likelihood of regression is 60%,persistence is 30%,progression to CIN3 is 10% and progression to invasive cancer is 1%. Similarly,corresponding approximations for CIN 2 are 40%,40%,20% and 5 %. Likelihood of CIN3 going for regression is 33% and progression to invasive cancer is 12%[24].So,if the cervical cancer is identified at an early micro invasive cervical cancer stage and confirmed by biopsy, it can be treated early and it does not present with metastatic disease. Whereas if the patients come with later stages treatment becomes difficult and it has a poor outcome[24].

Prevention and control of cervical cancer:

Owing to the huge burden of cervical cancer, prevention and control of cervical cancer becomes important. For this, different methods have been developed for the early diagnosis and treatment of the cervical precancerous lesions which has led to drastic reduction of the disease burden.

The screening can be done by several methods including cervical cytology (Pap smear and newly developed Liquid based cytology) and VIA or VILI and HPV DNA detection. So, awareness should be brought for reducing the incidence of cervical cancer worldwide.

Screening: definition and principle

Screening is to identify specific diseases or a condition among asymptomatic individuals[25]. So, screening tests have to be applied on a larger population and hence should be convenient , inexpensive ,safe and painless[26]. But the main disadvantage of the screening tests are the higher margins of error than the diagnostic tests and lesser accuracy.

Table 1 demonstrates the relationship between screening test parameters and screened disease and formula for calculating parameters of screening test.[27,28]

Screening test	Disease present	Disease absent
Positive	True positive(A)	False positive(B)
Negative	False negative(C)	True negative(D)

Sensitivity=proportion of persons with the disease in whom the screening test is also positive(A/A+C)

Specificity=proportion of those without the disease in whom the screening test is also negative(D/D+C)

Positive predictive value=probability of disease in subjects with a positive test result(A/A+B)

Negative predictive value=probability of absence of the disease in subjects with a negative test result

Efficacy of the screening test means that the test should be able to diagnose a disease earlier that it would be without screening and with sufficient accuracy that the screening test will produce less false positive and false negative results.

Effectiveness of early detection by screening test means that the persons with disease who are diagnosed early should have a better clinical outcome than those persons who are diagnosed without screening[29]. Screening service offered should not do any harm such as wrong diagnosis, False positive screening test result ,treatment may do more harm than good, labeling people and false assurance due to false negative screening test result[30]. False positive screening test result may cause unnecessary anxiety among healthy people and these persons may be exposed to risks by exposing the screened persons for further examination[26,28]. The screening test has some psychological harms on the people such as anticipated discomfort or perception of adverse effects of the screening test, unpleasant interactions with health care workers, anxiety over the results of the screening test, implications of positive screening test and consequence of labeling the persons as sick[29].The screening test is valuable only if the death of the persons due to disease is postponed after early diagnosis. The objective of the screening programs is to

reduce moratality and morbidity and improving the quality of the persons in the population[31].

Andermann et al[32] described a criteria for the screening program, (APPENDIX V). UK national screening committee[33] also provided a screening program(APPENDIX VI)

According to an article presented in the American journal of clinical pathology on the screening guidelines for cervical cancer the following recommendations were proposed according to evidence based systematic review[34](APPENDIX VII).

Impact of screening on the morbidity and mortality of cervical cancers:

After the introduction of screening programs by 1950 and 1960's and cytological investigation through PAP smear, effectiveness of the screening program was evaluated through many studies and calculated the reduction in the mortality and morbidity from various cancer and mortality registries[35]. A study conducted in Nordic countries for time trends in mortality from cervical cancer and in relation to the organized screening programs in these countries since 1950s which showed a decrease in cumulative mortality between 1965 and 1982. In Iceland, the screening program targeted a wider age group, so the decline in mortality was about 80% which was the greatest among these 5 countries. Sweden and Finland also had nationwide screening programs. So ,the decline in mortality was 34% and 50% respectively. Only 40% of the population was covered by

screening programs in Denmark, hence the fall in mortality rate was only 25%. Even worse was Norway where only 5% of the population was covered by screening program, the fall in mortality was also10%. So this study concluded that organized screening programs had a greater impact on the mortality from cervical cancers[36]. In Denmark, a high decrease in mortality was observed between ages 30-59[37]. In Sweden cervical cancer mortality trends in relation to age, calendar period, degree of screening programs showed that there was 53% reduction in the mortality rates and this was attributable to screening[38]. Cohort studies were done to estimate the probability of cervical cancer among screened and unscreened women and the age adjusted relative risk in the unscreened and screened women was found to be 6[39].In Finland protective effect of the screening program was found to be 58% in a National health screening programme[40].After PAP smear, for shorter intervals the protection from the disease is stronger[41]. There are many hurdles for the implementation of the screening program .If the screening program is well planned and managed and properly monitored and evaluated, program can be much more effective[42].There was decrease in the cervical cancer incidence by 25% and mortality by 35% in India due to the screening program through VIA[43].

CERVIAL CANCER SCREENING METHODS:

Most commonly used screening method is through cervical cytology which is done in asymptomatic populations or in cervical cancer patients who come for follow up[44]. This is done using routine PAP test or more improvised LBC.

Conventional cervical cytology:

In this, endo and ectocervix samples are taken, smeared onto a glass slide, fixed and stained by PAP stain[45] which reduced the mortality due to cervical cancer by more than 50 to 70%[46] and a false negative rate of 55%[47]. Errors can be due to poor sampling, sampling may be nonrepresentative and collected sample may be inadequately transferred onto the slide. Various new collection devices have enabled sampling of large and representative samples[48]. Depolymerisation of the cervical mucus by chemicals produces monolayer sheets of cells.[49]

LBC:

It is used as an alternative method for CP for improving the utility of the cervical specimens and detection. In CP,where the device is discarded, a portion of the sample is lost and slide may contain mucus and blood [50,51]. In LBC, the sample is collected in the special cytobrush. Representative samples are collected since the tip of the brush is placed into the vial. After removing the blood, mucus and debris, homogenous mixture of the sample is placed on the slide to get a monolayer and

stained with PAP. Though the cost of LBC is more, it improves the quality of screening by detecting more epithelial abnormality[52,53].

There are two types : first and second generation

First generation Liquid based cytology:

In mid 1990's liquid based cytology was introduced and after that many different LBC techniques were in use worldwide. Of these, Thin prep and Sure path were FDA approved and they were also used for non gynaecologic cytology[54].

1. Thin prep method:

In this method, cervix brushes were used for the collection of the sample after which the brush is rinsed in a vial containing preservyt solution ,a methanol based preservative and fixative by pressing the brush in the bottom of the vial. Next,the specimen is processed in the Thinprep automated processor in which by mechanical agitation, mucus and lumps of cells are broken. Then, the preservative fluid passes through a filter which has many pores with size designed to trap epithelial cells and other contaminants pass through. The membrane filter with the epithelial cells are transferred to a glass slide which has a diameter of 20mm. Automated stainer the stains the slides. Thin,monolayer like preparation is made[55,56]. Studies done for evaluating the efficacy of Thin prep showed that more number of adequate smears are made and more number of squamous epithelial

lesions are diagnosed[57].Another study shows that more number of intraepithelial lesions are detected than conventional smears.[58]

2. Sure path method:

Density gradient is the principle in this method. A broom like device with a detachable head is used. Here the vial contains a ethanol based fixative. The clumps and mucus are broken through syringe aspiration. By using the density gradient centrifugation, inflammatory cells and red blood cells are separated from the epithelial cells. Cell pellet formed is resuspended and transferred to a slide with 13mm circular area[59]. A study done by B.Kirshner et al[60] showed that there was reduced unsatisfactory smears in this method and there was increased detection of atypical cells and malignancy. Another study by Maurice Fremont-Smith et al (2004)[61] showed that intraepithelial lesions were detected more in Surepath than the conventional smears.

Staining:

Thinprep uses an automated staining machine and the procedure is similar to conventional smears .In surepath, it is an integral process and cytoplasmic staining is different from that of the conventional smears.

Table 2 depicts the differences between thinprep and surepath methods

	THINPREP	SUREPATH
Device for collection	Cervex brush.brush rinsed in fixative	Broom like brush.head is detached into the vial.
Fixative	Preservecyt fluid-methanol based fixative	Cytorich fluid-ethanol based fixative
Vortex	Not present	Vortex mixed
Gradient centrifuge	Not done	Done
Sedimentation	Not done	Done
Filter	Used	Not used
Staining	Standard automated staining	Integral part of the procedure
Staining protocol	Similar to Conventional smears	Cytoplasmic staining is different
Smear area	20mm	13mm

The two LBC methods were compared in few studies. A study by Fang-Hui Zhao[62], showed that cervical cancers were equally detected in both the methods. But, unsatisfactory smears were less in Surepath due to better enrichment process which removes more mucus and blood than Thin prep. Surepath also provided more material for HPV DNA testing.

Advantages:

Representative cells are transferred to slide, unsatisfactory smears are reduced, ancillary studies can be done with the residual material, epithelial cells can be better evaluated since inflammatory component is greatly reduced, interpretation time is reduced because of the smaller area.

Disadvantages:

Cost of the procedure is more, requires special equipments, interpretation differs from that of the conventional smears.

Second generation liquid based cytology:

Because of the disadvantages of the first generation, second generation LBC was introduced. In this, most of the instruments were eliminated and hence the cost is less and is much simpler[63].

Liquid prep (LP) system:

It consists of preservative for the specimen , specimen cleaner ,cell base reagent.

Procedure for specimen collection and processing:

Mucus is removed using a cotton swab and cervical brush used is inserted into the cervical canal and is rotated 3-5 times in clockwise direction. The head of the brush is detached into a vial containing 5ml of alcohol based preservative. The

preservative and cervical brush with the specimen is mixed with the vortex and a homogenous mixture is made. The entire content of the fixative vial is poured into a tube containing 4ml of the cleaning solution which is then centrifuged at 1000g for 10mins.The work of the cleaning solution is to separate mucus and blood from the cells. After discarding the supernatant, to the cell pellet,cell base is added(4-5 times of cell button) and suspended by mixing with vortex.Then,50µl of the mixture is pippetted and then placed onto a slide in circular motion(15-17mm) which are then dried and stained with pap stain[63,64].

Studies have been done to show the effectiveness of this system. A study by Roghaei et al[65] showed that LP had 62.4% satisfactory smears compared to 31.9% in conventional smears. Another study by Hao deshou et al[64] showed that LP detects more number of intraepithelial lesions than the conventional smears. Study by Mahmood khaniki et at [66] showed that more adequate samples were found in liquiprep than CP. Liquiprep had higher sensitivity than CP. M Tunc Canda et al[67] showed that unsatisfactory smears were less in LP and atypical squamous cells. HSIL and LSIL were detected more in LP. Different LBC techniques were compared by Alves et al[68] which showed that, in all these methods, cellular structure were adequately preserved and one can select the method according to the choice and availability.

Advantages of LP method are :[69,63,67] clean background ,the cell structure is preserved better,screening time is decreased,cost is less than first generation LBC , HPV testing can be done using the residual sample.

Manual liquid based cytology:

In this, processing of smears are done using own laboratory prepared fixative and cell encapsulating polymers. Vortex and centrifuge are enough for this method[70,71,72,73,74]. Maskem et al[70] uses alcohol agar solution for this method. A blend of nutrient agar, wetting agent linear alcoholic alkoxylate, polyethylene glycol, alcohol is mixed together to form a monolayer sheet of cells. Unsatisfactory smears were only 0.2% and also there was an increased detection of lesions.

Assessment of liquid based cytology smears:

Here, fields to be screened are round shaped and much smaller and hence advantageous than conventional smears.

A. Adequacy criteria for liquid based preparation specimens:

There should be 5000 well visualized and well preserved squamous cells. Atleast 10 microscopic fields should be seen before reporting.[75]

The number of cells required per field = 5000 /(area of preparation/area of field).

10 well preserved endocervical cells indicate adequate transformation zone.[76]

B. Cell morphology: Slight difference in cell morphology is seen between Conventional PAP and LBC

1. Clean background is seen in LBC which enables to visualize the cells clearly.
2. Necrotic debris ,blood and fibrin are less in LBC due to special processing
3. Linging diathesis – fine granules of fibrin, blood and necrotic debris seem to hang like a wall paper on the cell and cell structure surface
4. Due to immediate fixation, nuclear enlargement is less in LBC. There is less naked nuclei.
5. Metaplastic squamous cells look like HSIL due to increased N:C ratio and rounding up of the nuclei. But this can be differentiated using N:C ratio being less than 50%, chromatin distribution being even and smooth nuclear contour.[77]
6. Endometrial cells are larger with more prominent nucleoli and the chromatin details are better seen in LBC. It is seen as single cells or in groups with bean shaped nuclei and intracytoplasmic vacuoles with a clean background.
7. Atypical squamous cells – cells favouring SIL but no definitive qualitative and quantitative features[78] .ASC should be diagnosed with 3 features: Squamous differentiation , N:C ratio is increased to about 2-3 times the size of the intermediate cells , Minimal hyperchromasia, nuclear irregularity and multinuclearity.
8. ASC-US cells appear similar in both the methods

9. In ASC-US Cells are larger and flatter in CS whereas the cells are small but the nucleus is 2 to 3 times the size of the neutrophil.
10. Intermediate and superficial cells are affected in LSIL. Immature cells are affected in HSIL. HSIL cells are smaller than LSIL.
11. Morphology of the cells should be considered for the diagnosis of SCC. LBC smears have lower cellularity.[79]
12. Invasive features and tumour diathesis are difficult to find in LBC[80]

Papanicolaou developed a class system for reporting cervical smears: class I ,absence of atypical or abnormal cells; class II, atypical cytology but no evidence of malignancy; class III, cytology suggestive but not conclusive of malignancy; class IV, cytology strongly suggestive of malignancy; class V, cytology conclusive for malignancy. Later it underwent many changes. In 1960 Dr Ralph richart proposed a new term cervical intraepithelial neoplasia which was graded from grades 1 to 3. This grading remained for two decades. In 1989 the Bethesda system was introduced to standardize the reporting of cervical cytology. This underwent many modifications and finally in 2001 Bethesda system(APPENDIX IV) recommends a specific format for the cytology report ,starting with an explicit statement on the adequacy of the specimen, followed by general categorization and an interpretation/result.[21]

THE BETHESDA SYSTEM:

Specimen adequacy:[21]

In 1988, three categories were proposed "satisfactory,less than optimal,unsatisfactory". The middle category was renamed as "satisfactory but limited by…". In 2001 the middle category was eliminated but this system advocates the mentioning of the presence or absence of the transformation zone and presence of obscuring elements can be mentioned. In 1988 and 1991, for reporting it as adequate smear, there should be an adequate squamous component.

Types of preparation and minimal number of cells: (according to 2001 Bethesda system)

LBC: 5000

CP: 8000-12000

There should be 10 well preserved endocervical or metaplastic squamous cells. If the specimen contains even one abnormal cell, the slide is termed satisfactory. If the specimen has more than 75% of the squamous cells obscured by white blood cells,blood,drying artifact it is termed as unsatisfactory. When 50% to 75% of the cells are obscured, it is termed as satisfactory but partially obscured. Only if all the nuclei are devoid of cytoplasm the specimen is termed as unsatisfactory.

Cytology of normal cervical cells:[81]

Superficial squamous cell: 16to 20µm2,occurs singly and loose clusters, polygonal , translucent thin eosinophilic cytoplasm with brownish keratohyaline granules ,centrally located pyknotic nucleus

Intermediate squamous cells: 35µm , oval or polygonal , basophilic or eosinophilic translucent cytoplasm which may show folding , vesicular with fine chromatin nulclei

Herxheimer spirals: Seen in superficial and intermediate squamous cells with a spindle shape or can have a cytoplasmic extension or tail containing different types of intracytoplasmic filaments.

Parabasal squamous cells: 50µm2 seen in post menopausal atrophy occurring singly , oval in shape , Cytoplasm which is opaque,basophilic

Endocervical glandular cells: columnar , Cytoplasm which is pale,abundant and mucinous , Nuclei which is basally situated,vesicular,chromatin which is granular and micronuclei seen and ciliated cells. In CP , cells are present singly or can occur as monolayered sheets which can give a honeycomb and picket fence appearance. In LBC , single cells are seen.

Metaplastic squamous cells: originating from the endocervical columnar epithelial reserve cells , 50µm2 , Occuring singly or pavement like sheets, polygonal or oval , Cytoplasm varying with cell maturation, Immature cells have thin and vacuolated,can have extensions in the cytoplasm where as Mature cells

have cytoplasm which is eosinophilic or basophilic,waxy and Nuclei which is vesicular with granular chromatin.

Endometrial cells: Exfoliated more in the first 10 to 12 days of the menstrual cycle an seen as different size cohesive clusters. Endometrial cells are small cells,cuboidal in shape,round nuclei with chromatin clumping with a nucleoli which is small. Superficial stromal cells are seen singly or sheets which are loosely cohesive and they look like histiocytes. Deep stromal cells are spindle shapes cells which occur in loose clusters. Wreaths or exodus are masses of endometrial cells and histiocytes.

During pregnancy(4^{th}/5^{th} month), there is increased(80%) intermediate squamous cells due to estrogen and progesterone secretion. The PAP smear may show decidual cells which are also seen in persons taking containing progesterone. The cells are seen as clusters which have a granular thick cytoplasm with a round or oval large nuclei without prominent nucleoli.

Arias stella cells – large with cytoplasm which is vacuolated and they have multiple nuclei which are hyperchromatic and prominent nucleoli. They look like clear cell adenocarcinoma cells.

Cockleburrs – hematoidin crystal arrays with histiocytes surrounding them. They are upto 100µm in size most often seen in pregnant women.

Postpartum period – basal cells predominate in these smears.

Menopause :

Early – superficial cells predominate(due to nonovulated graffian follicle development).Intermediate or parabasal cells predominate as the menopause progresses.

Atrophic vaginitis – parabasal cells are seen. They have enlarged nuclei which mimic dysplastic squamous cells.

Negative for intraepithelial lesion or malignancy: infections and nonneoplastic conditions:[81]

Bacterial infections:

Gonorrhea: Caused by Neisseria gonnorhoea presenting as purulent discharge from the vagina with a burning sensation seen in the cytoplasm of neutrophilis confirmed only by bacterial culture

Bacterial vaginosis: Called as "shift in flora" , presenting as cervico vaginitis with characteristic clue cells present in the filmy background of coccobacilli.Smears contain superficial and intermediate cells covered by coccobacilli.False clue cells are squamous cells which are covered by the bacillary organisms

Actinomycosis: Caused by actinomyces israelii which is a normal commensal of the female genital tract , present in patients with IUDs or pessaries, present as foul smelling discharge from the vagina which contains sulfur granules. Gupta bodies are irregular, thick clusters or bundles of filaments.

Granuloma inguinale: Scraping of the ulcerated lesion shows inflammatory exudates with vacuolated macrophages which contains Donovan bodies which has safety pin shaped organism demonstrated by Giemsa stain.

Chlamydia trachomatis: Second most common sexually transmitted disease , asymptomatic. Sites affected are cervix, uterus and adnexal structures. Sites unaffected are vulva or vagina. Invades columnar cells of the endocervical region which can spread to the fallopian tubes and the endometrium present in ntracytoplasmic vacuoles which contains coccoid bodies.50% of patients present with follicular cervicitis which contains lymphoid cells.Tingible body macrophages are seen. Final diagnosis can be confirmed by molecular testing.

Viral infections:

HPV infection: Most common belonging to Papova virus , sexually transmitted exclusively , seen mostly in young women.Precancerous lesion is called as CIN.It begins as CIN 1 which progresses to CIN 2 and 3 which are higher grades. In some persistent HPV infection, it begins as CIN 2 and 3. Average age for CIN is 30 years and invasive cancer is 45 years. HPV (low risk) types 6 and 11 are responsible for condylomas or genital warts , HPV (high risk) types 16 and 18 are associated with 70% of cervical cancers. Most of the sexually transmitted women contract some type of HPV in their lifetime. So testing for HPV in the routine screening is of limited use. Gardasil ,Cervarix are HPV vaccines.

Herpes simplex virus: Cervix and vagina are the most commonly infected sites caused by HSV 1 and 2 presenting as inflammatory epithelial ulcers. Characteristic feature is multinucleated giant cells which has nuclear moulding, intranuclear inclusions or has chromatic liquefaction and also has ground glass appearance mostly seen at the ulcer borders. Commercially available antibodies can be used for identification and confirmation

Cytomegalovirus: Endometrial and endocervical cells are affected with large amphophilic or eosinophilic intranuclear inclusions

Fungal infections:

Candidiasis: Normally found in the vagina and cervix mostly caused by candida albicans , 3 to 7 µm present as yeasts and pseudohyphal forms which are grey brown to eosinophilic and they are formed by elongated budding and they have constrictions along their length

Parasitic infection:

Trichomonas vaginalis: In the lower female genital tract it is the most common STD , Pus balls are found on the dense inflammatory exudates and they are collection Trichomonas vaginalis organisms and polymorphonuclear leukocytes. Size of the organism is 15 to 30 µm which are pear shaped, round or oval cyanophilic organisms, eccentrically located nucleus,vesicular and pale with

intracytoplasmic eosinophilic granules. Seen mostly in LBC. Most commonly associated infection is leptothrix with "spaghetti and meat balls" configuration.

Inflammation associated cellular changes: Changes in squamous cells are seen which have cytoplasmic vacuolization or perinuclear halos and nucleus which are hyperchromatic , enlarged which have regular contours and with fuzzy or clumped chromatin with polymorphonuclear leukocytes in the background.

Non neoplastic epithelial changes: The squamous epithelium of the cervix are under the influence of inflammation, hormonal effects and external physical irritation. So,these effects can be seen as hyperplasia, metaplasia and keratinisation.

Reserve cell hyperplasia: Between the basement membrane and columnar cells of the endocervical region there are cells called reserve cells which can differentiate into squamous or endocervical glandular cells proliferating due to chemical or physical irritation. They occur in clusters and very rarely singly. The reserve cells have bland oval nuclei and with a illdefined,scant and vacuolated cytoplasm

Squamous cell metaplasia: Reserve cells proliferate and transform into immature squamous cells which occur in sheets or as single cells and cytoplasm of these cells is pale and vacuolated. The nuclei of these cells are slightly hyperchromatic oval with a fine chromatin and Spider cells are the cytoplasmic extensions or tails of these immature cells which can then transform into mature cells.

Hyperkeratosis: This is a protective process for injuries seen in prolapse uterus seen as layers of anucleated squamous cells are seen in the smears

Parakeratosis: In this, pyknotic nuclei is retained in the keratinized squamous cells which are small round or spindle shaped , occur in loose clusters or as single cells . Pseudokeratotic cells are also squamous cells which have basophilic or eosinophilic cytoplasm which have oval nonpyknotic nuclei which have condylomatous lesions.

Tubuloendometrial metaplasia: (30% of women). Seen in the deeper clefts of the endocervical regions upper portion. Smears show cells which are ciliated and with a clear cytoplasm and abundant secretory cells,apical ciliae and intercalated cells which have a scanty cytoplasm and long thin nuclei(peg cells)

Urothelial metaplasia: Seen in atrophic squamous containing elderly patients. These cells have longitudinal grooves and bland oval nuclei

Reactive cellular changes due to inflammation and repair cells: These changes are benign seen in radiation, inflammation, IUD usage and many other nonspecific causes

Repair cells : Seen in inflammatory epithelial ulcers with history of previous biopsy, cryosurgery and cautery of the uterine cervix .They are of glandular or squamous in type. Nucleus is 1,1.5,2 times the normal intermediate cell nucleus . Double or multiple nucleus may be seen and they have smooth contours with mild

hyperchromatism and fine chromatin with single or multiple prominent nucleoli and cytoplasm of these cells have polychromasia,perinuclear halo and vacuolization without thick rim of cytoplasm. Spider cells are seen.

Vitamin B12 and folic acid deficiency induced changes: Squamous cells enlargement with enlargement of their nuclei which may be single or double nuclei which have fine chromatin and slight hyperchromatism. Pernicious anaemia have polymorphonuclear leukocytes have hypersegmented nuclei in the smear

Radiation and chemotherapy effects:

Radiation effects: Intracytoplasmic molecules ionization seen due to destruction of cellular enzymes and proteins and inhibition of DNA synthesis. Highly susceptible cells are the rapidly dividing cells .These changes can be divided into acute and chronic

Acute radiation effects:

Few days after the completion of treatment, these changes appear which persists for about 6 to 8 weeks and then radiation effects gradually subside. Squamous cells are affected more than the endocervical cells. The effects are seen as inflammatory exudates.Pus balls and repair cells are seen. Nucleus have pallor, vacuolization, wrinkling and chromatin smudging with multinucleated/binucleated hyperchromatic nuclei with single or multiple prominent nucleoli. Cytoplasm may

show hyalinization, vacuolization, polychromasia and intracytoplasmic aggregation of leukocytes.

