DIAGNOSTIC VALUE OF IMMUNOMARKERS IN CERVICAL CANCER SRCEENING

Dissertation submitted in

partial fulfillment of the requirements for the degree of

M.D. (PATHOLOGY)

BRANCH - III

GOSCHEN INSTITUTE OF PATHOLOGY

MADRAS MEDICAL COLLEGE

CHENNAI – 600 003

THE TAMIL NADU

DR. M.G.R. MEDICAL UNIVERSITY

CHENNAI

APRIL 2015

CERTIFICATE

This is to certify that this Dissertation entitled **"DIAGNOSTIC VALUE OF IMMUNOMARKERS IN CERVICAL CANCER SCREENING"** is the bonafide original work of **Dr.RAMYA.N**, in partial fulfillment of the requirement for M.D., (Branch III) in Pathology examination of the Tamilnadu Dr. M.G.R Medical University to be held in April 2015.

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DECLARATION

I, **Dr. RAMYA.N**, solemnly declare that the Dissertation titled "**DIAGNOSTIC VALUE OF IMMUNOMARKERS IN CERVICAL CANCER SCREENING**" is the bonafide work done by me at Institute of Social Obstetrics and Govt. Kasturba Gandhi Hospital for Women and Children, Madras Medical College under the expert guidance and supervision of **Prof.Dr.K.RAMA,** M.D., Professor of Pathology, Institute of Social Obstetrics and Govt.Kasturba Gandhi Hospital for Women and Children, Madras Medical College. The dissertation is submitted to the Tamilnadu Dr. M.G.R Medical University towards partial fulfillment of requirement for the award of M.D., Degree (Branch III) in Pathology.

Place: Chennai

Date: **Dr. RAMYA. N**

ACKNOWLEDGEMENT

I express my sincere thanks to **Prof. Dr. R. VIMALA, M.D.**, Dean, Madras Medical College and Rajiv Gandhi Government General Hospital, for permitting me to utilize the facilities of the Institution.

I also thank my Director, **Prof. Dr. M. SARASWATHI, M.D.**, Professor and Director, Institute of Pathology, Madras Medical College, for her valuable opinions and encouragement throughout the study.

I am very grateful and indebted to **Prof. Dr. K. RAMA, M.D.**, Professor of Pathology, Institute of Social Obstetrics and Govt. Kasturba Gandhi Hospital for Women and Children, Madras Medical College, for her advice, suggestions, expert guidance, constant support, and encouragement throughout the study.

I am truly thankful to **Prof. Dr. P. KARKUZHALI, M.D**., former Director, Institute of Pathology, Madras Medical College for her valuable suggestions during the initial period of the study.

I am thankful to **Prof. Dr. SHANTHA RAVISANKAR** M.D., **Prof.Dr.R.PADMAVATHI** M.D., **Prof. Dr.V.RAMAMURTHY** M.D., **Prof. Dr. GEETHA DEVADAS** M.D.,D.C.P., **Prof. Dr. M.P.KANCHANA** M.D, **Prof. Dr. RAJAVELU INDIRA** M.D., **Prof. Dr. SUDHA**

VENKATESH M.D., **Prof. Dr. S. PAPPATHI** M.D.,D.C.H., for their valuable suggestions during the study.

I express my sincere thanks to all my Assistant Professors for their help and suggestions during the study.

I am thankful to my colleagues, friends, technicians and staff of the Institute of Social Obstetrics and Govt. Kasturba Gandhi Hospital for Women and Children and Institute of Pathology, Madras Medical College, Chennai for all their help and support they extended for the successful completion of this dissertation.

Last but not the least, I take this opportunity to express my heartfelt thanks to my parents Mr.S.Natarajan, Mrs.N.Santhi and my brother Mr.N.Karthik for their unconditional love and support.

INSTITUTIONAL ETHICS COMMITTEE
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CERTIFICATE OF APPROVAL

To
Dr. N. Ramya,
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Madras Medical College, Chennai-3.

Dear Dr. N. Ramya,

The Institutional Ethics Committee of Madras Medical College, reviewed and discussed your application for approval of the proposal entitled **"Diagnostic Value of Immunomarkers in Cervical Cancer Screening"** No.09022014

The following members of Ethics Committee were present in the meeting held on 04.02.2014 conducted at Madras Medical College, Chennai-3.

1. Dr. G. Sivakumar, MS FICS FAIS -- Chairperson
2. Prof. Kalaiselvi, MD -- Member Secretary
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3. Prof. Dr. K.Ramadevi, -- Member
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6. Thiru. S. Govindasamy, BABL -- Lawyer
7. Tmt. Arnold Saulina, MA MSW -- Social Scientist

We approve the proposal to be conducted in its presented form.

Sd/Chairman & Other Members

The Institutional Ethics Committee expects to be informed about the progress of the study, and SAE occurring in the course of the study, any changes in the protocol and patients information / informed consent and asks to be provided a copy of the final report.

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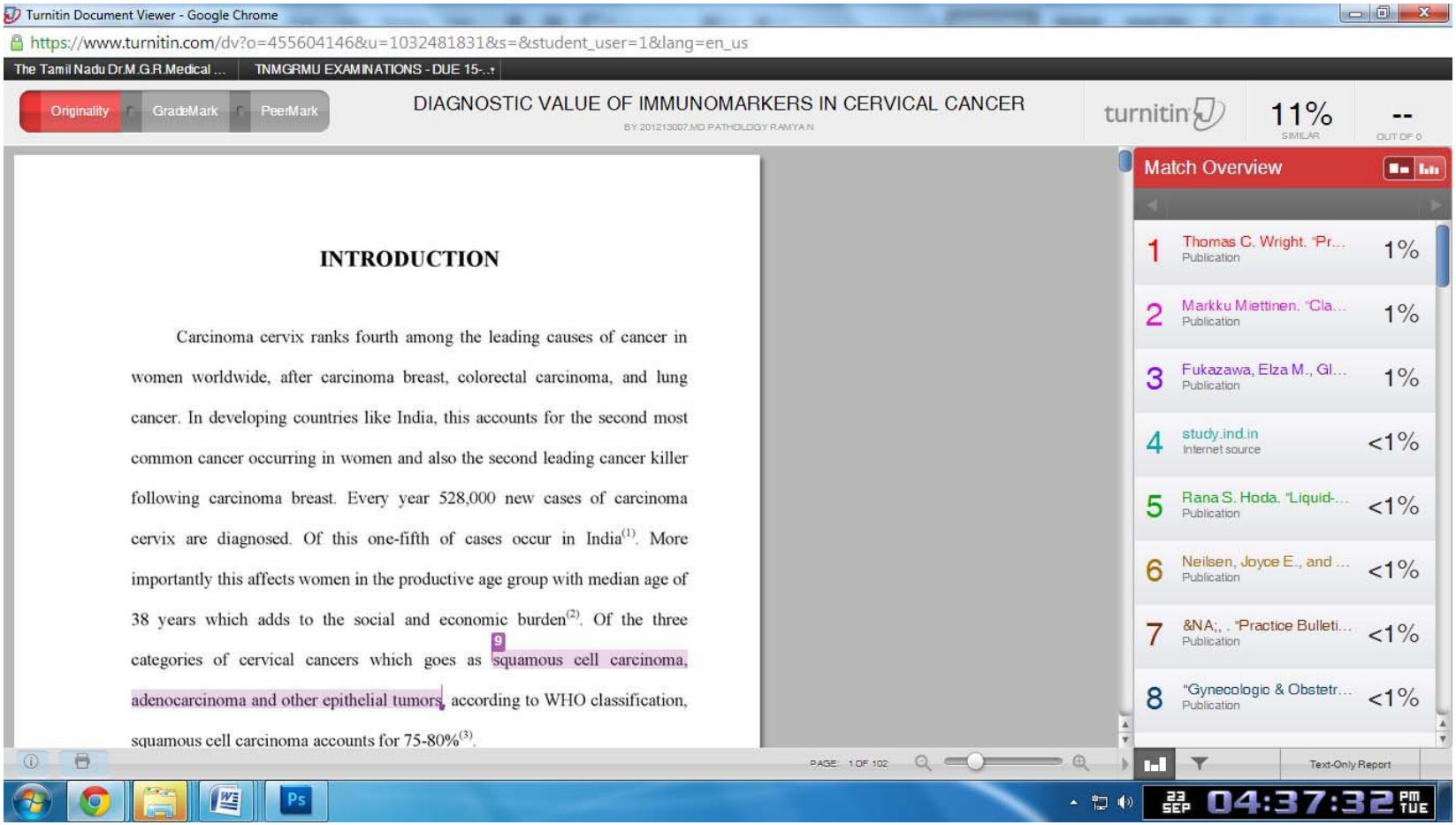
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Word count:	14,987
Character count:	77,921
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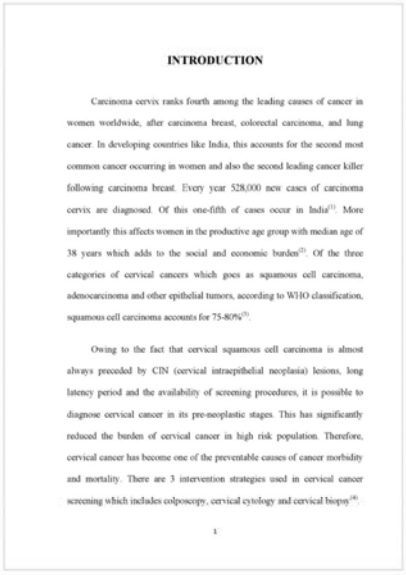

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Owing to the fact that cervical squamous cell carcinoma is almost always preceded by CIN (cervical intraepithelial neoplasia) lesions, long latency period and the availability of screening procedures, it is possible to diagnose cervical cancer in its pre-neoplastic stages. This has significantly reduced the burden of cervical cancer in high risk population. Therefore, cervical cancer has become one of the preventable causes of cancer morbidity and mortality. There are 3 intervention strategies used in cervical cancer screening which includes colposcopy, cervical cytology and cervical biopsy[4].

1

ABBREVIATIONS

WHO	:	World Health Organization
HPV	:	Human Papillomavirus
IUD	:	Intra Uterine device
OCP	:	Oral Contraceptive Pill
ASCCP	:	American Society for Colposcopy and Cervical Pathology
HPE	:	Histo-Pathologic Examination
LBC	:	Liquid Based Cytology
ICC	:	Immunocytochemistry
IHC	:	Immunohistochemistry
VIA	:	Visual Inspection with Acetic acid
VILI	:	Visual Inspection with Lugol's Iodine
LGL	:	Low Grade Lesion
HGL	:	High Grade Lesion
ASCUS	:	Atypical Squamous Cell of Undetermined Significance
ASC-H	:	Atypical Squamous Cell-cannot exclude HSIL
LSIL	:	Low grade Squamous Intraepithelial Lesion
HSIL	:	High grade Squamous Intraepithelial Lesion
CIN	:	Cervical Intraepithelial Neoplasia
SCC	:	Squamous Cell Carcinoma
SCC-WD	:	Squamous Cell Carcinoma-Well Differentiated
SCC-MD	:	Squamous Cell Carcinoma-Moderately Differentiated
SCC-PD	:	Squamous Cell Carcinoma-Poorly Differentiated

CONTENTS

ABSTRACT

DIAGNOSTIC VALUE OF IMMUNOMARKERS IN CERVICAL CANCER SCREENING

BACKGROUND: Carcinoma cervix is one of the leading causes of cancer deaths and lack of screening is found to be the major risk factor. Hence it is important to use screening methods which have better sensitivity.

OBJECTIVE: The objective of our current study is to evaluate the diagnostic value of use of immunomarkers like $p16^{INK4a}$ and Ki-67 in cervical smears with HPE as gold standard and to assess their sensitivity and specificity in relation to cervical cytology.

METHODS: We did immunostaining with $p16^{INK4a}$ and Ki-67 in liquid based cervical cytology sample and cervical biopsy specimens for 30 patients. $p16^{INK4a}$ is expressed in dysplastic cervical cells. Ki-67 is a proliferation marker. In cervical smears (immunocytochemistry), $p16^{INK4a}$ expression was graded as 0-negative, 1-weakly positive and 2-strongly positive. Ki-67 expression was scored according to percentage of dysplastic cells showing nuclear positivity as follows (0-negative, 1-<10%, 2-10-50% and 3->50% of dysplastic cells). In tissue sections (immunohistochemistry), p16 was interpreted as positive or negative and Ki-67 expression was interpreted according to the thickness of epithelium involved.

RESULTS: $p16^{INK4a}$ showed both nuclear and cytoplasmic staining and was positive in cervical smears of 81.8% of CIN I, 100% of CIN II, CIN III and malignant cases. All cervicitis cases were negative. All the CIN I cases showed weak staining, whereas high grade lesions and malignancy showed strong staining pattern. Ki-67 showed nuclear positivity. 54.5% of CIN I cases showed positivity in <10% of dysplastic cells, 60% of CIN II and 75% of CIN III cases showed positivity in 10-50% of dysplastic cells and 75% of malignant cases showed positivity in >50% of dysplastic cells. These associations were found to be statistically significant. The sensitivity for immunocytochemistry with $p16^{INK4a}$ and Ki-67 was 88.89% and 89.28% respectively, which were more than that for morphological interpretation in cervical cytology (77.78%).

CONCLUSION: The use of immunomarkers in cervical smears has significantly increased the sensitivity of cervical cancer screening in our study. Hence steps should be taken to incorporate immunocytochemistry in cervical cancer screening in areas with better financial and laboratory resources.

INTRODUCTION

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Owing to the fact that cervical squamous cell carcinoma is almost always preceded by CIN (cervical intraepithelial neoplasia) lesions, long latency period and the availability of screening procedures, it is possible to diagnose cervical cancer in its pre-neoplastic stages. This has significantly reduced the burden of cervical cancer in high risk population. Therefore, cervical cancer has become one of the preventable causes of cancer morbidity and mortality. There are 3 intervention strategies used in cervical cancer screening which includes colposcopy, cervical cytology and cervical biopsy[(4)].

According to ASCCP recommendations 2012, cancer screening should begin at the age of 21. This should be done every 3 years and continued upto the age of 65. HPV co-testing also can be included in screening. Despite the availability of these screening procedures, many are diagnosed only in the advanced stage. This is due to the limitations of cervical cytology (Pap smear) interpretation which are as follows,

- Lack of objectivity in the morphologic criteria for grading CIN
- Inter-observer variability

So to increase the diagnostic accuracy of the screening procedures, need for the use of biomarkers in cervical cytology has arisen now. The various biomarkers that can be used to improve the cervical cancer screening are as follows[4],

- $p16^{INK4a}$
- Ki-67
- Pro-Ex C
- HPV L1 capsid

Cervical cytology has a high specificity of 97%-100%, but a low sensitivity of 50 to 70%. When biomarkers like $p16^{INK4a}$ and Ki-67 are used in

cervical cytology, the sensitivity of the screening procedure is increased to 90%.

$p16^{INK4a}$ is a tumour suppressor protein and it inhibits cyclin-dependent kinase 4 and 6 which are regulatory proteins in cell cycle. So it is expressed in cervical dysplastic cells only. $p16^{INK4a}$ over-expression shows a positive correlation with HPV infection and degree of cervical neoplasia. Ki-67 is a marker of actively dividing cells. Therefore presence of $p16^{INK4a}$ and Ki-67 positivity in cervical smear is a marker of cervical dyskaryosis[5].

AIMS

AND

OBJECTIVES

AIMS AND OBJECTIVES

1. To study the incidence and distribution of cervical pre-neoplastic and neoplastic lesions diagnosed by HPE at the Institute of Social Obstetrics and Govt. Kasturba Gandhi Hospital for Women and Children, Madras Medical College, during February 2014 to July 2014.
2. To evaluate the diagnostic value of use of immunomarkers like $p16^{INK4a}$ and Ki-67in cervical smears with HPE as gold standard.
3. To compare the expression of these markers in cervical smears with the morphological diagnosis in cervical cytology.
4. To compare the expression of these markers and their inter-relationship in cervical pre-neoplastic and neoplastic lesions.
5. To assess the association of intensity of expression of $p16^{INK4a}$ for grading the cervical pre-neoplastic and neoplastic lesions.
6. To compare the expression of $p16^{INK4a}$ and Ki-67 in cervical smears (immunocytochemistry) in relation to expression of same markers in tissue sections (immunohistochemistry).

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Carcinoma cervix is one of the leading causes of cancer deaths occurring among Indian women. This mostly affects the reproductive age group with the median age of 38 years. Hence there is a must of preventing carcinoma cervix to reduce the socio-economic burden of developing countries.

It has been found that carcinoma cervix is almost always preceded by precursor lesions. This was first recognized by Sir John Williams in 1886 that areas adjacent to invasive squamous cell carcinoma show noninvasive dysplastic changes[6]. Later Cullen found that these noninvasive lesions histologically resemble the invasive cancer[7]. They are limited to the basement membrane and hence they are called as squamous intraepithelial lesions (SIL). They are graded according to the thickness of the squamous epithelium affected.

This led to the development of various screening procedures so that precancerous lesions of cervix can be detected at an early stage and treated and thus preventing the subsequent progression to invasive cancer in a wide range of population. Hence carcinoma cervix has now become one of the preventable causes of cancer morbidity and mortality. The incidence of carcinoma cervix also shows a steady decline worldwide owing to the availability of the screening procedures.

RISK FACTORS

The risk factors associated with precancerous lesions and carcinoma cervix are as follows:

- Infection with high risk types of Human papillomavirus[8]
- Age at first sexual intercourse
- More than one sexual partner
- Earlier age of first pregnancy
- Multi-parity
- Low socioeconomic status
- Nutritional deficiency
- Immunosuppression
- Sexually transmitted diseases
- Acquired immunodeficiency syndrome
- Smoking[9]
- Use of oral contraceptives
- Inadequate screening

Among these, HPV infection and inadequate screening are considered the most important risk factors.

Smoking, OCP use and immunosuppression when combined with HPV infection double the risk of cervical cancer[9].

Nutritional deficiency especially of micronutrients like folate, vitamin E is associated with increased cancer risk[13].

The mechanism behind the use of oral contraceptives is not clearly understood.

Low socioeconomic status is associated with early sexual intercourse, poor hygienic conditions, increased comorbid conditions and hence increases the risk of HPV infection. Presence of sexually transmitted disease favors the entry of Human papillomavirus into the basal cell layer of immature squamous epithelium. Use of condoms and male circumcision have shown to reduce the risk of HPV infection. Hence the incidence of cervical cancer is more in developing countries than developed countries.

Risk of persistence of HPV infection and its progression to neoplasia is increased in immunosuppressed individuals like post organ transplant recipients, patients affected by acquired immunodeficiency syndrome etc.

Thus all the risk factors mentioned above are interconnected and their combination further increases the cervical cancer risk. But adequate screening at regular intervals and proper treatment at right time can prevent cervical carcinoma.

ASSOCIATION OF HPV WITH CERVICAL CANCER

More than 80% of cervical squamous intraepithelial lesions are associated with Human papillomavirus infection. The association of cervical cancer with Human papillomavirus was first identified by Dr.Harald Zur Hausen who won Nobel prize in medicine in 2008[10]. Human papillomaviruses belong to the family Papillomaviridae. They have double stranded DNA containing 8000 base pairs, non-enveloped virion and a capsid. There are more than 118 types of papillomaviruses[10]. They are highly species specific and are relatively tissue and site specific. Human papillomavirus belongs to the genus alpha-papillomavirus. Papillomaviruses are epitheliotrophic. They infect skin and mucous membrane and cause epithelial proliferations at the infection site. They are known to be associated with cancer cervix in most of the cases. They are also known to be associated with vulval, anal, penile and some of the head and neck squamous cell carcinomas[26].

They are classified as following according to their oncogenic potential,

Low risk types - 6, 11, 42, 43, 44, 53

High risk types -16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66

Unclear oncogenic risk – 26, 68, 73, 82

Among these, HPV 16 is the most common type encountered in LSIL (26%), HSIL (45.3%) and invasive cancers (70%). Next most common is HPV 18. The other types associated are HPV 31, 33, 58 and 52.

The viral genome is composed of three regions such as

- Upstream regulatory region or Long Control Region (LCR)
- Early region – E1, E2, E5, E6, E7
- Late region – L1, L2

LCR is a non-coding region which controls the transcription of codons in the early region. Early region codes for the proteins required for viral replication and also codes for transforming genes like E5, E6 and E7[10]. The late region codes for the structural proteins like L1 and L2[11].

E1 is an ATP dependent helicase which is required for initiation of viral replication in association with E2. E2 also acts as transcriptional repressor of E6 and E7 proteins.

E6 protein binds to p53 and causes its proteolytic degradation. p53 is a protein that causes cell cycle arrest at G1/S checkpoint by inducing the cell cycle inhibitor p21. So E7 protein by causing proteolytic degradation of p53, inhibits apoptosis and leads to cell cycle progression.

E7 protein binds to Rb gene product which is a tumour suppressor protein, inhibits its function and thus leading to progression of cell cycle in an uncontrolled manner. It activates cyclin A and E and also inhibits cyclin dependent kinase inhibitors WAF-1 and p27, thus further augmenting the cell cycle progression. So overexpression of both E6 and E7 leads to uncontrolled cell proliferation and blockage of apoptosis.

Late coding region contains L1 and L2 which are viral capsid proteins and are produced late in the course of infection.

Human papillomavirus enters into the basal cell layer of squamous epithelium through defects in the epithelium. HPV may infect

- Basal cells of exocervical portio
- Basal cells of transformation zone
- Reserve cells of endocervix

But mostly they begin in the squamo-columnar junction of the transformation zone. It involves the posterior lip than the anterior lip of the cervix. Those that involve the exocervical portio are of low grade and that of the endocervical canal are of high grade.

HPV produces two types of infections which are as follows,

- Latent infection
- Productive infection

Latent infection means the HPV genome resides freely inside the nucleus in circular form called episome and there is no replication. This does not induce any morphological changes in the cervical epithelium. The viral genome can only be detected by molecular methods.

Productive infection means the viral genome replicates independently that of the host genome and produces infectious virions and this occurs in the intermediate and superficial layers of the squamous epithelium, because HPV-DNA replicates heavily in highly differentiated cells, that is the superficial layer. This produces HPV associated effects in the cells which can be seen both grossly and microscopically. The characteristic microscopic changes are as follows,

- Acanthosis
- Koilocytosis
- Cytoplasmic vacuolization
- Nuclear atypia
- Multinucleation

Most of the HPV infections are transient and resolve within one or two years[12]. The persistence of HPV infections and its progression to cancer is associated with the following factors,

- Persistence of HPV for longer duration
- Infection with high risk types[9]

This strong association of carcinoma cervix with HPV led to the development of vaccine against HPV and HPV targeted therapies to cure even advanced stages of carcinoma cervix.

PRECURSOR LESIONS OF SQUAMOUS CELL CARCINOMA OF CERVIX

The three systems of classification of cervical pre-neoplastic lesions are compared in the following table:

Older classification	WHO classification	The Bethesda system
Mild dysplasia	CIN 1	LSIL(low grade squamous intraepithelial lesion)
Moderate dysplasia	CIN 2	HSIL(high grade squamous intraepithelial lesion)
Severe dysplasia/ Carcinoma in situ	CIN 3	HSIL

Initially it was thought that dysplasia and carcinoma in situ were two different entities stating that dysplasia is a reversible condition and carcinoma in situ is a true neoplastic process.

Later, Richart said that all precursor lesions are monoclonal proliferations and are a single disease process and hence introduced the term cervical intraepithelial neoplasia (CIN)[14]. CIN 1 corresponds to mild dysplasia, CIN 2 to moderate dysplasia, and CIN 3 to severe dysplasia and carcinoma in situ.

Zur Hausen et al found that Human papillomavirus is involved in the pathogenesis of cervical cancer and its precursor lesions[15]. According to him, infection with HPV especially with high risk types leads to a precursor lesion and with persistence of infection, progresses to invasive cancer[16,17]. Thus CIN 1 indicates a productive viral infection that regresses in majority of cases, whereas CIN 2 or 3 indicates true neoplastic process, confined within the epithelium. Moreover LSIL is polyclonal and HSIL is monoclonal. This indicates that cervical carcinoma does not involve a step like process, progressing from CIN 1, to CIN 2, to CIN 3, and to invasive carcinoma.

CIN 1 or productive viral infection exhibits cytological changes commonly referred to as koilocytotic changes. This also includes flat and exophytic condylomas. They have high viral load. They share a common

ploidy status[19,20]. Most of the cases are self-limited HPV infections even without treatment. 57% of CIN 1 cases regress spontaneously without treatment and 11% only progress to carcinoma. CIN 2 and CIN 3 lesions show high grade dysplastic changes and have the potential to progress to invasive squamous cell carcinoma if not treated[18]. 43% of CIN 2 lesions regress and 22% progress to carcinoma. With regard to CIN 3 lesions, 32% regress and 12% progress to carcinoma[24].

Hence the new Bethesda system which is a two tier system came into vogue which categorized the lesions as LSIL (low grade squamous intraepithelial lesion) and HSIL (high grade squamous intraepithelial lesion). LSIL indicates CIN 1 or mild dysplasia and HSIL includes CIN 2 and CIN 3, otherwise called moderate dysplasia and severe dysplasia/ carcinoma insitu[21,22].

LSIL is most prevalent in the adolescent age group (15-19 years) and decreases in 25-40 year age group. Prevalence of HSIL steadily increases upto 25-29 years and then decreases[23]. Invasive carcinoma shows increased prevalence in 40-65 year age group.

PATHOLOGY OF PRECURSOR LESIONS

CIN lesions are graded as follows,

- CIN 1 – Neoplastic cells occupying the lower one-third of the epithelium.
- CIN 2 - Neoplastic cells occupying lower one-third to two-third of the epithelium.
- CIN 3 - Neoplastic cells occupying two-third to full thickness of the epithelium.

Neoplastic cells show the following nuclear features,

- Irregular nuclear membrane
- Increased N:C ratio
- Nuclear pleomorphism
- Nuclear hyperchromatism
- Coarse chromatin

LSIL

The features of LSIL are as follows,

- Nuclear atypia in the lower one third of the epithelium.
- Koilocytotic changes like cytoplasmic vacuolation, bi and multinucleation.
- Architectural changes like papillomatosis and acanthosis.

Koilocytotic changes are also seen in trichomoniasis, candidiasis etc., but they are not associated with nuclear atypia.

HSIL

The features of HSIL are as follows,

- Nuclear atypia in more than one-third to full thickness of the epithelium
- Nuclear crowding
- Loss of polarity
- Increased nuclear pleomorphism
- Anisonucleosis
- Abnormal mitotic figures (AMF)
- Indistinct cell borders

Reparative and atrophic changes resemble HSIL and they are distinguished by strong and diffuse expression of p16 in all HSIL lesions.

