HISTOPATHOLOGICAL ANALYSIS AND ROLE OF $p16^{INK4a}$ AS A DIAGNOSTIC MARKER IN UTERINE CERVICAL NEOPLASMS

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CERTIFICATE

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Dr .AL.SANTHI, M.D.,D.G.O.
Professor& Head of the Department,
Department of Pathology,
Thanjavur Medical College,
Thanjavur.

DEAN
Thanjavur Medical College,
Thanjavur.

Place : Thanjavur

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This is to certify that this dissertation entitled **"HISTOPATHOLOGICAL ANALYSIS AND ROLE OF $p16^{INK4a}$ AS A DIAGNOSTIC MARKER IN UTERINE CERVICAL NEOPLASMS"** is the original and bonafide work done by **Dr.R.Shobana** under my guidance and supervision at the Thanjavur Medical College & Hospital, Thanjavur, during the tenure of her course in M.D. Pathology from May-2012 to April-2015 held under the regulation of the Tamilnadu Dr. M.G.R. Medical University, Guindy, Chennai – 600032.

Dr.K.G.PADMANABAN, M.D
Associate Professor,
Department of Pathology,
Thanjavur Medical College,
Thanjavur.

Place: Thanjavur

Date: .09.2014

Thanjavur Medical College

THANJAVUR, TAMILNADU, INDIA-613 001

(Affiliated to the T.N.Dr.MGR Medical University, Chennai)

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submitted by Dr. R. SHOBANA **of**

Dept. of PATHOLOGY **Thanjavur Medical College, Thanjavur**

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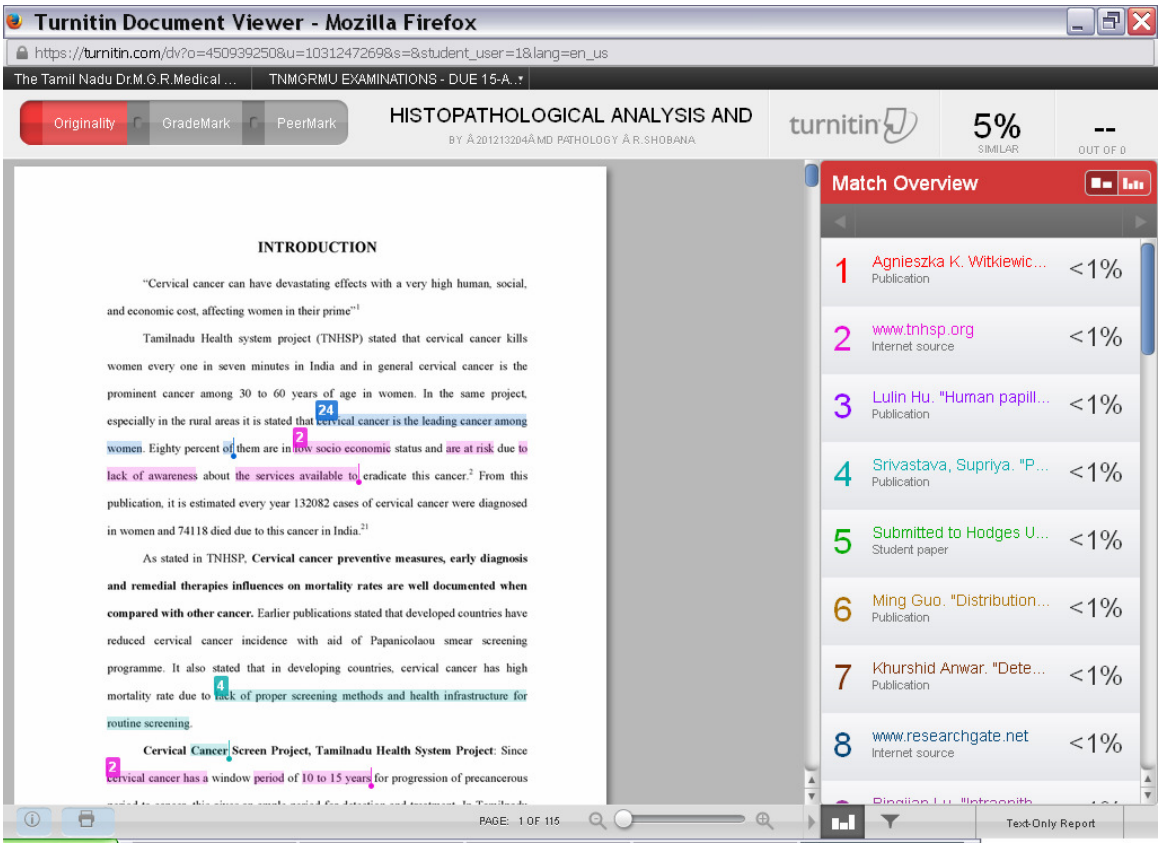
TMC, Thanjavur.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INTRODUCTION
"Cervical cancer can have devastating effects with a very high human, social, and economic cost, affecting women in their prime"[1]
Tamilnadu Health system project (TNHSP) stated that cervical cancer kills women every one in seven minutes in India and in general cervical cancer is the prominent cancer among 30 to 60 years of age in women. In the same project, especially in the rural areas it is stated that cervical cancer is the leading cancer among women. Eighty percent of them are in low socio economic status and are at risk due to lack of awareness about the services available to eradicate this cancer.[2] From this publication, it is estimated every year 132082 cases of cervical cancer were diagnosed in women and 74118 died due to this cancer in India.[21]
As stated in TNHSP, Cervical cancer preventive measures, early diagnosis and remedial therapies influences on mortality rates are well documented when compared with other cancer. Earlier publications stated that developed countries have reduced cervical cancer incidence with aid of Papanicolaou smear screening programme. It also stated that in developing countries, cervical cancer has high mortality rate due to lack of proper screening methods and health infrastructure for routine screening.
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CONTENTS

ABSTRACT

INTRODUCTION:

Uterine cervical cancer is the most common cancer cause of death in the developing countries. Human papilloma virus (HPV) infection is the most important risk factor for cervical intraepithelial neoplasms and invasive cervical cancer. HPV oncogene expression and evidence of its deregulation can be monitored through direct detection of the cellular protein p16.p16 (INK4a) immunostaining shows great promise as a marker of lesions associated with high-risk HPV, and it may assist in improving the reproducibility of diagnoses in cervical dysplastic and reactive lesions.

AIM AND OBJECTIVES:

To determine the incidence,age distribution, histopathological features of uterine cervical neoplasms and observe the frequency and distribution of $p16^{INK4a}$ protein expression as a diagnostic marker in uterine cervical neoplasms using immunohistochemical staining and relating its expression with the different histological grades of uterine cervical neoplasms.

MATERIALS AND METHODS:

This prospective study, analyses 608 cases of uterine cervical neoplasms diagnosed in Thanjavur medical college during the one year period from January 2013 to December 2013.Among which 60 cases of uterine cervical lesions selected randomly for $p16^{INK4a}$ immunohistochemical expression by immunoperoxidase method.

RESULTS:

- Uterine cervical neoplasms accounts for 19% of total number of cervical biopsies.The mean age for cervical intraepithelial neoplasms is 39.8 years and cervical carcinoma is 52.61 years.
- CIN 1 constitutes majority (77%) of total cervical intraepithelial neoplasms cases.Squamous cell carcinoma constitutes themost common subtype (95.8%) in the total cervical carcinoma cases.
- $p16^{ink4a}$immunoreactivity waspositive in 100% of all cervical neoplasms cases included in the study ($p<0.0001$) and increasing intensity of staining & percentage of positive tumour cells with increasing grades of cervical neoplasms.

CONCLUSIONS:

The pattern of over expression demonstrates the potential use of p16 as a diagnostic marker for cervical squamous and glandular neoplastic lesions.

Key words: Cervical neoplasms, Human papilloma virus and $p16^{INK4a}$.

INTRODUCTION

"Cervical cancer can have devastating effects with a very high human, social, and economic cost, affecting women in their prime"[1]

Tamilnadu Health system project (TNHSP) stated that cervical cancer kills women every one in seven minutes in India and in general cervical cancer is the prominent cancer among 30 to 60 years of age in women. In the same project, especially in the rural areas it is stated that cervical cancer is the leading cancer among women. Eighty percent of them are in low socio economic status and are at risk due to lack of awareness about the services available to eradicate this cancer.[2] From this publication, it is estimated every year 132082 cases of cervical cancer were diagnosed in women and 74118 died due to this cancer in India.[21]

As stated in TNHSP, **Cervical cancer preventive measures, early diagnosis and remedial therapies influences on mortality rates are well documented when compared with other cancer.** Earlier publications stated that developed countries have reduced cervical cancer incidence with aid of Papanicolaou smear screening programme. It also stated that in developing countries, cervical cancer has high mortality rate due to lack of proper screening methods and health infrastructure for routine screening.

Cervical Cancer Screen Project, Tamilnadu Health System Project: Since cervical cancer has a window period of 10 to 15 years for progression of precancerous period to cancer, this gives an ample period for detection and treatment. In Tamilnadu (India), cervical cancer screening project was started on February 2007 and Tamilnadu Health system project experts developed a screening protocol for cancer cervix. These were scrutinized by Tata Memorial Cancer Institute, Mumbai and peer reviewed by Madurai Medical College Institutional Board, Madurai. Theni and Thanjavur districts

in Tamilnadu were selected as pilots for this screening due to high proportion of rural population. This cancer screening programme ongoing in 58 Primary Health Centres, 13 Government Hospitals and 1 Government Medical College and Hospital in Thanjavur District. In this programme, women were screened between 30 to 60 years for Cancer Cervix using Visual Inspection Method (VIA /VILI). If positive, biopsy done under colposcopy and specimen sent for Histopathological examination.

Histopathological diagnosis is considered as "**gold standard"** method for diagnosis of cervical neoplasms. However due to **interobserver variability** this diagnosis still deemed to consider an element of risk. These limitations highlight the need to identify the biomarkers for dysplastic epithelial cells to assist in primary screening and lesion diagnosis. Wide range of biomarkers are currently been evaluated by many studies and researchers for its diagnostic effectiveness of cervical cancer and its precursors. **Potential biomarker should distinguish between cervical intraepithelial neoplasms (CIN) and other non-neoplastic cervical lesions to avoid any under treatment (Al Nafussi et al., 1990) or over treatment (Creagh et al., 1995).**

An infectious agent (HPV) is being a prime cause for cervical carcinogenesis, detection of HPV is done by using immunomarker **$p16^{INK4a}$, a surrogate marker of HPV** in immunohistochemical staining method. $p16^{INK4a}$ is expressed only in dysplastic cervical epithelial cells and is associated with high-risk HPV. In order to evaluate the usefulness of potential biomarker for Cervical neoplasm diagnosis, this study used $p16^{INK4a}$ biomarker in immunohistochemical staining as diagnostic marker to show the varied intensity and expression in uterine cervical neoplasms, which was diagnosed during one year period of 2013 under Pilot screening project of cervical cancers at TMCH.

AIM OF THE STUDY

1. To determine the incidence of uterine cervical neoplasms in our institution during the one year period from January 2013 to December 2013.
2. To evaluate the age distribution of uterine cervical neoplasms.
3. To determine the histopathological features of uterine cervical neoplasms.
4. To observe the frequency and distribution of $p16^{INK4a}$ protein expression as a diagnostic marker in uterine cervical neoplasms using immuno histochemical staining and relating its expression with the different histological grades of uterine cervical neoplasms.

MATERIALS AND METHODS

This is a prospective study, done to analyse the uterine cervical neoplasms for a period of 1 year, i.e. from January 2013 to December 2013 in the Department of Pathology, TMCH (Thanjavur Medical College Hospital), where Pilot Project for Screening of Cervical Cancer programme for cervical cancer is an ongoing process.

Local Ethical clearance was obtained from TMCH Ethical Review committee.

3.1 INCLUSION CRITERIA:

- Females after the age of menarche
- Family history of cervical cancer
- VIA/VILI positive

3.2 EXCLUSION CRITERIA:

- Inflammatory lesions and any other benign lesions were excluded.
- HPV vaccinated patient and patient on chemotherapy and radiotherapy
- Hysterectomy specimens were excluded.

3.3 SAMPLE SIZE:

A total of 3198 cervical biopsies were received, diagnosed and reported during the study period. 608 cases of which diagnosed as uterine cervical neoplasms were taken as the sample size.

3.4 METHODS OF COLLECTION OF DATA:

Patients clinical information, demographic data, socio economic history, VIA/VILI positivity and abnormal colposcopic findings data were collected from the PILOT PROJECT SCREENING proforma [Annexure I] .Cervical biopsies specimens received were either punch or wedge biopsies.

3.5 FIXATION AND GROSSING:

All the specimens obtained were fixed in 10% buffered formalin for a period of 24 hours and were submitted in Toto for routine histopathological examination.

3.6 HISTOPATHOLOGICAL STUDY OF CERVICAL NEOPLASMS:

Formalin fixed paraffin embedded tissues were sectioned of 3-5 micron thickness and staining with Haematoxylin and eosin (Annexure-II) was done. The H&E stained slides were reviewed and the interobserver variations in diagnosing the cervical intraepithelial neoplasms were noted. Histologic findings like basal cell hyperplasia, reactive atypia were downgraded. Some cases reported as HSIL were categorized as CIN2 and CIN3 separately during review.

3.7 IMMUNOHISTOCHEMISTRY:

For Immunohistochemistry, 5µ sections of formalin fixed paraffin embedded tissue floated on chrom alum coated slides were utilized. After heat fixation at 37 degree Celsius overnight and 60 degree Celsius for ten minutes, deparaffinization was done. The antigen retrieval was done by heat using Tris buffer. The sections were stained using a standard peroxidase - antiperoxidase technique. The slides were

incubated with primary monoclonal antibody - p16 (a mouse monoclonal anti-p16 antibody, Fremont, CA, 94538, Biogenex, USA) for one and half hour. The chromogen used was DiaminoBenzidine (DAB), after incubation with secondary polymer antibody for 30 minutes. The slides were counterstained with haematoxylin (30 seconds to 1 min).

3.8 ASSESSMENT OF IMMUNOSTAINS:

EVALUATION OF IMMUNOHISTOCHEMICAL MARKER-$p16^{INK4a}$

The immunostaining was considered positive when the nucleus and/or cytoplasm take chest nut brown colour. Various researchers have used different methods for scoring $p16^{INK4a}$ immunostaining, but in this study four parameters were considered for scoring which will eventually increase the specificity of the results, and the parameters are as follows:

1. Percentage of proportion of positive tumour cells[42, 43, 44, 45, 46] were graded as 0, 1+, 2+, 3+ when 0%, 1-5%, 5-25%, >25% of tumour cell shows positivity respectively.
2. Intensity of staining[42, 44, 47, 48, 49]was graded between 0-3 (negative - 0, weak – 1+, moderate – 2+ and Strong – 3+).
3. $p16^{INK4a}$ staining in cellular reaction patterns[42, 44, 45, 49] showing only cytoplasmic positivity, Nucleo:cytoplasmic and Nuclear positivity.
4. Patterns of $p16^{INK4a}$ staining expression within epithelium in different grades of CIN[42, 45, 50] as Diffuse full thickness, Diffuse basal and Patchy.

3.9 STATISTICAL METHODS:

Descriptive statistics were derived using mean and percentages. Fisher exact test and Chi square test were used to assess the association between their trends in their subgroups. Value < 0.05 was considered as significant p value. All statistical analysis was done using SPSS version -18 (Statistical Package for Social Sciences) Inc., Chicago, USA.

3.10 LITERATURE SOURCES:

Research papers and review articles were accessed through PubMed and Google /Google scholar websites.

REVIEW OF LITERATURE

4.1 HISTORICAL REVIEW:

In 400 BC, Hippocrates, the Greek physician diagnosed warts and wrote about cervical carcinoma. Although he could do nothing to treat the cancer, he attempted to treat it with the procedure known as trachelectomy. In 25 AD, Aulus Cornelius Celsus identified distinct types of warts: Acrochordon (skin tags), Thymion (genital warts) and Myremecia (non-genital warts).[28]

Aretaeus, an ancient Greek physician probably of 2nd or 3rd century BC, described uterine cancers as superficial and deep ulcers, which would later infiltrate the uterus or as a growth in uterus. He distinguished between the two lesions and acknowledged that the symptoms and prognosis of cancer with ulcers was the most negative.[28]

Rigoni-Stern, a surgeon in Padua in the mid-19th century had an amateur interest in epidemiology. He observed that uterine cancer was rare in celibate nuns following his study of the death certificates of women dying due to cervical carcinoma.[29]

In early 20th century, as stated in previous studies epidemiologists observed that among female sex workers cervical carcinoma is common. In women whose husbands with high number of sexual partners or were regular customers of prostitutes as well had commonly noted and less common in Jews.[29]

In 1976, Human papilloma virus (HPV) 16 was identified in precursor lesions of genital cancer by Zur Hausen, Giesmann and their co-workers and in 1985, they demonstrated the presence of HPV DNA in cervical carcinoma cells. These findings created a basis for subsequent studies, which led to the development of Gardasil and Cervarix, the two preventive vaccines that were approved by FDA in 2006.[2]

In the prevention of cervical carcinoma, the important milestones discoveries were: the invention of colposcopy in 1925 by Hinselmann, the development of pap technique by Papanicolaou, the launch of pap screening by Papanicolaou and Traut and the invention of a specific spatula by Ayre in 1946 to scrape the cervix. The standardization of screening results by Bethesda system in 1988 and further improvisation in 2001 was another important achievement.[29]

4.2 INCIDENCE AND PREVALENCE

Cervical carcinoma is the fifth common tumour in human being. It ranks second among the cancers in women, worldwide and is the leading cancer among Indian women. Cervical carcinoma is the leading cause of cancer deaths in second world countries. Every year around 510,000 new cases occur throughout the world and 288,000 deaths globally.[30]A report published in the American Cancer Society's journal for clinicians says that "the vast majority of deaths due to cervical cancer occur in the developing world, with India contributing to 27% of its mortality."[31]

The age group affected is the productive one with the starting age group of 30-34 years and peaking in 55-65 years. Median age of occurrence is 38 years (21-67). Most of the sexually active females (80%) get HPV infection in the genital tract.

Around 365.71 million women (>15 years) in India form the risk group for cervical cancer. About one lakh thirty two thousand new cases occur in India contributing greater than one fifth of total new cases worldwide. Around one third of the death due to cervical cancer throughout the world is from India (74,000 deaths annually). Lifetime cumulative risk for Indian female is 2.5 and it is 1.4% cumulative death risk from the cervical carcinoma. About seventy percent of the world's burden falls in the underdeveloped countries.

There is a drastic difference between the incidence and death rate among the developed and underdeveloped countries. In sub-Saharan Africa the incidence is 34.8 per 100000 women and in North America it is 6.6 per 100000 women. The death rate is twenty two per lakh in Africa and only 2.5 per lakh in North America. Poor access for proper screening, early identification and treatment explains this difference.[1]

There is significant decrease in occurrence of new cases in major cities like Delhi, Bengaluru, Chennai and Mumbai which is evident from the respective urban registries.

Cancer cervix is still the number one cancer in India as majority of the population (>70%) are in rural area. In 2007 incidence is 90/708 (PBCR) and relative 5 year survival is reported as forty eight percent.

HBCR reveals that cervix is the most common site for cancer in Chennai and Bengaluru, second most common site in Trivandrum and Mumbai and third most common in Dibrugarh. Age of occurrence is later in Trivandrum. Around forty percent of patients did not get treatment in spite of having diagnosed cancer cervix. Incidence in 5 districts recorded higher than Chennai and of them 4/5 are from north east Tamilnadu and Pondicherry.

Each year around one lakh thirty four thousand, four hundred and twenty cases are estimated to occur (crude incidence rate 23.5) giving rise to 203,757 by 2025 and increase in death to 115,751 in 2025.[32]

4.3 EMBRYOLOGY[3, 4]

At the 6th week of intrauterine life, the coelomic epithelium of the mullerian duct invaginates and fuses to form the uterus. From coelomic epithelium, basal cells are derived which lines the cervix. From the basal cells, some uncommitted stem cells (reserve cells) in the cervix is capable of differentiate into both columnar and squamous epithelium.

4.4 GROSS ANATOMY

The term CERVIX (Latin word) means NECK. Cervix is the lower most part of the uterus which measures 2.5 -3 cm in length in nulliparous women. The cervical canal opens into vagina by an opening called external os and is continuous above with the cavity of the uterus through an opening called internal os. The posterior aspect of the cervix is covered by peritoneum of the pouch of Douglas. The cellular connective tissue present anterior and lateral to the supravaginal portion is the parametrium. The ureter runs about 1 cm lateral to the supravaginal portion.

Arterial supply

Uterine arteries, branches of anterior division of internal iliac artery form the arterial supply. Uterine artery runs medially towards cervix and runs upwards to supply the uterus after giving a branch in the supravaginal part which supplies the cervix.[3]

Venous drainage

Veins from plexus along the lateral border of the uterus drains through the uterine, ovarian and vaginal veins into iliac veins.[3]

Nerve supply

Dense uterovaginal plexus or paracervical plexus of nerves consisting of visceral afferent and sympathetic efferent fibres passes to the uterus through the lateral cervical ligament and at the junction of cervix and body gives abundant supply to the cervix.

Cervical pain fibres pass from inferior hypo gastric plexus along with pelvic splanchnic nerves into S2-S4 segments of spinal cord.[3]

4.5 HISTOLOGY

Cervix is made up of connective tissue, muscle and elastic fibres. The epithelial lining is of columnar and squamous type. Major part is formed by supportive connective tissue. Around fifteen percent tissue is contributed by smooth muscle and that too concentrated in the endocervix. Isthmus contains fifty to sixty percent smooth muscles acting as a sphincter but portiovaginalis almost lacks smooth muscle fibres.[6]

Cervico vaginal epithelium of late foetal life

The original squamocolumnar junction is situated just above the external cervical os in foetal life which at term, changes its position, caudally to the external os, usually on the ectocervix or rarely on the vaginal fornix walls.

Anatomy of Cervical Epithelium:

There are 3 types of epithelium seen in the cervix of adult women.1) Normally occurring original squamous or columnar epithelium, 2) metaplastic squamous, and 3) atypical one having pathological lining.

Microscopically squamous cell line contains stratified epithelium with 5 distinct layers or zones. They are[6, 7]

Zone 1: basal cells or stratum cylindricum.

Zone 2: parabasal cells

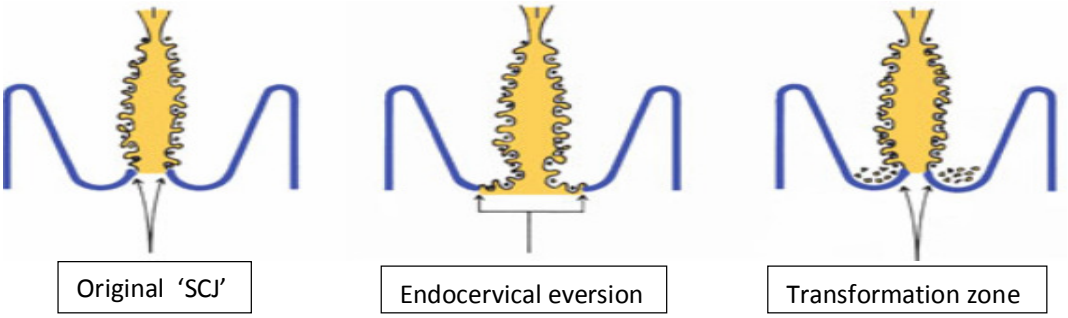
Zone 3: intermediate cells

Zone 4: interepithelial zone or condensation zone

Zone 5: also known as stratum corneum, representing keratinisation.[6]

Columnar epithelium is found lining the endocervical glands. The cytoplasm is granular having mucin droplets and tall uniform basally placed nuclei. They are also known as picket cells.

Pictorial representation of squamocolumnar junction



Transformation zone

The zone where the squamous epithelium meets columnar cells is known as squamocolumnar junction (SCJ). Morphogenically, there are two different squamocolumnar junction - the original squamocolumnar junction and physiological squamocolumnar junction. The original one is the place where squamous cells meet the columnar cells of endocervix at birth.

Overtime, the columnar epithelium gets remodelled and metaplastic squamous cells occupy the place of columnar cells pushing the SCJ towards external cervical os. This new junction is known as the physiological squamocolumnar junction.

The region between the original and the newly formed functional squamocolumnar junction is known as Transformation zone. Histologically this zone contains metaplastic epithelium. This zone is very important because all squamous tumours in cervix begin in new junction and extension also depends upon the distribution of this zone. All over the reproductive period the movement of the functional junction continues making the transformation zone to lie on exposed areas. This makes punch biopsy a useful tool for histological diagnosis.

Metaplastic squamous epithelium

In around 90% of post menarche cervix, squamous metaplasia is seen most commonly at the transformation zone. It is a normal phenomenon taking place during menarche and pregnancy. This does not get converted back to columnar cells and the metaplasia is due to the acidic pH in the vagina.[6, 7, 8]

The Cervical Stroma

The connective tissue stroma of cervix is mainly composed of collagen fibres that are dense in the region of ectocervix and loosely surround the endocervical glands.[6, 7] Inflammatory infiltrate, suggested as an immunological response to the cell necrosis and regeneration associated with metaplastic change is seen deep to the epithelium in normal cervix particularly in the transformation zone. [6, 7]

4.6 ETIOLOGY

Human papilloma virus

Role of HPV in carcinoma cervix was first proposed by Dr.Harald zur Hausen in late 1970s. This invention fetched him a Nobel Prize in 2008.[6]

HPV is linked with variety of cervical lesions from benign condyloma to malignant carcinoma.[9]

Incidence and Prevalence[14, 15, 16]

The incidence of HPV virus infections varies according to age, sexual activity, the number of times tested and the laboratory technique used. M. El Mzibri et al stated that "incidence rates, vary from about 5 per 100 000 women per year in many industrialized countries to more than 50 per 100 000 in some developing nations". The probability rate of HPV transmission of the transmission rate from male to female per coital act has been estimated for genital HPVs to be between 0.4 and 0.8.

Cervical HPV prevalence peaks at young ages on sexual initiation and population-based prevalence remains low across the lifespan after about the age of 30 years. HPV prevalence curves are U-shaped characterized by high cervical HPV

prevalence at young ages followed by a significant decline and subsequent increase in prevalence beginning at different later ages. Life time risk of becoming infected with HPV in sexually active women is more than 50%. The greatest risk of HPV infection is in women aged 25 years and younger. The second peak of infection occurs after the age of 55 yrs.

Morphology [3, 6, 7, 12]

HPV is a small nonenveloped DNA virus. The genome of this virus contains 3 regions for regulation, replication, oncogenesis and capsid formation.

HPV Life cycle

They are host specific. HPV has a great affinity towards cervical transformation zone. To start they infect the basal cells through a mild abrasion. Viral receptor for them is not clearly identified with many proposed ones. Irrespective of the grade they are mostly seen as episome in the benign and premalignant lesions. In true neoplasms they get integrate to host genome, thus promoting oncogenesis.

HPV subtypes[14, 15]

Currently, there are 148 HPV types with 33 species. Types infecting genital tract are classified in to high risk and low risk types. Forty HPV types infect mucosal epithelium with around 12 high risk subtypes that are etiologically linked to cervical cancer and its immediate pre-malignant precursors. Warts in the genital tract and low grade dysplasia are caused by low risk strains such as HPV6 and 11. High risk genotypes are responsible for 95% of cervical cancers. HPV 16 and 18 are found in most of the carcinoma.

Low risk HPV subtypes – 6, 11, 42 and 44

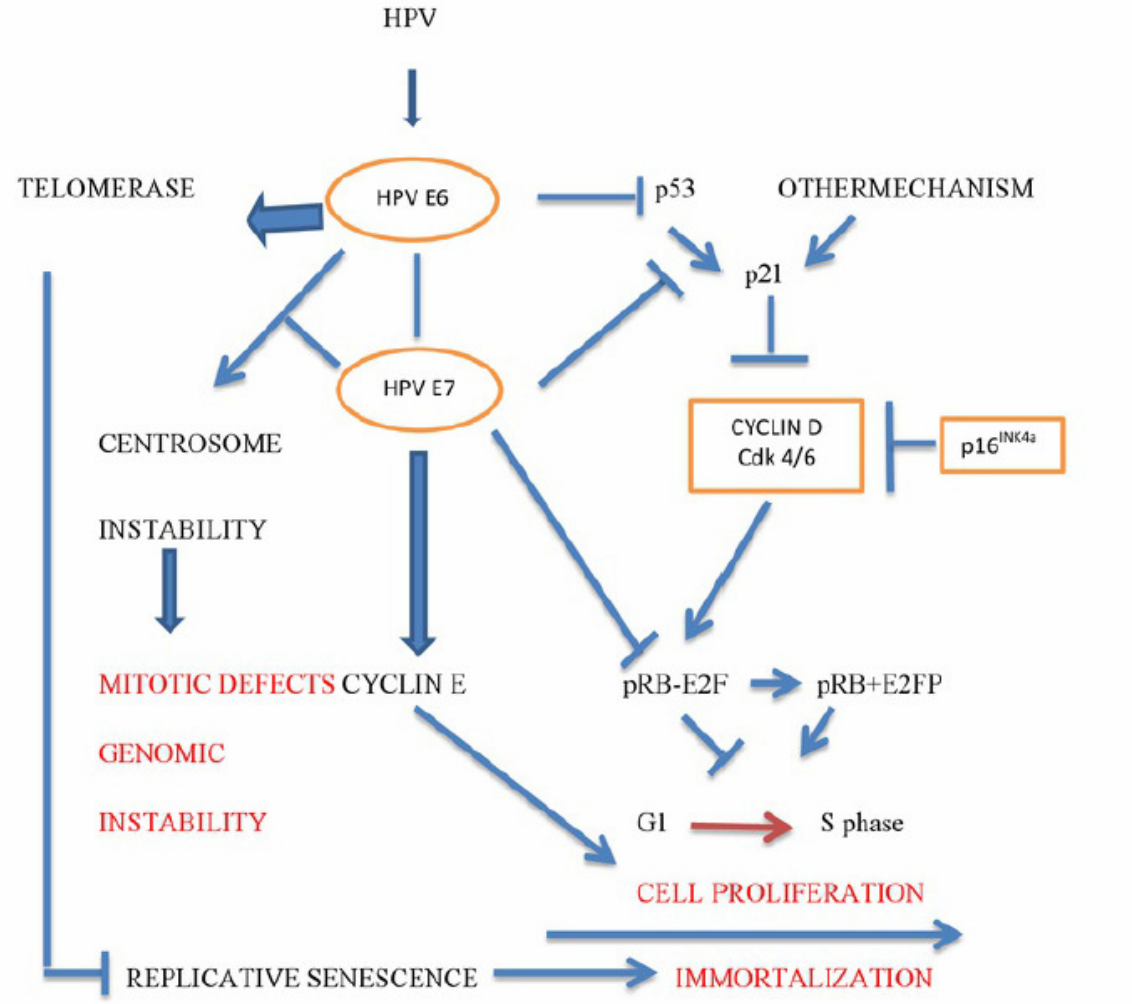
High risk HPV subtypes – 16,18,31,33,35,39,45,51,52,56,58,59,66,68 etc

HPV 16 is found in around sixty percent of squamous carcinoma and HPV 18 is found in around fifteen percent. Either of them is found in around fifty percent of high-grade intraepithelial lesions (CIN 3).[17]

4.7 PATHOGENESIS OF HPV[13]

HPV are epitheliotrophic. Initially they enter the basal cells via micro abrasions. They lie within the cells as episome for years together. E1 and E2 genes help for this long survival by maintaining replication. Viral multiplication depends upon the host environment.

In high risk HPV types E7 protein degrades pRb and p130 proteins. It drives the cell in to S phase. E6 protein helps E7 protein in its action. Both of them jointly inhibit p53 protein action so that it decreases apoptosis which in turn facilitate tumour growth.



Integration of the high risk genome represents an important event in the etiopathogenisis of carcinoma cervix, closely linked to progression to carcinoma from intraepithelial lesions.[11]

HPV infection is essential for the occurrence of dysplasia and carcinoma in cervix, but most of the females with HPV infection have least of these lesions. Some risk factors other than HPV infections play as cofactor in development of cervical carcinoma.[1]

4.8 RISK FACTORS[3]

It includes both host and viral factors.

1) HPV infection.

2) Oncogenecity of virus.

3) Immunodeficiency

4) Co-carcinogens

High risk group includes:

1. Multiple sexual partners.

2. Male partner with multiple previous or current sexual partners.

3. Young age at first intercourse.

4. High parity.

5. Persistent infection with a high oncogenic risk HPV.

6. Immunosuppression.

7. Certain HLA subtypes.

8. Use of oral contraceptives.

9. Use of nicotine.

HPV prevalence increases at young ages and then remains constant with increasing age. However, the second peak occurs at old age

Factors contributing to these age-specific regional HPV prevalence differences at older ages are

- Continuing new sexual exposures.
- Reappearance of quiescent/latent HPV.
- Changes in immune status, such as decline in immune function and hormonal milieu at older ages or immunosuppression caused by coinfection with other viral or parasitic agents.
- Cofactors including the cervicovaginal microbiota and pH or factors yet to be determined.

In the first 2 instances, region-specific and age-specific patterns of sexual behaviour would be expected to contribute to the differences in HPV prevalence. While, in the third and fourth instances, changes in immune responsiveness and the local microenvironment could modify the risk of acquisition of new HPV infection, activation of previously acquired but quiescent or latent HPV infections, which lead to persistence of both.

4.9 WHO CLASSIFICATION OF CERVICAL NEOPLASMS (ANNEXURE IV)

4.10 CERVICAL INTRAEPITHELIAL NEOPLASIA

Sir John Williams in 1886 was the first to report non-invasive intraepithelial lesions nearer to the invasive squamous ones in the cervix. Various classification systems have been evolved later to describe these preinvasive cervical squamous lesions.

Initial classification used the degree of dysplasia and development of in situ carcinoma as severe degree. Richard used the term intraepithelial neoplasia and it is now used in WHO classifications. Bethasda classified them in to low grade and high grade lesion (table no-1).

CIN lesions shall remain non-invasive for more than two decades shedding dysplastic cells that can be identified by cytology. These lesions not always progress to invasive one but can regress to lower grade on their own. HPV infection always co-exists with these lesions and the types vary according to the grade as discussed elsewhere. They always occur in the epithelial junction and the differentiation pattern may reflect the differentiation "plasticity" of the transformation zone.

TABLE NO - 1

DIFFERENT TERMINOLOGIES USED IN VARIOUS CLASSIFICATIONS

Old classification-1950. Dysplasia/carcinoma in situ	**Richart -1960/WHO classification-2008**	**Bethasda system terminology-1988**
Mild dysplasia	CIN1	LSIL
Moderate dysplasia	CIN2	HSIL
Severe dysplasia/carcinoma in situ	CIN3	HSIL

When assessing CIN lesions, the histological features to be taken into account are: [6, 7, 18]

1) Differentiation (maturation, stratification)

a) Presence or absence

b) Proportion of epithelium showing differentiation

2) Nuclear abnormalities

a) Nucleus: cytoplasm ratio

b) Hyperchromasia

c) Nuclear pleomorphism and anisokaryosis

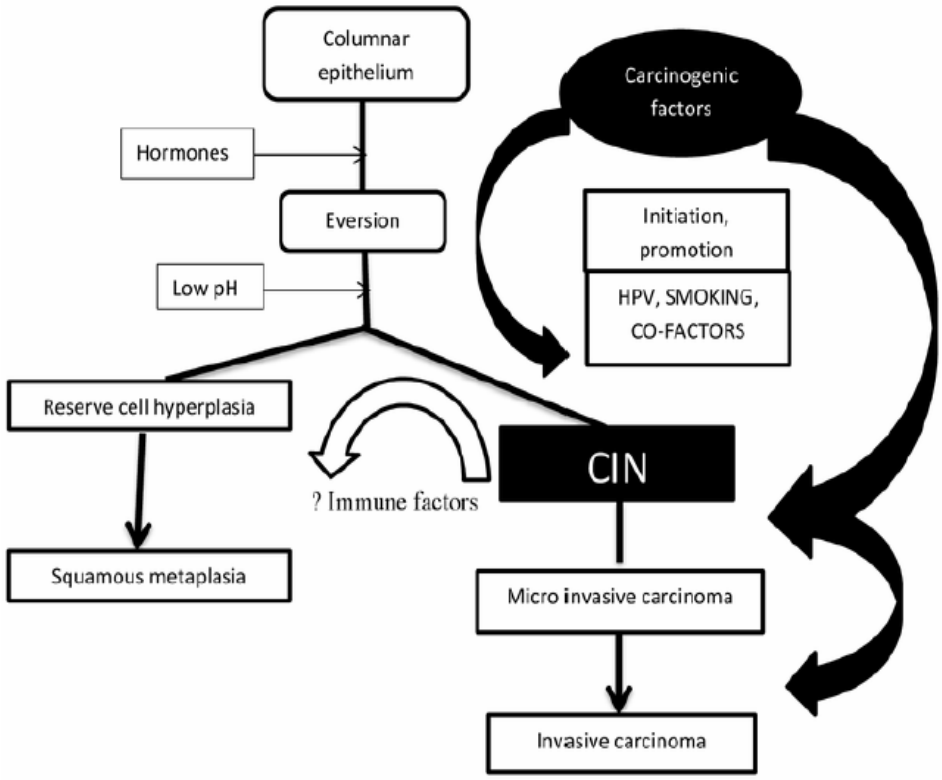
3) Mitotic activity

a) Number of mitotic figures

b) Height in epithelium

c) Abnormal configuration.

HISTOGENESIS OF CERVICAL SQUAMOUS NEOPLASIA[7]



The grades of CIN may be characterised as follows[3, 4, 18]

CIN1

Abnormal cells are limited to lower 1/3rd of the epithelium, though slight nuclear atypia persist up to the surface, representing a delay in nuclear maturation.

Nuclear abnormalities are slight and more marked in the basal third.

Koilocytotic atypia or Koilocytosis

- Koilos is a Greek word, meaning holes.[25]
- It is pathognomonic of a productive HPV infection.

- The cellular changes presumably reflect the impact of replicating virus on the cells including cell cycle stimulation by the viral proteins.[13]
- Differential diagnosis includes inflammatory atypia and reparative changes.

CIN2

Here abnormal cells are present up to ½ of the epithelial area. Atypia in the nucleus may extend until the surface. Increased mitotic activity is limited to the basal 2/3rd of the epithelial lining.

CIN3

Immature cells extend up to or more than lower 2/3rd of the cell column. Almost all of the epithelium has abnormalities in the nucleus. Increased mitotic activities are seen at all levels.

Understanding mainly HPV types 16 and 18 and a minority of other types are identified in CIN and that the greatest risk of progression is strongly associated with higher-grade lesions—the pathologist can appreciate that most lesions falling between the limits of "suggestive of LSIL" to moderate dysplasia (CIN 2) impose little immediate risk to the patient, a realization that will progressively diminish the frequency of cone or LEEP biopsy.[13]

Most of the new HPV infections are self-limited and are cleared spontaneously. Around seventy percent gets cleared by one year and ninety percent by two years. High risk types take long time than the lower one for clearance. The clearance rate of HPV infections also decrease with time. In women with CIN1, clearance rate of HPV is up to seventy to ninety percent as compared to forty percent with CIN2.

The following table no-2 shows the metaanalysis of population based on cervical cancer progression from cervical dysplasia. Here, CIN 3 has a higher risk to get into invasive cancer.

TABLE NO - 2

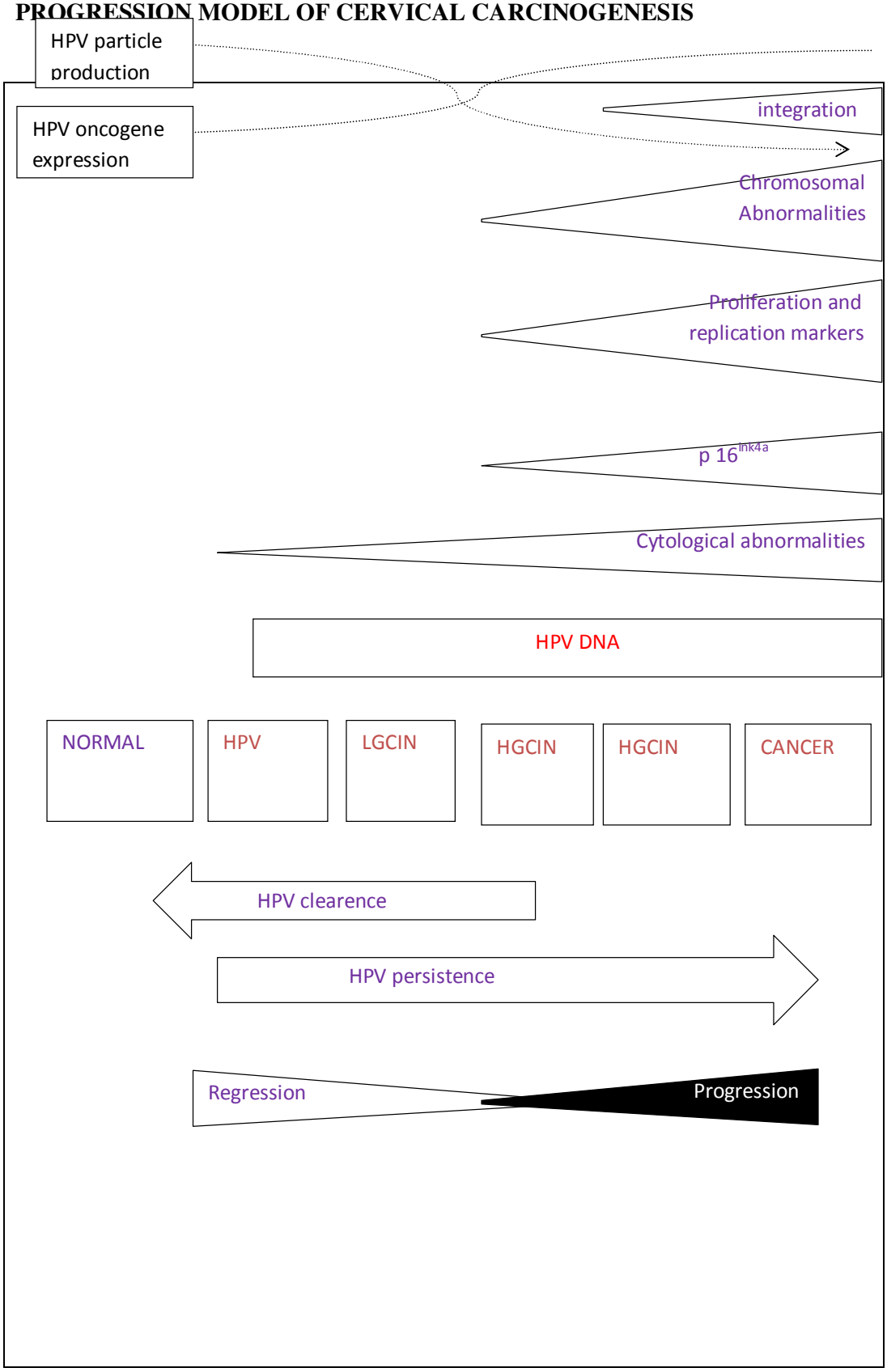
Grade of CIN and Risk of Progression over Life Time: metaanalysis[14]

Degree of CIN	Risk of persistent HPV	Risk of progression to CIN3	Risk of progression to cervical cancer
CIN 1	30%	10%	1%
CIN 2	40%	20%	5%
CIN 3	n/a	n/a	12%

PROGRESSION MODEL OF CERVICAL CARCINOGENESIS

The high risk HPV infection namely HPV-16 and HPV -18 get integrated into the genome of human. Then there is a dysregulation of cell cycle leading to the uninterrupted proliferation of malignant cells. The following schematic diagram encloses the entire three elements which plays a major role of cervical carcinogenesis. The progression and regression depends mainly on the virulence of strains and immunological genetic factors of host.

PROGRESSION MODEL OF CERVICAL CARCINOGENESIS



4.11 SQUAMOUS CELL CARCINOMA[18, 88, 89, 94, 95]

Squamous cell carcinoma is the most common carcinoma of cervix which constitutes 80-90% of total carcinoma of cervix. Nowadays frequencies have been reduced in developed countries due to early detection, whereas adenocarcinoma of cervix incidence is in increasing mode.