Chronic radiation changes:

Appear late i.e after 6 months of completion of treatment but these changes remain for years. There is an atrophic pattern in the smear with intermediate and parabasal cells predominating in the smear and pleomorphic giant cells are seen.

Chemotherapeutic effects:

Alkylating agents are the most common offending drugs. DNA,RNA and proteins are altered by many different mechanisms. These changes are similar to those caused by radiation but they are systemic and they are less marked and there is less number of cells in PAP smear.
Benign appearing endometrial cells in women over 40 years of age(1% of smears):

In PAP smears these are seen as three dimensional small round clusters. In most of the patients these cells are from normal and cycling endometrium and in small number of cases, these cells are seen in cases with endometrial polyp and in patients using intrauterine devices and in those patients receiving hormone replacement therapy. Endometrial hyperplasia and carcinoma are seen in less than 1% of patients.

Glandular cells in post hysterectomy PAP smear:

The benign glandular cells are similar to the endocervical cells of the cervix and seen in patients after total hysterectomy and after postoperative radiotherapy. Squamous epithelium of the vagina undergoes glandular or mucinous metaplasia and these cells are the glandular cells seen in PAP smear.

Abnormal shedding of normal appearing endometrial cells:

Endometrial cells appear in small clusters or groups which have a small oval or round bland nucleus and with scant cytoplasm.

In the premenopausal women,only upto 10 to 12 days of the menstrual cycle the endometrial cells appear in the smear.The causes are patients with IUD ,anovulatory cycle, endometritis, prior uterine endoscopy or endometrial curettage, hormonal therapy, endometrial polyp, submucosal fibroid, hyperplasia of the endometrium or endometrial carcinoma rarely.

In post menopausal women,the most important cause for this abnormal shedding is hormonal replacement therapy. Second important cause is endometrial polyp and others being endometrial hyperplasia and carcinoma.

Vaginal endometriosis:

In smears ,endometrial epithelial fragments,endometrial stromal cell clusters, degenerated erythrocytes and hemosiderin laden macrophages are seen.

Vaginal adenosis:

Presence of tuboendometrial epithelium or endocervical glandular epithelium in the vagina. Upper one third of the anterior vaginal wall is the most commonly affected . DES exposure in utero is considered to be an important contributing factor. In the smears, benign endocervical glandular cells can occur in loose clusters, monolayered sheets or singly. There may also be metaplastic squamous cells in the smears.

IUD induced cellular changes:

In IUD users there are atypical cellular changes involving the metaplastic squamous cells of the cervix and endometrial glandular cells. In smears, regenerative and reactive endometrial cells seen in clusters can be seen due to mechanical effects. There is enlargement of the cytoplasm in these cells. The cytoplasm show vacuolization, prominent nucleoli which mimic malignant cells of the glandular epithelium. The metaplastic squamous cells of the cervix show nucleoli which is prominent. The endometrial cells may mimic HSIL/CIN 3 and hence they show high N/C ratio, irregular nuclear contours or membrane with nuclear hyperchromatism.

Other cytologic findings:

Cornflakes – This is a brown artifact and is caused due to xylene deposition before application of the coverslip and there is air deposition in the superficial squamous cells.

Blue blobs – It is condensed mucus, degenerated bare nuclei and haematoxylin precipitation. It represents degenerating intermediate/parabasal cells in post menopausal women. They appear in the smear as oval to round dark blue amorphous masses.

Psammoma bodies – They are calcified laminated round bodies which are rarely seen in conventional PAP smears.

Carpet beetle part – It is a contaminant from tampon or cotton application

Curshmann spirals – They are mucous threads which are inspissated within cervical glands or clefts. It has no significance.

Squamous cell abnormalities:[81]

Squamous intraepithelial lesions:(SIL)

These lesions are considered to be the precursor lesions for squamous cell carcinoma and these lesions are predominantly seen in the reproductive age group women. There are 3 grades in CIN lesions with mild dysplasia in grade 1 and moderate and severe dysplasia is grade 2 and 3 respectively. Flat condyloma and CIN 1 is equal to LSIL and CIN 2 and 3 or CIS is equal to HSIL.

LSIL:

It includes flat condyloma,mild dysplasia and CIN 1. The most important cause is infection with low risk and high risk HPV. In PAP smears, it is found only

in 2% of cases. Majority of cases with LSIL in PAP smear have LSIL in cervical biopsy but in 18% of the cases have HSIL in cervical biopsy.

LSIL/flat condyloma:

Koilocytes are present with dyskaryotic nuclei and they are present in sheets or singly. Koilocytes are intermediate and superficial squamous cells which have multiple or single hyperchromatic and enlarged nuclei with smudged or granular chromatin and their nuclear membrane is irregular. There is a perinuclear halo which is a clear space surrounded by thick well defined rim of cytoplasm. This halo is caused by perinuclear cytoplasmic microorganelles degeneration which is caused by HPV infection.

LSIL/mild dysplasia/CIN 1:

In the smears , there are intermediate and superficial squamous cells which have hyperchromatic and enlarged nuclei and they have irregular nuclear membrane. koilocytes are common and absent nucleoli.

HSIL:

In this category, moderate dysplasia,severe dysplasia,CIS,CIN 2 and 3 are included. It is present in about 0.5% in PAP smears. Many of them almost 97% are positive for high risk HPV. HSIL lesions if not treated may develop into invasive carcinomas.

CIN II:

These are parabasal cells which are seen in sheets or singly. These have well defined, thick cytoplasm and their nucleus have hyperchromatic and enlarged nucleus with irregular or smooth nuclear membranes. The chromatin is fine or coarsely granular and it is evenly distributed. Koilocytic changes are not that prominent and they have absent nucleoli.

CIN III:

In this category there are 3 histologic patterns: Large cell non keratinizing ,Keratinizing , Small cell patterns

Non keratinizing CIN3:

The cells which are exfoliated are large and show pleomorphism. They have abundant,ill defined or well defined cytoplasm. The nucleus is irregular and are enlarged and hyperchromatic. The nucleus have a finely or coarsely granular chromatin and this chromatin is evenly distributed. These cells are exfoliated in syncitial clusters.fragments of epithelium is also seen. They do not have nucleoli and the necrotic debri is not seen in the smear background. The smear is composed fully of spindle shaped cells very rarely.

Keratinizing CIN3:

The smear is composed of spindle shaped cells. These cells have thick,well defined orangeophilic cytoplasm with nucleus showing hyperchromatism. Mostly they are seen singly and rarely clusters of cells are seen in the smear

Small cell CIN 3:

The cells exfoliated in this type have hyperchromatic nucleus and illdefined scant cytoplasm. They are exfoliated in loose clusters or singly. They may or may not have nuclear moulding.

Atypical squamous cells:

This is seen in about less than 5% in PAP smears.ASC/SIL ratio is 3:1.in about 10 to 20% of cases,patients who are diagnosed as having ASC have CIN in colposcopy directed biopsy. This includes 2 categories: ASC – US and ASC – H.

ASC-US:

They are intermediate or superficial in type. The size of the nucleus is 2.5 to 3 times larger than that of the 35μm^2 size of the intermediate cells of the squamous epithelium. They are bi-nucleated or multinucleated with enlargement and N/C ratio is slightly increased. They have irregular chromatin and slight hyperchromatism is seen in the nucleus. The nuclear contours are regular. They have keratinized orangeophilic or eosinophilic dense cytoplasm and perinuclear halo may be seen. ASC-US changes may also be seen in conditions such as atypical parakeratosis,atrophic vaginitis and atypical repair.

ASC-H: (5 -10 % of cases)

They are the metaplastic squamous cells which show nuclear atypia but they do not fit into the category of HSIL. These cells are found in small numbers. They

are seen in small groups or singly. They can be seen in epithelial fragments and less than 10 cells are seen.The cells are polygonal in shape,with squamous metaplastic cells size and cytoplasm which is dense. The nuclear size is 1.5 to 2.5 times the squamous metaplastic cell nuclear size which have irregular nuclear membrane and chromatin which are irregular and no nucleoli. These cells have increased N/C ratio and is equal that of that of HSIL. There is nuclear polarity loss in those cells occurring in epithelial fragments or crowded sheets. The smears have vague palisading in the peripheral cells which may mimic cervical AIS.

Cervical invasive SCC: (60 – 80 % of cervical malignancies)

5^{th} to 6^{th} decade of patients are affected presenting mostly with post coital bleeding.

They are classified into 2 types: Keratinizing and Non keratinizing SCC .

These are single cells with cytoplasm which is keratinized. The cells with non keratinized cytoplasm are seen in smaller aggregates and also in clusters which have prominent nucleoli. Necrotic debris due to tumour diathesis is prominent in conventional PAP smears. The necrotic debris is minimal in liquid based cytological preparations and is collected at the periphery of the tumour cells known as clinging diathesis.

Cervical microinvasive SCC:

These are single cells with or without keratinisation.These cells occur in syncitia,loose clusters, hyperchromatic groups or tissue fragments. The nucleus of these cells have nuclear clumping and irregular distribution of chromatin having macro or micro nucleoli.

Other rare variants of cervical SCC are Verrucuous carcinoma , Papillary squamous (transitional) cell carcinoma , Lymphoepithelioma like carcinoma , Sarcomatous squamous carcinoma.

Glandular cell abnormalities:

Multipotential reserve cells in the subcolumnar region is responsible for this lesion.The most common one is adenocarcinoma of the endocervical type.

Cervical AIS:

It is common in women in their 3 rd or 4^{th} decade. Transformation zone is the most commonly involved area in majority of cases. There are 3 types: Endocervical , Endometriod , Intestinal. In this,endocervical type is the most common classified into well and poorly differentiated.

Well differentiated-endocervical type:

The glandular epithelial cells which are malignant occur in large sheets. The columnar cells are crowded with stratification of nuclei which is more prominent at the edges of the large sheets.The cytoplasm of the short strips of tumour cells extend off the tumour cell sheet edges known as feathering which have nuclear

palisading. Tumour cell rosettes are seen. The tumour cells are about two to three times that of normal endocervical glandular cells Tthe nucleus of these cells are hyperchromatic, enlarged and the nuclear chromatin is coarsely or finely granular with absent nucleoli in 50% of cases.

AIS - intestinal variant: Most commonly seen in association with endocervical type. The cells are large and they occur singly, in large epithelial fragments or in clusters. Intracytoplasmic vacuoles are seen. The vacuoles contain mucin and these cells resemble epithelial cells of the colon

Atypical glandular cells:

In this category are cells which are inbetween benign reactive changes and AIS and adenocarcinoma. It is divided into 2 types: Atypical glandular cells, NOS type and Atypical glandular cells,favour neoplastic . It is again divided into two types: endocervical and endometrial types

Atypical endocervical cells,NOS type: These cells occur in strips or sheets with some crowding of cells. The smears have enlarged nucleus and is about 3 to 5 times that of nucleus of endocervical cells. It has well defined distinct cytoplasm and it is abundant. N/C ratio is increased and hyperchromatic nuclei.

Atypical endocervical cells,favour neoplastic:

These include cells that do not fit into criteria of invasive or AIS but fall short in some features. The cells are seen in clusters,sheets and strips with nuclear

overlap and crowding . Nucleus are enlarged and hyperchromatic. N/C ratio is increased. The cell borders are illdefined with scanty cytoplasm.

Atypical endometrial glandular cells: Single or rounded clusters of small glandular cells are seen with cytoplasm is scant or moderate and some cells have cytoplasm which is vacuolated . Nucleus has irregular membrane and hyperchromatic and enlarged and nucleoli is prominent. Chronic endometritis,polyp of the endometrium,IUD,endometrial hyperplasia are the causes. During menstruation, reactive changes may be seen in normal endometrium with enlargement of nucleus and pleomorphism. About 50% of women in the postmenopausal age group with atypical endometrial cells have endometrial pathology which include atypia or hyperplasia. Tumour diathesis or high maturation index of squamous cells also indicates malignancy.

Invasive cervical adenocarcinoma:

It is most common in 5^{th} decade of life and 25% of all cases are adenocarcinoma. ovarian mucinous or endometriod carcinoma are associated with these lesions.HPV 16 and 18,P16,ER and PR are positive in majority of adenocarcinomas. The most common type is endocervical mucinous carcinomas. The second common type is carcinoma of endometriod type. Very rare types are the signet ring adenocarcinoma and intestinal adenocarcinoma.

Well differentiated cervical adenocarcinoma:

It has features similar to that of the AIS endocervical type.

Moderately differentiated cervical invasive adenocarcinoma:

In this category,large number of malignant cells are seen and they are seen as acini and they form papillary clusters,sheets,balls,rosettes,syncytia or strips. The tumour cells are dyscohesive with oval hyperhromatic pleomorphic nuclei.The nuclear chromatin is fine or coarsely granular with single or multiple macronucleoli. The cytoplasm is ill defined and sometimes vacuolated. In about 30% of cases,tumour diathesis may be present.

Poorly differentiated cervical adenocarcinoma:

Clusters or single malignant glandular cells may be seen in the smear. The tumour cells have cytoplasm which is illdefined showing nuclear pleomorphism and nucleoli which is prominent.

Cervical adenocarcinoma variants:

Glassy cell carcinoma: It is an adenosquamous carcinoma which is poorly differentiated and is present in less than 1 to 2% of the cervical smears. It is seen more commonly in younger patients (mean age 41). It is positive for HPV 16 and 18.The patients present with a exophytic bulky mass. The polygonal tumour cells occur in masses and nests. The cells have the characteristic glassy cytoplasm with an oval nuclei and nucleoli which is prominent. Peripheral blood shows prominent eosinophilia. In PAP smears, large malignant cells occur as single cells or in

clusters. They have oval nuclei and nucleoli which is prominent similar to squamous cell carcinoma-non keratinizing type. The ground glass cytoplasm may be found in some cells of the smear.

Adenoid cystic carcinoma: (1% of adenocarcinoma patients)

It is more common in elderly patients presenting with a friable polypoidal structure. The tumour cells are small and they occur in clusters,trabeculae and cords which have lumens filled with eosinophilic hyaline material. It may be admixed with SCC of cervix. It is positive for HPV 16 and it is associated with poor prognosis. In PAP smears, smaller cells with scanty cytoplasm and the nucleus is small,hyperchromatic and oval.basophilic globules can be seen.

Minimal deviation adenocarcinoma: There are 3 types:

Cervical minimal deviation adenocarcinoma,mucinous type.

It is the most common of the 3 types. It is seen in younger women(32 to 42 years). Mostly it is sporadic or sometimes it is preceded by an ovarian mucinous tumour with HPV negativity . They are diagnosed at a higher stage and hence associated with poor prognosis. An area of poorly or moderately differentiated adenocarcinoma may be present adjacent to the tumour. In PAP smear,glandular cells occur in clusters or sheets,the nucleus is monomorphic and a nucleoli which is small and a clear cytoplasm. Cytoplasm may have tails or extensions.

Mucinous adenocarcinoma,endometriod type:

These occur in sheets with oval nuclei which is of low grade and there is palisading at the free borders. The tumour cells form cell stripes have pseudostratified nuclei and form rosettes.

Mucinous adenocarcinoma-nonspecific type: Not clearly known manifestations.

Clear cell carcinoma: (4% of adenocarcinoma)

It occurs in older patients but with DES exposure vaginal adenosis develops. The patients present with a nodular or ulcerated lesion. There are microcystic, tubular,solid and papillary patterns. In PAP smears, epithelial cells are seen in irregular clusters or sheets.The tumour cells have a granular eosinophilic to clear cytoplasm and hobnail configuration is seen. They have oval nuclei and nucleoli which is prominent.

Villoglandular carcinoma:

Papillae covered by epithelium is seen with central fibrovascular core. In PAP smears, malignant epithelial cells occur in monolayered sheets with nuclear crowding and they show folding. They have oval nucleus with hyperchromatism and nucleoli is inconspicuous.

Papillary serous carcinoma : (1% of carcinoma) : It is an aggressive tumour with metastasis to pelvic and periaortic lymphnode groups occur early. HPV positive cases are seen in young women and older patients are HPV negative. In PAP smears,malignant glandular cells are seen in three dimensional clusters or

monolayered sheets. Papillary structures are seen with a thin fibrovascular core. Malignant epithelial cells cover the papillae.

HISTOPATHOLOGY: [8]

Squamous lesions of the uterine cervix:

Basal cell hyperplasia: There is increased thickness of the parabasal and basal layers. There is loss of picket fence appearance with nuclei that are vertically oriented,oval and enlarged with normal squamous cell maturation.

Hyperkeratosis and parakeratosis:

Chronic irritation causes these changes which can be focal or diffuse. Increase in the thickness of the keratin layer is called as hyperkeratosis. Retention of the nuclei in the superficial layers is called as parakeratosis. The epithelium has rete edges elongation with acanthosis.

Atrophy:

It occurs due to hormonal stimulation,there is altered maturation of the squamous epithelium which manifests as:

1. Typical atrophy – thin epithelium,total maturation loss with parabasal and basal cells, N/C ratio is increased

2. Atrophy with partial maturation – the cells have glycogen,enlarged nuclei and mitosis

3. pseudokoilocytosis – these cells contain cytoplasmic halos but these cells unlike koilocytic cells have uniform appearance. Binucleation can be seen with hyperchromatic nuclei and size variation.

HSV cervicitis:

The biopsy should be taken during infection. There is basal layer nuclei homogenisatiom and cytoplasm/nuclear vacuolization in the initial phases. Later,intermediate layer is also affected which finally forms bullae which rupture and there is formation of ulcers. There is epithelial cell necrosis. The cells which are infected have a multiple/single nuclei and ground glass chromatin.

Reactive and reparative squamous atypia:

These changes are seen with infections such as Chlamydia,trichomonas and candida. The changes are enlargement of nuclei,hyperchromatic/vesicular nuclei,spongiosis,variation in shape and size of nuclei. Binucleation and halos in the cytoplasm are seen. Ulceration,post erosion,post biopsy leads to reparative changes. The changes are atypical nuclei and hence should be differentiated from dysplasia. This can be differentiated using Ki 67 and P16.

Radiation induced atypia:

The changes occur some years after exposure. Squamous epithelium shows cytomegaly and due to swelling of the nucleus and cytoplasm. The cytoplasm shows vacuolization,erosion and nucleoli which is prominent. The stroma shows

necrosis and edema with leukocyte infiltration. The epithelium becomes atrophic and hyalinised collagen and necrotic foci are seen in the stroma by sixth week. Reactive fibroblasts,endothelial cell enlargement, blood vessel dilatation which is followed by thickening of intima,thrombosis with obliteration of lumen.

Koilocytosis(LSIL , condyloma): There is three fold variation in the size of the nuclei with raisinoid nuclei,nuclear membrane irregularity , darker chromatin . Halos/peri nuclear cytoplasmic clearing are seen and nuclear spacing is uneven.

CIN 1(mild squamous dysplasia/LSIL):

In this lesion,the lower one third of the epithelium of the cervix is affected. Picket fence appearance of the basal cells is lost. The atypia is mild to moderate which includes enlargement of the nuclei and size and shape of nucleus variation. Chromatin pattern alteration and mitosis are seen. The superficial and intermediate cell layers show koilocytosis.

CIN 2(moderate squamous dyaplasia/HSIL):

In this category,the mild to moderate atypia changes are seen in the lower two thirds of the epithelium. If there is marked atypia in the lower thirds of the epithelium,it is also included in this category.

CIN 3(severe squamous dysplasia/squamous CIS/HSIL):

In this category,there is marked to moderate atypia which is seen involving full thickness of the epithelium. Distinction between severe squamous dysplasia and in situ lesion, flattened superficial layer is seen in in situ lesions.

Microinvasive squamous carcinoma: (superficially invasive squamous carcinoma/squamous carcinoma with superficial invasion)

Different staging system (society of gynaecologic oncologists and FIGO) is used for microinvasive carcinoma. FIGO staging system gives the definition as follows:

Stage IA: invasive carcinoma diagnosed by microscopic examination, macroscopically visible lesions even with superficial invasion is stage IB

Stage IA1:<3mm of stromal invasion in depth and horizontal spread of <7mm

Stage IA2: >3mm but <5mm of stromal invasion and horizontal spread of <7mm

Venous,lymphatic and vascular involvement do not change the classification.

SGO definition is as follows: “A microinvasive lesion should be defined as one in which neoplastic epithelium invades the stroma in one or more places to a depth <3mm below the basement membrane of the epithelium and in which lymphatic and blood vascular involvement is not demonstrated”

Features:

There are neoplastic epithelium tongues which penetrates through the basement membrane of the epithelium. The cells near the focus of invasion is differentiated better than the other places(in situ/high grade dyaplasia). The

eosinophilic cytoplasm is increased,polarity loss,keratin production occasionally. There is ragged border at the invasive foci.There is scalloping at the epithelial and stromal junction. The stromal reaction is desmoplastic reaction and lymphoplasmacytic infiltrate. Microinvasive carcinoma occurs when the surface epithelium shows high grade SIL changes , when the glands show necrosis within the lumen , when there is aberrant differentiation to squamous epithelium within the epithelium.The measurement of invasion is made at the point of origin in the basement membrane to the deepest part of the invasion.

Recognition of vascular and lymphatic invasion:

The epithelium which is neoplastic is present within the lymphatic/vascular spaces. Invasive squamous carcinoma,usual type: Irregular/ragged malignant nests of cells with eosinophilic cytoplasm in the stroma showing desmoplasia with epithelium reduplication extending below the endocervical glands. There is loss of polarity of the epithelial lining with pseudogland formation within the tumour cell nests with necrosis and debri of keratinous material. Tumour is present adjacent to the vessels.

There are 3 grades according to the degree of differentiation:

Poorly differentiated, moderately differentiated and well differentiated

Unusual variants of squamous carcinoma:

Verrucuous carcinoma: The surface has a broad papillae and invasion has a pushing pattern with bulbous nests. The cells have no atypia and the cytoplasm is abundant.

Squamous carcinomas with a papillary pattern: The cells have a basaloid/squamoid which looks like SIL cells or transitional cells with oval nucleus perpendicular to the papillae. These cases are associated with recurrences.

Basaloid squamous carcinoma: There are epithelial cell islands which are mitotically active and they have nuclear hyperchromatism and peripheral palisading associated with poor prognosis

Lymphoepithelioma like carcinoma: Undifferentiated cells are present in trabeculae or nests with an inflammatory infiltrate. The cells are polygonal or larger with eosinophilic cytoplasm,vesicular nuclei and nucleoli which is prominent. Lymphocytes,eosinophils and plasma cells are present. These cases have a good prognosis.

Spindle cell carcinoma (sarcomatoid carcinoma) : This aggressive tumour have spindle cells. Osteoclastic type of giant cells are seen. The spindle cells express vimentin and keratin focally and EMA.

Glandular lesions of the uterine cervix:

Benign lesions that mimic adenocarcinoma in situ:

Reactive atypia: These cells have enlargement of nuclei,nuclear size variation,nuclear membrane irregularity and psuedostratification of nuclei.

Atypical oxyphilic metaplasia: These cells are cuboidal to polygonal cells with eosinophilic cytoplasm and nuclear hyperchromatism and multinucleation. Cytoplasmic vacuolization,apical snouts are seen. The glands are dilated lined by a single layer of cells with tufting occasionally.

Endometriosis: There are two forms: deep and superficial. Deep lesion is associated with pelvic endometriosis. Superficial lesion is associated with trauma to the cervix. Macroscopically, it presents as friable red patches, blebs filled with blood and measures upto 2cm. The entire epithelium is sometimes replaced with small or medium sized oval or round glands. The glandular epithelium is columnar with pesudostratification or single row. It has an elongated or round nuclei and even distribution of chromatin. Hyperplastic or secreatory changes may be seen. There is inflammation, fragmentation and haemorrhage. Bcl 2 staining is useful for diagnosis.

Intestinal metaplasia: This is a rare metaplasia with globlet cells.This metaplasia is associated with in situ or invasive lesions. Atypia and mitosis not seen.

Benign lesions that mimic invasive adenocarcinoma:

Microglandular hyperplasia:

Reproductive age group women are affected more presenting with polyps,friable masses or erosions. These lesions can be localized or multifocal which can involve the endocervical glands or the surface epithelium. It is composed of variably sized and shaped closely placed glands with little stroma in between and inflammation. The glands are lined by mucin producing columnar epithelium with vacuoles present sub or supranuclearly with uniform nuclei. Squamous metaplasia and hyperplasia of the reserve cells can be present. It can have reticular or pseudoinfiltrative pattern and signet ring or hobnail cells or hyalinised stroma.