These lesions can be managed colposcopically using cryosurgery, laser ablation and LEEP (loop electrosurgical excision procedure), thus preventing their progression to invasive carcinoma. For this screening becomes essential.

INVASIVE SQUAMOUS CELL CARCINOMA

Carcinoma cervix most commonly affects women in their 40's and 50's, that is 20 years older than those affected by intraepithelial lesions[25]. Human

papillomavirus infection is the most common risk factor and the most common types are HPV 16 and 18. There is a latency of 10 years between initial HPV infection and development of carcinoma cervix[26].

According to WHO classification, carcinoma cervix is divided into three categories as follows,

- Squamous cell carcinoma
- Adenocarcinoma
- Other epithelial tumours

Of the above categories, squamous cell carcinoma accounts for more than 70-80% of cases.

Squamous cell carcinoma is further divided into following types,

- Microinvasive squamous cell carcinoma
- Invasive squamous cell carcinoma
    - Keratinizing
    - Non keratinizing
    - Verrucous
    - Papillary
    - Warty
    - Basaloid
    - Squamo-transitional
    - Lymphoepithelioma like carcinoma

There is often a long latency period between initial HPV infection and development of full fledge cervical cancer. Hence adequate screening and appropriate treatment at the initial stages can prevent the progression of precursor lesions to cervical cancer.

CERVICAL CANCER SCREENING

Inadequate screening is the most important contributing factor to cervical cancer at the present scenario. Hence it is high time to make cervical cancer screening, a must for all women past 21 years to bring down the incidence of cervical cancer especially in developing countries.

The objectives of cervical cancer screening are as follows,

- To prevent morbidity and mortality of cervical cancer
- To prevent over management of precursor lesions that in most cases regress

The various screening modalities commonly used in developing countries at present are the following,

- Cervical cytology – conventional
- Cervical cytology – liquid based preparation
- HPV testing
- Colposcopy – VIA, VILI
- Cervical biopsy
- Immunomarkers

ASCCP 2012 GUIDELINES FOR CERVICAL CANCER SCREENING[30]

The current guidelines for cervical cancer screening according to ASCCP are as follows,

- Screening should start at the age of 21 regardless of the age of sexual onset.
- For ages 21-29 years, cytology alone is recommended every 3 years. HPV testing is neither employed as co-test nor as a primary screening test.
- For ages 30-64 years, cytology and HPV co-testing together every 5 years is preferable. Cytology alone every 3 years is also acceptable. But HPV testing alone is not recommended.
- Screening should stop at 65 years even if the woman resumes sexual life with a new sexual partner, in case of
    - Adequate negative screening - 3 consecutive negative cytology or 2 consecutive negative HPV testing
    - No CIN II within the last 20 years
- Screening can be stopped before 65 years in case of hysterectomy with removal of cervix without any prior history of CIN II. Adequate negative screening is not required. This is because, risk of Pap abnormality following hysterectomy is only 1%.

- In the presence of CIN II, CIN III, adenocarcinoma insitu, continue routine screening for atleast 20 years even past 65 years.
- The above screening guidelines should not change for women who are vaccinated against HPV.

Most of the HPV infections are transient at 1-3 years interval and only persistent infection has the risk of progression to cervical cancer. Risk of HSIL/cancer after 3 years is not significantly higher than the risk after 1 year. Hence 3 years interval can be given between screening visits.

HPV testing is not needed in the 21-29 year age group because the prevalence of carcinogenic HPV in early 20's is 20%, most of which disappear without intervention. HPV testing in this age group creates unnecessary anxiety and intervention and hence avoided.

HPV co-testing is added in 30-64 year age group because of increased prevalence of CIN III in this age group. Addition of HPV co-testing also reduces the number of colposcopies. However this cannot be done in all areas because of financial restrictions and hence cytology alone every 3 years is also acceptable in this age group.

Screening should stop at 65 years because of the following reasons,

- Risk of HPV infection is 5-10%

- Incident HPV infection is less likely to progress to cancer within the remaining lifetime.
- Colposcopy/biopsy/treatment is difficult after this age group.

MANAGEMENT ALGORITHMS ACCORDING TO ASCCP GUIDELINES 2012: [27]

- For women with unsatisfactory cytology/ HPV negative, repeat cytology after 2-4 months. If again unsatisfactory, go for colposcopy. If unsatisfactory cytology/HPV positive, directly go for colposcopy.

- For women with negative cytology/ HPV positive, go for either repeat co-testing after 12 months or can immediately go for HPV genotype testing for HPV 16/18. Direct colposcopy is not indicated because most infections regress within 12 months. If either the repeat co-test or HPV genotyping is positive, they are referred to colposcopy. If HPV genotyping is negative for 16/18, repeat co-test after 12 months. If both are negative, they can return to routine screening.

- Women of 21-24 year age group with ASCUS or LSIL in cytology, should undergo repeat cytology yearly for 2 years. If both are negative, can continue with routine screening. If positive, should go for colposcopy. Even if reflex HPV test is positive, they should go for yearly cytology for 2 years.

- For women with ASCUS cytology/ HPV negative, continue routine screening because risk of CIN III in these patients is less than 2%. If ASCUS cytology/ HPV positive, go for colposcopy. If no HPV test is available, repeat cytology after 1 year and if negative, can continue with routine screening.

- For women with LSIL/ HPV negative, go with repeat co-testing after 1 year. If either of them is positive, go for colposcopy. If both are negative, continue with routine screening. If LSIL/ no HPV or positive HPV, go with colposcopy.

- For pregnant women with LSIL, colposcopy is preferred. But deferring colposcopy till 6 weeks postpartum also is acceptable.

- For women with ASC-H, go for colposcopy.

- For women with HSIL, go either for colposcopy or directly immediate loop electrosurgical excision. LEEP is not recommended for women in 21-24 year age group.

- For women with biopsy proven CIN I preceded by lesser abnormalities, go for co-testing after 1 year. If either test is positive, go for colposcopy.

- For women with biopsy proven CIN I preceded by ASC-H or HSIL, go for diagnostic excision procedure.

- For women with biopsy proven CIN 2 or 3, go for either excision or ablation of T-zone if adequate colposcopy. For inadequate colposcopy or recurrent CIN 2 or 3, go for excision. Then repeat co-testing at 12 and 24 months. If 2 negative results, go for routine screening.

- For young women with biopsy proven CIN 2 or 3, repeat colposcopy at 6 months interval for 12 months. If abnormal, repeat biopsy. If CIN 2 or 3 persists, go for treatment.

CERVICAL CYTOLOGY

Cervical cytology is the most common and the most cost effective screening procedures available. Cervical cytology done twice in lifetime reduces risk of cervical cancer by 43% and yearly screening reduces risk by more than 90%[28,29]. Despite screening, many women develop cervical cancer. The sensitivity of Pap smear is 40.2% and specificity is 90.6%[43]. As for any other diagnostic or therapeutic techniques, cervical cytology also has its merits and demerits. There are two methods of cervical cytology, one is the conventional Pap smear and the other is the liquid based preparation.

METHODS OF CERVICAL CYTOLOGY

The two methods of cervical cytology are as follows,

- Conventional Pap smear
- Liquid based cytology

CONVENTIONAL PAP SMEAR

It was George Papaniculaou who first discovered that in patients with cervical carcinoma, tumour cells can be detected in vaginal fluid[32]. Papaniculaou published in 1928, "New cancer diagnosis"[33]. The merits and demerits of Pap smear are as follows,

MERITS

- No injury to the tissue even with repeated sampling.
- Smears cover wider surface area than biopsy.
- The procedure causes minimum shrinkage and distortion of cells and hence cellular details are clearly made out.
- Effects of infection and irradiation on cellular morphology are easily made out.

DEMERITS

- The interpretation of morphology of cells is subjective and hence leads to inter-observer variation.

- The diagnosis in cytology is not taken as the final take. It has to be confirmed by histopathology for further management.
- Large area has to be screened and hence it is time consuming.
- The study is based on the morphology of exfoliated cells only. Hence it may not represent the lesion many a times.
- The cells are often obscured by the inflammatory cells and also by haemorrhage related to the procedure.
- Cellular arrangement or their inter-relationship cannot be studied.
- The extent of invasion cannot be evaluated as only the cellular details are studied.
- Size and site of the lesion cannot be assessed by cytology.
- The smears cannot be used for further ancillary studies.

LIQUID BASED CYTOLOGY

Here the material is not smeared onto the slide but rinsed into a liquid collection media containing fixatives, so that 100% of cells are preserved. The advantages of liquid based preparation over conventional Pap smear are as follows,

MERITS OF LBC OVER CONVENTIONAL PAP SMEAR

- Almost 100% of the collected cells are captured.
- Immediate liquid fixation prevents air-drying.

- Easier to review slides.
- Smaller screening area.
- Reduced debris, cell clumps and obscuring elements and hence a cleaner background. So unsatisfactory cases are significantly reduced.
- Increased detection of high-grade squamous intra-epithelial lesions and above.
- Ancillary testing such as reflex Human papillomavirus (HPV) test, other molecular tests (Chlamydia/gonorrhoea) and immunocytochemistry can be performed from the residual material.
- The residual material can also be processed as cell block.

TRANSFORMATION ZONE

This is the area where the ectocervix meets the endocervix. This is the site of origin of squamous intra-epithelial lesions and squamous cell carcinomas. In adolescents and young adults, the transformation zone is on the surface of the ectocervix called as ectropion. In the reproductive age group, it is seen near the external os. In post-menopausal women, the transformation zone recedes into the endocervical canal.

NORMAL CELLS IN CERVICAL CYTOLOGY

- The squamous epithelium of cervix and vagina are composed of three layers of cells
  - Superficial cells – large and flat with eosinophilic cytoplasm and pyknotic nuclei
  - Intermediate cells – large cells with cyanophilic to eosinophilic cytoplasm and small vesicular nuclei
  - Parabasal cells – singly dispersed with large uniform nuclei and distinct cytoplasmic borders

- Squamous metaplasia – the cells have cytoplasmic processes and uniform pale nuclei with small nucleoli.

- Endocervical cells – these cells are arranged in honeycomb sheet and when viewed from side, show picket fence appearance. They are columnar cells with vacuolated basophilic cytoplasm and basally located nuclei with fine chromatin and small nucleoli.

- Endometrial cells – the cells are arranged in tight three-dimensional clusters. The cells have scant cytoplasm, small round dark nuclei showing no nucleoli. Superficial stromal cells are round and histiocytic. Deep stromal cells are spindled and dark. Exodus is seen

in 6 to 10 days of menstrual cycle. They consist of central core of stromal cells surrounded by epithelial cells.

THE BETHESDA SYSTEM 2001(ANNEXUE IV)

In 1988, the Bethesda system was introduced so that uniform guidelines can be provided for reporting cervical cytology. This was later revised in the years 1991 and 2001[31]. Terence J. Colgan has discussed the various sections of the Bethesda system 2001[34]. The Bethesda system 2001 is given in annexure IV.

SPECIMEN ADEQUACY[34]

- **SATISFACTORY**
  - Conventional – minimum 8000-12000 well preserved and well visualised squamous cells
  - Liquid based cytology - ≥5000 well preserved and well visualised squamous cells
  - For representation of transformation zone, 10 endocervical or squamous metaplastic cells should be present, not necessarily in clusters. But absence of this does not indicate it is unsatisfactory.

- **UNSATISFACTORY**
  - Unlabelled vial or slide

- Broken slide
- Blood, inflammation and other elements obscuring more than 75% of the cells

GENERAL CATEGORISATION

- Negative for intraepithelial lesion
- Epithelial cell abnormalities
- Others

NEGATIVE FOR INTRAEPITHELIAL LESION

According to Terence J. Colgan, >90% of Pap smears are negative for intraepithelial lesion[34]. The causes are as follows.

- Organisms
  - Trichomonas vaginalis
  - Candida
  - Bacterial vaginosis
  - Actinomyces
  - Cellular changes related to Herpes simplex virus

- Reactive cellular changes associated with
  - Inflammation
  - Radiation
  - Atrophy
  - Intrauterine contraceptive device

EPITHELIAL CELL ABNORMALITIES[34]

- Squamous cell abnormalities
- Glandular cell abnormalities
- Other

SQUAMOUS CELL ABNORMALITIES[34]

The squamous cell abnormalities according to the Bethesda system 2001 are classified as follows,

- Atypical squamous cells
  - ASC-US
  - ASC-H
- Low grade squamous intra-epithelial lesion
- High grade squamous intra-epithelial lesion
  - HSIL with features suspicious for invasion
- Squamous cell carcinoma

ATYPICAL SQUAMOUS CELL OF UNDETERMINED SIGNIFICANCE (ASCUS)

ASCUS is a diagnosis of exclusion. This should not exceed >5% of all cytology specimens and should be more than twice of the SIL samples. ASC-

US is the least reproducible diagnosis[37,38,39,40]. The criteria for ASCUS according to the Bethesda system 2001are as follows[36].

- Cells resemble superficial or intermediate squamous cells
- Nuclei 2-3 times the size of a normal intermediate cell
- Nuclei are normochromatic to slightly hyperchromatic with minimum irregularities.

ATYPICAL SQUAMOUS CELLS, CANNOT EXCLUDE HSIL (ASC-H)

The features are suggestive of HSIL, but not all criteria are met to call it as HSIL[35]

- Cells resemble basal or parabasal cells
- Nuclei are irregular and hyperchromatic

LOW GRADE SQUAMOUS INTRAEPITHELIAL LESION (LSIL)[36]

This includes both the HPV effects and mild dysplasia.

- The cells resemble superficial or intermediate cell
- Nuclei are 4-6 times the size of a normal intermediate cell nucleus
- Bi- and multi-nucleation are common
- Chromatin is finely granular and uniformly distributed

HIGH GRADE SQUAMOUS INTRAEPITHELIAL LESION (HSIL)

This includes moderate dysplasia, severe dysplasia and also carcinoma in situ[35]. The diagnosis of HSIL almost always indicates a risk for significant cervical disease[41,42].

- Cells resemble basal or parabasal cells
- Greatly increased nuclear cytoplasmic ratio
- Hyperchromatic nucleus
- Irregular nuclear contour

INVASIVE SQUAMOUS CELL CARCINOMA

There are 2 types of squamous cell carcinoma, keratinizing and non-keratinizing types.

NON-KERATINIZING CARCINOMAS

- Large number of cells that form syncytial sheets and loose clusters
- Nuclei 2-3 times that of the intermediate cell nucleus
- Coarse chromatin with focal clearing
- Prominent macronucleoli
- Tumour diathesis

KERATINIZING CARCINOMAS

- Pleomorphic and tadpole shaped malignant cells
- Abundant orangophilic cytoplasm
- Hyperkeratosis and parakeratosis
- No tumour diathesis

HPV TESTING

The persistent detection of HPV in cervical cancer is a consistent feature[43]. The risk of cervical cancer is increased with prolonged duration of HPV infection and specifically infection by high risk types like 16, 18 etc. The sensitivity of HPV DNA testing is 68.2% and specificity is 91.6%[43]. In women under 30, HPV infection is transient and regresses and hence HPV co-testing is done only after 30 years according to ASCCP guidelines 2012[30].

The molecular techniques currently used are as follows,

- Amplified methods
  - Signal amplification
    - The hybrid capture 2 HPV DNA assay
  - Target amplification
    - Polymerase chain reaction (PCR)
- Non-amplified methods
  - The Southern blot hybridisation

COLPOSCOPY

According to ASCCP guidelines 2012, colposcopy is indicated only after repeated positive cytology especially in younger women[30]. Hans Heinselmann was the one to introduce colposcopy in 1925, a method of identifying cervical lesions by illumination and magnification of the cervix[44]. Schiller later introduced the concept of identifying cervical lesions by iodine application in 1928. This procedure has high sensitivity but low specificity.

The merits of colposcopy as a screening procedure are as follows,

- It is a relatively reliable procedure in developing countries
- Simple and easy to perform
- Maintenance is cheaper
- Both diagnostic and therapeutic

Visual inspection in colposcopy can be done by two methods,

- VIA – Visual inspection with acetic acid
- VILI – Visual inspection with Lugol's iodine

After application of acetic acid, the abnormal cervical lesions turn acetowhite while the normal areas remain pink. This is because the density of white is proportional to the amount of cellular proteins. So the areas with highest proliferation and hence DNA content appear denser than the other

areas. The change in colour reverses quickly in case of inflammation, whereas slowly in case of CIN.

Similarly after application of Lugol's iodine, abnormal areas do not take up the mahogany brown but take up saffron-yellow or mustard-yellow colour. The glycogen rich normal squamous epithelium takes up the mahogany brown colour.

The dense acetowhite area with VIA or the mustard yellow colored area with VILI are taken as positive when the lesion is in the transformation zone or around the squamo-columnar junction. Leopard skin like lesions (as seen in Trichomonas infection) and satellite lesions seen away from the squamo-columnar junction are not taken as positive.

They can also be classified as low grade and high grade lesions. The acetowhite areas in high grade lesions are opaque and the iodine non-uptake lesions are thick and dense.

CERVICAL BIOPSY

Cervical biopsy always remains the gold standard and as a confirmatory procedure for cervical pre-neoplastic and neoplastic lesions. This can be both diagnostic and therapeutic, because in many a cases, smaller abnormal lesions are completely removed. There are various methods of cervical biopsies which are as follows,

- Punch biopsy
- Conisation
- LLETZ (Large Loop Excision of the Transformation Zone)

In punch biopsy, small amount of tissue is removed with the help of a biopsy forceps. In conisation procedure, laser or a scalpel is used to remove large portions of cervix in the shape of a cone. In LLETZ, electric current through a wire loop is used for excision of cervical tissue. Thus these procedures are diagnostic as well as therapeutic and also remain the gold standard for the diagnosis of cervical pre-neoplastic and neoplastic lesions.

IMMUNOMARKERS

The use of immunomarkers in cervical cytology is going to be the promising tool in the future in the screening for cervical cancer[45]. Though there are certain morphological criteria laid down for grading the lesions according to the Bethesda system, this still lacks accuracy because of the inter-observer variation. Immunocytochemistry can be used for triaging women with cervical dysplasia with increased accuracy.

The basic principle behind the use of immunomarkers in cytology and biopsy is unmasking the antigen in the cells and detecting them with the help of specific primary antibodies.

The various biomarkers that can be used in cervical cancer screening are,

- $p16^{INK4a}$
- Ki-67
- Pro-Ex C
- HPV L1 Capsid

$p16^{INK4a}$

$p16^{INK4a}$ is a cellular protein involved in cell cycle control. Normally the Retinoblastoma protein is bound to elongation factor 2 and this pRb-E2F complex keeps the cell cycle in check. When they are bound together, the cells are in rest. They come apart to turn on the cell cycle and leads to mitosis. The cell cycle regulatory protein p16 is now expressed at lower levels, facilitates the binding of pRb to E2F and put the cell cycle back to rest[46].

With HPV infection, the viral DNA is introduced into the basal cells. But the viral DNA replicates in the superficial (more differentiated) cell layers and produces infectious virions. Most of the infections are transient and they do not affect the cell cycle control. But in transforming infections, the viral oncoprotein E7 begins to interfere in the cell cycle control. E7 protein binds to pRb, thus preventing the binding of E2F to pRb and holds the cell cycle permanently in the turn-on position and the cells continue to divide without any control. The cell cycle controller protein p16 is over-produced. Hence

increased expression of p16 has now become the marker of cervical carcinogenesis[46,47].

Ki-67

Ki-67 is a nuclear antigen and is expressed during all phases of the cell cycle[48]. Hence it is a proliferation marker that is normally expressed in parabasal cells in normal squamous epithelium. But in CIN lesions they get expressed in the stratified squamous epithelium according to the level of disordered maturation[46]. Ki-67 expression is also seen in reactive changes and hence dual staining with p16 and Ki-67 is suggested. This provides higher sensitivity.

Pro-Ex C

Pro-Ex C detects minichromosome maintenance proteins (MCMs)[46]. They are required for DNA replication and hence are expressed in cervical pre-neoplastic and neoplastic conditions. They are found to be negative in normal squamous epithelium and consistently positive in high grade cervical lesions[49,50].

HPV L1 Capsid

The HPV proteins L1 and L2 are structural proteins. Of these L1capsid protein is the major protein on the surface of the virus. They are often expressed in productive viral infections[51,52], which usually occurs in

terminally differentiated cells. In CIN III and carcinomas, there is arrest of squamous maturation and hence are not expressed. HPV L1 capsid is usually used to assess the disease progression[53,54].

The other markers that can be used for cervical cancer diagnosis are as follows,

- Loss of E-cadherin expression [48]
- CK17 negativity [55]
- ER, PR [56]
- Laminin[57]
- Survivin[58]

IMMUNOCYTOCHEMISTRY

According to a study done by Yoshida et al[59], the use of p16 as a biomarker in cervical cytology is more sensitive and specific than detecting HPV status. Hence immunocytochemistry using p16 can be used as a screening procedure in cervical pre-neoplastic and neoplastic lesions. For this liquid based preparations can be satisfactorily used. Dual staining with p16 and Ki-67 can also be done in cytology to increase the sensitivity. This allows early diagnosis even before colposcopy and cervical biopsy. This is a non-invasive procedure with high sensitivity and specificity. Hence

immunocytochemistry is the future promising tool in cervical cancer screening.

IMMUNOHISTOCHEMISTRY

All the above said markers can be used in tissue sections to confirm the morphological diagnosis. This is because many of the reactive process resemble dysplastic changes. To rule out all these reactive causes, immunohistochemistry, which has revolutionized the field of pathology is used in the diagnosis of cervical pre-neoplastic and neoplastic conditions.

MATERIALS AND METHODS

MATERIALS AND METHODS

This was a prospective study, conducted at the Institute of Social Obstetrics and Govt. Kasturba Gandhi Hospital for Women and Children, Madras Medical College from February 2014 to July 2014.

This study involved 30 women who had been diagnosed with positive VIA/VILI in colposcopy. These women were subjected to liquid based cervical cytology and cervical punch biopsy. Immunocytochemistry in the liquid based cytology sample and immunohistochemistry in cervical biopsy specimens using $p16^{INK4a}$ and Ki-67 were performed. The results of the above tests were compared with histopathology as the gold standard.

Also the incidence and distribution of cervical pre-neoplastic and neoplastic lesions diagnosed by HPE in our Institute from February 2014 to July 2014 were studied.

INCLUSION CRITERIA

1. Women in the age group of 21-65 years.
2. Non-pregnant women.
3. Women with positive VIA/VILI.
4. Women without any prior treatment for cervical pre-neoplastic and neoplastic lesions.

EXCLUSION CRITERIA

1. Pregnant women.
2. Menstruating women.
3. IUD user.
4. Sexual intercourse with spermicidal jelly, douches/tampons 24 hours prior.
5. Women who have undergone prior treatment for cervical pre-neoplastic and neoplastic lesions like cryotherapy, LLETZ, cervical biopsy, conisation, hysterectomy or radiation.

VISUAL INSPECTION WITH ACETIC ACID AND LUGOL'S IODINE PROCEDURE

1. The patient was put in lithotomy position and good visualization ensured.
2. Any abnormality in external genitalia was recorded.
3. Cusco's speculum was inserted into the vagina and cervix was clearly visualised.
4. Any discharge or mucus if present was wiped using a cotton swab wet with normal saline.
5. External appearance of the cervix was recorded.
6. Cervix was washed with freshly prepared 5% acetic acid using a syringe.

7. Then the cervix was observed for acetowhite areas after a one minute wait.
8. Lugol's iodine was applied onto the cervix with a syringe or a cotton swab.
9. Cervix was observed for iodine uptake and non-uptake areas.
10. All the findings were recorded.

REPORTING

NEGATIVE VIA/VILI

- VIA - retention of pink hue
- VILI - iodine uptake areas taking mahogany brown colour

POSITIVE VIA/VILI

- Low grade lesion
    - VIA - any acetowhite area
    - VILI - any iodine non-uptake area
- High grade lesion
    - VIA - opaque acetowhite patches abutting the squamo-columnar junction
    - VILI - thick dense saffron-yellow or mustard-yellow iodine non-uptake areas around the squamo-columnar junction

- Invasive cancer
    - Irregular, ulcerative, proliferative growth which changes white with acetic acid and yellow with iodine application

LIQUID BASED CYTOLOGY

1. The patient was put in lithotomy position.
2. Cervix was visualised using a Cusco's speculum.
3. The sample was taken using a cervical brush by rotating it 360° clockwise at the cervical os with the brush in contact with the ectocervix. The tip of the brush was broken and dropped into the alcohol based preservative fluid.
4. The vial containing the tip of the brush was shaken with a vortex for 10 seconds.
5. Samples containing excess of mucus or blood were cleared with 4ml of clearing solution.
6. Then the contents were poured into a 15ml centrifuge tube.
7. Centrifugation was done for 1000g for 10 min.
8. The supernatant was poured off and cell base reagent was added to the sample proportional to the cell pellet formed.
9. A vortex was used to suspend the cell pellet.
10. Then 50 microlitres of suspended cell pellet was pipetted onto a clean slide in the form of a circle.

11. The slides were air-dried and routine pap staining done.

CYTOLOGYREPORTING

The cytology reporting was done based on the Bethesda system 2001as given in annexure IV.

CERVICAL BIOPSY

Cervical biopsy was taken using punch biopsy forceps and put in 10% neutral buffered formalin and sent for histopathologic examination. The reports were given as

- Normal
- Chronic non-specific cervicitis
- CIN I
- CIN II
- CIN III
- Invasive squamous cell carcinoma
    - Well differentiated
    - Moderately differentiated
    - Poorly differentiated
- Adenocarcinoma

IMMUNOCYTOCHEMISTRY

The remaining material in liquid based preparation was centrifuged and slides were prepared. Then immunocytochemistry was done using $p16^{INK4a}$ and Ki-67 in two separate slides using the procedure given in annexure V. The scoring was given as below.

REPORTING

$p16^{INK4a}$ scoring

p16 showed nuclear or cytoplasmic positivity or both. Scoring was given as follows based on intensity of staining[89].

- 0 - Negative
- 1 – Weakly positive in dysplastic cells
- 2 – Strongly positive in dysplastic cells

Ki-67 scoring

Ki-67 showed nuclear positivity. Scoring was based on the percentage of dysplastic cells taking up the marker[88].

- 0 – Negative
- 1 –<10% of dysplastic cells positive
- 2 - 10-50% of dysplastic cells positive
- 3 - >50% of dysplastic cells positive

IMMUNOHISTOCHEMISTRY

The unstained sections were prepared from the cervical biopsy specimens and subjected to immunohistochemistry using the procedure given in annexure VI.