The peak age of presentation to the OPD (outpatient department) is 60-64 years.

Clinical features – most common complaint is abnormal vaginal bleeding after intercourse, chronic pelvic pain and chronic illness symptoms like weight loss and loss of appetite.

Gross features - tumour grow either as exophytic with papillary/ polypoid excrescences or endophytic with ulceroinfiltrative/nodulo-infiltrative.

Wentz and Reagan (1958) classified SCC into three types-LCKSCC, LCNKSCC AND SCNKSCC. Immunohistochemistry and electron microscopy pictures revealed that, the term Small cell carcinoma includes Small cell neuroendocrine carcinoma, Small cell anaplastic carcinoma and Small cell squamous cell carcinoma. Nowadays the term “Small cell carcinoma” is many used for neuroendocrine neoplasms.

Numerous systems for histologic grading have been proposed, based on the type and the degree of predominant cell differentiation, modified Broder’s classification is used commonly for grading the squamous cell carcinoma of cervix (table no-3).

TABLE NO - 3

GRADING OF SQUAMOUS CELL CARCINOMA

Grade of conventional SCC	Amount of keratin	Degree of nuclear atypia	Mitotic figures
Grade 1	Abundant intercellular bridges, keratin pearls and cytoplasmic keratinization	Mild atypia	<2/hpf
Grade 2	Individual cell keratinization	Moderate atypia	4/hpf
Grade 3	Cells are immature, little evidence of squamous differentiation, cytoplasm is scant.	Marked atypia	>4/hpf

SQUAMOUS CELL CARCINOMA (NOS TYPE)

Microscopic features of SCC composed of varying pattern of growth, type of cell and differentiation. Malignant cells infiltrate the stroma with anastomosing cord and bands, looks like irregular islands. Necrosis may be present and it mostly of comedo type. SCC with micro invasive type is classified based on infiltration of tumour cell in the stroma by FIGO classifications. Here, invasive malignant cells have tongue like contour in the invading stroma.

To say invasion of SCC in the stroma, any one of the following features should be present.

- Nest of malignant cells is present near the blood vessel.
- Presence of desmoplastic reaction in the stroma.
- Malignant cell nest shows loss of polarity and comeodo necrosis.
- Tongue like projection of tumour cells extending deeper with adjacent endocervical gland.

- Nest of malignant cells with features of abundant eosinophilic cytoplasm within the stroma.
- Reduplication of epithelium.

LARGE CELL KERATINIZING SQUAMOUS CELL CARCINOMA

To diagnosis this tumour presence of keratin pearl is must. The pearl comprise concentric whorls of squamous cell with central nest of acellular keratin. Individual tumour cells are large mature with abundant eosinophilic cytoplasm, pleomorphic nuclei and prominent intercellular bridges. Less frequently mitosis is seen.

LARGE CELL NON KERATINIZING SQUAMOUS CELL CARCINOMA

Absence of keratin pearl, increased cytological and nuclear pleomorphism and increased mitotic activity is the diagnosing features used to differentiate it from large cell keratinizing squamous cell carcinoma.

BASALOID SQUAMOUS CELL CARCINOMA

It is a very aggressive tumour composed of nest of basaloid squamous cell with peripheral palisading. Some nests shows keratinization in the centre of nest.

VERRUCOUS SQUAMOUS CELL CARCINOMA

This is a rare tumour and it is caused mostly by HPV 6 infection. It has an indolent nature and local recurrence. Tumour present with warty and exophytic growth. Microscopically, tumour shows large ‘bulbous’ rete pegs, surface epithelium are hyperplastic and hyperkeratotic. Individual squamous cell with low mitotic activity at the basal are arranged in a papillary process.

WARTY CARCINOMA

It is also known as 'condylomatous squamous cell carcinoma'. It associated with high HR-HPV infection.

PAPILLARY SQUAMOUS CELL CARCINOMA

The tumour present as papillary growth. Microscopically, the tumour malignant cells arranged are perpendicular to the fibrovascular core. The malignant cells shows moderate to severe dysplasia. Differential diagnosis includes warty carcinoma, verrucous carcinoma and squamous transitional growth.

LYMPHOEPITHELIOMA-LIKE CARCINOMA

This is a rare neoplasms caused by EBV infection. Microscopically, tumour cells are arranged in a syncytial pattern, large cells with indistinct cell borders, and vesicular nuclei with prominent nucleoli. Inflammatory infiltrate mainly T-lymphocyte obscure the background. Its histologic feature is similar to that nasopharyngeal carcinoma, this tumour may have favourable prognosis.

SQUAMOTRANSITIONAL CARCINOMA

It is important to differentiate it from transitional cell carcinoma deposit by detecting strain of HPV 16 and allelic loss in chromosome 3q. Histologically, malignant cell gets differentiate into both squamous and transitional malignant cells arranged over the papillary core. Since this tumour is aggressive, care should be taken while diagnosing it from the biopsy material.

SPINDLE CELL CARCINOMA

It is a rare variant composed of malignant spindle cells arranged in the fascicles with foci of squamous differentiation. IHC shows positivity to epithelial markers.

4.12 ADENOCARCINOMA OF CERVIX[18, 88, 89, 95, 96]

The incidence of adenocarcinoma is in increasing mode from 5% to 15 % in recent decades. Cytological diagnosis of adenocarcinoma is still being challenge. The age of incidence is 50-55 years; the commonest cause for adenocarcinoma is HPV infection mainly due to 16 and 18 strains. Apart from HPV infection, oestrogen hormonal dysregulation have been implicated in association with adenocarcinoma occurrence.

80% of cervical adenocarcinomas are mucinous adenocarcinoma, of which endocervical subtype is common.

Grossly – present as ulcerative or exophytic growth.

ADENOCARCINOMA IN SITU

ACIS was first described in 1953, and its frequency has been increasing steadily since then.

Christopherson WM et al described the average age of presentation as 35 to 40 years, approximately 10 years earlier than invasive adenocarcinoma;[39]More than 90% of cases of adenocarcinoma and ACIS have detectable HPV.

Boon ME et al. stated that individual cases of ACIS have been observed to progress towards invasive adenocarcinoma and that ACIS is usually present adjacent to most very early invasive adenocarcinomas.[38]

Bertrand M et al described that ACIS arises either from columnar epithelium or, more likely, from reserve cells that have the capacity to undergo columnar cell differentiation. Evidence for the role of reserve cells is the common coexistence of ACIS with HSIL and its frequent location near the squamocolumnar junction. The lesion may arise on the surface, followed by extension into crypts and replacement of normal mucosa.[40]

Histologically, the lining epithelium is stratified and crowded, and consists of moderately enlarged nuclei with coarse chromatin. Mitotic figures are easily found, often suspended on or near the luminal surface of the gland and are essential to the diagnosis. Nucleoli are usually small and inconspicuous, but prominent in some cases. A periglandular inflammatory reaction may be present. Apoptotic bodies, present in 80% of cases. Uncommonly, ACIS will mimic a reactive epithelial process, with prominent multinucleation. So any columnar cell atypia should be carefully evaluated.

The most common subtype of ACIS is the endocervical type. Other subtypes are the Endometrioid pattern and the Intestinal type.[13]

GRADING OF ADENOCARCINOMA[37]

Cervical adenocarcinoma is graded based on two factors which include the pattern (% of solid growth) and cytological nuclear feature. It has been graded in to three grades from 1to 3. Grade 1 is well differentiated, grade 2 is moderately differentiated and Grade 3 is poorly differentiated.

Grade 1 carcinoma shows following features- malignant glands are regular with papillae, individual columnar cells shows minimal stratification with uniform oval nuclei. Mitotic figures are rare. Solid growth area of atleast ≤ 10%should be present.

Grade 2 carcinoma shows following features- malignant glands are complex with bridging, nuclei become rounded and irregular with micro nucleoli. Mitotic figures are frequently seen and Solid growth pattern constitutes 10-50%.

Grade 3 carcinoma shows following features - only few malignant glands are seen. Individual cells are large with high pleomorphism, mitotic figures are more common and necrosis is present. Solid pattern of growth is >50%.

MUCINOUS ADENOCARCINOMA

There are five types of mucinous adenocarcinoma which includes endocervical, intestinal, signet ring cell type, minimal deviation and villoglandular type.

ENDOCERVICAL ADENOCARCINOMA

CGIN is a premalignant condition. The morphological feature is characterized by malignant glands which are lined by tall columnar cells with hyperchromatic nuclei. The cells have cytoplasmic mucin which is inconspicuous. Mitotic figures are prominent in the luminal cells. Apoptotic bodies are seen in the dysplastic glands. The glands are arranged in back to back fashion with complex architecture such as intraluminal papillae, budding and branching patterns. Desmoplastic reaction is noted around the tumour cells in the stroma. Endocervical adenocarcinoma is otherwise called as colloid carcinoma, since the malignant cells secrete large amount of mucin forming lakes in the stroma.

Different variants documented are Microcystic variant and dedifferentiated variant.

INTESTINAL VARIANT OF ADENOCARCINOMA

This type of carcinoma resembles the morphological features of intestinal adenocarcinoma. This variant is uncommon. HPV infection is not associated with the risk factors of intestinal variant. Some foci show intestinal epithelial cells like goblet cells, less frequently endocrine cells and paneth cells. Necrosis is a prominent feature observed.

Before diagnosing as an intestinal variant, metastasis from the intestine should be ruled out.

SIGNET RING CELL VARIANT

Pure form of signet cell carcinoma is uncommon. Both poorly differentiated adenocarcinoma and adenosquamous carcinoma exhibits some foci of signet ring cell changes. Differential diagnosis includes metastatic tumour and squamous cell carcinoma with signet ring cell change.

ADENOMA MALIGNUM/ MINIMAL DEVIATION VARIANT

This is an uncommon tumour, the incidence being 1-3 %. This variant is associated with Peutz- Jeghers syndrome. Malignant glands are arranged haphazardly that extend beyond the normal endocervix, which shows moderate nuclear atypia and desmoplastic reaction. Diagnosis cannot be made out with punch biopsy, since the depth of penetration is the criteria. Differential diagnosis includes endocervical glandular hyperplasia- laminar& lobular, endocervicosis, deep seated Nabothian cyst and adenomyoma. In endometrioid adenocarcinoma, this variant is also seen.

VILLOGLANDULAR VARIANT

This is an uncommon type, occurs in premenopausal age group which ranges from 33 to 39 years whose comes with the complaints of vaginal bleeding. There is an association with the oral contraceptives usage. Microscopic features are surface papillae mostly tall, thin papillae intermixed with broad short papillae. Since tumour have frond like arrangement which resembles villoglandular adenoma of the colon. The lining epithelium may be intestinal type, endocervical type and endometrioid type. It has a better prognosis, since there is no vascular invasion.

ENDOMETRIOID ADENOCARCINOMA

These tumours constitute 30% of total adenocarcinoma of cervix. Microscopically, the malignant cell resembles endometrial adenocarcinoma cells, but the differentiating point is minimal squamous metaplasia, focal cytoplasmic mucin, adjacent CGIN and absence of complex endometrial hyperplasia. The cells are columnar shape with large elongated hyperchromatic nuclei. The cells arranged in sheets with 2D rosettes like pattern. Here, immunohistochemistry places the main role that is vimentin is more specific, positive in endometrial adenocarcinoma whereas diffuse positivity of p16 in cervical neoplasm.

Differential diagnosis for adenoma malignum of endometrioid variant are, Endometriosis, tuboendometrioid metaplasia and endometrioid endometrial adenocarcinoma.

IHC – vimentin-, CEA+, ER-, HPV in situ +, p16+.

CLEAR CELL ADENOCARCINOMA

This is another uncommon tumour, constitutes about 2-4%, age of occurrence shows bimodal pattern. Younger age group presentation is usually associated with intra uterine diethylstilboestrol consumption, whereas old age presentation is not associated with DES exposure. Microscopically the tumour composed of clear polygonal cells with hobnail nuclei arranged in a papillary, tubulocystic or solid pattern. Stroma may be scanty. Similar morphological features are also seen in clear cell adenocarcinoma of ovary, vagina, and endometrium.

The differential diagnosis includes mesonephric hyperplasia, aria stella change, and microglandular hyperplasia with clear cell change.

SEROUS ADENOCARCINOMA

It is an uncommon tumour constitutes about 3%. The age of presentation is bimodal type with the range from 26 to 70 years; one peak is at 45 years and other at 65 years. Histologically, malignant cells are arranged in a complex papillary pattern with cellular stratification, budding and tufting. There are gland formation in solid areas and slit like spaces are also seen. The cells show moderate to marked nuclear atypia with increased mitotic figures, psammoma bodies is the characteristic feature noted. Histologic feature resembles the serous papillary ovarian, endometrial and peritoneal tumour. This tumour is very aggressive and have very poor prognosis.

MESONEPHRIC ADENOCARCINOMA

It arises from the mesonephric remnants; most common site is lateral wall of cervix. This is a rare tumour with peak age of incidence is 52 years with a range of 34 to 84 years. Grossly it present as exophytic lesions with circumferential involvement

and the tumour size range from 2 to 8 cm. Microscopically, pattern of arrangement of malignant cells in this adenocarcinoma varies which includes retiform, solid, tubular, ductal and sex cord like. Most common pattern is tubular in which glands are lined by cuboidal epithelium with mucin free and the lumen is filled with eosinophilic hyaline material.

Differential diagnosis includes Endometroid variant adenocarcinoma of cervix, endometrial adenocarcinoma and diffuse mesonephric hyperplasia.

IHC- pancytokeratin+,CK7+, CAM 5.2+, EMA+, calretinin+ and vimentin+. CK20 and monoclonal CEA negative.

4.13 NEUROENDOCRINE TUMOURS[94, 95, 96]

WHO classifies neuroendocrine tumours into four type's such as carcinoid, atypical carcinoid, small cell neuroendocrine carcinoma and large cell neuroendocrine carcinoma.

SMALL CELL NEUROENDOCRINE CARCINOMA

Incidence is 1-6% with age occurrence is similar to that age group of squamous cell carcinoma (21 to 87 years). Mean age is fifth decade. Most common cause is HPV 16 and 18. Grossly, the tumour is large ulcerating mass extend up to the parametrium, vagina and uterus. Microscopically, tumour is highly cellular composed of solid sheets, trabeculae, single cells and ill-defined or with sharply outlined nests. Small rosette-like or acini areas are seen. Individual malignant cells are oval to spindle in shape with scant cytoplasm. Nucleus is hyperchromatic, moulded with salt and pepper chromatin and inconspicuous nucleoli. Mitotic figures are high up to >50/ hpf. Adjacent foci of precancerous and cancerous lesion of both squamous and glandular type may be

present. The tumours typically have a delicate fibrovascular stroma. The tumour is aggressive and has a high propensity for metastasis.

The cells are argyropilic and show immunoreactivity for Neuron specific enolase.

Differential diagnosis includes small cell squamous cell carcinoma, lymphoma, and adenoid basal carcinoma.

Classification of endocrine tumours other than small cell carcinoma of the uterine cervix based on mitoses, nuclear atypia and necrosis

Typical carcinoid tumour- rare, 0-1+ and necrosis.

Atypical carcinoid tumour-=< 10 MFs/10hpf, 1+-2+ and focal.

Large Cell Neuroendocrine carcinoma - >10 MFs/10hpf, 2+-3+ and geographic necrosis.

LARGE CELL NEUROENDOCRINE ADENOCARCINOMA

This is a rare carcinoma, less common than small cell carcinoma. Age of occurrence is wide range with mean age of 34 years. Grossly, tumour is large and more deeply invasive than other endocrine tumour. Microscopically, the pattern of malignant cells arrangement is insular, trabecular and solid with areas of geographic necrosis. Individual tumour cells are medium to large size with abundant cytoplasm containing eosinophilic granules, nucleus shows high grade and high mitotic figures >20/hpf. Single cell infiltration is absent in this tumour. Adenocarcinomatous differentiation is common.

Differential diagnosis includes poorly differentiated squamous cell carcinoma.

Argyrophilia and immunoreactivity for chromogranin are present.

4.14 OTHER EPITHELIAL TUMOURS[18, 94, 95, 96]

ADENOSQUAMOUS CARCINOMA

It is defined as tumour composed of mixture of squamous and glandular elements recognizable on H&E slides. Thus it should be differentiated from endometrioid adenocarcinoma which contains benign squamous differentiation. Signet ring cell type and clear cell type variant is also seen.

Differential diagnosis includes scattered mucin containing cells in poorly differentiated squamous cell carcinoma.

GLASSY CELL CARCINOMA VARIANT

This rare tumour is a poorly differentiated adenosquamous cell carcinoma which accounts 1-2%. It occurs in the age group younger (30-44) than other tumour occurrence. Grossly, the tumour is bulky and exophytic mass measures 3 -7cm. microscopically, malignant neoplasm composed of sheets and nest of malignant cells. Individual cells are large with abundant eosinophilic cytoplasm which is ground glass appearance. The cell border is distinct. The nucleus is large with macro nucleoli and prominent mitotic figures. The stroma has plasma cell and eosinophil inflammatory infiltrate. It is highly aggressive tumour and unresponsive to radiotherapy.

Differential diagnosis includes poorly differentiated squamous cell carcinoma and Lymphoepithelioma like carcinoma.

IHC- low and high molecular weight keratin +, MUC-1& MUC-2+ and ER & PR negative.

ADENOID CYSTIC CARCINOMA

Adenoid cystic carcinoma of cervix has similar histological features of salivary gland tumours. It occurs in the postmenopausal females with mean age of 60 years particularly in black women. The cell of origin is reserve cell in the cervix. Grossly, the tumour is either small polypoid growth to large exophytic/endophytic friable mass. Microscopically, malignant neoplasms composed of cribriform pattern of nest and cords of small basaloid cells with scant cytoplasm. Unlike salivary gland, the neoplasms in cervix shows marked nuclear atypia, high mitotic activity and necrosis. The cystic space is filled with hyaline eosinophilic secretion or basophilic mucin. The hyalinised stroma situated within the tumour nest contains basement membrane material positive for PAS. Presence of squamous intraepithelial neoplasia above the tumour nest is frequently observed. This tumour is aggressive and got poor prognosis.

Differential diagnosis includes small cell Nonkeratinizing squamous cell carcinoma, small cell neuroendocrine carcinoma and adenoid basal carcinoma.

IHC- MNF116, CAM 5.2 and EMA markers are positive.

ADENOID BASAL CARCINOMA

This is a rare neoplasm; occur in the post-menopausal females with the range 19-91 years. The HPV DNA most commonly encountered here is HPV16 strain. Since adenoid basal carcinoma and adenoid cystic carcinoma has similar histiogenesis, microscopic features and epidemiology, it is difficult to differentiate between them. The main differentiating features are absence of hyaline basement material; mitosis and nuclear atypia are rare; it is less aggressive and never metastasized to lymph node. Microscopically, malignant neoplasms composed of nest of uniform basaloid cell with

peripheral palisading with no stromal response. It is associated with cervical intraepithelial neoplasms and micro invasive squamous cell carcinoma with focal squamous cell or glandular differentiation. Thus, it is named as 'Epithelioma' due to its benign nature and good prognosis.

Differential diagnosis includes Adenoid cystic carcinoma, Basaloid squamous cell carcinoma and adenoid basal hyperplasia.

IHC – CAM 5.2, CK7, EMA, CEA, p53 and p63 marker shows positivity.

4.15 MESENCHYMAL TUMOURS

This tumour incidence is very less common. Both benign and malignant tumours are noted in which smooth muscle tumours are the most common.

BENIGN TUMOURS

Leiomyoma is the most common tumour and constitute <2%. The microscopic feature is similar to that of uterine leiomyoma.

Genital rhabdomyoma presents as polypoid lesions, microscopic features shows rhabdomyoblast with benign nature which is dispersed in the edematous and myxoid stroma. Cambium layer is absent.

Other benign tumours like haemangioma, schwannoma, granular cell tumour, lipoma, glomus tumour, localised neurofibromatosis, ganglioneuroma and paragangiloma have also been reported.

MALIGNANT TUMOURS

Leiomyosarcoma present as polypoid growth with soft, fleshy consistency. Microscopically, it is composed of interlacing bundles of smooth muscle fibres with marked atypia and necrosis.

Endometrioid stromal sarcoma, low grade is a rare tumour arising from outside the uterus and histologic features are similar to that of uterus neoplasm.

Undifferentiated endocervical sarcoma histologically composed of malignant cells with ill-defined cell borders. Individual cells are spindle to stellate shape arranged in a fasciculate or storiform pattern. The nucleus is hyperchromatic.

Sarcoma botryoides has been associated with sertoli-leydig cell tumour which composed of individual cells with spindle, oval, small nuclei with some foci show differentiation to skeletal muscles.

Alveolar soft part sarcoma present as polyp, histologic features shows individual cells with large epithelial like cell with eosinophilic, granular cytoplasm arranged in the solid or alveolar pattern. It has the better prognosis than other site.

Other tumour includes malignant peripheral nerve sheath tumour, liposarcoma, osteosarcoma and malignant fibrous histiocytoma.

4.16 MALIGNANT MIXED EPITHELIAL MESENCHYMAL TUMOURS

Carcinosarcoma is rare neoplasms composed of both malignant epithelial component (squamous cell, adenoid basal carcinoma and adenoid cystic carcinoma) and homologous mesenchymal component. Most common strain of HPV isolated here

is HPV 16. Age of occurrence is in postmenopausal (61years) age group. This is a very aggressive tumour.

Other malignant tumour like adenosarcoma, Wilms tumour is also reported.

Benign neoplasms like adenofibroma, adenomyoma with variant have also being reported.

HAEMATOPOIETIC LESIONS like lymphoma and leukaemia involving the cervix is rare.

MELANOCYTIC LESIONS including both benign lesion like blue naevus and malignancy like melanoma is also reported.

4.17 MISCELLANEOUS TUMOURS AND METASTASES

Germ cell tumours like dermoid cyst, yolk sac tumour have also been reported. Other rare tumours like Ewing sarcoma and trophoblastic tumour incidence have also been reported.

Metastases from breast, ovary, peritoneal and pancreas are also reported.

4.18 STAGING OF CERVICAL NEOPLASMS (ANNEXURE V)

4.19 SCREENING OF CERVICAL CARCINOMA

Most of the cervical carcinoma (around eighty percent) are identified in later stage leading to decreased survival rates (5yr survival< forty percent).[33,34]

The important aim of screening is to decrease the incidence and mortality of cervical carcinoma. Identifying pre-neoplastic stage early is an effective measure of reducing mortality due to carcinoma cervix.

Methods of screening

Though there are different methods of screening, the most effective has been Pap smear.[35]In 1939, Papanicolaou and Herbert Traut initiated systematic evaluation of vaginal smears and it became apparent that abnormal cells could be found in several of the asymptomatic patients.[36]

The other methods used for screening are as follows[35]

- Unaided visual inspection
- Visual inspection after application of acetic acid (VIA)
- VIA with magnification
- Visual inspection after application of Lugol's iodine (VILI)
- HPV DNA testing screening

Screening plays an important role in early detection of cervical neoplasm. Pap smear helps in early detection of premalignant and early cervical carcinoma. But it has false negative rate of about fifteen to fifty percent and false positivity rate of about thirty percent[25]

Methods involving visual inspection

VIA -visual inspection with acetic acid (white vinegar) -involves the application of 4-5% acetic acid on the cervix and the test result is based on the colour and margin of the aceto-white epithelium, the surface contour, the arrangement of the blood vessels.

VILI (Visual inspection with lugol's iodine) - Cervical neoplasia fails to stain deeply with iodine due to the lack of glycogen.

- It is cheap and simple alternative to Pap smear.
- Both this technique help to identify precancerous lesions by naked eye
- There is no need for separate lab and can be used in a simple clinic setup.
- Any trained medical and paramedical persons can be trained to perform this test.

Dr.Shastri and colleagues initiated a randomized control trial on usage of VIA on large scale. They found that it decreased the mortality to eleven deaths per one lakh women years of observation. It amounts to thirty one percent reductions in comparison with the control group.

Colposcopy

The colposcopy is a non-invasive binocular instrument designed to examine the cervix with 6 to 40-fold magnification. Most cervical neoplasia arises in the transformation zone, hence the relevant colposcopic signs are observed within its limits. [28]

Abnormal colposcopic findings include

- white keratotic lesions apparent before the application of acetic acid , termed "leukoplakia"
- aceto-white epithelium,
- punctuation,
- mosaic pattern,
- Atypical tortuous vessels.

A variation in quality and quantity of the above atypical appearances helps to differentiate cervical neoplasia from physiological, benign, infective, inflammatory and reactive changes in the cervix. Colposcopy and histopathology are complementary to the diagnosis and management of CIN.[18]

p16INK4A

$p16^{INK4a}$ is a promising biomarker for cervical neoplasm screening. Murphy et al reported a sensitivity of ninety nine percent and specificity of hundred percent.[25] Recent recommendations from ACS and ASCP also included HPV and its biomarker as an important screening tool. It is given as grade A recommendation.

4.20 PROGNOSTIC MARKERS

The prognosis of cervical carcinoma is related to the following parameters: [9]

1. Clinical stage: is the most important prognostic determinator.

2. Nodal status: is a crucial predictor found to be an independent prognostic marker.

3. Size of the largest involved node and number of positive nodes.

4. Size of the primary tumour as determined by measurement of the tumour's greatest diameter or by volumetric techniques.

5. Depth of invasion.

6. Endometrial extension: decreases the survival rate by a factor of 10-20%.

7. Parametrial involvement detected microscopically.

8. Blood vessel invasion.

9. Microscopic grade: Whether the degree of tumour differentiation as evaluated in routinely stained sections correlated with survival independently from staging is a controversial issue. The two types of grading used is Reagan-Ng and Broder's method.

10. Microscopic type: Some authors have found a better prognosis with large cell non-keratinizing type and a worse prognosis with the small cell type but others found no correlation.

11. Tumour associated tissue eosinophilia (TATE): Presence of numerous mature eosinophils in the inflammatory infiltrate of cervical carcinoma has been associated with improved survival in one study and poor survival in other.

12. Keratin profile: No predictive value seems to be attached.

13. Cell proliferation index: High S-phase rates as determined by flow cytometry are correlated with both a poorly differentiated histologic type and decreased short term survival.

14. Angiogenesis: There is no evidence of a correlation between micro vessel density and prognosis.

15. HPV: It has been claimed that HPV is a major determinant of the course of cervical cancer.

16. Others: Stromal infiltration by S-100 protein positive Langerhans cells, allelic loss of chromosome 1, expression of HER2/neu, RAS oncogene and Tn antigen have all been found to related to an unfavourable outcome.

4.21 BIOMARKERS

Different classes of biomarkers are categorized at the molecular level which has a promising effect in diagnosing carcinoma of cervix. The markers are

- Apoptotic markers
- Epigenetic regulations-imaging marker, methylation and metabolic markers

- Cell cycle check points
- Angiogenetic parameters
- Chromosomal anomalies
- Tumour suppressor gene expression

p16^{INK4a}

p16^{INK4A} gene is situated in the 9p21chromosome which transcript the cell-cycle inhibitor protein .i.e.CDK 4 and 6 [24]. It is a negative regulatory protein and product of CDKN2A gene. Serrano et al reported that, it regulates the progression of eukaryotic cells through the G1 phase of the cell cycle.[16]

In normal quiescent cell, retinoblastoma protein is in active state (hypophosphorylated form) and it is bound to E2F (transcription factors) thus preventing it to regulate the progression of cell cycle. In bounded form Rb is a negative regulator of p16. p16 prevents the Rb protein phosphorylation by inhibiting the cyclin dependant kinase 4 and 6 and keeping it in active form.

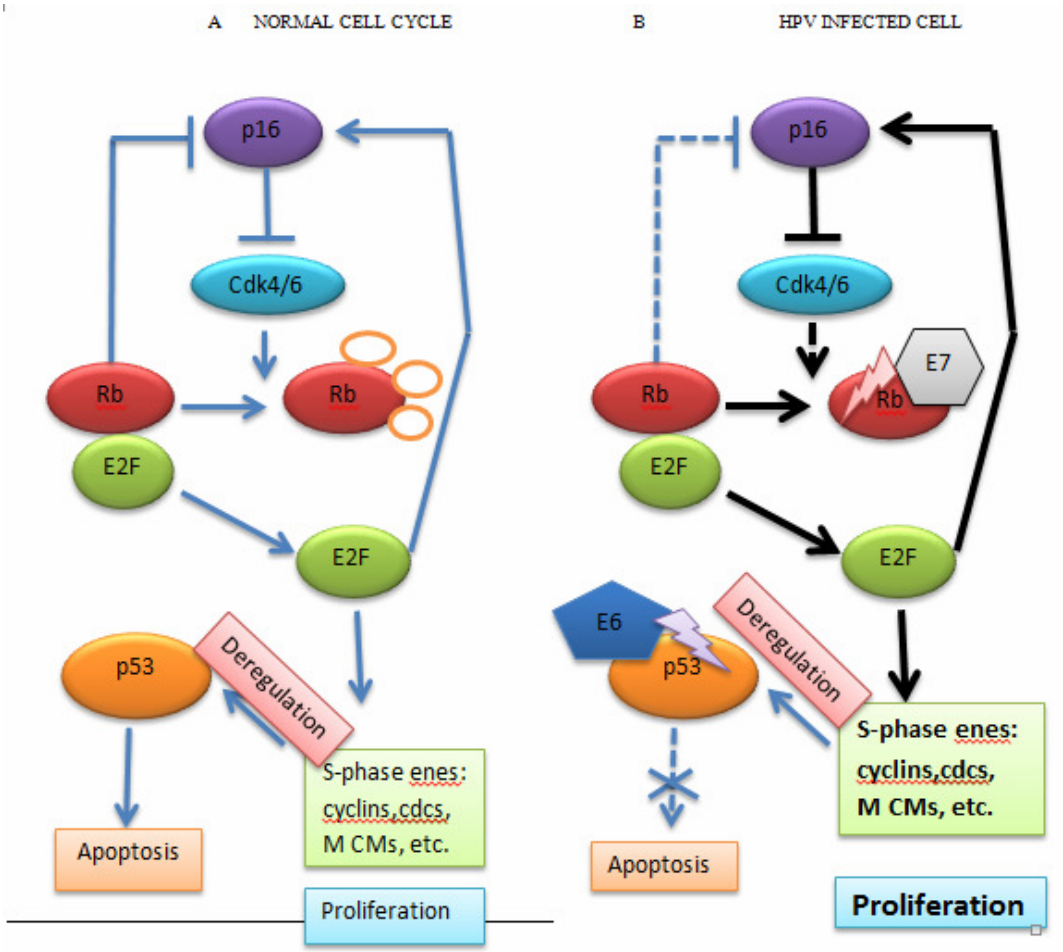
In HR-HPV infected cell, the viral transcription factors gets bind to Rb protein to make it inactive form. So Rb protein cannot able to inhibit the transcription of p16 tumour suppressor protein. Thus p16 is over expressed in dysplastic cells not in the normal epithelium.

Identification of biomarker p16 by IHC in dysplastic cells is an ongoing study all over the world to reduce the interobserver variations and to improve the reproducibility in diagnosing fields[16, 24].

The results of hr-HPV genotyping were compared to the expression levels of p16INK4a mRNA. It was determined that all HR-HPV types cause the increased

p16INK4a mRNA expression; however, the correlation between the particular HR-HPV type and the p16INK4a mRNA level is obscure. This suggests that all HR-HPV types may dysregulate the cell cycle by a similar molecular mechanism. Therefore, it can be concluded that the oncogenic potential of HPV but not a particular HR-HPV type is the main cause of enhanced p16INK4a mRNA expression indicating the dysregulation of cell cycle and leading to high-grade cervical lesions.[23]

Comparative study model of cell cycle mechanism both in normal cell and Hpv infected cell



Ki67/MIB-1

A Dysplastic cell usually shows increased cell cycling. Ki67 and MIB-1 are markers of proliferation; they are strongly expressed in CIN lesions and normal basal cells that have proliferation capacity.

MYC

This oncogene is frequently amplified and over expressed in cervical cancer.

Survivin

Survivin is an another independent marker of high-risk HPV type which is an anti-apoptotic marker. It might be an early predictor of cervical carcinogenesis.

Phosphatase and tensine homologue (PTEN)

This anti-oncogene is negatively correlated with survivin expression in CIN and cervical cancer.

Telomerase

In cervical dysplasia particularly in CIN 3, it was found that there is an increased telomerase activity with varying sensitivity.

The mini chromosome maintenance (MCM)

This is a class of proteins of the DNA pre-replication complex. Early publications have shown that the MCM proteins and in particular MCM5 are useful for detection of cervical diseases.

Topoisomerase II-α (TOP2A)

Key proteins like MCM and TOP2A are expressed during this aberrant gene transcriptional activation which is over expressed in cervical diseases.

ProExC

It is an anti-topoisomerase II alpha antibody mainly used to distinguish squamous metaplasia and dysplasia. Thus it mainly helps in cytological smears.

Aneuploidy, chromosomal imbalances

Many studies have found that, with the integration of HPV genome with the human gene will lead to chromosomal imbalances mainly on the 3q chromosome, which is usually seen in the higher grades of CIN and also in transition to invasive carcinoma.

p53 and pRB

These are the targets of HPV oncogenes E6 and E7. Loss of p53 and pRB represents an indirect sign of HPV E6 and E7 expression. This loss is not specific for HPV and is present also in other tumours. In HPV infection, there is an inverse relationship with progression and frequency of pRB and p53

Cyclins

Are regulatory proteins involved in cell cycle regulation. A strong association was found between Cyclin E and HPV induced cell changes. Cyclins A and B are over expressed in cervical adenocarcinoma and its malignant precursors.

Angiogenetic markers

Obermair et al. demonstrated the angiogenetic parameters such as ‘micro vessel density and the expression of vascular endothelial growth factor (VEGF)’ increase with

the grade of CIN, Suggesting that angiogenesis might be used as a prognostic factor in patients with squamous cell carcinoma.

nm23-H1

It is a human antimetastatic gene. In carcinoma of cervix it is found that the levels are inversely related to cervical neoplasm grade.

4.22 IHC-REVIEW

Immunohistochemistry (IHC) is a technique which identifies cellular proteins (antigen) by its ability to bind monoclonal antibody. The importance gained by this method is that it identifies the reaction by light microscope. Though Coons introduced it before 70 years, it is widely used in the past twenty years only. The enzymatic label (horseradish peroxidase) developed by Avrameas and by Nakane and colleagues helps in identifying the labelled antibody by microscope itself.[17]

In Oxford, Taylor and Burns in 1974 made IHC more useful by making it possible in normal paraffin embedded tissue. With this, the issue of greater sensitivity was raised and subsequently solved by series of development of enzymes and labelling systems. Now it becomes a routine to do one or more IHC stains for tumour diagnosis.

Enzyme digestion was introduced by Hung as a pre-treatment to IHC staining to unmask some antigens that had been altered by formalin fixation. Enzyme digestion was followed by invention of antigen retrieval method (AR). In contrast to enzyme digestion, the AR technique is a simple method. The intensity of IHC staining was increased dramatically after AR pre-treatment.

$p16^{INK4a}$ is a useful IHC marker with high sensitivity and specificity for cervical carcinoma diagnosis.

S.NO	OP NO	HP NO	AGE	WHO CLASSIFICATION	GRADE OF DIFFERENTIATION
1	158440	3/13	45	LCNKSCC	GRADE 2
2	158427	5/13	55	LCNKSCC	GRADE 2
3	158351	11/13	59	CIN 1	NA
4	131213	12/13	45	LCNKSCC	GRADE 2
5	144762	15/13	70	SCNKSCC	GRADE 3
6	144967	20/13	26	CIN 1	NA
7	144057	22/13	60	LCKSCC	GRADE 1
8	144775	23/13	55	LCKSCC	GRADE 1
9	243983	29/13	70	LCKSCC	GRADE 1
10	1447121	34/13	73	SCNKSCC	GRADE 3
11	152439	37/13	70	LCNKSCC	GRADE 2
12	131164	38/13	55	LCKSCC	GRADE 1
13	1847	41/13	55	CIN 1	NA
14	134633	44/13	30	CIN 1	NA
15	2233	50/13	65	LCNKSCC	GRADE 2
16	2239	52/13	50	LCKSCC	GRADE 1
17	2006	54/13	30	CIN 3	NA
18	2040	59/13	23	CIN 1	NA
19	14934	61/13	46	CIN 2	NA
20	149063	63/13	38	CIN 1	NA
21	1834	66/13	30	CIN 2	NA
22	512201	71/13	35	CIN 1	NA
23	7290	80/13	60	ADENO SCC	
24	7298	82/13	47	LCNKSCC	GRADE 2
25	7300	85/13	50	CIN 1	NA
26	176631	94/13	45	LCKSCC	GRADE 1
27	737	95/13	60	CIN 2	NA
28	176626	96/13	40	LCNKSCC	GRADE 2
29	281	97/13	55	LCNKSCC	GRADE 2
30	246665	98/13	48	CIN 1	NA
31	1819	102/13	50	LCNKSCC	GRADE 2
32	2474	104/13	50	ADENO SCC	
33	18294	115/13	60	LCNKSCC	GRADE 2
34	246664	117/13	47	LCKSCC	GRADE 1
35	2488	118/13	32	CIN 1	NA
36	19320	120/13	45	CIN 2	NA
37	18557	126/13	67	SCC WITH MICROINVASION	
38	140144	129/13	55	LCNKSCC	GRADE 2
39	1853	144/13	38	CIN 1	NA
40	19366	150/13	60	LCNKSCC	GRADE 2
41	19432	151/13	40	SCNKSCC	GRADE 3
42	2317	158/13	42	CIN 2	NA

43	23131	160/13	60	LCKSCC	GRADE 1
44	25288	162/13	43	ADENO SCC	
45	23406	171/13	68	SCNKSCC	GRADE 3
46	23427	172/13	39	CIN 1	NA
47	24142	176/13	55	ADENOID BASAL CARCINOMA	
48	24213	183/13	37	CIN 3	NA
49	24484	190/13	30	CIN 1	NA
50	24284	195/13	65	LCNKSCC	GRADE 2
51	24285	198/13	51	LCNKSCC	GRADE 2
52	19554	201/13	45	LCNKSCC	GRADE 2
53	19638	202/13	26	CIN 1	NA
54	28474	214/13	55	LCNKSCC	GRADE 2
55	23354	215/13	45	LCNKSCC	GRADE 2
56	28286	216/13	55	CIN 1	NA
57	10180	218/13	38	LCNKSCC	GRADE 2
58	28331	219/13	60	LCNKSCC	GRADE 2
59	28210	220/13	34	CIN 1	NA
60	24933	221/13	43	CIN 1	NA
61	28212	223/13	53	LCNKSCC	GRADE 2
62	1437668	224/13	60	LCNKSCC	GRADE 2
63	28177	225/13	50	LCNKSCC	GRADE 2
64	201844	227/13	27	CIN 2	NA
65	19941	228/13	75	LCNKSCC	GRADE 2
66	19999	229/13	30	CIN 1	NA
67	19917	235/13	38	CIN 2	NA
68	19838	236/13	37	LCNKSCC	GRADE 2
69	18985	241/13	40	CIN 2	NA
70	28107	242/13	45	CIN 3	NA
71	28112	245/13	28	CIN 1	NA
72	19841	247/13	51	CIN 1	NA
73	222606	249/13	40	CIN 1	NA
74	18273	258/13	47	LCNKSCC	GRADE 2
75	28682	261/13	55	LCNKSCC	GRADE 2
76	28924	268/13	38	LCNKSCC	GRADE 2
77	28904	273/13	58	LCNKSCC	GRADE 2
78	28121	275/13	55	LCNKSCC	GRADE 2
79	258181	276/13	65	LCNKSCC	GRADE 2
80	2886	277/13	44	LCNKSCC	GRADE 2
81	28899	278/13	34	CIN 2	NA
82	27558	287/13	45	LCNKSCC	GRADE 2
83	24158	288/13	70	LCNKSCC	GRADE 2
84	78889	289/13	50	CIN 2	NA
85	27603	296/13	40	LCNKSCC	GRADE 2
86	1439751	297/13	55	LCKSCC	GRADE 1
87	27657	303/13	35	CIN 3	NA
88	27902	318/13	37	LCKSCC	GRADE 1

89	41085	319/13	55	LCNKSCC	GRADE 2
90	24156	321/13	45	SCNKSCC	GRADE 3
91	7433	324/13	33	CIN 1	NA
92	4114	326/13	29	CIN 1	NA
93	41100	328/13	37	CIN 1	NA
94	41183	329/13	55	CIN 3	NA
95	41111	331/13	38	CIN 1	NA
96	41091	334/13	45	CIN 1	NA
97	41245	337/13	65	LCKSCC	GRADE 1
98	1276	338/13	40	CIN 3	NA
99	412334	343/13	45	CIN 1	NA
100	23009	346/13	55	LCNKSCC	GRADE 2
101	41231	348/13	30	CIN 1	NA
102	48011	357/13	37	CIN 3	NA
103	48059	358/13	27	CIN 1	NA
104	48101	364/13	26	CIN 1	NA
105	48056	369/13	48	LCKSCC	GRADE 1
106	251626	374/13	32	LCNKSCC	GRADE 2
107	481044	380/13	50	LCNKSCC	GRADE 2
108	481019	381/13	25	CIN 1	NA
109	28532	387/13	55	CIN 1	NA
110	28891	396/13	47	LCKSCC	GRADE 1
111	48181	397/13	40	LCNKSCC	GRADE 2
112	48058	403/13	45	CIN 1	NA
113	252133	407/13	65	LCNKSCC	GRADE 2
114	44550	410/13	71	CIN 1	NA
115	48236	412/13	36	CIN 1	NA
116	44823	431/13	40	CIN 1	NA
117	21250	436/13	49	CIN 1	NA
118	44798	439/13	47	LCNKSCC	GRADE 2
119	44661	445/13	70	CIN 1	NA
120	44673	447/13	37	LCNKSCC	GRADE 2
121	44601	452/13	45	LCNKSCC	GRADE 2
122	252805	455/13	48	CIN 2	NA
123	252179	457/13	60	LCNKSCC	GRADE 2
124	54310	461/13	32	CIN 3	NA
125	44862	473/13	45	CIN 2	NA
126	44954	483/13	62	CLEARCELL AC	
127	54423	488/13	35	LCNKSCC	GRADE 2
128	54371	489/13	50	ADENO SCC	
129	54431	491/13	45	LCKSCC	GRADE 1
130	54458	494/13	30	CIN 1	NA
131	41286	496/13	45	CIN 1	NA
132	61551	499/13	65	LCNKSCC	GRADE 2
133	61519	502/13	60	CIN 1	NA
134	59200	508/13	27	CIN 1	NA

135	59056	513/13	50	LCKSCC	GRADE 1
136	61763	524/13	50	LCKSCC	GRADE 1
137	61770	526/13	35	CIN 1	NA
138	2065	535/13	40	SCC WITH MICROINVASION	
139	61822	539/13	32	CIN 1	NA
140	61940	540/13	65	LCNKSCC	GRADE 2
141	61894	541/13	38	CIN 1	NA
142	41134	546/13	28	LCNKSCC	GRADE 2
143	41267	548/13	70	LCNKSCC	GRADE 2
144	62365	555/13	50	CIN 1	NA
145	62337	556/13	39	CIN 1	NA
146	62332	557/13	45	CIN 1	NA
147	61597	562/13	35	CIN 1	NA
148	61602	566/13	46	CIN 1	NA
149	61600	567/13	27	CIN 2	NA
150	54291	571/13	55	LCNKSCC	GRADE 2
151	254988	576/13	60	LCNKSCC	GRADE 2
152	68171	592/13	50	CIN 1	NA
153	68441	594/13	62	LCNKSCC	GRADE 2
154	23087	601/13	60	CIN 1	NA
155	68457	605/13	45	LCNKSCC	GRADE 2
156	41139	612/13	28	LCNKSCC	GRADE 2
157	164678	621/13	45	CIN 1	NA
158	68715	622/13	44	CIN 1	NA
159	68676	623/13	50	LCNKSCC	GRADE 2
160	68677	624/13	50	SCNKSCC	GRADE 3
161	68754	625/13	62	VILLOGLANDULAR AC	
162	68795	631/13	40	CIN 1	NA
163	68962	640/13	48	CIN 1	NA
164	68975	641/13	50	LCNKSCC	GRADE 2
165	68972	642/13	55	LCNKSCC	GRADE 2
166	73599	656/13	60	LCNKSCC	GRADE 2
167	79530	658/13	77	LCNKSCC	GRADE 2
168	79660	666/13	40	CIN 1	NA
169	79650	667/13	55	LCNKSCC	GRADE 2
170	79680	682/13	60	LCNKSCC	GRADE 2
171	1129	691/13	37	CIN 2	NA
172	81648	694/13	38	LCNKSCC	GRADE 2
173	81678	697/13	65	LCNKSCC	GRADE 2
174	1902803	701/13	50	LCNKSCC	GRADE 2
175	81586	706/13	37	CIN 1	NA
176	79928	714/13	45	SCNKSCC	GRADE 3
177	79924	716/13	45	CIN 1	NA
178	81828	723/13	27	CIN 1	NA