Lobular endocervical glandular hyperplasia:

Average age of presentation is 45 years. The patients present with discharge,abdominal discomfort or polypoidal mass. There is variably sized round glands proliferation in lobules, small cystic lined by mucin producing columnar cells with a basally situated bland nuclei. Atypia may be seen in some glands with irregular contour. Mitosis may be seen. The stroma is hypercellular with inflammatory cells.There is no invasion.

Diffuse laminar endocervical glandular hyperplasia:

Mostly seen in premenopausal patients presenting with mucoid/watery discharge. It consists of medium sized abnormally or round shaped glands

detached from the stroma. Columnar cells which are mucin producing lines the glands. Reactive changes may be seen with or without nflammation.

Endocervical gland hyperplasia,NOS: Endocervical glands increased.

Benign lesions mimicking either insitu or invasive adenocarcinoma:

Tubal,endometriod and tuboendometriod metaplasia: Tubal metaplasia is the replacement of the epithelium of the endocervix with ciliated,intercalated cells. It usually presents as a polyp or a spongy mucoid material. The glands are medium sized with variation in shape and size with bland cells and atypia seen. The stroma is hypercellular,myxoid,edematous with focal calcifications.In endometriod metaplasia,there is replacement of the epithelium with columnar cells.

Arias stella reaction: It is more commonly associated with pregnancy and rarely in patients who are consuming oral contraceptives. It involves superficial and deep glands either focally/extensively (confluent). The cells show enlargement, cytoplasmic vacuolization, eosiniphilia, irregular nuclei, hob nailing. Marked proliferation of the epithelial cells leads to cribriform and solid pattern and papillae with tufting.

Radiation induced atypia of endocervical epithelium: The changes can be acute or chronic. Acutely induced changes are immediately apparent which shows epithelial cells swelling and vacuolization, necrosis and edema of the stroma with vessel dilatation and infiltration by inflammatory cells. Chronic changes appear

late and show the following features – endocervical glands are decreased in number with shape and size variation. The cell shape can vary from flat to columnar with enlargement seen in the nucleus and cells.Vacuolisation and dense eosinophilia seen in the cytoplasm,nuclear membrane is slightly irregular or smooth with chromatin can be smudged,dense,vesicular or finely granular . Intranuclear eosinophilic inclusions/multi nucleated cells can be rarely seen. The stromal cells may show the following changes – edema, hyalinization,fibrosis,fibroblasts which are bizarre, myxoid areas, calcifications and inflammation. Blood vessels changes include hyalinised intima , sclerosis, endothelial cell atypia. There is focal expression of CEA.

AIS and glandular dysplasia:

In situ lesions have enlargement of nucleus, stratification, nucleoli(small), chromatin which is coarse with increased mitotic activity. Apoptotic bodies are seen. It first involves the hyperplastic/distended glands. This lesion involves both the endocervical glands and the surface epithelium of the transformation zone. This lesion can be seen focally or multifocally with glands showing cribriform pattern or tufting. There are various subtypes: clear cell , adenosquamous , endometriod , tubal , intestinal , endocervical(most common). The endocervical type has decreased mucin and moderate cytoplasm.The globlet cells are seen in intestinal type and vacuolated cytoplasm. The glands resemble endometrial glands with

pseudostratification in the endometriod type. A mixture of intercalated cells,non ciliated and ciliated cells are seen with pseudostratification,enlargement of nuclei and with mitotic figures. In the adenosquamous type,there is mixture of glandular and squamous cells or with cellular features intermediate inbetween. There is cuboidal,polyhedral or flattened cells with eosinophilic to clear cytoplasm.

Glandular dysplasia is defined as cells having nuclear features inbetween atypia in the glandular cells and AIS.

Superficially invasive adenocarcinoma(microinvasive adenocarcinoma):

In this, there is invasion into the stroma at more than one place to <3mm below the basement membrane without lymphatic/vascular involvement according to SGO. According to FIGO, two categories are described – stage IA1 is when there is <3mm depth and <7mm width stromal invasion , stage IA2 is when there is >3mm depth and <5mm and >7mm stromal invasion.

Invasive adenocarcinoma,common and uncommon types:

Adenocarcinoma, usual endocervical type: (80% of adenocarcinoma)

They are moderately differentiated and they contain eosinophilic cytoplasm,apoptosis and mitosis.

Villoglandular adenocarcinoma:

It is seen in premenopausal women and a villoglandular pattern is seen. They present with cervical erosion or mass. They have tall thin papillae and short broad papillae. It has atypical epithelium. The invasion is seen as branching glands.

Mucinous adenocarcinoma: They have cells having mucin production.

Minimal deviation adenocarinoma(adenoma malignum):

Premenopausal women present with watery to mucoid discharge and bleeding from the vagina. The patients have a firm friable mass which may be endophytic/ exophytic. There is gland proliferation diffusely which is lined by columnar mucin producing cells with basally situated nuclei. The glands show branching with angulation. There is perineural,vascular/lymphatic invasion in some cases.

Intestinal type adenocarcinoma,not otherwise specified: (signet ring cell adenocarcinoma and colloid carcinoma).

These tumours have globlet cells with neuroendocrine and paneth cells. Signet ring cell carcinoma is associated with adenosquamous or poorly differentiated carcinomas. Mucin pools separate the stroma in the colloid carcinoma.

Endometriod carcinoma: This shows similar features like that of endometrium. The difference of this from the primary endometrial carcinoma is the in situ adenocarcinoma lesion, no endometrial tissue and CEA positivity,vimentin,ER and P16 negativity.

Clear cell carcinoma: Common in women with inutero exposure to DES. This cells are flattened,cuboidal or polygonal with eosinophilic to clear cytoplasm in a solid,tubulocystic or papillary patterns. Atypia is marked.

Serous carcinoma: Common in the age group of 26 to70 years with patients presenting with watery discharge and bleeding from the vagina. The patients have ulcer or mass in the cervix. It is mixed with other types. The papillary structures have stratification, cell detachment, tufting with solid areas. Cytologic atypia is marked and mitosis is seen. >2cm size,invasion >10mm.Metastasis to lymph node is associated with recurrence.

Adenosquamous carcinoma: Both glandular and squamous elements seen and is associated with bad prognosis.

Glassy cell carcinoma:

One of the poorly differentiated variant of adenosquamous carcinoma which occurs in the age group of 30 to 44 years and uterine bleeding is the most common presentation. It presents as a bulky mass wit size ranging from 3 to 7 cm. Large cells occur in sheets and nests with abundant cytoplasm which is amphophilic to eosinophilic and ground glass cytoplasm with a large nuclei and nucleoli. Eosinophil and plasma cell infiltration is seen.

Adenoid basal carcinoma(epithelioma) : It consists of basaloid cells which are bland and uniform. The tumour occur as well defined nests which are round with

peripheral palisading in the stroma. It is seen most commonly in post menopausal women(19-91 years). Mostly it is asymptomatic. Usually,tumour is not visible grossly. The cells can be cuboidal,spindled or oval. Squamous metaplasia or glands can be seen. Intracytoplasmic vacuoles are due to glycogen. This is benign and no metastasis is seen.

Adenoid cystic carcinoma: Post menopausal women are commonly affected presenting with bleeding uterus.Iit may present as polyp,friable exophytic mass to endophytic lesions. Small basaloid cells are arranged as sheets,nests,trabeculae and cords. It presents with cribriform pattern with basement membrane like eosinophilic hyaline material or mucin or secretions which are eosinophilic with variable mitotic activity and necrosis. Hyalinization or myxoid or fibroblastic changes can be seen in the stroma. A solid variant is there in which there is absence of cribriform pattern . Diagnosis made with PAS positive basement membrane like material. This is an aggressive tumour.

Microcystic adenocarcinoma: Cystic pattern is seen. The patient usually present with vaginal bleeding and they may have mass or asymmetry of the cervix. 1 to 8 mm sized cysts with eosinophili material is seen occupying 50 to 90% of the tumour. Lobular or diffuse cysts may be seen deep in the wall. Pseudostratified or cuboidal epithelium is seen with a fibrotic stroma. Typical endocervical carcinomatous areas are found within the tumour with mitosis.

Mesonephric adenocarcinoma and malignant mixed mesonephric tumours:

These rare tumours have mesonephric duct remnants. The malignant mixed mesonephric tumour have heterologous or homologous sarcomatous areas . They present in age group 34 to 84 years who present with grossly visible lesion or bleeding. They present as polyp or cervical wall is enlarged diffusely. The adenocarcinomatous component occur in various patterns - tubular, glandular(ductal),solid,retiform and sex cord like. These cuboidal or columnar cells have moderate pleomorphism and mitosis is variable. The luminal spaces are filled with eosinophilic material and papillary structures. Spindle cells(non malignant) may be seen in biphasic cases. Other heterologous elements are also described.

MASTER CHART

MASTER CHART

S.NO	PSP NO	AGE/SEX	OP NO	PAP	LBC	HPE
1.	16/15	28/F	5500497	ASC	LSIL	CC
2.	17/15	30/F	5500306	N	N	LSIL
3.	18/15	29/F	5500502	N	N	CC
4.	20/15	40/F	5500632	N	LSIL	LSIL
5.	21/15	30/F	5500381	N	N	CC
6.	22/15	47/F	5503603	ASC	ASC	CC
7.	23/15	65/F	5500323	HSIL	SCC	SCC
8.	24/15	44/F	5503687	ASC	ASC	CC
9.	26/15	27/F	5500578	N	N	CC
10.	27/15	38/F	5500617	US	LSIL	HSIL
11.	31/15	40/F	5508140	ASC	N	CC
12.	32/15	38/F	5502052	N	LSIL	CC
13.	37/15	40/F	5562073	N	N	CC
14.	41/15	38/F	5502098	US	US	SCC
15.	43/15	42/F	5508239	LSIL	LSIL	LSIL
16.	45/15	35/F	5500382	N	N	CC
17.	46/15	37/F	5500397	ASC	ASC	LSIL
18.	53/15	40/F	5502063	N	N	CC
19.	56/15	40/F	5508363	N	N	CC
20.	58/15	32/F	0000647	N	N	CC
21.	59/15	35/F	0000737	N	LSIL	LSIL
22.	62/15	36/F	5500476	N	N	CC
23.	64/15	35/F	104063	N	N	CC
24.	65/15	50/F	870	LSIL	N	CC
25.	67/15	47/F	55608463	LSIL	N	CC
26.	68/15	35/F	5500478	N	N	CC
27.	74/15	42/F	66134	N	HSIL	CC
28.	77/15	37/F	5500527	N	N	CC
29.	83/15	34/F	0001493	N	N	CC
30.	85/15	37/F	5504777	N	N	CC
31.	87/15	42/F	5500611	N	N	CC
32.	88/15	50/F	0001198	N	N	CC

33.	94/15	35/F	0003672	US	US	SCC
34.	99/15	32/F	1095	N	N	CC
35.	101/15	35/F	957	N	N	CC
36.	105/15	40/F	3443	N	N	CC
37.	110/15	41/F	3644	N	N	CC
38.	111/15	32/F	3519	N	N	CC
39.	112/15	41/F	1458	N	N	CC
40.	114/15	42/F	3488	N	N	CC
41.	116/15	37/F	3494	N	N	CC
42.	199/15	47/F	13812	N	N	CC
43.	203/15	43/F	14270	N	N	CC
44.	204/15	49/F	1092	N	N	CC
45.	207/15	52/F	351281	LSIL	HSIL	SCC
46.	214/15	30/F	17742	N	N	CC
47.	224/15	41/F	17658	ASC	HSIL	HSIL
48	225/15	29/F	335801	N	N	LSIL
49.	226/15	47/F	17663	N	N	CC
50.	227/15	27/F	17670	N	N	CC
51.	231/15	35/F	17651	N	N	CC
52.	232/15	35/F	14045	N	N	CC
53.	245/15	42/F	17901	LSIL	LSIL	LSIL
54.	246/15	45/F	17789	N	N	CC
55.	247/15	45/F	371256	N	N	CC
56.	248/15	46/F	14258	N	N	CC
57.	257/15	35/F	3672	US	US	SCC
58.	259/15	30/F	17944	N	N	CC
59.	261/15	49/F	17916	N	N	CC
60.	262/15	35/F	17955	N	N	CC
61.	263/15	30/F	17939	N	N	CC
62.	264/15	40/F	3443	N	N	CC
63.	265/15	35/F	17929	N	N	CC
64.	268/15	35/F	7952	N	LSIL	CC
65.	271/15	40/F	352013	N	N	CC
66.	272/15	45/F	18133	N	N	CC
67.	276/15	40/F	18145	ASC	N	CC
68.	278/15	40/F	18059	N	N	CC
69.	280/15	39/F	17773	N	N	CC
70.	281/15	33/F	9347	N	N	CC
71.	282/15	30/F	9344	N	N	CC

72.	283/15	45/F	14207	N	N	CC
73.	284/15	45/F	14040	ASC	ASC	CC
74.	286/15	43/F	9272	US	N	LSIL
75.	290/15	50/F	121095	N	N	CC
76.	291/15	45/F	6071	N	HSIL	CC
77.	292/15	35/F	9612	N	LSIL	LSIL
78.	293/15	46/F	17957	ASC	ASC	CC
79.	294/15	43/F	9491	ASC	HSIL	HSIL
80.	298/15	37/F	9431	ASC	ASC	LSIL
81.	302/15	35/F	376693	N	LSIL	LSIL
82.	303/15	42/F	9570	LSIL	N	CC
83.	306/15	35/F	9677	US	US	SCC
84.	308/15	37/F	353052	N	N	CC
85.	310/15	34/F	9642	US	LSIL	HSIL
86.	311/15	60/F	786	HSIL	SCC	POORLY DIFFERENTI--ATED SCC
87.	314/15	35/F	9694	N	HSIL	HSIL
88.	356/15	55/F	14133	N	N	CC
89.	372/15	62/F	38203	LSIL	HSIL	SCC
90.	375/15	31/F	30523	N	N	CC
91.	385/15	25/F	41570	ASC	LSIL	CC
92.	389/15	36/F	30500	N	N	CC
93.	392/15	38/F	38183	N	N	CC
94.	402/15	33/F	38201	N	N	CC
95.	407/15	37/F	41616	N	N	CC
96.	409/15	49/F	37570	LSIL	N	CC
97.	417/15	40/F	37791	N	N	CC
98.	439/15	67/F	52049	N	N	CC
99.	451/15	40/F	48569	N	HSIL	HSIL
100.	452/15	45/F	355301	US	N	LSIL

OBSERVATION AND RESULTS

OBSERVATION AND RESULTS

This prospective study was conducted on 100 patients who attended the pilot screening project programme being organized by Tamilnadu health screening project conducted at the department of Obstetrics and Gynaecology,Raja Mirasudar hospital affiliated to Thanjavur medical college,Thanjavur.

Clinical history regarding age, socioeconomic status, parity and complaints were obtained from the patient, and thorough physical examination was done. Per speculum examination was done. Exfoliative cytology specimens were collected for conventional PAP smear and Liquid based cytology. Colposcopy was done using Sim's speculum and biopsy was taken from the same patient.

Most of the cases who attended the screening programme were in the fourth decade of life(50 cases , 50%) followed by 32 cases(32%) in the fifth decade. Minimum age of the patient screened was 25 years of age and the maximum age was 67 years. About 61.5% of cases who was diagnosed with LSIL and HSIL were in the age group of 21 – 40 years. Age wise distribution of cases are shown in table 3 and chart 1.

TABLE 3: Age wise distribution of cases:

AGE	TOTAL	NORMAL	ABNORMAL	LSIL	HSIL	CARCINOMA
25-30	12	10	2	2	-	-
31-35	24	16	8	3	2	3
36-40	26	20	6	3	2	1
41-45	20	14	6	4	2	-
46-50	12	12	-	-	-	-
51-55	2	1	1	-	-	1
56-60	1	-	1	-	-	1
>60	3	1	2	-	-	2
TOTAL	100	74	26			

Out of 100 cases,36 cases(36%) of cases belonged to class II of modified Prasad's classification[82] followed by 24 cases(24%) of cases in class III. Out of 26 cases with dysplasia/carcinoma, 12 cases (46.1 %) of cases belonged to class III.(Table 4 & chart 2)

Chart 1: Age wise distribution of cases

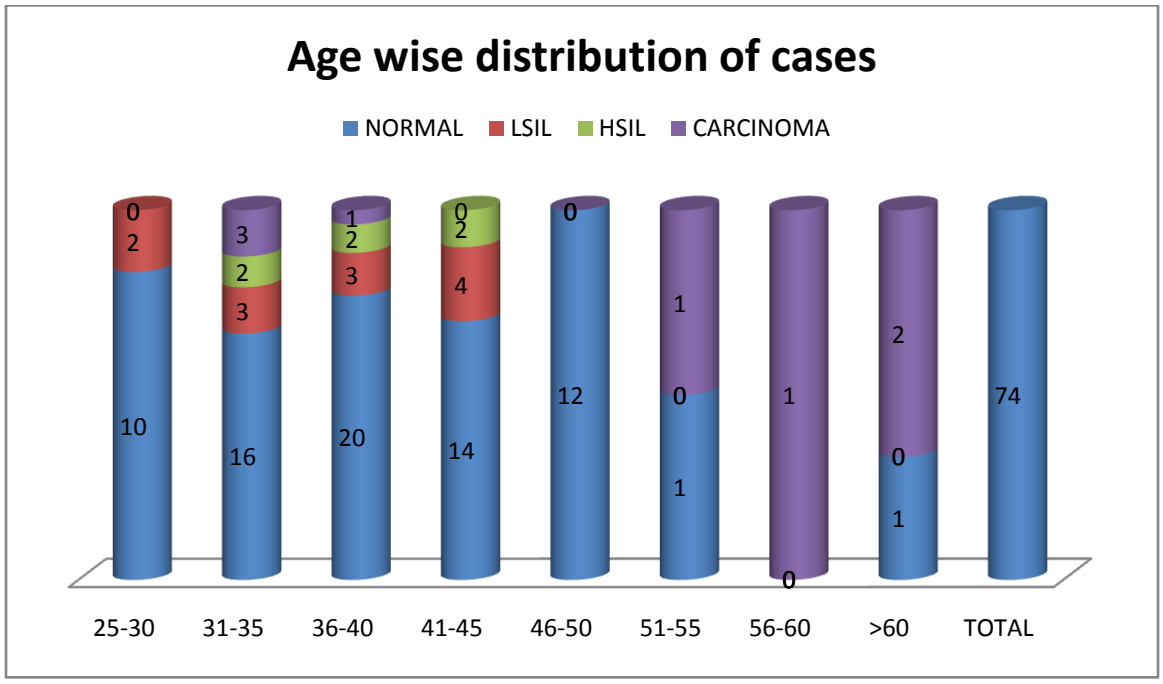


TABLE 4: Case distribution according to socio-economic status (Modified prasad's classification)[82]

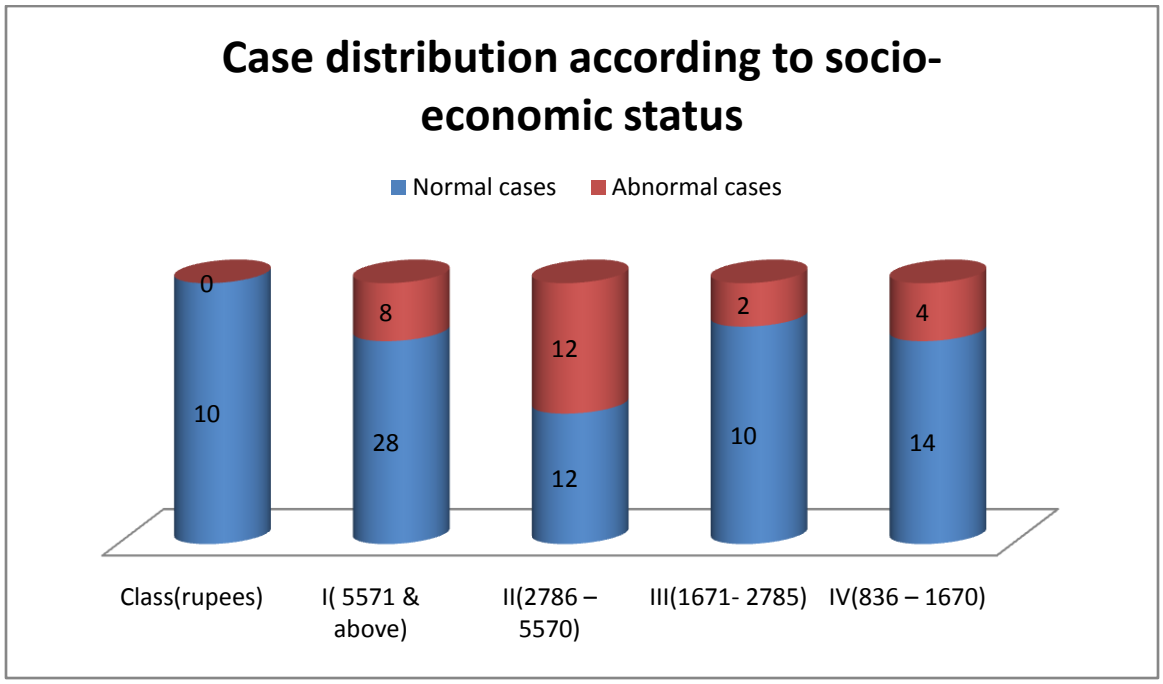
Class(rupees)	Total no. of cases	Normal cases	Abnormal cases
I(5571 & above)	10	10	-
II(2786 – 5570)	36	28	8
III(1671- 2785)	24	12	12
IV(836 – 1670)	12	10	2
V(Below 836)	18	14	4
Total	100	74	26

90 cases(90%) started sexual activity before 25 years of age and out of these 90 patients,92.3% had dysplasia but out of the remaining 10 cases,only 2 cases(2% of total number of cases) showed dysplasia. (Table 5).

Table 5: Case distribution according to the onset of sexual activity:

Age	Total number of cases	% of patients with dysplasia
<25 years	90	92.3% of 90 cases
>25 years	10	2% of 100 cases

Chart 2: Case distribution according to Socioeconomic status



In this study, about 46 cases (46%) had 2 children and 34 cases (34%) had 3 children. Most of the cases with dysplasia were seen when patients had 3 children(14 cases , 53.8% of the abnormal smears)(Table 6 & chart 3).

Table 6: Case distribution according to parity:

Gravida	Total number of cases	Number of cases with dysplasia
Nulligravida	6	2(2%)
1	8	-
2	46	7(7%)
3	34	14(14%)
>3	6	3(3%)

Most common presenting complaint was white discharge per vaginum (46 cases,46%),followed by lower abdominal pain(26 cases,26%) and bleeding per vaginum(16 cases,16%).other minor complaints were dysfunctional uterine bleeding(4 cases,4%),itching(4 cases,4%),difficulty in micturition(2 cases,2%),post coital bleeding(2 cases,2%).(Table 7 & chart 4).