REPORTING

- **$p16^{INK4a}$**

  The expression in cytoplasm or in nucleus or both were taken as positive[90], regardless of the intensity of staining.

  - 0 - negative
  - 1 - positive

- **Ki-67**

  Ki-67 expression showed strong nuclear positivity in varying thickness of the stratified squamous epithelium and were graded as follows[91,92,93],

  - 0 – negative
  - 1 –nuclear positivity in lower-third of epithelium
  - 2 – nuclear positivity in lower two-thirds of epithelium
  - 3 – nuclear positivity in lower two-thirds to full thickness
  - 4 – diffuse nuclear positivity in the malignant cells

STATISTICAL ANALYSIS

The information collected and the reports of various screening modalities like colposcopy, cervical cytology, immunocytochemistry, cervical biopsy and immunohistochemistry were recorded in a master chart and they were compared with each other.

The P value was calculated for liquid based cervical cytology, immunocytochemical expression of p16 and Ki-67 in cervical non-neoplastic, pre-neoplastic and neoplastic lesions, showing their statistical significance in correlation with biopsy. The sensitivity, specificity, positive predictive value and negative predictive value were also calculated and they were compared with other similar studies.

The incidence and distribution of cervical pre-neoplastic and neoplastic lesions diagnosed by HPE from February 2014 to July 2014 in our Institute were studied. The distribution of cervical pre-neoplastic and neoplastic lesions in relation to various clinicopathological factors like age, menstrual status, quadrant of cervix involved were also studied.

OBSERVATION AND RESULTS

OBSERVATION AND RESULTS

This was a prospective study conducted in the Institute of Social Obstetrics and Govt. Kasturba Gandhi Hospital for Women and Children, Madras Medical College, from February 2014 to July 2014.

EPIDEMIOLOGY OF CERVICAL PRE-NEOPLASTIC AND NEOPLASTIC LESIONS IN OUR INSTITUTE (FEBRUARY 2014 TO JULY 2014)

In a 6 months period from February 2014 to July 2014, totally 202 cases were reported as cervical pre-neoplastic and neoplastic lesions by HPE. Of the 202 cases, 139(68.8%) were CIN I, 19(9.4%) were CIN II, 11(5.4%) were CIN III and 33(16.3%) were malignancies (table 1 and chart 1).

TABLE 1

DISTRIBUTION OF CERVICAL PRE-NEOPLASTIC AND NEOPLASTIC LESIONS

n=202

BIOPSY	CIN I	CIN II	CIN III	MALIGNANCY
FREQUENCY	139	19	11	33
PERCENTAGE	68.8%	9.4%	5.4%	16.3%

The distribution of subtypes of SCC and adenocarcinoma among the malignant cases is given in table 2 and chart 2. SCC-WD constitutes 63.6%, SCC-MD constitutes 30.3%, SCC-PD constitutes 3% and adenocarcinoma (adenoca) constitutes 3%.

TABLE 2

DISTRIBUTION OF MALIGNANT CERVICAL LEISONS

n=33

BIOPSY	SCC-WD	SCC-MD	SCC-PD	ADENOCA
FREQUENCY	21	10	1	1
PERCENTAGE	63.6%	30.3%	3%	3%

Age-wise distribution of cervical pre-neoplastic and neoplastic lesions is given in table 3 and chart 3. All CIN lesions were common in the 31-40 year age group. All malignant cases were common in the 51-60 year age group.

TABLE 3

AGE-WISE DISTRIBUTION OF CERVICAL PRE-NEOPLASTIC AND NEOPLASTIC LESIONS

n=202

BIOPSY	AGE (YEARS)				
	21-30	**31-40**	**41-50**	**51-60**	**61-70**
CIN I - 139	**27**(19.4%)	**70**(50.4%)	**38**(27.3%)	**4**(2.9%)	**0**
CIN II – 19	**5(**26.3%)	**9**(47.4%)	**2**(10.5%)	**2**(10.5%)	**1**(5.3%)
CINIII - 11	**1**(9.1%)	**6**(54.5%)	**2**(18.2%)	**2**(18.2%)	**0**
MALIG - 33	**0**	**3**(9.1%)	**12**(36.4%)	**14**(42.4%)	**4**(12.1%)

The distribution of menstrual status in cervical pre-neoplastic and neoplastic lesions is given in table 4. More than 70% of CIN lesions occur in reproductive women. More than 90% of malignant lesions occur in post-menopausal women.

TABLE 4

DISTRIBUTION OF CERVICAL PRE-NEOPLASTIC AND NEOPLASTIC LESIONS – MENSTRUAL STATUS WISE

n=202

BIOPSY	MENSTRUAL STATUS	
	REPRODUCTIVE	**POST-MENOPAUSAL**
CIN I - 139	**123**(88.5%)	**16**(11.5%)
CIN II – 19	**14**(73.7%)	**5**(26.3%)
CINIII - 11	**9**(81.8%)	**2**(18.2%)
SCC - 32	**3**(9.4%)	**29**(90.6%)
ADENOCA - 1	**0**	**1**(100%)

The distribution of cervical pre-neoplastic and neoplastic lesions in colposcopic biopsies according to quadrant of cervix involved is given in table 5 and chart 4**.** Most of the lesions were seen around 12 o' clock position. All invasive carcinomas were seen circumferentially abutting the os.

TABLE 5

DISTRIBUTION OF CERVICAL PRE-NEOPLASTIC AND NEOPLASTIC LESIONS IN COLPOSCOPIC BIOPSIES – QUADRANT OF CERVIX INVOLVED WISE

n=170

BIOPSY	QUADRANT OF CERVIX (o' clock)				
	12-3	3-6	6-9	9-12	CIRCUM
CIN I	**70**(56.9%)	**21**(17.1%)	**11**(9%)	**21**(17.1%)	**0**
CIN II	**11**(73.3%)	**2**(13.3%)	**0**	**1**(6.7%)	**1**(6.7%)
CIN III	**3**(50%)	**0**	**1**(16.7%)	**1**(16.7%)	**1**(16.7%)
SCC	**1**(4%)	**0**	**0**	**0**	**24**(96%)
ADENOCA	**0**	**0**	**0**	**0**	**1**(100%)

CHARACTERISTICS OF THE CURRENT STUDY GROUP

30 VIA/VILI positive cases were randomly selected from the patients attending the colposcopy department for our study. Of these, 15 were low grade lesions (LGL), 10 were high grade lesions (HGL) and 5 were invasive carcinoma (inv ca) by VIA/VILI (table 6).

TABLE 6

DISTRIBUTION OF VIA/VILI DIAGNOSIS IN THE STUDY GROUP

n=30

VIA/VILI	LGL	HGL	INV CA
FREQUENCY	15	10	5
PERCENTAGE	50%	33.3%	16.7%

These 30 patients were subjected to liquid based cytology and cervical biopsy. The biopsy report was the confirmatory diagnosis. So it was taken as the gold standard. Of these 30 VIA/VILI positive cases, the biopsy results turned out to be 2 chronic cervicitis, 11 CIN I, 5 CIN II, 4 CIN III and 8 SCC cases (table 7 and chart 5).

TABLE 7 : BIOPSY RESULTS

n=30

BIOPSY DIAGNOSIS	NUMBER OF CASES	PERCENTAGE
CHRONIC CERVICITIS	2	6.7%
CIN I	11	36.7%
CIN II	5	16.7%
CIN III	4	13.3%
SCC	8	26.7%

In this study group, the age wise distribution of biopsy diagnosis is as follows. 2 patients diagnosed with chronic cervicitis were in the 21-30 year age group. Majority of CIN I (54.5%) and CIN III (50%) lesions were in the 31-40 year age group. CIN II cases were more or less equally distributed among all the age groups here. All cases of squamous cell carcinomas were above 40 years (table 8 and chart 6).

TABLE 8

AGEWISE DISTRIBUTION OF BIOPSY RESULTS IN THE STUDY GROUP

n=30

BIOPSY DIAGNOSIS	**AGE(YEARS)**			
	21-30	**31-40**	**41-50**	**51-60**
CERVICITIS	**2**(100%)	**0**	**0**	**0**
CIN I	**2**(18.2%)	**6**(54.5%)	**3**(27.3%)	**0**
CIN II	**2**(40%)	**1**(20%)	**1**(20%)	**1**(20%)
CIN III	**0**	**2**(50%)	**1**(25%)	**1**(25%)
SCC	**0**	**0**	**4**(50%)	**4**(50%)

Majority of the cervical non-neoplastic and pre-neoplastic lesions were seen in reproductive women in this study group. But all the squamous cell carcinoma cases were seen in the post-menopausal women (table 9).

TABLE 9

DISTRIBUTION OF BIOPSY RESULTS IN THE STUDY GROUP – MENSTRUAL STATUS WISE

n=30

BIOPSY	MENSTRUAL STATUS	
	REPRODUCTIVE	**POST-MENOPAUSAL**
CERVICITIS	**2**(100%)	**0**
CIN I	**10**(90.9%)	**1**(9.1%)
CIN II	**3**(60%)	**2**(40%)
CIN III	**3**(75%)	**1**(25%)
SCC	**0**	**8**(100%)

The distribution of cervical non-neoplastic, pre-neoplastic and neoplastic lesions according to the quadrant of cervix involved is given in table 10. Majority of lesions were in the 12-3 o' clock quadrant. All the carcinoma cases were seen all around abutting the os.

TABLE 10

DISTRIBUTION OF BIOPSY RESULTS IN THE STUDY GROUP – QUADRANT OF CERVIX INVOLVED WISE

n=30

BIOPSY	QUADRANT OF CERVIX INVOLVED (o' clock)				
	12-3	3-6	6-9	9-12	Circum-ferential
CERVICITIS	1	0	0	1	0
CIN I	8	2	0	1	0
CIN II	3	0	0	1	1
CIN III	2	0	1	0	1
SCC	0	0	0	0	8

15 low grade lesions (LGL) in VIA/VILI turned out to be cervicitis – 2, CIN I – 10, CIN II – 3 in biopsy. So 2 were false negative by VIA/VILI.10 high grade lesions (HGL) in VIA/VILI turned out to be CIN I – 1, CIN II – 2, CIN III – 4 and SCC – 3 in biopsy. 3 squamous cell carcinomas were underestimated as high grade lesions in VIA/VILI. All the 5 invasive carcinomas were reported as squamous cell carcinoma in biopsy. This is given in table 11 and chart 7.

TABLE 11

COMPARISON OF VIA/VILI WITH BIOPSY DIAGNOSIS

n=30

VIA/VILI	BIOPSY REPORT				
	CERVICITIS	CIN I	CIN II	CIN III	SCC
LGL=15	2(100%)	10(90.9%)	3(60%)	0	0
HGL=10	0	1(9.1%)	2(40%)	4(100%)	3(37.5%)
INV CA=5	0	0	0	0	5(62.5%)

The percentage of concordance for VIA/VILI with biopsy was 66.7% for LGL, 90% for HGL and 100% for SCC (table 12).

TABLE 12

PERCENTAGE OF CONCORDANT CASES IN VIA/VILI

VIA/VILI	LGL	HGL	INV CA
TOTAL	15	10	5
CONCORDANT	10	9	5
% OF AGREEMENT	66.7%	90%	100%

These patients were subjected to liquid based cervical cytology. The reports were given as NIL – 6, LSIL – 10, HSIL – 8, SCC – 6 (table 13).

TABLE 13 : LBC RESULTS

n=30

LBC	NIL	LSIL	HSIL	SCC
FREQUENCY	**6**	**10**	**8**	**6**
PERCENTAGE	**20%**	**33.3%**	**26.6%**	**20%**

Of the 15 low grade lesions reported by VIA/VILI, 6 were reported as NIL and 9 as LSIL in LBC. Of the 10 high grade lesions, 1 was reported as LSIL, 8 as HSIL and 1 as SCC. All the 5 invasive carcinomas in VIA/VILI were reported as SCC in LBC (table 14).

TABLE 14 : COMPARISON OF LBC WITH VIA/VILI

n=30

VIA/VILI	LBC REPORTS			
	NIL	LSIL	HSIL	SCC
LGL=15	**6**	**9**	**0**	**0**
HGL=10	**0**	**1**	**8**	**1**
INV CA=5	**0**	**0**	**0**	**5**

The 6 NIL cases turned out to be 2 cervicitis and 4 CIN I by biopsy. 10 LSIL cases turned out to be 7 CIN I and 3 CIN II. 9 HSIL cases turned out to be 2 CIN II, 4 CIN III and 3 SCC in biopsy. All 5 SCC cases were proved to be SCC only (table 15 and chart 8).

TABLE 15

COMPARISON OF LBC WITH BIOPSY

n=30

LBC	BIOPSY DIAGNOSIS				
	CERVICITIS	CIN I	CIN II	CIN III	SCC
NIL	2(100%)	4(36.4%)	0	0	0
LSIL	0	7(63.6%)	3(60%)	0	0
HSIL	0	0	2(40%)	4(100%)	2(25%)
SCC	0	0	0	0	6(75%)

The percentage of concordance for LBC with biopsy was 33.3% for NIL, 70% for LSIL, 75% for HSIL and 100% for SCC (table 16).

TABLE 16

PERCENTAGE OF CONCORDANT CASES IN LBC

LBC	NIL	LSIL	HSIL	SCC
TOTAL	6	10	8	6
CONCORDANT	2	7	6	6
% OF AGREEMENT	33.3%	70%	75%	100%

TABLE 17 : NUMBER OF TRUE POSITIVES, FALSE POSITIVES, TRUE NEGATIVES AND FALSE NEGATIVES IN LBC

LBC	BIOPSY	
	POSITIVE	NEGATIVE
POSITIVE	TP(14)	FP(0)
NEGATIVE	FN(4)	TN(2)

Sensitivity = 77.78%

Specificity = 100%

Positive predictive value = 100%

Negative predictive value = 33.33%

The correlation of LBC report with biopsy diagnosis showed a P value of 0.000 (table 18).

TABLE 18

CORRELATION OF LBC INTERPRETATION WITH HPE DIAGNOSIS

LBC	BIOPSY DIAGNOSIS					P value
	CERVICITIS	CIN I	CIN II	CIN III	SCC	
NIL	2	4	0	0	0	0.000
LSIL	0	7	3	0	0	
HSIL	0	0	2	4	2	
SCC	0	0	0	0	6	

p16 showing expression in nucleus or cytoplasm or both were taken as positive. The expression of p16 in cervical smears (ICC) was reported as negative, weakly positive and strongly positive. The results were 4 negative cases, 12 weakly positive cases and 14 strongly positive cases (table 19).

TABLE 19

IMMUNOCYTOCHEMISTRY - p16 RESULTS

n=30

ICC – p16	NEGATIVE	WEAKLY +	STRONGLY +
FREQUENCY	4	12	14
PERCENTAGE	13.3%	40%	46.7%

Among the 6 NIL cases diagnosed by LBC, 4 cases showed negativity with p16 and 2 showed weak positivity. Among the 10 LSIL cases, 9 cases showed weak positivity and 1 showed strong positivity. Among the 8 HSIL cases, 1 showed weak positivity and 7 cases showed strong positivity. All 6 carcinoma cases showed strong positivity with p16 (table 20 and chart 9).

TABLE 20 : COMPARISON OF EXPRESSION OF p16 IN CERVICAL SMEARS WITH MORPHOLOGICAL DIAGNOSIS IN LBC

n=30

LBC	ICC – p16		
	NEGATIVE	WEAK +	STRONG +
NIL	4(66.7%)	2(33.3%)	0
LSIL	0	9(90%)	1(10%)
HSIL	0	1(12.5%)	7(87.5%)
SCC	0	0	6(100%)

All cervicitis cases showed negativity with p16 in cervical smears. 81.81% of CIN I and 100% of CIN II, CIN III and SCC cases showed positivity with p16 in ICC (table 21 and chart 10).

TABLE 21

COMPARISON OF p16 EXPRESSION (ICC) WITH BIOPSY

n=30

ICC – p16	BIOPSY				
	CERVICITIS	CIN I	CIN II	CIN III	SCC
NEGATIVE	2(100%)	2(18.18%)	0	0	0
WEAK +	0	9(81.81%)	2(40%)	1(25%)	0
STRONG +	0	0	3(60%)	3(75%)	8(100%)

TABLE 22 : NUMBER OF TRUE POSITIVES, FALSE POSITIVES, TRUE NEGATIVES AND FALSE NEGATIVES IN ICC-p16

ICC – p16	BIOPSY	
	POSITIVE	NEGATIVE
POSITIVE	TP(16)	FP(0)
NEGATIVE	FN(2)	TN(2)

Sensitivity = 88.89%

Specificity = 100%

Positive predictive value = 100%

Negative predictive value = 50%

The association of p16 expression in cervical non-neoplastic, pre-neoplastic and neoplastic lesions showed P value of 0.000 (table 23).

TABLE 23

CORRELATION OF p16 EXPRESSION IN CERVICAL SMEARS (ICC) WITH BIOPSY DIAGNOSIS

ICC – p16	BIOPSY					P value
	CERVICITIS	CIN I	CIN II	CINIII	SCC	
NEG	2	2	0	0	0	0.000
WEAK +	0	9	2	1	0	
STRONG+	0	0	3	3	8	

All the 9 CIN I cases showed weak positivity with p16 in dysplastic cells. Among the 5 CIN II cases, 2 were weakly positive and 3 were strongly positive. Among the 4 CIN III cases, 1 was weakly positive and 3 were strongly positive. All the 8 SCC cases showed strong positivity. The correlation of increasing intensity of p16 expression in cervical pre-neoplastic and neoplastic lesions showed P value of 0.000 (table 24).

TABLE 24 : CORRELATION OF INTENSITY OF p16 EXPRESSION (ICC) WITH INCREASING GRADES OF CERVICAL LESIONS

ICC – p16	BIOPSY				P value
	CIN I	CIN II	CIN III	SCC	
WEAK	9	2	1	0	0.000
STRONG	0	3	3	8	

The results of Ki-67 expression in cervical smears are given in table 25. Ki-67 showed nuclear positivity. As even normal proliferating basal cell layer of cervical lining shows positivity with Ki-67, <1% of normal cells showing positivity was taken as negative. The scores were given as 0 if negative, score of 1 if <10% of dysplastic cells were positive , score of 2 if 10-50% of dysplastic cells were positive and score of 3 if >50% of dysplastic cells were positive. Here, 5 cases were negative, 7 cases showed positivity in <10% of dysplastic cells, 10 cases showed positivity in 10-50% of dysplastic cells and 8 cases showed positivity in >50% of dysplastic cells.

TABLE 25 : IMMUNOCYTOCHEMISTRY – Ki67 RESULTS

n=30

ICC - Ki-67	NEG	<10%	10-50%	>50%
FREQUENCY	5	7	10	8
PERCENTAGE	16.7%	23.3%	33.3%	26.7%

Among the 6 NIL cases diagnosed by LBC, 5 were negative for Ki-67 and 1showed positivity in <10% of dysplastic cells. Among the 10 LSIL cases, 6 showed positivity in <10% of dysplastic cells and 4 showed positivity in 10-50% of dysplastic cells. Among the 8 HSIL cases, 5 showed positivity in 10-50% of dysplastic cells and 3 showed positivity in >50% of dysplastic cells. Among the 6 SCC cases, 1 showed positivity in 10-50% of dysplastic cells and 5 showed positivity in >50% of dysplastic cells (table 26 and chart 11).

TABLE 26 : COMPARISON OF EXPRESSION OF Ki-67 IN CERVICAL SMEARS WITH MORPHOLOGICAL DIAGNOSIS IN LBC

LBC	ICC – Ki67			
	NEGATIVE	<10%	10-50%	>50%
NIL	5(83.3%)	1(16.7%)	0	0
LSIL	0	6(60%)	4(40%)	0
HSIL	0	0	5(62.5%)	3(37.5%)
SCC	0	0	1(16.7%)	5(83.3%)

2 cervicitis cases diagnosed by biopsy were negative. Of the 11 CIN I cases, 8 cases (72.7%) showed positivity, of which 6 showed positivity in <10% of dysplastic cells and 2 showed positivity in 10-50% of dysplastic cells. All the CIN II cases showed positivity, of which 1showed positivity in <10% of dysplastic cells, 3showed positivity in 10-50% of dysplastic cells and 1 showed positivity in >50% of dysplastic cells. All the 4 CIN III cases showed positivity, of which 3 showed positivity in 10-50% of dysplastic cells and 1 showed positivity in >50% of dysplastic cells. All the 8 SCC cases were positive, of which 2 showed positivity in 10-50% of dysplastic cells and 6 showed positivity in >50% of dysplastic cells (table 27 and chart 12).

TABLE 27 : COMPARISON OF Ki67 EXPRESSION IN CERVICAL SMEARS (ICC) WITH HPE

ICC – Ki67	BIOPSY				
	CERVICITIS	CIN I	CIN II	CIN III	SCC
NEG	2(100%)	3(27.3%)	0	0	0
<10%	0	6(54.5%)	1(20%)	0	0
10-50%	0	2(18.2%)	3(60%)	3(75%)	2(25%)
>50%	0	0	1(20%)	1(25%)	6(75%)

TABLE 28: NUMBER OF TRUE POSITIVES, FALSE POSITIVES, TRUE NEGATIVES AND FALSE NEGATIVES IN ICC-Ki67

ICC – Ki67	BIOPSY	
	POSITIVE	NEGATIVE
POSITIVE	TP(25)	FP(0)
NEGATIVE	FN(3)	TN(2)

Sensitivity = 89.28%

Specificity = 100%

Positive predictive value = 100%

Negative predictive value = 40%

The association of percentage of dysplastic cells expressing Ki-67 in cervical smears in cervical non-neoplastic, pre-neoplastic and neoplastic lesions showed P value of 0.000 (table 29).

TABLE 29 : CORRELATION OF Ki67 EXPRESSION IN CERVICAL SMEARS (ICC) WITH HPE

ICC – Ki67	BIOPSY					P value
	CERVICITIS	CIN I	CIN II	CINIII	SCC	
NEG	2	3	0	0	0	0.000
<10%	0	6	1	0	0	
10-50%	0	2	3	3	2	
>50%	0	0	1	1	6	

The results of p16 expression in tissue sections is given in table 30. p16 showed nuclear or cytoplasmic positivity or both. 26 cases were positive and 4 were negative.

TABLE 30 : IMMUNOHISTOCHEMISTRY – p16 RESULTS

n=30

IHC – p16	POSITIVE	NEGATIVE
FREQUENCY	26	4
PERCENTAGE	86.7%	13.3%

All cervicitis cases showed negativity with p16 in IHC. Among the 11 CIN I cases, 9 showed positivity and 2 were negative. All the 5 CIN II cases showed positivity for p16. All the 4 CIN III cases showed positivity for p16. All the 8 SCC cases showed positivity for p16 (table 31 and chart 13).

TABLE 31 : COMPARISON OF p16 EXPRESSION (IHC) WITH BIOPSY

n=30

IHC – p16	BIOPSY				
	CERVICITIS	CIN I	CIN II	CIN III	SCC
NEGATIVE	2(100%)	2(18.2%)	0	0	0
POSITIVE	0	9(81.8%)	5(100%)	4(100%)	8(100%)

The association of immunohistochemical expression of p16 in cervical non-neoplastic, pre-neoplastic and neoplastic lesions showed P value of 0.003 (table 32).

TABLE 32
CORRELATION OF IMMUNOHISTOCHEMICAL
p16 EXPRESSION WITH BIOPSY

IHC – p16	BIOPSY					P value
	CERVICITIS	CIN I	CINII	CINIII	SCC	
NEGATIVE	2	2	0	0	0	0.003
POSITIVE	0	9	5	4	8	

The positivity and negativity of p16 expression in ICC and IHC were compared in relation to cervical lesions. All cases showed 100% concordance with IHC (table 33).

TABLE 33 : COMPARISON OF EXPRESSION OF p16 IN ICC AND IHC IN RELATION TO CERVICAL LESIONS

p16	BIOPSY									
	CERVICITIS		CIN I		CIN II		CIN III		SCC	
	1CC	IHC	ICC	IHC	ICC	IHC	ICC	IHC	ICC	IHC
POS	0	0	9	9	5	5	4	4	8	8
NEG	2	2	2	2	0	0	0	0	0	0
% of agreement	100%		100%		100%		100%		100%	

The results of Ki67 expression in tissue sections is given in table 34. Cases expressing Ki-67 nuclear positivity in the basal layer was taken as negative as it is normal. Only those cases expressing Ki-67, 2 layers above the basal layer were considered positive.

TABLE 34 : IMMUNOHISTOCHEMISTRY – Ki-67 RESULTS

n=30

IHC – Ki67	Neg	Lower 1/3	Lower 2/3	Full thickness	Diffuse
FREQUENCY	4	9	5	4	8
PERCENTAGE	13.3%	30%	16.7%	13.3%	26.7%

2 cases of cervicitis were negative. Of the 11 CIN I cases, 2cases were negative and the remaining 9 cases showed nuclear positivity in lower 1/3rd of the epithelium. All the 5 CIN II cases showed nuclear positivity in lower 2/3rd of the epithelium. All the 4 CIN III cases showed nuclear positivity in full thickness of the epithelium. All the 8 SCC cases showed diffuse nuclear positivity in the malignant cells including the invasive component (table 35 and chart 14).

TABLE 35

COMPARISON OF Ki67 EXPRESSION IN TISSUE SECTIONS (IHC) WITH BIOPSY

n=30

IHC – Ki67	BIOPSY				
	CERVICITIS	CIN I	CIN II	CIN III	SCC
Neg	2(100%)	2(18.2%)	0	0	0
lower 1/3	0	9(81.9%)	0	0	0
lower 2/3	0	0	5(100%)	0	0
Full thickness	0	0	0	4(100%)	0
Diffuse	0	0	0	0	8(100%)

The association of thickness of epithelium showing Ki-67 expression in tissue sections in cervical non-neoplastic, pre-neoplastic and neoplastic lesions showed P value of 0.000 (table 36).

TABLE 36

CORRELATION OF Ki-67 EXPRESSION IN TISSUE SECTIONS (IHC) WITH BIOPSY

IHC – Ki67	BIOPSY					P value
	CERVICITIS	CIN I	CIN II	CINIII	SCC	
Neg	2	2	0	0	0	0.000
lower 1/3	0	9	0	0	0	
lower 2/3	0	0	5	0	0	
Full thickness	0	0	0	4	0	
Diffuse	0	0	0	0	8	

The Ki-67 expression in ICC and IHC were compared in relation to cervical lesions. All cervicitis cases showed concordance in ICC and IHC. Among the 11 CIN I cases, 8 cases showed positivity in ICC, and 9 cases showed positivity in IHC. All CIN II, CIN III and SCC cases showed 100% concordance in ICC and IHC (table 37).