179	68630	730/13	70	LCKSCC	GRADE 1
180	2541532	741/13	50	SCC WITH MICROINVASION	
181	81992	758/13	31	CIN 1	NA
182	88079	765/13	30	CIN 1	NA
183	68846	766/13	50	CIN 2	NA
184	88527	769/13	30	CIN 1	NA
185	88154	777/13	45	SQUAMOTRANSITIONAL SCC	
186	88257	778/13	70	LCNKSCC	GRADE 2
187	88258	779/13	32	CIN 1	NA
188	81710	780/13	55	LCNKSCC	GRADE 2
189	88423	792/13	42	CIN 2	NA
190	82532	800/13	31	CIN 1	NA
191	82573	807/13	40	CIN 1	NA
192	82829	810/13	37	CIN 1	NA
193	32622	814/13	50	CIN 1	NA
194	82562	815/13	41	LCNKSCC	GRADE 2
195	82865	828/13	36	CIN 1	NA
196	82862	835/13	25	CIN 1	NA
197	82836	837/13	40	LCKSCC	GRADE 1
198	83147	859/13	50	LCNKSCC	GRADE 2
199	83059	862/13	38	CIN 1	NA
200	83051	864/13	60	LCNKSCC	GRADE 2
201	83028	865/13	60	LCNKSCC	GRADE 2
202	83106	876/13	60	LCNKSCC	GRADE 2
203	83252	885/13	65	LCNKSCC	GRADE 2
204	83445	891/13	35	LCNKSCC	GRADE 2
205	259784	893/13	50	LCNKSCC	GRADE 2
206	87733	897/13	60	LCKSCC	GRADE 1
207	87539	899/13	57	LCKSCC	GRADE 1
208	88323	908/13	39	CIN 1	NA
209	87533	913/13	42	LCKSCC	GRADE 1
210	87731	918/13	40	CIN 1	NA
211	4480	919/13	45	CIN 3	NA
212	87738	924/13	37	CIN 1	NA
213	78555	930/13	26	LCKSCC	GRADE 1
214	87862	931/13	50	LCNKSCC	GRADE 2
215	87653	938/13	40	CIN 1	NA
216	89082	943/13	60	CIN 3	NA
217	260402	956/13	50	LCKSCC	GRADE 1
218	83017	958/13	35	LCNKSCC	GRADE 2
219	87959	964/13	65	LCNKSCC	GRADE 2
220	89198	965/13	60	LCNKSCC	GRADE 2
221	89328	970/13	45	CIN 3	NA
222	87622	975/13	52	CIN 1	NA

223	87673	976/13	50	LCNKSCC	GRADE 2
224	89203	978/13	50	LCNKSCC	GRADE 2
225	88086	985/13	45	CIN 1	NA
226	88151	987/13	32	CIN 1	NA
227	88247	1002/13	55	LCNKSCC	GRADE 2
228	83248	1005/13	50	LCNKSCC	GRADE 2
229	89026	1007/13	60	LCKSCC	GRADE 1
230	88461	1013/13	45	LCNKSCC	GRADE 2
231	88493	1016/13	60	CIN 2	NA
232	99535	1025/13	30	CIN 1	NA
233	259833	1028/13	49	LCNKSCC	GRADE 2
234	89392	1029/13	40	CIN 1	NA
235	41358	1031/13	32	CIN 1	NA
236	99787	1038/13	40	LCNKSCC	GRADE 2
237	99570	1044/13	60	LCNKSCC	GRADE 2
238	99672	1045/13	60	LCNKSCC	GRADE 2
239	88177	1050/13	40	CIN 1	NA
240	99613	1052/13	40	CIN 3	NA
241	99624	1055/13	33	SCC WITH MICROINVASION	
242	88213	1059/13	46	CIN 2	NA
243	99892	1064/13	49	SCC WITH MICROINVASION	
244	99981	1065/13	42	LCNKSCC	GRADE 2
245	68643	1075/13	45	LCNKSCC	GRADE 2
246	99710	1076/13	40	CIN 1	NA
247	76123	1080/13	50	LCNKSCC	GRADE 2
248	99013	1085/13	50	LCNKSCC	GRADE 2
249	87739	1102/13	46	LCNKSCC	GRADE 2
250	82023	1105/13	50	LCNKSCC	GRADE 2
251	200836	1109/13	30	CIN 1	NA
252	280617	1111/13	32	CIN 1	NA
253	89666	1128/13	60	CIN 1	NA
254	1196	1170/13	60	LCNKSCC	GRADE 2
255	1207	1171/13	65	LCNKSCC	GRADE 2
256	1255	1172/13	60	LCNKSCC	GRADE 2
257	1234	1179/13	35	LCNKSCC	GRADE 2
258	264835	1180/13	60	LCNKSCC	GRADE 2
259	264855	1181/13	55	LCKSCC	GRADE 1
260	264321	1187/13	50	LCNKSCC	GRADE 2
261	17911	1188/13	40	CIN 1	NA
262	1916	1191/13	40	LCNKSCC	GRADE 2
263	1948	1196/13	60	LCKSCC	GRADE 1
264	1247	1201/13	32	CIN 1	NA
265	1119	1210/13	50	LCNKSCC	GRADE 2
266	6595	1219/13	35	CIN 1	NA

267	6685	1234/13	37	CIN 1	NA
268	6584	1239/13	50	LCNKSCC	GRADE 2
269	6772	1245/13	35	LCNKSCC	GRADE 2
270	6853	1255/13	48	LCNKSCC	GRADE 2
271	6838	1258/13	39	CIN 1	NA
272	78965	1268/13	45	LCKSCC	GRADE 1
273	6867	1269/13	45	CIN 1	NA
274	6993	1274/13	29	LCNKSCC	GRADE 2
275	6785	1282/13	37	CIN 3	NA
276	6987	1283/13	50	CIN 3	NA
277	17109	1295/13	40	LCNKSCC	GRADE 2
278	17226	1302/13	60	LCNKSCC	GRADE 2
279	17298	1304/13	48	LCNKSCC	GRADE 2
280	17365	1309/13	35	CIN 1	NA
281	17646	1310/13	40	LCNKSCC	GRADE 2
282	6636	1321/13	60	CIN 3	NA
283	17646	1337/13	34	CIN 1	NA
284	17385	1341/13	32	CIN 2	NA
285	17600	1352/13	60	LCNKSCC	GRADE 2
286	17765	1363/13	50	LCNKSCC	GRADE 2
287	17762	1364/13	65	LCKSCC	GRADE 1
288	17910	1371/13	70	LCNKSCC	GRADE 2
289	17915	1373/13	30	CIN 1	NA
290	17865	1381/13	60	LCNKSCC	GRADE 2
291	20758	1396/13	39	LCNKSCC	GRADE 2
292	20700	1401/13	40	LCNKSCC	GRADE 2
293	20650	1402/13	85	LCNKSCC	GRADE 2
294	264797	1405/13	40	LCKSCC	GRADE 1
295	24567	1418/13	55	LCNKSCC	GRADE 2
296	24546	1432/13	50	LCNKSCC	GRADE 2
297	267427	1438/13	34	LCNKSCC	GRADE 2
298	24717	1457/13	65	LCNKSCC	GRADE 2
299	24847	1464/13	42	LCNKSCC	GRADE 2
300	24956	1471/13	47	CIN 1	NA
301	31383	1489/13	39	CIN 2	NA
302	21379	1496/13	55	LCNKSCC	GRADE 2
303	128274	1498/13	35	CIN 1	NA
304	126753	1501/13	52	CIN 3	NA
305	31598	1504/13	33	SCC WITH MICROINVASION	
306	31632	1506/13	30	CIN 3	NA
307	31520	1515/13	31	CIN 1	NA
308	31555	1521/13	35	CIN 1	NA
309	31660	1527/13	39	CIN 3	NA
310	31784	1545/13	48	CIN 1	NA
311	31757	1546/13	31	CIN 1	NA

312	24014	1548/13	40	LCNKSCC	GRADE 2
313	24242	1559/13	40	LCNKSCC	GRADE 2
314	24111	1562/13	55	LCNKSCC	GRADE 2
315	24098	1563/13	72	LCNKSCC	GRADE 2
316	24234	1577/13	51	CIN 2	NA
317	24222	1579/13	70	LCNKSCC	GRADE 2
318	24382	1593/13	40	CIN 1	NA
319	24455	1605/13	30	CIN 1	NA
320	6902	1612/13	50	LCNKSCC	GRADE 2
321	34588	1613/13	50	LCNKSCC	GRADE 2
322	34754	1622/13	36	CIN 1	NA
323	24436	1630/13	55	LCNKSCC	GRADE 2
324	24391	1637/13	60	LCKSCC	GRADE 1
325	1407	1639/13	50	CIN 3	NA
326	34890	1648/13	65	LCNKSCC	GRADE 2
327	34857	1654/13	39	CIN 1	NA
328	34832	1658/13	30	CIN 1	NA
329	34198	1689/13	36	CIN 1	NA
330	34060	1694/13	45	LCNKSCC	GRADE 2
331	34057	1700/13	45	CIN 3	NA
332	34166	1715/13	45	CIN 3	NA
333	34388	1721/13	46	LCNKSCC	GRADE 2
334	34406	1723/13	43	LCKSCC	GRADE 1
335	34446	1724/13	30	CIN 1	NA
336	269863	1725/13	43	ENDOCERVICAL AC	GRADE 2
337	34103	1732/13	50	LCNKSCC	GRADE 2
338	38595	1735/13	39	CIN 2	NA
339	265273	1740/13	53	CIN 1	NA
340	268032	1741/13	40	CIN 1	NA
341	34222	1755/13	70	LCNKSCC	GRADE 2
342	38980	1759/13	55	LCNKSCC	GRADE 2
343	38934	1763/13	67	LCNKSCC	GRADE 2
344	38891	1765/13	55	LCNKSCC	GRADE 2
345	38993	1775/13	34	CIN 2	NA
346	43641	1776/13	55	LCNKSCC	GRADE 2
347	43633	1785/13	47	LCNKSCC	GRADE 2
348	38860	1789/13	32	CIN 2	NA
349	43830	1793/13	65	LCNKSCC	GRADE 2
350	43972	1810/13	30	LCNKSCC	GRADE 2
351	126295	1813/13	35	LCNKSCC	GRADE 2
352	44330	1821/13	35	CIN 2	NA
353	44243	1822/13	90	LCNKSCC	GRADE 2
354	1905756	1832/13	27	CIN 3	NA
355	44211	1838/13	55	LCNKSCC	GRADE 2
356	44173	1843/13	37	CIN 2	NA

357	44289	1847/13	31	CIN 3	NA
358	44493	1856/13	50	CIN 2	NA
359	44521	1858/13	35	CIN 1	NA
360	38656	1861/13	35	LCNKSCC	GRADE 2
361	1881	1872/13	47	LCNKSCC	GRADE 2
362	44887	1875/13	45	LCNKSCC	GRADE 2
363	44547	1876/13	56	LCNKSCC	GRADE 2
364	44659	1897/13	45	LCNKSCC	GRADE 2
365	44580	1899/13	40	CIN 1	NA
366	51002	1903/13	70	LCNKSCC	GRADE 2
367	44601	1907/13	55	LCNKSCC	GRADE 2
368	44754	1910/13	70	LCKSCC	GRADE 1
369	56074	1916/13	37	LCNKSCC	GRADE 2
370	50080	1920/13	34	CIN 1	NA
371	51004	1921/13	65	LCNKSCC	GRADE 2
372	50312	1928/13	44	SCNKSCC	GRADE 3
373	273730	1930/13	50	LCKSCC	GRADE 1
374	50215	1934/13	70	SCNKSCC	GRADE 3
375	50338	1936/13	55	LCNKSCC	GRADE 2
376	44617	1943/13	66	LCNKSCC	GRADE 2
377	34055	1958/13	30	CIN 1	NA
378	51148	1961/13	55	LCNKSCC	GRADE 2
379	50561	1978/13	60	LCNKSCC	GRADE 2
380	50749	2011/13	35	CIN 1	NA
381	50987	2020/13	50	LCNKSCC	GRADE 2
382	50968	2023/13	60	LCNKSCC	GRADE 2
383	50966	2041/13	60	LCKSCC	GRADE 1
384	53646	2043/13	35	LCNKSCC	GRADE 2
385	53573	2045/13	40	LCNKSCC	GRADE 2
386	53809	2060/13	50	LCNKSCC	GRADE 2
387	53026	2067/13	50	LCNKSCC	GRADE 2
388	1473659	2070/13	50	LCKSCC	GRADE 1
389	1234	2074/13	40	CIN 1	NA
390	53897	2076/13	65	LCNKSCC	GRADE 2
391	53830	2083/13	60	LCNKSCC	GRADE 2
392	53786	2086/13	30	CIN 1	NA
393	80455	2090/13	75	LCNKSCC	GRADE 2
394	136459	2097/13	40	SCNKSCC	GRADE 3
395	27228	2103/13	37	LCNKSCC	GRADE 2
396	53219	2108/13	43	LCNKSCC	GRADE 2
397	53171	2111/13	35	CIN 1	NA
398	50766	2113/13	60	LCNKSCC	GRADE 2
399	274097	2114/13	27	ADENOID SCC	
400	287288	2122/13	37	CIN 1	NA
401	53309	2126/13	65	LCKSCC	GRADE 1
402	53318	2129/13	30	CIN 1	NA

403	53322	2132/13	39	CIN 1	NA
404	53342	2135/13	45	CIN 1	NA
405	53292	2137/13	46	LCNKSCC	GRADE 2
406	56199	2151/13	38	CIN 3	NA
407	53416	2155/13	55	LCKSCC	GRADE 1
408	56409	2176/13	54	CIN 1	NA
409	56160	2189/13	45	CIN 1	NA
410	1474657	2194/13	50	LCNKSCC	GRADE 2
411	56007	2204/13	60	LCNKSCC	GRADE 2
412	56077	2211/13	53	CIN 1	NA
413	1906965	2216/13	60	SCC WITH MICROINVASION	
414	56161	2217/13	50	LCKSCC	GRADE 1
415	56211	2218/13	40	CIN 1	NA
416	56057	2220/13	24	CIN 1	NA
417	56147	2228/13	43	CIN 1	NA
418	56342	2233/13	45	SCC WITH MICROINVASION	
419	56147	2247/13	55	LCNKSCC	GRADE 2
420	277347	2254/13	55	SCNKSCC	GRADE 3
421	56449	2261/13	70	CIN 2	NA
422	56781	2275/13	37	CIN 1	NA
423	56757	2277/13	37	SCC WITH MICROINVASION	
424	56707	2281/13	65	LCNKSCC	GRADE 2
425	274398	2282/13	66	LCNKSCC	GRADE 2
426	56689	2283/13	45	LCKSCC	GRADE 1
427	56670	2284/13	60	SCC WITH MICROINVASION	
428	56649	2286/13	50	LCKSCC	GRADE 1
429	56625	2294/13	40	CIN 1	NA
430	110597	2301/13	32	CIN 1	NA
431	53487	2310/13	35	CIN 1	NA
432	531167	2311/13	50	LCNKSCC	GRADE 2
433	53118	2314/13	50	SCC WITH MICROINVASION	
434	56951	2315/13	30	CIN 1	NA
435	160864	2320/13	48	CIN 1	NA
436	57893	2322/13	38	CIN 1	NA
437	60554	2324/13	40	CIN 1	NA
438	60786	2345/13	52	CIN 1	NA
439	60817	2347/13	37	CIN 2	NA
440	60711	2353/13	35	LCNKSCC	GRADE 2
441	622183	2367/13	34	CIN 1	NA
442	62141	2369/13	35	CIN 1	NA
443	62274	2371/13	40	LCNKSCC	GRADE 2

444	62034	2372/13	43	CIN 1	NA
445	62027	2376/13	45	LCNKSCC	GRADE 2
446	62013	2379/13	45	CIN 1	NA
447	60861	2382/13	43	SCC WITH MICROINVASION	
448	60723	2383/13	29	CIN 1	NA
449	277539	2385/13	40	LCNKSCC	GRADE 2
450	62375	2396/13	37	LCNKSCC	GRADE 2
451	62401	2408/13	37	CIN 2	NA
452	111061	2416/13	30	CIN 1	NA
453	62114	2422/13	52	LCNKSCC	GRADE 2
454	65387	2429/13	75	ENDOCERVICAL AC	GRADE 3
455	65382	2432/13	65	LCKSCC	GRADE 1
456	62467	2434/13	55	LCNKSCC	GRADE 2
457	62111	2438/13	70	LCNKSCC	GRADE 2
458	65295	2443/13	60	LCNKSCC	GRADE 2
459	65307	2444/13	60	LCKSCC	GRADE 1
460	65503	2462/13	70	LCKSCC	GRADE 1
461	23037	2475/13	60	CIN 1	NA
462	65576	2476/13	60	LCKSCC	GRADE 1
463	65065	2492/13	51	LCNKSCC	GRADE 2
464	65527	2496/13	55	CGIN	NA
465	62485	2502/13	47	LCNKSCC	GRADE 2
466	241812	2506/13	57	LCNKSCC	GRADE 2
467	65590	2513/13	47	LCNKSCC	GRADE 2
468	65619	2515/13	45	CIN 1	NA
469	65752	2528/13	40	CIN 1	NA
470	54123	2537/13	46	LCNKSCC	GRADE 2
471	65853	2539/13	45	LCNKSCC	GRADE 2
472	65897	2543/13	40	CIN 2	NA
473	250580	2544/13	50	ENDOMETRIOID AC	
474	65976	2557/13	21	CIN 1	NA
475	65800	2572/13	55	CIN 2	NA
476	65682	2577/13	38	LCNKSCC	GRADE 2
477	65764	2581/13	70	LCNKSCC	GRADE 2
478	65809	2582/13	60	CIN 2	NA
479	65825	2585/13	40	LCKSCC	GRADE 1
480	65936	2603/13	58	LCNKSCC	GRADE 2
481	275949	2617/13	67	LCNKSCC	GRADE 2
482	258544	2628/13	45	LCNKSCC	GRADE 2
483	68472	2638/13	35	CIN 1	NA
484	68476	2641/13	50	CIN 1	NA
485	281198	2651/13	59	LCNKSCC	GRADE 2

486	68575	2656/13	39	CIN 1	NA
487	56401	2662/13	30	CIN 1	NA
488	68250	2663/13	35	CIN 1	NA
489	279839	2667/13	40	ENDOCERVICAL AC	GRADE 1
490	281821	2668/13	45	LCNKSCC	GRADE 2
491	74536	2670/13	62	LCNKSCC	GRADE 2
492	68879	2676/13	49	LCNKSCC	GRADE 2
493	68781	2681/13	55	LCKSCC	GRADE 1
494	68855	2684/13	40	PAPILLARY SCC	
495	28005	2687/13	44	ENDOCERVICAL AC	GRADE 3
496	283235	2697/13	57	ENDOCERVICAL AC	GRADE 2
497	74580	2698/13	60	CIN 1	NA
498	74619	2701/13	34	CIN 1	NA
499	68642	2703/13	50	CIN 1	NA
500	68693	2716/13	40	CIN 1	NA
501	65094	2717/13	50	CIN 1	NA
502	60861	2720/13	55	LCNKSCC	GRADE 2
503	74693	2721/13	55	LCNKSCC	GRADE 2
504	6867	2729/13	38	CIN 1	NA
505	68422	2730/13	50	LCNKSCC	GRADE 2
506	68720	2732/13	37	CIN 1	NA
507	74694	2734/13	45	PAPILLARY SCC	
508	74728	2735/13	32	CIN 1	NA
509	283926	2737/13	50	LCNKSCC	GRADE 2
510	65655	2739/13	48	LCNKSCC	GRADE 2
511	34366	2745/13	47	CIN 1	NA
512	65595	2747/13	65	LCKSCC	GRADE 1
513	80785	2750/13	50	BASALOID SCC	
514	80784	2751/13	38	CIN 1	NA
515	80778	2752/13	45	CIN 1	NA
516	87854	2754/13	35	CIN 2	NA
517	80700	2765/13	50	LCNKSCC	GRADE 2
518	80795	2766/13	36	CIN 1	NA
519	74518	2767/13	30	CIN 1	NA
520	80821	2772/13	38	LCNKSCC	GRADE 2
521	284131	2773/13	40	CIN 1	NA
522	80912	2781/13	56	LCNKSCC	GRADE 2
523	87079	2798/13	35	CIN 1	NA
524	156151	2804/13	67	CIN 1	NA
525	155781	2805/13	50	LCNKSCC	GRADE 2
526	87474	2811/13	42	CIN 1	NA
527	90006	2812/13	55	LCNKSCC	GRADE 2
528	87439	2816/13	70	LCNKSCC	GRADE 2

529	87401	2817/13	55	LCNKSCC	GRADE 2
530	87431	2828/13	80	LCNKSCC	GRADE 2
531	90098	2837/13	60	BASALOID SCC	
532	90102	2838/13	55	LCNKSCC	GRADE 2
533	90182	2848/13	43	LCNKSCC	GRADE 2
534	90129	2851/13	52	LCNKSCC	GRADE 2
535	27402	2854/13	53	CIN 1	NA
536	90271	2855/13	50	LCNKSCC	GRADE 2
537	90876	2857/13	30	LCNKSCC	GRADE 2
538	31554	2859/13	60	LCNKSCC	GRADE 2
539	90373	2865/13	65	LCNKSCC	GRADE 2
540	90436	2868/13	80	LCNKSCC	GRADE 2
541	90387	2871/13	60	LCNKSCC	GRADE 2
542	90727	2884/13	50	LCNKSCC	GRADE 2
543	74688	2886/13	31	CIN 1	NA
544	90807	2893/13	35	CIN 1	NA
545	90819	2896/13	50	LCNKSCC	GRADE 2
546	90657	2903/13	45	CIN 1	NA
547	90456	2904/13	55	LCKSCC	GRADE 1
548	90868	2907/13	30	CIN 1	NA
549	90914	2908/13	60	LCNKSCC	GRADE 2
550	90873	2909/13	32	CIN 1	NA
551	90871	2910/13	32	CIN 1	NA
552	90976	2915/13	45	ENDOCERVICAL AC	GRADE 2
553	1488634	2922/13	38	CIN 1	NA
554	100256	2923/13	46	CIN 1	NA
555	100293	2925/13	40	CIN 1	NA
556	90887	2927/13	41	CIN 1	NA
557	100101	2930/13	45	CIN 1	NA
558	100083	2931/13	41	CIN 2	NA
559	90730	2936/13	52	CIN 2	NA
560	100352	2937/13	45	CIN 1	NA
561	100075	2945/13	41	CIN 1	NA
562	113512	2947/13	48	CIN 1	NA
563	121470	2955/13	36	CIN 1	NA
564	87304	2961/13	48	CIN 3	NA
565	91003	2962/13	60	LCNKSCC	GRADE 2
566	287602	2971/13	43	CIN 3	NA
567	91101	2972/13	34	CIN 1	NA
568	91187	2980/13	40	CIN 1	NA
569	91267	2991/13	50	LCNKSCC	GRADE 2
570	286904	2992/13	55	LCNKSCC	GRADE 2
571	91325	2996/13	40	CIN 1	NA
572	91337	2998/13	34	CIN 1	NA
573	91297	3004/13	35	CIN 1	NA

574	91391	3005/13	38	CIN 1	NA
575	91345	3008/13	35	CIN 1	NA
576	100230	3009/13	40	CIN 1	NA
577	91287	3018/13	45	LCNKSCC	GRADE 2
578	91620	3024/13	50	LCNKSCC	GRADE 2
579	83770	3028/13	62	LCNKSCC	GRADE 2
580	91785	3039/13	37	CIN 1	NA
581	91783	3043/13	40	CIN 1	NA
582	91844	3046/13	27	CIN 1	NA
583	91897	3049/13	31	CIN 1	NA
584	91860	3052/13	65	LCNKSCC	GRADE 2
585	91949	3053/13	33	CIN 1	NA
586	91935	3058/13	50	CIN 1	NA
587	91972	3059/13	58	LCNKSCC	GRADE 2
588	100201	3066/13	35	CIN 1	NA
589	691847	3073/13	39	CIN 1	NA
590	1546	3084/13	40	CIN 3	NA
591	105002	3093/13	46	CIN 1	NA
592	287691	3100/13	45	SCC WITH MICROINVASION	
593	105024	3101/13	62	LCNKSCC	GRADE 2
594	105139	3108/13	30	CIN 1	NA
595	105094	3118/13	50	CIN 2	NA
596	105689	3124/13	68	LCKSCC	GRADE 1
597	105218	3125/13	30	CIN 1	NA
598	105369	3146/13	27	CIN 1	NA
599	105340	3149/13	60	CIN 1	NA
600	105331	3152/13	34	CIN 1	NA
601	112598	3159/13	60	LCNKSCC	GRADE 2
602	665	3160/13	48	CIN 2	NA
603	112624	3161/13	31	CIN 1	NA
604	112773	3167/13	21	CIN 1	NA
605	289690	3168/13	44	LCNKSCC	GRADE 2
606	112802	3169/13	45	PAPILLARY SCC	
607	112728	3170/13	65	LCNKSCC	GRADE 2
608	112836	3174/13	31	CIN 1	NA

IMMUNOHISTOCHEMICAL ANALYSIS OF $p16^{INK4a}$ EXPRESSIONS IN CERVICAL LESIONS

S. NO	HP .NO	AGE	WHO CLASSIFICATION	TYPE OF STAINING	PROPORTION OF POSITIVE TUMOR CELLS	INTENSITY OF STAINING	PATTERN OF STAINIG
1	P910/13	30	CNSC	NEGATIVE	-	-	-
2	P1528/13	39	CNSC	NEGATIVE	-	-	-
3	P1596/13	34	CNSC	NEGATIVE	-	-	-
4	P2055/13	30	CNSC	NEGATIVE	-	-	-
5	P2061/13	40	CNSC	CYTOPLASMIC	2	2	DIFFUSE BASAL
6	P2123/13	26	CNSC	NEGATIVE	-	-	-
7	P2133/13	27	CNSC	N:C	1	1	PATCHY
8	P2988/13	33	CNSC	NEGATIVE	-	-	-
9	P3126/13	30	CNSC	NEGATIVE	-	-	-
10	P1234/13	37	CIN 1	N:C	2	1	DIFFUSE BASAL
11	P1605/13	30	CIN 1	N:C	1	2	PATCHY
12	P1689/13	36	CIN 1	N:C	2	2	DIFFUSE BASAL
13	P1740/13	53	CIN 1	N:C	1	1	PATCHY
14	P1858/13	35	CIN 1	N:C	1	1	DIFFUSE BASAL
15	P2310/13	35	CIN 1	N:C	1	3	PATCHY
16	P2656/13	39	CIN 1	N:C	1	2	DIFFUSE BASAL
17	P2893/13	35	CIN 1	CYTOPLASMIC	2	2	DIFFUSE BASAL
18	P3005/13	38	CIN 1	CYTOPLASMIC	1	1	PATCHY
19	P3073/13	39	CIN 1	CYTOPLASMIC	2	2	DIFFUSE BASAL
20	P1341/13	32	CIN 2	N:C	2	3	DIFFUSE BASAL
21	P1489/13	39	CIN 2	N:C	1	1	PATCHY
22	P1775/13	34	CIN 2	N:C	2	3	DIFFUSE BASAL
23	P1789/13	32	CIN 2	N:C	2	2	DIFFUSE BASAL
24	P1856/13	50	CIN 2	N:C	2	2	DIFFUSE BASAL
25	P2261/13	70	CIN 2	N:C	2	2	DIFFUSE BASAL
26	P2347/13	37	CIN 2	N:C	2	1	DIFFUSE BASAL
27	P2408/13	37	CIN 2	N:C	2	3	DIFFUSE BASAL

28	P2582/13	60	CIN 2	CYTOPLASMIC	1	1	DIFFUSE BASAL
29	P3160/13	48	CIN 2	N:C	2	2	DIFFUSE BASAL
30	P183/13	37	CIN3	N:C	3	3	DIFFUSE FULL THICK
31	P970/13	45	CIN 3	N:C	2	2	DIFFUSE BASAL
32	P1283/13	50	CIN 3	CYTOPLASMIC	2	1	DIFFUSE FULL THICK
33	P1527/13	39	CIN 3	N:C	3	3	DIFFUSE FULL THICK
34	P1700/13	45	CIN 3	N:C	2	2	DIFFUSE BASAL
35	P1715/13	45	CIN 3	N:C	2	3	DIFFUSE FULL THICK
36	P1832/13	27	CIN 3	N:C	3	3	DIFFUSE BASAL
37	P1847/13	31	CIN 3	N:C	1	2	PATCHY
38	P2151/13	38	CIN 3	N:C	3	3	DIFFUSE FULL THICK
39	P2961/13	48	CIN 3	CYTOPLASMIC	2	1	DIFFUSE BASAL
40	P1055/13	33	SCC WITH MI	N:C	3	3	NA
41	P2233/13	45	SCC WITH MI	N:C	3	2	NA
42	P2277/13	37	SCC WITH MI	N:C	3	3	NA
43	P3100/13	45	SCC WITH MI	N:C	3	3	NA
44	P913/13	42	LCKSCC	N:C	3	3	NA
45	P1181/13	55	LCKSCC	CYTOPLASMIC	3	2	NA
46	P546/13	28	LCNKSCC	N:C	3	3	NA
47	P899/13	57	LCNKSCC	N:C	3	3	NA
48	P958/13	35	LCNKSCC	CYTOPLASMIC	2	2	NA
49	P1005/13	50	LCNKSCC	N:C	3	3	NA
50	P1180/13	60	LCNKSCC	N:C	3	3	NA
51	P1295/13	40	LCNKSCC	N:C	3	3	NA
52	P1310/13	40	LCNKSCC	N:C	3	3	NA
53	P1765/13	55	LCNKSCC	CYTOPLASMIC	3	3	NA
54	P1943/13	66	LCNKSCC	N:C	3	3	NA
55	P2812/13	55	LCNKSCC	N:C	3	3	NA
56	P3018/13	45	LCNKSCC	N:C	3	3	NA
57	P1928/13	44	SCNKSCC	N:C	3	2	NA

58	P1934/13	70	SCNKSCC	CYTOPLASMIC	3	2	NA
59	P162/13	43	ADENOSCC	N:C	3	3	NA
60	P2750/13	50	BASALOID SCC	N:C	3	3	NA
61	P3169/13	45	PAPILLARY SCC	N:C	3	3	NA
62	P777/13	45	ST SCC	NUCLEAR	2	2	NA
63	P2429/13	75	ENDO AC	N:C	3	3	NA
64	P483/13	62	CLEAR CELL AC	N:C	3	3	NA
65	P2544/13	50	ENDOMETRIOID AC	N:C	3	3	NA
66	P625/13	62	VILLOGLANDULAR AC	CYTOPLASMIC	3	3	NA
67	P2687/13	44	ENDO AC	N:C	3	3	NA
68	P2915/13	45	ENDO AC	N:C	3	3	NA
69	P2496/13	55	CGIN	N:C	3	3	NA

OBSERVATION AND RESULTS

The present study was carried out to assess the prevalence of uterine cervical neoplasms in patients diagnosed at TMCH during the one year period from January 2013 to December 2013.

In one year study period, a **total of 3198 cervical biopsies** were received. In which 656 cases were diagnosed as uterine cervical neoplasms. All 656 cases were stained with Haematoxylin and eosin and reviewed; interobserver variations in diagnosing the cervical intraepithelial neoplasms were noted. Histologic findings like basal cell hyperplasia, reactive atypia were downgraded; cases reported as HSIL were categorized as CIN2 and CIN3 separately during review. After review, **608 cases were diagnosed as cervical neoplasms which constitute our study sample.**

5.1 INCIDENCE

Among 3198 cervical biopsies received during the one year study period, 608 cases of uterine cervical neoplasms were reported, which constitutes **19%** of the total cervical biopsies.

The incidence of **uterine cervical carcinoma is 10.38%** and that **of cervical intraepithelial neoplasia is 8.6%.**

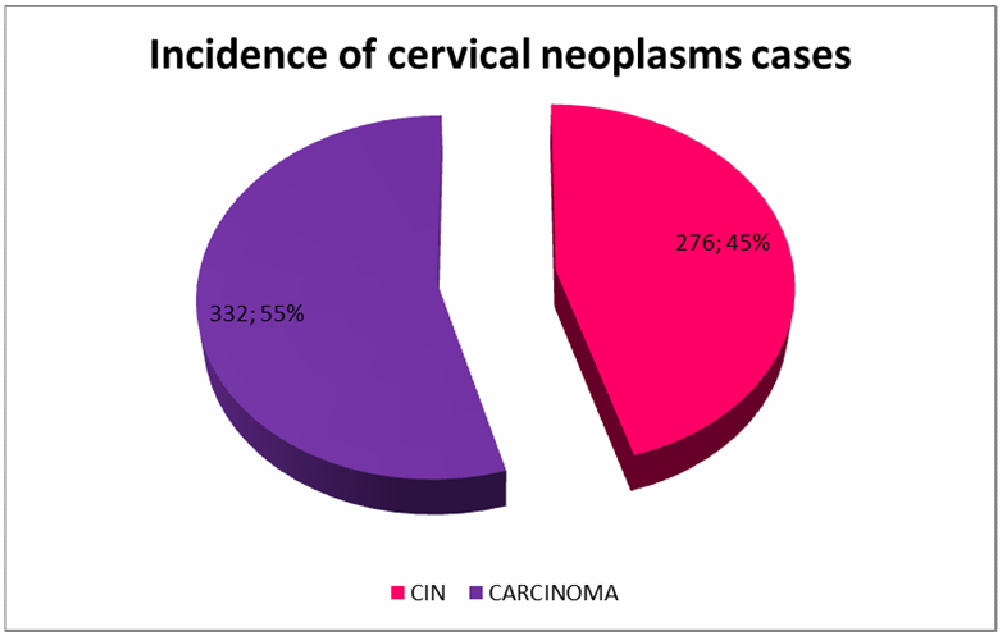
The proportion of uterine cervical carcinoma (332 cases-54.61%) and cervical intraepithelial neoplasms (276 cases-45.39%) are in the ratio of 1.22:1. (table no-4, graph no-1)

TABLE NO-4

INCIDENCE OF UTERINE CERVICAL NEOPLASMS

HISTOPATHOLOGICAL DIAGNOSIS	NO. OF CASES	PERCENTAGE
CIN	276	45.39
CARCINOMA	332	54.61
TOTAL	608	100

GRAPH NO- 1



5.2 HISTOPATHOLOGICAL EXAMINATION AND CLASSIFICATION

The tumours were typed according to the WHO classification system (ANNEXURE IV).

5.2.a CERVICAL INTRAEPITHELIAL NEOPLASIA

Cervical intraepithelial neoplasia were graded as CIN 1,2 and 3 based on the thickness of the epithelium showing loss of differentiation/ orderly maturation (basal 1/3, 1/3 to 2/3 and >2/3),distribution of mitotic figures in the epithelium with presence of koilocytic atypia in CIN 1. The histopathological pictures of CIN 1, CIN 2, CIN 3 and CGIN are depicted in figures: 5, 7, 9, 11 respectively.

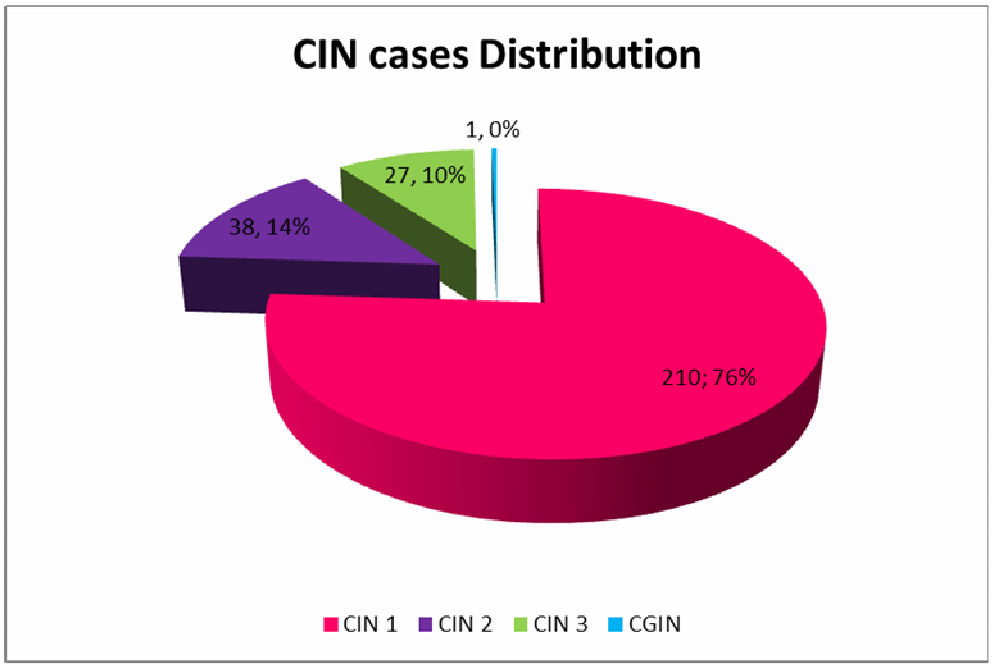
Among 276 cases, 210 cases (76%), 38 cases (14%), 27 cases (10%) and one case (0.37%) were classified as CIN1, CIN2, CIN3 and CGIN respectively. (Table no-5, graph no-2)

TABLE NO-5

DISTRIBUTION OF DIFFERENT TYPES OF CERVICAL INTRAEPITHELIAL NEOPLASMS

HISTOPATHOLOGICAL DIAGNOSIS	NO. OF CASES	PERCENTAGE
CIN 1	210	76
CIN 2	38	14
CIN 3	27	9.6
CGIN	1	0.4
TOTAL	276	100

GRAPH NO-2



5.1.b MALIGNANT LESION OF CERVIX

After analysing the data **332(55.44%) cases** of cervical carcinoma were identified.

Out of 332 cases, 318(95.79%) cases were Squamous cell carcinoma, 9(2.7%) cases were Adenocarcinoma and other epithelial tumours constitutes 4(1.2%) cases of AdenoSCC and one (0.3%) case of Adenoid Basal carcinoma. (table no-6, graph no- 3)

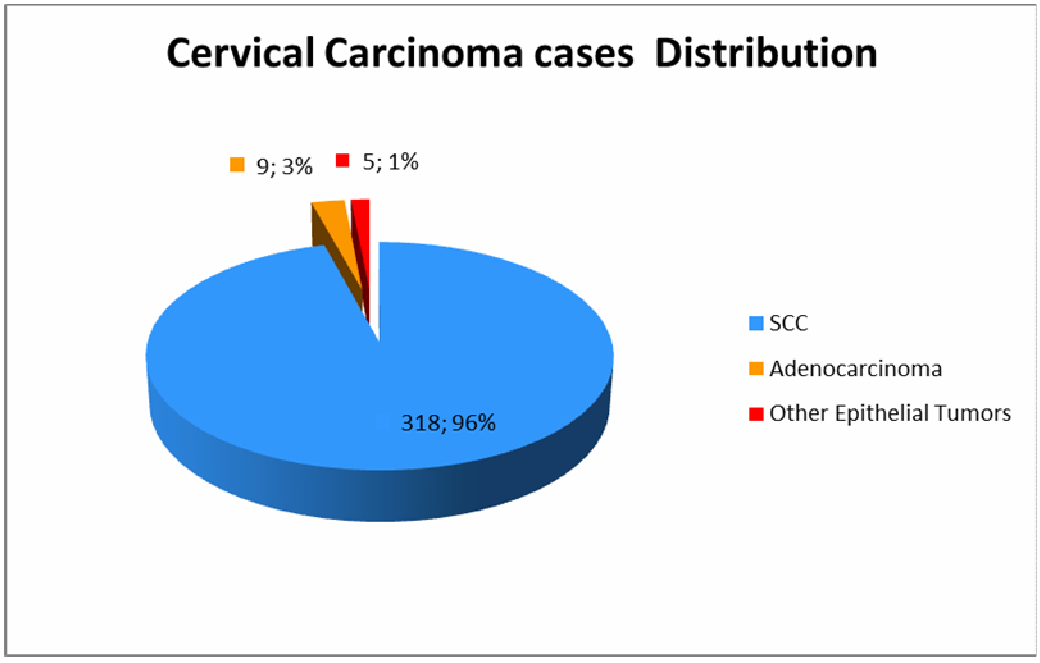
The most of the malignant lesions occurred in the 5th and 6th decades. Mean age of malignant cases was 52.6 years.