Chart 3: Case distribution according to parity

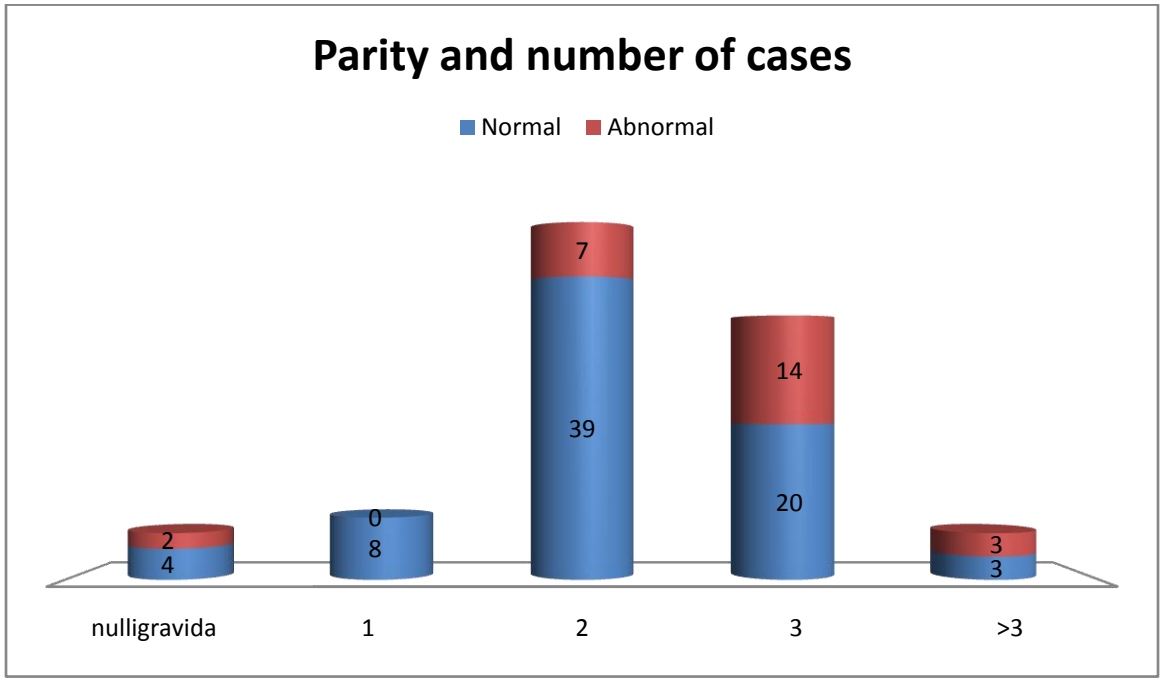


Table 7: Case distribution according to the presenting complaints:

Complaints	Number of cases
White discharge P/V	46 cases (46%)
Lower abdominal pain	26 cases (26%)
Bleeding P/V	16 cases (16%)
Dysfunctional uterine bleeding	4 cases (4%)
Dfficulty in micturition	2 cases (2%)
Post coital bleeding	2 cases (2%)

Out of the 100 cases studied, conventional PAP smear detected abnormality in 22 cases(22%) whereas LBC detected abnormality in 28 cases(28%) of cases.(Table 8 & chart 5)

Table 8: number of abnormal cases

Study	Abnormal cases
Conventional PAP	22(22%)
LBC	28(28%)

Chart 4: Case distribution according to presenting complaints

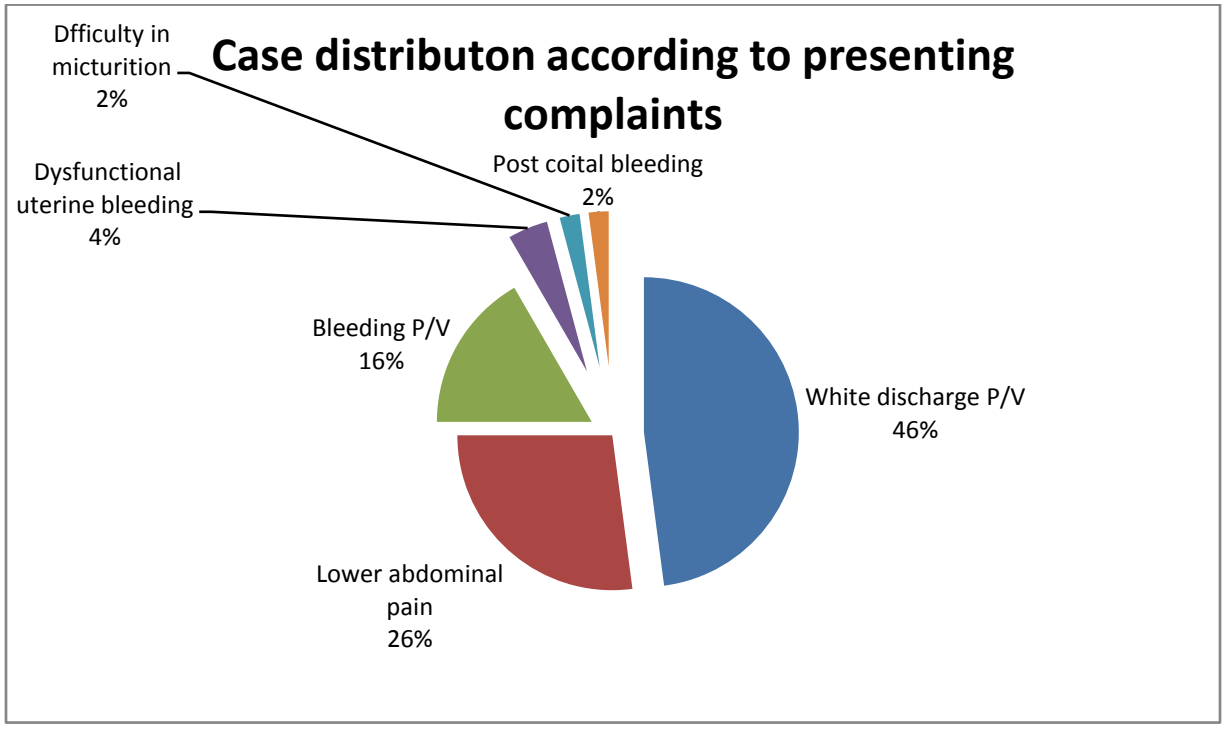
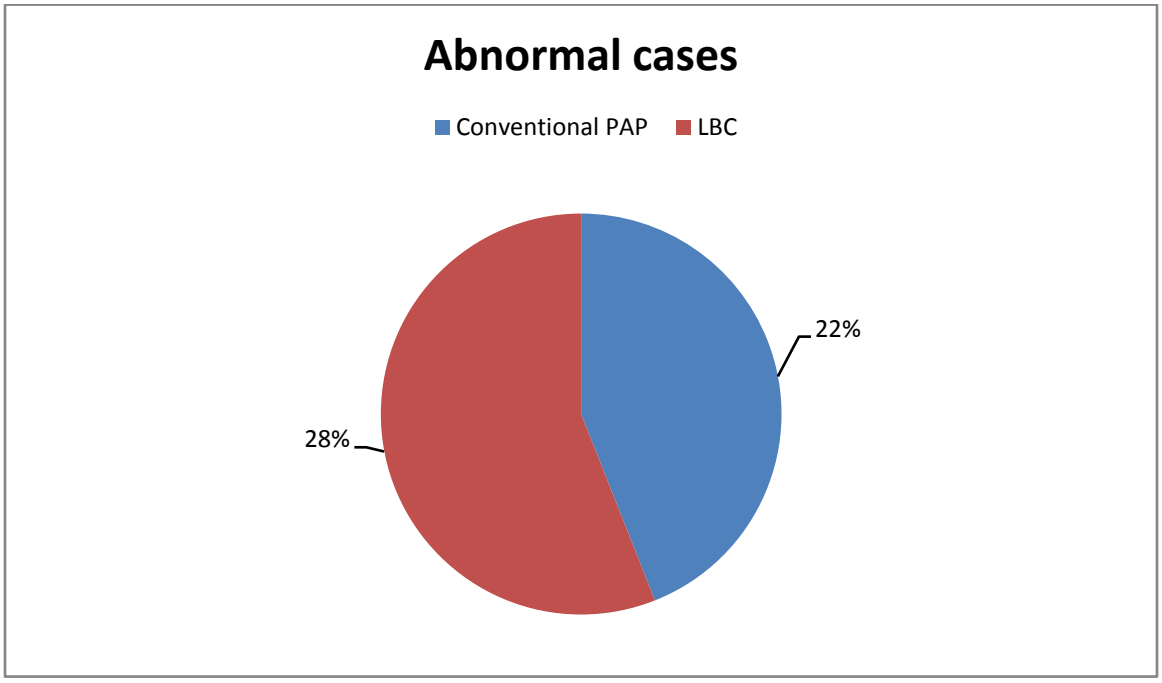


Chart 5: Number of abnormal cases



Out of the 100 cases , 92 cases(92%) were satisfactory for evaluation in conventional PAP smear whereas 96 cases(96%) were satisfactory in LBC. About 60 cases(60%) in conventional PAP smear and 12 cases(12%) were satisfactory but limited by factors such as blood and inflammatory cells, air drying. 8 cases(8%) and 4 cases(4%) were unsatisfactory. The most common cause for unsatisfactoriness in conventional PAP smear is thick smear and reduced cell number in LBC. Table 9 & chart 6 shows the comparison of results of both Conventional PAP and LBC.

TABLE 9: comparison of PAP and LBC results

Category	PAP(number)	PAP (%)	LBC(number)	LBC (%)
Unsatisfactory	8	8%	4	4%
Normal	70	70%	68	68%
ASC	12	12%	6	6%
LSIL	8	8%	12	12%
HSIL	2	2%	8	8%
Carcinoma	-	-	2	2%
Total	100	100%	100	100%

Chart 6: Comparison of Conventional PAP and LBC results

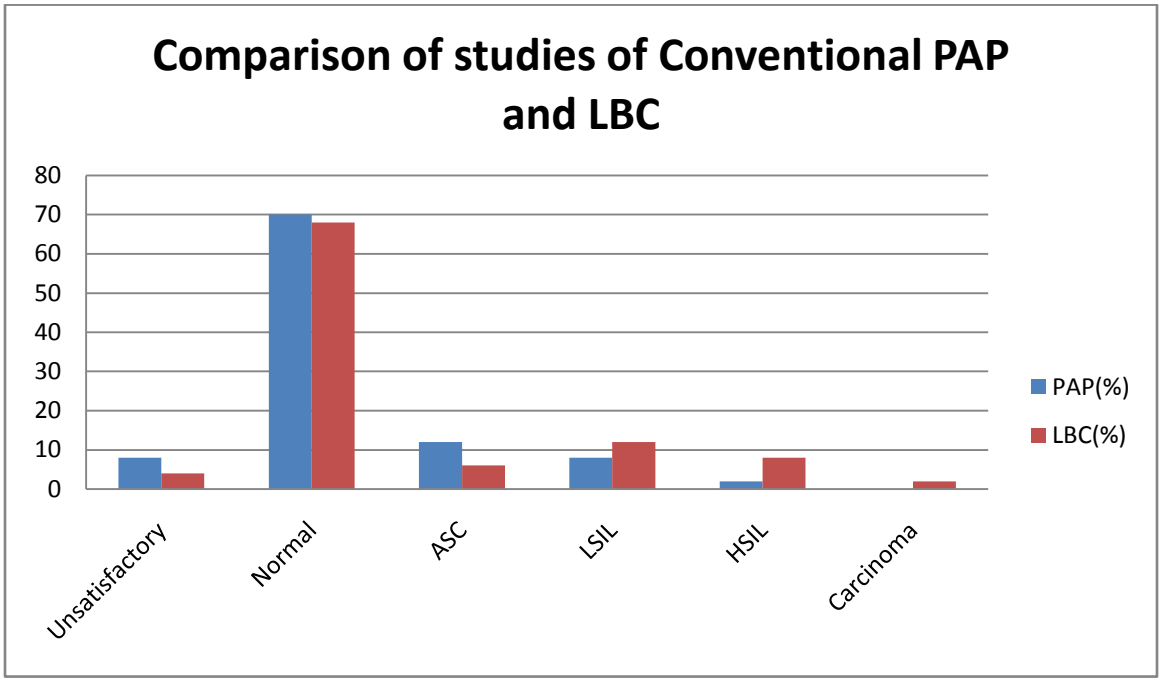


Chart 7: Results of Conventional PAP smear

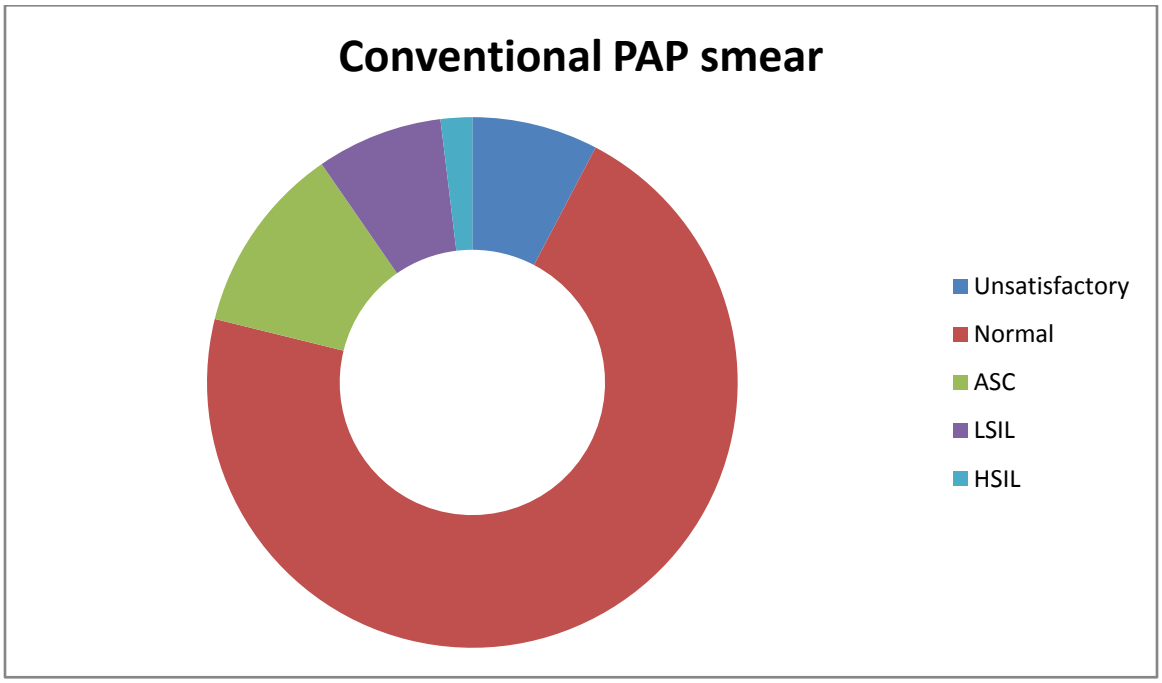


Chart 8: Results of LBC

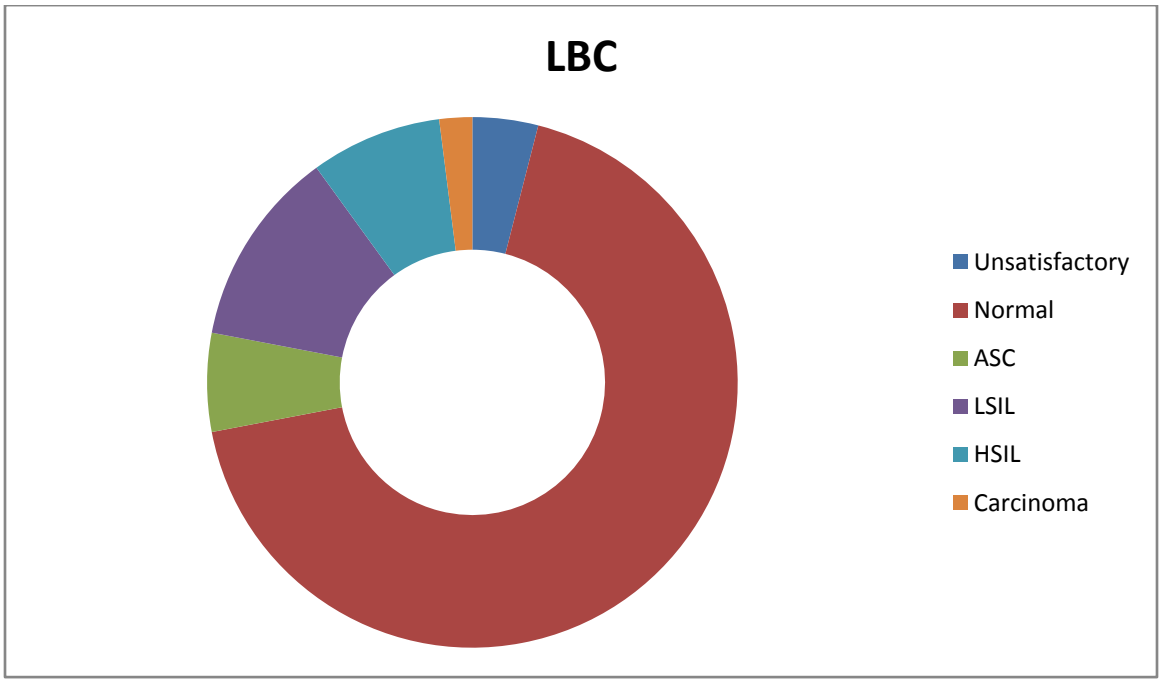
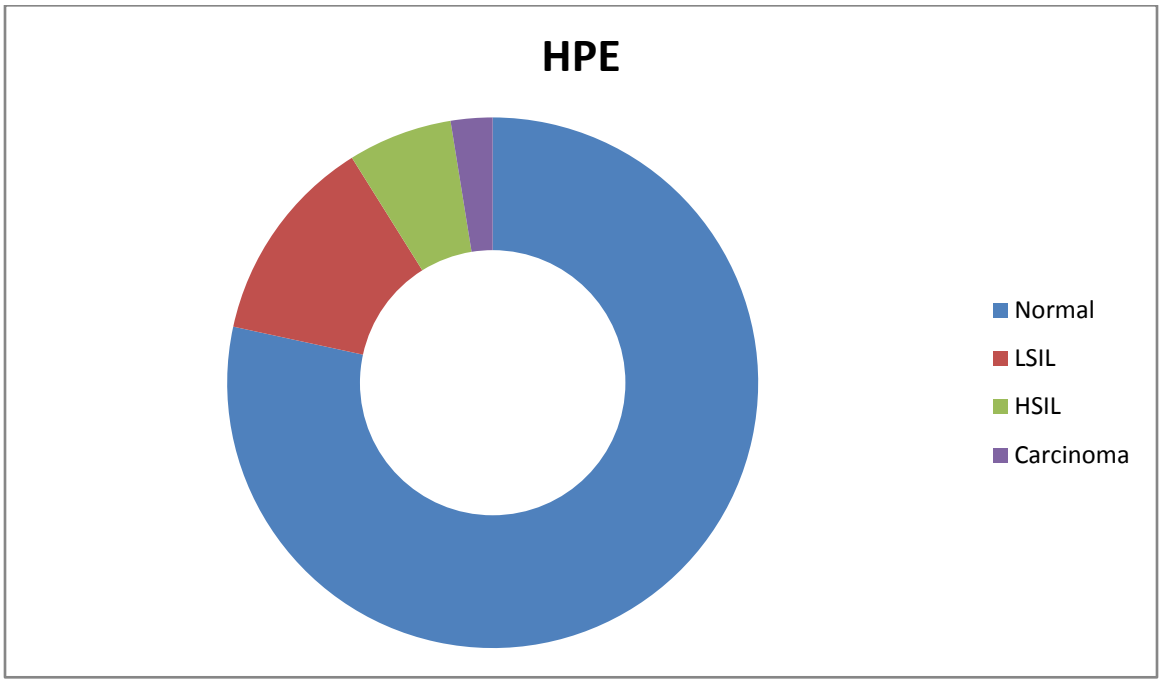


Chart 9: Results of biopsy specimens.



A comparative study between the findings of conventional PAP smear and the biopsy results were made. Out of 100 cases 92 cases were satisfactory for evaluation. Out of the 92 cases,70 cases were found to be normal. Out of the 70 normal cases in PAP, 62 cases had normal histology in the biopsy obtained, 6 cases had LSIL changes and 2 cases had HSIL change histopathologically. Atypical squamous cells(ASC) were found in 12 cases in PAP. Out of 12 ASC cases in PAP,8 had normal histology,2 cases each had LSIL change and HSIL change in biopsy. 8 cases had LSIL features in conventional PAP smear. Out of these 8 cases,4 cases had normal histology and 2 case had LSIL features and carcinoma was found in 2 cases histopathologically. 2 cases showed HSIL features in PAP which turned out to be carcinoma histopathologcally. Results of PAP smear is shown in chart 7 and its correlation with histopathology is shown in table 10.

TABLE 10: Comparison of conventional PAP and histopathology results:

HPE / PAP	**PAP results**	**Normal**	**LSIL**	**HSIL**	**Carcinoma**
Unsatisfactory	8	-	2	2	4
Normal	70	62	6	2	-
ASC	12	8	2	2	-
LSIL	8	4	2	-	2
HSIL	2	-	-	-	2
Carcinoma	-	-	-	-	-
Total	100	74	12	6	8

Similarly, comparative study was also found between LBC and biopsy results. 96 cases were found to be satisfactory for evaluation. Out of the 96

cases,68 cases were found to be normal. Out of the 68 normal cases, 64 cases also had normal histology on biopsy, 4 cases had LSIL features. 6 cases had atypical squamous cells in LBC, but out of these , 4 cases had normal histology and 2 cases had LSIL features on histopathology. 12 cases had LSIL features on LBC and out of the 12 cases, 4 cases had normal histology,6 cases also had LSIL features and 2 cases had HSIL features in histopathology. 8 cases had HSIL features on LBC. Out of the 8 cases, 2 cases had normal histology,4 cases had HSIL features and 2 cases had carcinoma. 2 cases which had carcinoma on LBC also had similar features in biopsy. The results of LBC are shown in chart 8 and its comparison with HPE is shown in table 11.

TABLE 11: Comparison of LBC and HPE results

HPE / LBC	**LBC results**	**Normal**	**LSIL**	**HSIL**	**Carcinoma**
Unsatisfactory	2	-	-	-	2
Normal	34	32	2	-	-
ASC	3	2	1	-	-
LSIL	6	2	3	1	-
HSIL	4	1	-	2	1
Carcinoma	1	-	-	-	1
Total	50	37	6	3	4

Statistics:

Sensitivity and specificity of PAP smear in detecting LSIL:

PAP / HPE	POSITIVE	NEGATIVE
POSITIVE	4(TP)	4(FP)
NEGATIVE	6(FN)	62(TN)

Sensitvity = TP /TP + FN x 100 = 4/4+6 x 100 = 40 %

Specificity = TN/TN + FP x 100 = 62/ 62 + 4 x 100 = 93%

Sensitivity and specificity of PAP smear in detecting HSIL:

PAP / HPE	POSITIVE	NEGATIVE
POSITIVE	2(TP)	0(FP)
NEGATIVE	6(FN)	62(TN)

Sensitivity = TP /TP + FN x 100 = 2/2+2 x 100 = 50%

Specificity = TN/TN + FP x 100 = 62/ 62 + 0 x 100 = 100%

Sensitivity and specificity of LBC in detecting LSIL:

LBC / HPE	POSITIVE	NEGATIVE
POSITIVE	8(TP)	4(FP)
NEGATIVE	4(FN)	64(TN)

Sensitvity = TP /TP + FN x 100 = 8/8+4 x 100 = 66%

Specificity = TN/TN + FP x 100 = 64/ 64 + 4 x 100 = 94%

Sensitivity and specificity of LBC in detecting HSIL:

LBC / HPE	POSITIVE	NEGATIVE
POSITIVE	6(TP)	2(FP)
NEGATIVE	0(FN)	64(TN)

Sensitvity = TP /TP + FN x 100 = 6/6+0 x 100 = 100%

Specificity = TN/TN + FP x 100 = 64/ 64 + 2 x 100 = 96%

Sensitivity and specificity of LBC in detecting carcinoma:

LBC / HPE	POSITIVE	NEGATIVE
POSITIVE	2(TP)	0(FP)
NEGATIVE	0(FN)	64(TN)

Sensitvity = TP /TP + FN x 100 = 2/2+0 x 100 = 100%

Specificity = TN/TN + FP x 100 = 64/ 64 + 0 x 100 = 100%

Overall sensitivity and sensitivity of PAP smear:

PAP / HPE	POSITIVE	NEGATIVE
POSITIVE	10(TP)	6(FP)
NEGATIVE	8(FN)	62(TN)

Sensitvity = TP /TP + FN x 100 = 5/5+4 x 100 = 55.5%

Specificity = TN/TN + FP x 100 = 62/ 62 + 6 x 100 = 83.7%

Overall sensitivity and specificity of LBC:

LBC / HPE	POSITIVE	NEGATIVE
POSITIVE	20(TP)	10(FP)
NEGATIVE	4(FN)	64(TN)

Sensitvity = TP /TP + FN x 100 = 20/20+4 x 100 = 83%

Specificity = TN/TN + FP x 100 = 64/ 64 + 10 x 100 = 86.5%

STATISTICAL CORRELATION

Controlling for Age Factor partial correlation co-efficient shows:

1. The LBC Vs HPE (r = 0.617) - High level of correlation
2. LBC Vs Papsmear (r= 0.59) – Medium level of correlation
3. Papsmear Vs HPE (r = 0.4651) – Medium level of correlation.