TABLE 37

COMPARISON OF EXPRESSION OF Ki-67 IN ICC AND IHC IN RELATION TO CERVICAL LESIONS

Ki67	BIOPSY									
	CERVICITIS		CIN I		CIN II		CIN III		SCC	
	1CC	IHC	ICC	IHC	ICC	IHC	ICC	IHC	ICC	IHC
NEG	2	2	3	2	0	0	0	0	0	0
POS	0	0	8	9	5	5	4	4	8	8
% of agreement	100%		88.9%		100%		100%		100%	

The efficacy of LBC and immunocytochemistry with p16 and Ki-67are compared in table 38 and chart 15.

TABLE 38

COMPARISON OF EFFICACY OF LBC AND IMMUNOCYTOCHEMISTRY WITH p16 AND Ki-67

	LBC	ICC-p16	ICC-Ki67
Sensitivity	77.78%	88.89%	89.28%
Specificity	100%	100%	100%
PPV	33.3%	50%	40%
NPV	100%	100%	100%
P value	0.000	0.000	0.000

CHARTS AND PICTURES

Chart 1 : Distribution of cervical pre-neoplastic and neoplastic lesions in our Institute

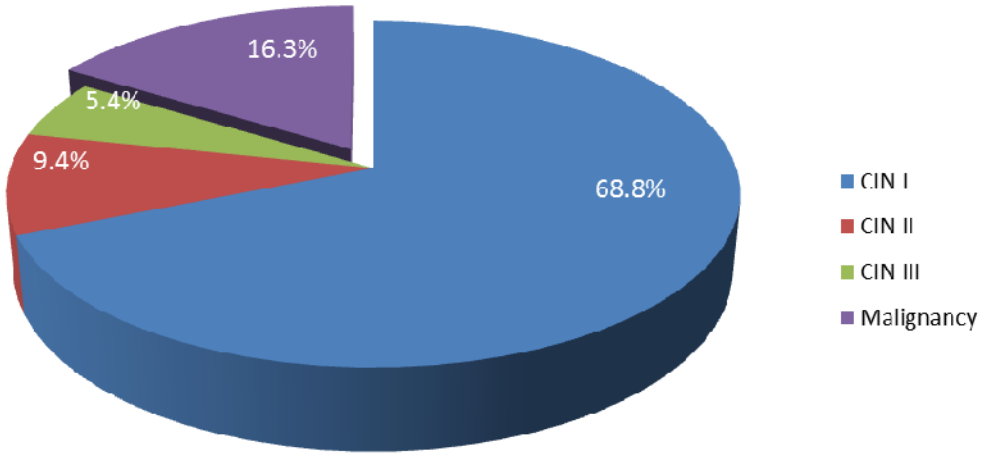


Chart 2 : Distribution of malignant cervical leisons in our Institute

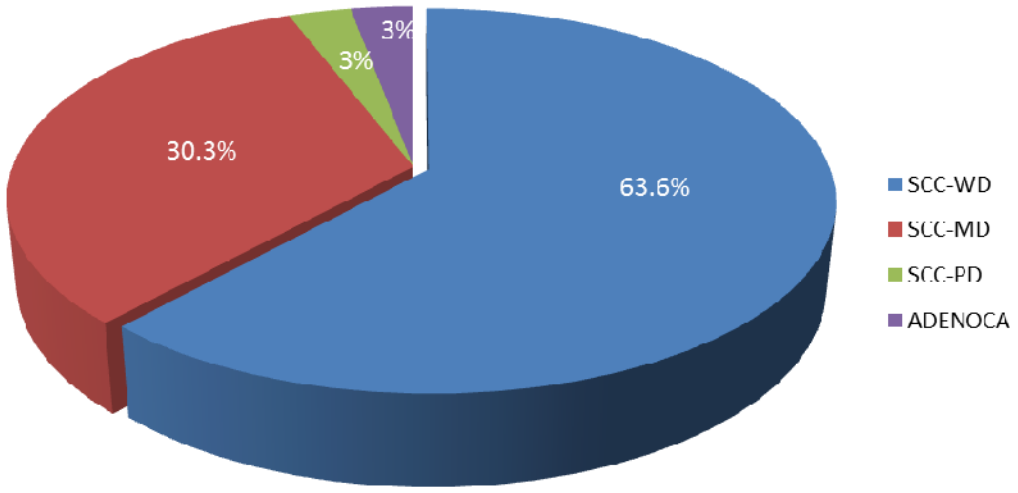


Chart 3 : Age-wise distribution of cervical pre-neoplastic and neoplastic lesions in our Institute

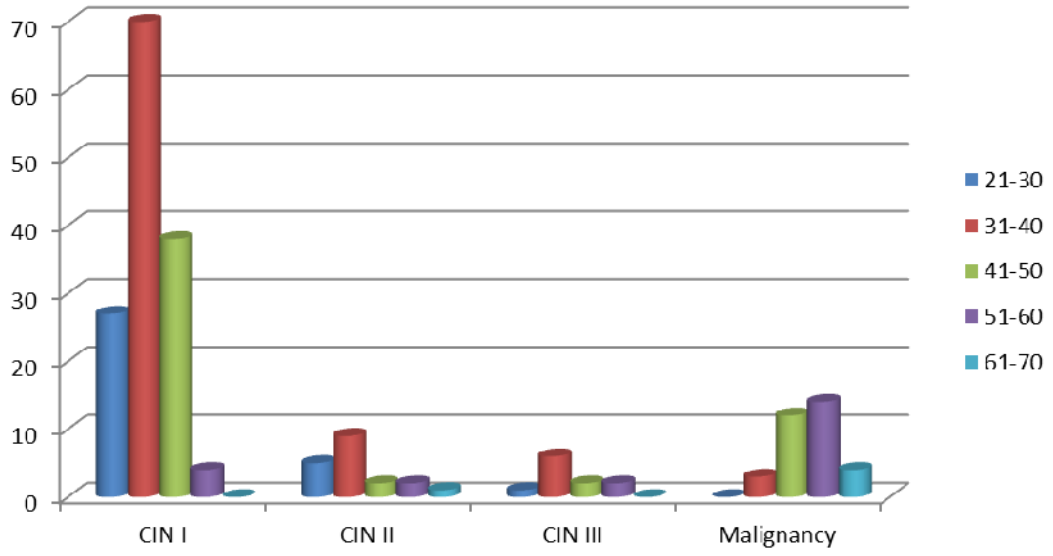


Chart 4 : Distribution of cervical lesions in colposcopic biopsies in our Institute – quadrant of cervix involved wise

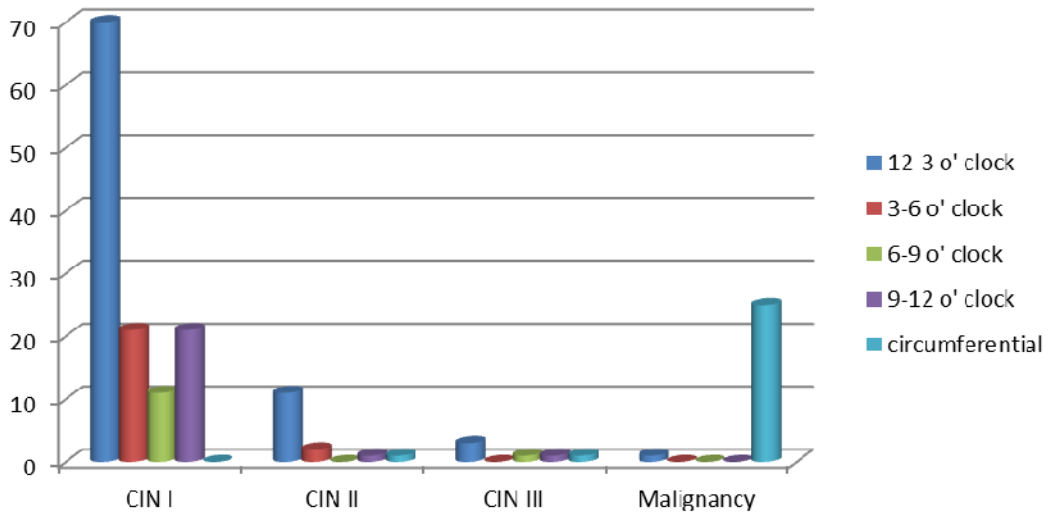


Chart 5 : Distribution of cervical lesions in the study group

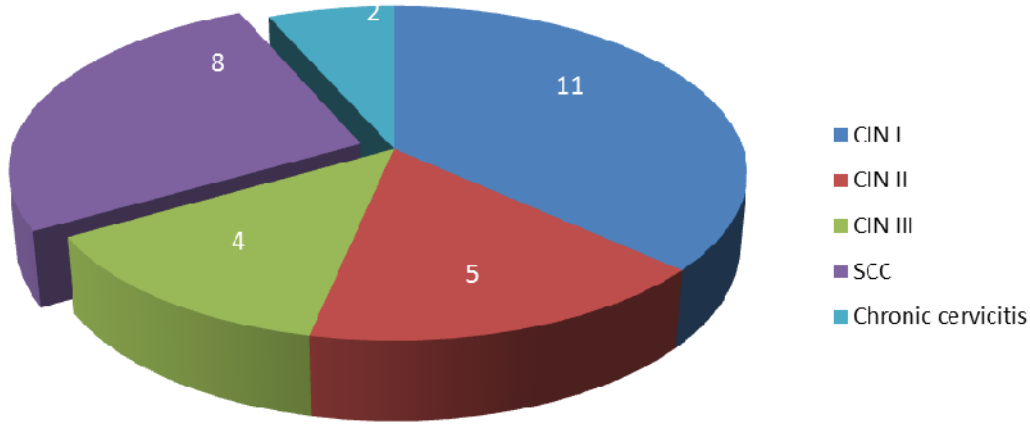
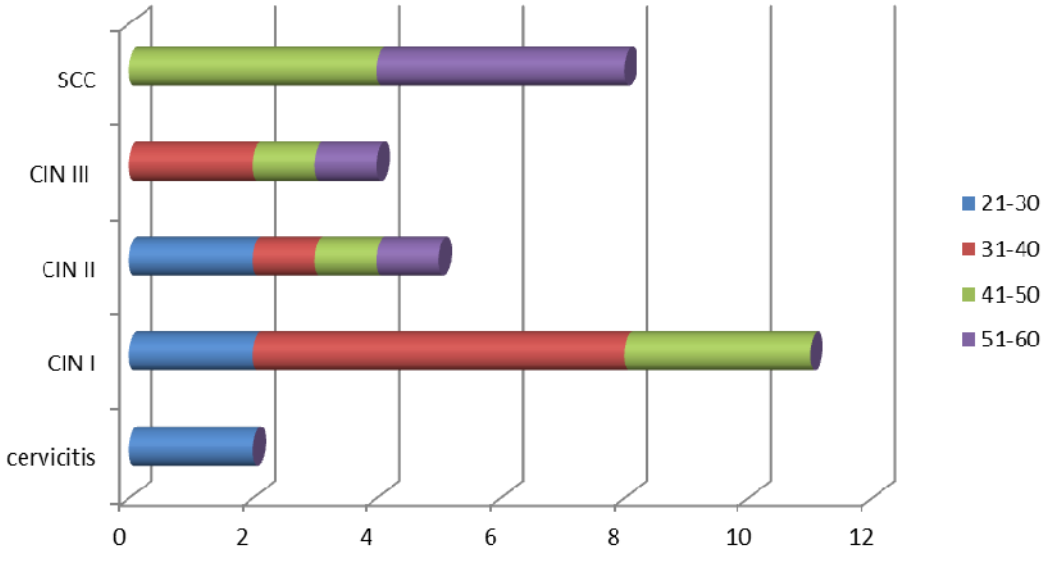


Chart 6 : Agewise distribution of cervical lesions in the study group



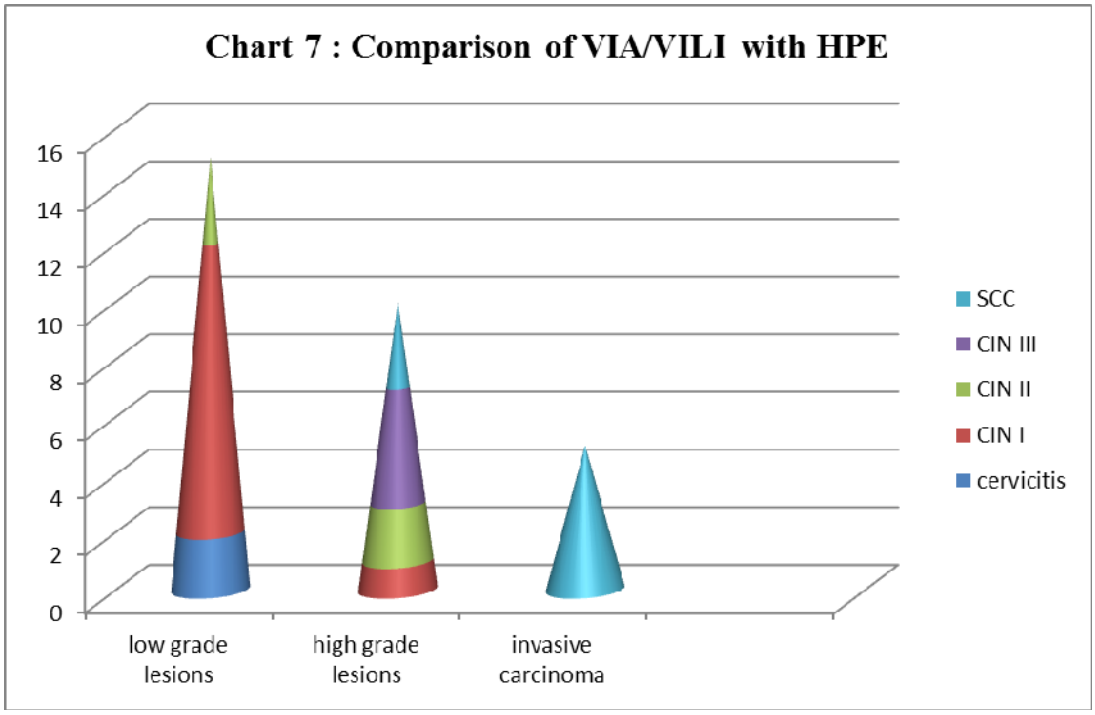


Chart 7 : Comparison of VIA/VILI with HPE
16
14
12
10
8
6
4
2
0
low grade lesions
high grade lesions
invasive carcinoma
SCC
CIN III
CIN II
CIN I
cervicitis

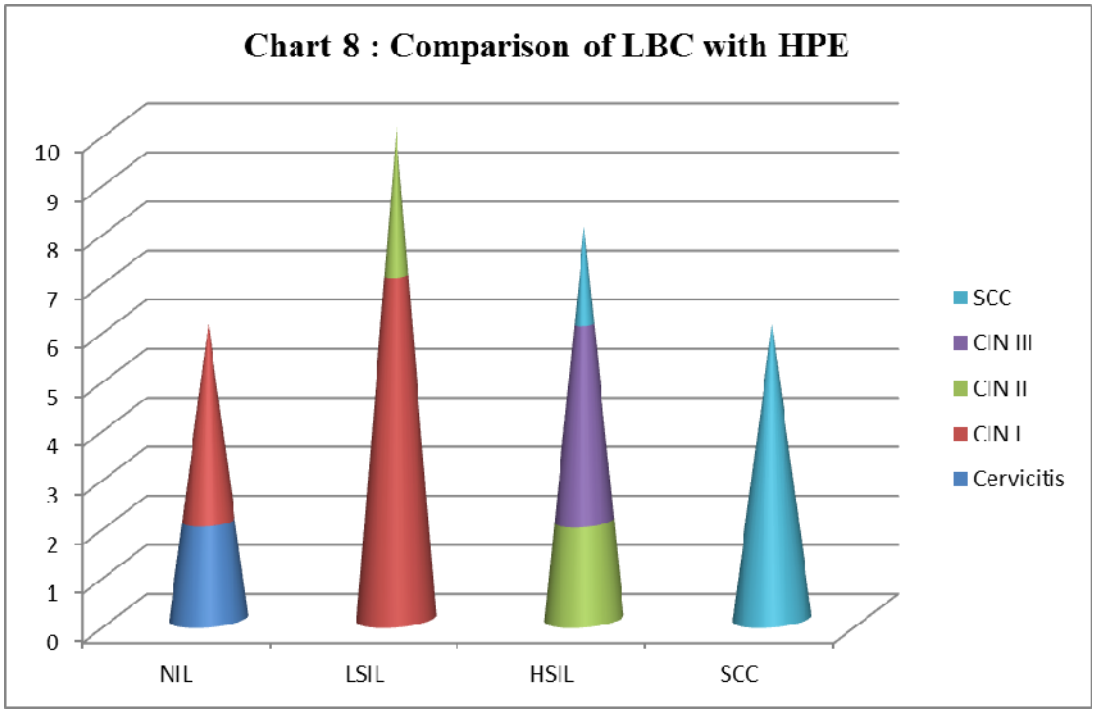


Chart 8 : Comparison of LBC with HPE
10
9
8
7
6
5
4
3
2
1
0
NIL
LSIL
HSIL
SCC
SCC
CIN III
CIN II
CIN I
Cervicitis

Chart 9 : Comparison of p16 expression in cervical smears with morphologic diagnosis in LBC

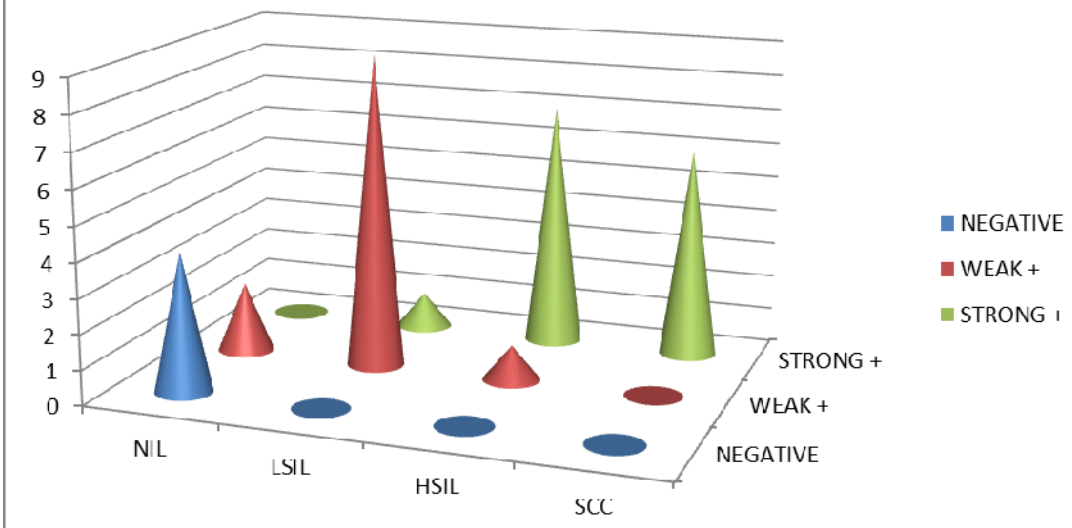


Chart 10 : Comparison of p16 expression in cervical smears with HPE

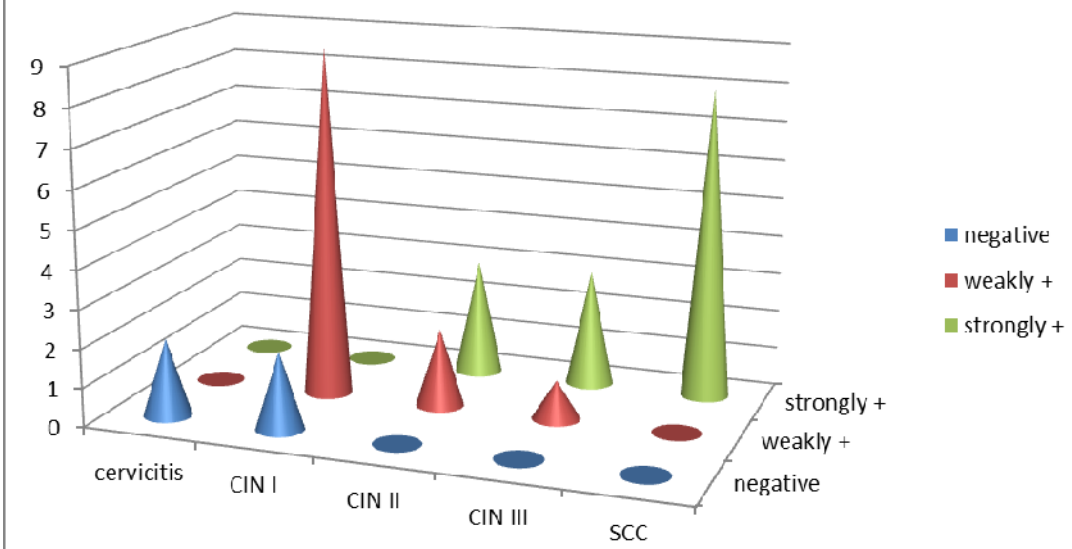


Chart 11 : Comparison of Ki-67 expression in cervical smears with morphologic diagnosis in cytology