TABLE NO - 6

DISTRIBUTION OF DIFFERENT TYPES OF CERVICAL CARCINOMA

HISTOPATHOLOGICAL DIAGNOSIS	NO. OF CASES	PERCENTAGE
SQUAMOUS CELL CARCINOMA	318	95.8
ADENOCARCINOMA	9	2.7
OTHER EPITHELIAL TUMORS	5	1.5
TOTAL	332	100

GRAPH NO - 3



5.1.c SQUAMOUS CELL CARCINOMA OF THE UTERINE CERVIX:

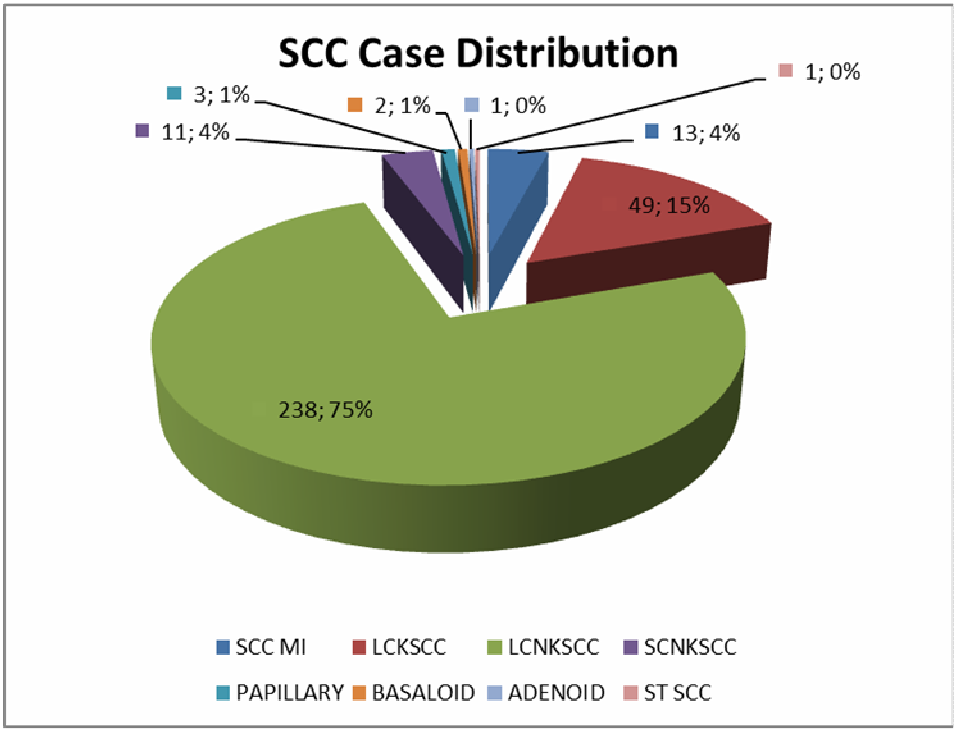
In this present study, SCC constitutes the majority of malignant lesions. Out of 318 cases, 13 cases were SCC with microinvasion and 298 cases were conventional SCC. In conventional SCC, 49(15.41%) cases were Large cell Keratinizing SCC, 238(74.84%) cases were Large cell Nonkeratinizing SCC, 11(3.46%) cases were Small cell Nonkeratinizing SCC. The histopathological pictures of squamous cell carcinoma with microinvasion and variants of SCC are depicted in figures: 13, 21, 23, 25, 39. Distribution of the histopathological variants of SCC encountered in our study are given in (table no-7, graph no -4)

TABLE NO - 7

DISTRIBUTION OF DIFFERENT TYPES OF SQUAMOUS CELL CARCINOMA

HISTOPATHOLOGICAL DIAGNOSIS	NO. OF CASES	PERCENTAGE
SCC WITH MICROINVASION	13	4
LCKSCC	49	15.4
LCNKSCC	238	74.8
SCNKSCC	11	3.5
PAPILLARY SCC	3	0.9
BASALOID SCC	2	0.6
SQUAMOTRANSITIONAL SCC	1	0.3
ADENOID SCC	1	0.3
TOTAL	318	100

GRAPH NO - 4



5.1.d GRADING OF CONVENTIONAL SCC:

In this study, all the conventional SCC were graded based on modified Broder's method into three grades namely well, moderately and poorly differentiated carcinomas. As Keratinizing SCC (16.44%) was graded as Grade1, Large cell Nonkeratinizing SCC (79.86%) as Grade 2 and Small cell Nonkeratinizing (3.6%) as Grade 3. The histopathological pictures of LCKSCC, LCNKSCC and SCNKSCC are depicted in figures: 15, 17, 19. The distribution of different grades of conventional SCC is given in table no - 8

TABLE NO - 8

DISTRIBUTION OF GRADE OF DIFFERENTIATION OF CONVENTIONAL SCC

HISTOPATHOLOGICAL DIAGNOSIS	GRADE OF DIFFERENTIATION	NO. OF CASES	PERCENTAGE
LCKSCC	1	49	16.44
LCNKSCC	2	238	79.86
SCNKSCC	3	11	3.6
TOTAL		298	100

5.1.e ADENOCARCINOMA

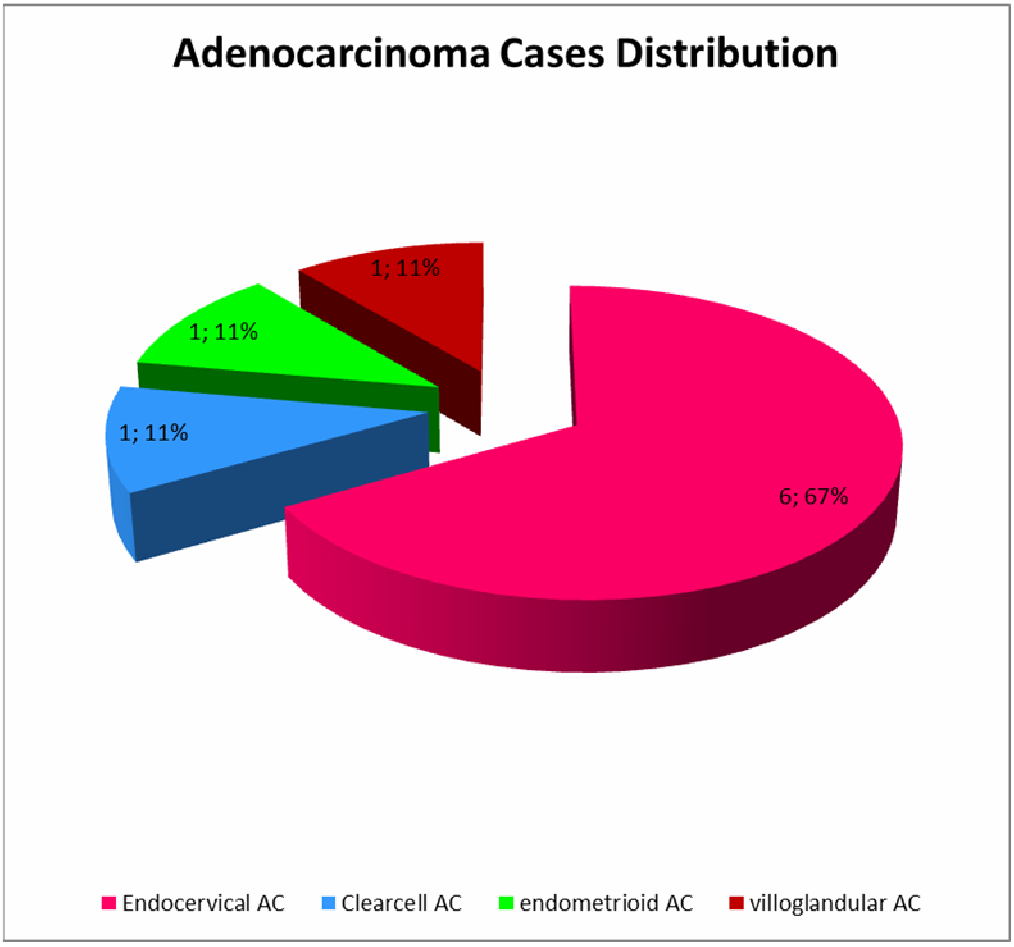
In this present study out **of 9 cases** of adenocarcinoma, 6 (66.7%) cases were of Mucinous adenocarcinoma of endocervical type were and the other three cases include **Villoglandular adenocarcinoma, clear cell adenocarcinoma and Endometrioid adenocarcinoma** (table no-9, graph no-5). The histopathological pictures of different types of adenocarcinoma are depicted in figures: 27, 29, 31, 33, 35.

TABLE NO - 9

DISTRIBUTION OF DIFFERENT TYPES OF ADENOCARCINOMA

HISTOPATHOLOGICAL DIAGNOSIS	NO. OF CASES	PERCENTAGE
VILLOGLANDULAR	1	11.1
ENDOCERVICAL TYPE	6	66.7
CLEAR CELL TYPE	1	11.1
ENDOMETRIOID TYPE	1	11.1
Total	9	100

GRAPH NO - 5



5.1.f GRADING OF ADENOCARCINOMA

In this study, Mucinous adenocarcinoma of endocervical type was graded, based on the complex architecture and the nuclear features into three grade i.e. well, moderately and poorly differentiated carcinoma as Grade 1, Grade 2 and Grade 3 respectively.

Out of 6 cases of Mucinous adenocarcinoma of endocervical type, one case (16.66%) of Grade 1, three cases (50%) of Grade 2 and two cases (33.3%) of Grade 3.(table no-10) were observed. The histopathological pictures of grade 2 and grade 3 are depicted in figures: 31, 33.

TABLE NO - 10

DISTRIBUTION OF GRADE OF DIFFERENTIATION OF ENDOCERVICAL ADENOCARCINOMA

GRADE OF DIFFERENTIATION	NO. OF CASES	PERCENTAGE
Grade 1	1	16.7
Grade 2	3	50
Grade 3	2	33.3
Total	6	100

5.1.g OTHER EPITHELIAL TUMOURS

In this present study, apart from SCC and adenocarcinoma, **4 cases of Adeno SCC and one case of Adenoid basal carcinoma** were noted. The histopathological pictures of these tumours are depicted in figure: 37, 40.

5.2 DISTRIBUTION OF CERVICAL NEOPLASMS ACCORDING TO AGE

The present study statistical study data inferred that majority of the uterine cervical neoplasms belonged to **30-60 years age group (510 cases-75.45%**).

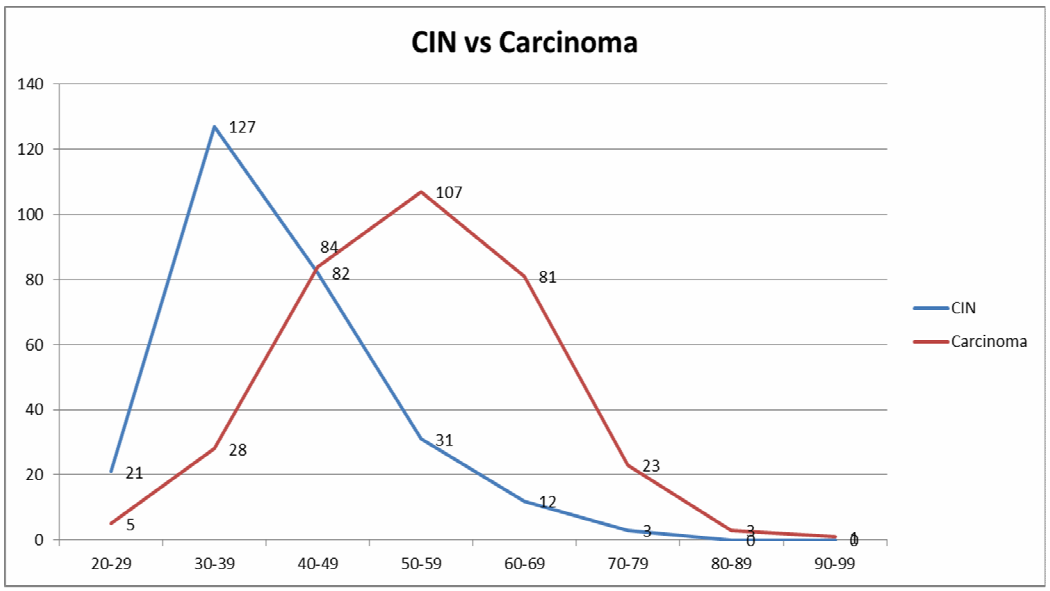
Among individual age groups most of the patients were in the 40-49 age group (166 cases-27.3%), followed by those belonging to 30-39 years age group (155 cases-25.49%).

In this present study, the age ranges from 21-90 years, with mean age of 46.8 years. In one end of the spectrum, two cases were reported as CIN 1 at the age of 21 years and in another end of the spectrum, a case has been reported as carcinoma at the age of 90 years.

The peak incidence of cervical intraepithelial neoplasms of cervix was seen in the **fourth decade** which was two decades earlier than that observed in the carcinoma group of patients seen in the following graph no-6

GRAPH NO – 6

AGE-GROUP WISE INCIDENCE



The age wise distribution pattern of CIN and CARCINOMA are given in table no – 11 and graph no – 7.

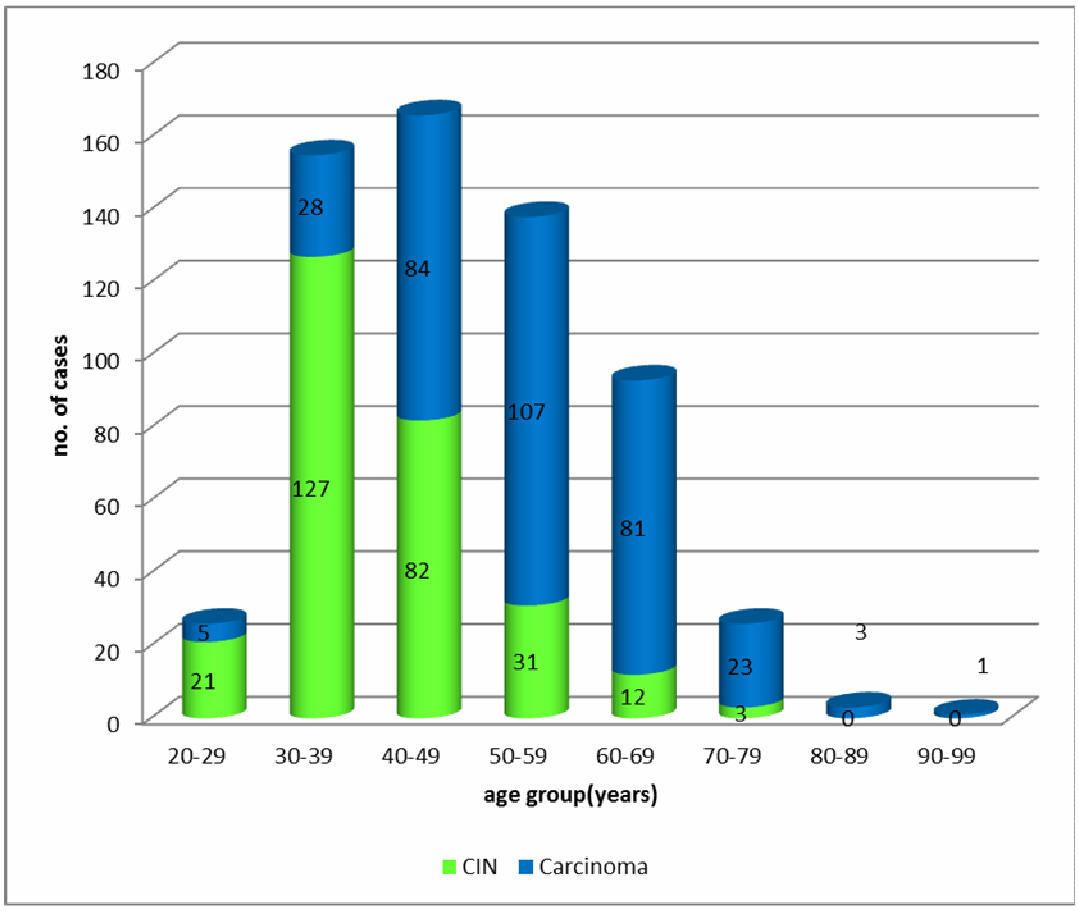
TABLE NO - 11

AGE GROUP WISE DISTRIBUTION PATTERN OF CIN AND CARCINOMA

AGE GROUP(YEARS)	NO. OF CIN CASES n (%)		NO. OF CARCINOMA CASES n (%)		TOTAL CASES n (%)	
20-29	21	(7.6%)	5	(1.5%)	26	(4.27%)
30-39	127	(46%)	28	(8.43%)	155	(25.49%)
40-49	82	(29.7%)	84	(25.3%)	166	(27.3%)
50-59	31	(11.2%)	107	(32.22%)	138	(22.69%)
60-69	12	(4.3%)	81	(24.39%)	93	(15.29%)
70-79	3	(1%)	23	(6.9%)	26	(4.27%)
80-89	0	00	3	(0.9%)	3	(0.49%)
90-99	0	00	1	(0.3%)	1	(0.16%)
TOTAL	276	100	332	100	608	100

GRAPH NO – 7

AGE GROUP WISE DISTRIBUTION PATTERN OF CIN AND CARCINOMA



5.2.b CERVICAL INTRAEPITHELIAL NEOPLASMS

In the present study, the age group of two hundred and seventy six patients of cervical intraepithelial neoplasms of cervix aged from **21 to 71 years with a mean age of 39.8years.**(table no-12, graph no-8)

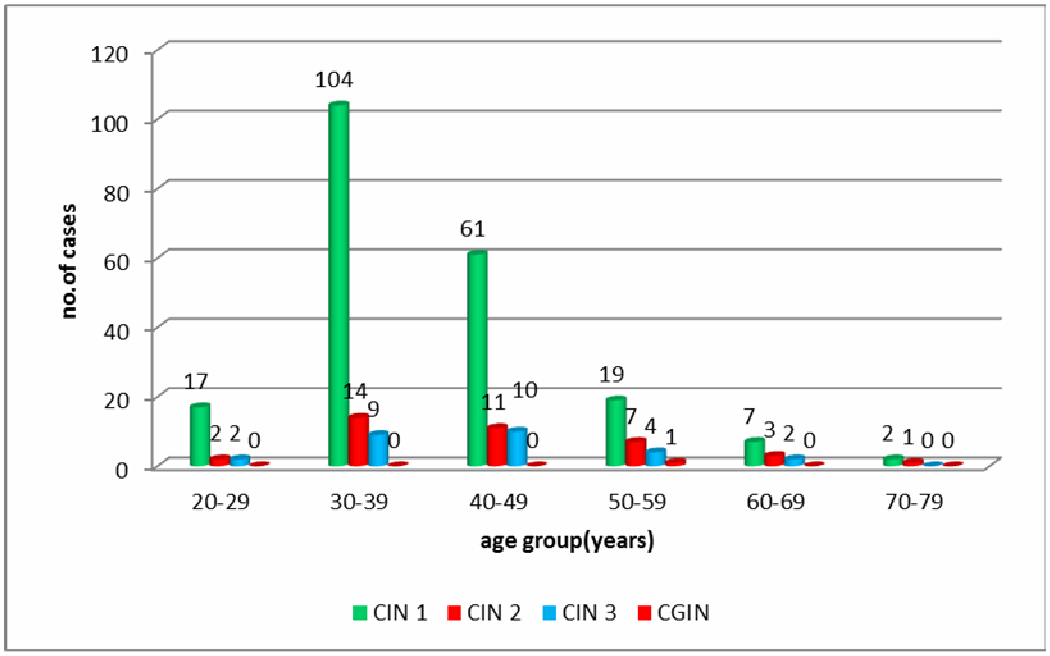
TABLE NO - 12

AGE GROUP WISE DISTRIBUTION PATTERN OF CERVICAL INTRAEPITHELIAL NEOPLASMS

Age group(years)	CIN 1	CIN 2	CIN 3	CGIN	Total	Percentage
20-29	17	2	2	0	21	7.6
30-39	104	14	9	0	127	46
40-49	61	11	10	0	82	29.7
50-59	19	7	4	1	31	11.2
60-69	7	3	2	0	12	4.3
70-79	2	1	0	0	3	1
Total	210	38	27	1	276	100

GRAPH NO – 8

AGE GROUP WISE DISTRIBUTION PATTERN OF CERVICAL INTRAEPITHELIAL NEOPLASMS



CIN 1

The majority of CIN 1 cases were found between **30-39 years (49.5%),** with the minimum and maximum age being 21 years and 71 years respectively. The mean age for CIN 1 was **38.8 years**

CIN 2

The maximum no. of CIN 2 cases were found between **30-39 years (36.84%),** with the minimum and maximum age being 27 years and 70 years respectively. The mean age for CIN 2 was **43 years**

CIN 3

The most cases of CIN 3 were found between **40-49 years (37%),** with the minimum and maximum age being 27 years and 60 years respectively. The mean age for CIN 3 was **42 years.**

CGIN

In this present study, one case of CGIN was noted in a **55 years**.

5.2.c MALIGNANT LESIONS OF UTERINE CERVIX

The age of the patients with carcinoma cervix ranged from **26 to 90 years** with mean age of **52.6 years.** The peak incidence of carcinoma of cervix was seen in the **sixth decade.**

5.2.d SQUAMOUS CELL CARCINOMA

The maximum no. of SCC was found between 50-59 years age group (102 cases-31.9%), with the minimum and maximum age being **26 years and 90 years** respectively. The mean age for SCC was **52.6 years.** (table no-13, graph no-9)

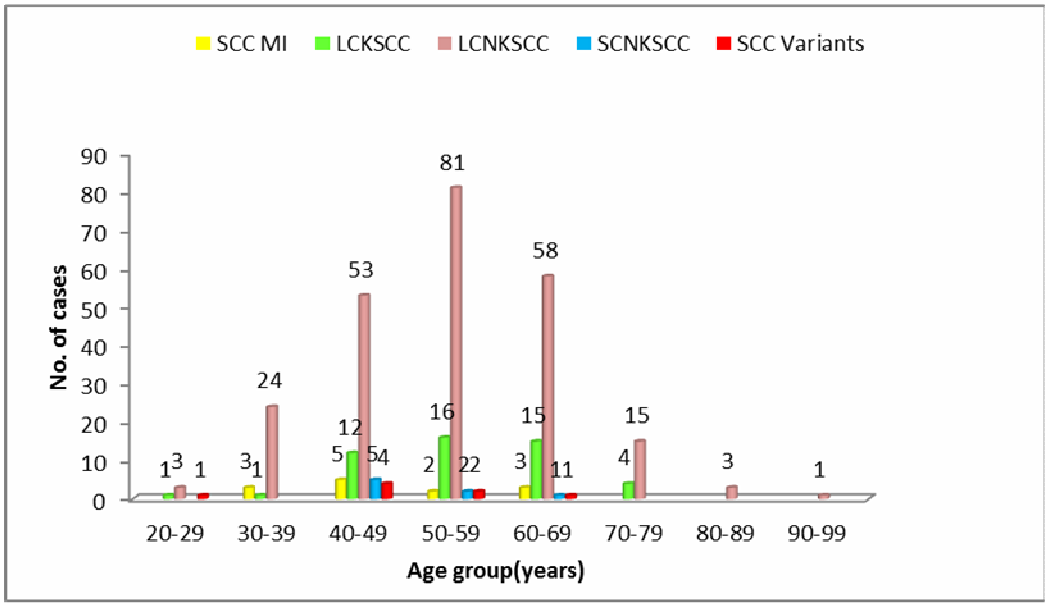
TABLE NO - 13

AGEGROUP WISE DISTRIBUTION PATTERN OF SQUAMOUS CELL CARCINOMA

Age group(years)	SCC MI	LCKSCC	LCNKSCC	SCNKSCC	SCC Variants	Total	Percentage
20-29	0	1	3	0	1	5	1.5
30-39	3	1	24	0	0	28	8.8
40-49	5	12	53	5	4	79	24.8
50-59	2	16	81	2	1	102	32
60-69	3	15	58	1	1	78	24.5
70-79	0	4	15	3	0	22	6.9
80-89	0	0	3	0	0	3	0.9
90-99	0	0	1	0	0	1	0.3
Total	13	49	238	11	7	318	100

GRAPH NO – 9

AGE GROUP WISE DISTRIBUTION PATTERN OF SQUAMOUS CELL CARCINOMA



SCC WITH MICROINVASION

Majority of SCC with micro invasion was found between 40-49 years (38.46%), with the minimum and maximum age being **33 years and 67 years** respectively. The mean age for SCC with microinvasion was **47 years.**

LARGE CELL KERATINIZING SQUAMOUS CELL CARCINOMA

Most of LCKSCC cases were found between 50-59 years (32.65%), with the minimum and maximum age being **26 years and 70 years** respectively. The mean age for LCKSCC was **53.9 years.**

LARGE CELL NON KERATINIZING SQUAMOUS CELL CARCINOMA

The most of LCNKSCC cases was found between 50-59 years (34%), with the minimum and maximum age being **28 years and 90 years** respectively. The mean age for LCNKSCC was **52.7 years.**

SMALL CELL NON KERATINIZING SQUAMOUS CELL CARCINOMA

Most of SCNKSCC cases were found between 40-49 years (45.45%), with the minimum and maximum age being **40 years and 73 years** respectively. The mean age for SCNKSCC was **54.5 years**.

5.2.e HISTOLOGIC VARIANTS OF SCC

Three cases of Papillary variant of SCC were noted in the **fifth decade**, two cases of Basaloid variant of SCC were seen in **50 and 60 years** , one case of Adenoid SCC was seen in **27 years** and a case of squamoustransistional variant was seen in **45 years.**

5.2.f ADENOCARCINOMA

The most of the Adenocarcinoma cases were found between 40-49 years (44.4%), with the minimum and maximum age being **40 years and 75 years** respectively. The mean age for Adenocarcinoma **was 53 years.** (table no-14, graph-10)

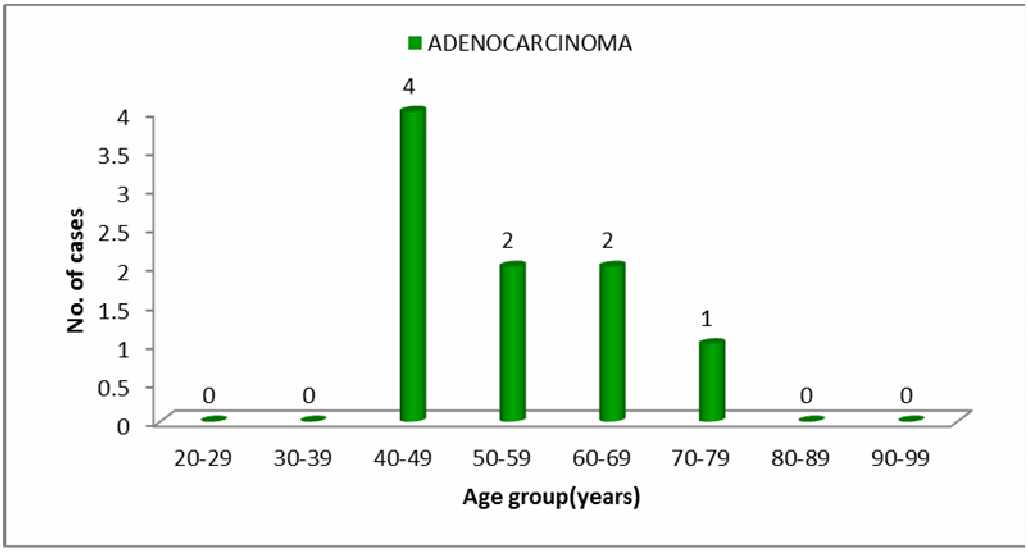
TABLE NO - 14

AGE GROUP WISE DISTRIBUTION PATTERN OF ADENOCARCINOMA

Age group(years)	No. of cases	percentage
20-29	0	00
30-39	0	00
40-49	4	44.4
50-59	2	22.2
60-69	2	22.2
70-79	1	11.1
Total	9	100

GRAPH NO – 10

AGE GROUP WISE DISTRIBUTION PATTERN OF ADENOCARCINOMA



5.2.g HISTOLOGIC VARIANTS OF ADENOCARCINOMA

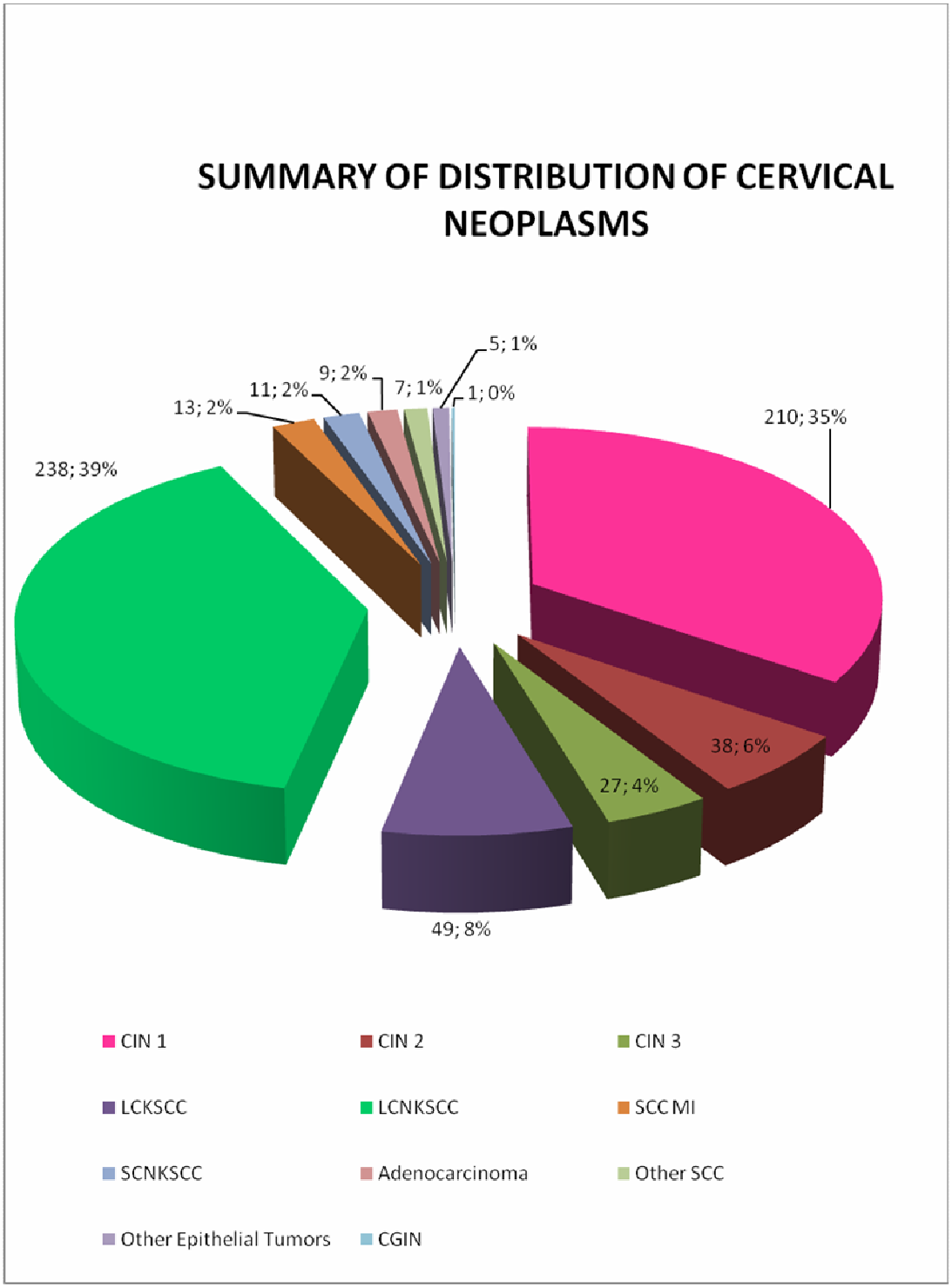
A case of Villoglandular adenocarcinoma and one case of Clear cell adenocarcinoma was seen in **62 years** and a case of Endometroid adenocarcinoma was seen in **50 years.**

TABLE NO - 15

DISTRIBUTION OF CASES ACCORDING TO DIFFERENT HISTOPATHOLOGICAL DIAGNOSIS

HISTOPATHOLOGICAL DIAGNOSIS				FREQUENCY	PERCENTAGE
CERVICAL INTRAEPITHELIAL NEOPLASMS		CIN1		210	34.54
		CIN2		38	6.25
		CIN3		27	4.44
		CGIN		1	0.16
CARCINOMA	SCC	SCC with microinvasion		13	2.14
		CONVENTIONAL SCC	LCKSCC	49	8
			LCNKSCC	238	39.14
			SCNKSCC	11	1.8
		PAPILLARY SCC		3	0.49
		BASALOID SCC		2	0.32
		SQUAMOTRANSITIONAL SCC		1	0.17
		ADENOID SCC		1	0.17
	ADENO CARCINOMA	VILLOGLANDULAR AC		1	0.17
		ENDOCERVICAL TYPE		6	0.99
		CLEAR CELL AC		1	0.17
		ENDOMETRIOID AC		1	0.16
	OTHER EPITHELIAL TUMOURS	ADENO SCC		4	0.66
		ADENOID BASAL CARCINOMA		1	0.16
TOTAL				608	100

GRAPH NO - 11


SUMMARY OF DISTRIBUTION OF CERVICAL NEOPLASMS
5; 1%
9; 2%
11; 2%
7; 1%
1; 0%
13; 2%
210; 35%
238; 39%
38; 6%
27; 4%
49; 8%
CIN 1
CIN 2
CIN 3
LCKSCC
LCNKSCC
SCC MI
SCNKSCC
Adenocarcinoma
Other SCC
Other Epithelial Tumors
CGIN

5.3 IMMUNOHISTOCHEMICAL ANALYSIS

$p16^{INK4a}$ IMMUNOEXPRESSION IN CERVICAL NEOPLASMS

Immunohistochemical staining using advanced polymer staining systems (a mouse monoclonal anti-p16 antibody, Fremont, CA, 94538, Biogenex, USA) $p16^{INK4a}$ monoclonal antibody was used for **69 cases** of uterine cervical biopsies according to the protocol (ANNEXURE III).

The study group composed of randomly selected 69 cases, which represents 10% of the sample size. Out of 69 cases, 9 cases of chronic non-specific cervicitis, 10 cases of CIN 1, 10 cases of CIN 2, 10 cases of CIN 3, a case of CGIN, 22 cases of SCC, 6 cases of Adenocarcinoma and a case of Adenosquamous carcinoma were taken.

Squamous cell carcinoma cases includes 4 cases of SCC with microinvasion, 15 cases of Conventional Squamous cell carcinoma and 3 cases of its variants which includes Papillary SCC, Basaloid SCC and Squamotransitional cell carcinoma.

Among the conventional squamous cell carcinoma, 2 cases were Large cell keratinizing SCC, 11 Large cell non keratinizing SCC and 2 Small cell non keratinizing SCC which was graded into grade 1, grade 2 and grade 3 respectively, according to modified Broder's classification.

Adenocarcinoma and its variants taken for IHC includes, clear cell adenocarcinoma, Endometrioid adenocarcinoma, Villoglandular adenocarcinoma, endocervical mucinous adenocarcinoma of grade 2 and 2 cases of endocervical mucinous adenocarcinoma of grade 3.

5.3.a EVALUATION OF IMMUNOHISTOCHEMICAL MARKER-p16^{INK4a}

The immunostaining was considered **positive when the nucleus and/or cytoplasm take chest nut brown colour**. Various researchers have used different methods for scoring p16^{INK4a} immunostaing, but in this study two different protocols were considered: 1) positive vs negative p16 immunostaining; 2) a semi-quantitative method based on four parameters for scoring which will eventually increase the specificity of the results and the parameters are as follows:

1. **Percentage of proportion of positive tumour cells[42, 43, 44, 45, 46] were graded as**:
    - 0% - negative staining - 0
    - 1-5% - 1+
    - 5-25% - 2+
    - > 25% - 3+.
2. **Intensity of staining[42, 44, 47, 48, 49]-(0-3 points**)
    - Negative - 0
    - Weak – 1+
    - Moderate - 2+
    - Strong - 3+
3. **p16^{INK4a} staining in cellular reaction pattern[42, 44, 45, 49, 62]-**

    Only cytoplasmic positivity

    Nucleo: cytoplasmic positivity

    Nuclear positivity

4. **$p16^{INK4a}$ pattern staining expression in epithelium of different CIN grades** [42, 45, 50] as stated in Lulin Hu publications**,**

- Negative -- no positive cells or <1% positive cells.
- Patchy -- focally aggregated positive cells contain <25% of epithelium.
- Diffuse basal -- In lower half of broad area of epithelium if positive cells present in continuity.
- Diffuse full thickness -- In lower half of broad area of epithelium if positive cells present in continuity with each other and in full thickness.

5.3.b PROPORTION OF POSITIVE TUMOUR CELLS AMONG THE DIFFERENT GROUPS OF UTERINE CERVICAL LESIONS

Based on the above parameters mentioned, 69 cases were studied and the results were shown in the following table no-16, graph-12, 13.

TABLE NO - 16

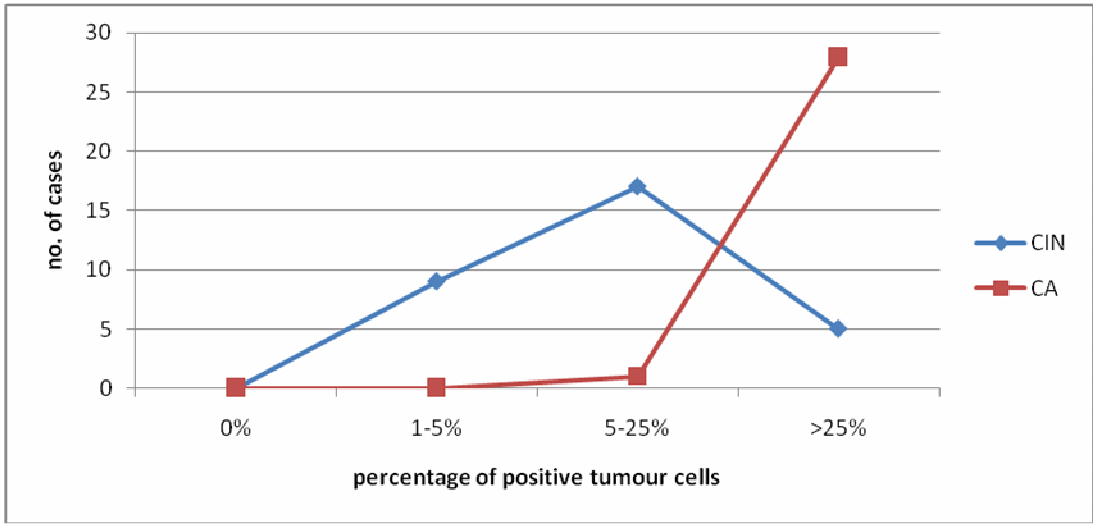
PROPORTION OF POSITIVE TUMOUR CELLS AMONG THE DIFFERENT GROUPS

CATEGORY		**NO.OF CASES (n)**	**PROPORTION OF POSITIVE CELLS: n (%)**			
			0% (grade 0)	**1-5% (grade 1)**	**5-25% (grade 2)**	**>25% (grade 3)**
CNSC		9	7(77.7%)	1(11%)	1(11%)	-
CIN	**CIN 1**	10	-	6(60%)	4(40%)	-
	CIN 2	10	-	2(20%)	8(80%)	-
	CIN 3	10	-	1(10%)	5(50%)	4(40%)
	CGIN	1	-	-	-	1(100%)
CA	**SCC**	22	-	-	1(4.5%)	21(95.45%)
	AC	6	-	-	-	6(100%)
	ASC	1	-	-	-	1(100%)

The above table shows seven cases of CNSC were negative (Figure-4) for p16.

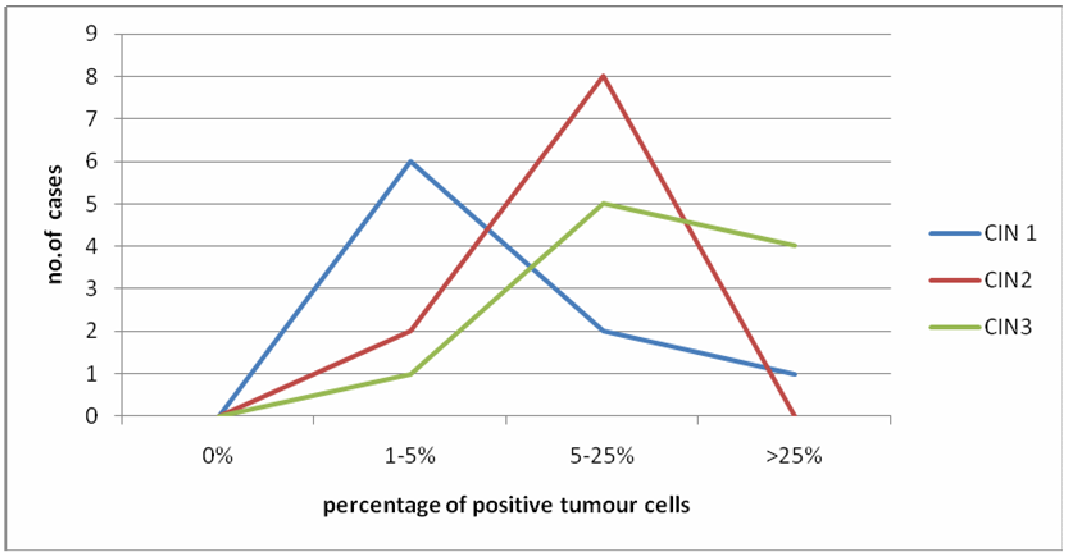
GRAPH NO - 12

PROPORTION OF POSITIVE TUMOUR CELLS BETWEEN CIN AND CARCINOMA



GRAPH NO – 13

PROPORTION OF POSITIVE TUMOUR CELLS AMONG CIN CASES



5.3.c STAINING INTENSITY OF p16 AMONG THE DIFFERENT GROUPS OF UTERINE CERVICAL LESIONS

The staining intensity was scored between 0-3 and the results are shown in

table no-17, graph-14, 15.

TABLE NO - 17

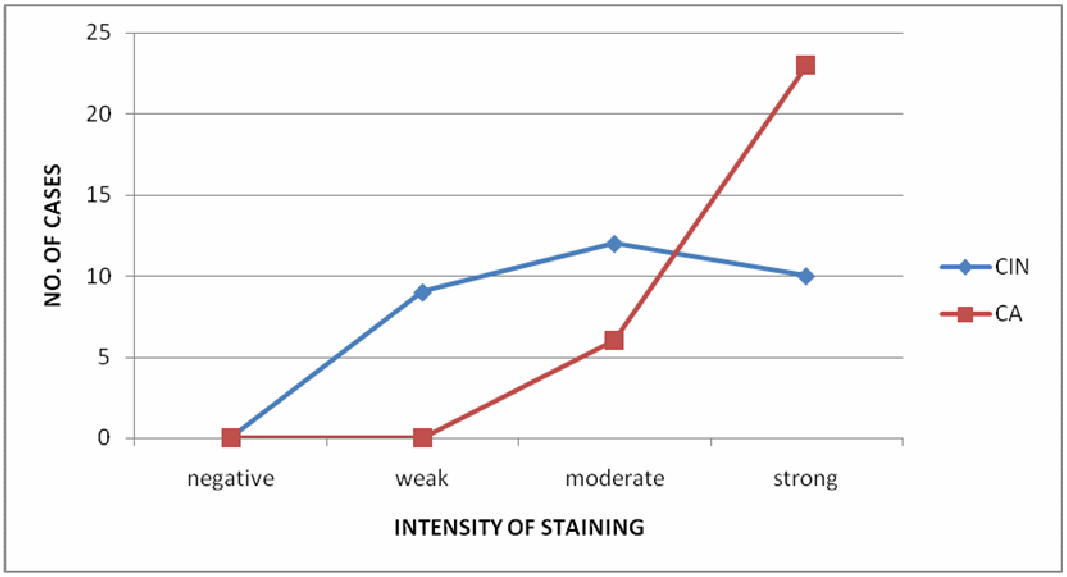
STAINING INTENSITY OF p16 AMONG THE DIFFERENT GROUPS

CATEGORY		**INTENSITY OF STAINING: n (%)**			
		0 (negative)	**1+(weak)**	**2+(moderate)**	**3+(strong)**
CNSC		7(77.7%)	1(11%)	1(11%)	-
CIN	**CIN 1**	-	4(40%)	5(50%)	1(10%)
	CIN 2	-	3(30%)	4(40%)	3(30%)
	CIN 3	-	2(20%)	3(30%)	5(50%)
	CGIN	-	-	-	1(100%)
CA	**SCC**	-	-	6(27%)	16(72.72%)
	AC	-	-	-	6(100%)
	ASC	-	-	-	1(100%)

Interpretation of these data reveals that all adenocarcinoma cases (figure-28, 30, 32, 34, 36), a case of Adenosquamous cell carcinoma (figure-38) and 73% cases of SCC have shown strong intensity.