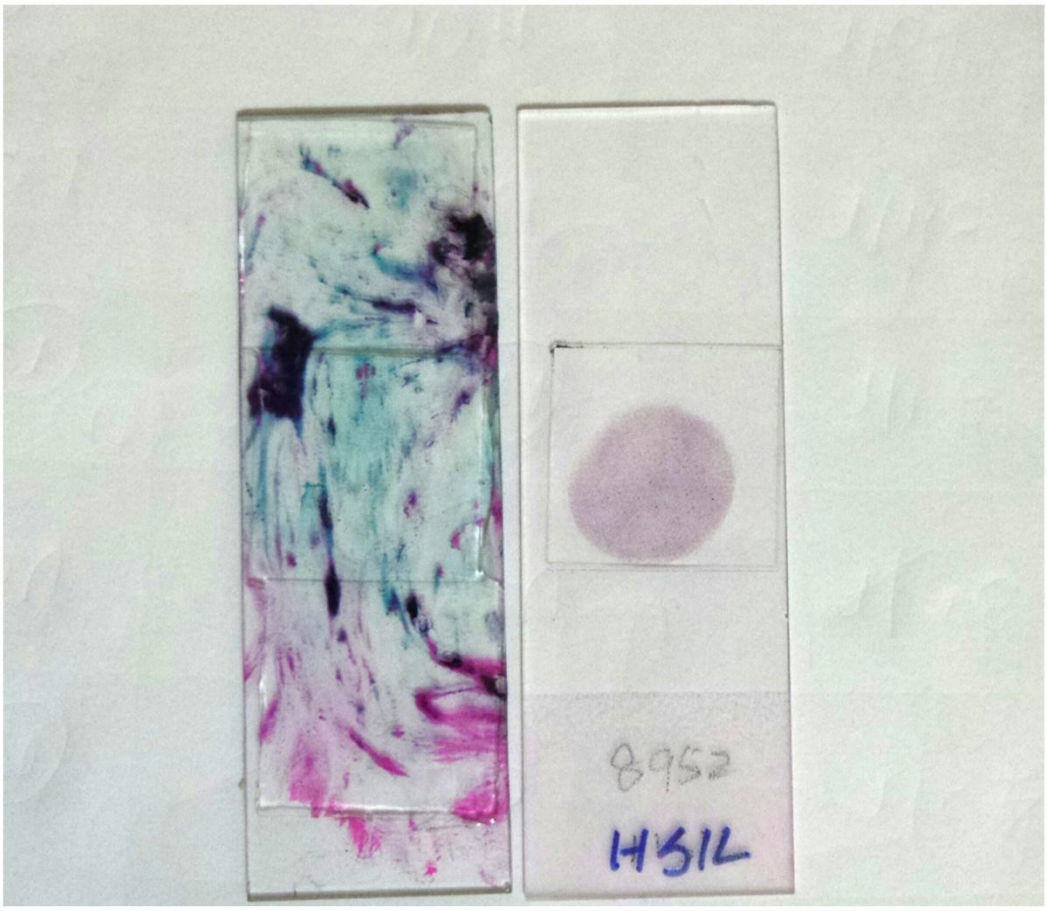
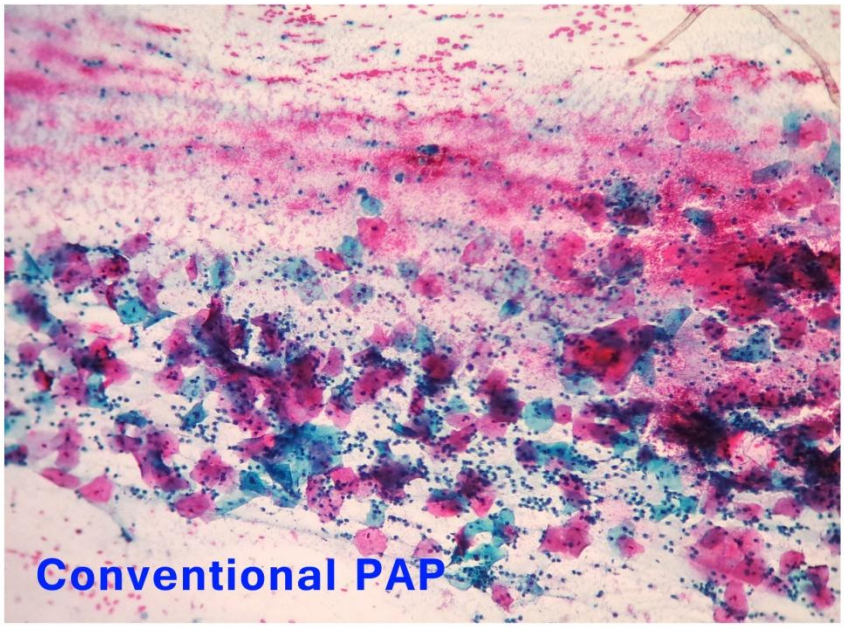


FIG 4: Comparison of slides of Conventional PAP smear(left) and Liquid based cytology(right). In PAP smear, sample is spread throughout the slide with thick and bloody smear whereas in LBC, smear is thin and sample is found in a small well circumscribed area.



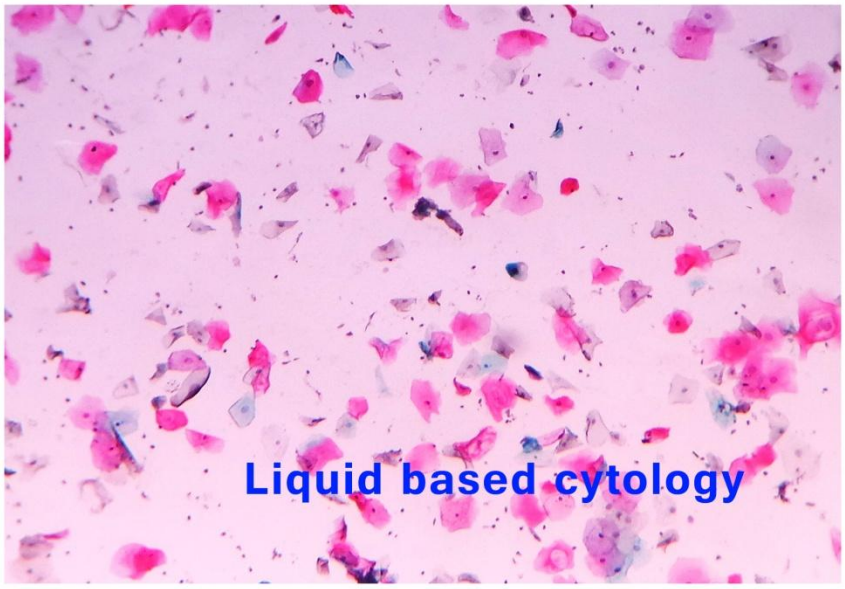


FIG 5: Comparison of microscopy of Conventional PAP and Liquid based cytology. In PAP smear, cells are obscured by blood, mucous and inflammatory cells whereas LBC has a clean background with monolayering of cells

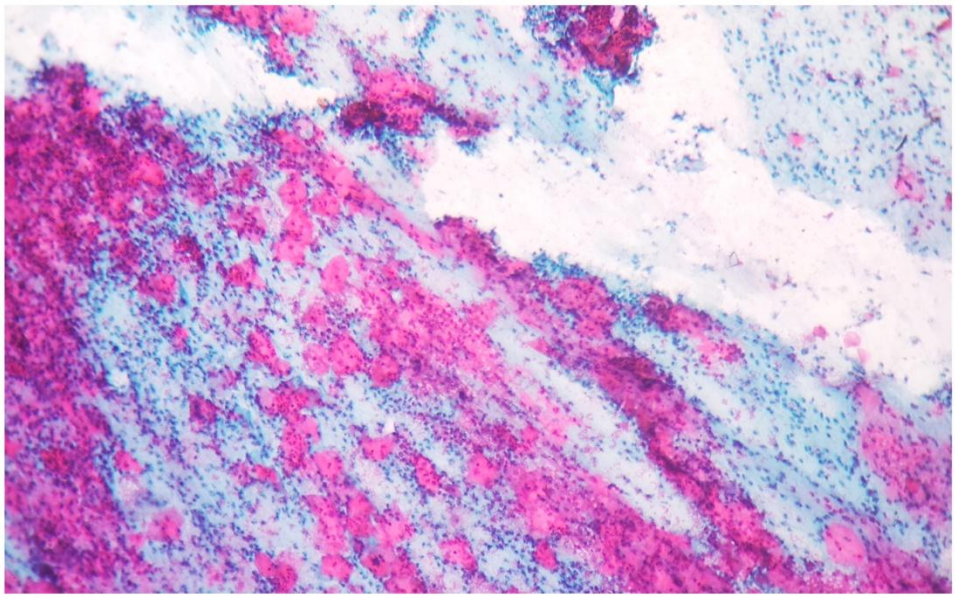


FIG 6: Unsatisfactory smear – Conventional PAP.

Cells are obscured by blood and inflammatory cells.

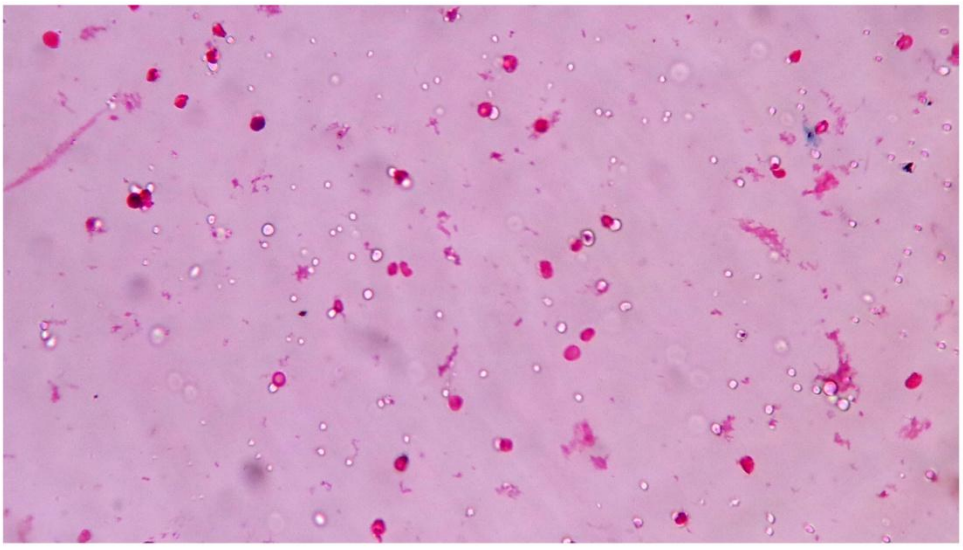


FIG 7:Unsatisfactory smear – LBC .

Only blood components found with no diagnostic cells.

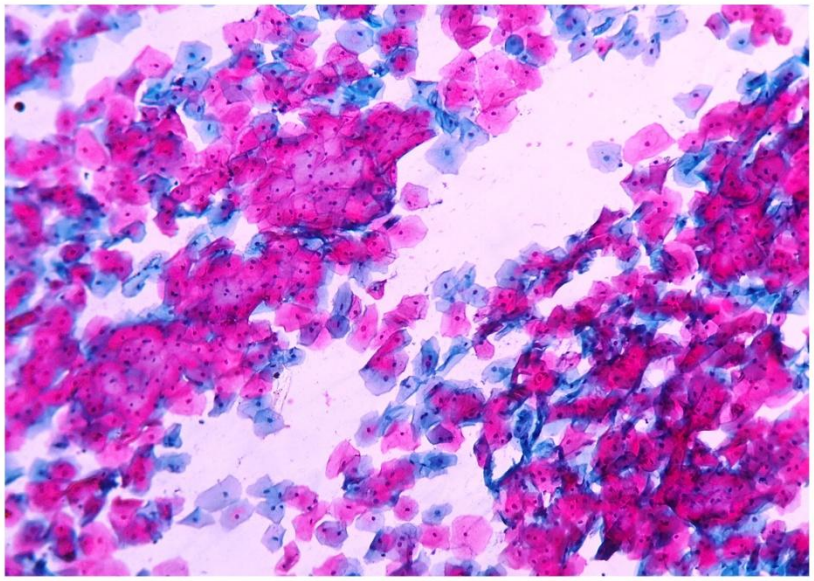


FIG 8:Satisfactory smear – Conventional PAP.

This slide shows approximately 100 cells. Entire slide covered at this level of cellularity have 10000 cells.

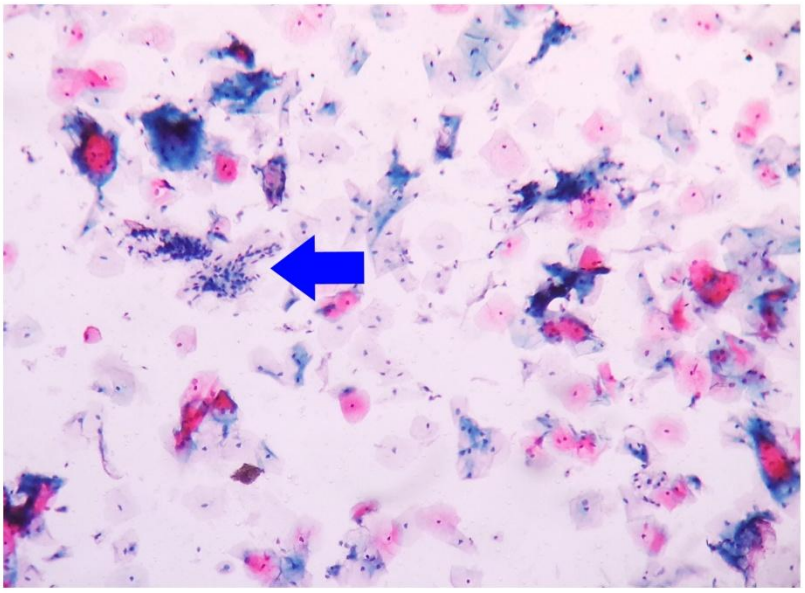


FIG 9:Satisfactory smear – LBC.

This slide has approximately 50 cells. Entire slide covered at this level of cellularity have 5000 cells. Few endocervical clusters found(arrow).

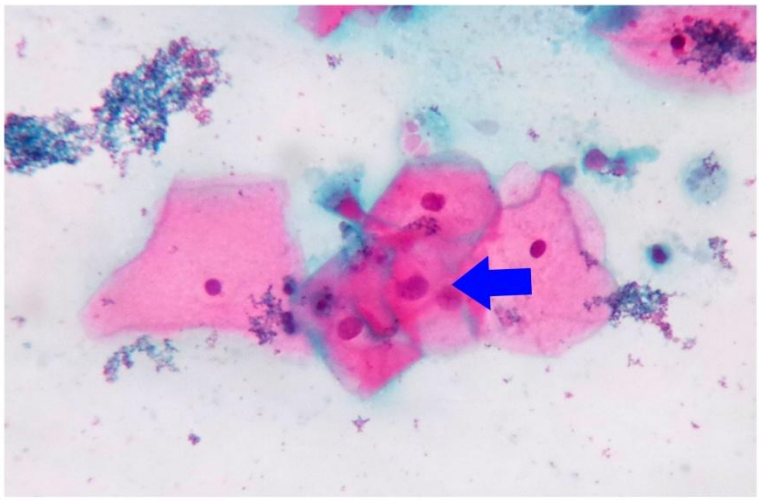


FIG 10: Atypical squamous cells –PAP.

Superficial cells are seen with enlarged nuclei , slightly increased N/C ratio , regular nuclear contour with focal irregularity(arrow), dense and orangeophilic cytoplasm.

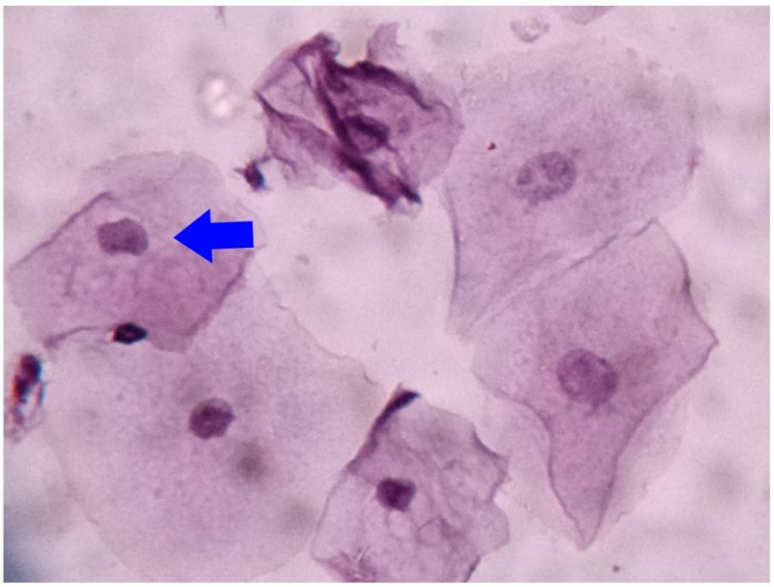


FIG 11: Atypical squamous cells – LBC.

Focal irregularity is shown by arrow.

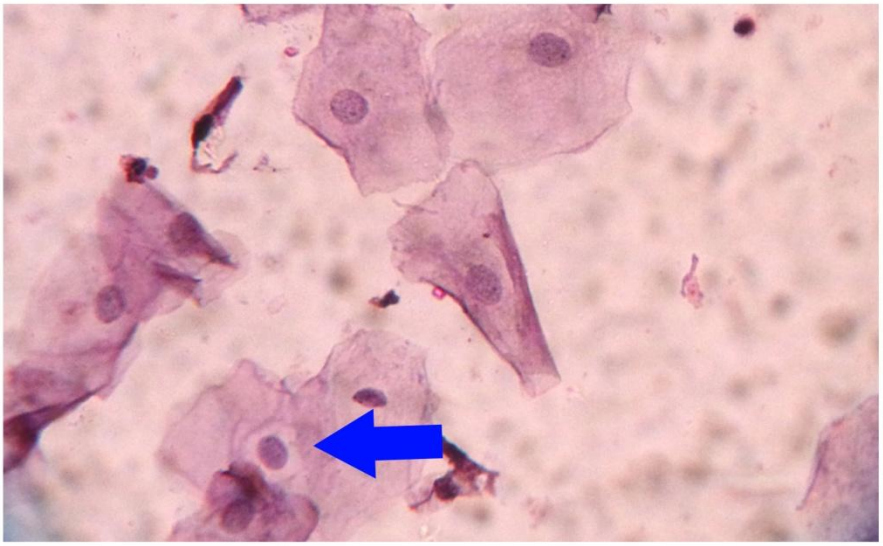


FIG 12:LSIL(koilocytosis) – Conventional PAP.

Koilocytes are large cells with sharply defined perinulear cytoplasmic cavities surrounded by dense rim of cytoplasm with enlarged nuclei and irregular nuclear membrane

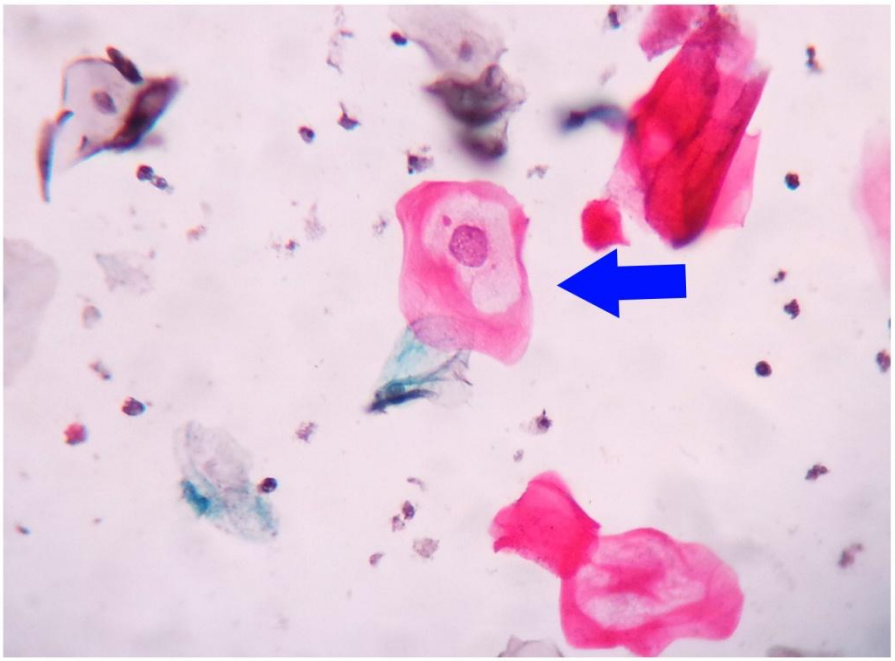


FIG 13:LSIL(koilocytes) – LBC.

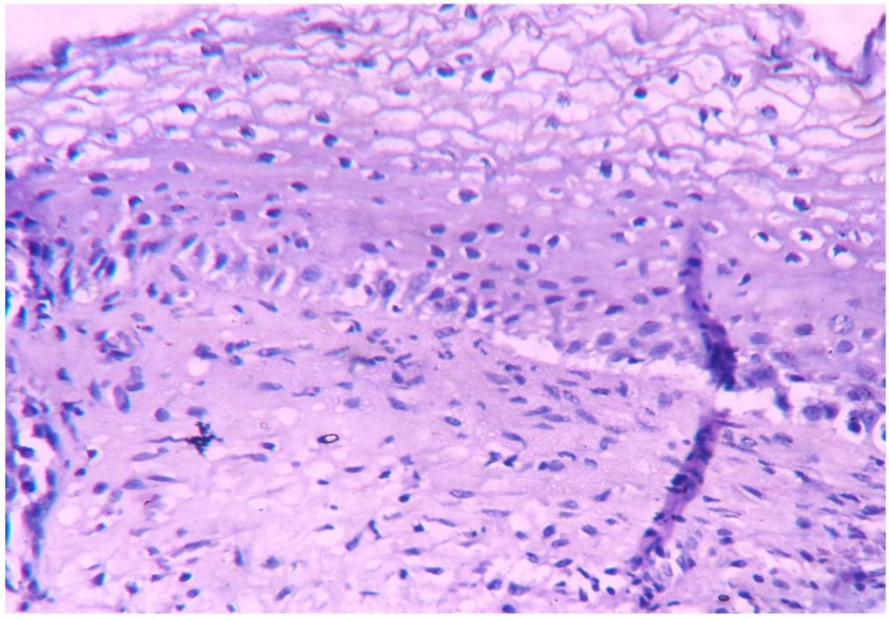


FIG 14:LSIL(koilocytes) – HPE.

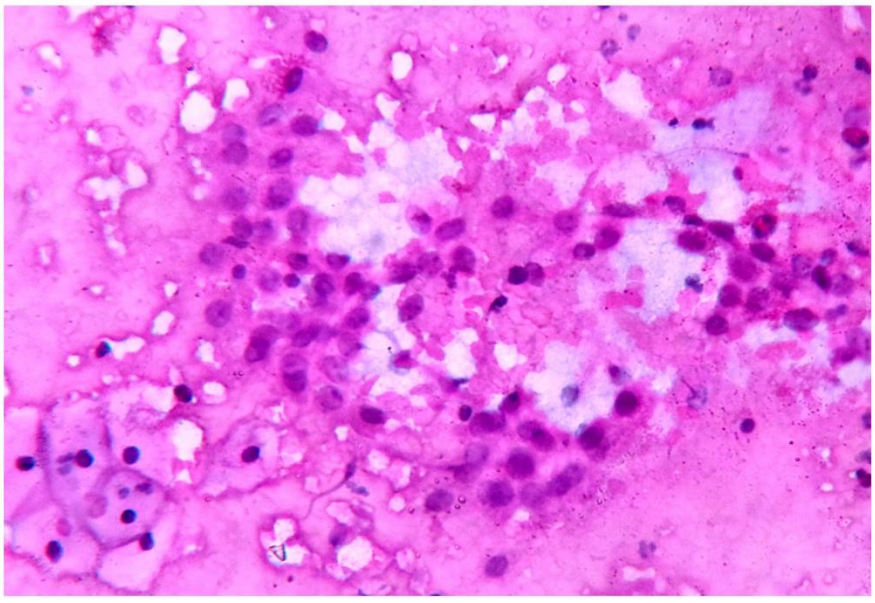


FIG 15:LSIL – Conventional PAP.

Intermediate sized cells with enlarged nuclei, mild hyperchromasia and nuclear contour irregularity.