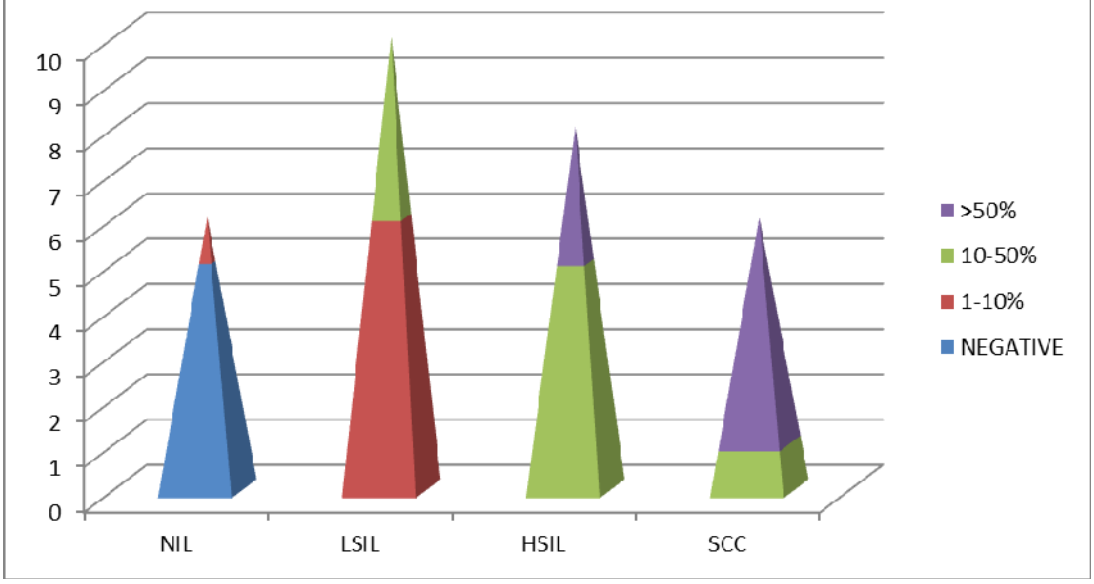


Chart 12 : Comparison of Ki-67 expression in cervical smears with HPE

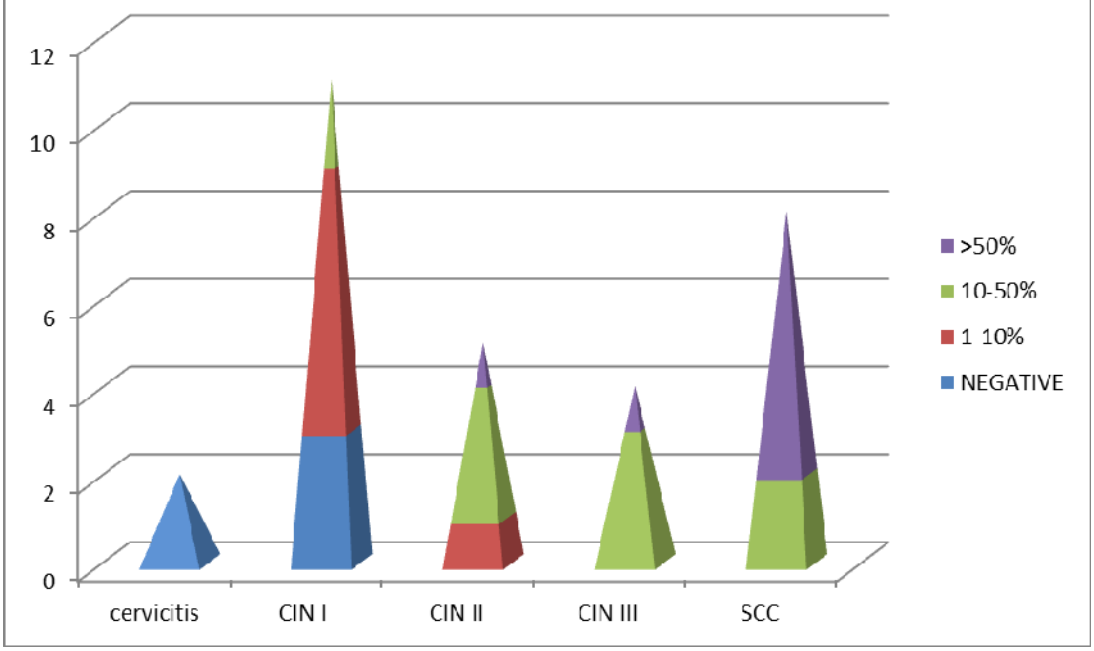


Chart 13 : Comparison of p16 expression in tissue sections with HPE

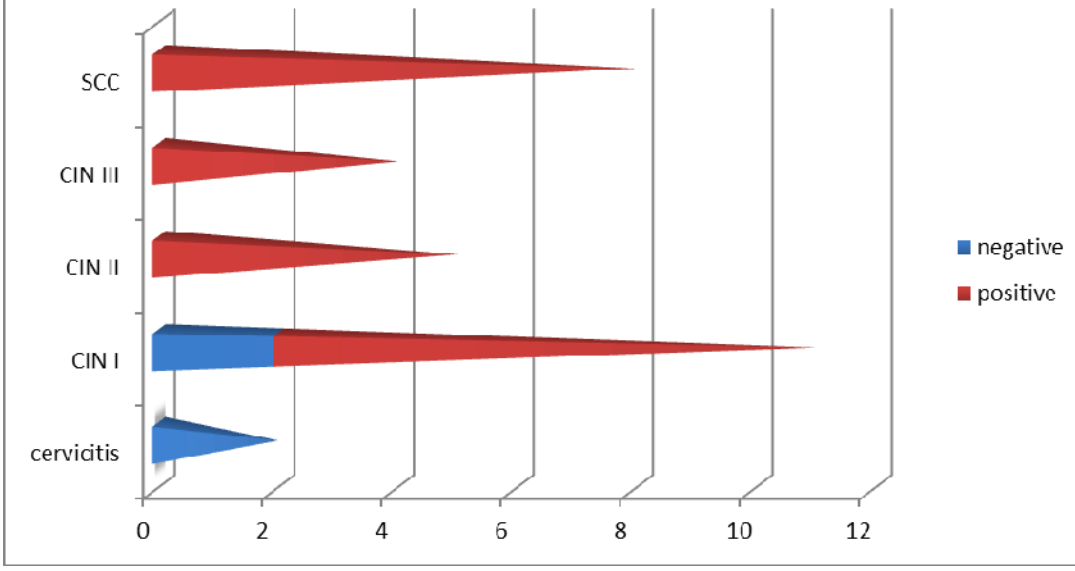


Chart 14 : Comparison of Ki-67 expression in tissue sections with HPE

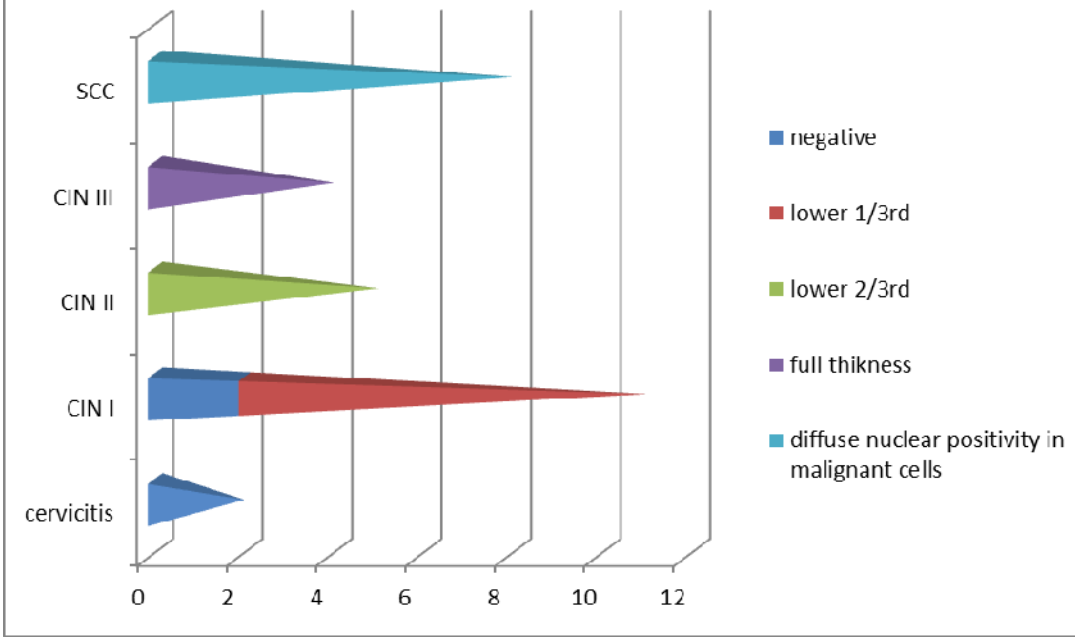
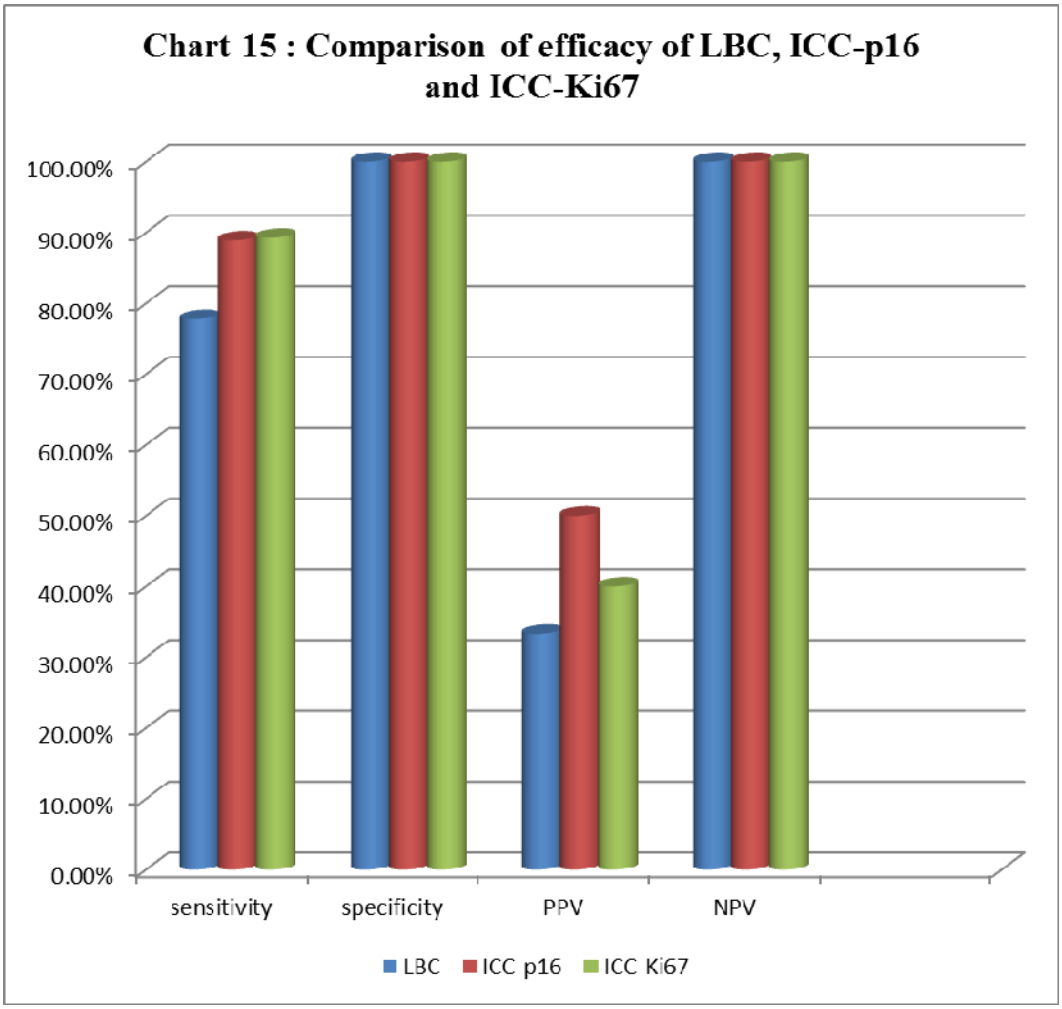


Chart 15 : Comparison of efficacy of LBC, ICC-p16 and ICC-Ki67



CHRONIC CERVICITIS

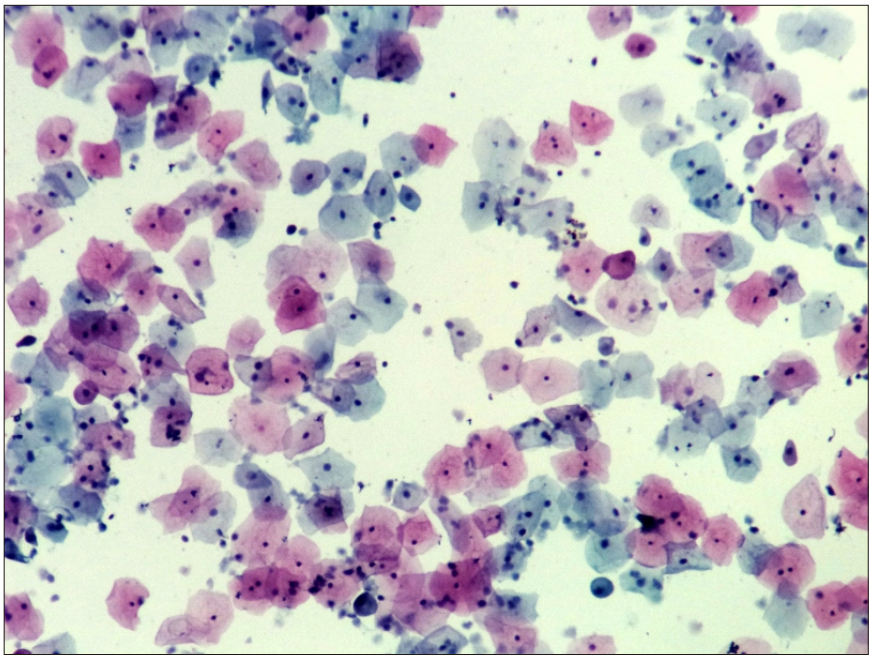


Figure 1 : LBC - Mature squamous cells in a background of inflammatory cells (Pap stain, 100x)

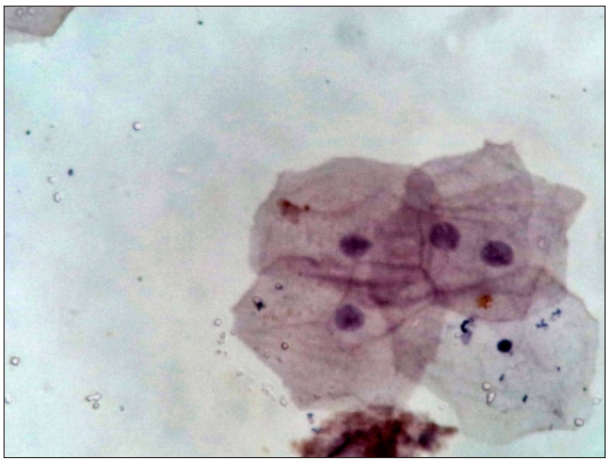


Figure 2 : Immunocytochemistry p16 negative, 400x

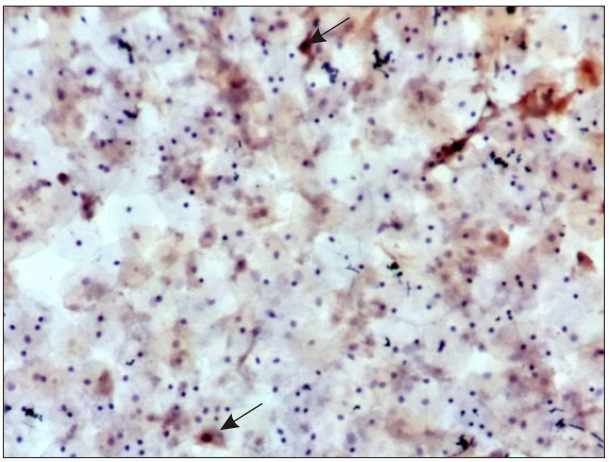


Figure 3 : Immunocytochemistry Ki-67 nuclear positivity in normal cells, 100x

CHRONIC CERVICITIS

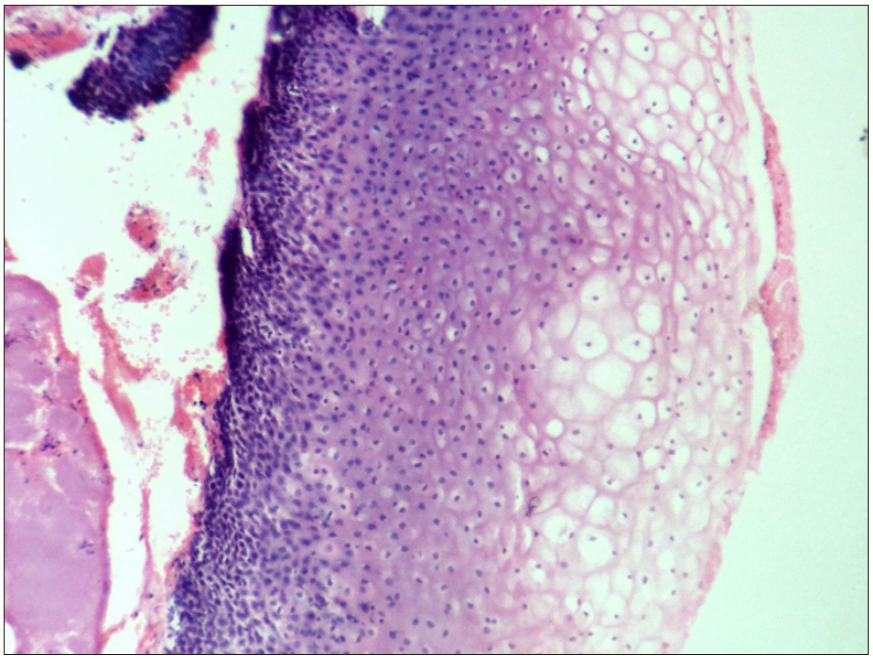


Figure 4 : Cervical punch biopsy. (H&E stain, 100x)

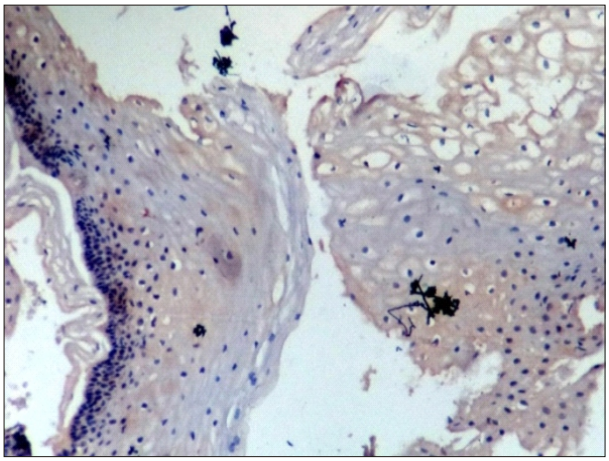


Figure 5 : Immunohistochemistry p16 negative, 100x

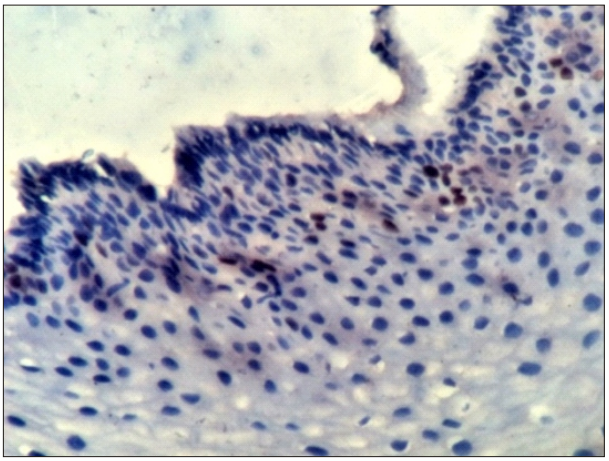


Figure 6 : Immunohistochemistry Ki-67 nuclear positivity in normal basal cells, 400x

LOW GRADE SQUAMOUS INTRAEPITHELIAL LESION

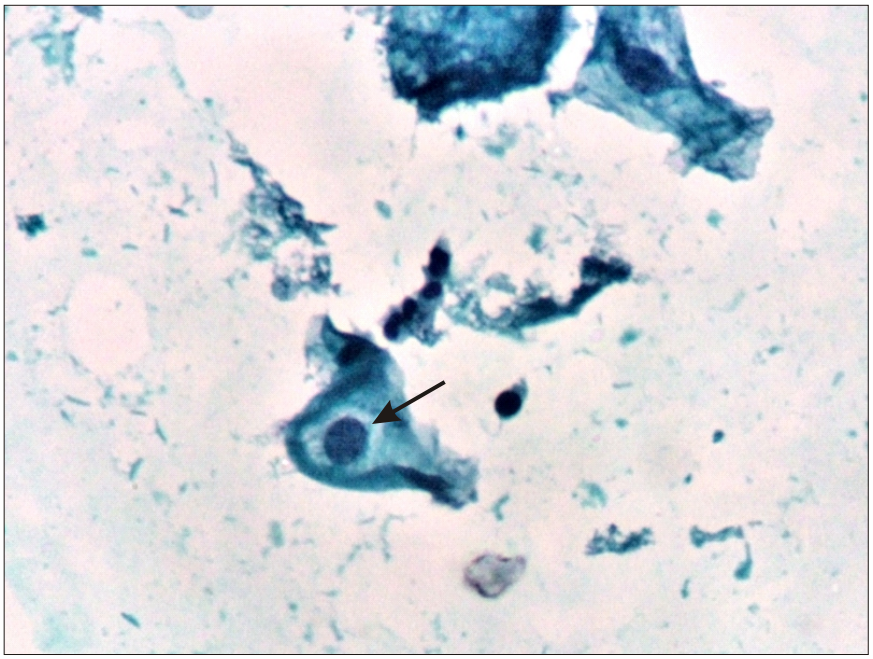


Figure 7 : LBC - Koilocytotic atypia with perinuclear halo. (Pap stain, 400x)

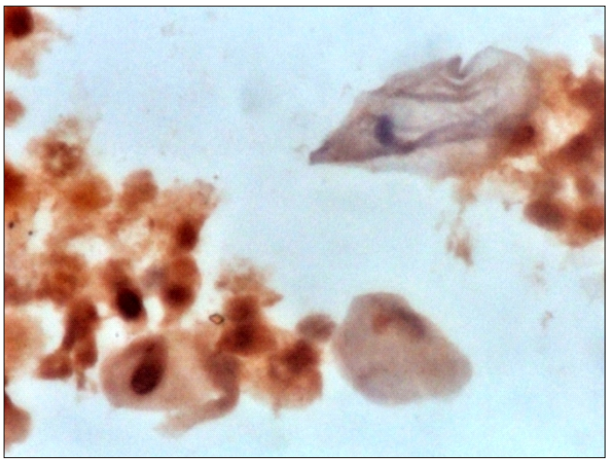


Figure 8 : Immunocytochemistry p16 weakly positive, 400x

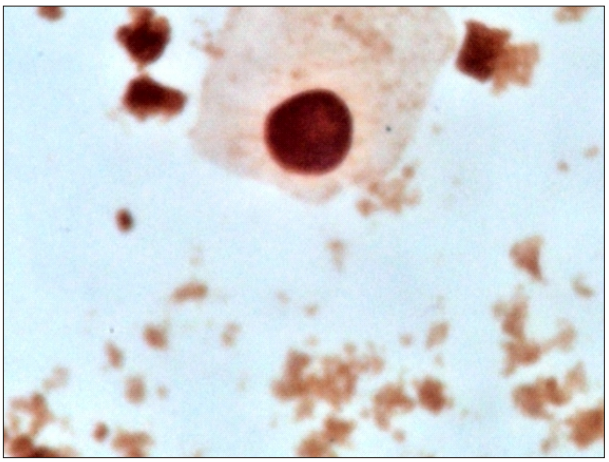


Figure 9 : Immunocytochemistry Ki-67 nuclear positivity, 400x

CERVICAL INTRAEPITHELIAL NEOPLASIA - I

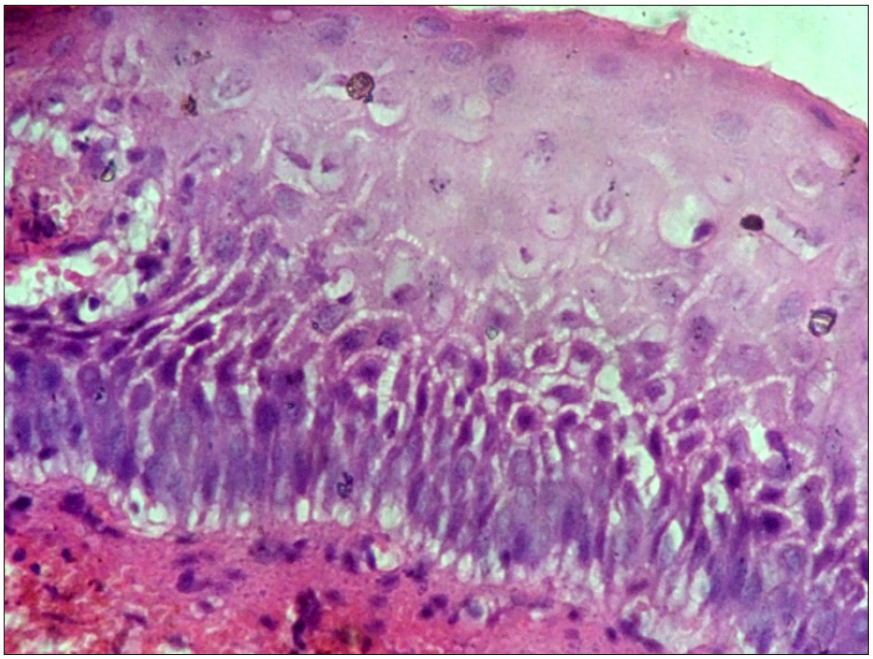


**Figure 10 : Cervical punch biopsy -
Dysplasia in lower 1/3rd (H&E, 400x)**

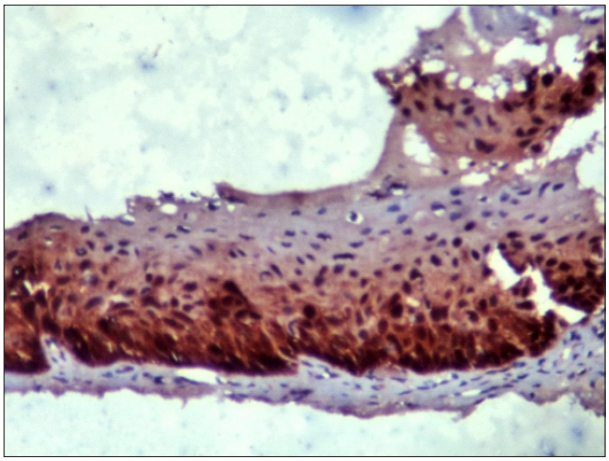


**Figure 11 : Immunohistochemistry
p16 band like positivity
in lower 1/3rd, 100x**

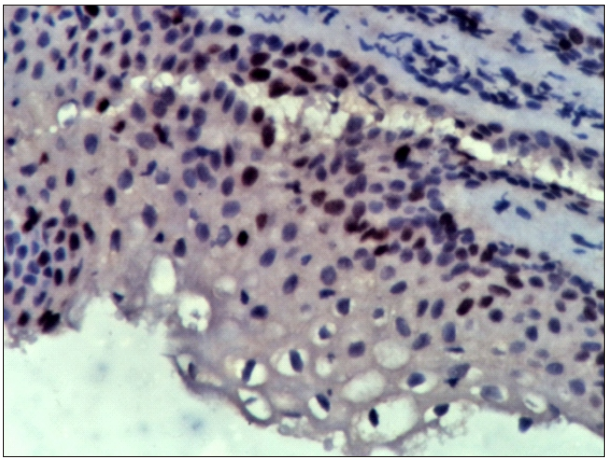


**Figure 12 : Immunohistochemistry
Ki-67 nuclear positivity
in lower 1/3rd, 400x**

HIGH GRADE SQUAMOUS INTRAEPITHELIAL LESION

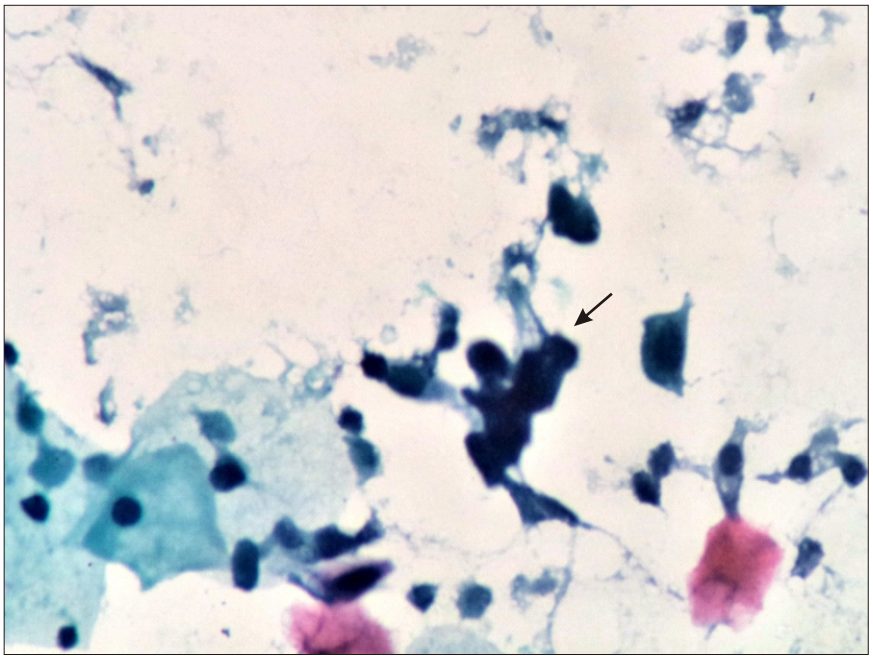


Figure 13 : LBC - Cells with increased N:C Ratio and irregular nuclear contour (Pap stain, 400x)

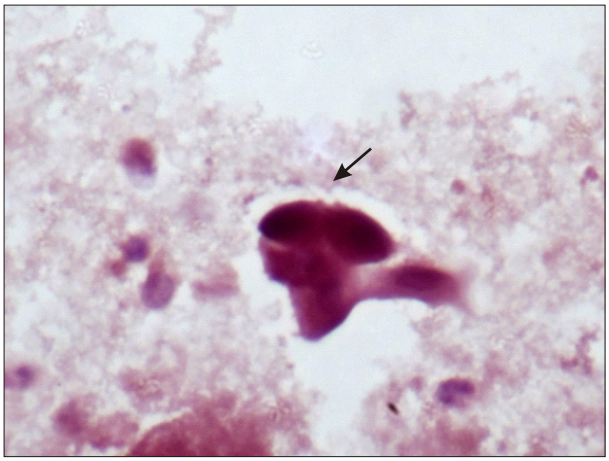


Figure 14 : Immunocytochemistry p16 strongly positive, 400x

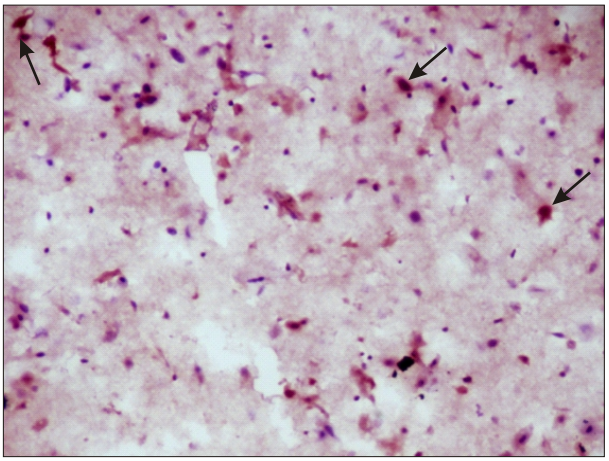


Figure 15 : Immunocytochemistry Ki-67 nuclear positivity in 10 to 50% of dysplastic cells, 100x