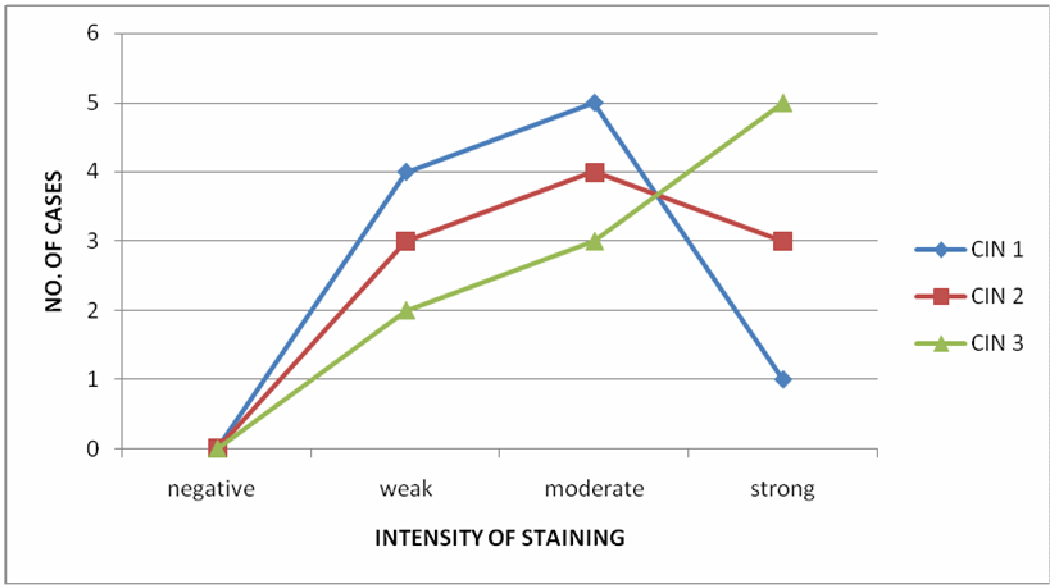
GRAPH NO - 14

STAINING INTENSITY OF p16 BETWEEN CIN AND CARCINOMA



GRAPH NO - 15

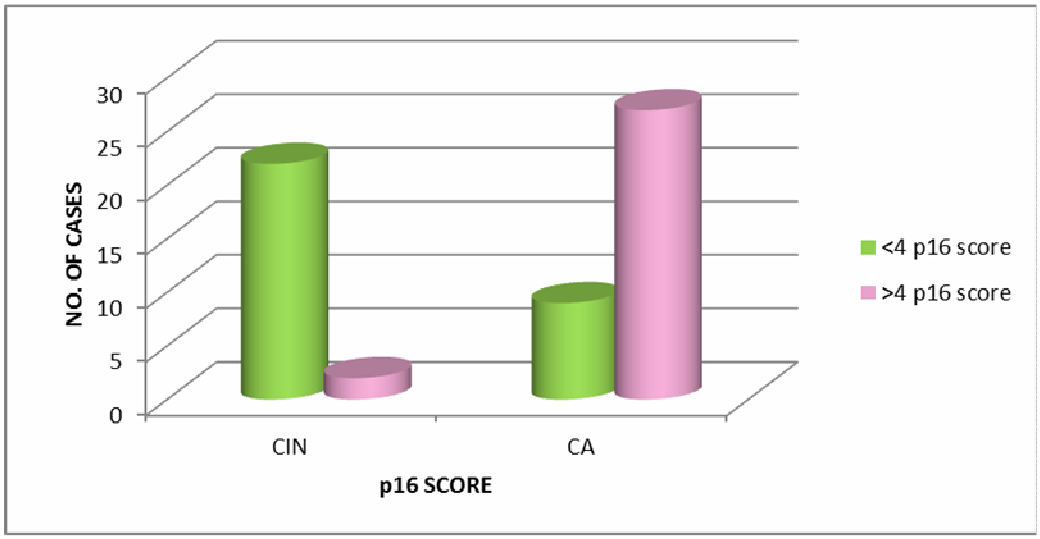
INTENSITY OF p16 STAINING AMONG CIN CASES



Hence p16 expression scoring was calculated by the product of percentage of positive tumour cell and intensity of grading and plotted in the following graph. The values obtained are 1, 2, 3, 4, 6 and 9. The value 4 is taken as cut off value. (Graph- 16, 17)

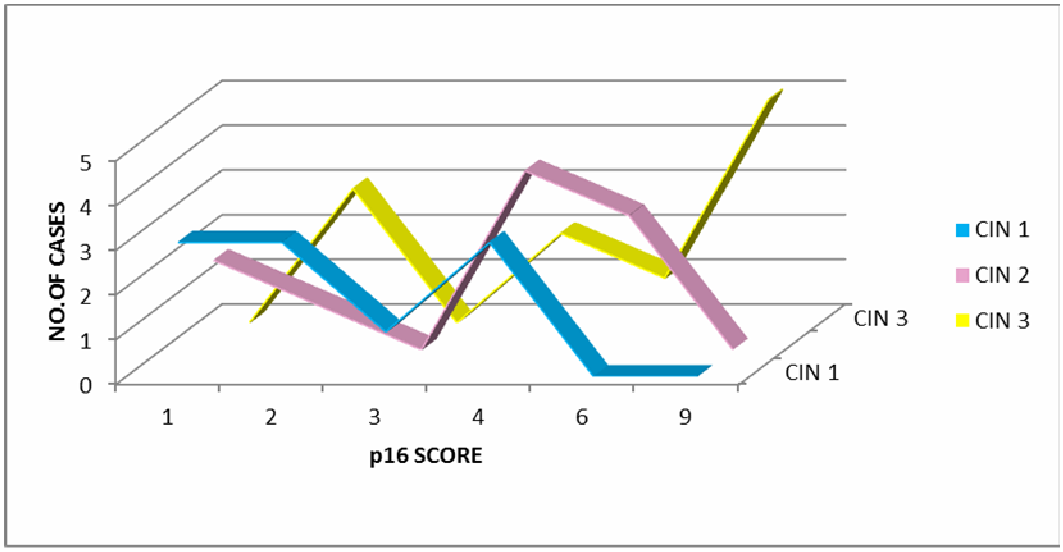
GRAPH NO - 16

p16 SCORE BETWEEN CIN AND CARCINOMA



GRAPH NO - 17

p16 SCORE AMONG CIN CASES



5.3.d $p16^{INK4A}$ STAINING IN CELLULAR REACTION PATTERN AMONG UTERINE CERVICAL LESIONS

Various cellular reaction patterns were observed in our study which includes, cytoplasmic positivity in 16 cases, Nucleo-cytoplasmic positivity in 50 cases and one case showed nuclear positivity (Squamotransitional SCC i.e.figure-26) as illustrated in the following table no-18

TABLE NO – 18

STAINING OF CELLULAR PATTERNS AMONG THE DIFFERENT GROUPS

TYPES	CNSC	CIN				CA		
		CIN 1	CIN 2	CIN 3	CGIN	SCC	AC	ASC
NEGATIVE (-VE)	7	-	-	-	-	-	-	-
POSITIVE(+VE)	2	10	10	10	1	22	6	1
CYTOPLASMIC POSITIVITY	1	3	1	2	-	4	1	-
NUCLEO:CYTOPLASMIC POSITIVITY	1	7	9	8	1	17	5	1
NUCLEAR POSITIVITY	-	-	-	-	-	1	-	-

5.3.e PATTERNS OF $p16^{INK4a}$ STAINING EXPRESSION WITHIN EPITHELIUM OF DIFFERENT GRADES OF CIN

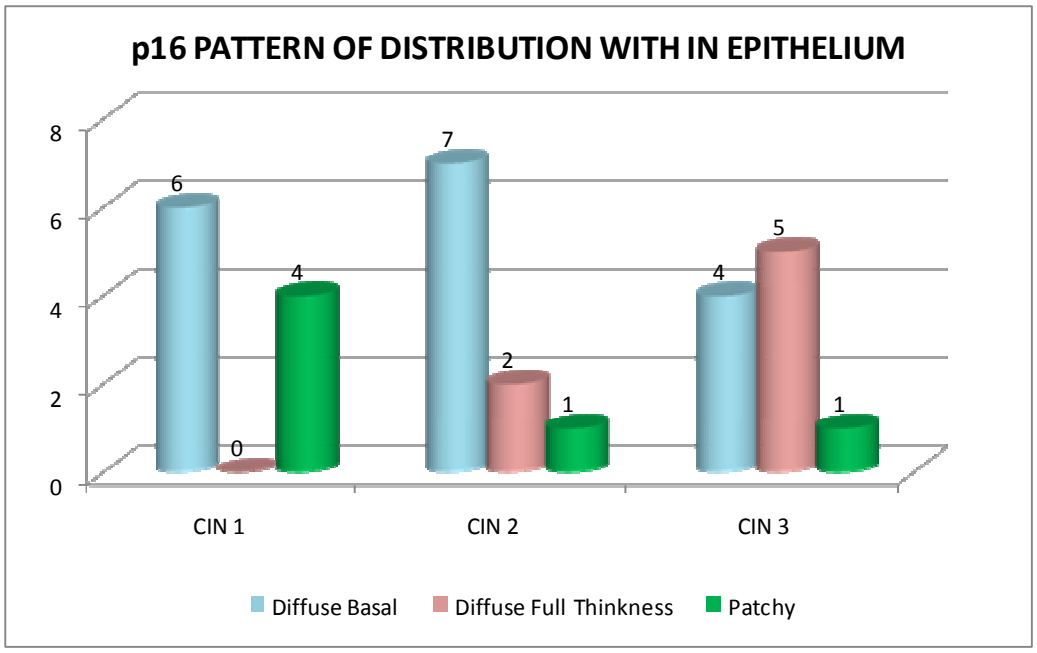
Among the cervical intraepithelial neoplasia cases different patterns of staining was observed. The following table no- 19, graph-18 shows the percentage of cases showing patchy, diffuse basal and diffuse full thickness. (Figure- 6A,6B,8,10)

TABLE NO - 19

PATTERN OF p16^{INK4a} EXPRESSION IN DIFFERENT GRADES OF CIN

CATEGORY	N	PATCHY n (%)	DIFFUSE BASAL n (%)	DIFFUSE FULL THICKNESSn (%)
CIN 1	10	4(40)	6(60)	-
CIN 2	10	1(10)	7(70)	2(20)
CIN 3	10	1(10)	4(40)	5(50)

GRAPH NO - 18



Above table concludes that p16 immunostaining positivity found to be both nuclear and/or cytoplasmic. The chronic non-specific cervicitis cases was predominantly negative for p16 (7/9) immunostaining. The four parameters used for analysis of p 16 immunostaining includes the proportion of positive tumour cells, intensity, pattern of p16 immunostaining with in the epithelium and cellular reaction pattern. Based on the four parameters it has been inferred that, there is an increase in progression of p16 expression with increase in the grades of CIN and also from CIN to carcinoma.

5.4 STATISTICAL ANALYSIS

pValue was calculated to find the significant correlation of p16 overexpression within the sub groups of cervical lesions by statistical analysis.

Test used - Fisher exact test and Extended Mantel-Haenszel chi square test for linear trend

pValue = 0.0001.

From the tabulated data, p value was calculated. The value obtained was 0.0001. Since the p value is smaller (<0.05), it is evident that rejecting the null hypothesis can be possible. This infers that $p16^{INK4a}$ expression can be directly correlated with the increasing grades of cervical intraepithelial neoplasia and carcinoma.

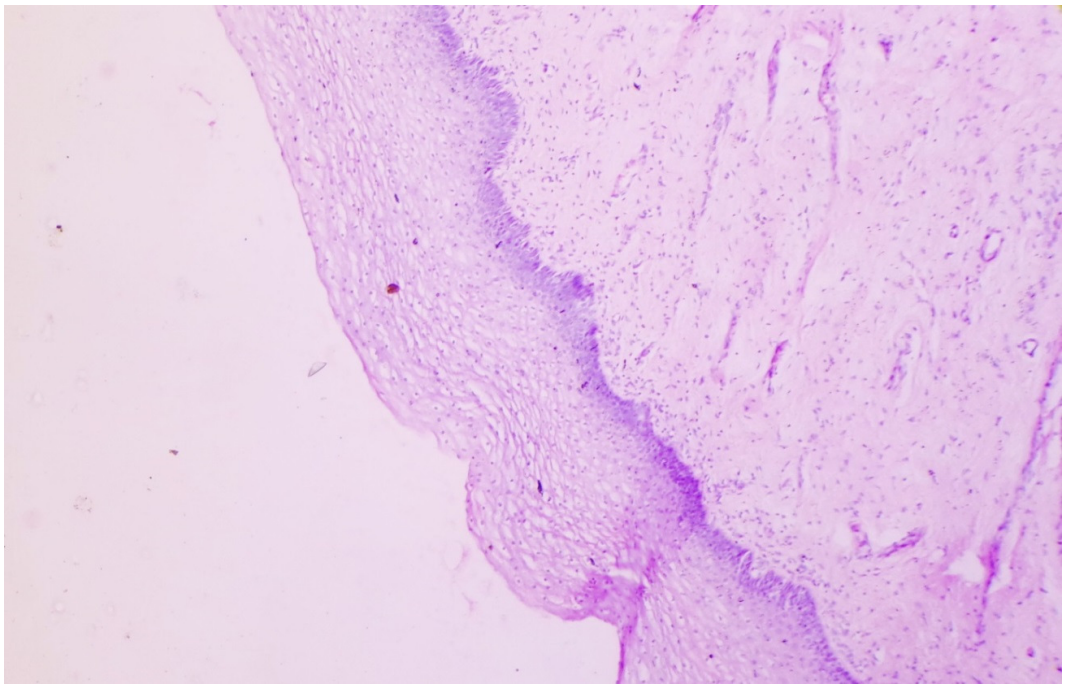


FIGURE- 1.Histology of normal cervical non-keratinizing squamous epithelium.

The squamous cells show maturation from basal layer to the surface (H&E, 40X)

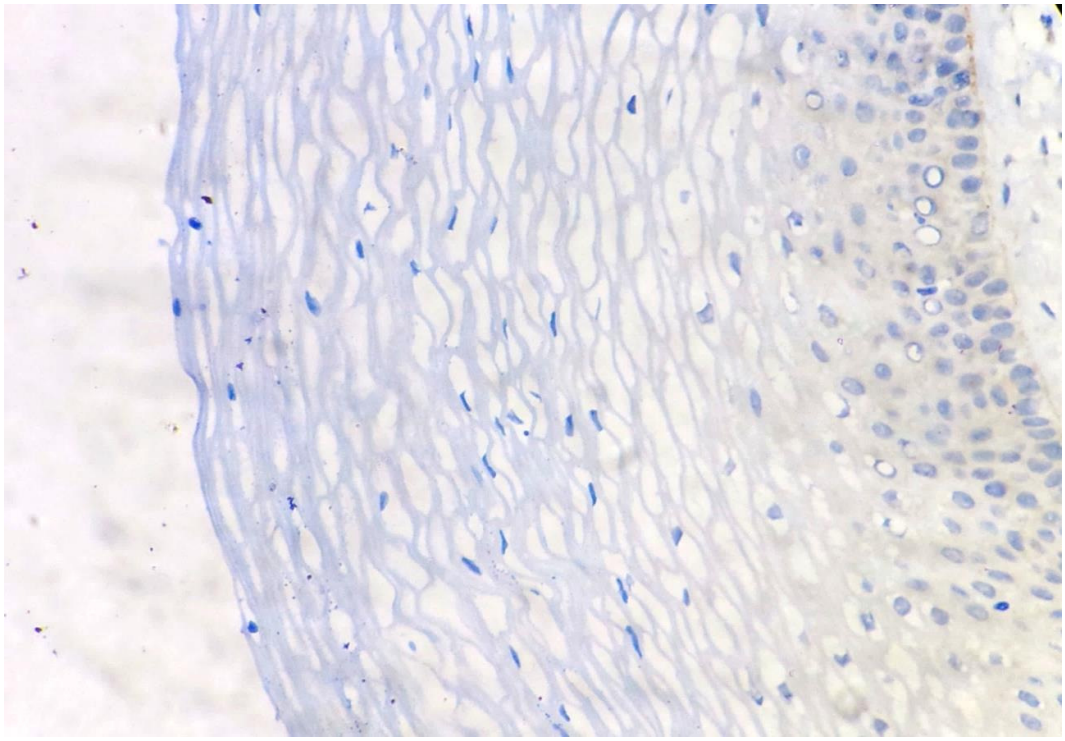


FIGURE-2.Histology of normal cervix (IHC p16, 40X)-NEGATIVE STAINING

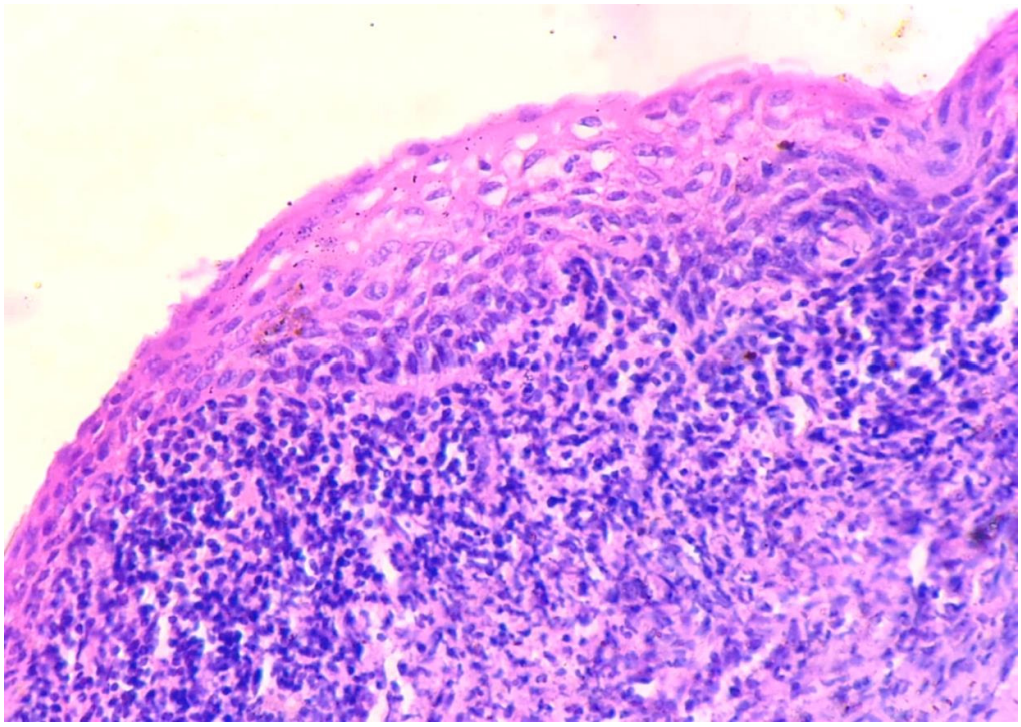


FIGURE-3.Chronic non-specific cervicitis composed of reactive atypia of squamous epithelium with inflammatory infiltrate in the stroma (H&E, 40x)

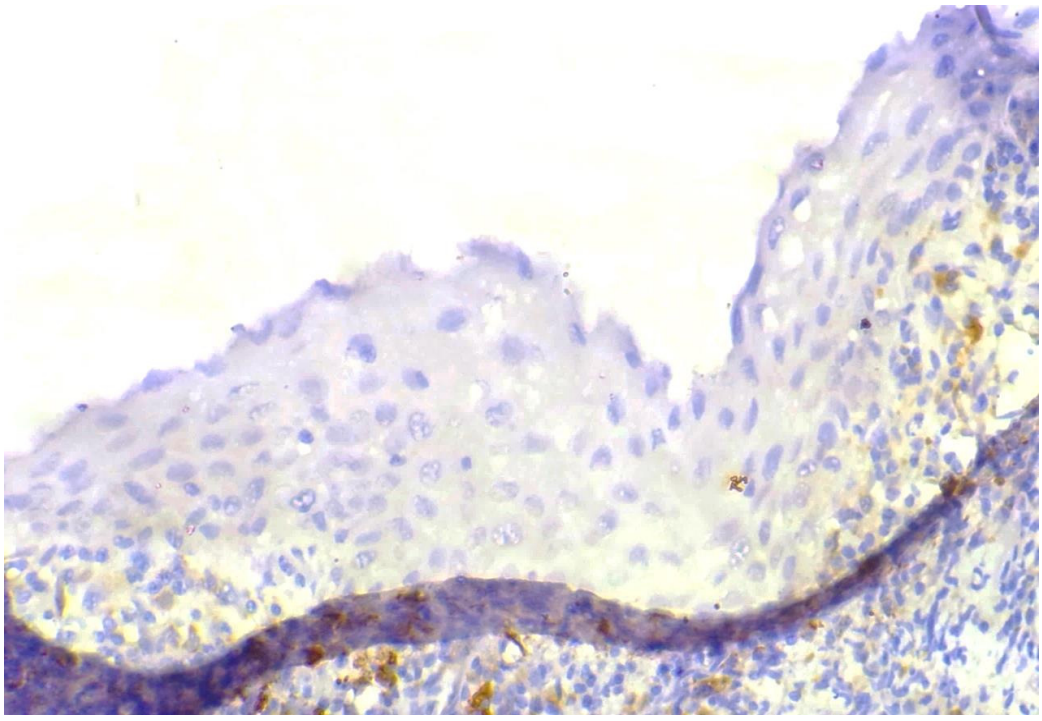


FIGURE- 4.Chronic non specific cervicitis (IHC p16, 40X) – NEGATIVE STAINING

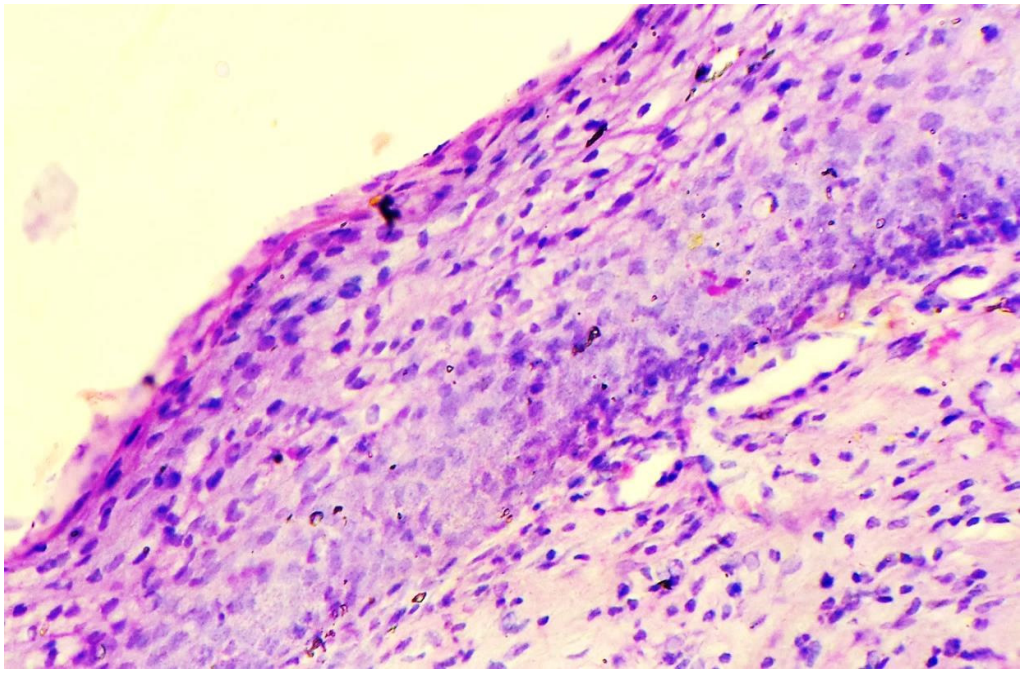


FIGURE- 5.Cervical intraepithelial neoplasia 1 composed of mild dysplasia confined to basal 1/3 of the epithelium (H&E, 40X)

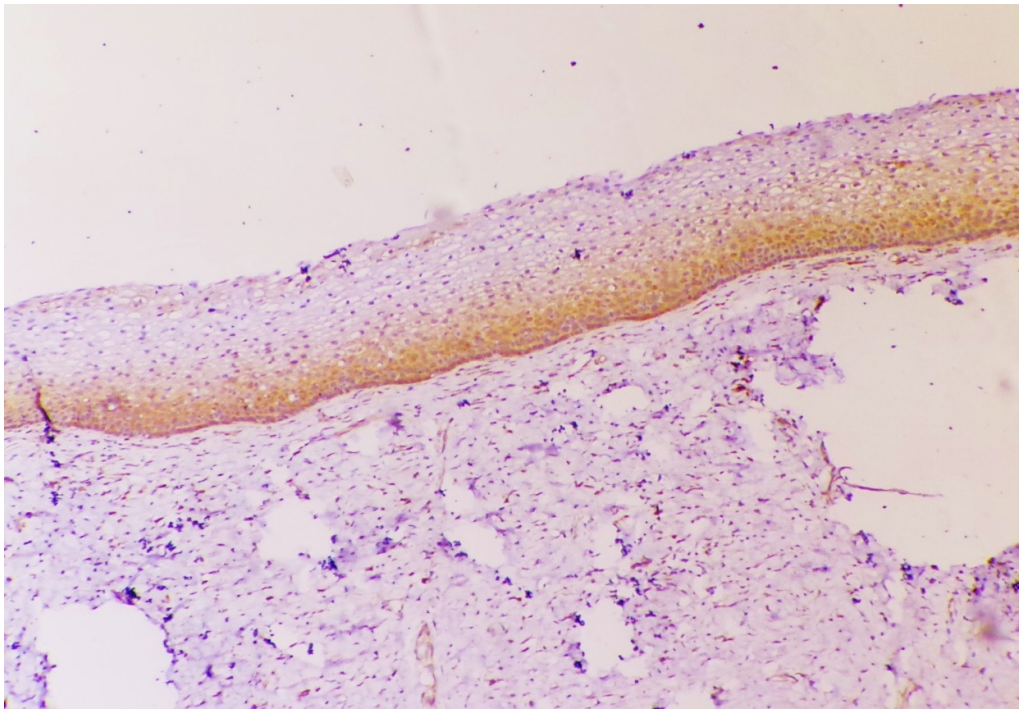


FIGURE- 6A.Cervical intraepithelial neoplasia 1(IHC P16, 40X) – DIFFUSE BASAL

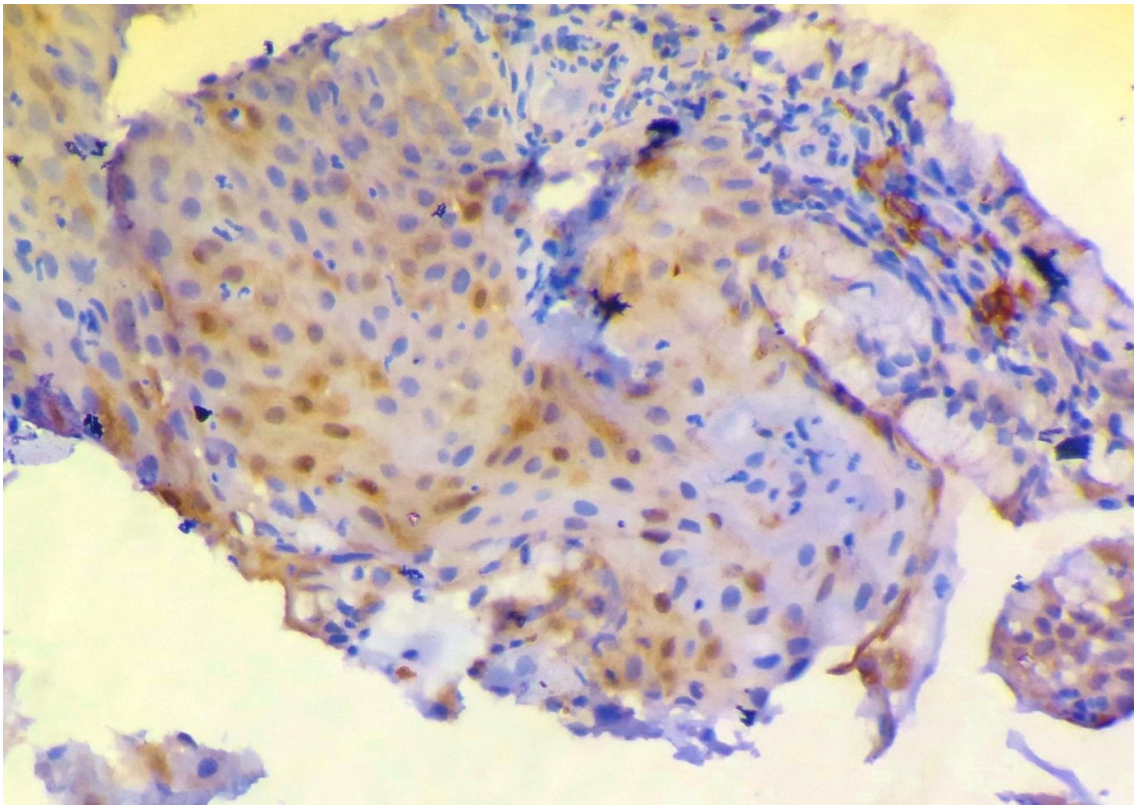


FIGURE – 6B, Cervical intraepithelial neoplasia 1 (IHC p16, 40X,) PATCHY DISTRIBUTION

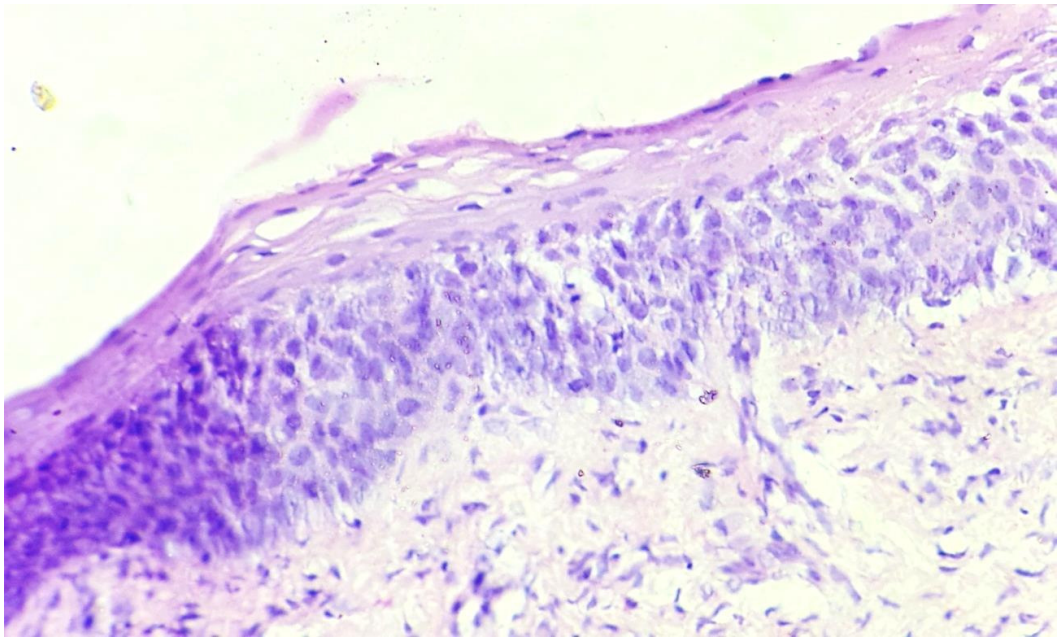


FIGURE-7.Cervical intraepithelial neoplasia 2 composed of moderate dysplasia confined to basal 2/3 of the epithelium (H&E, 40X)

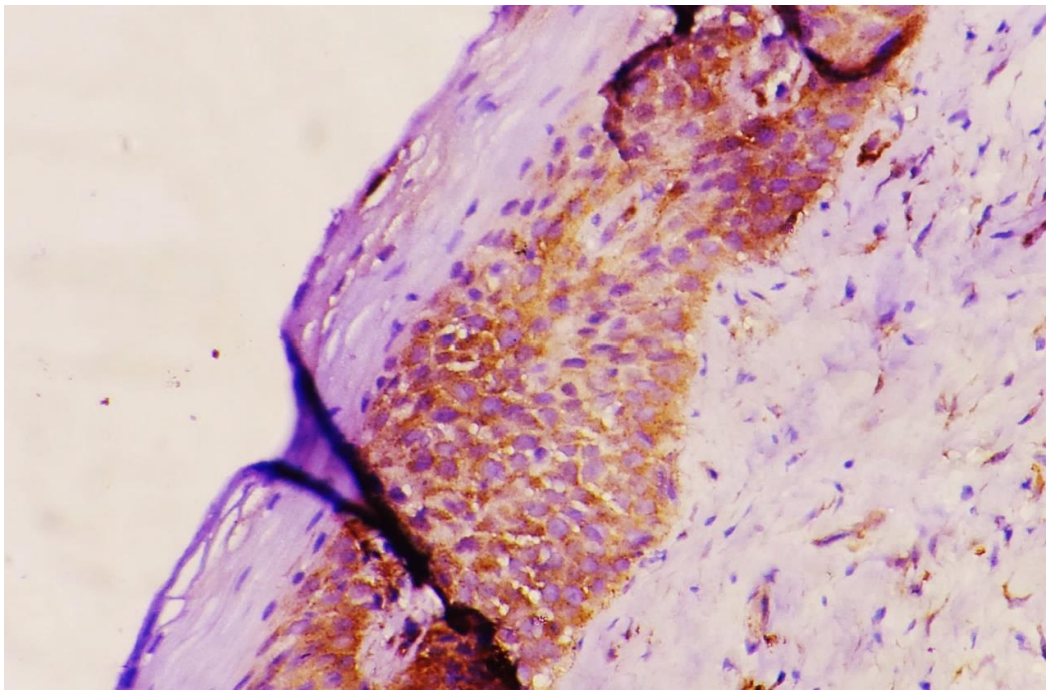


FIGURE-8.Cervical intraepithelial neoplasia -2(IHC p16, 40X)

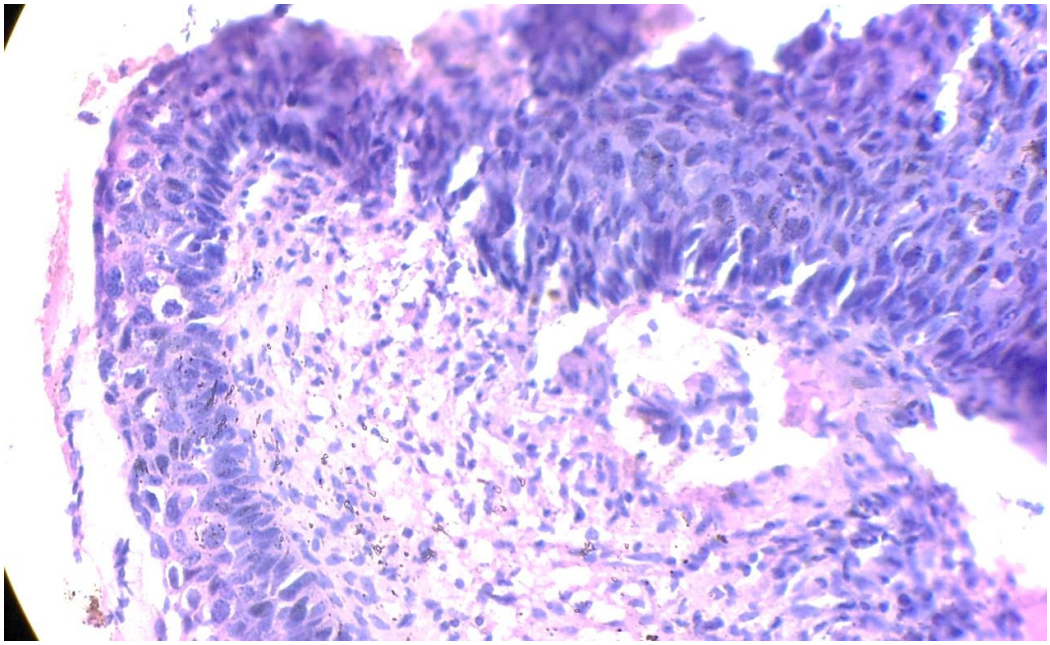


FIGURE-9.Cervical intraepithelial neoplasia 3 composed of severe dysplasia involving the full thickness epithelium (H&E, 40X)

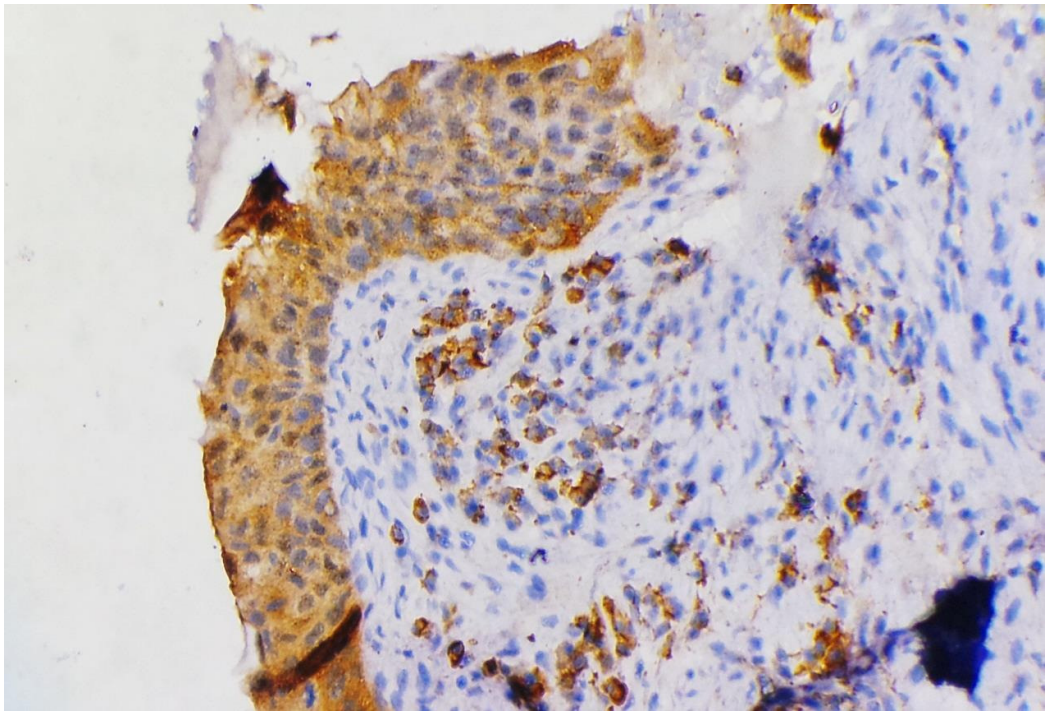


FIGURE-10.Cervical intraepithelial neoplasia 3 (IHC P16, 40X)- DIFFUSE FULL THICKNESS, NUCLEO: CYTOPLASMIC POSITIVITY

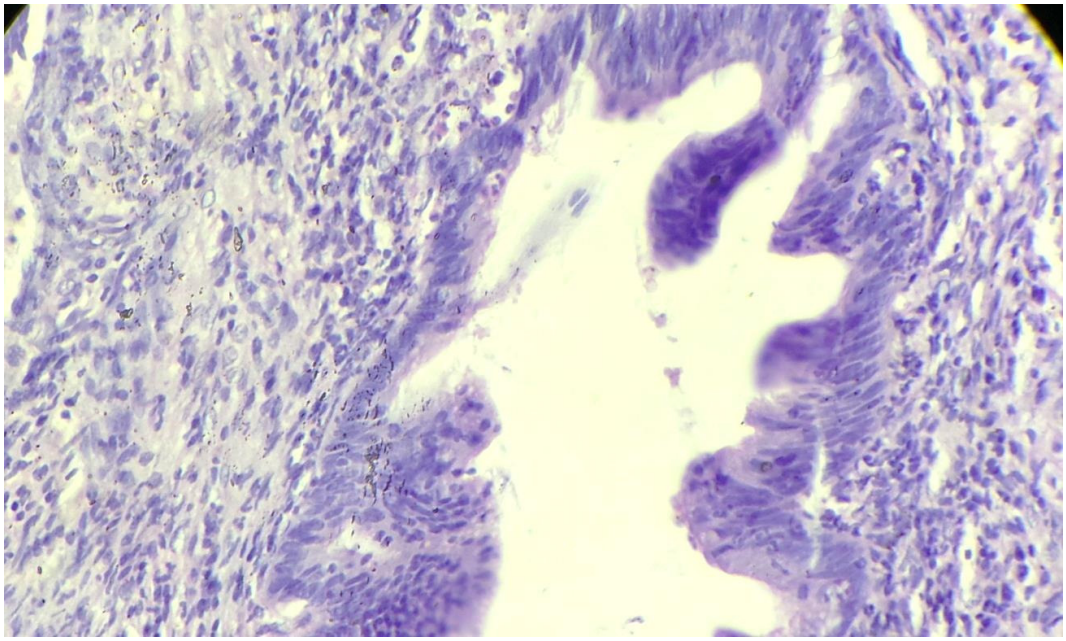


FIGURE- 11.Cervical glandular intraepithelial lesions composed of dysplastic glands with intraluminal papillary projections (H&E, 10X)

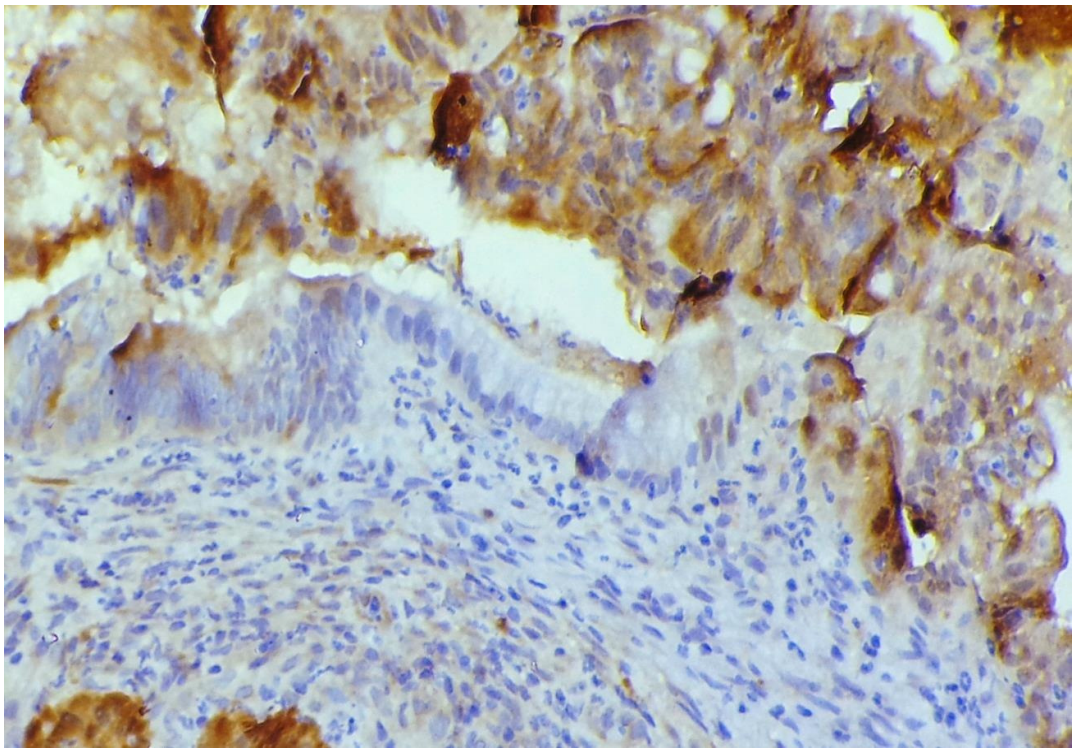


FIGURE-12. Cervical glandular intraepithelial neoplasia

(IHC p16, SCANNER VIEW)

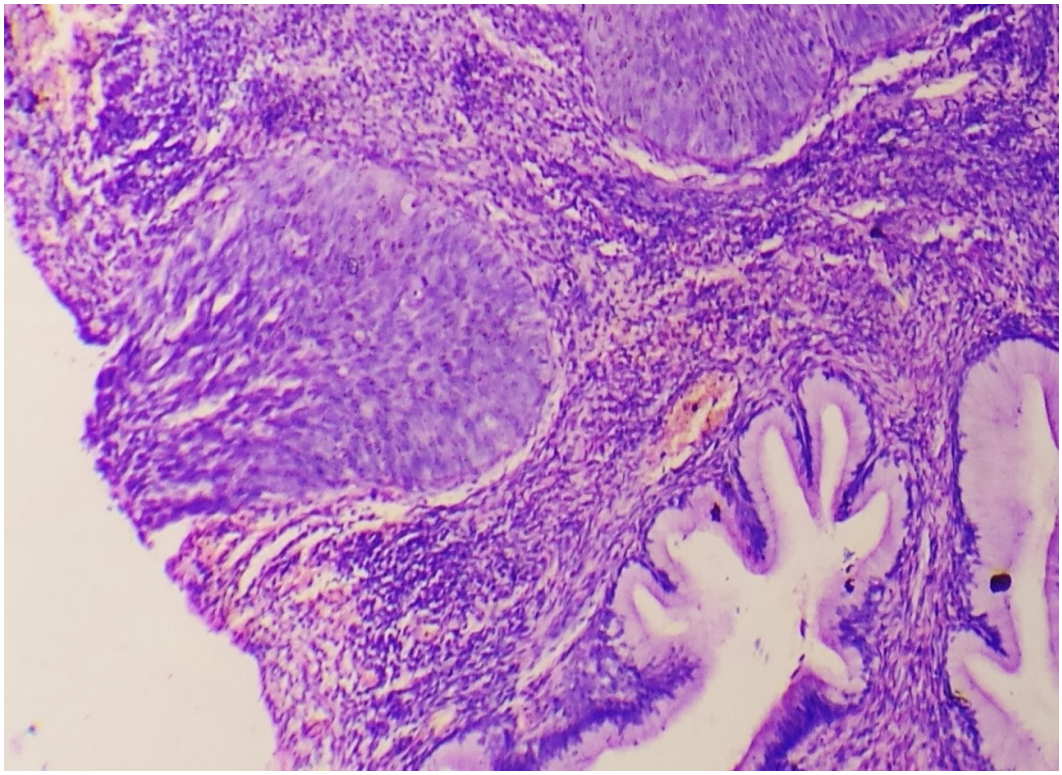


FIGURE-13.Squamous cell carcinoma with microinvasion characterized by tongue shaped malignant epithelial nest invading the stroma (H&E, 40X)