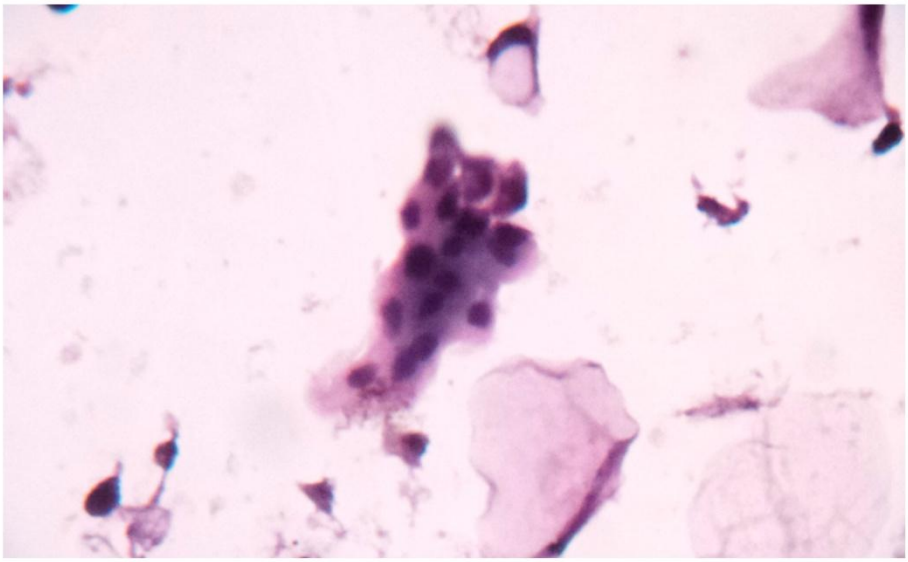


FIG 16:LSIL – LBC

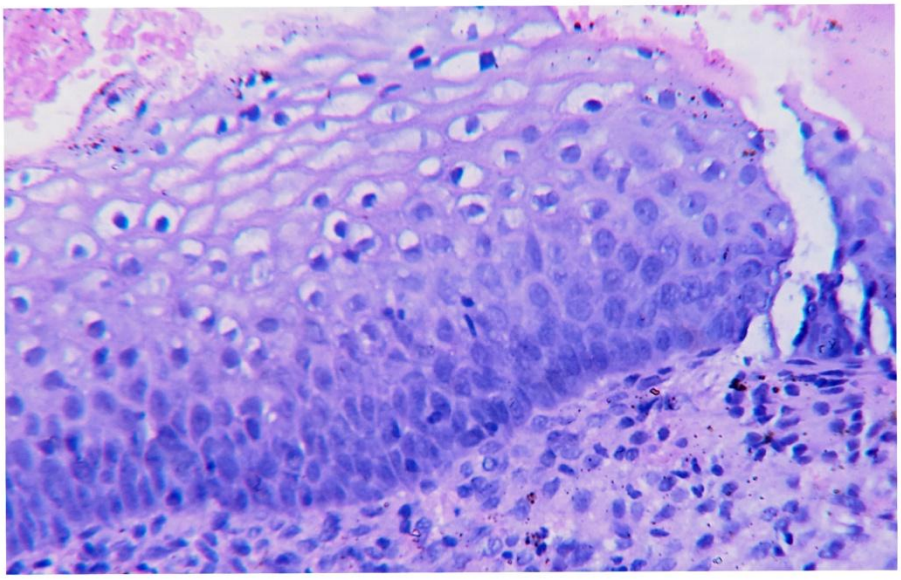


FIG 17:LSIL – HPE.

Lower 1/3rd of the epithelium shows loss of picket fence appearance of basal cells with enlarged nuclei showing mild to moderate atypia.

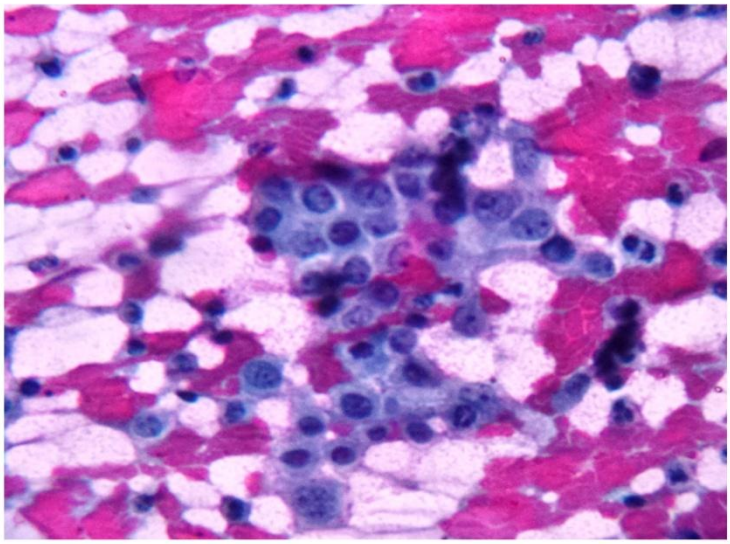


FIG 18:HSIL – Conventional PAP.

Parabasal sized cells which have enlarged markedly hyperchromatic nuclei,highly irregular outlines and scant cytoplasm.

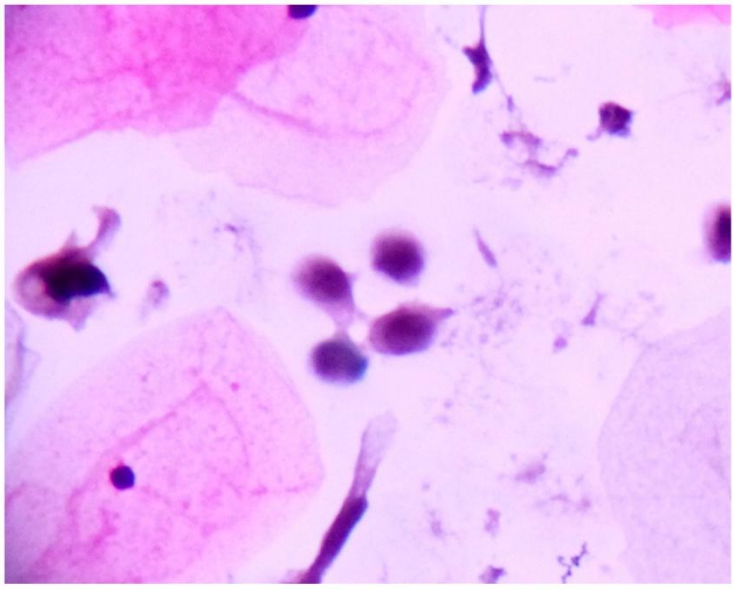


FIG 19: HSIL – LBC

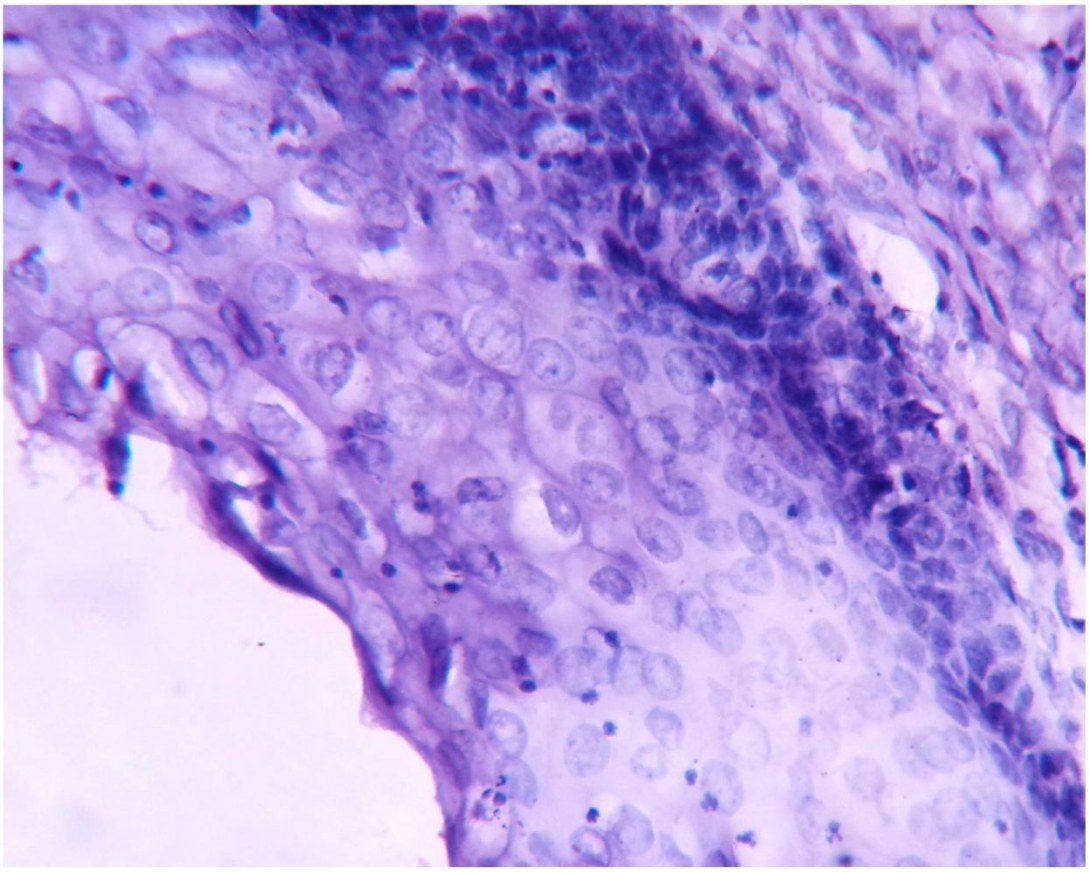


FIG 20:HSIL – HPE

More than 2/3rd of the epithelium shows nuclear pleomorphism with moderate atypia.

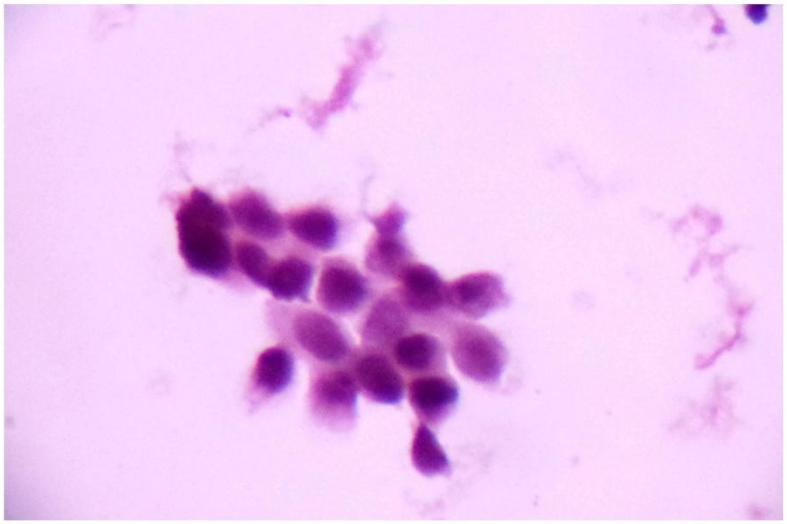


FIG 21:Squamous cell carcinoma – LBC

This smear shows pleomorphic malignant cells in syncytial clusters with scant cytoplasm and markedly hyperchromatic nuclei and irregular nuclear outlines.

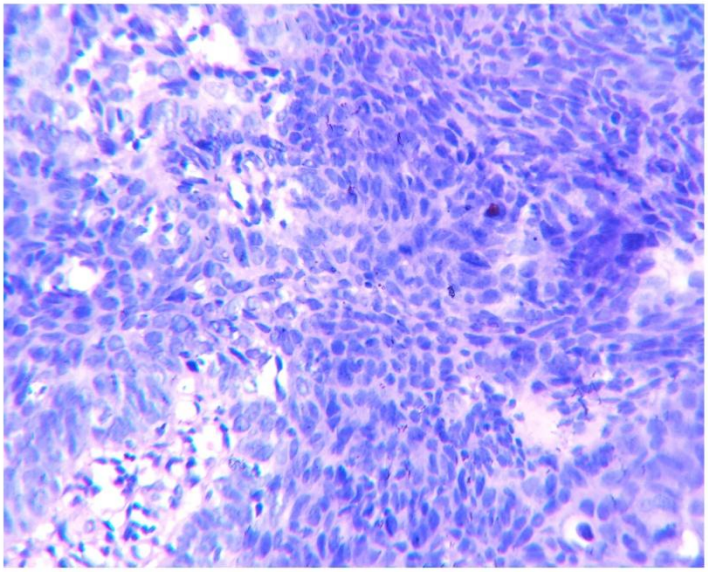


FIG 22: Squamous cell carcinoma-HPE

This section shows sheets of malignant cells with markedly pleomorphic hyperhromatic nuclei with increased N/C ratio and scant cytoplasm.

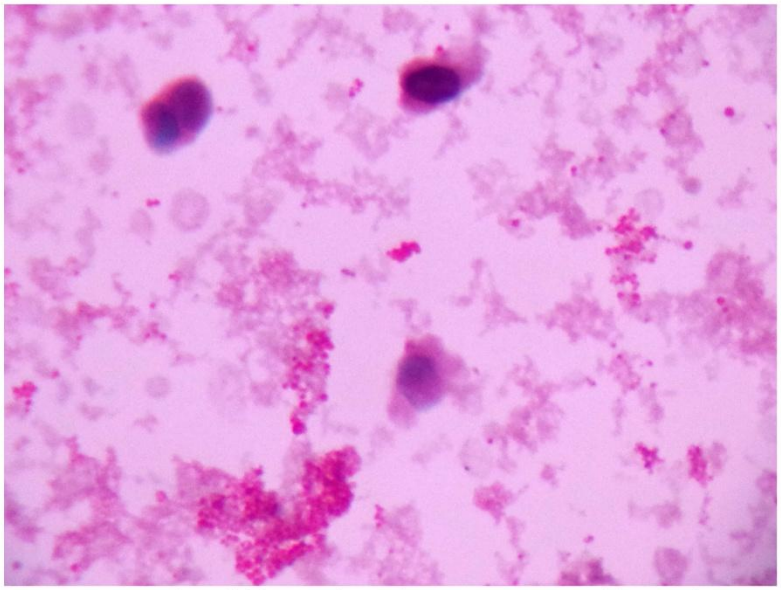


FIG 23: Poorly differentiated squamous cell carcinoma – LBC

This smear shows tumour cells arranged singly with markedly pleomorphic nuclei with prominent nucleoli and scant illdefined non keratinized cytoplasm.

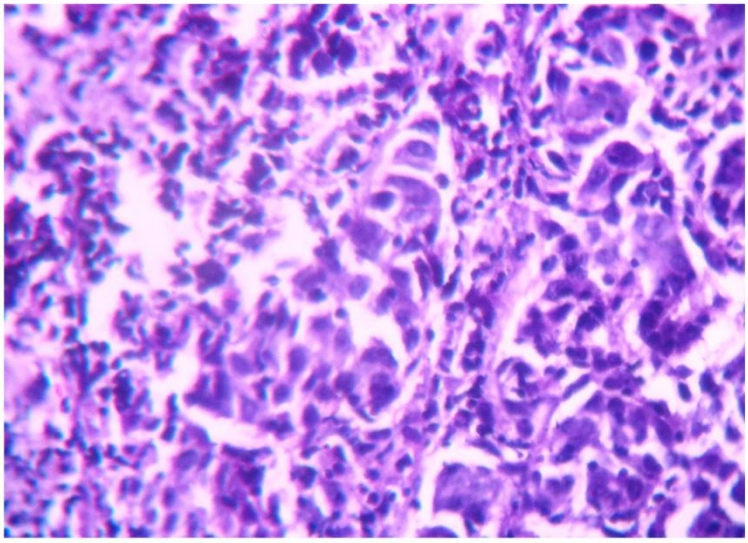


FIG 24: Poorly differentiated squamous cell carcinoma – HPE

This section shows sheets of malignant cells with nuclei showing marked pleomorphism and prominent nucleoli and illdefined scant cytoplasm.

DISCUSSION

DISCUSSION

For more than 50 years, PAP smear remained the only modality for screening which had a high false positive rate. Due to this, liquid based cytology was developed. This study was done to compare both the methods by correlating it with the biopsy taken from the same patient.

100 patients were randomly selected from those attending the Pilot screening project conducted at the Department of Obstetrics and Gynaecology, Thanjavur medical college for the study and all the three samples were taken from all the cases and the results analysed.

Out of the 100 cases, 50 cases (50%) of cases were in the fourth decade of life and most of the LSIL and HSIL cases were in the 4th decade ,a finding similar to Sherwani RK et al.[83], Richart et al.,[84],Jie Zhu et al.,[98] , Erdin ITLER et al.,[92] ,Dr. Shubhangi et al.,[114], Macharid H.C et al[118], M.Almonte et al.,[121.] . But studies by Ahmed Ibrahim[123] , Chinaka et al.[120], and S.E Nigerio Justus et al.[112], reported cases mostly in the 5th decade of life which in contrast to a study by Pragya Sharma et al.[113], who reported most number of cases in the 3rd decade of life.Invasive cancer was diagnosed in 35 years of age in our study which was similar to that of Sherwani RK et al.[83], but contrast to Parker et al.[85] who reported carcinomas above the age of 70 years. Early marriage and early sexual activity in this part of the

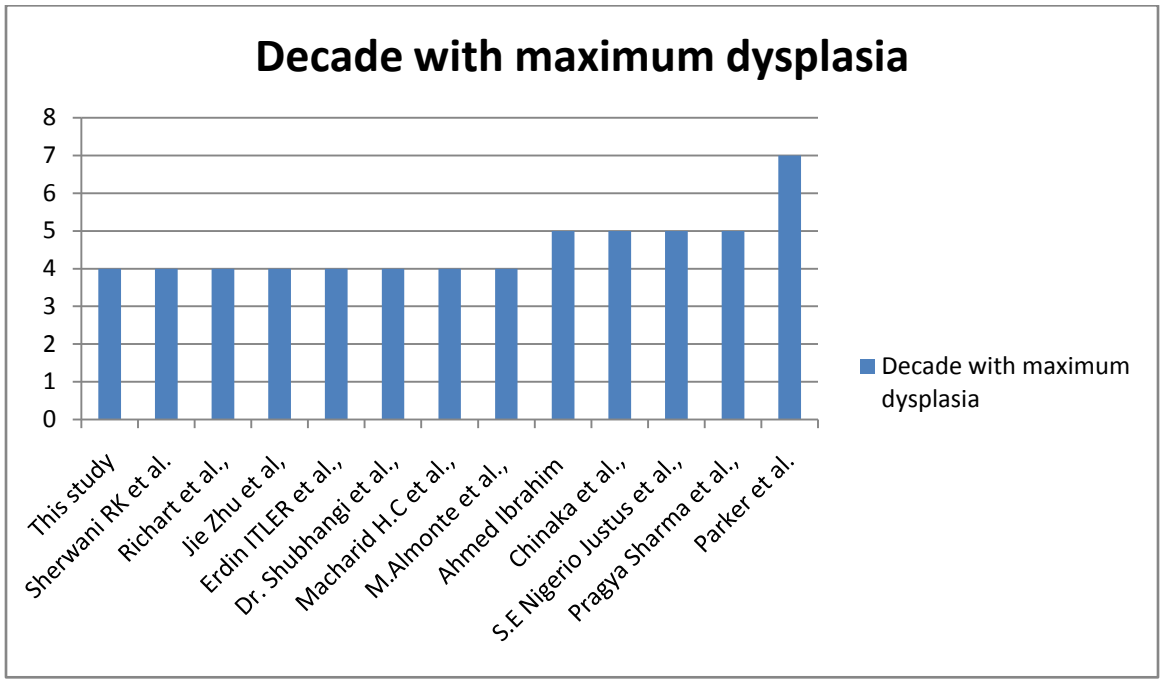
country may be responsible for the early onset of invasive cancer. Age with maximum dysplasia is shown in table 12 and chart 10.

Table 12 : Studies showing their ages with maximum dysplasia:

Studies	Decade with maximum dysplasia
This study	4th decade
Sherwani RK et al.[83]	4th decade
Richart et al.,[84]	4th decade
Jie Zhu et al,[98]	4th decade
Erdin ITLER et al.,[92]	4th decade
Dr. Shubhangi et al.,[114]	4th decade
Macharid H.C et al.,[118]	4th decade
M.Almonte et al., [121]	4th decade
Ahmed Ibrahim [123]	5th decade
Chinaka et al.,[120]	5th decade
S.E Nigerio Justus et al.,[112]	5th decade
Pragya Sharma et al.,[113]	3rd decade
Parker et al.[85]	>70 years

About 46.1% of the abnormal smears belonged to class III socioeconomic status and most of the dysplasia cases were observed in this group which was similar to Sherwani RK et al.,[83] and Christopherson and Parker [89],Pragya Sharma[113] . Parker noted that lower socio economic status women had marriage at a younger

Chart 10 : showing decade with maximum dysplasia.



age and child birth. Latest WHO report shows that 70% of cases are from the lower socio economic status due to lack of access to screening programs and late detection of diagnosis and treatment.[1] . A thesis done by Ahmed Ibrahim[123]., showed that uneducated and unemployed from the lower socioeconomic status showed more dysplasia. Also S.E. Nigerio Justus et al.[112], postulated that illiteracy, poverty, nonuse of screening methods and lack of communication after referral among lower socio economic status persons were responsible for the increased number of dysplasia among these persons.

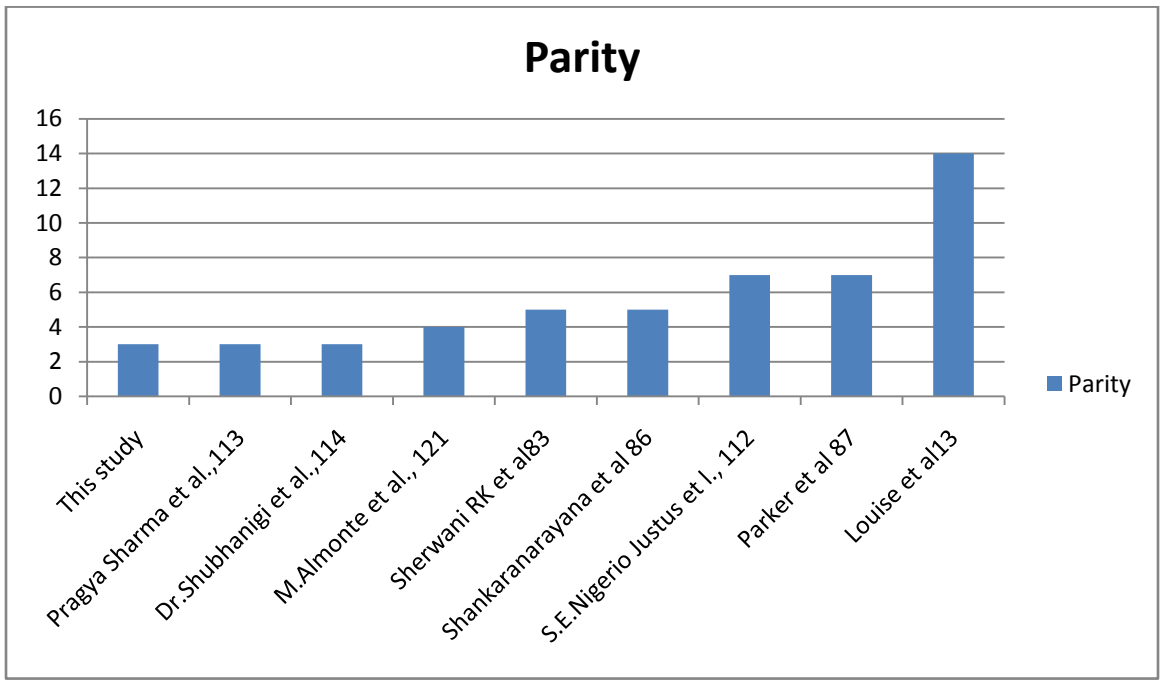
Carcinoma and dysplasia were mostly diagnosed when the parity was 3 or more in this study (53.8% of abnormal smears) similar to Pragya Sharma[113] and Dr.Shubanigi et al.,[114]. M.Almonte et al.,[121] reported more incidence of dysplasia when the parity was four. Sherwani RK et al[83] and Shankaranarayana et al [86] reported high incidence of dysplasia when the parity was more than five. Parker et al [87] showed four fold increase in incidence of dysplasia when the parity was seven or more similar to S.E Nigerio Justus et al.,[112]. Louise et al[13] found a 5 fold increase in risk of dysplasia when the parity was 14 or more. This is shown in table 13 and chart 11.

Table 13 : Studies showing parity with maximum dysplasia

Studies	Parity
This study	3
Pragya Sharma et al.,[113]	3
Dr.Shubhanigi et al.,[114]	3
M.Almonte et al., [121]	4
Sherwani RK et al[83]	5
Shankaranarayana et al [86]	5
S.E.Nigerio Justus et l., [112]	7
Parker et al [87]	7
Louise et al[13]	14

About 90%(90 cases) of cases in this study had onset of sexual activity before 25 years of age where majority of dysplasia was noted. Only 2 patients with the start of sexual activity above the age of 25 years had dysplasia. This finding was similar to Sherwani RK et al.,[83] and Rotkin et al.,[88]. Rotkin postulated that during intercourse, there is higher probability of transmission of infections and hence dysplasia is more common when there is early onset of sexual activity.[88] S.E Nigerio Justus et al., [112] and Pragya Sharma et al., [113] also postulated that early

Chart 11 : studies showing parities with maximum dysplasia



marriage and early onset of sexual activity were responsible for increased dysplasia.