CERVICAL INTRAEPITHELIAL NEOPLASIA - II

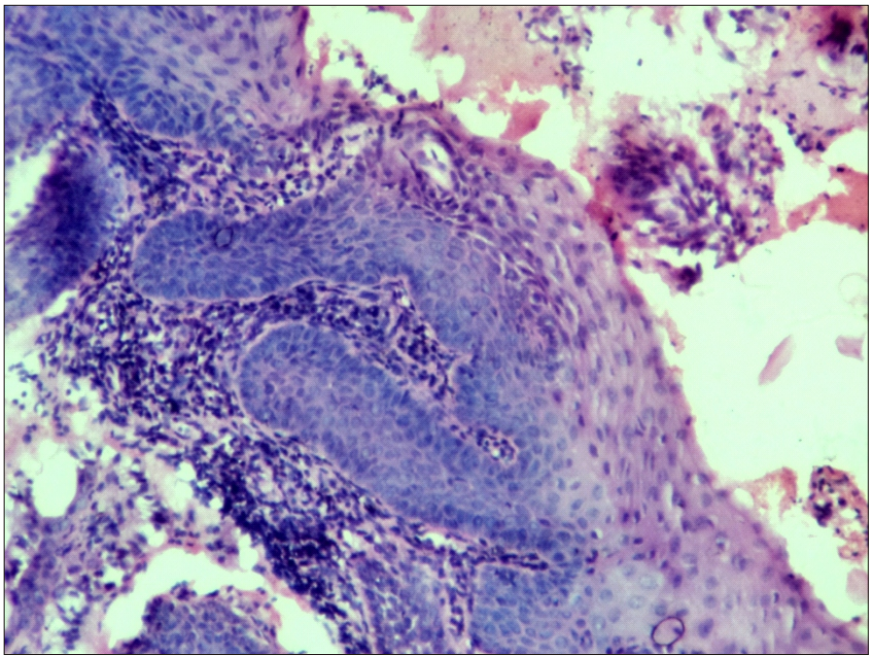


Figure 16 : Cervical punch biopsy - dysplasia in lower 2/3rd (H&E stain, 100x)

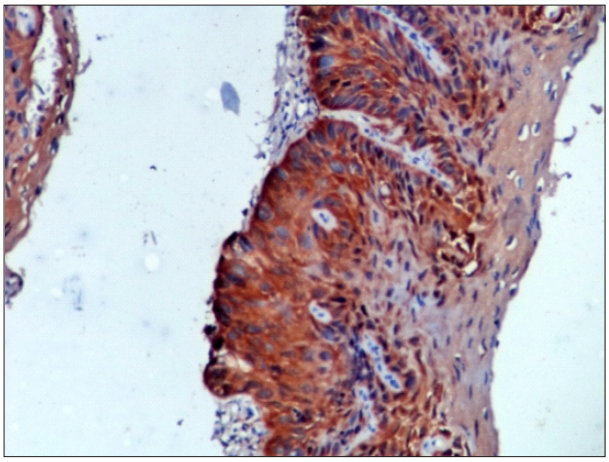


Figure 17 : Immunohistochemistry p16 positive in lower 2/3rd, 100x

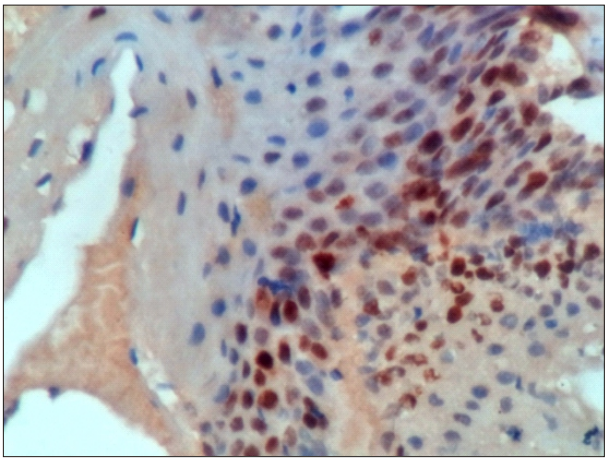


Figure 18 : Immunohistochemistry Ki-67 nuclear positivity in lower 2/3rd, 400x

CERVICAL INTRAEPITHELIAL NEOPLASIA - III

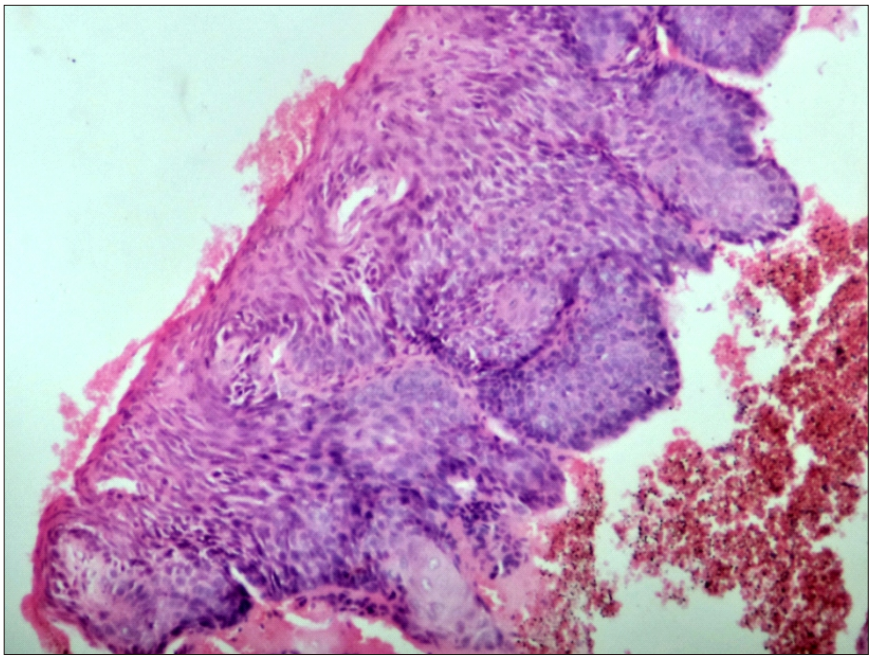


**Figure 19 : Cervical punch biopsy -
Dysplasia in full thickness of epithelium (H&E, 100x)**

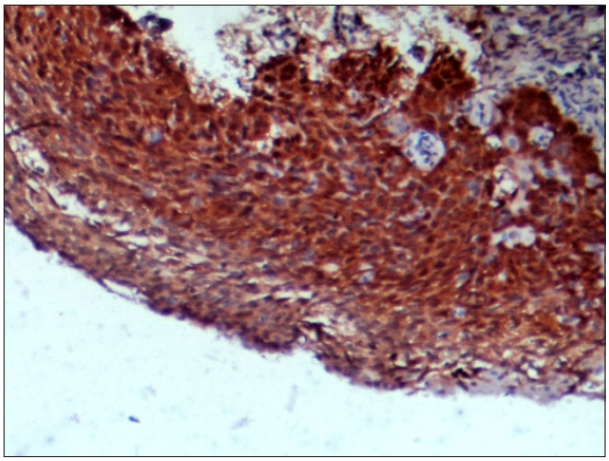


**Figure 20 : Immunohistochemistry
p16 positive in full thickness, 100x**

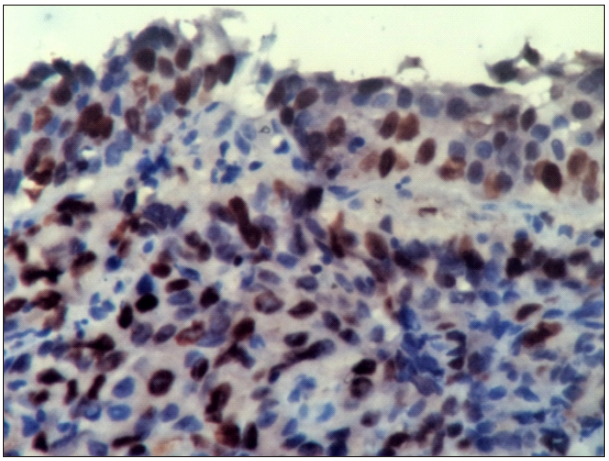


**Figure 21 : Immunohistochemistry
Ki-67 nuclear positivity
in full thickness, 400x**

SQUAMOUS CELL CARCINOMA

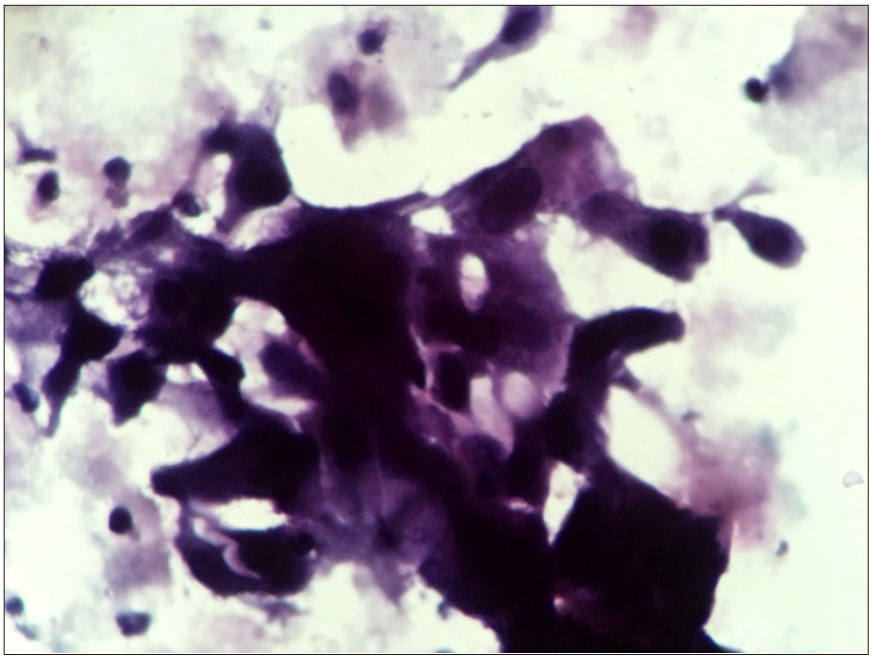


Figure 22 : LBC - Cluster of atypical malignant cells (Pap stain, 400x)

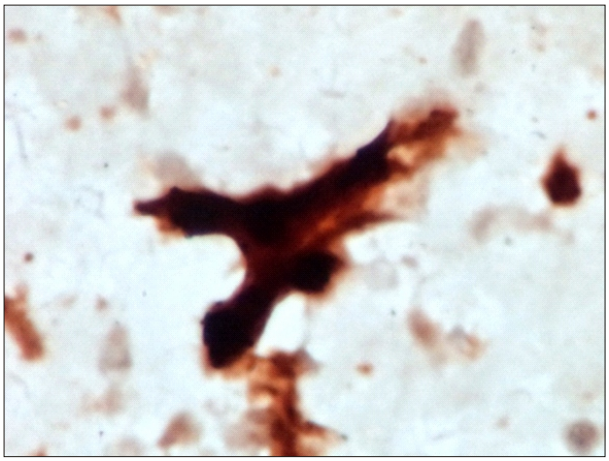


Figure 23 : Immunocytochemistry p16 strongly positive, 400x

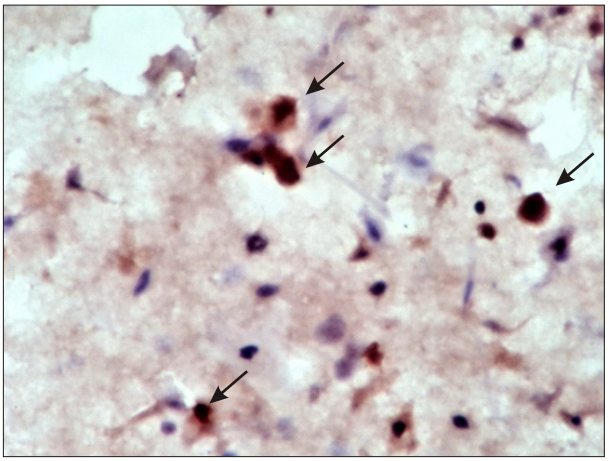


Figure 24 : Immunocytochemistry Ki-67 nuclear positivity in >50% of dysplastic cells, 400x

SQUAMOUS CELL CARCINOMA

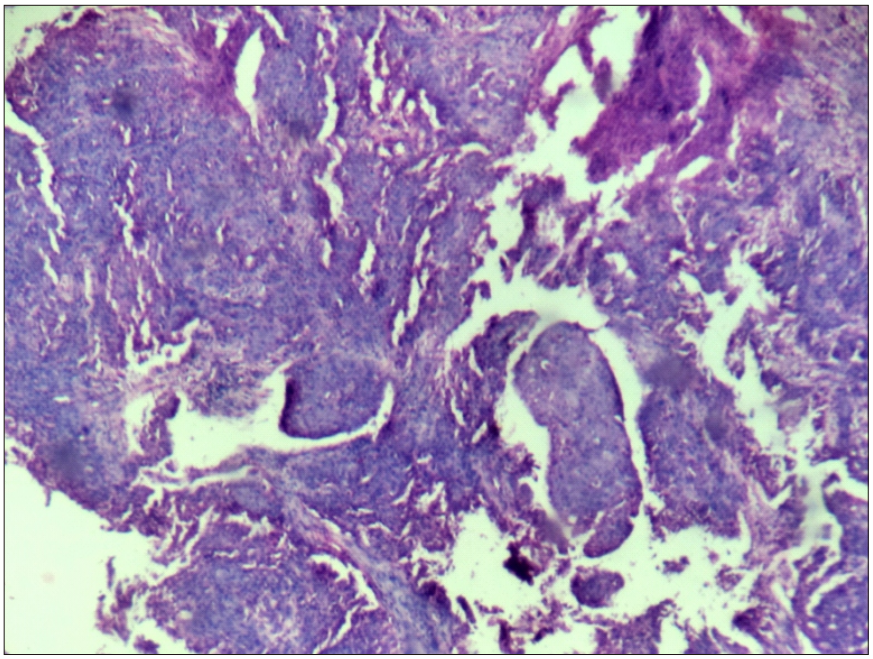


Figure 25 : Cervical punch biopsy -
Invasive squamous cell carcinoma (H&E, 100x)

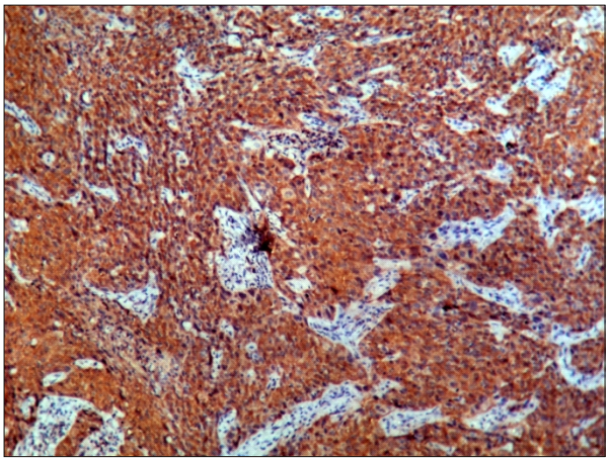


Figure 26 : Immunohistochemistry
p16 diffuse positivity, 100x

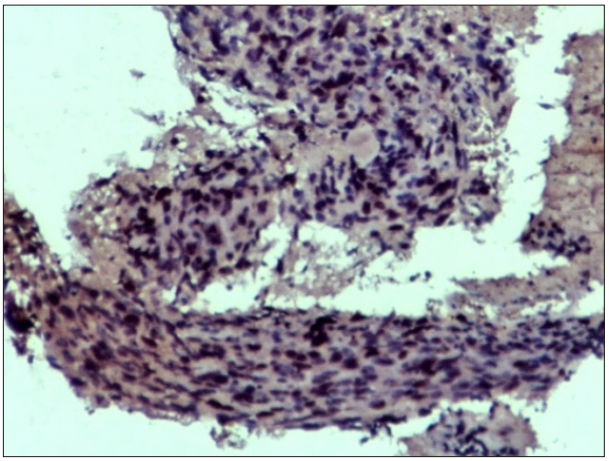


Figure 27 : Immunohistochemistry
Ki-67 nuclear positivity, 100x

DISCUSSION

DISCUSSION

Cervical cancer in developing countries like India is still a major socio-economic burden, despite the availability of screening procedures. It has been said that routine screening can bring down the incidence of carcinoma cervix to a great extent[60]. And it has also been said that lack of screening is the major risk factor for cervical cancer. Hence there is a need for better screening procedures.

Cervical cytology is complicated by inter-observer variability. Though there are specific morphological criteria to diagnose cervical precancerous and cancerous lesions, this is complicated by inter-observer variability and hence lacks sensitivity[61]. Co-testing with HPV PCR was introduced. But this lacks specificity because most of the HPV infections are transient[62,63,64,65,66,67,68]. Hence use of immunomarkers in cervical cytology by immunocytochemistry was introduced to overcome the problems faced by the above said screening procedures. This has been proved to be both highly sensitive and specific[69]. There are various markers to diagnose cervical precancerous and cancerous lesions like p16, HPV L1 capsid, CDC6, MCM5 etc[69,70]. Of these, p16 has been proved to be the most useful and reliable marker[70].

In our current study, we studied the association of p16 and Ki-67 expression in cervical smears of cervical precancerous and cancerous lesions and compared their expression with cervical cytology, HPE and the expression of same markers in tissue sections (immunohistochemistry). Of these, histopathologic examination was taken as the gold standard and the efficacy of various screening procedures like cervical cytology, immunocytochemistry with p16 and immunocytochemistry with Ki-67 were compared.

EPIDEMIOLOGY OF CERVICAL PRE-NEOPLASTIC AND NEOPLASTIC LESIONS IN OUR INSTITUTE (FEBRUARY 2014 TO JULY 2014)

Totally, 202 cases of cervical pre-neoplastic and neoplastic lesions were diagnosed during a 6 months period from February 2014 to July 2014 at the Institute of Social Obstetrics and Govt. Kasturba Gandhi Hospital for Women and Children, Madras Medical College. Of these, 170 were colposcopic biopsies, 9 were LLETZ specimens, 13 were random ectocervix biopsy specimens and 10 were hysterectomy specimens. The statistics for 6 months period were as follows,

1. Of the 202 cases, 139 were CIN I, 19 were CIN II, 11were CIN III and 33 were malignancies. So CIN I (68.8%) constituted majority of cases, followed by malignancy (16.3%), CIN II (9.4%) and CIN III (5.4%).

2. Of the 33 malignant cases, 21 were SCC-WD, 10 were SCC-MD, 1 was SCC-PD and 1 was adenocarcinoma. So SCC-WD constituted majority of cases (63.6%), followed by SCC-MD (30.3%).

3. Of the 139 CIN I cases, 70 cases (50.4%) were in the 31-40 year age group, followed by 38 cases (27.3%) in the 41-50 year age group, 27 cases (19.4%) in the 21-30 year age group and 4 cases (2.9%) in above 50 year age group.

4. Of the 19 CIN II cases, 9 (47.4%) were in the 31-40 year age group. Of the 11 CIN III cases, 6 (54.5%) were in the 31-40 year age group. So the incidence of all the cervical precancerous lesions was common in the age group of 31-40 years. According to a study conducted by Bhojani[71], the cervical precancerous lesions were more common in the 30-39 year age group.

5. Of the 33 malignant cases, 14 (42.4%) were in the 51-60 year age group, followed by 12 cases (36.4%) in the 41-50 year age group, 4 (12.1%) in the above 60 year age group. Only 3 cases (9.1%) were in the 31-40

year age group. So malignancy was common in women above 40 years. According to a study conducted by Satya B. Paul et al[72], the incidence of cervical carcinoma was more common in the 41-50 year age group.

6. Cervical precancerous lesions were found to be more common in the reproductive women (CIN I – 88.5%, CIN II – 73.7%, CIN III – 81.8%), whereas malignancy was found to be more common in the post-menopausal women (90.9%).

7. Majority (58.3%) of cervical precancerous lesions were seen in the 12-3 o' clock quadrant of the cervix. Majority of the carcinoma cases (96.1%) were seen circumferentially abutting the os.

THE CURRENT STUDY GROUP

This was a prospective study involving 30 patients. 30 VIA/VILI positive cases were randomly selected from the patients attending the colposcopy department of the Institute of Social Obstetrics and Govt. Kasturba Gandhi Hospital for Women and Children, Madras Medical College for our study. These 30 patients were then subjected to liquid based cervical cytology and cervical biopsy. Immunocytochemistry with p16 and Ki-67 in cervical smears and immunohistochemistry with p16 and Ki-67 in tissue sections were performed. These procedures were compared with each other taking biopsy diagnosis as the gold standard.

Of the 30 VIA/VILI positive cases, 15 were low grade lesions, 10 were high grade lesions and 5 were interpreted as invasive carcinoma. The 15 low grade lesions (LGL) in VIA/VILI turned out to be cervicitis – 2, CIN I – 10, CIN II – 3 by histopathologic examination. Thus 2 cases which were VIA/VILI positive turned out to be cervicitis (false positive). 10 high grade lesions (HGL) in VIA/VILI turned out to be CIN I – 1, CIN II – 2, CIN III – 4 and SCC – 3 in biopsy. 3 squamous cell carcinomas were underestimated as high grade lesions in VIA/VILI. All the 5 invasive carcinomas were reported as squamous cell carcinoma in biopsy.

The percentage of concordance for VIA/VILI with biopsy was 66.7% for LGL, 90% for HGL and 100% for SCC in this study group. Thus VIA/VILI was more sensitive in detecting high grade lesions and malignancy than low grade lesions.

CHARACTERISTICS OF THE STUDY GROUP

1. Of the 30 cases, 2 patients diagnosed with chronic cervicitis by biopsy were in the 21-30 year age group. Majority of CIN I lesions were in the 31-40 year age group (54.5%). CIN II/III lesions were also common in 31-40 year age group (33.3%), followed by equal distribution among the 21-30, 41-50 and 51-60 year age groups. All 8 cases of squamous cell carcinomas were in the 41-60 year age group.

2. Majority of the cervical non-neoplastic and pre-neoplastic lesions (81.8%) were seen in reproductive women. But all the squamous cell carcinoma cases (100%) were seen in the post-menopausal women.

3. Majority of cervical precancerous lesions (63.6%) were in the 12-3 o' clock quadrant of the cervix. All the carcinoma cases were seen circumferentially abutting the os.

LIQUID BASED CERVICAL CYTOLOGY

The cervical cytology reports of the 30 cases were given as negative for intraepithelial lesion (NIL) – 6, low grade squamous intraepithelial lesion (LSIL) – 10, high grade squamous intraepithelial lesion (HSIL) – 8 and squamous cell carcinoma (SCC) – 6. These reports were then compared with the HPE diagnosis.

The 6 NIL cases turned out to be 2 cervicitis and 4 CIN I by biopsy. Thus 4 NIL cases were false negative. 10 LSIL cases turned out to be 7 CIN I and 3 CIN II by biopsy. 9 HSIL cases turned out to be 2 CIN II, 4 CIN III and 3 SCC in biopsy. All 5 SCC cases were proved to be SCC in biopsy. The percentage of concordance for LBC with biopsy was 33.3% for NIL, 70% for LSIL, 75% for HSIL and 100% for SCC. Thus the cytology reports were more concordant for high grade lesions than low grade lesions.

The correlation of grading cervical lesions by morphological criteria in cervical cytology with the biopsy diagnosis was statistically significant showing P value of 0.000

The sensitivity of liquid based cervical cytology in diagnosing the cervical pre-neoplastic and neoplastic lesions was 77.78%, specificity was 100%, positive predictive value was100% and negative predictive value was 33.33% in our study. This is compared with other studies in table 39.

TABLE 39

COMPARISON OF EFFICACY OF LBC WITH OTHER STUDIES

	SENSITIVITY	**SPECIFICITY**	**PPV**	**NPV**
Our study	77.78%	100%	100%	33.33%
Singh KN et al [73]	70.02%	97.2%	51.2%	97%
Divya Hedge et al [74]	83%	98%	97.9%	80%
Rana T et al [75]	83.3%	97%	83%	97%
Shankaranarayanan et al [78]	86%	91%	22%	99%

IMMUNOCYTOCHEMISTRY

$p16^{INK4a}$ EXPRESSION

$p16^{INK4a}$ showed nuclear or cytoplasmic positivity or both. This was reported as positive or negative. But in some studies [89], all low grade lesions showed faint or weak positivity and all high grade lesions showed strong and diffuse positivity. And hence in our study, an attempt to study the significance of correlation of intensity of p16 staining with increasing grades of cervical lesions was done. Hence scoring was given as follows based on intensity of staining.

- 0 – negative
- 1 – weakly positive in dysplastic cells
- 2 – strongly positive in dysplastic cells

The results were 4 negative cases, 12 weakly positive cases and 14 strongly positive cases.

COMPARISON WITH CYTOLOGY

The expression of p16, when compared with morphologic diagnosis of cells in cervical smears showed the following results. Among the 6 NIL cases, 4 (66.7%) were negative and 2 were weakly positive. Among the 10 LSIL cases, 9 (90%) cases showed weak positivity and 1 showed strong positivity. Among the 8 HSIL cases, 1 showed weak positivity and 7 cases (87.5%)

showed strong positivity. All 6 carcinoma cases in cytology showed strong positivity with p16.

Carmen Ungureanu et al[77] found p16 positivity in 16.66% of ASCUS (1/6), 40.62% of LSIL (13/32), 96.29% of HSIL (26/27) and 100% of SCC cases. All NIL cases were negative with p16.

According to a study conducted by Ingrid Norman et al[80], 33% of ASCUS cases showed weak positivity with p16, 17% and 9.8% of LSIL cases showed weak and moderate staining with p16 respectively and 20%, 49% and 20% of HSIL cases showed weak, moderate and strong staining with p16 respectively.

In a study conducted by CD Izaaks et al[82], 5/28 (17.9%) cases with ASCUS, 7/9 (77.8%) cases with ASC-H, 50/96 (52.1%) cases with LSIL and 51/54 (94.4%) cases with HSIL showed positivity with p16 in cervical smears.

In our study, 4 cases diagnosed as NIL by cytology turned out to be CIN I in biopsy. Of these, 2 have been detected by p16 in cervical smear thus increasing the sensitivity of cervical cytology.