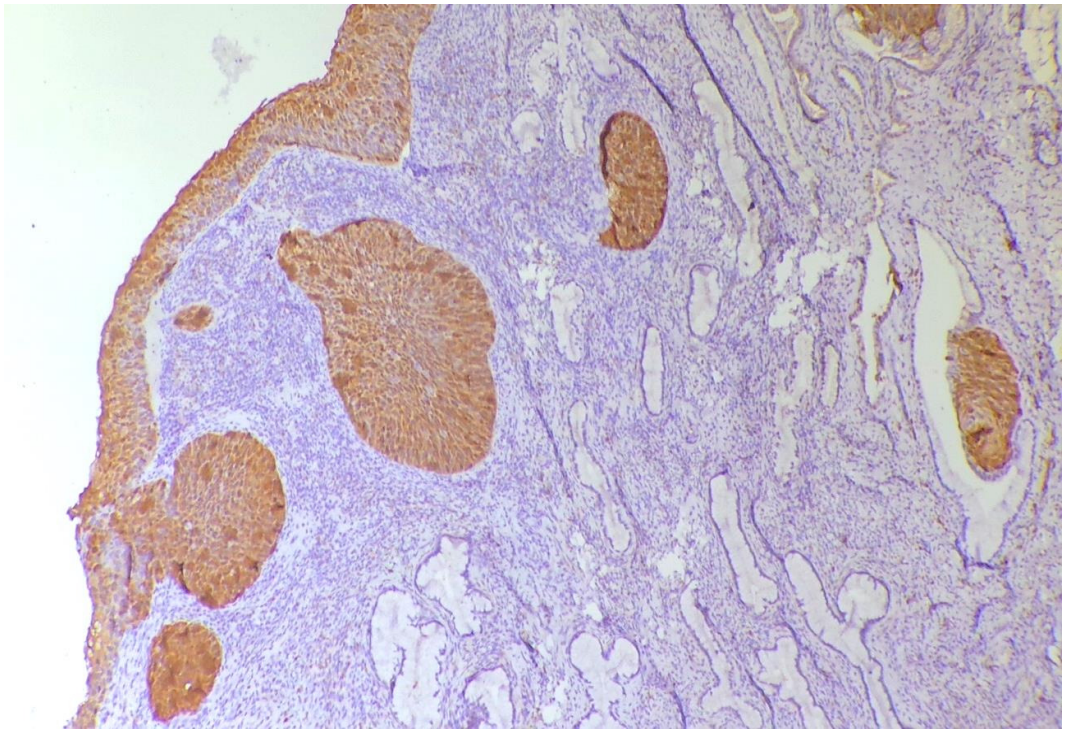


FIGURE- 14. Squamous cell carcincoma with microinvasion (IHC p16, SCANNER VIEW)

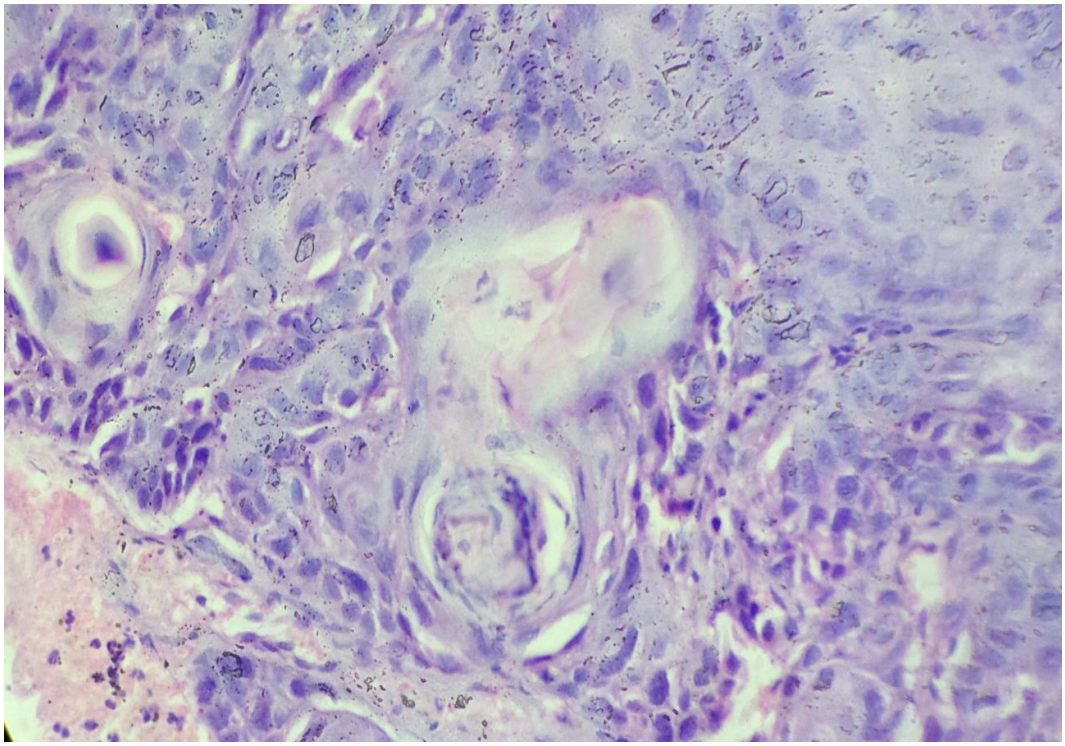


FIGURE- 15.Large cell keratinizing squamous cell carcinoma characterized by malignant keratin pearl. (H&E, 40X)

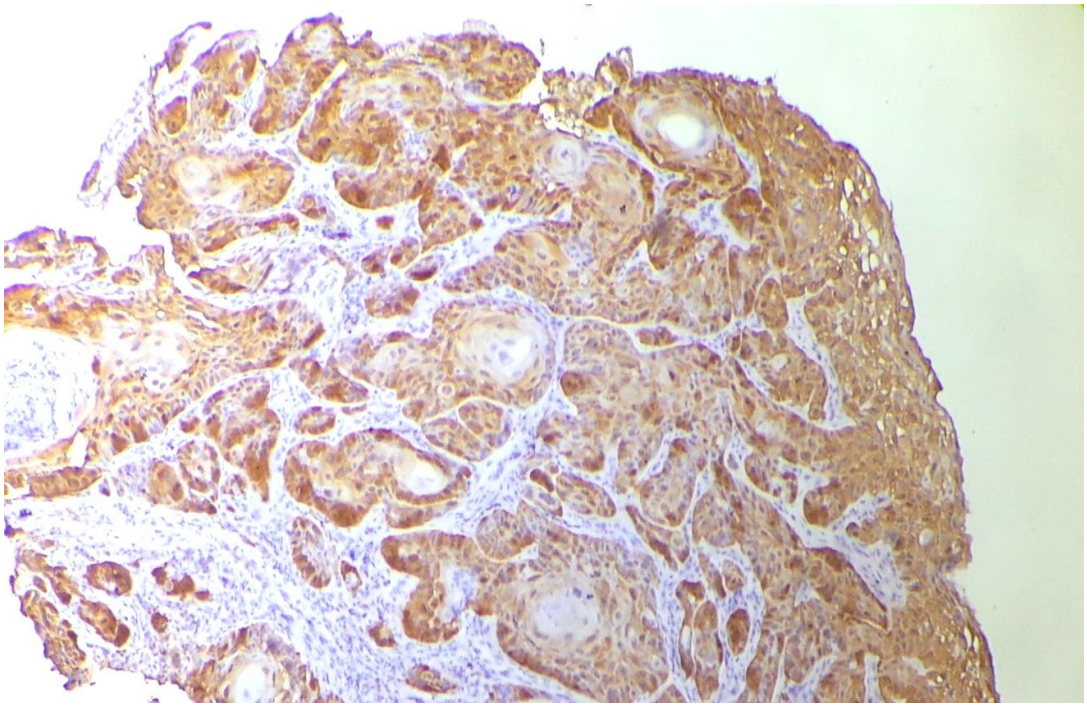


FIGURE- 16.Large cell keratinizing squamous cell carcinoma (IHC p16, 40X)

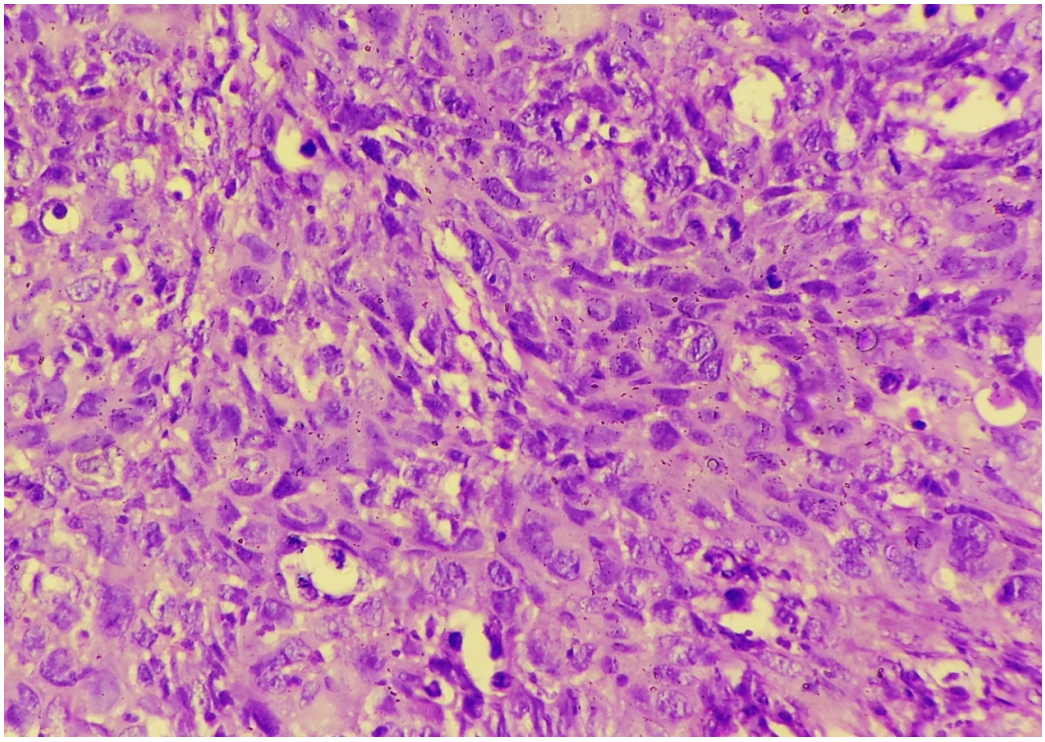


FIGURE- 17.Large cell Non keratinizing Squamous cell Carcinoma composed of irregular nest of large malignant cells (H&E, 40X)

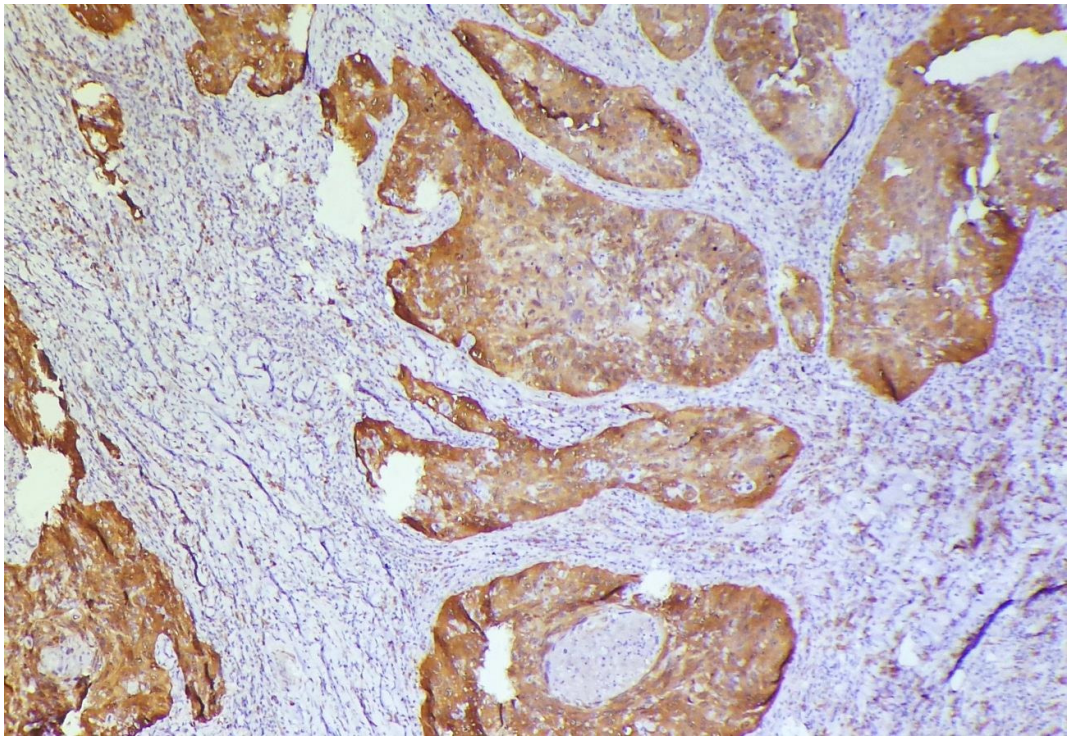


FIGURE-18. Large cell non keratinizing squamous cell carcinoma (IHC p16, 40X)

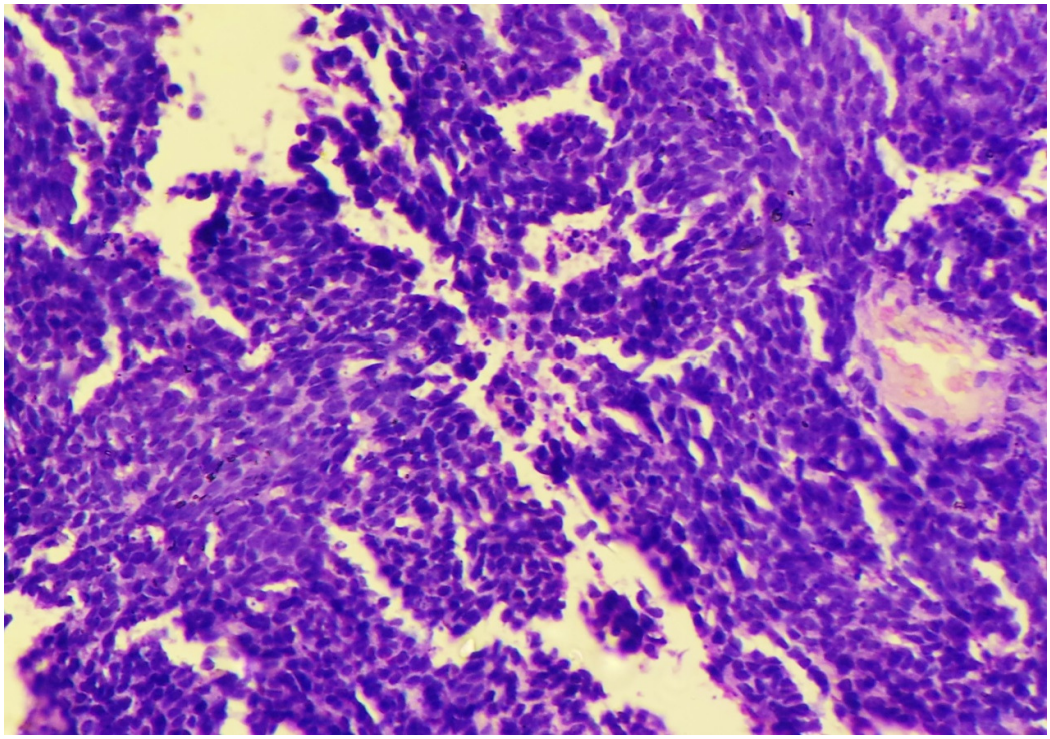


FIGURE-19. Small cell Non keratinizing Squamous cell Carcinoma composed of sheets of small malignant cells(H&E, 40X)

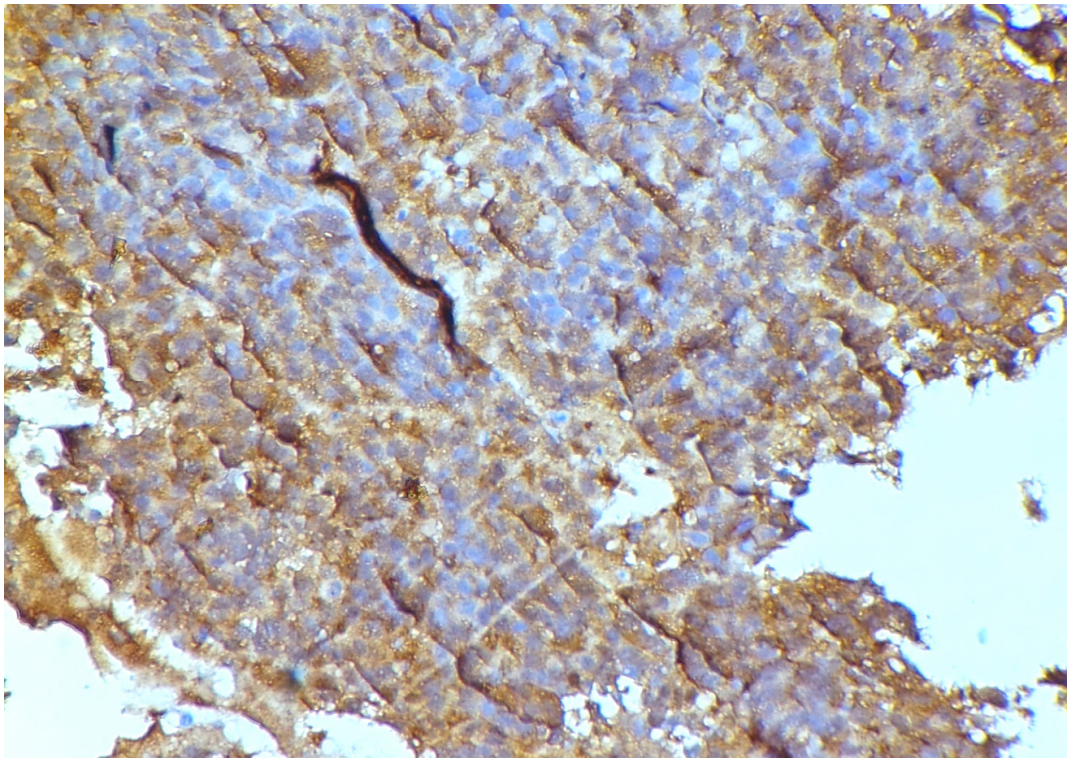


FIGURE- 20.Small cell non keratinizing squamous cell carcinoma (IHC p16, 40X)

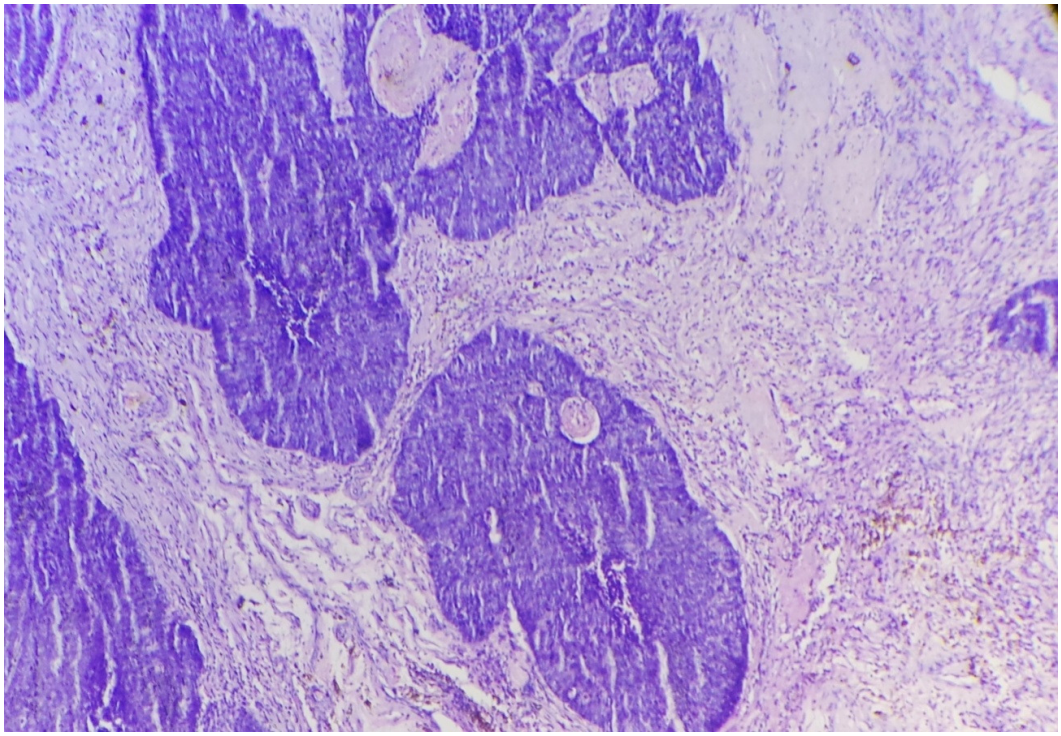


FIGURE- 21.Basaloid squamous cell carcinoma composed of nests of small basal type of squamous cell with peripheral palisading. (H&E, SCANNER VIEW)

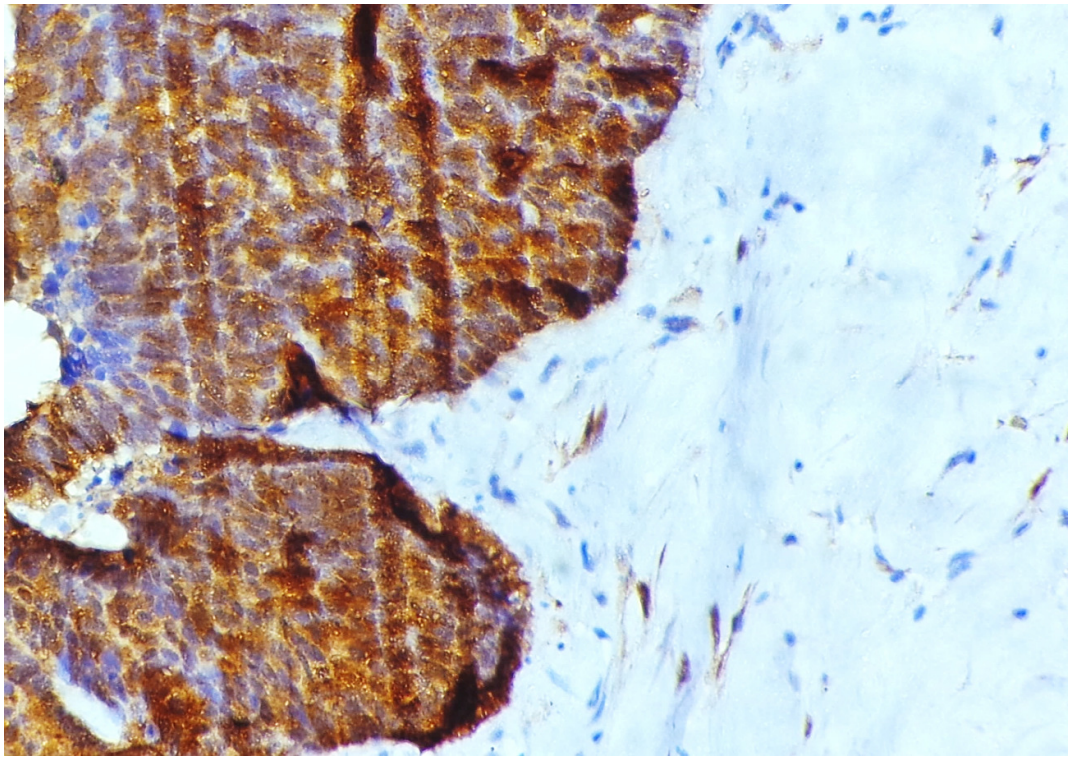


FIGURE-22. Basaloid squamous cell carcinoma (IHC p16, 40X)

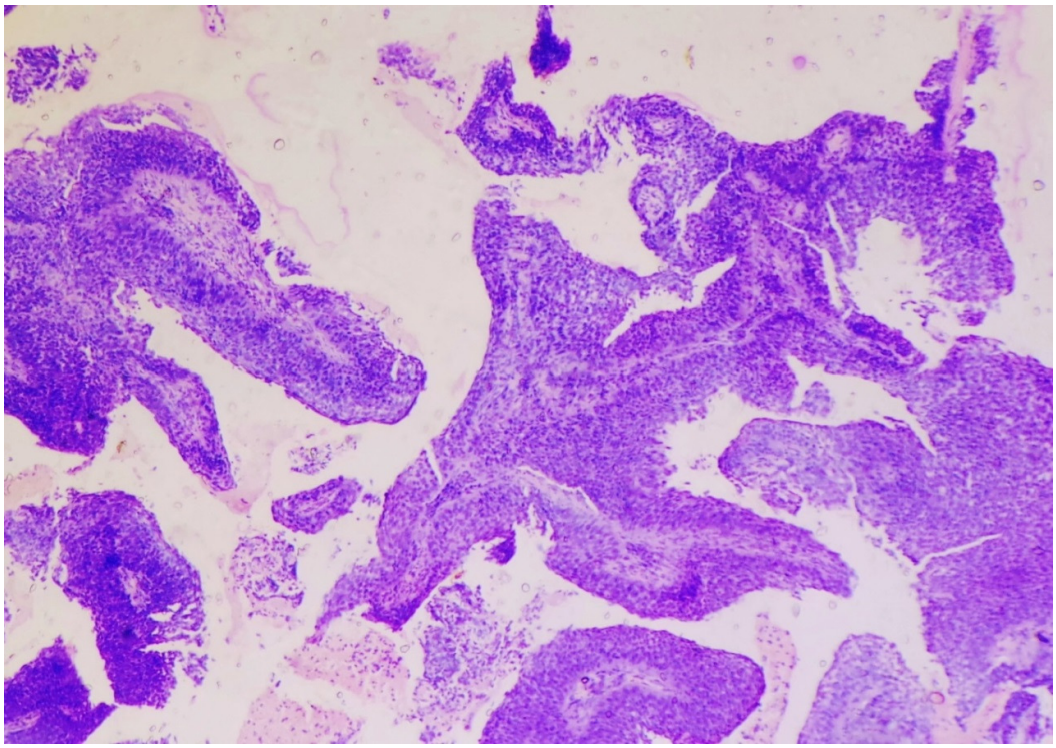


FIGURE- 23.Papillary Squamous cell carcinoma composed of fibrovascular core lined by squamous epithelium resembles CIN (H&E, 40X)

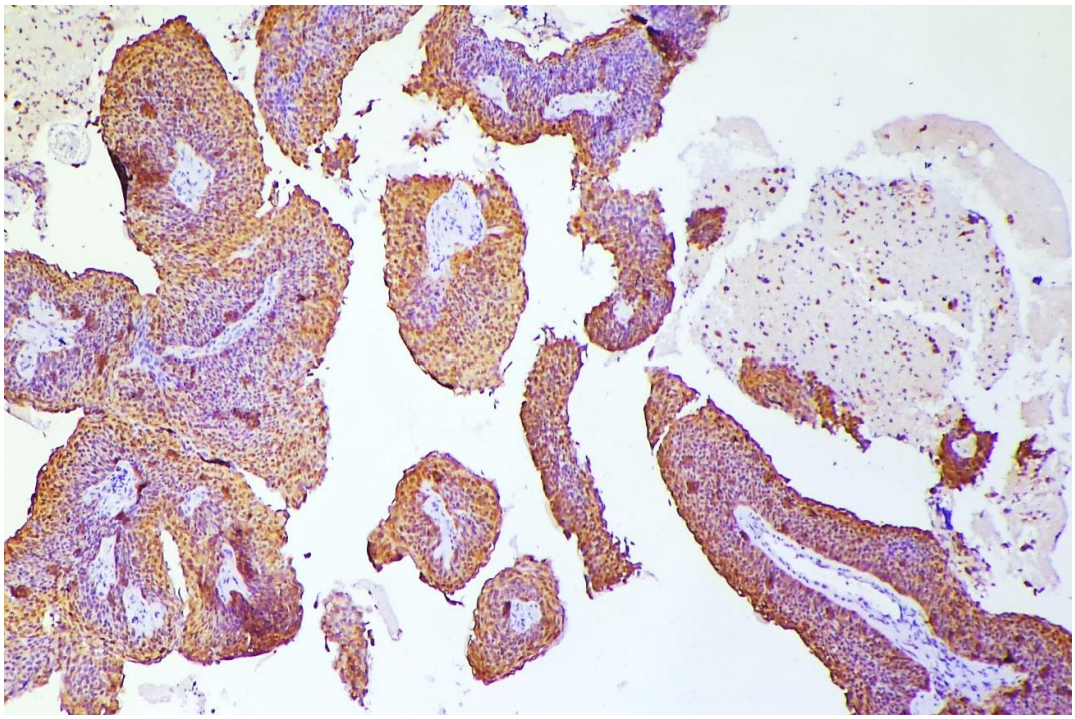


FIGURE- 24. Papillary squamous cell carcinoma (IHC p16, 40X)

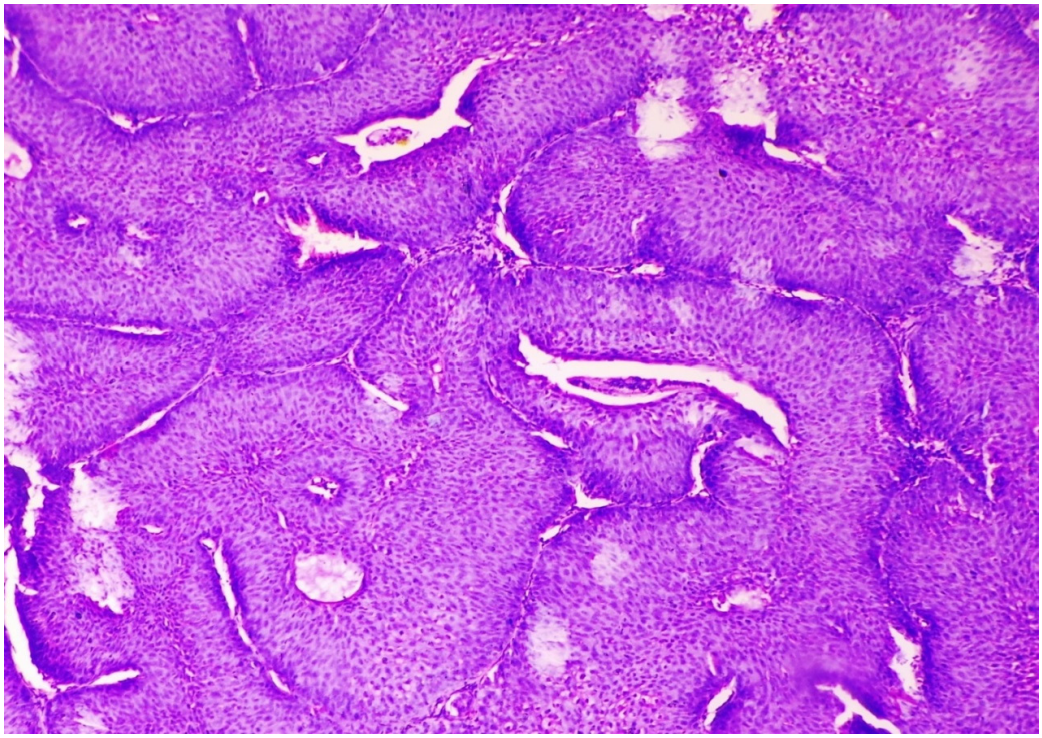


FIGURE- 25, Squamotransitional cell carcinoma composed of papillary architecture with fibrovascular cores lined by a multi-layered, atypical epithelium resembling CIN3 (H&E, SCANNER VIEW)

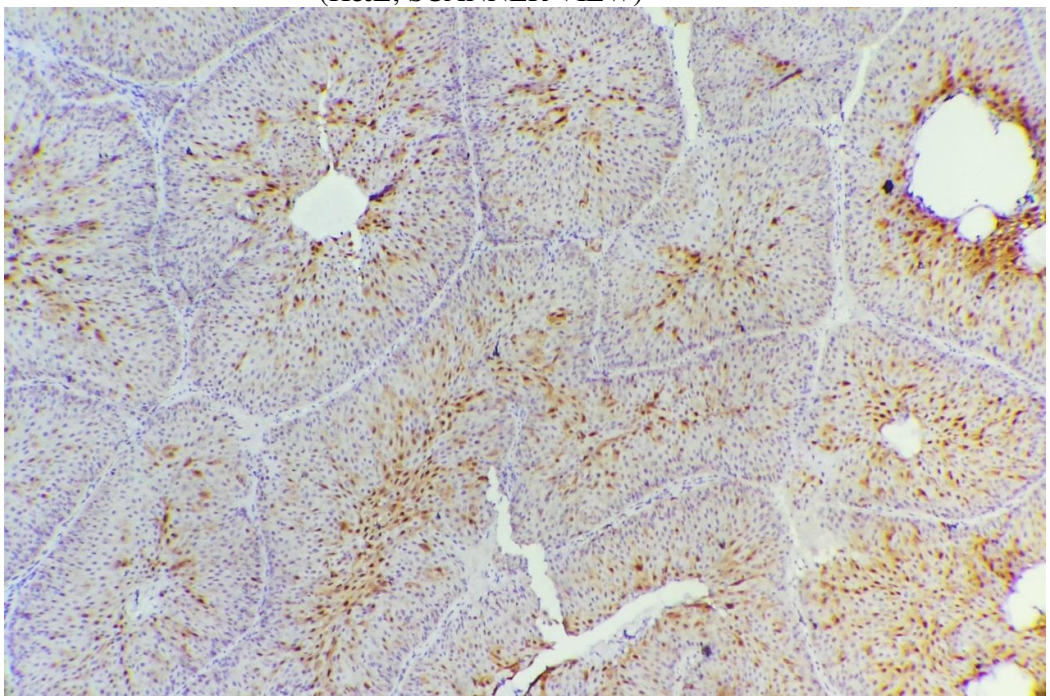


FIGURE- 26.Squamotransistional cell carcinoma (IHC p16, 40X) – NUCLEAR POSITIVITY

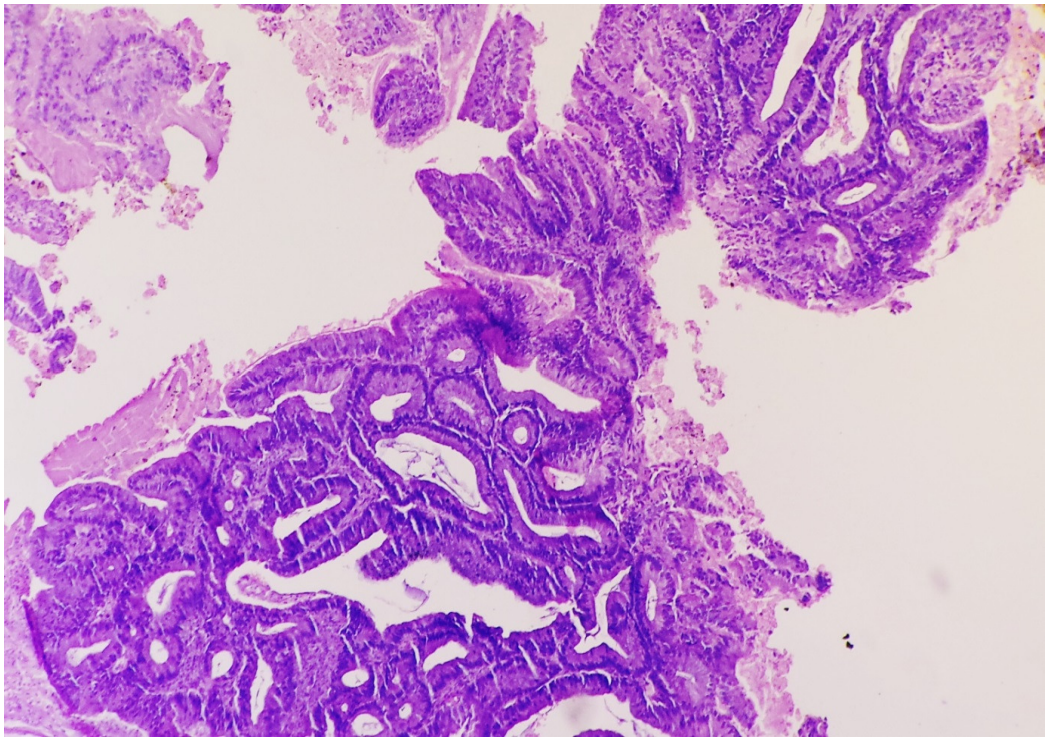


FIGURE-27. Villoglandular Adenocarcinoma composed of fronded growth of thick or thin papillae, which are covered by endocervicaltype of epithelium.(H&E, 40X)

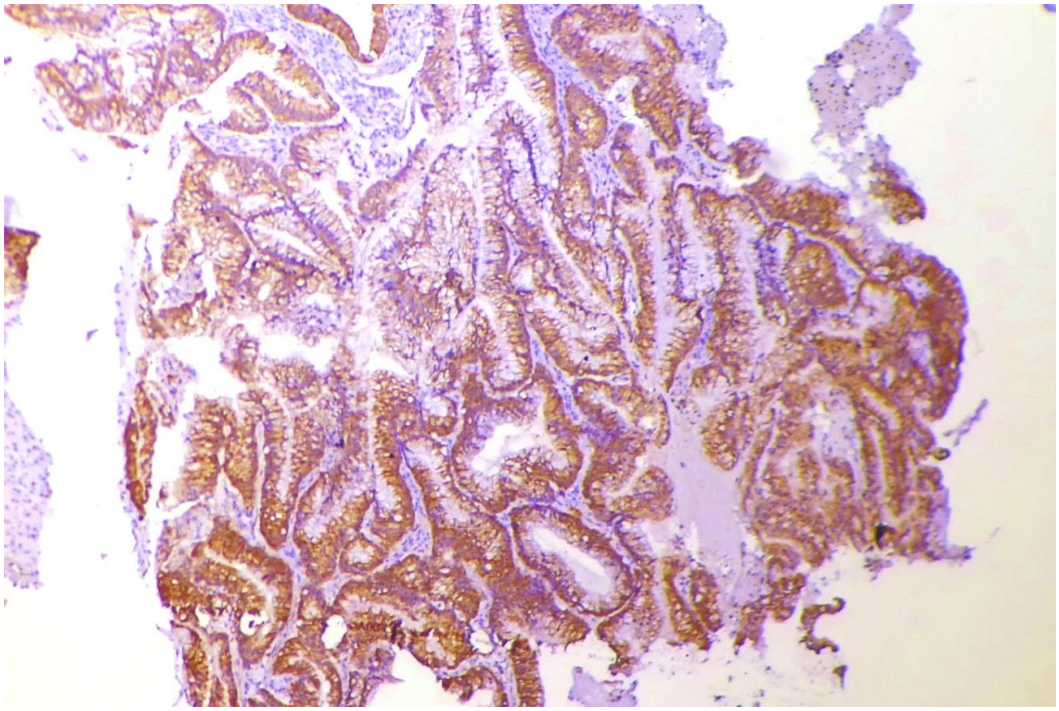


FIGURE-28. Villoglandular adenocarcinoma (IHC p16, 40X) – CYTOPLASMC POSITIVITY

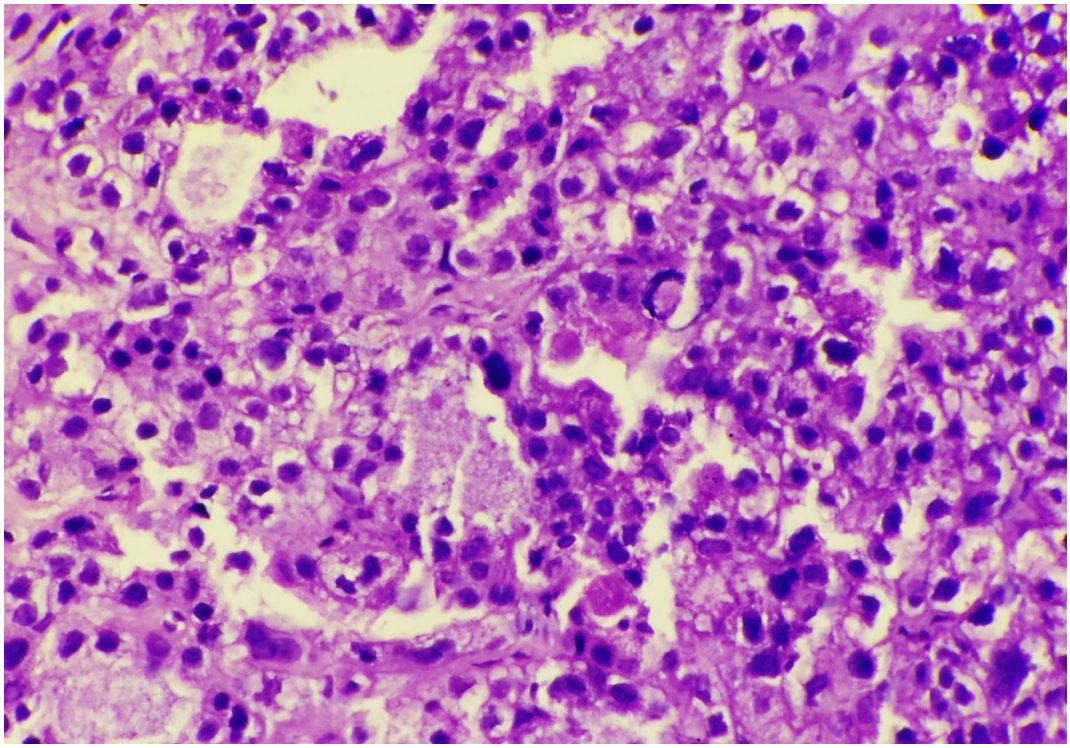


FIGURE- 29.Clear cell adenocarcinoma composed of clear cells or hob nail cells arranged in solid pattern (H&E, 40X)