Most of our cases complained of white discharge PV (46 cases,46%) followed by lower abdominal pain and bleeding PV(26 cases (26%) and 16 cases(16%) respectively). Sherwani et al [83] , Kenneth and Yao [90] , S.E Nigerio Justus et al., [112] ,Pragya Sharma et al.,[113] and Dr.Shubhanigi et al., [114] also had patients with similar complaints. Kenneth and Yao noted that white discharge was associated with neoplastic changes in cervix similar to our study where most of the dysplastic changes were in this subset of patients. In a study done by Robert ME et al., [91] Post coital bleeding was noted in many patients and all these had dysplasia(66.7%) and carcinoma (33.3%) . In contrast, only 2 cases in this study had this complaint and similar to Robert ME et al[91] this patient had carcinoma. Study done by M.Tarney et al .,[121], also had more number of patients with complaints of post coital bleeding in contrast to our study.(table 14)

Table 14 : studies and their most common complaints

Studies	Most common complaint
This study	White discharge P/V
Sherwani et al [83]	White discharge P/V
Kenneth and Yao [90]	White discharge P/V
S.E. Nigerio Justus et al., [112]	White discharge P/V

Pragya Sharma et al., [113]	White discharge P/V
Dr.Shubanigi et al., [114]	White discharge P/V
M.Tarney et al., [121]	Post coital bleeding
Robert ME et al.,[91]	Post coital bleeding

In our study, the number of satisfactory smears were 92%(92 cases) in Conventional PAP smear compared to(96 cases) 96% in Liquid based cytology. Most of the unsatisfactory smears in conventional PAP were due to thick and bloody smears whereas in LBC, it is due to reduced cell number. Percentages of satisfactory smears in other studies are shown in Table 15 and chart 12.

Table 15: studies and their percentage of satisfactory smears

	Conventional PAP(%)	**LBC(%)**
This study	92	96
Erdin ITLER et al.,[92]	99.50	99.95
Beerman et al.,[93]	99.1	99.87
Monsonego et al.,[94]	99.52	99.47
Sykes et al.,[95]	97.3	98.9
Longatto et al,[96]	89.6	98.6
Weintraub and Morabia et al.,[97]	72.2	92
Sherwani RK et al.,[83]	31.9	83.1
Chinaka et al., [120]	53.3	83.3
Guidelines for use of LBC in cervical cancer screening [117]	90.9	98.4

GP notebook [119]	91	98.6
M.Almonte et al., [121]	88.6	94.5
Singh VB et al., [116]	95.7	98.3

In all these studies, liquid based cytology had more number of satisfactory smears than the conventional PAP smear. In our study, the reason for unsatisfactoriness in conventional PAP smear is thick smear and obscuring blood and inflammatory cells and LBC is reduced number of cells similar to Monsanego et al.,[94]. According to Sherwani et al.,[83] in Liquid based cytology cytolysis and drying artifact is minimal or absent due to immediate fixative in a liquid fixative and lesser limited factors such as inflammatory cells, blood and mucus and in Conventional PAP is due to thick smear.

In the present study , number of ASC cases in CP was 12%(12 cases) and 6% in LBC(6 cases). The number of ASC cases in other studies are shown in table 16 and chart 13:

Table 16 : studies with their percentage of atypical squamous cells

Studies	CP(%)	LBC(%)
This study	12	6
Davey et al., [131]	3.8	4
ERDIN itler et al., [92]	2.1	2.6
Jie Zhu et al., [98]	8	4
O Abulafia et al., [55]	7.35	8.31

Bolick et al., [103]	2.42	2.97
Diaz –Rosario et al., [99]	4.76	4.53
Weintraub et al., [97]	1.50	2.40
Hatch et al., [111]	7.04	8
Guidos et al., [110]	2.03	3.40

The number of smears diagnosed as ASC was more in Conventional PAP smear (12 cases,12%) compared to Liquid based cytology(6 cases,6%) similar to that of Jie Zhu et al[98] and Diaz Rosario et al., [99] but contrast to studies by Davey et al, [131] , ERDIN itler et al., [92] ,Jie Zhu et al., [98] , O Abulafia et al., [55] , Bolick et al., [103] , Weintraub et al., [97] , Hatch et al., [111] , Guidos et al., [110] who showed that LBC was a better test for diagnosis of ASC.

In the present study number of LSIL increased from 8% in Conventional PAP to 12% in LBC. Other studies with similar results are shown in table 17 and chart 14:

Table 17 : studies with their percentage of LSIL cases

	Conventional PAP(%)	**LBC(%)**
This study	8	12
Sherwani RK et al[83]	10.6	18.1
Hutchinson et al 1992[48]	9	10.6
Diaz-Rosario and Kabawat et al[99]	1.6	2.7
Beerman et al [93]	0.22	0.27

Monsonego et al [94]	1.2	1.84
Hutchinson et al 1999[100]	3.03	3.40
Jie Zhu et al [98]	29	32
Sykes et al [95]	21	24.4
O.Abulafia et al [55]	6.24	7.15
M.Almonte et al., [121]	0.9	13.8
Chinaka et al., [120]	10.6	12.6

In all these studies, it can be seen that the rate of detection of LSIL is higher in LBC than Conventional PAP smears.

In our present study, rate of detection of HSIL was more with LBC(6 cases, 6%) compared to that of Conventional PAP(2 cases, 2%).Many studies have found similar results and these are shown in table 18 and chart 15.

Table 18 : studies with their percentage of HSIL cases

	Conventional PAP(%)	**LBC(%)**
My study	2	6
Sherwani RK et al [83]	0.6	4.3
Diaz-Rosario and Kabawat et al[99]	0.3	0.5
Beerman et al[93]	0.56	0.64
Monsonego et al [94]	0.52	0.60
Hutchinson et al 1999[100]	1.54	1.60
O.Abulafia et al [55]	4.24	4.45
M.Almonte et al., [121]	0.9	3.1
Chinaka et al., [120]	8.0	10.0

Similar to LSIL, LBC detected more HSIL lesions than conventional PAP smear.

In our study,1 frank carcinoma was detected in LBC, whereas no case were detected in Conventional PAP because the carcinoma cases in Conventional PAP smears were bloody and hence unsatisfactory for evaluation. In contrast to our study, Beerman et al.[93], Hutchinson et al.[100],Sykes et al.[95], O.Abulafia et al [55] reported higher detection of carcinoma in Conventional PAP than LBC.

Chart 12: studies showing percentage of satisfactory smears

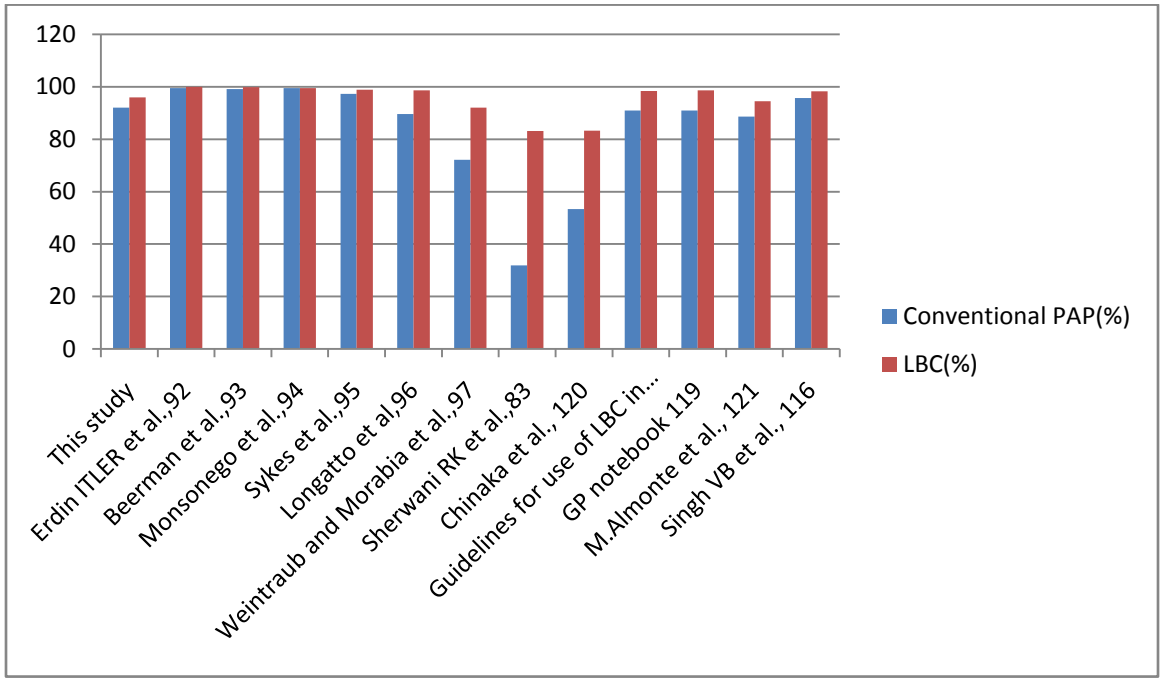


Chart 13: studies with their percentage of atypical squamous cells.

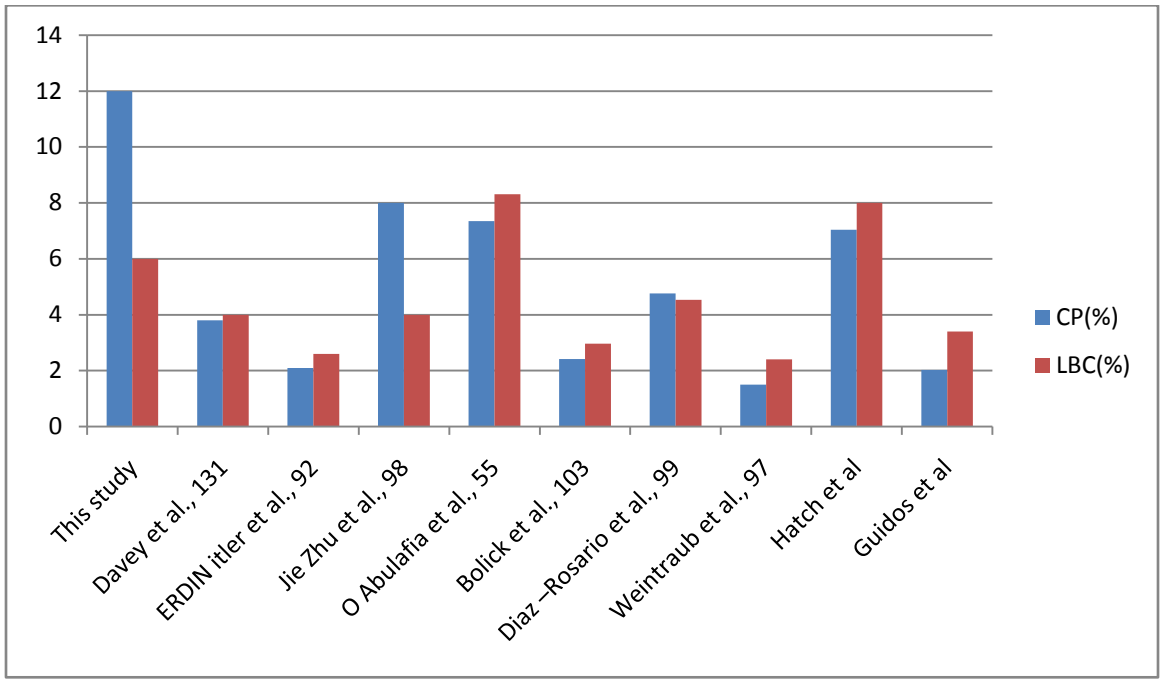


Chart 14: studies with their percentage of LSIL cases

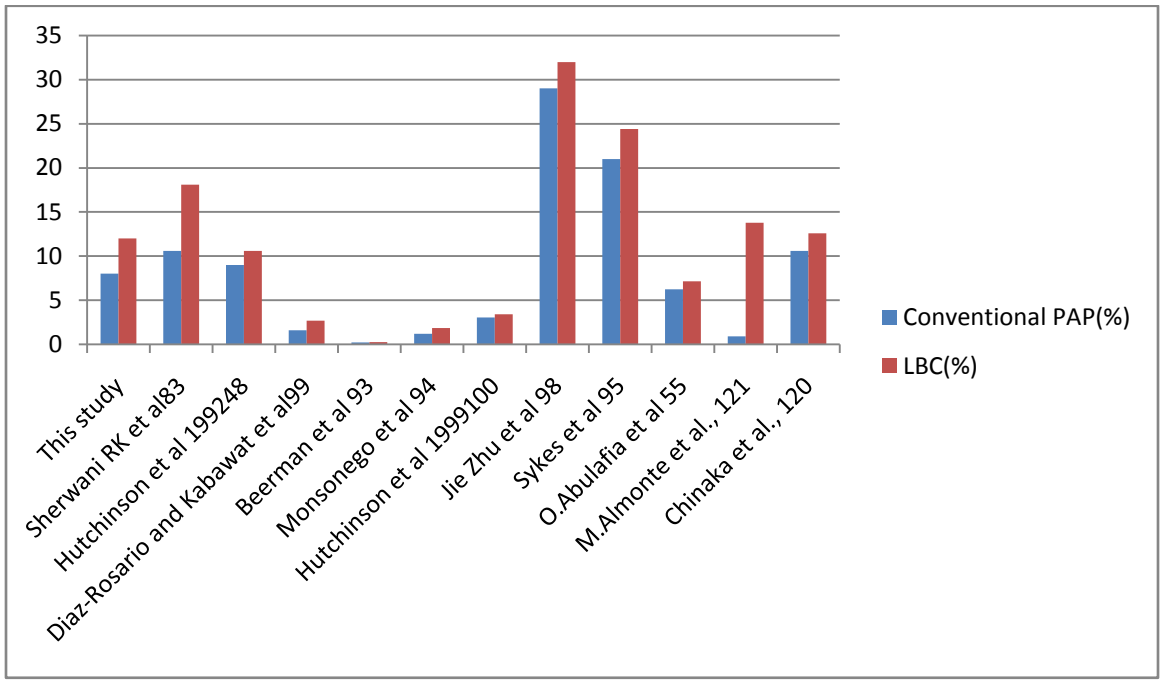
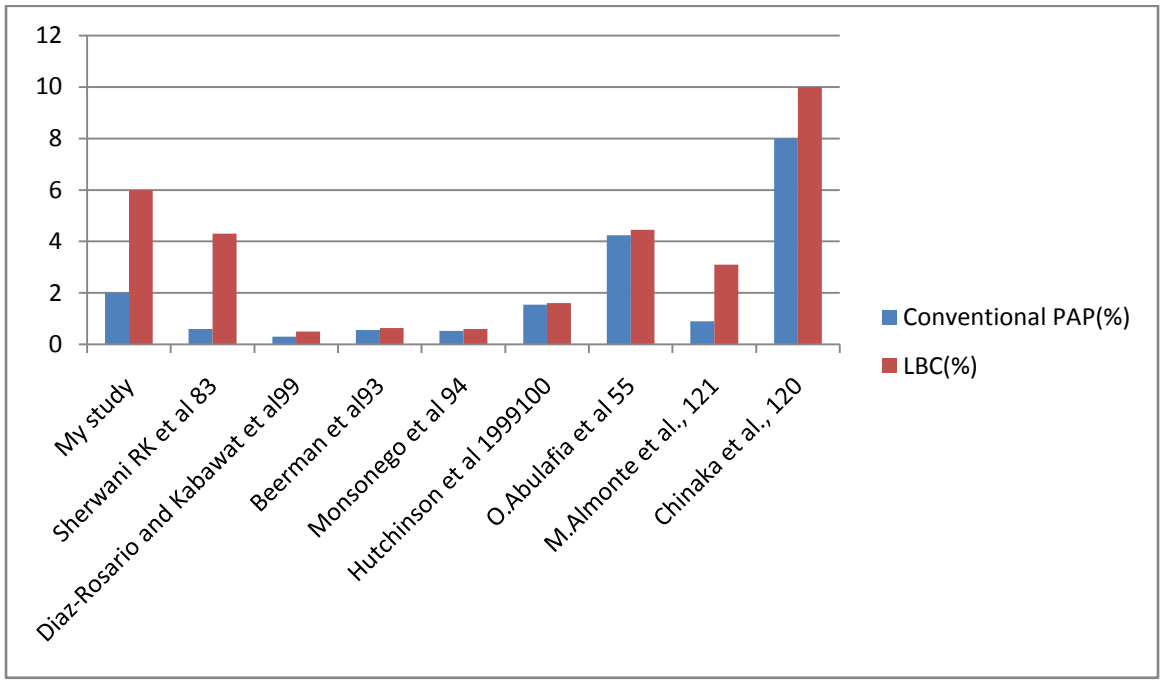


Chart 15 : studies with their percentage of HSIL cases..



Concordance between CP and LBC:

This study showed 84% concordance between Conventional PAP and LBC. Quite similarly, Hussein et al [101] showed 73% agreement and O.Abulafia et al [55] study, which is a comparison of 17 paired studies showed in general 90% concordance and 10% discordance. He showed that in various studies, discordance was as low as 1% and as high as 20%.

Concordance with histological findings:

Sensitivity:

Sensitivity of the screening tests in detecting low grade SIL:

Our study showed sensitivity of 40% in CP and 66% n LBC for detecting LSIL. Sensitivity of other studies are shown in table 19 and chart 16.

Table 19: Sensitivity of the screening tests in various studies in detecting low grade SIL

Studies	CP(%)	LBC (%)
This study	40	66
Lee K C et al., [124]	62.6	91.7
Kim Y R et al., [125]	64	86
Jeon Y K et al., [126]	73.7	78.9
Lim Y K et al., [127]	87.2	94.9
Park IA et al., [128]	89.6	82.8

Arbyn et al., [122]	75.6	79.1
ERDIN Itler et al., [92]	37.5	54.5
M.Almonte et al., [121]	26.21	69.66

Except for a study done by Park IA et al., [128], in all the other studies LBC was more sensitive than CP in detecting LSIL.

Sensitivity of the screening tests in detecting high grade SIL:

Our study showed sensitivity of 50% in CP and 100% in LBC. Sensitivity of other studies is shown in table 20 and chart 17.

Table 20 : Sensitivity of the screening tests in various studies in detecting high grade SIL

Studies	CP(%)	LBC(%)
This study	50	100
Lee K C et al., [129]	62	85.1
Jim K Young oh et al., [130]	76	92
Arbyn et al., [122]	55.2	57.1
ERDIN itler et al., [92]	50	61
Jie Zhu et al., [98]	47	66

In all these studies, LBC was a better test for diagnosing HSIL lesions.

Chart 16 : Sensitivity of the screening tests in various studies in detecting low grade SIL:

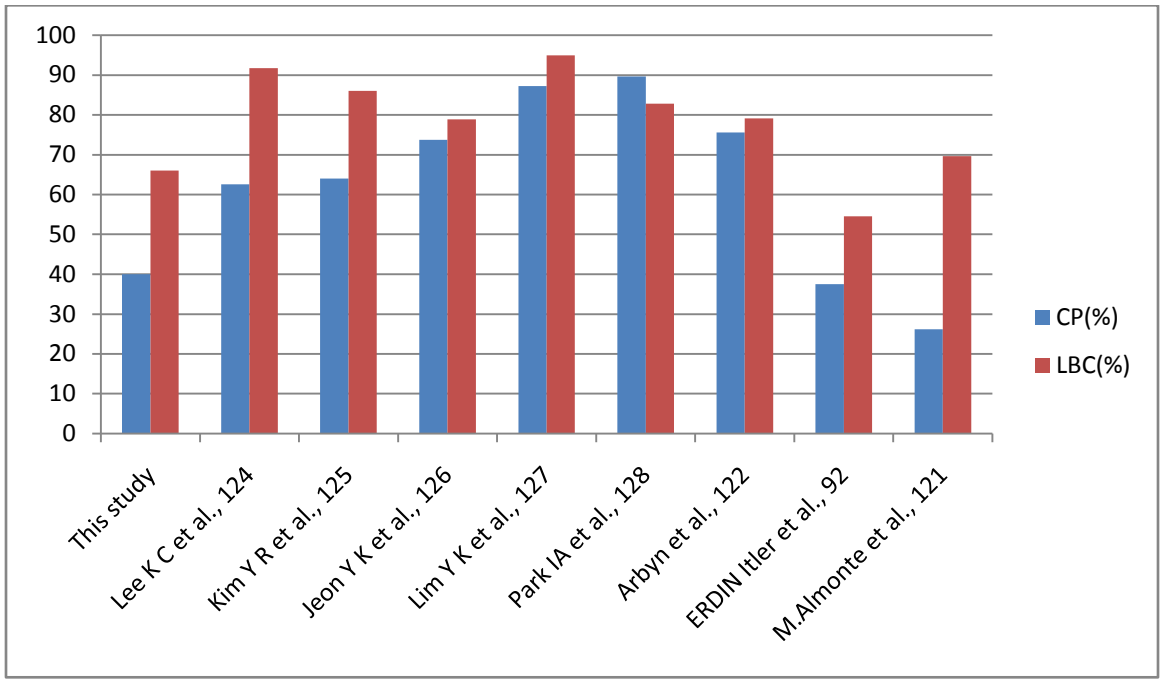
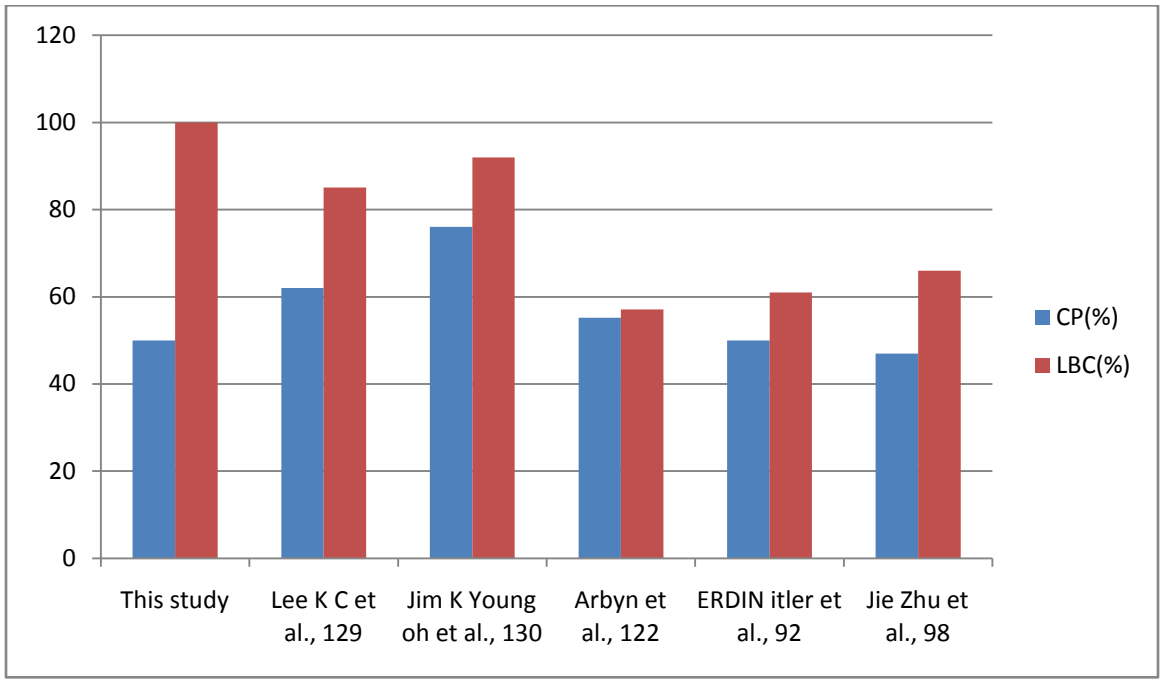


Chart 17 :Sensitivity of the screening tests in various studies in detecting high grade SIL:



Overall sensitivity:

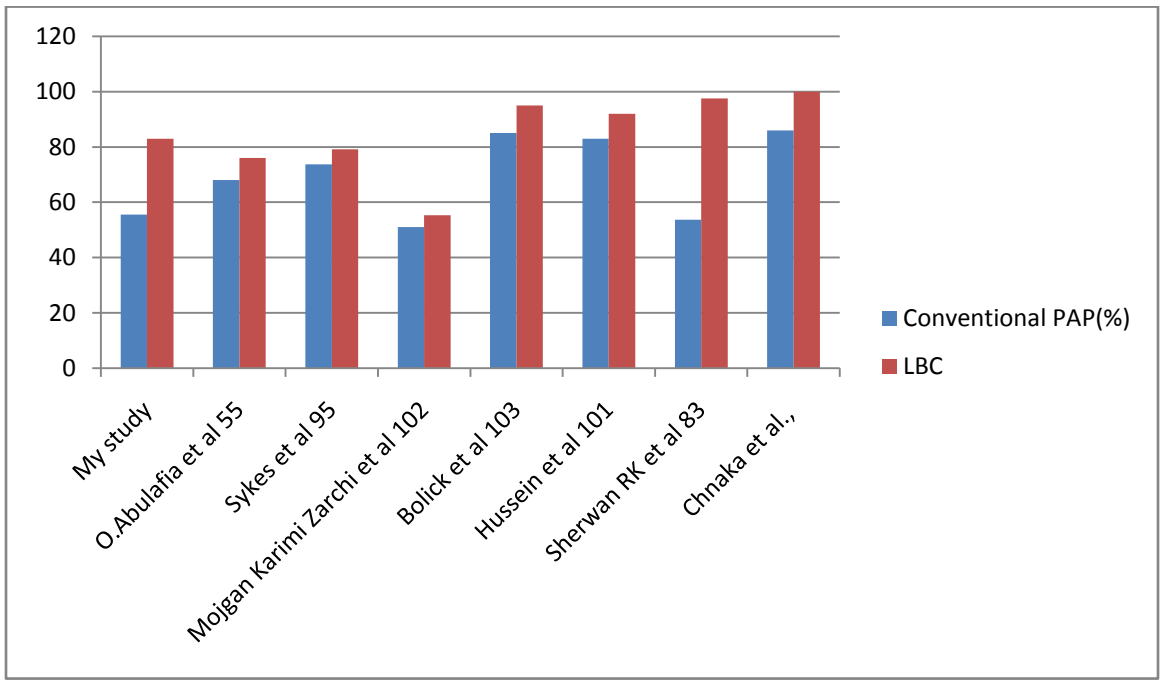
Our study showed sensitivity of 55.5% in CP and 83% in LBC. Sensitivity of other studies are shown in table 21 and chart 18.