COMPARISON WITH HPE

The expression of p16 in cervical smears was compared with biopsy diagnosis. All cervicitis cases showed negativity with p16. 81.8% of CIN I and

100% of CIN II, CIN III and SCC cases showed positivity with p16. This association was statistically significant (P value = 0.000).

According to a study conducted by Tsoumpou I et al[79], 2% of normal cervix and 38% of CIN I, 68% of CIN II and 82% of CIN III showed positivity with p16 in cervical smears.

In a study conducted by N Murphy et al[81], all 12 normal smears were negative for p16, all 5 CIN I, 6 of 7 CIN II and all 8CIN III cases showed positivity with p16 in Thin Prep smears.

In a study conducted by Nicolas Wetzensen et al[83], p16/Ki-67 showed positivity in 26.8% of normal smears, 46.5% of CIN I, 82.8% of CIN II and 92.8% of CIN III cases.

In our study, the sensitivity (88.89%) and the negative predictive value (50%) for the use of p16 in cervical smears (ICC) were increased when compared to morphological diagnosis of cells in cervical smears (cervical cytology). The specificity and the positive predictive value remained 100% for immunocytochemistry with p16, similar to that of cytology. This is compared with other studies in table 40.

TABLE 40 : COMPARISON OF EFFICACY OF ICC WITH p16 WITH OTHER STUDIES

	SENSITIVITY	SPECIFICITY	PPV	NPV
Our study	88.89%	100%	100%	50%
Carmen et al[77]	100%	76.74%	76.7%	100%
Indigo Norman et al[80]	60%	100%	100%	-
CD Izaaks et al[82]	96.4%	54%	60.4%	40%
Nicolas et al [83]	90.6%	-	48.6%	-

INTENSITY OF p16 EXPRESSION

In addition to this, the intensity of expression of p16 also showed an association with increasing grades of cervical pre-neoplastic and neoplastic lesions. Majority of the cases diagnosed as LSIL in cytology showed weak positivity (90%) and 1 showed strong positivity. Majority of cases (87.5%) diagnosed as HSIL and 100% of cases diagnosed as malignancy in cytology showed strong positivity with p16. Thus the intensity of expression of p16 in cervical smears increased with increasing grades of cervical pre-neoplastic and neoplastic lesions in cytology.

When compared with biopsy, all the 9 CIN I cases showed weak positivity with p16 in dysplastic cells in cervical smears. Among the 5 CIN II cases, 3 (60%) were strongly positive and 2 were weakly positive. Among the

4 CIN III cases, 3(75%) were strongly positive and 1 was weakly positive. All the 8 SCC cases (100%) showed strong positivity. The correlation of increasing intensity of p16 expression in cervical smears with HPE diagnosis of cervical lesions showed P value of 0.000 which was statistically significant.

Thus the increasing intensity of p16 expression in cervical smears was found to have an association with increasing grades of cervical lesions.

This was also proved by Ingrid Norman et al[80] who found a correlation between intensity of p16expression and CIN grade. Benign cases and most of the low grade lesions showed no or weak reactivity and most of the high grade lesions showed moderate or strong reactivity with p16 in cervical smears.

COMPARISON WITH IMMUNOHISTOCHEMISTRY

In tissue sections, p16 expression was reported as positive or negative[90]. All cervicitis cases showed negativity with p16 in tissue sections. Among the 11 CIN I cases, 9 showed positivity and 2 were negative. All the 5 CIN II cases, all the 4 CIN III cases and all the 8 SCC cases showed positivity for p16. The correlation of immunohistochemical p16 expression with biopsy showed a P value of 0.003 which was statistically significant.

Also the expression of p16 in cervical smears (ICC) showed 100% concordance with their expression in tissue sections (IHC).

It was found that majority of CIN I cases which were positive showed moderate p16 expression in lower 1/3rd of the epithelium like a band. And all high grade lesions showed diffuse and strong positivity occupying the lower 2/3rd to full thickness of the epithelium. The malignant cases showed diffuse and strong positivity in almost all of the malignant cells. This was also proved by Maria Carolina et al(90).

Ki-67 EXPRESSION

Ki-67 showed nuclear staining. As even normal proliferating basal cell layer of cervical lining shows positivity with Ki-67, positivity in <1% of normal cells was taken as negative. Hence Ki-67 positivity in dysplastic cells was counted. Ki-67 expression in cervical smears were graded as follows(88),

- 0 – negative
- 1 – <10% of dysplastic cells
- 2 – 10-50% of dysplastic cells
- 3 – >50% of dysplastic cells

In our study, 5 cases were negative, 7 cases showed positivity in <10% of dysplastic cells, 10 cases showed positivity in 10-50% of dysplastic cells and 8 cases showed positivity in >50% of dysplastic cells.

COMPARISON WITH CYTOLOGY

Among the 6 NIL cases diagnosed by LBC, 5 (83.3%) showed negativity (<1% of normal cells positive) with Ki-67 and 1 showed positivity in <10% of dysplastic cells. Among the 10 LSIL cases, 6 (60%) showed positivity in <10% of dysplastic cells and 4 (40%) showed positivity in 10-50% of dysplastic cells. Among the 8 HSIL cases, 5 (62.5%) showed positivity in 10-50% of dysplastic cells and 3 (37.5%) showed positivity in >50% of dysplastic cells. Among the 6 SCC cases, 5 (83.3%) showed positivity in >50% of dysplastic cells and 1 showed positivity in 10-50% of dysplastic cells. Thus with increasing grades, the percentage of dysplastic cells expressing Ki-67 in cervical smears also increased.

COMPARISON WITH HPE

2 cervicitis cases diagnosed by biopsy showed positivity in <1% of normal cells in cervical smears which was taken as negative. Of the 11 CIN I cases, 3 cases were negative and 8 cases (72.7%) showed positivity. Of the 8 positive cases, 6 showed positivity in <10% of dysplastic cells and 2 showed positivity in 10-50% of dysplastic cells. All the CIN II cases showed positivity, of which 1showed positivity in <10% of dysplastic cells, 3showed positivity in 10-50% of dysplastic cells and 1 showed positivity in >50% of dysplastic cells. All the 4 CIN III cases showed positivity, of which 3 showed positivity in 10-50% of dysplastic cells and 1 showed positivity in >50% of dysplastic cells.

All the 8 SCC cases were positive, of which 2 showed positivity in 10-50% of dysplastic cells and 6 showed positivity in >50% of dysplastic cells. Thus the percentage of dysplastic cells expressing Ki-67 increased with increasing grades of cervical lesions. This was statistically significant showing P value of 0.000

In a study conducted by Nicolas Wetzensen et al [(83)], p16/Ki-67 showed positivity in 26.8% of normal smears, 46.5% of CIN I, 82.8% of CIN II and 92.8% of CIN III cases.

The sensitivity of use of Ki-67 in cervical smears was 89.28% and negative predictive value was 40%, which were more than that of cervical cytology. The specificity and the positive predictive value were 100%, similar to that of cervical cytology. This is compared with other studies in table 41.

TABLE 41 : COMPARISON OF EFFICACY OF ICC WITH Ki-67 WITH OTHER STUDIES

	SENSITIVITY	**SPECIFICITY**	**PPV**	**NPV**
Our study	89.28%	100%	100%	40%
Nicolas et al[(83)]	90.6%	-	48.6%	-
S Sahebali et al[(84)]	95%	95%	-	-
S W Byun et al[(85)]	76.2%	51.4%	48.5%	78.3%

Many of the inflammatory cases, reactive atypias and even normal smears showed positivity in less than 1% of normal cells. But p16 showed no such expression in non-neoplastic lesions. This was also proved by S. Nicholas Agoff et al [85]. So Ki-67 expression in <1% of normal cells was taken as negative.

Ki-67, when done along with p16 as dual staining in cervical smears can help to distinguish cervicitis and low grade lesions like CIN I. This dual staining with p16 and Ki-67 was studied in cervical smears by Nicolas Wetzensen et al [83]. It showed positivity in 19.6% of NIL, 40.2% of ASC-US, 68.8% of LSIL, 82.5% of ASC-H, and 95.3% of HSIL cases. A study conducted by Petry KU et al [87] showed 91.9% sensitivity and 82.1% specificity for p16/Ki-67 dual staining. This was also studied by Seung Won Byun et al [85].

Lesions above CIN II till malignancy showed a mixture of cases expressing Ki-67 in 10-50% and >50% of dysplastic cells.

Thus Ki-67 (ICC) can be used to distinguish high grade lesions from low grade lesions in cervical smears. But it is difficult to distinguish cervicitis from CIN I using Ki-67 alone in some cases.

COMPARISON WITH IMMUNOHISTOCHEMISTRY

In tissue sections, cases expressing Ki-67 in nuclei of the basal cells were taken as negative as it is normal. Only those cases expressing Ki-67, 2 layers above the basal layer were considered as positive[91,92,93]. The 2 cases of cervicitis showed Ki-67 expression only in the basal layer and hence reported as negative. Of the 11 CIN I cases, 2cases were negative and the remaining 9 cases showed nuclear positivity in lower 1/3rd of the epithelium. All the 5 CIN II cases showed nuclear positivity in lower 2/3rd of the epithelium. All the 4 CIN III cases showed nuclear positivity in full thickness of the epithelium. All the 8 SCC cases showed nuclear positivity diffusely in the malignant cells including the invasive component. The association of thickness of epithelium showing Ki-67 expression in tissue sections (IHC) in cervical non-neoplastic, pre-neoplastic and neoplastic lesions showed P value of 0.000 which was statistically significant. This was also proved by Maria Carolina et al [90].

18.2% of CIN I lesions were negative for Ki-67 in tissue sections, like that in cervical smears overlapping with the features of cervicitis. This when combined with p16 helped to distinguish cervicitis and CINI.

The percentage of concordance for Ki-67 expression in ICC with IHC was 100% for cervicitis, 88.9% for CIN I, 100% for CIN II, 100% for CIN III and 100% for SCC cases.

COMPARISON OF EFFICACY OF LBC AND IMMUNOCYTOCHEMISTRY IN OUR STUDY

The efficacy of cervical cytology and immunocytochemistry with p16 and Ki-67 are compared in table 42.

TABLE 42

COMPARISON OF EFFICACY OF LBC AND IMMUNOCYTOCHEMISTRY WITH p16 AND Ki-67

	LBC	ICC-p16	ICC-Ki67
Sensitivity	**77.78%**	**88.89%**	**89.28%**
Specificity	**100%**	**100%**	**100%**
PPV	**33.3%**	**50%**	**40%**
NPV	**100%**	**100%**	**100%**
P value	**0.000**	**0.000**	**0.000**

Thus the use of immunomarkers in cervical smears was found to definitely increase the sensitivity from 77.78% for cervical cytology interpreted by morphological criteria to 89%. The specificity was found to be 100% for both in our study.

As lack of diagnosis of cervical carcinogenesis in early stages is currently the major risk factor for cervical cancer, there is a need to use screening procedures with better sensitivity for early detection. In our study, immunocytochemistry using $p16^{INK4a}$ and Ki-67 has been proved to hold a higher sensitivity. Hence immunocytochemistry can be used as a screening tool in routine cervical cancer screening.

SUMMARY

SUMMARY

1. Totally, 202 cases of cervical pre-neoplastic and neoplastic lesions were diagnosed during a 6 months period from February 2014 to July 2014 at the Institute of Social Obstetrics and Govt. Kasturba Gandhi Hospital for Women and Children, Madras Medical College.

2. Of these, 170 were colposcopic biopsies, 9 were LLETZ specimens, 13 were random ectocervix biopsy specimens and 10 were hysterectomy specimens.

3. Of the 202 cases, CIN I (68.8%) constituted majority of cases, followed by malignancy (16.3%), CIN II (9.4%) and CIN III (5.4%).

4. Of the 33 malignant cases, SCC-WD constituted majority of cases (63.6%), followed by SCC-MD (30.3%), SCC-PD (3%) and adenocarcinoma (3%).

5. The incidence of all the cervical precancerous lesions was common in the 31-40 year age group. Malignant cases were common in the 51-60 year age group.

6. Cervical precancerous lesions were found to be more common in the reproductive women (CIN I – 88.5%, CIN II – 73.7%, CIN III – 81.8%),

whereas malignancy was more common in the post-menopausal women (90.9%).

7. Majority (58.3%) of cervical precancerous lesions were seen in the 12-3 o' clock quadrant of the cervix. Majority of the carcinoma cases (96.1%) were seen circumferentially abutting the os.

8. In our study group involving 30 patients, the percentage of concordance for VIA/VILI with biopsy was 66.7% for LGL, 90% for HGL and 100% for SCC.

9. The percentage of concordance for LBC with biopsy was 33.3% for NIL, 70% for LSIL, 75% for HSIL and 100% for SCC. Thus the cytology reports were more concordant for high grade lesions than low grade lesions.

10. The correlation of grading cervical lesions by morphological criteria in cervical cytology with the biopsy diagnosis was statistically significant showing P value of 0.000

11. The sensitivity of liquid based cervical cytology was 77.78%, specificity was 100%, positive predictive value was100% and negative predictive value was 33.33%

12. By immunostaining with p16 in cervical smears, 4 (66.7%) NIL cases were negative and 2 were weakly positive. 90% of LSIL cases showed weak positivity. 87.5% of HSIL and100% of SCC cases showed strong positivity. Among the 4 CIN I cases diagnosed as NIL by cytology, 2 have been detected by p16 in cervical smear thus increasing the sensitivity of cervical cytology.

13. When compared with HPE, all cervicitis cases showed negativity with p16 in cervical smears (ICC). 81.8% of CIN I and 100% of CIN II, CIN III and SCC cases showed positivity with p16 in cervical smears. This association was statistically significant (P value = 0.000).

14. All the 9 p16 positive CIN I cases showed weak positivity in dysplastic cells in cervical smears (ICC). 60% of CIN II, 75% of CIN III and 100% of SCC cases showed strong positivity. The correlation of increasing intensity of p16 expression in cervical smears with HPE diagnosis in cervical lesions showed P value of 0.000 which was statistically significant.

15. The sensitivity (88.89%) and the negative predictive value (50%) for immunocytochemistry with p16 increased when compared to cervical cytology. The specificity and the positive predictive value remained 100%, similar to that of cytology.

16. In tissue sections, p16 expression in CIN I showed weak band like positivity in the lower third of epithelium. In high grade lesions, p16 showed strong and diffuse positivity in 2/3rd to full thickness of epithelium. All the malignant cases showed diffuse and strong positivity in the malignant cells.

17. The correlation of immunohistochemical p16 expression with biopsy showed a P value of 0.003 which was statistically significant.

18. The expression of p16 in cervical smears (ICC) showed 100% concordance with their expression in tissue sections (IHC).

19. By immunostaining with Ki-67 in cervical smears, 83.3% of NIL cases showed negativity (positive in <1% of normal cells). 60% of LSIL cases showed positivity in <10% of dysplastic cells and 40% in 10-50% of dysplastic cells. 62.5% of HSIL cases showed positivity in 10-50% of dysplastic cells and 37.5% in >50% of dysplastic cells. 83.3% of SCC cases showed positivity in >50% of dysplastic cells. Thus with increasing grades, the percentage of dysplastic cells expressing Ki-67 in cervical smears also increased.

20. When compared with biopsy, 2 cervicitis cases were negative with Ki-67 (positive in <1% of normal cells) in cervical smears (ICC). 72.7% of CIN I cases showed positivity, most of them in <10% of dysplastic cells.

60% of CIN II and 75% CIN III cases showed positivity in 10-50% of dysplastic cells. 75% of SCC cases showed positivity in >50% of dysplastic cells. Thus with increasing grades, the percentage of dysplastic cells expressing Ki-67 in cervical smears also increased. This was statistically significant showing P value of 0.000

21. The sensitivity of use of Ki-67 in cervical smears was 89.28% and negative predictive value was 40%, which were more than that of cervical cytology. The specificity and the positive predictive value were 100%, similar to that of cervical cytology.

22. In tissue sections, 2 cases of cervicitis showed Ki-67 nuclear expression only in the basal layer and hence reported as negative. Of the 11 CIN I cases, 2 cases were negative and the remaining 9 cases showed nuclear positivity in lower 1/3rd of the epithelium. All the 5 CIN II cases showed nuclear positivity in lower 2/3rd of the epithelium. All the 4 CIN III cases showed nuclear positivity in full thickness of the epithelium. All the 8 SCC cases showed diffuse nuclear positivity in the malignant cells including the invasive component. The association of thickness of epithelium showing Ki-67 expression in tissue sections (IHC) in cervical non-neoplastic, pre-neoplastic and neoplastic lesions showed P value of 0.000 which was statistically significant.

23. The percentage of concordance for Ki-67 expression in ICC with IHC was 100% for cervicitis, CIN II, CIN III and malignancy, but 88.9% for CIN I.

24. Many of the inflammatory cases, reactive atypias and even normal smears showed Ki-67 nuclear positivity in less than 1% of normal cells. But p16 showed no such expression in non-neoplastic lesions. Ki-67 when combined with p16 helped to distinguish cervicitis and CIN I.

25. Thus the use of immunomarkers in cervical smears was found to definitely increase the sensitivity from 77.78% for cervical cytology by routine pap staining to 89%. The specificity was found to be 100% for both in our study.

CONCLUSION

CONCLUSION

The sensitivity of cervical cytology was 77.78% and specificity was 100% in our study. As lack of cervical cancer screening is the major risk factor for cervical carcinoma now, it is of utmost importance to increase the sensitivity of present day screening procedures, so that cervical precancerous lesions can be diagnosed at an early stage and appropriately treated, thus reducing the incidence of cervical carcinoma.

The use of immunomarkers like $p16^{INK4a}$ and Ki-67 in cervical smears showed sensitivity of 88.89% and 89.28% respectively and specificity of 100%. Thus even cases that cannot be morphologically diagnosed in cytology can be detected by immunocytochemistry.

In addition to this, the intensity of p16 expression and percentage of cells expressing Ki-67 in cervical smears can be used to grade cervical pre-neoplastic and neoplastic lesions whose association was found to be statistically significant in our study.

The limitation of use of Ki-67 in cervical smears is that they show positivity even in normal basal cells and in cases of inflammation, reactive atypia etc. p16 showed no such expression. By using dual staining for p16 and Ki-67 or by simply combining the results of p16 and Ki-67 expression, this

limitation can be overcome and cervical non-neoplastic and low grade lesions can be distinguished.

The use of immunomarkers in cervical smears has significantly increased the sensitivity of cervical cancer screening in our study as well as in many other studies all over the world. Hence steps should be taken to incorporate immunocytochemistry in cervical cancer screening in areas with better financial and laboratory resources, thus further bringing down the incidence and burden of cervical carcinoma.

ANNEXURES

ANNEXURE I

CONSENT FORM

Name and address of the sponsor / agency (ies) (if any) :

Documentation of the informed consent

I _____ have read the information in this form (or it has been read to me). I was free to ask any questions and they have been answered. I am over 18 years of age and, exercising my free power of choice, hereby give my consent to be included as a participant in "**DIAGNOSTIC VALUE OF IMMUNOMARKERS IN CERVICAL CANER SCREENING".**

1. I have read and understood this consent form and the information provided to me.
2. I have had the consent document explained to me.
3. I have been explained about the nature of the study in which the pap smears collected from cervix will be subjected to conventional smear preparation and immunocytochemistry.
4. I have been explained about my rights and responsibilities by the investigator. I have the right to withdraw from the study at any time.
5. I have been informed the investigator of all the treatments I am taking or have taken in the past _____ months including any native (alternative) treatment.
6. I hereby give permission to the investigators to release the information obtained from me as result of participation in this study to the sponsors, regulatory authorities, Govt. agencies, and IEC. I understand that they are publicly presented.
7. I have understand that my identity will be kept confidential if my data are publicly presented
8. I have had my questions answered to my satisfaction.
9. I have decided to be in the research study.

I am aware that if I have any question during this study, I should contact the investigator. By signing this consent form I attest that the information given in this document has been clearly explained to me and understood by me, I will be given a copy of this consent document.

For adult participants:

Name and signature / thumb impression of the participant (or legal representative if participant incompetent)

Name _____ Signature_____ Date_____

Name and Signature of impartial witness (required for illiterate patients):

Name _____ Signature_____ Date_____

Address and contact number of the impartial witness:

Name and Signature of the investigator or his representative obtaining consent:

Name _____ Signature_____ Date_____

INFORMATION SHEET

- Your sample has been accepted.
- We are conducting a study on diagnostic value of immunomarkers in cervical cancer screening, among patients attending Institute of Social Obstetrics and Govt. KasturbaGandhi hospital, Madras Medical College,Chennai and for that your sample may be valuable to us.
- The purpose of this study is evaluating the diagnostic value of immunomarkers in cervical cancer screening.
- We are selecting certain cases and if your sample is found eligible, we may be using your sample to perform immunocytochemistry which in any way do not affect your final report or management.
- The privacy of the patients in the research will be maintained throughout the study. In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared.
- Taking part in this study is voluntary. You are free to decide whether to participate in this study or to withdraw at any time; your decision will not result in any loss or benefits to which you are otherwise entitled.
- The results of the special study may be intimated to you at the end of the study period or during the study if anything is found abnormal which may aid in the management or treatment.

Signature of investigator Signature of participant

Date:

சுய ஒப்புதல் படிவம்

ஆய்வு செய்யப்படும் தலைப்பு:

கர்பப்பை வாய் புற்றுநோய் பரிசோதனையில் காப்பணுவூட்டியின் பயன் தகுதியைக் கணித்தல்

ஆய்வாளரின் பெயர் :

பங்கேற்பவரின் பெயர் :

சென்னை மருத்துவக் கல்லூரி, நோய்க்குறியியல் துறையில் பயிலும் முதுகலை மருத்துவர். N. ரம்யா, அவர்கள் மேற்கொள்ளும் இந்த ஆய்வில் பங்குகொள்ள ..... ஆகிய நான் முழுமனதுடன் சம்மதிக்கிறேன். இந்த ஆய்வை மேற்கொள்ளும் மருத்துவர் என் மருத்துவ விவரங்கள் மற்றும் மருத்துவ ஆய்வின் முடிவுகள் ஆகியவற்றை தெரிந்துகொள்ளவும், என்னுடைய கர்பப்பை வாயில் இருந்து எடுக்கப்படும் ஸ்மியர் மாதிரியை பரிசோதனைக்கு எடுத்துக்கொள்ளவும் முழுமனதுடன் சம்மதிக்கிறேன். இந்த ஆய்வினால் எந்த தீங்கும் ஏற்படாது என்பதையும் அறிவேன். மேலும் இந்த ஆய்வின் முடிவுகளை பிரசுரிக்கவும் சம்மதிக்கிறேன்.

பங்கேற்பவரின் கையொப்பம்..... இடம் :..... தேதி.....
கட்டைவிரல் ரேகை

பங்கேற்பவரின் பெயர் மற்றும் விலாசம்.....

ஆய்வாளரின் கையொப்பம்.....இடம் :..... தேதி.....

ஆய்வாளரின் பெயர்.....

தகவல் படிவம்

ஆய்வு செய்யப்படும் தலைப்பு:

கர்ப்பப்பை வாய் புற்றுநோய் பரிசோதனையில் காப்பணுவூட்டியின் பயன் தகுதியைக் கணித்தல்.

ஆய்வாளர்: **மருத்துவர். N. ரம்யா,**
நோய்க்குறியியல் துறை,
சென்னை மருத்துவக் கல்லூரி,
சென்னை - 600003.

கர்ப்பப்பை வாய் புற்றுநோயானது இந்தியாவில் உள்ள பெண்களில் மூன்றில் ஒரு பங்கினரை பாதிக்கிறது. இது இந்திய பெண்களுக்கு உண்டாகும் புற்றுநோய்களுள் இரண்டாவது இடம் வகிக்கிறது. எனவே, கர்ப்பப்பை வாய் புற்றுநோயை அதன் ஆரம்ப நிலையிலேயே கண்டறிய 20 வயதுக்கு மேற்பட்ட பெண்கள் வருடம் ஒரு முறை **"பாப் ஸ்மியர்"(Pap Smear)** பரிசோதனையை மேற்கொள்ள வேண்டும். அதன் தனிப்பயன் உடைமை 56% மட்டுமே ஆகும். எனவே, பாப் ஸ்மியரில் புற்றுநோய்க்கான காப்பணுவூட்டியைக் கண்டறிவதன் மூலம் இந்த பரிசோதனையின் தனிப்பயன் உடைமை அதிகரிக்கும். இதன் மூலம் கர்ப்பப்பை வாய் புற்றுநோயை ஆரம்ப நிலையிலேயே துல்லியமாக கண்டறியலாம்.

எனது ஆய்வு கர்ப்பப்பை வாய் புற்றுநோய் பரிசோதனையில் காப்பணுவூட்டியின் பயன் தகுதியைக் கணித்தலே ஆகும்.

இந்த ஆய்வில் பங்கு பெறுவது நோயாளிகளின் சொந்த விருப்பத்திலேயே ஆகும். எந்த நேரத்திலும் நோயாளிகள் இந்த ஆய்விலிருந்து விலகிக் கொள்ளலாம். இந்த ஆய்வினால் நோயாளிகளுக்கு எந்த செலவும் இருக்காது. இந்த ஆய்வையொட்டி எந்தவிதமான சந்தேகங்களுக்கும் விளக்கம்பெற நோயாளிகளுக்கு உரிமை உள்ளது. இந்த ஆய்வின் முடிவுகள் இறுதியில் பிரசுரிக்கப்படும்.

இந்த ஆய்வை பற்றி சந்தேகங்களுக்கு தொடர்பு கொள்ள வேண்டியவர். மரு. N. ரம்யா, செல் : 9360570339

பங்கேற்பவரின் கையொப்பம்..... இடம் :..... தேதி.....
கட்டைவிரல் ரேகை

பங்கேற்பவரின் பெயர் மற்றும் விலாசம்.....

ஆய்வாளரின் கையொப்பம்.....இடம் :..... தேதி.....

ஆய்வாளரின் பெயர்.....