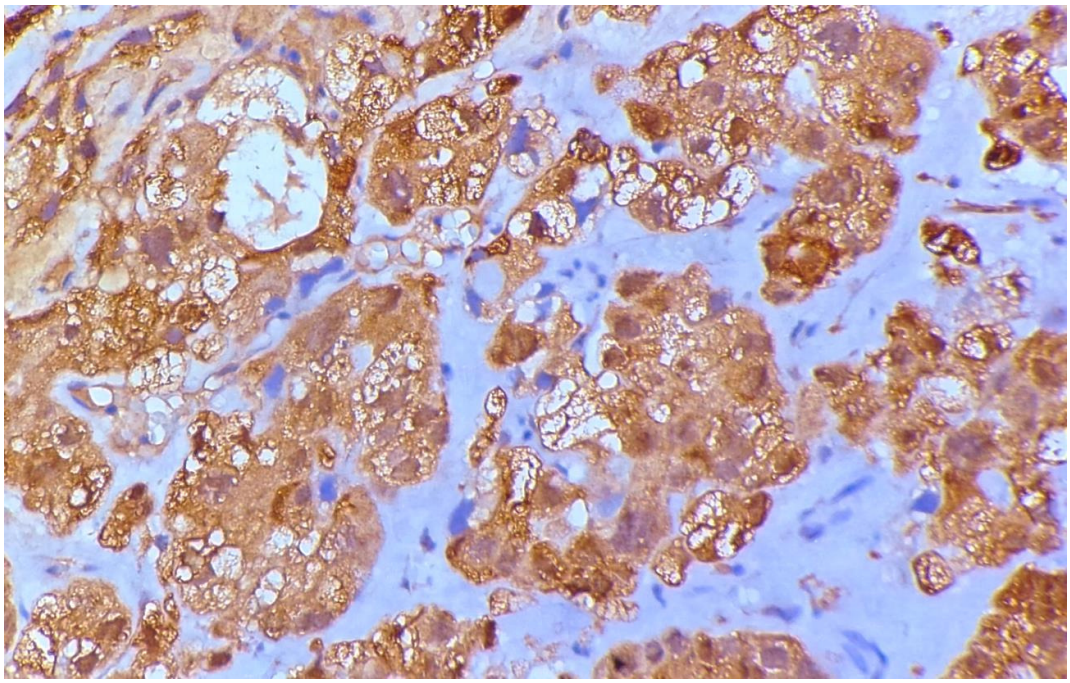


FIGURE- 30.Clear cell adenocarcinoma (IHC p16, 40X)-STRONG INTENSITY OF STAINING

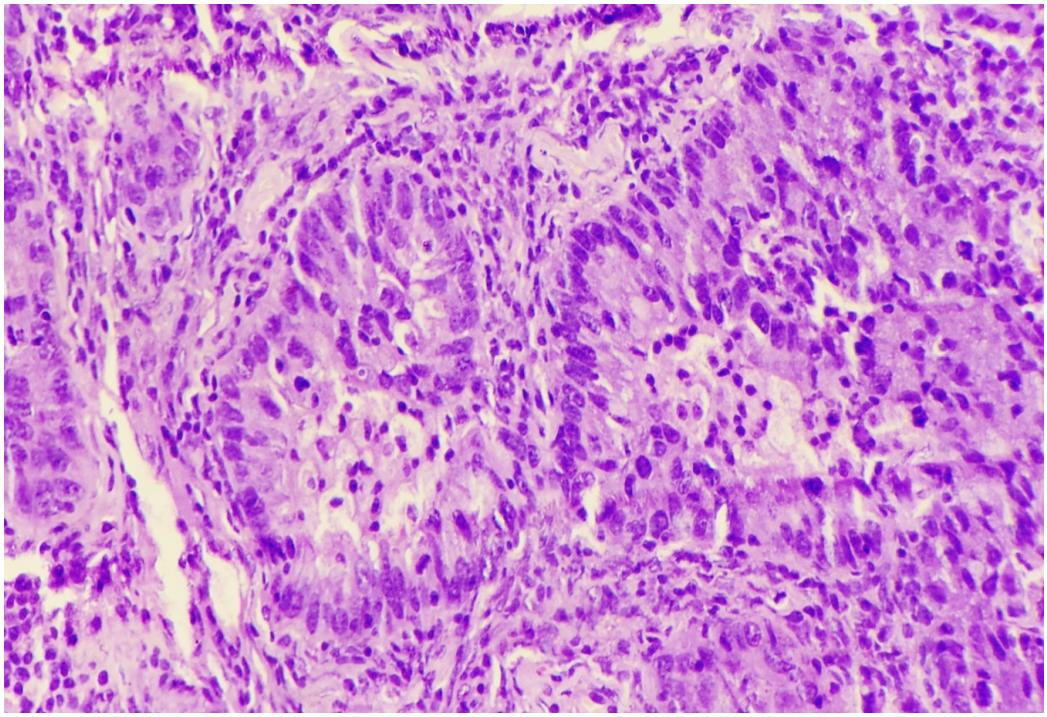


FIGURE- 31.Endocervical adenocarcinoma (grade 2) composed of complex malignant glands lined by tall columnar cells with hyperchromatic nuclei. There is eosinophilic granular cytoplasm and loss of mucin. (H&E, 40X)

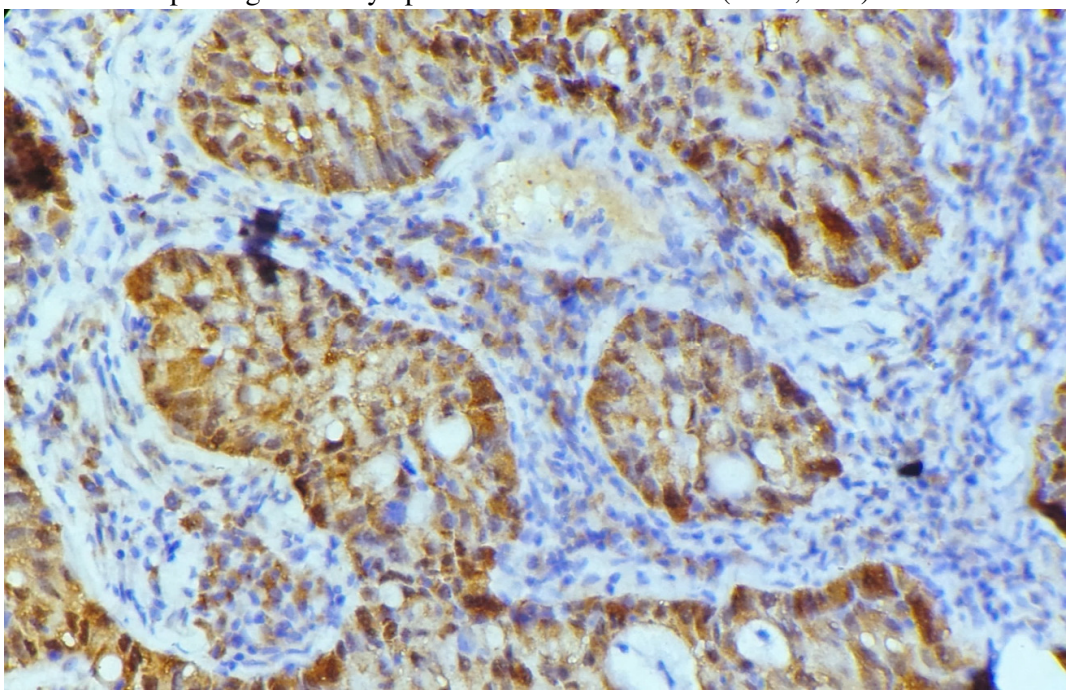


FIGURE- 32.Endocervical adenocarcinoma (GRADE 2) (IHC p16, 40X))

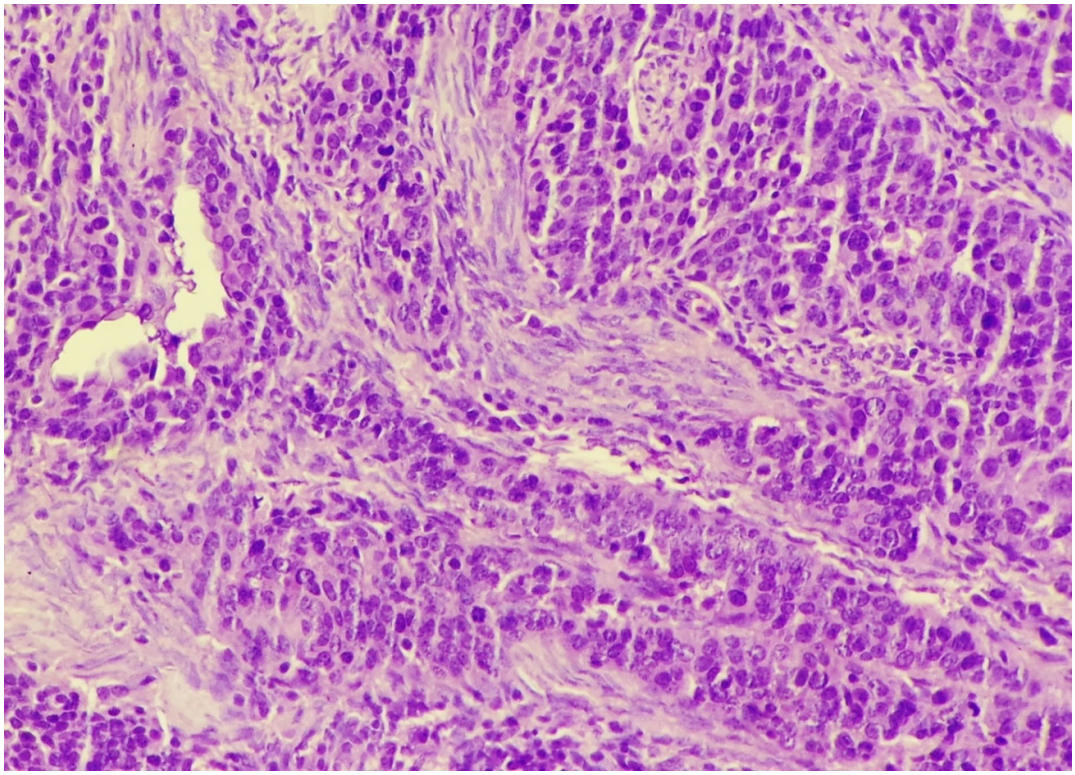


FIGURE- 33.Poorly differentiated Endocervical Adenocarcinoma composed of sheets of malignant cells arranged in ill-defined glandular pattern (H&E, 40X)

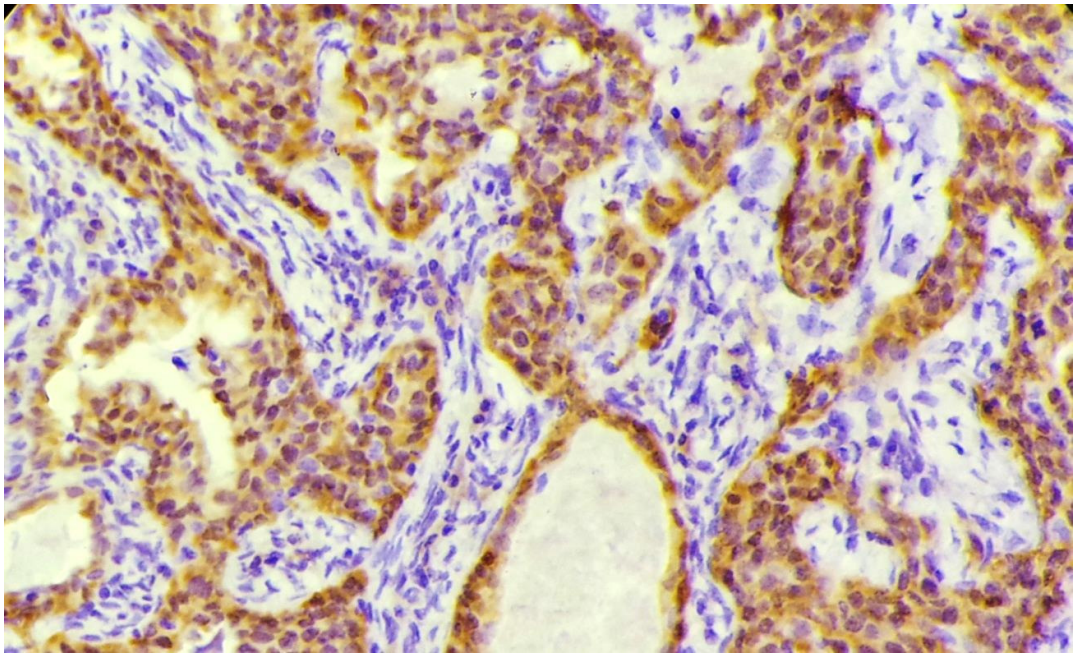


FIGURE- 34.Poorly differentiated endocervical adenocarcinoma (IHC p16, 40X)

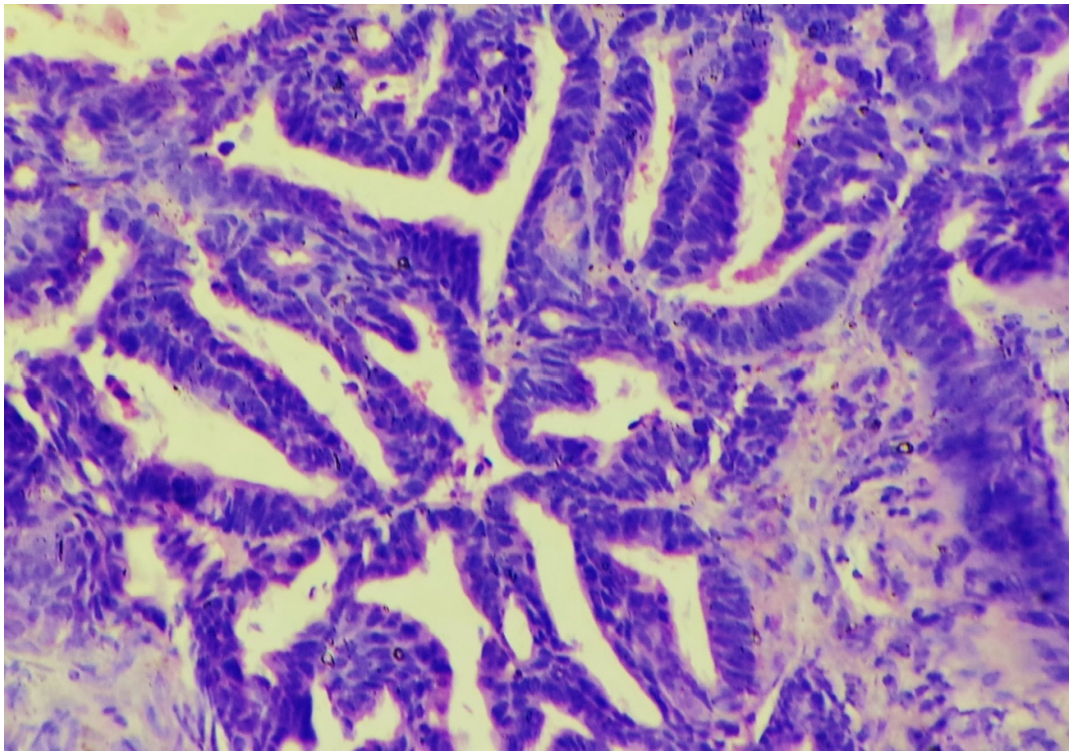


FIGURE- 35.Endometrioid Adenocarcinoma composed of complex glands that are linedby endometrioid-type epithelium with stratified nuclei and minimal intracytoplasmic mucin. (H&E, 40X)

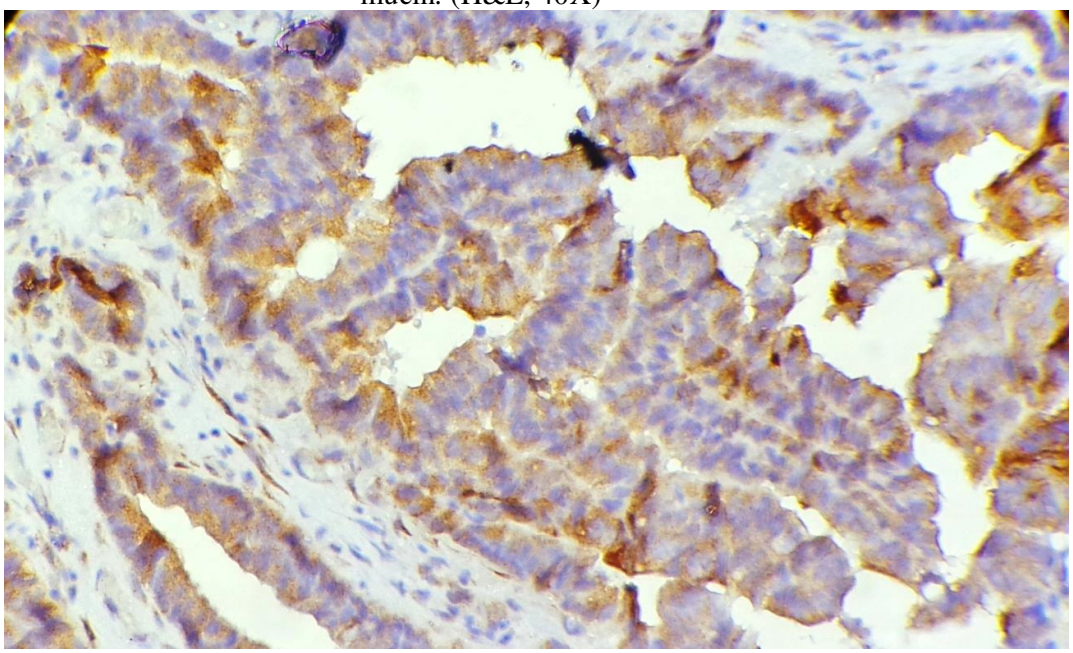


FIGURE- 36.Endometroid adenocarcinoma (IHC p16, 40X)

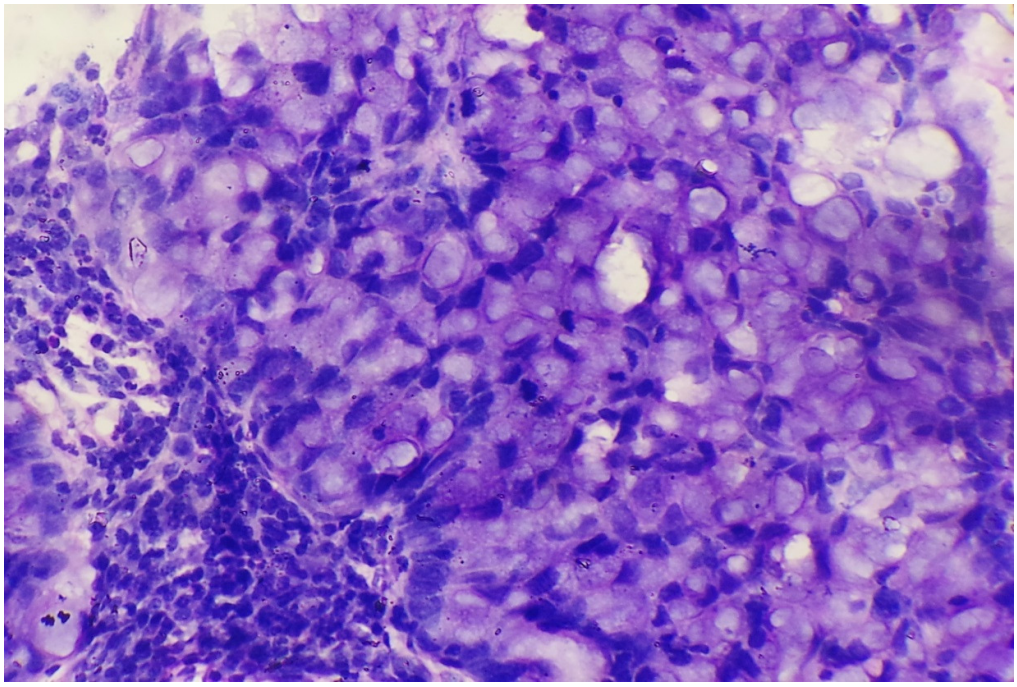


FIGURE-37.Adenosquamous cell carcinoma composed of mixture of malignant glandular and squamous epithelial elements (H&E, 40X)

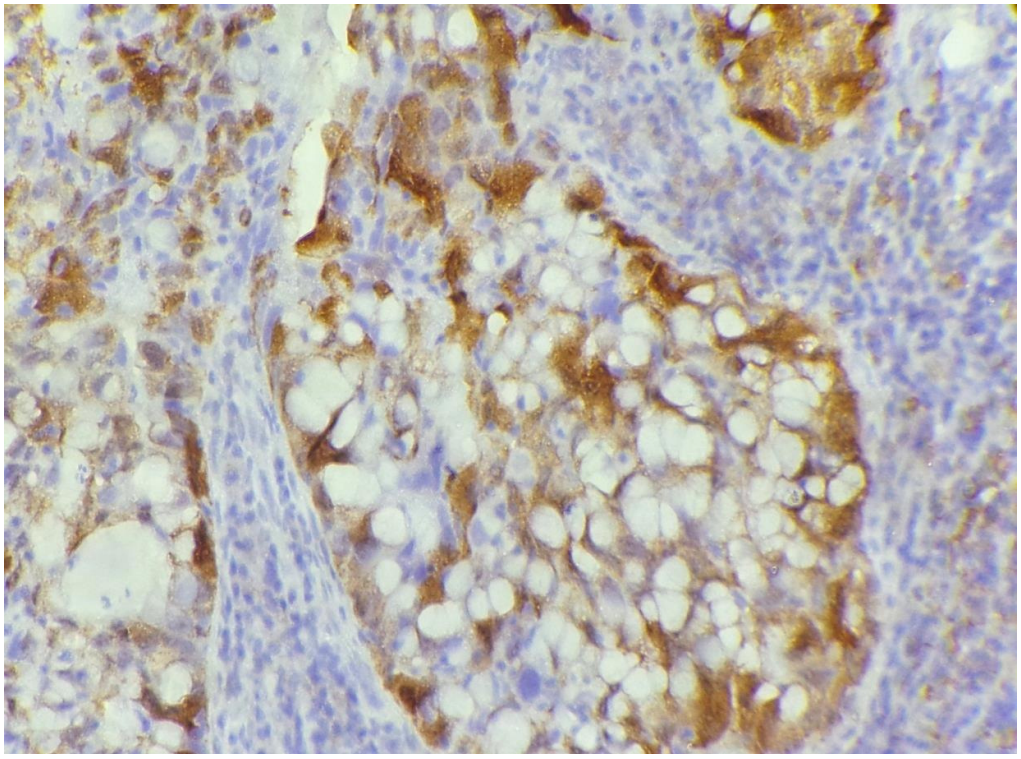


FIGURE-38. Adenosquamous cell carcinoma (IHC p16, 40X)

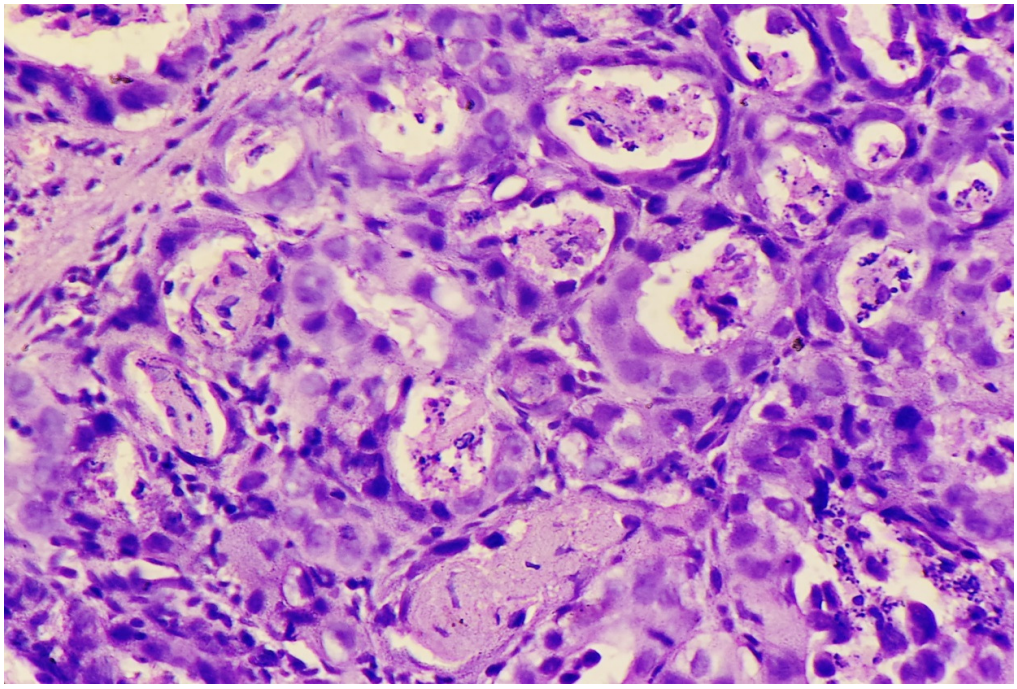


FIGURE- 39.Adenoid squamous cell carcinoma composed of tubular or pseudoglandularpattern,polygonal cells with glassy eosinophilic cytoplasm and focal squamous pearl formation.(H&E, 40X)

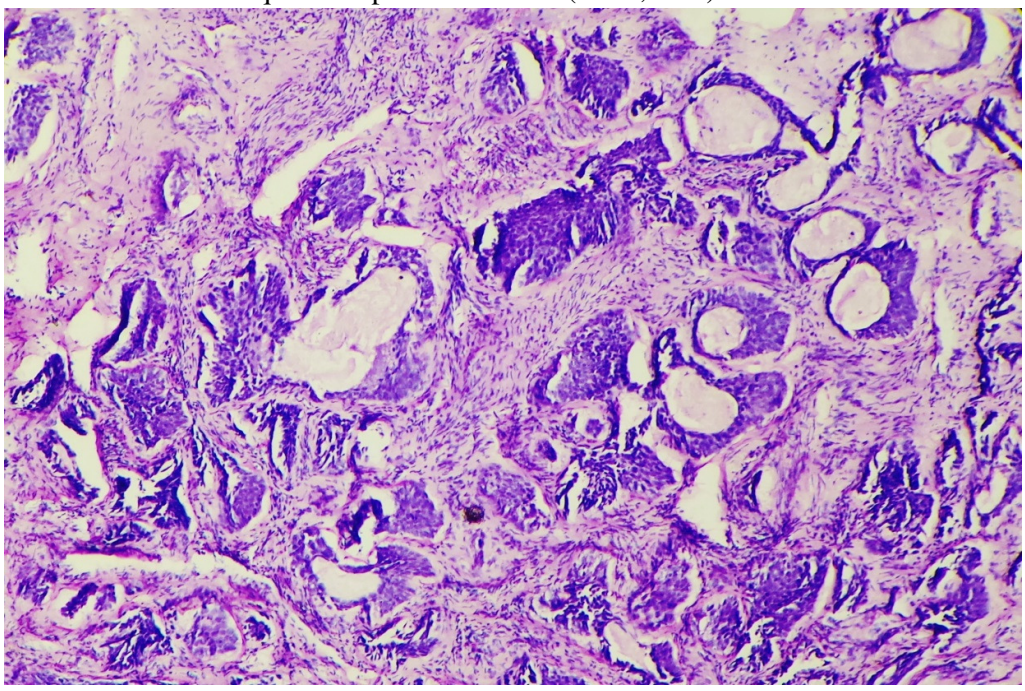


FIGURE- 40.Adenoid basal carcinoma composed of nest of basaloid cells show focal gland formation (H&E, 40X)

DISCUSSION

In Thanjavur District, Tamil Nadu Health System Project - Pilot screening project for cervical cancer is an ongoing programme. Under this programme, 3198 cervical biopsies specimen from various health centres has been received during the year 2013. Present study was carried out on 608 cervical neoplasms cases out of 3198 cervical biopsies for a period of one year from January 2013 to December 2013 in the Department of Pathology, Thanjavur medical college.

In this study, Histologic subtyping of the neoplasms was assigned according to the WHO classification of tumours. Cervical neoplasms comprises 210 cases of CIN 1, 38 cases of CIN 2, 27 cases of CIN 3, a case of CGIN, 318 cases of Squamous cell carcinoma, 9 cases of Adenocarcinoma and 5 cases of other epithelial tumours.

INCIDENCE

India contributes twenty seven percent (77,100) of the total cervical cancer deaths. Globally, age standardized death rate was about 9.5/10,000 population (WHO2009b), which is about one third of global cervical cancer death. In India years of life lost (YLL) were 936.3 in 2000 due to cervical cancer. This reflects that death rate due to cervical cancer in India is highest among the world[84].

6.1 INCIDENCE, AGE GROUP AND BIOMARKER P16^{INK4A} EXPRESSION IN CERVICAL NEOPLASMS IN THE PRESENT STUDY

The following tables and graph shows the comparative study regarding incidence, age group and expression of p16 biomarker. The distribution of cervical neoplasms in different study groups is given table no-20 and graph-19.

TABLE NO - 20

DISTRIBUTION OF CERVICAL NEOPLASMS IN DIFFERENT STUDY GROUPS

STUDY (AUTHORS)	CERVICAL INTRAEPITHELIAL LESIONS		INVASIVE CARCINOMA		TOTAL	
	NO. OF CASES	%	NO. OF CASES	%	NO. OF CASES	%
Grubb&janota et al[53]	49	50.5	48	49.5	97	100
Klaes et al[43]	139	69.8	60	30.1	199	100
Agoff et al[54]	169	76.1	53	23.8	222	100
Nigatu et al[55]	358	13.4	2312	86.5	2670	100
K gupta et al[56]	60	42.5	81	57.4	141	100
Van bogaert et al[57]	439	42.9	584	57	1023	100
Present study	276	45.4	332	54.6	608	100

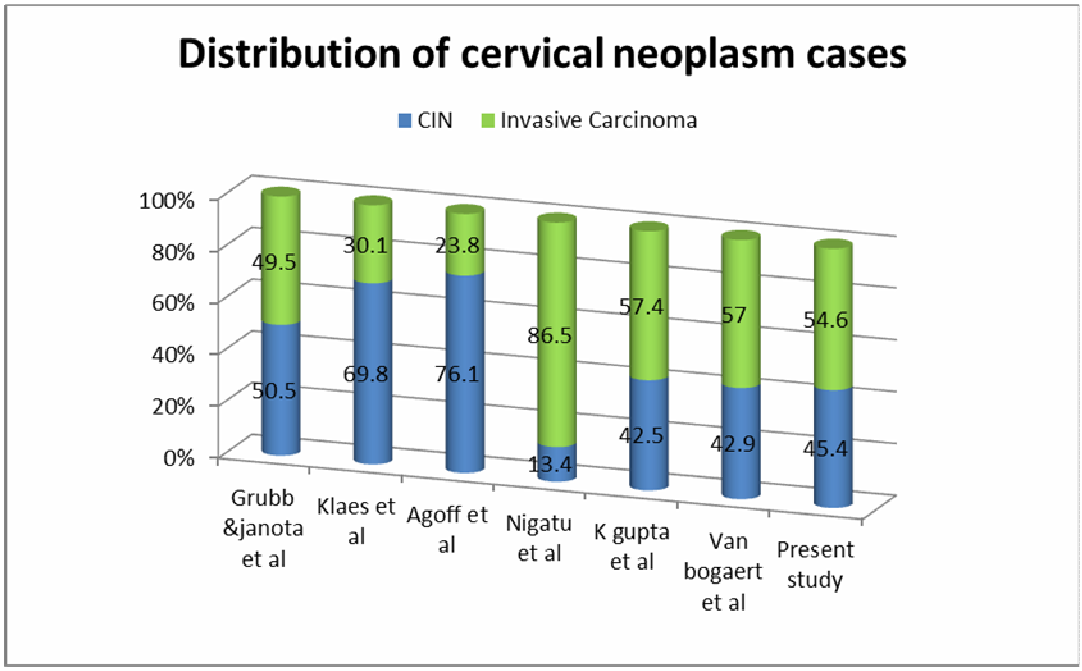
The incidence of cervical neoplasms varies from time to time and place to place in different parts of world.

K gupta et al[56]in their study of 141cases of cervical lesions found 57.4% with invasive carcinoma and 42.5 % with intraepithelial lesions.

In another study by Van bogaert et al[57] CIN 1, CIN 2, CIN 3 cases together constituted 42.9% and invasive carcinoma was 57%.

The present study incidence correlates with Van bogaert et al[57] and K gupta et al[56] studies.

GRAPH NO - 19



AGE-WISE DISTRIBUTION PATTERN

In this present study, age ranges from 21-90 years, with mean age of 46.8 years. In one end of the spectrum, two cases were reported as CIN1 at the age of 21 years and in other end, a case has been reported as carcinoma at the age of 90 years. The peak incidence of cervical intraepithelial neoplasms of cervix was seen in the fourth decade which was two decades earlier than that observed in the carcinoma group of patients.

Munhoz et al[87]studied 54 cases with the age ranges from 22 to 90 years and reported mean age is 45.74 years, which correlates closely with our study

MEAN AGE OF PRESENTATION OF CERVICAL NEOPLASMS

Branca et al[68] in their study found a mean age was 35.5 years and 59.2 years for the occurrence of both CIN and Carcinoma, in patients whose age ranged from 18-79 years. In the present study, mean age of patients with CIN and Carcinoma was 39.7 years and 52.61 years respectively with the range of 21-90 years. The present study is very close to Branca et al[68]study. (Table no-21, graph- 20)

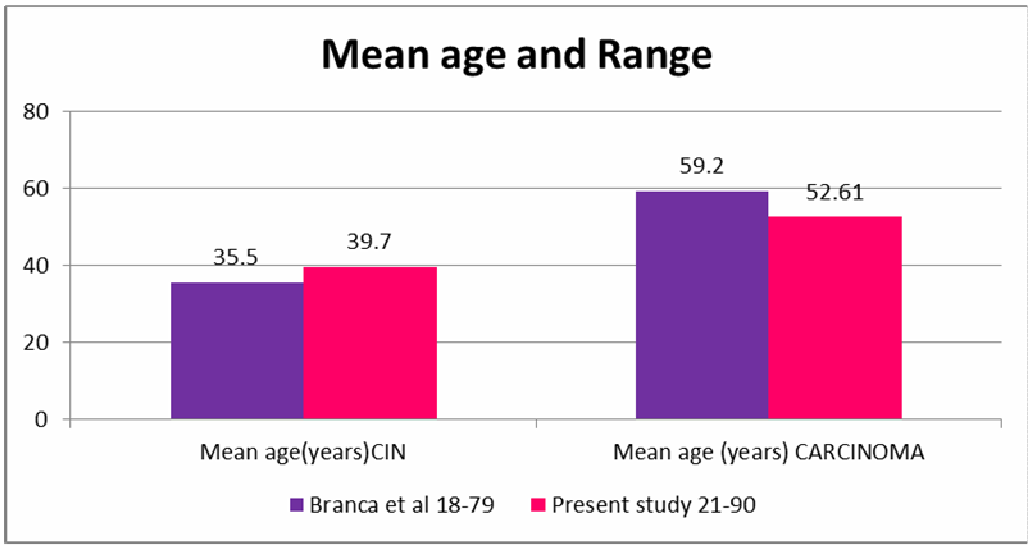
TABLE NO - 21

RANGE AND MEAN AGE OF OCCURRENCE OF CERVICAL NEOPLASMS IN DIFFERENT STUDIES

STUDY (AUTHOR)	RANGE (YEARS)	MEAN AGE (YEARS) CIN	MEAN AGE (YEARS) CARCINOMA
Branca et al[68]	18-79	35.5	59.2
Present study	21-90	39.7	52.61

Thus, the above table suggest that there is a window period of 10 years progression of CIN to carcinoma. As there is a long time interval for progression to malignancy, appropriate intervention strategies like screening with VIA/VILI or PAP smears examination in this intervening period helps in early diagnosis and management of cervical neoplasms.

GRAPH NO - 20



6.2 CERVICAL INTRAEPITHELIAL NEOPLASMS

The distribution of grades of CIN in various studies is given in table no-22, graph- 21, 22.

TABLE NO - 22

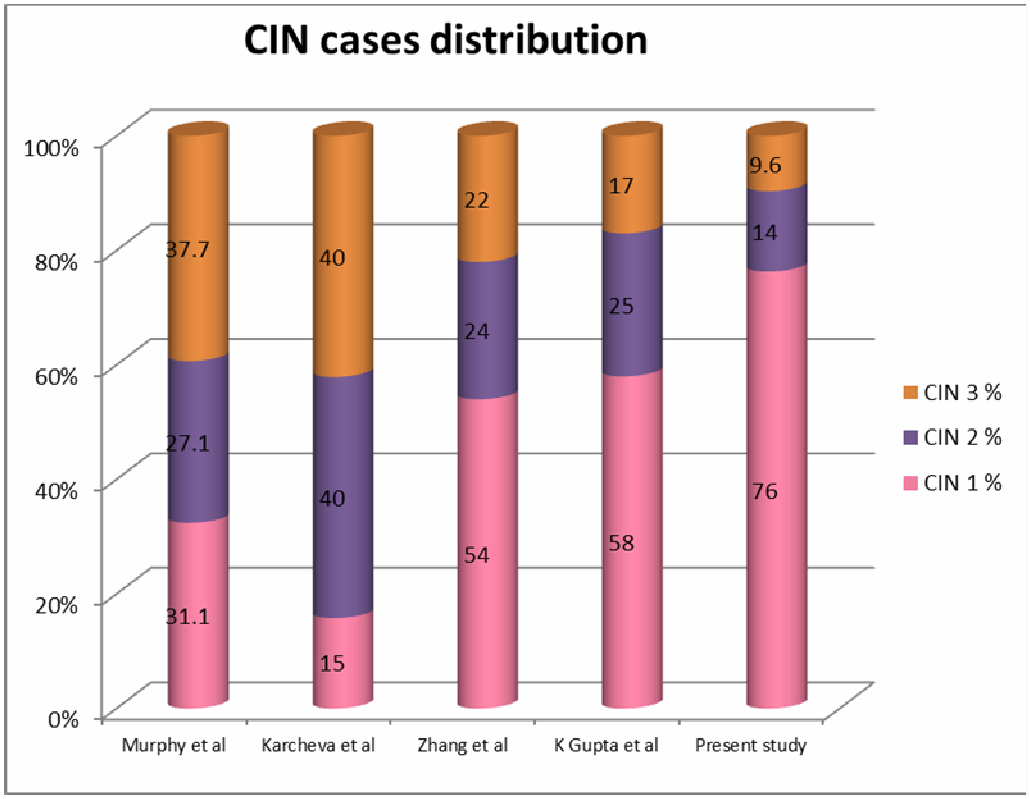
DISTRIBUTION OF GRADES OF CIN IN VARIOUS STUDY GROUPS

STUDIES (AUTHORS)	CIN 1		CIN 2		CIN 3		TOTAL	
	No. of cases	%	No. of cases	%	No. of cases	%	No. of cases	%
Murphy et al[62]	38	32.4	33	28.2	46	39.3	117	100
Karcheva et al[86]	3	15.7	8	42.1	8	42.1	19	100
Zhang et al[58]	157	54	70	24	65	22	292	100
K Gupta et al[56]	35	58	15	25	10	17	60	100
Present study	210	76	38	14	27	10	275	100

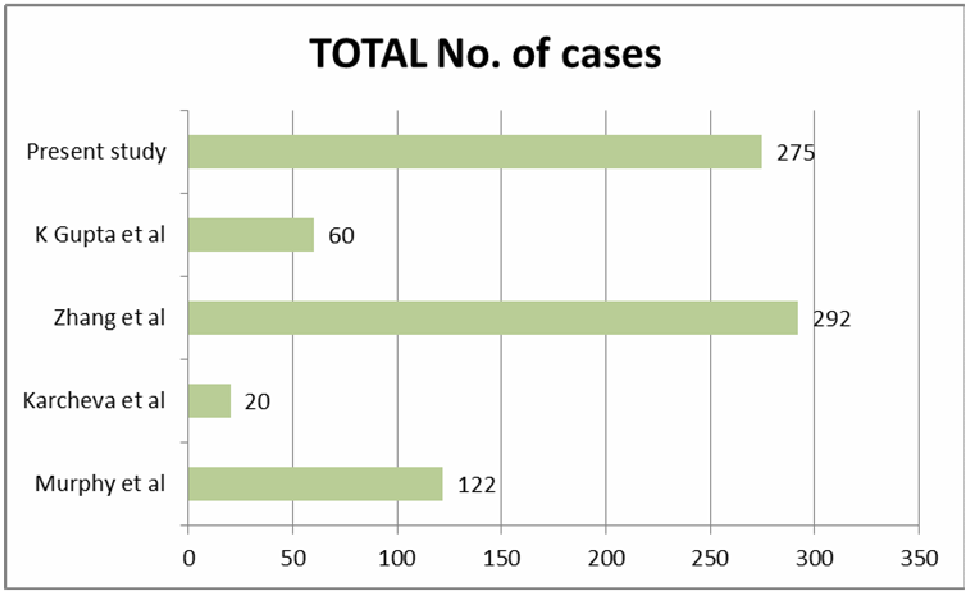
Zhang et al[58]. Reported 54%, 24%, 22% cases of CIN 1, CIN 2, CIN 3 respectively out of 292 cases, whereas K gupta et al[56] reported 58%, 25% and 17% of CIN 1, CIN 2 and CIN 3 out of 60 cases respectively. Murphy et al and karcheva et al also noted varied distribution in cervical intraepithelial neoplasms.

In the present study CIN 1, CIN 2 and CIN 3 constituted 76%, 14% and 10% cases respectively. This comparative study reveals that CIN 1 lesion constituted the majority.

GRAPH NO - 21



GRAPH NO – 22



p16^{INK4a}EXPRESSION

Based on the four parameters (percentage of positive tumour cells, intensity of staining, cellular pattern staining , pattern of staining within epithelium), the present study assessed the p16 immunostaining and observed that, with increasing grades of intra epithelial squamous neoplasia, the percentage of positivity and the intensity of p16 staining also increased.

Analysis of first parameter based on the (table no-16) it will endorse the percentage of positive tumour cells correlates directly with the CIN grading. Here, 60% of CIN 1 cases exhibit 1-5% (1+) tumour cell positivity, 80% of CIN 2 cases exhibiting 5-25%(2+) positivity whereas in 90% of CIN 3 cases 50% exhibiting 5-25%(2+) and 40% exhibiting >25%(3+) positivity.

Analysis of intensity of staining among the different groups based on the (table no-17) 50% cases of CIN 1 lesions show moderate intensity(2+), among CIN 2 lesion 40% exhibits moderate intensity(2+) and 30% exhibits strong intensity, in CIN 3 lesions 50% of cases express strong(3+) positivity. Hence the intensity of staining also correlates directly with the increasing grades CIN lesions.

Based on the distribution of p16 expression with in epithelium, CIN cases were graded as diffuse full thickness, diffuse basal and patchy. Among CIN 1cases, 4 cases showed patchy staining, 6 cases showed diffuse basal staining, In CIN 2 cases, predominantly diffuse basal pattern was observed (7/10). In CIN 3 cases, 50% cases expressed diffuse full thickness staining. when compared with Kumar et al study and Lulinhu et al showed that CIN 1 cases expressed predominantly patchy staining whereas CIN3 exhibited diffuse full thickness staining pattern.

Hence the above factors can be taken for standardization of p16 immunostaining as supported by other studies done by Kumari et al[42], Klaes et al[43] and R gupta et al[44]

6.3 CERVICAL GLANDULAR INTRAEPITHELIAL NEOPLASIA

Murphy et al[62] studied 5 cases of CGIN out of 153 cases, where Karcheva et al[86]reported a case of CGIN out of 54 case. From the present study a case of CGIN was reported out of 608 cases.

Mean age of presentation –CGIN

Kurian and Nafussi et al[69] studied 27 cases of CGIN and reported mean age for low grade CGIN was 39 years whereas Plaxe and saltzstein et al[64] conducted study in a large series of 5845 patients and found that mean age of CGIN was 38.8 years. Fadwa J.et al[86] studied 167 cases of cervical carcinoma and the age incidence was 58 years. But in this study, single case of CGIN was reported and the age was 55 years which coincides with the age group of Fadwa J. et al[86](table no-23)

TABLE NO - 23

MEAN AGE FOR CGIN IN DIFFERENT STUDIES

STUDY (AUTHOR)	MEAN AGE(YEARS)
Kurian and nafussi et al[69]	39
Brown and wells et al[70]	36.9
Plaxe and saltzstein et al[64]	38.8
Fadwa J.et al[86]	58
Present study	55

p16^{ink4a} in cervical glandular intraepithelial neoplasia

The p16 immunostaining showed strong intensity of staining (figure-12). The distinction of carcinoma in situ from benign mimics, especially tubo-endometrial metaplasia, endometriosis, and micro glandular hyperplasia is difficult. Here, p16 plays a major role by differentiating the dysplastic cells[45].

In this study, a case of CGIN expressed >25% positive tumour cells with p16, similar results were found in Negri et al[80], Karcheva et al[86], Murphy et al study. In klaes et al[43] reported 85% positivity. The intensity of staining showed strong diffuse nuclear cytoplasmic positivity.

6.4 CERVICAL CARCINOMA

The distribution of cervical carcinoma in different study groups is given table no-24, graph-23.

TABLE NO - 24

DISTRIBUTION OF CERVICAL CARCINOMA IN DIFFERENT STUDY GROUPS

Study(author)	Squamous cell carcinoma		Adenocarcinoma		Adenosquamous cell carcinoma		Total	
	No. of cases	%	No. of cases	%	No. of cases	%	No. of cases	%
Balkachewnigatu et al[55]	2182	94.3	104	4.4	26	1.1	2312	100
K gupta et al[56]	75	92.5	3	3.7	3	3.7	81	100
Takaakisano et al[59]	39	72.2	9	16.6	6	11	54	100
Morelva et al[76]	62	76.5	19	23.4	ND		81	100
Present study	318	95.8	9	2.7	4	1	331	100

The invasive carcinoma of cervix is classified into three main group's namely Squamous cell carcinoma, adenocarcinoma and other epithelial tumours.