Table 21 : Overall sensitivity of the screening tests in various studies

	Conventional PAP(%)	**LBC(%)**
My study	55.5	83
O.Abulafia et al [55]	68	76
Sykes et al [95]	73.7	79.1
Mojgan Karimi Zarchi et al [102]	51	55.3
Bolick et al [103]	85	95
Hussein et al [101]	83	92
Sherwan RK et al [83]	53.7	97.6
Chinaka et al., [120]	86	100

Sheets et al.[104], Sherman et al 1997 [105],Roberts et al 1997 [106], Papillo et al98 [107],Sherman et al 1998 [108] , yeoh et al99 [109], also showed higher sensitivity for LBC than CP and higher dectection rate similar to our study. O.Abulafia et al [55] compared 10 studies and showed that most of the studies had higher sensitivity for LBC and wide range of sensitivity (50%-90%).

Chart 18 : overall sensitivity of screening tests in various studies



Specificity:

Specificity of the screening tests for detection of LSIL:

Our study showed specificity of 93% for CP and 94% for LBC. Specificity of other studies are shown in table 22 and chart 19

Table 22: Specificity of the screening tests for detection of LSIL in various studies

Studies	CP(%)	LBC(%)
This study	93	94
Lee K C et al., [124]	96.1	75.9
Kim Y R et al., [125]	79.5	66
Jeon Y K et al., [126]	90.9	81.6
Lim Y K et al., [127]	87.2	92.3
Park IA et al., [128]	69.8	83
Arbyn et al., [122]	81.2	78.8

Our study showed increased specificity for LBC than CP similar to studies done by Lim YK et al., [127] and Park IA et al., [128] but contrast to Lee KC et al.,[124] , Kim YR et al., [125] ,Jeon YK et al., [126] and Arbyn et al., [122] who showed that CP is more specific than LBC.

Specificity of the screening tests for detection of HSIL:

This study showed specificity of 100% for CP and 96% for LBC. Specificity of other studies are shown in table 23 and chart 20.

Table 23 : Specificity of the screening tests for detection of HSIL in various studies

Studies	CP(%)	LBC(%)
This study	100	96
Lee KC et al., [129]	96.5	98.3
Jim K Young oh et al. [130]	76	79
Arbyn et al., [122]	96.7	97

In contrast with other studies, our study showed that CP was more specific than LBC in detection of HSIL.

Overall specificity:

Our study showed specificity of 83.7% and 86.5% in CP and LBC respectively. Specificites of other studies are shown in table 24 and chart 21.

Table 24 :Overall specificity of screening tests in various studies

	Conventional PAP(%)	LBC(%)
My study	83.7	86.5
O.Abulafia et al [55]	79	86
Sykes et al[95]	69	69
Mojgan Karimi Zarchi et al [102]	66	77.7
Bolick et al [103]	36	58
Sherwan RK et al [83]	50	50
Chinaka et al., [120]	97	100
Macharid et al., [118]	11	75

Other studies which shows higher specificity of LBC Sheets et al.[104], Sherman et al 1997[105],Roberts et al 1997 [106] , Papillo et al 98 [107] ,Sherman et al 1998 [108] , yeoh et al99 [109], Guidos et al 1995[110],Hatch et al 00 [111],Park et al2001 [56] Chinaka et al.,[120] , Macharid et al.,[118] similar to our study. But a study done by Hussein et al.,[101] showed high specificity for CP(82% vs & 76%).

Chart 19 : Specificity of the screening tests for detection of LSIL in various studies:

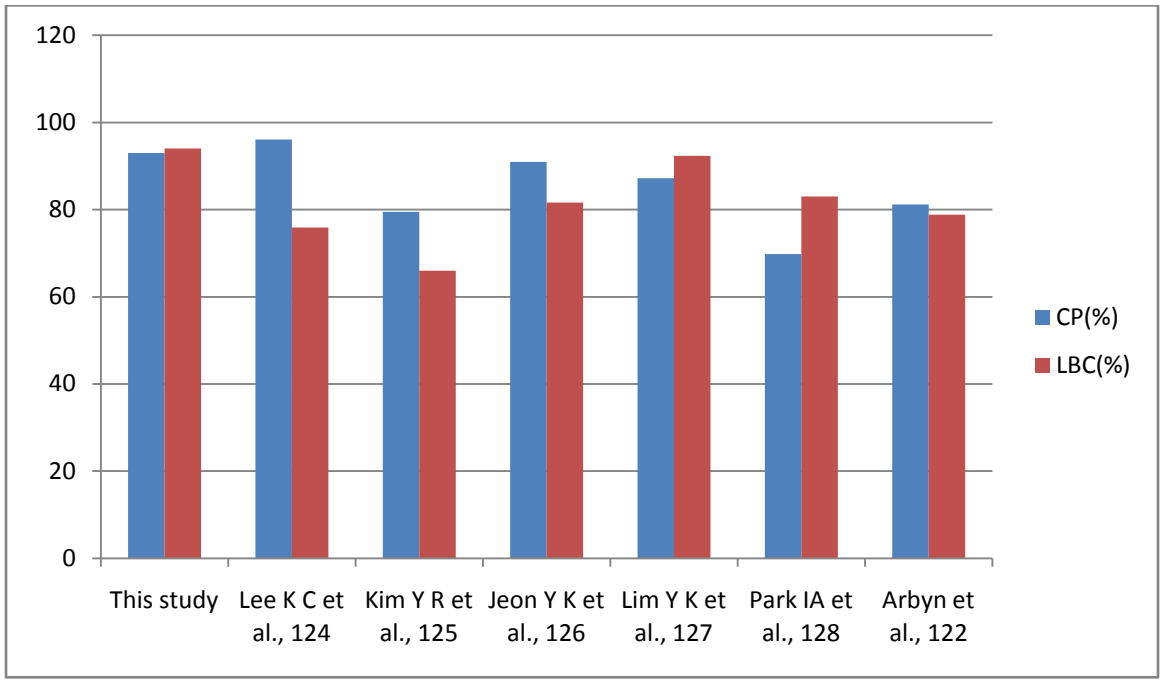


Chart 20 :Specificity of the screening tests for detection of HSIL in various studies:

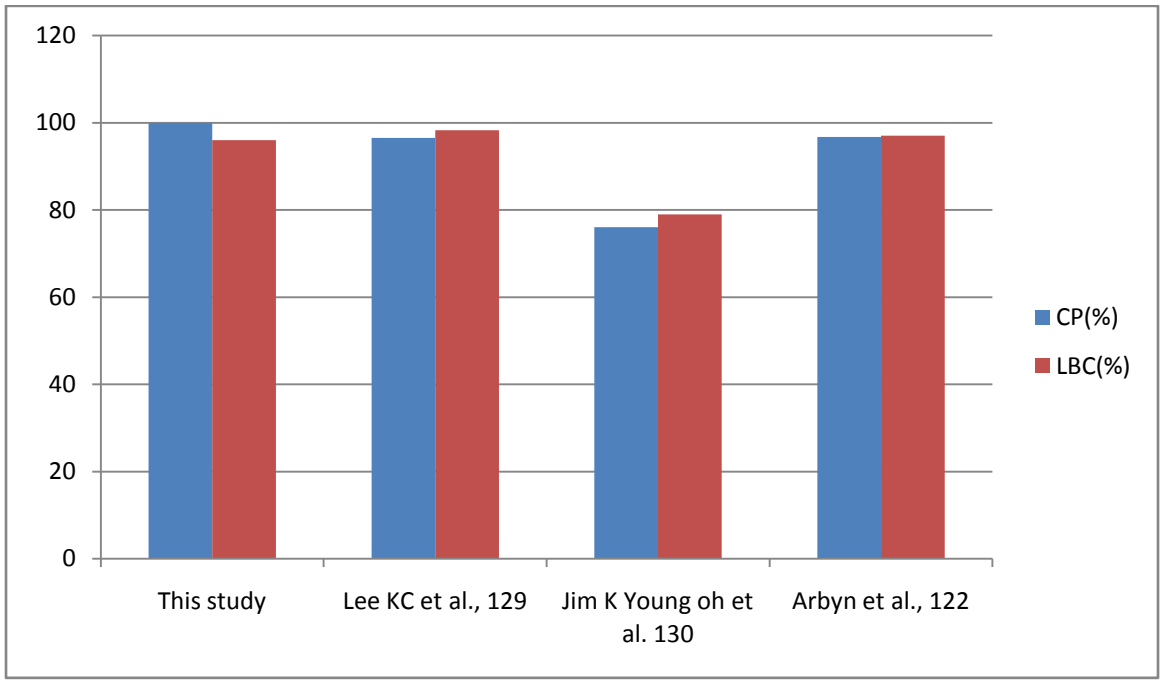
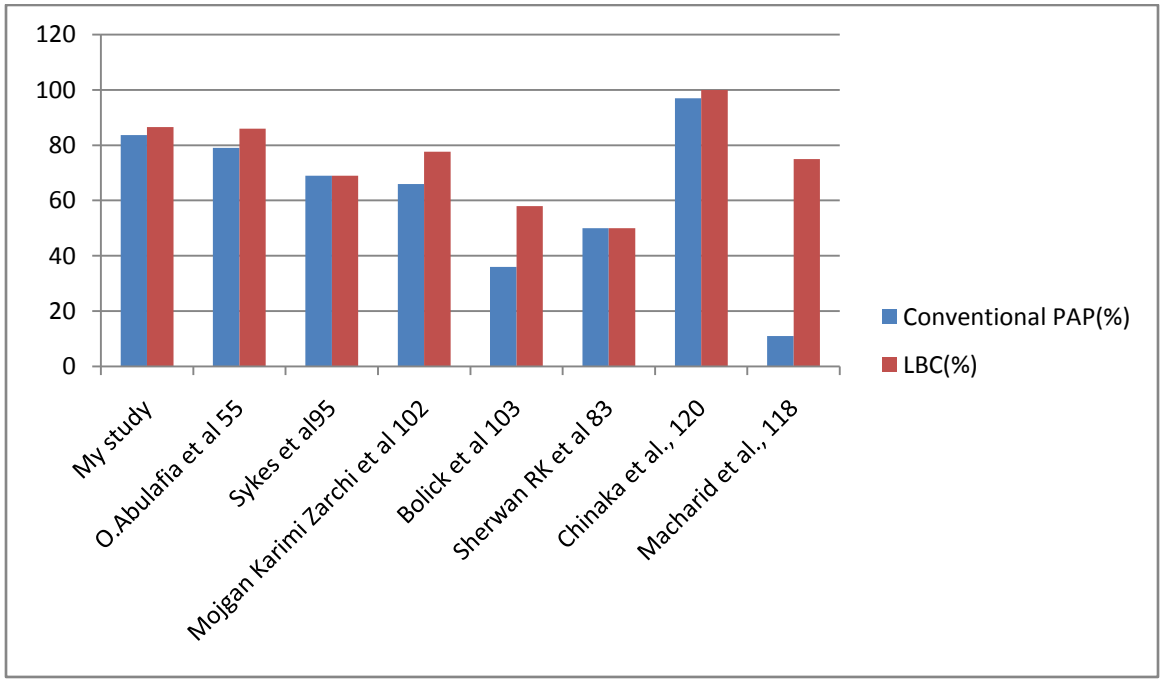


Chart 21 : Overall specificity of screening tests in various studies:



CONCLUSION

SUMMARY AND CONCLUSION

This study was conducted to compare the efficacy of CP and LBC in detecting intraepithelial lesions by comparing with the biopsy result which is considered the gold standard.

The results of the study is as follows:

1. 50% of cases who attended the screening program were in the fourth decade of life and LSIL and HSIL cases were mostly in the age group of 21-40 years of age.
2. Out of 90% cases who started sexual activity before 25 years of age, 92.3% cases showed dysplasia.
3. 46% of cases had parity 2. Dysplasia was reported more in cases when the parity was more than 3 (53.8% of the abnormal smears).
4. Most of the cases(36%) were in the class II of modified Prasad classification.46.1% of the 13 cases with dysplasia were in the class III of modified Prasad classification.
5. About 46% of the cases presented with white discharge per vaginum.
6. LBC detected abnormality in 28% of cases whereas CP detected in only 22% of cases.

7. 96% of cases were satisfactory for evaluation in LBC whereas 92% of cases were satisfactory in CP.
8. ASC cases was detected more in CP than LBC(12% vs 6%)
9. LSIL was detected more in LBC than CP(12% in LBC vs 8% in CP)
10. HSIL was deteted more in LBC than CP (8% in LBC vs 2% in CP)
11. LBC detected 1 case of carcinoma whereas none was detected in CP.
12. In our study, concordance between CP and LBC was 84%
13. Sensitivity for detection of LSIL was more for LBC than CP(66% in LBC vs 40% in PAP)
14. Sensitivity for detection of HSIL is more for LBC than CP(50 % for CP vs 100% for LBC)
15. LBC was more specific than CP for detection of LSIL(93% for CP vs 94% for LBC)
16. Specificity was more for CP than LBC for detection of HSIL cases(100% for CP vs 96% for CP)
17. Our study showed sensitivity of 55.5% in CP and 83% in LBC
18. Our study showed specificity of 83.7% and 86.5% in CP and LBC respectively

19. Controlling for Age Factor The LBC Vs HPE level is highly correlated (r = 0.617), LBC Vs PAP smear (r= 0.59) and Medium level of Correlated level of PAP ssmear Vs HPE (r = 0.4651)

So, the risk factors for the development of dysplasia according to our study is older age, early sexual activity, increasing parity and lower socio economic status.

Though CP is a simple and cost effective method of cervical screening which has been in use for more than 50 years, false negative rate is an important disadvantage. To overcome this,LBC was developed in which obscuring materials such as blood and mucus was less (which was more in CP) and provided slides with a monolayer of well preserved cells which was easier to interpret. So, we compared both these methods in our study and compared it with the biopsy result. LBC provided more representative samples and morphology of the cells were better visualized due to less obscuring materials. LBC generated more number of satisfactory smears and intraepithelial lesions were detected more in LBC. Sensitivity and specificity was more in LBC than CP. LBC showed better correlation with HPE than CP statistically.

To conclude, in a country where more number of people belong to lower socio – economic status and with higher incidence of cervical cancer , screening

plays an important role in prevention . so awareness should be created about the screening programs and government should take adequate measures to improve the quality of the screening procedures by introducing improved methods like LBC, since cervical cancer is preventable by early detection and intervention.

APPENDIX

APPENDIX I

PROFORMA

NAME:

AGE: SEX:

INCOME: WORK:

AGE OF MENARCHE: AGE OF MARRIAGE:

YEARS OF SEXUAL LIFE: AGE OF MENOPAUSE:

NO OF CHILDREN:

AGE OF FIRST CHILD BIRTH:

AGE OF LAST CHILD BIRTH:

PRESENTING COMPLAINTS:

LOWER ABDOMINAL PAIN YES/NO

SPOTTING YES/NO

WHITE DISCHARGE YES/NO

DIFFICULTY IN MITURITION YES/NO

POST COITAL BLEEDING YES/NO

ITCHING YES/NO

IRREGULAR PERIODS YES/NO

APPENDIX II

RAPID PAP STAIN

REQUIREMENTS:

1. Harris haematoxylin
2. EA and OG combo
3. 100% alcohol

PROCEDURE:

1. Stain in harris haemotoxylin for 3 minutes
2. Wash in 100% alcohol
3. Rinse in running tap water for blueing for 5 minutes
4. Stain in EA and OG combo for 3 minutes
5. Wash in 100% alcohol – 3 times till all the stain is washed off.
6. Dry the slides and mount with DPX.

APPENDIX III

HAEMATOXYLIN AND EOSIN STAIN

PREPARATION OF SOLUTION:

HARRIS HAEMATOXYLIN:

- Distilled water -1000ml
- Ammonium alum – 100gm
- Absolute ethyl alcohol – 50ml
- Mercuric oxide – 2.5gm
- 100 gm of ammonium alum dissolved in 1000ml of distilled water by heating at 60 degree centigrade. Add solution of 5 gm of haematoxylin in 50ml of ethylalcohol and bring rapidly to boil. When it begns to boil,remove from flame and add 2.5gm of mercuric oxide . Mix by swirling gently.

EOSIN STAIN:

- Eosin Y – 1gm
- Distilled water – 20ml
- 95% ethanol – 80ml
- Glacial acetic acid – 0.2ml
- Dissolve 1gm of eosin Y in 20ml of water,then add 80ml of 95% ethylalcohol and 0.2ml of glacial acetic acid.

PROCEDURE:

1. Bring the sections to water
2. Dip in Harris haematoxylin for 15minutes
3. Rinse in tap water
4. Differentiate in 1% acid alcohol – 3-4 quick dips
5. Wash in tap water briefly
6. Dip in ammonia water or saturated lithium carbonate until the sections are blue.
7. Wash in running tap water for 10-20 minutes
8. Stain with eosin for 15seconds to 2 minutes depending on the age of the eosin and the depth of counter stain.
9. Rinse in tap water.
10. Dip in 95% alcohol.
11. 3 changes in absolute alcohol.
12. Xylene - 2 changes.
13. Mount in DPX mountant.

APPENDIX IV

BETHESDA SYSTEM OF CLASSIFICATION (2001)

The Bethesda System - 2001

The Bethesda System-2001 consists of several components, as outlined below:

SPECIMEN TYPE

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

SPECIMEN ADEQUACY

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

• Unsatisfactory for evaluation (specify reason)

• Specimen rejected/not processed (specify reason)

• Specimen processed and examined, but unsatisfactory for evaluation of epithelial abnormality because of (specify reason)

GENERAL CATEGORIZATION (Optional)

• Negative for Intraepithelial Lesion or Malignancy

• Epithelial Cell Abnormality: See Interpretation/Result

• Other: see Interpretation/Result (e.g. endometrial cells in a woman ≥40 yrs)

INTERPRETATION/RESULT

A. Negative for Intraepithelial Lesion or Malignancy

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

1. 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

2. **Other Non-neoplastic Findings** (Optional to report; list not inclusive):

• Reactive cellular changes associated with

- inflammation (includes typical repair)

- radiation

- intrauterine device (IUD)

• Glandular cells status posthysterectomy

• Atrophy

3. Other

• Endometrial cells (in a woman ≥40 years of age) (specify if "negative for squamous intraepithelial lesion")

B. Epithelial Cell Abnormalities

1. Squamous cell:

• Atypical squamous cells

- of undetermined significance (ASC-US)

- cannot exclude HSIL (ASC-H)

• Low-grade squamous intraepithelial lesion (LSIL) (encompassing: HPV/mild dysplasia/CIN1)

• High-grade squamous intraepithelial lesion (HSIL)(encompassing: moderate and severe dysplasia, CIS, CIN 2 and CIN 3) with features suspicious for invasion (if invasion is suspected)

• Squamous cell carcinoma

3. Glandular Cell:

• Atypical

- endocervical cells (NOS or specify in comments)

- endometrial cells (NOS or specify in comments)

- glandular cells (NOS or specify in comments)

• Atypical

- endocervical cells, favor neoplastic

- glandular cells, favor neoplastic

• Endocervical adenocarcinoma in situ

• Adenocarcinoma

- endocervical
- endometrial
- extrauterine
- not otherwise specified (NOS)

C. Other Malignant Neoplasm: (specify)

ANCILLARY TESTING

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

AUTOMATE REVIEW

If specimen was examined by automated device, specify the device and the result.

EDUCATIONAL NOTES AND SUGGESTIONS (optional)

Suggestions should be concise and consistent with clinical follow-up guidelines published by professional organizations (references to relevant publications may be included).

APPENDIX V

ANDERMANN ET AL SCREENING CRITERIA.

A.The Wilson jungner criteria for appraising the validity of a screening program:

- The disease condition screened should be an important health problem
- Natural history of the disease condition should be well understood
- The disease should have an detectable early stage
- Early stage diagnosis and treatment should have more benefits than treatment at a later stage
- A suitable test should be developed for the early stage
- The screening test should be acceptable by the population
- The interval for repeating the test should be determined
- Health care services should be made adequate for the extra clinical workload resulting from screening
- Both physical and psychological risks should be less than the benefits
- Financially, there should be a balance between the costs and benefits

B. The criteria of screened condition

- The disease condition should be an important health problem
- The epidemiology and natural history of the disease, including development from latent to declared disease, should be adequately understood and there should be a detectable risk factor, disease marker, latent period or early symptomatic stage.
- All the cost-effective primary prevention intervention should have been implemented as far as practicable
- If the carriers of a mutation are identified as a result of screening the natural history of people with this status should be understood, including the psychological implications

C. Criteria of screening test:

- Screening test should be simple, safe, precise and validated

- The distribution of test values in the target population should be known and a suitable cutoff level defined and agreed
- The screening test should be acceptable by the population
- There should be an agreed policy on the further diagnostic investigation of individuals with a positive test result and on the choices available to those individuals

If the screening test is done for mutations, the criteria to select the subset of mutations to be covered by screening, if all possible mutations are not being tested for, should be clearly set.

D. The treatment for screened condition:

- An effective treatment or intervention should be available for patients who are identified through early detection, with evidence that the early diagnosis and treatment will lead to better outcomes than late treatment
- An evidence based policies should be there covering which individuals should be offered treatment and the appropriate treatment to be offered
- All healthcare providers should be optimized about the clinical management of the condition and patient outcomes prior to participation in a screening program.

APPENDIX – VI

UK NATIONAL SCREENING COMMITTEE SCREENING PROGRAM.

- High quality randomized control trials should have been conducted in the past to provide evidence that the mortality and morbidity is reduced by screening tests. Where the persons undergoing screening can get an informed choice about the screening procedure evidence must be there from the high quality trials that the screening procedure accurately measures risk. The information that is provided about the screening procedure must be of value and the information that is provided should be readily understood by the person undergoing screening.
- Evidence should be there that the complete screening procedure is ethically, clinically and socially acceptable to the public and health care providers
- The screening programs benefit should be more than the psychological and physical harm caused due to screening procedure, diagnostic test and the treatment given after the screening procedure
- The expenditure for the screening program which will include testing, diagnosis ,treatment, administration, training and quality assurance should be correctly balanced in relation to the cost of medical care as a whole
- A plan for the monitoring and management of the screening procedure and a set of quality assurance standards.
- The staffs and facilities required for the screening test, diagnosis, treatment and program management should be readily available before the commencement of the screening program.

- The other options available for managing the condition should be thought about before starting the screening program so that no more cost effective intervention is introduced or new intervention in the screening procedure is introduced within the available resources after the start of the screening program.
- For assisting the participants in the screening program to make an informed choice, there should be an evidence based information about the potential consequences of testing, investigation and treatment
- Public pressure for reducing the screening interval, for eligibility criteria widening and for sensitivity improvement of the screening test process should be anticipated. Decisions about the parameters should justified scientifically to the public.
- The program should be acceptable to the people identified as carriers and to the family members if the screening test is done for identifying mutations.

APPENDIX – VII

THE AMERICAN JOURNAL OF CLINICAL PATHOLOGY SCREENING GUIDELINES FOR CERVICAL CANCER:

- 21 years should be the age at which screening should be started.
- Screening periodicity – Now, annual screening is not recommended
    1. <21 years – screening is not recommended
    2. 21-29 years – cytology should be done every 3 years
    3. 30 to 64 years – every 3 years cytology alone is acceptable, contesting (HPV and cytology) every 5 years is preferred
    4. >65 years – if the patient has adequate prior negative screening, screening is not recommended
    5. After hysterectomy – screening is not necessary
    6. After HPV vaccination – screening as in unvaccinated women

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