ANNEXURE II : PROFORMA

NAME:

AGE:

CYTOLOGY NO. :

BIOPSY NO. :

PRESENTING COMPLAINTS:

H/o white discharge : Yes/No

H/o bleeding PV : Yes/No

H/o post coital bleeding : Yes/No

MENSTRUAL HISTORY:

Age at menarche :

Age at marriage :

H/o pregnancy :

LMP :

Menstrual cycle :

Age at menopause :

PRIOR TREATMENT HISTORY:

H/o prior Cryotherapy/ Cervical biopsy/ LLETZ/ Conisation/

Hysterectomy/ Radiation : Yes/No

COITAL HISTORY:

H/o IUD use : Yes/No

H/o Sexual intercourse with spermicidal jelly,

douches/tampons 24 hours prior : Yes/No

COLPOSCOPY FINDINGS:

Gross :

VIA :

VILI :

Quadrant of cervix :

ANNEXURE III

WHO CLASSIFICATION OF TUMOURS OF THE UTERINE CERVIX – EPITHELIAL TUMOURS

SQUAMOUS TUMOURS AND PRECURSORS

- SQUAMOUS CELL CARCINOMA, NOT OTHERWISE SPECIFIED
  - Keratinizing
  - Non-keratinizing
  - Basaloid
  - Verrucous
  - Warty
  - Papillary
  - Lymphoepithelioma-like
  - Squamotransitional

- EARLY INVASIVE (MICROINVASIVE) SQUAMOUS CELL CARCINOMA

- SQUAMOUS INTRAEPTHELIAL NEOPLASIA
  - Cervical intraepithelial neoplasia (CIN) 3/ squamous cell carcinoma in situ

- BENIGN SQUAMOUS CELL LESIONS
  - Condyloma acuminatum
  - Squamous papilloma
  - Fibroepithelial polyp

ANNEXURE IV

THE BETHESDA SYSTEM 2001

Specimen Type:

Conventional Smear (Pap Smear) vs Liquid-Based Preparations vs Other

Specimen adequacy

Satisfactory for evaluation

Unsatisfactory for evaluation

General categorization (optional)

Negative for intra-epithelial lesion or malignancy

Epithelial cell abnormality

Other

Automated review

Ancilliary testing

Interpretation/result

Negative for intra-epithelial lesion or malignancy

Epithelial cell abnormalities

Squamous cell

- Atypical squamous cells

  -of undetermined significance

  -cannot exclude high-grade squamous intra-epithelial lesion (HSIL)
- Low-grade squamous intra-epithelial lesion
- High-grade squamous intra-epithelial lesion
- Squamous-cell carcinoma

Glandular cell

- Atypical
- Endocervical cells (not otherwise specified [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
- NOS

Other malignant neoplasms: (*specify*)

Educational notes and suggestions (*optional*)

ANNEXURE V

IMMUNOCYTOCHEMISTRY

MATERIALS REQUIRED

- PBS (phosphate buffer solution)

  Sodium chloride – 8g

  Disodium hydrogen phosphate – 8.3g

  Potassium dihydrogen phosphate – 1.25g

  Distilled water – 1000ml

PROCEDURE

1. Position slides in slide holders with filter cards and sample chambers and attach to cytocentrifuge rotor.
2. Prepare cell suspension of 500 cells/mm3 with PBS (Preparations that are too dilute for cytocentrifugation may be dropped with a pipet using the location of the filter card as a guide and allowed to dry).
3. Add 0.1 ml cell suspension to chamber and centrifuge at 1000 rpm for 5 minutes (It is important to get just the right amount of speed for cytocentrifugation, as too much speed will flatten the cell, and too little will not allow the cells to adequately bind to the slide).
4. Remove slide and immediately dip in 95% ethanol, 5% glacial acetic acid fixative for 2 minutes.

5. Rinse 3x5 min with PBS to remove fixative.
6. Incubate the slides with 0.25-0.5% Triton X-100 in PBS for 10 minutes to permeabilize the membranes (There is no need for a permeabilization step following acetone or methanol fixation).
7. Rinse 3x5min in PBS to remove detergents.
8. Blocking endogenous peroxidase using 3% H2O2 for 10-30 minutes.
9. Rinse 3x5 min in PBS.
10. Incubate with power block for 10 minutes.
11. Incubate with primary antibody for 1 hour.
12. Rinse 3x5 min in PBS.
13. Incubate with antibody amplifier for 15 minutes.
14. Rinse 3x5 min in PBS.
15. Incubate with HRP polymer for 30 minutes.
16. Rinse 3x5 min in PBS.
17. Incubate with freshly prepared chromogen for 5 minutes.
18. Rinse for 2 minutes in distilled water.
19. Dip in Harris Hematoxylin and keep in running tap water for bluing.
20. Slides are cleared and mounted and analysed under light microscopy.

ANNEXURE VI

IMMUNOHISTOCHEMISTRY

MATERIALS REQUIRED

- Phosphate buffer solution
- Retrieval buffer - Tris buffered solution
    - Tris – 6.05g
    - EDTA – 0.744g
    - Distilled water – 1000ml

PROCEDURE

1. Clear in xylene for 30 minutes and dehydrate in alcohol for 10 minutes.
2. Wash in running tap water for 10 minutes.
3. Rinse in distilled water for 5 minutes.
4. Place the slides in Tris buffer and keep it in microwave oven in the following temperatures for antigen retrieval
    - 640 watts – 5 minutes
    - 800 watts – 5 minutes
    - 800 watts – 5 minutes
    - 800 watts – 3 minutes
5. Take out the bowl and cool the slides to room temperature.
6. Rinse 2x5 min in distilled water.

7. Block endogenous peroxidase using 3% H_2O_2 for 10-30 minutes.
8. Rinse 3x5 min in PBS.
9. Incubate with power block for 10 minutes.
10. Incubate with primary antibody for 1 hour.
11. Rinse 3x5 min in PBS.
12. Incubate with antibody amplifier for 15 minutes.
13. Rinse 3x5 min in PBS.
14. Incubate with HRP polymer for 30 minutes.
15. Rinse 3x5 min in PBS.
16. Incubate with freshly prepared chromogen for 5 minutes.
17. Rinse for 2 minutes in distilled water.
18. Dip in Harris Hematoxylin and keep in running tap water for bluing.
19. Slides are cleared and mounted and analysed under light microscopy.

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MASTER CHART

FOR

CURRENT STUDY

MASTER CHART FOR CURRENT STUDY

S.NO.	Biopsy No	Age	Menstrual status	VIA/VILI	Site	Cytology	Type of specimen	HPE diagnosis	ICC - p16	ICC-Ki67	IHC-p16	IHC-Ki67
1	240/14	29	mensturating	low grade lesion	I	LSIL	I	CIN I	1	1	1	1
2	243/14	45	mensturating	low grade lesion	I	LSIL	I	CIN I	1	1	1	1
3	250/14	27	mensturating	low grade lesion	I	LSIL	I	CIN I	1	1	1	1
4	259/14	43	mensturating	low grade lesion	I	LSIL	I	CIN I	1	2	1	1
5	272/14	38	mensturating	low grade lesion	IV	LSIL	I	CIN I	1	1	1	1
6	287/14	38	mensturating	high grade lesion	I	LSIL	I	CIN I	1	2	1	1
7	350/14	50	menopause	high grade lesion	V	HSIL	I	SCC-WD	2	3	1	4
8	352/14	53	menopause	high grade lesion	IV	HSIL	I	CIN II	2	3	1	2
9	419/14	32	mensturating	high grade lesion	I	HSIL	I	CIN III	2	2	1	3
10	420/14	60	menopause	high grade lesion	V	HSIL	I	SCC-WD	2	2	1	4
11	422/14	25	mensturating	high grade lesion	I	HSIL	I	CIN II	2	2	1	2
12	435/14	33	mensturating	low grade lesion	I	LSIL	I	CIN I	1	1	1	1
13	518/14	31	mensturating	low grade lesion	II	NIL	I	CIN I	1	0	1	1
14	545/14	42	mensturating	low grade lesion	II	NIL	I	CIN I	1	1	1	1
15	596/14	38	mensturating	low grade lesion	I	NIL	I	CIN I	0	0	0	0
16	601/14	38	mensturating	low grade lesion	I	LSIL	I	CIN II	2	1	1	2
17	627/14	35	mensturating	low grade lesion	I	NIL	I	CIN I	0	0	0	0
18	647/14	49	menopause	invasive carcinoma	V	SCC	I	SCC-MD	2	3	1	4
19	651/14	22	mensturating	low grade lesion	V	LSIL	I	CIN II	1	2	1	2
20	710/14	55	menopause	invasive carcinoma	V	SCC	I	SCC-MD	2	3	1	4
21	746/14	47	menopause	low grade lesion	I	LSIL	I	CIN II	1	2	1	2
22	995/14	51	menopause	high grade lesion	III	HSIL	I	CIN III	2	3	1	3
23	1009/14	30	mensturating	low grade lesion	I	NIL	I	Cervicitis	0	0	0	0
24	1010/14	28	mensturating	low grade lesion	IV	NIL	I	Cervicitis	0	0	0	0
25	1031/14	55	menopause	invasive carcinoma	V	SCC	I	SCC-MD	2	3	1	4
26	1126/14	42	mensturating	high grade lesion	I	HSIL	I	CIN III	1	2	1	3
27	1159/14	50	menopause	invasive carcinoma	V	SCC	I	SCC-WD	2	3	1	4
28	1177/14	38	mensturating	high grade lesion	V	HSIL	I	CIN III	2	2	1	3
29	1213/14	54	menopause	invasive carcinoma	V	SCC	I	SCC-WD	2	3	1	4
30	1276/14	45	menopause	high grade lesion	V	SCC	I	SCC-WD	2	2	1	4

MASTER CHART FOR EPIDEMIOLOGY

MASTER CHART FOR EPIDEMIOLOGY

S.NO.	Biopsy No	Age	Menstrual status	VIA/VILI	Site	Cytology	Type of specimen	HPE diagnosis	ICC - p16	ICC-Ki67	IHC-p16	IHC-Ki67
1	230/14	42	mensturating	low grade lesion	IV		I	CIN I				
2	236/14	49	menopause				IV	CIN I				
3	240/14	29	mensturating	low grade lesion	I	LSIL	I	CIN I	1	1	1	1
4	243/14	45	mensturating	low grade lesion	I	LSIL	I	CIN I	1	1	1	1
5	244/14	27	mensturating	high grade lesion	II		I	CIN II				
6	247/14	27	mensturating	high grade lesion	II		I	CIN II				
7	249/14	36	mensturating	low grade lesion	I		I	CIN II				
8	250/14	27	mensturating	low grade lesion	I	LSIL	I	CIN I	1	1	1	1
9	259/14	43	mensturating	low grade lesion	I	LSIL	I	CIN I	1	2	1	1
10	269/14	62	menopause	invasive carcinoma	V		I	SCC-MD				
11	272/14	38	mensturating	low grade lesion	IV	LSIL	I	CIN I	1	1	1	1
12	280/14	40	mensturating	high grade lesion	I		I	SCC-WD				
13	287/14	38	mensturating	high grade lesion	I	LSIL	I	CIN I	1	2	1	1
14	301/14	70	menopause	invasive carcinoma	V		I	SCC-WD				
15	303/14	50	menopause	low grade lesion	III		I	CIN I				
16	305/14	30	mensturating	low grade lesion	I		I	CIN I				
17	325/14	40	mensturating	low grade lesion	II		I	CIN II				
18	336/14	28	mensturating	low grade lesion	II	LSIL	I	CIN I				
19	337/14	30	mensturating	low grade lesion	IV	ASCUS	I	CIN I				
20	350/14	50	menopause	high grade lesion	V	HSIL	I	SCC-WD	2	3	1	4
21	352/14	53	menopause	high grade lesion	IV	HSIL	1	CIN II	2	3	1	2
22	353/14	44	post TAH BSO				IV	SCC-WD				
23	364/14	46	mensturating	low grade lesion	I		I	CIN I				
24	387/14	40	mensturating	low grade lesion	I		I	CIN I				
25	389/14	22	mensturating	low grade lesion	II		I	CIN I				
26	390/14	37	mensturating	low grade lesion	I		I	CIN I				
27	419/14	32	mensturating	high grade lesion	I	HSIL	I	CIN III	2	2	1	3
28	420/14	60	menopause	high grade lesion	V	HSIL	I	SCC-WD	2	2	1	4
29	422/14	25	mensturating	high grade lesion	I	HSIL	I	CIN II	2	2	1	2
30	435/14	33	mensturating	low grade lesion	I	LSIL	I	CIN I	1	1	1	1
31	488/14	40	mensturating	low grade lesion	IV		I	CIN I				
32	511/14	38	mensturating				IV	CIN II				
33	518/14	31	mensturating	low grade lesion	II	NIL	I	CIN I	1	0	1	1
34	519/14	35	mensturating	low grade lesion	I		I	CIN I				
35	531/14	29	mensturating				II	CIN III				
36	545/14	42	mensturating	low grade lesion	II	NIL	I	CIN I	1	1	1	1

37	596/14	38	mensturating	low grade lesion	I	NIL	I	CIN I	0	0	0	0
38	601/14	38	mensturating	low grade lesion	I	LSIL	I	CIN II	2	1	1	2
39	627/14	35	mensturating	low grade lesion	I	NIL	I	CIN I	0	0	0	0
40	647/14	49	menopause	invasive carcinoma	V	SCC	I	SCC-MD	2	3	1	4
41	651/14	22	mensturating	low grade lesion	V	LSIL	I	CIN II	1	2	1	2
42	659/14	35	mensturating	low grade lesion	I		I	CIN I				
43	660/14	35	mensturating	high grade lesion	I		I	CIN III				
44	702/14	53	menopause				IV	CIN III				
45	709/14	48	menopause	invasive carcinoma	V		I	SCC-MD				
46	710/14	55	menopause	invasive carcinoma	V	SCC	I	SCC-MD	2	3	1	4
47	712/14	50	menopause	polyp			III	SCC-MD				
48	713/14	62	menopause	polyp			III	SCC-WD				
49	723/14	30	mensturating	low grade lesion	I	LSIL	I	CIN I				
50	726/14	43	mensturating				III	CIN I				
51	745/14	32	mensturating				II	CIN III				
52	746/14	47	menopause	low grade lesion	I	LSIL	I	CIN II	1	2	1	2
53	748/14	50	menopause				III	CIN I				
54	788/14	39	mensturating				IV	CIN III				
55	796/14	35	mensturating	low grade lesion	I		I	CIN I				
56	812/14	30	mensturating	low grade lesion	I		I	CIN I				
57	815/14	38	mensturating	low grade lesion	I		I	CIN I				
58	821/14	38	mensturating				II	CIN II				
59	877/14	27	mensturating	low grade lesion	I		I	CIN I				
60	881/14	35	mensturating	high grade lesion	IV		I	CIN III				
61	888/14	50	menopause	low grade lesion	IV		I	CIN I				
62	899/14	40	mensturating	low grade lesion	IV		I	CIN I				
63	900/14	42	mensturating	low grade lesion	I		I	CIN I				
64	931/14	60	menopause	invasive carcinoma	V		I	SCC-WD				
65	935/14	23	mensturating	low grade lesion	I		I	CIN I				
66	937/14	35	mensturating	low grade lesion	II		I	CIN I				
67	959/14	42	mensturating	low grade lesion	III		I	CIN I				
68	980/14	38	mensturating	low grade lesion	IV	LSIL	I	CIN I				
69	986/14	50	menopause	low grade lesion	II		I	CIN I				
70	995/14	51	menopause	high grade lesion	III	HSIL	I	CIN III	2	3	1	3
71	1006/14	34	mensturating	low grade lesion	IV		I	CIN I				
72	1029/14	42	mensturating	low grade lesion	I	LSIL	I	CIN I				
73	1030/14	45	menopause	low grade lesion	IV		I	CIN I				

74	1031/14	55	menopause	invasive carcinoma	V	SCC	I	SCC-MD	2	3	1	4
75	1032/14	32	mensturating	low grade lesion	I		I	CIN I				
76	1041/14	50	menopause	invasive carcinoma	V		I	SCC-MD				
77	1052/14	40	mensturating				IV	CIN I				
78	1100/14	45	mensturating	low grade lesion	I		I	CIN I				
79	1101/14	31	mensturating	low grade lesion	IV		I	CIN I				
80	1103/14	53	menopause	low grade lesion	IV		I	CIN I				
81	1104/14	43	mensturating	low grade lesion	I		I	CIN I				
82	1105/14	34	mensturating	low grade lesion	III		I	CIN I				
83	1126/14	42	mensturating	high grade lesion	I	HSIL	I	CIN III	1	2	1	3
84	1129/14	40	mensturating	low grade lesion	I		I	CIN I				
85	1147/14	31	mensturating	low grade lesion	I		I	CIN I				
86	1148/14	30	mensturating	low grade lesion	II		I	CIN I				
87	1155/14	47	menopause	low grade lesion	I		I	CIN I				
88	1156/14	35	mensturating	low grade lesion	II		I	CIN I				
89	1157/14	28	mensturating	low grade lesion	I		I	CIN I				
90	1159/14	50	menopause	invasive carcinoma	V	SCC	I	SCC-WD	2	3	1	4
91	1177/14	38	mensturating	high grade lesion	V	HSIL	I	CIN III	2	2	1	3
92	1181/14	46	menopause	invasive carcinoma	V		I	Adenocarcinoma				
93	1209/14	60	menopause	low grade lesion	II		I	CIN I				
94	1211/14	45	menopause	invasive carcinoma	V		I	SCC-WD				
95	1213/14	54	menopause	invasive carcinoma	V	SCC	I	SCC-WD	2	3	1	4
96	1219/14	40	mensturating				III	SCC-WD				
97	1235/14	24	mensturating	low grade lesion	I		I	CIN II				
98	1241/14	38	mensturating	low grade lesion	I		I	CIN I				
99	1242/14	42	mensturating	low grade lesion	II		I	CIN I				
100	1244/14	38	mensturating				II	CIN I				
101	1248/14	28	mensturating	low grade lesion	I		I	CIN I				
102	1276/14	45	menopause	high grade lesion	V	SCC	I	SCC-WD	2	2	1	4
103	1278/14	35	mensturating	low grade lesion	IV		I	CIN I				
104	1313/14	32	mensturating	low grade lesion	IV	unsatisfac	I	CIN I				
105	1315/14	60	menopause	low grade lesion	I		I	CIN II				
106	1327/14	42	mensturating				II	CIN I				
107	1328/14	51	menopause				III	SCC-WD				
108	1348/14	40	mensturating	high grade lesion	I		I	CIN II				
109	1352/14	38	mensturating	low grade lesion	I	LSIL	I	CIN I				

110	1370/14	45	mensturating	low grade lesion	I		I	CIN I				
111	1398/14	31	mensturating				II	CIN I				
112	1400/14	60	menopause	invasive carcinoma	V		I	SCC-WD				
113	1426/14	60	menopause				III	SCC-WD				
114	1442/14	42	mensturating				IV	CIN I				
115	1448/14	32	mensturating	low grade lesion	IV		I	CIN I				
116	1458/14	45	mensturating	low grade lesion	IV		I	CIN I				
117	1459/14	32	mensturating	low grade lesion	I		I	CIN I				
118	1466/14	32	mensturating	low grade lesion	I		I	CIN I				
119	1474/14	39	mensturating				II	CIN II				
120	1491/14	60	menopause				III	CIN I				
121	1501/14	40	mensturating	low grade lesion	I		I	CIN I				
122	1502/14	25	mensturating	low grade lesion	I		I	CIN I				
123	1503/14	42	mensturating	low grade lesion	II		I	CIN I				
124	1505/14	48	menopause	low grade lesion	I		I	CIN I				
125	1507/14	45	mensturating	low grade lesion	I		I	CIN I				
126	1509/14	42	mensturating	low grade lesion	IV		I	CIN I				
127	1533/14	35	mensturating	low grade lesion	II		I	CIN I				
128	1535/14	30	mensturating	low grade lesion	IV		I	CIN I				
129	1558/14	40	mensturating	invasive carcinoma	V		I	SCC-WD				
130	1560/14	23	mensturating	low grade lesion	I		I	CIN I				
131	1570/14	34	mensturating	low grade lesion	I		I	CIN I				
132	1572/14	34	mensturating	low grade lesion	III		I	CIN I				
133	1576/14	25	mensturating	low grade lesion	I		I	CIN I				
134	1597/14	33	mensturating	low grade lesion	II		I	CIN I				
135	1602/14	36	mensturating	low grade lesion	I		I	CIN I				
136	1619/14	65	menopause				IV	CIN II				
137	1621/14	42	mensturating				V	CIN III				
138	1645/14	33	mensturating	low grade lesion	I		I	CIN II				
139	1672/14	60	menopause	invasive carcinoma	V		I	SCC-WD				
140	1685/14	40	mensturating	low grade lesion	I		I	CIN I				
141	1696/14	47	mensturating	low grade lesion	I		I	CIN I				
142	1771/14	40	mensturating				III	CIN I				
143	1782/14	28	mensturating	low grade lesion	I		I	CIN I				
144	1793/14	55	menopause				III	SCC-PD				
145	1815/14	32	mensturating	low grade lesion	I		I	CIN I				
146	1819/14	42	mensturating	low grade lesion	II		I	CIN I				

147	1820/14	34	mensturating	low grade lesion	I		I	CIN I				
148	1821/14	35	mensturating	low grade lesion	I		I	CIN I				
149	1830/14	33	mensturating	low grade lesion	I		I	CIN I				
150	1833/14	65	menopause	invasive carcinoma	V	unsatisfac	I	SCC-WD				
151	1840/14	38	mensturating				III	CIN I				
152	1845/14	44	mensturating			NIL	IV	CIN I				
153	1846/14	27	mensturating	low grade lesion	II		I	CIN I				
154	1859/14	43	mensturating	low grade lesion	III		I	CIN I				
155	1864/14	32	mensturating				II	CIN I				
156	1870/14	42	menopause	invasive carcinoma	V		I	SCC-MD				
157	1892/14	30	mensturating	low grade lesion	IV		I	CIN I				
158	1910/14	35	mensturating	low grade lesion	I		I	CIN I				
159	1914/14	34	mensturating	low grade lesion	III		I	CIN I				
160	1915/14	31	mensturating	low grade lesion	I		I	CIN I				
161	1921/14	32	mensturating	low grade lesion	I		I	CIN I				
162	1928/14	24	mensturating	low grade lesion	IV		I	CIN I				
163	1933/14	38	mensturating	low grade lesion	II		I	CIN I				
164	1934/14	40	mensturating	low grade lesion	I		I	CIN I				
165	1935/14	54	menopause	invasive carcinoma	V		I	SCC-MD				
166	1943/14	35	mensturating	low grade lesion	I		I	CIN I				
167	1947/14	37	mensturating	low grade lesion	I		I	CIN I				
168	1957/14	38	mensturating				IV	CIN I				
169	1958/14	45	menopause	low grade lesion	IV		I	CIN I				
170	1974/14	32	mensturating	low grade lesion	I		I	CIN I				
171	1989/14	39	mensturating	low grade lesion	III		I	CIN I				
172	1990/14	35	mensturating	low grade lesion	III		I	CIN I				
173	2004/14	48	menopause	low grade lesion	I		I	CIN I				
174	2014/14	25	mensturating	low grade lesion	I		I	CIN I				
175	2015/14	60	menopause	invasive carcinoma	V		I	SCC-WD				
176	2018/14	30	mensturating	low grade lesion	I		I	CIN I				
177	2058/14	40	mensturating	low grade lesion	II		I	CIN I				
178	2104/14	33	mensturating	low grade lesion	I		I	CIN I				
179	2105/14	50	menopause	invasive carcinoma	V		I	SCC-MD				
180	2130/14	45	menopause	low grade lesion	I		I	CIN II				
181	2147/14	50	menopause	low grade lesion	II		I	CIN I				
182	2149/14	37	mensturating	low grade lesion	I		I	CIN II				

183	2155/14	34	mensturating	low grade lesion	III		I	CIN I				
184	2164/14	55	menopause	invasive carcinoma	V		I	SCC-MD				
185	2172/14	38	mensturating	low grade lesion	I		I	CIN I				
186	2189/14	30	mensturating	low grade lesion	I		I	CIN I				
187	2208/14	52	menopause	low grade lesion	II		I	CIN I				
188	2210/14	34	mensturating	low grade lesion	III		I	CIN I				
189	2226/14	35	mensturating	low grade lesion	I		I	CIN I				
190	2254/14	27	mensturating	high grade lesion	III		I	CIN II				
191	2255/14	30	mensturating	low grade lesion	I		I	CIN I				
192	2262/14	45	mensturating	low grade lesion	I	LSIL	I	CIN I				
193	2263/14	38	mensturating	low grade lesion	II		I	CIN I				
194	2264/14	30	mensturating	low grade lesion	I		I	CIN I				
195	2268/14	40	mensturating				III	CIN I				
196	2277/14	55	menopause	invasive carcinoma	V		I	SCC-WD				
197	2279/14	31	mensturating	low grade lesion	IV		I	CIN I				
198	2280/14	32	mensturating	low grade lesion	I		I	CIN I				
199	2288/14	40	mensturating	low grade lesion	I		I	CIN I				
200	2290/14	36	mensturating	low grade lesion	I		I	CIN I				
201	2299/14	45	mensturating				II	CIN I				
202	2306/14	45	menopause	low grade lesion	I		I	CIN I				

KEY TO MASTER CHART

AGE: Entered in years

SITE OF LESION:

I	– 12-3 o' clock quadrant
II	– 3-6 o' clock quadrant
III	– 6-9 o' clock quadrant
IV	– 9-12 o' clock quadrant
V	– Circumferential

CYTOLOGY

NIL	– Negative for Intra-epithelial Lesion
LSIL	– Low grade Squamous Intra-epithelial Lesion
HSIL	– High grade Squamous Intra-epithelial Lesion
SCC	– Squamous Cell Carcinoma

TYPE OF SPECIMEN

I	– Colposcopy
II	– LLETZ
III	– Ectocervix biopsy
IV	– Hysterectomy

HPE DIAGNOSIS

CIN I	– Cervical Intra-epithelial Neoplasia I
CIN II	– Cervical Intra-epithelial Neoplasia II
CIN III	– Cervical Intra-epithelial Neoplasia III
SCC-WD	– Squamous Cell Carcinoma-Well Differentiated
SCC-MD	– Squamous Cell Carcinoma-Moderately Differentiated
SCC-PD	– Squamous Cell Carcinoma-Poorly Differentiated

ICC – IMMUNOCYTOCHEMISTRY – p16

0 – negative

1 – weakly positive in dysplastic cells

2 – strongly positive in dysplastic cells

ICC – IMMUNOCYTOCHEMISTRY – Ki-67

0 – negative

1 – <10% of dysplastic cells positive

2 – 10-50% of dysplastic cells positive

3 – >50% of dysplastic cells positive

IHC – IMMUNOHISTOCHEMISTRY – p16

0 – negative

1 – positive

IHC – IMMUNOHISTOCHEMISTRY – Ki-67

0 – negative

1 – nuclear positivity in lower-third of epithelium

2 – nuclear positivity in lower two-thirds of epithelium

3 – nuclear positivity in lower two-thirds to full thickness of epithelium

4 – diffuse nuclear positivity in the malignant cells