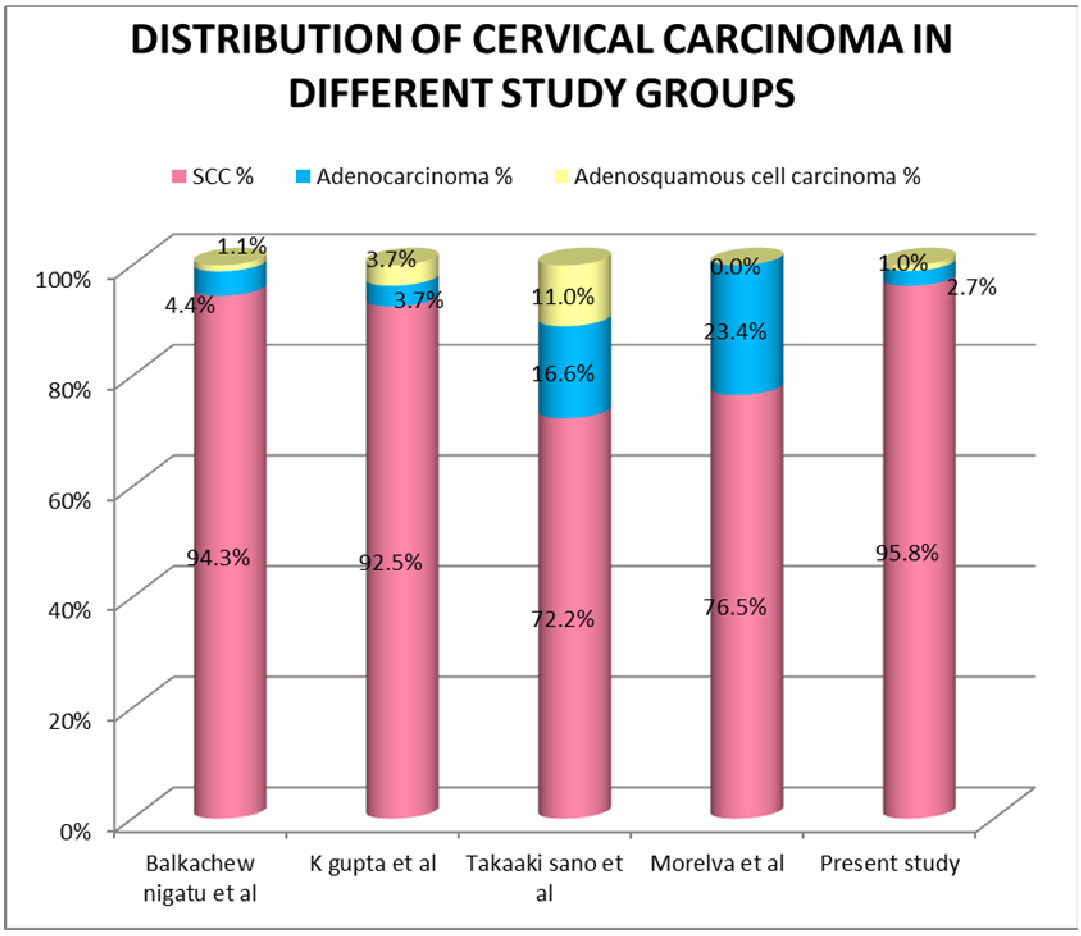
Balkachew nigatu et al[55], in their study found 94.3% (2182) of SCC, 4.4% (104) of Adenocarcinoma and 1.1% (26) of Adenosquamous cell carcinoma in total number of 2312 cases.

K gupta et al[56]also had similar observations in their study with 92.5% of SCC a, 3.7% of Adenocarcinoma and 3.7% of Adenosquamous cell carcinoma.

Takaakisano et al[59] and Morelva et al[76] also found varied incidence in carcinoma of cervix.

In the present study, 95.8% (318) cases of SCC group, 2.7% (9) cases of Adenocarcinoma group and 1 %(4) case of Adenosquamous cell carcinoma. This is in accordance with other studies, where SCC is the predominant histological type.

GRAPH NO - 24



DISTRIBUTION OF PATIENTS ACCORDING TO AGE GROUP:

COMPARISON OF PATIENTS ACCORDING TO INDIVIDUAL AGE-GROUP DISTRIBUTION

Comparison of cervical cancer patients among individual age groups between the present study at Thanjavur and a cancer registry in AIIMS[75] showed similar incidence trends with respect to age group. In both studies majority of cases belonged to 50-59 years age group followed in descending order by 40-49 years, 60-69 years, 30-39 years and 70-79 years.(table no-25, graph-24).

TABLE NO - 25

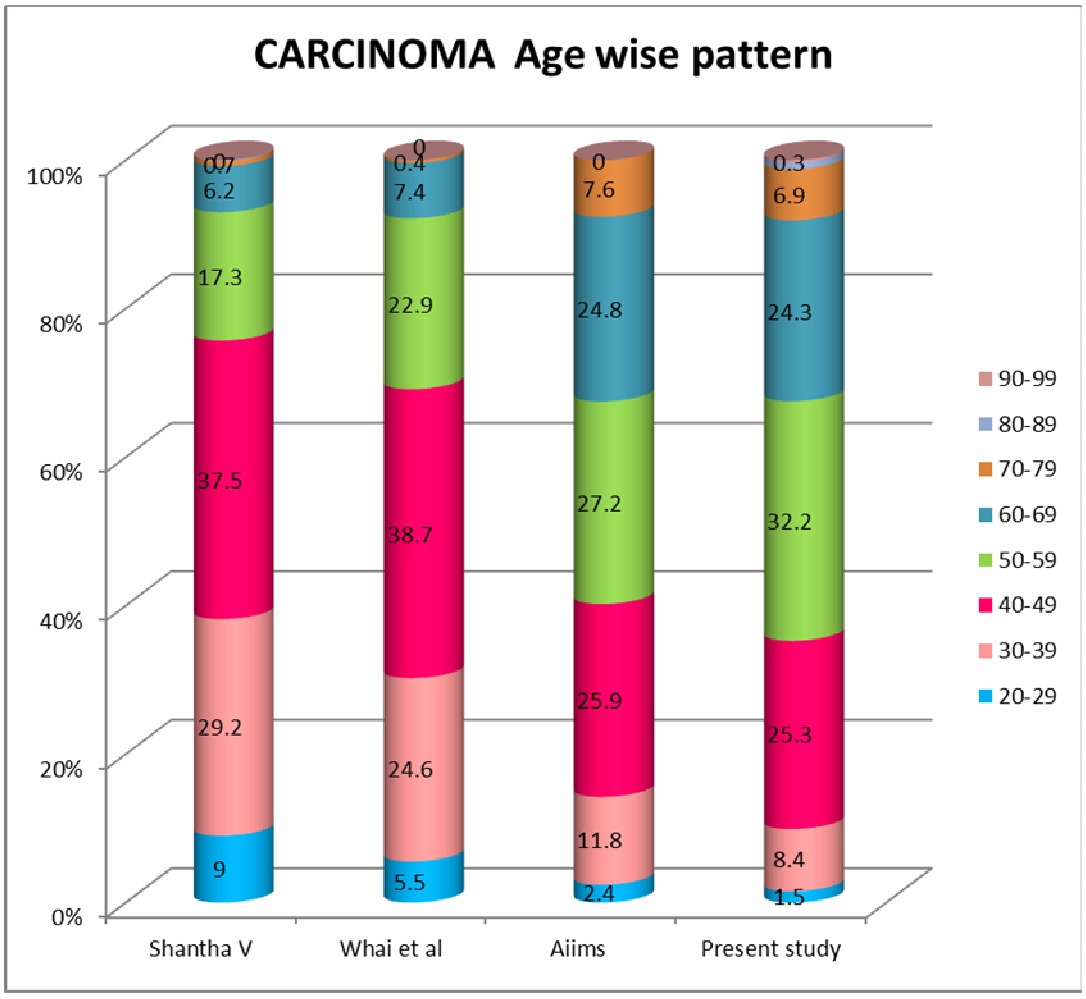
AGE WISE INCIDENCE PATTERN OF INVASIVE CARCINOMA IN VARIOUS STUDIES

AGE (YEARS)	SHANTHA V[60]		WHAI ET AL[67]		AIIMS [75]		PRESENT STUDY	
	NO. OF CASES	%	NO. OF CASES	%	NO. OF CASES	%	NO. OF CASES	%
20-29	93	9	38	5.5	22	2.4	5	1.5
30-39	299	29.2	170	24.6	106	11.8	28	8.4
40-49	384	37.5	267	38.7	233	25.9	84	25.3
50-59	176	17.3	158	22.9	245	27.2	107	32.2
60-69	63	6.2	51	7.4	223	24.8	81	24.3
70-79	8	0.7	3	0.4	69	7.6	23	6.9
80-89	2	0.1	-	-	-	-	3	0.9
90-99	-	-	-	-	-	-	1	0.3
Total	1025	100	689	100	898	100	332	100

- All the fact shows that cervical neoplasms are common in 5^{th} to 7^{th} decades.

These findings are in accordance with already existing fact that cervical cancer is due to persistence of high risk human papilloma virus. Thus persistence of integrated HPV infection results in carcinogenic transformation.

GRAPH NO - 25



In this study vast majority of patients 81% (272) belonged to 5th to 7th decade of life. This is in line with AIIMS data (78%) [75].

C.S. Herrington et al[81] reports that peak age incidence for invasive carcinoma is 60-64 years.

TABLE NO - 26

AGE GROUP WITH MAXIMUM INCIDENCE OF INVASIVE CARCINOMA CERVIX IN DIFFERENT STUDY GROUP

STUDIES (AUTHORS)	AGE GROUP WITH MAJORITY OF CASES (YEARS)
Shantha V[60]	40-49
Chaudhary et al[61]	41-50
Wahi et al[67]	45-54
AIIMS[75]	50-59
Present study	50-59

From the above data it has been inferred that invasive carcinoma occurs predominantly in the age group of 50-59 years. Similar findings were seen in studies done by Wahi et al[67] and AIIMs cancer registry[75.](table no-26)

Shantha V[60] et al reported that maximum number of patients belonged to 40-49 years. However Chaudhary et al[61] showed majority of cases belonged to 41-50 years age group similarly.

6.5 SQUAMOUS CELL CARCINOMA

Squamous cell carcinoma constitutes 80-90% on cervical carcinoma. It is graded into three grade based on Modified Broder's grade. Distribution of modified Broder's grading of squamous cell carcinoma in different study groups is given in table no-27, graph-25, 26.

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TABLE NO - 27

DISTRIBUTION OF MODIFIED BRODER'S GRADING OF SQUAMOUS CELL CARCINOMA IN DIFFERENT STUDY GROUPS

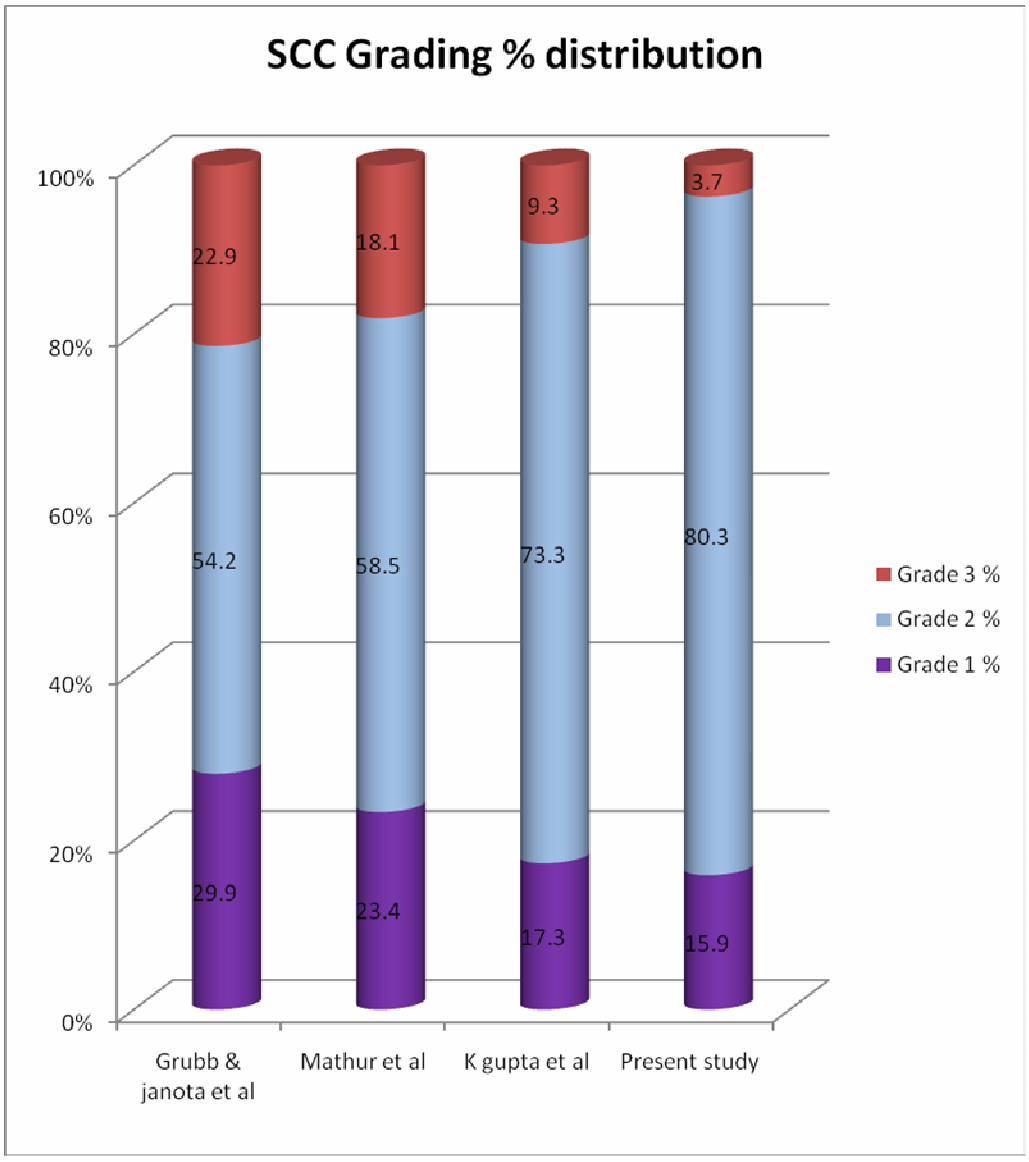
STUDY (AUTHOR)	GRADE 1		GRADE 2		GRADE 3		TOTAL	
	NO. OF CASES	%	NO. OF CASES	%	NO. OF CASES	%	NO. OF CASES	%
Grubb &janota et al[53]	11	29.9	26	54.2	11	22.9	48	100
Mathur et al[77]	66	23.4	165	58.5	51	18.1	282	100
K gupta et al[56]	13	17.3	55	73.3	7	9.3	75	100
Present study	48	15.9	242	80.3	11	3.7	301	100

K gupta et al[56] in their study of 75 cases of conventional SCC reported 13(17.3%), 55(73.3%) and 7(9.3%) cases of well, moderately and poorly differentiated types respectively.

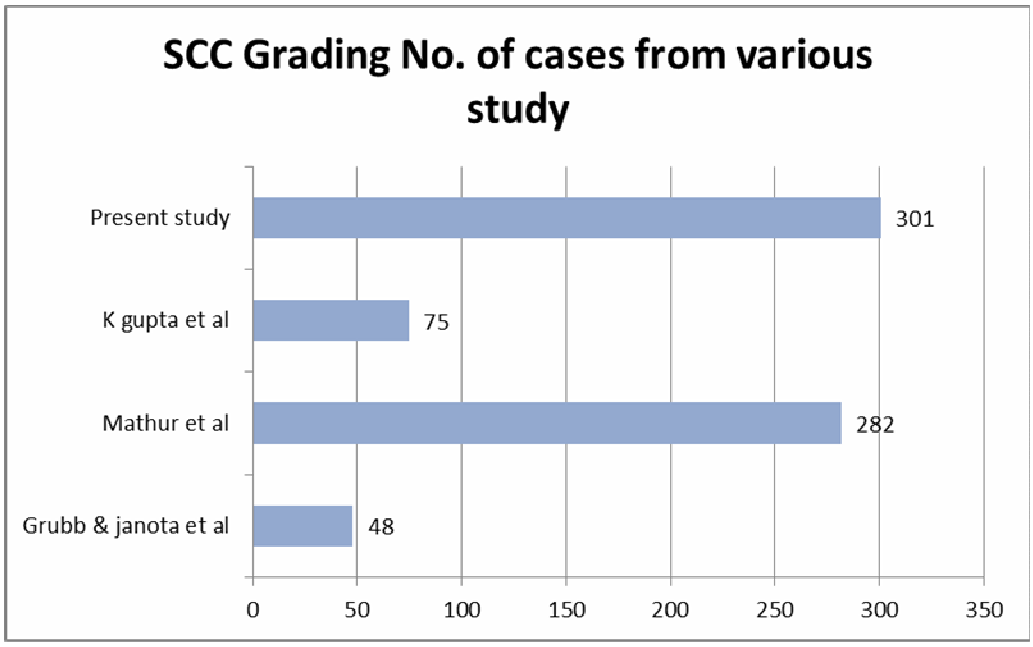
Grubb & janota et al[53] and Mathur et al[77] study also showed moderately differentiated SCC being the predominant histological type.

In this present study, moderately differentiated carcinoma constitutes 80 %(242) whereas 15.9% (48) of well differentiated and 3.7 %(11) cases of poorly differentiated SCC were observed.

GRAPH NO – 25


SCC Grading % distribution
100%
80%
60%
40%
20%
0%
22.9
54.2
29.9
18.1
58.5
23.4
9.3
73.3
17.3
3.7
80.3
15.9
Grade 3 %
Grade 2 %
Grade 1 %
Grubb & janota et al
Mathur et al
K gupta et al
Present study

GRAPH NO - 26



MEAN AGE FOR SCC IN DIFFERENT STUDIES

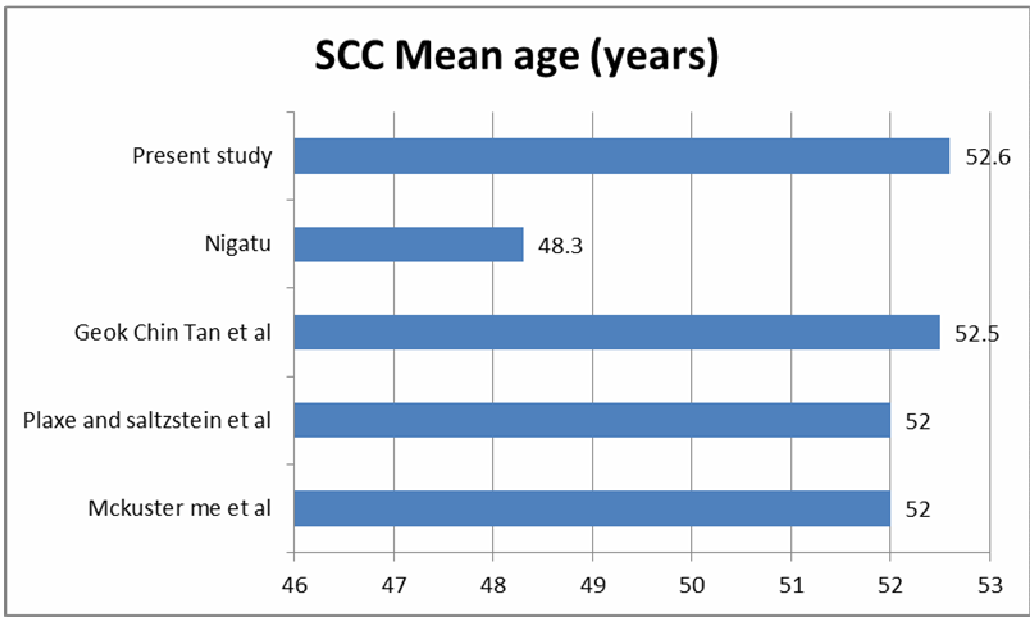
Several studies such as Mckuster me et al[65], Plaxe and saltzstein et al[64], Geok Chin Tan et al[63] have reported that the mean age for SCC was 52 years which is in close proximity to our study.(table no-28, graph-27)

TABLE NO - 28

MEAN AGE FOR SCC IN DIFFERENT STUDIES

STUDIES (AUTHOR)	MEAN AGE (YEARS)
Mckuster me et al[65]	52
Plaxe and saltzstein et al[64]	52
Geok Chin Tan et al[63]	52.5
Nigatu et al[55]	48.3
Present study	52.6

GRAPH NO - 27



p16 EXPRESSION IN SQUAMOUS CELL CARCINOMA

In the present study, analysis of cellular patterns of p16 staining (nuclear: cytoplasmic staining, cytoplasmic staining, and nuclear staining) in SCC (22 cases) showed 4 cases of cytoplasmic positivity (1- LCKSCC, 2-LCNKSCC, 1-SCNKSCC), 17 cases expressed nuclear: cytoplasmic positivity (4- SCC MI, 1-LCKSCC, 9-LCNKSCC, 1-SCNKSCC, 1-basaloid, 1-papillary) and a case of Squamotransitional carcinoma expressed nuclear positivity.

Analysis of intensity of staining in SCC 72.72% cases show strong (3+) positivity and 27% shows moderate (2+) positivity (single case of SCC with microinvasion, LCKSCC, LCNKSCC, Squamotransitional SCC and 2 cases of

SCNKSCC) and percentage of positive tumour cell showed 95.45% cases of SCC expresses >25% positive tumour cells (Squamotransitional SCC-5-25%).

Hence above factors can be taken for standardization of p16 immunostaining as also supported by other studies such as Kumari et al[42], Klaes et al[43] and R gupta et al[44].

The p16 immunostaining of SCC in various grades are depicted in figure - 16, 18, 20.

6.6 SQUAMOUS CELL CARCINOMA WITH MICROINVASION AND ITS VARIANTS

SQUAMOUS CELL CARCINOMA WITH MICROINVASION

In the present study, 14 cases (4.2%) were reported out of 332 cancer cases where Fadwa et al[86]reported a case out of 167 cases.

Costa et al[92] reported median age is 37 years with in the range of 20-69 years in 230 total cases whereas mean age is 47 years with in the range of 33-67 years in this study.

Biomarker p16 expression study was done on four cases and all cases showed >25% positive tumour cells, nucleo: cytoplasmic postivity and strong intensity of staining, expect one case showed moderate intensity.

PAPILLARY SQUAMOUS CELL CARCINOMA

Microscopically, the tumour composed of thin or broad fibrovascular septa lined by multi-layered squamous epithelium.

Fadwa et al[86] reported a case(0.6%) out of 167 total cases and in the present study, 3 cases (0.9%) of papillary squamous cell carcinoma out of 608 cases were reported which has a similar incidence of Fadwa et al study.

Michal odida et al[91] studied 20 cases of papillary SCC and found the age ranges from 22 to 70 years with mean age of 46.6years which go in line with the mean age of 45 years in the present study.

Immunostaining with p16 biomarker express strong intensity, >25% positive tumour cells and showed Nucleo: cytoplasmic positivity. (Figure-24)

BASALOID SQUAMOUS CELL CARCINOMA

This is a rare variant of SCC with an aggressive nature, which has the characteristic histologic features of Basaloid cells arranged in nests, groups, trabeculae and lobules with peripheral palisading. In the present study 2 cases of Basaloid squamous cell carcinoma was reported, whose age is 50 & 60 years.

IHC staining with p16 showed strong intensity of staining, >25% positive tumour cells and showed nuclear: cytoplasmic positivity. (Figure-22)

SQUAMOTRANSITIONAL CELL CARCINOMA

It's a rare variant tumour which is potentially aggressive; it occurs predominantly in the age group of post-menopausal women[89]. Mani Anand et al[90] studied 9 cases of Squamotransitional cell carcinoma and the age group of patients in that study range from 35 to 75 years. This study reported a case and its age is 45 years which falls within this age group.

p16^{INK4a} immunostaining showed moderate intensity of staining, 5-25% positive tumour cells and nuclear positivity. (Figure-26)

ADENOID SQUAMOUS CELL CARCINOMA

A single case was noted and the age was 27 years in the present study. Horie Y et al[93] reported the similar case in cervix at 60 years.

6.7 ADENOCARCINOMA

The distribution of adenocarcinoma and its variants cases in various studies is given in table no-29, graph-28.

TABLE NO - 29

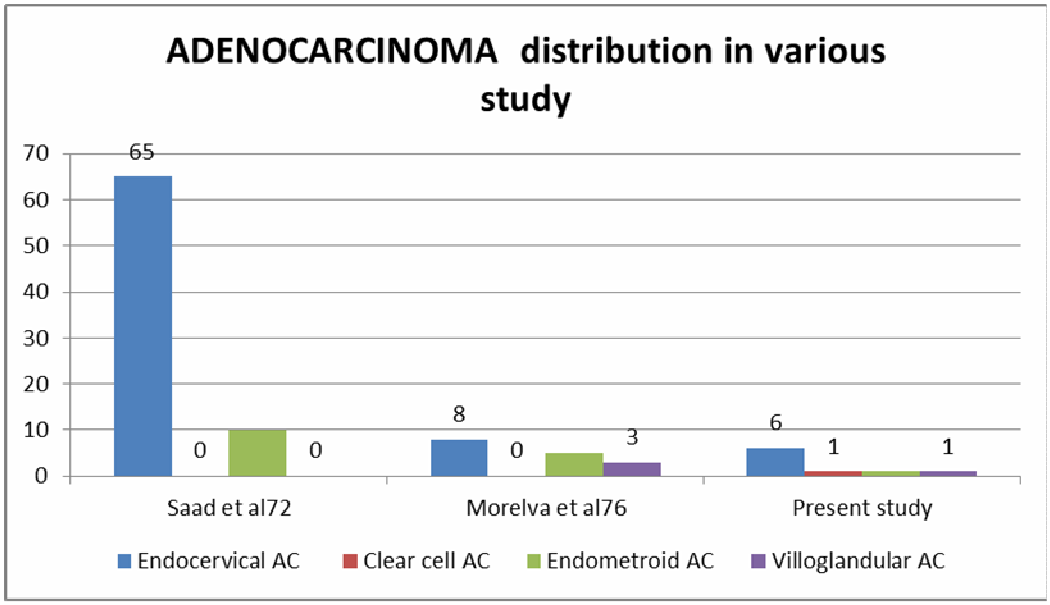
DISTRIBUTION OF ADENOCARCINOMA AND ITS VARIANTS IN VARIOUS STUDIES

Studies (author)	Endocervical AC	Clear cell AC	Endometrioid AC	Villoglandular AC
Saad et al[72]	65	-	10	-
Morelva et al[76]	8	-	5	3
Present study	6	1	1	1

Saad et al[72]in their study of 75 cases of Adenocarcinoma found 65 cases of endocervical and 10 cases of Endometroid AC whereas Morelva et al[76] in their study of 16 cases of Adenocarcinoma found 8, 5 and 3 cases of Endocervical, Endometroid and Villoglandular types respectively.

In the present study, Endocervical Adenocarcinoma being predominant, constitutes about 67%(6) whereas other variants like clear cell Adenocarcinoma, Endometroid Adenocarcinoma and Villoglandular Adenocarcinoma constitutes 11%(1) each.

GRAPH NO - 28



The mean age for adenocarcinoma in different studies is given in table no-30, graph-29.

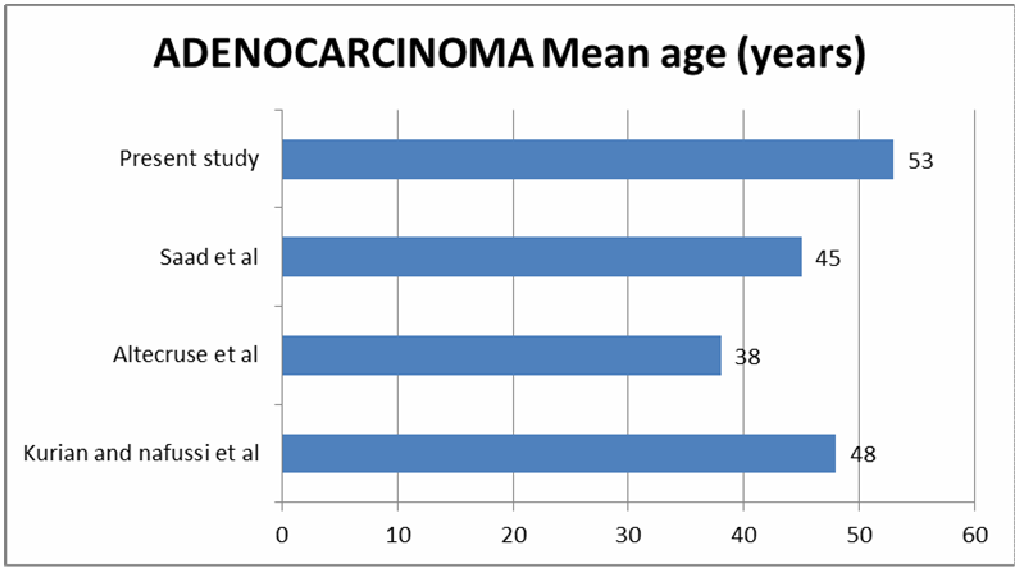
TABLE NO - 30

MEAN AGE FOR ADENOCARCINOMA IN DIFFERENT STUDIES

STUDY (AUTHORS)	MEAN AGE (YEARS)
Kurian and nafussi et al[69]	48
Altecruse et al[66]	38
Saad et al[72]	45
Nigatu et al[55]	48.5
Present study	53

The mean age of Adenocarcinoma of cervix has shown to be 48 years in a study by Kurian and Nafussi et al[69] and 45 years in a study by saad et al[72] .In the present study the mean age is 53 and closely correlates with kurain and nafussi study and Nigatu et al[55]

GRAPH NO - 29



VILLOGLANDULAR ADENOCARCINOMA

WHO[18] states that there is a possible link with occurrence of Villoglandular adenocarcinoma and oral contraceptives usage.

Jones et al study states that it occurs in women aged between 23 to 57 years and in the present study a case of Villoglandular Adenocarcinoma in a 60 years old lady was documented.

INVASIVE ENDOCERVICAL ADENOCARCINOMA

Fadwa j et al studied 12 cases of endocervical Adenocarcinoma and found that mean age of occurrence is 48 years, similar mean age group of 50 years among 6 endocervical adenocarcinoma cases was observed in the present study.

ENDOMETRIOID ADENOCARCINOMA

Endometroid adenocarcinoma resembles endometrial adenocarcinomas so closely. The age group of presentation in the present study was 50 yrs.

CLEAR CELL ADENOCARCINOMA

Histologically the tumour cells are clear or hobnail in appearance. Its peak frequency occurs in bimodal age groups, that is in young age group (DES exposure) and in postmenopausal age group[18]. Here, a case of clear cell adenocarcinoma in 62 years old female was reported.

$p16^{INK4a}$ EXPRESSION

All cases of adenocarcinoma expressed 100% positivity in both intensity and percentage of positive cells; similar results were found in Negri et al[80], Karcheva et al[86], Murphy et al study. In klaes et al[43] showed 85% positivity.

Among 6 cases of adenocarcinomas, one case of Villoglandular type expressed cytoplasmic positivity, and others showed nuclear: cytoplasmic positivity

6.8 OTHER EPITHELIAL TUMOURS

ADENOSQUAMOUS CELL CARCINOMA

Adenosquamous cell carcinoma comprises 3–5% of cervical tumours. Cell of origin is columnar cells of the cervical mucosa. This reserve cells simultaneously differentiate towards columnar and squamous cells. Hence the tumour contains an admixture of histologically malignant squamous and glandular cells which has a significantly worse prognosis than other glandular lesions.

TABLE NO - 31

INCIDENCE OF ADENOSQUAMOUS CELL CARCINOMA IN VARIOUS STUDIES

STUDIES (AUTHOR)	PERCENTAGE(N)
K gupta et al[56]	3.7%(3)
Balkachewnigatu et al[55]	1.1%(26)
Present study	1.2%(4)

From this comparative study (table no- 31), our incidence of Adenosquamous carcinoma correlates with Balkachewnigatuetal[55] study. The mean age is 50 years in our study.

Immunostaining with p16 showed strong intensity of staining, >25% positive tumour cells and Nucleo: cytoplasmic positivity. (figure-38)

ADENOID BASAL CARCINOMA

It accounts for< 1 % of cervical carcinoma[6]. In this present study, it constitutes 0.17% i.e. <1%.

Baggish and Woodruff [74] et al studied 100 cases of adenoid basal carcinoma and reported that mean age is ≥45 years of age (post-menopausal age group).

WHO[18] reports Adenoid basal carcinoma patients age group is usually more than 50 years. Similar age group incidence also found in our study i.e. 55 years.

6.9 BIOMARKER $p16^{INK4a}$ IMMUNOEXPRESSION IN CERVICAL NEOPLASMS

Here, the main diagnostic role of immunohistochemistry in evaluation of cervical neoplastic lesions is to distinguish dysplasia from benign lesions and to evaluate the cauterised margins.

Grading the dysplasia remains an issue for morphological criteria. However in the histologic diagnosis of these reports shows repeated absence of interobserver reproducibility.

There is wide array of biomarkers which have been used in diagnosis of cervical neoplasms. Objective biomarkers could result in improvement of diagnostic specificity as a result of clear identification of accurate dysplastic cells.

One of the ideal biomarker used extensively is p16, which is a tumour suppressor protein located in the nucleus, so nuclear staining in IHC is expected. But both nucleus and cytoplasm stain positively in dysplasia has been observed possibly because of post transcriptional modification or over production of p16 overflowing into the cytoplasm. Various research papers supported that there is an up regulation of p16 in HPV related cervical lesions. Hence this study used this marker as a diagnostic tool.

The following table no- 32, graph-30, compares the present study with the recent studies done on p16 expression that is number of positive cases in the total number of cases in cervical lesions.

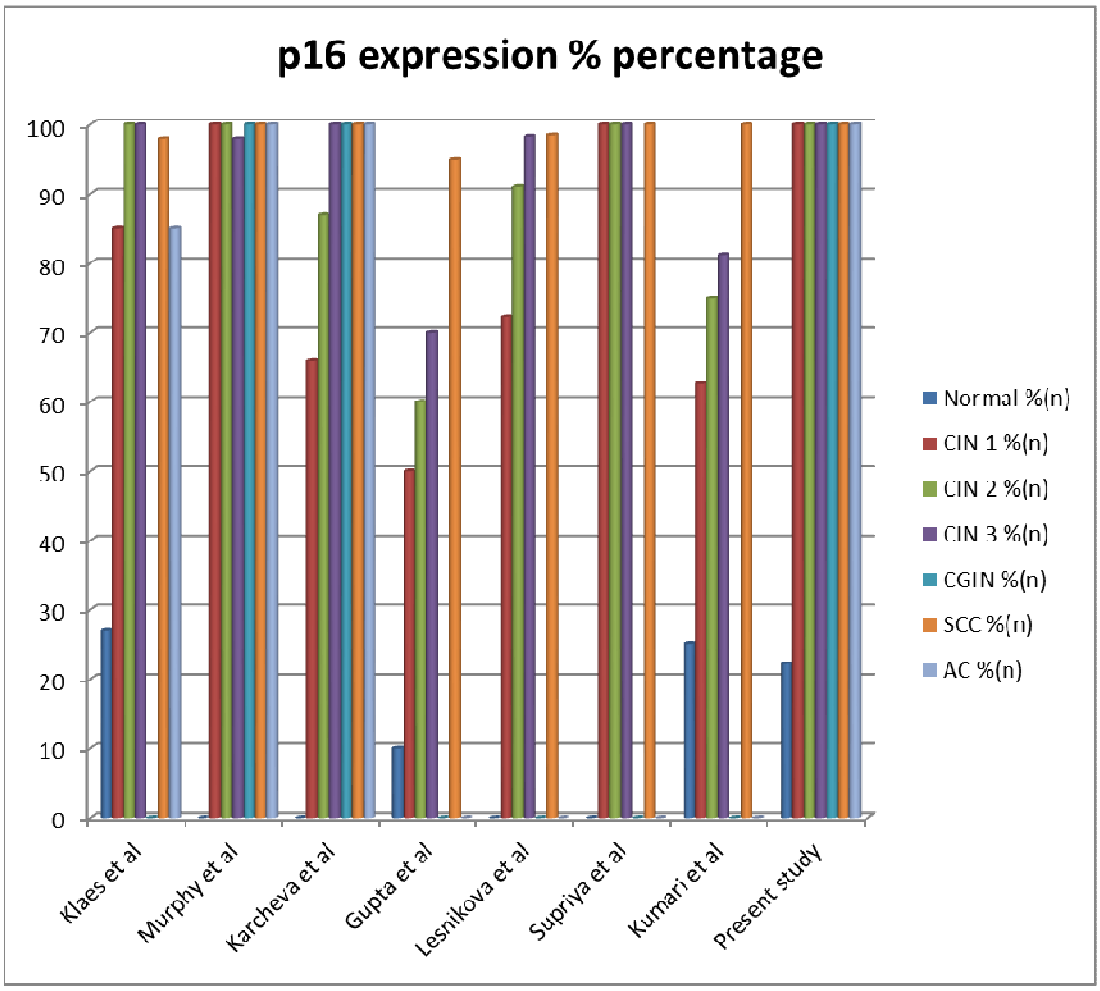
TABLE NO - 32

STUDIES PUBLISHED ON $p16^{INK4A}$ EXPRESSION

Studies(years)	**Normal %(n)**	**CIN 1 %(n)**	**CIN 2 %(n)**	**CIN 3 %(n)**	**CGIN %(n)**	**SCC %(n)**	**AC %(n)**
Klaes et al[43] (2001)	27 (13/48)	85 (40/47)	100(32/32)	100 (60/60)	ND	98 (52/53)	85 (6/7)
Murphy et al[62] (2003)	0 (0/21)	100 (38/38)	100 (33/33)	98 (45/46)	100 (5/5)	100 (8/8)	100 (2/2)
Karchev a et al[86] (2007)	0 (0/13)	66 (2/3)	87 (7/8)	100(8/8)	100 (1/1)	100 (16/16)	100 (5/5)
Gupta et al[44] (2007)	10 (2/20)	50 (10/20)	60 (12/20)	70 (14/20)	ND	95 (19/20)	ND
Lesniko va et al[47] (2009)	ND	72.3 (180/249)	91 (212/233)	98.3 (178/181)	ND	98.5 (131/133)	ND
Supriya et al[22] (2010)	0(0/15)	100 (15/15)	100 (15/15)	100(3/3)	ND	100 (15/15)	ND
Kumari et al[42] (2013)	25(4/16)	62.6 (10/16)	75(12/16)	81.2 (13/16)	ND	100 (16/16)	ND
Present study	22.2 (2/9)	100 (10/10)	100(10/10)	100 (10/10)	100 (1/1)	100 (23/23)	100 (6/6)

ND - Not done.

GRAPH NO – 30


p16 expression % percentage
100
90
80
70
60
50
40
30
20
10
0
Klaes et al
Murphy et al
Karcheva et al
Gupta et al
Lesnikova et al
Supriya et al
Kumari et al
Present study
Normal %(n)
CIN 1 %(n)
CIN 2 %(n)
CIN 3 %(n)
CGIN %(n)
SCC %(n)
AC %(n)

From the above data all precancerous lesions and the carcinomas have shown 100% positivity for p16 in our study. In comparison with other studies like Supriya et al (100%)[22] and Murphy et al(100%)[62] that as well documented similar results.

Since p16 immunostaining positivity directly correlates with the HPV induced morphological changes and expected 0 % positivity in chronic non-specific cervicitis but found that 2 /9 cases (22%) showed p16 positivity. Likewise, few other studies like Kumari et al[42] (25%), Gupta et al[44] (10%) and Klaes et al[43] (27%) have also observed p16 positivity in chronic non-specific cervicitis. Such cases were reviewed and classified into CIN 1. It infers difficulty to diagnosis the subtle histopathological features only by analysing the haematoxylin and eosin stained slides as either cervicitis or CIN. Hence, p16 immunostaining can serve as diagnostic tool assisting the difficult cases to diagnose correctly.

Murphy et al.[62] performed p16 immunostaining in 154 cases and found negative staining in all normal cervical tissue and 100% positivity in all cervical neoplasm expect a case of CIN 3. In this study some CIN1cases showed nuclear staining whereas in our study a case of Squamotransitional cell carcinoma showed nuclear staining. The study by Murphy et al. found precancerous lesions showed predominantly cytoplasmic pattern staining. All cancerous lesions including both squamous cell carcinoma and adenocarcinoma showed strong nuclear and cytoplasmic staining.

Supriya et al[22]studied 63 cases with immunostaining markers p16 and MIB1. All normal cervical tissue including normal epithelium, metaplastic, endocervical reactive and inflammatory regions showed negative p16 immunostaining whereas all precancerous and cancerous lesions showed 100 % positivity.

The present study showed similar results to Murphy et al[62]and Supriya et al[22] study.

Gupta et al[44] studied 100 cases, in that 18/20 cases of normal cervical epithelium showed negative staining with anti-p16 antibody. Intraepithelial neoplasms showed progressive increase in expression with p16 immunostaining. In his study CIN 1, CIN 2, CIN 3 and SCC cervical lesions expressed p16 positivity of about 50%, 45%, 55% and 90% showed a strong nuclear or Nucleo : cytoplasmic positivity respectively. Whereas Kumari et al[42] study showed p16 positivity of 25% CNSC, 62.2% CIN 1, 75% CIN 2, 81% CIN 3 and 100% SCC.

Observed statistical analysis in this study showed p value <0.05 which was considered very significant and signifies p16 is ideal marker for cervical dysplasia.

Thus this study highlights the increasing expression of $p16^{INK4a}$ in higher grades of CIN and cervical carcinoma concurrent with many studies (Table - 32).This finding emphasizes the role played by this marker in early carcinogenesis and progressive accumulation of nuclear protein as the tumour progresses. The association of this marker seen in high grade CIN and cervical carcinoma suggests their association with infection by high risk HPV types.

CONCLUSION

- Uterine cervical neoplasms accounts for 19% of total number of cervical biopsies received at Thanjavur Medical College & Hospital, Pathology Department, for a period of one year from January 2013 to December 2013.
- The incidence of uterine cervical carcinoma is 10.41%, while cervical intraepithelial neoplasm is 8.44%, with a ratio of 1.23:1.
- Most of the patients age group with cervical carcinoma (27.52%) fall under 5th decade of life.
- Majority of Cervical intraepithelial neoplasms patients (45.6%) falls in the age group between 30-39 years which is two decades earlier than the cervical carcinoma patients (32.13%) age group (50-59 years).
- The mean age for cervical intraepithelial neoplasms is 39.8 years and cervical carcinoma is 52.61 years. This confirms window period of ten years to intervene by screening tests for early detection and treatment. It prevents the progression of disease process from cervical intraepithelial neoplasia to cervical carcinoma.
- CIN 1 constitutes 77% of total cervical intraepithelial neoplasia cases.
- Squamous cell carcinoma constitutes 95.8% in the total cervical cancer cases which is the most common subtype.
- In squamous cell carcinoma, large cell non keratinizing squamous cell carcinoma (Grade 2) predominate the profile based on grade of differentiation.

- Immunostaining with $p16^{ink4a}$ was performed on 69 cases of cervical lesions, in that 9 cases of chronic non-specific cervicitis were taken as control. 60 cases were randomly selected from 608 cases, which constitute 10% of the sample size. Cervical neoplasms showed 100% positivity.
- Four parameters were used to score the p16 expression in cervical neoplasm and found there is progressive increase in the percentage of positive tumour cells and intensity of staining from CIN to cervical carcinoma.
- Thus, overexpression of p16 immunostaining serves as a potential diagnostic tool in cervical neoplasms.

The present study signifies the usefulness of an ideal immunomarker $p16^{INK4a}$ as a diagnostic tool and emphasizes the importance of incorporating the HPV cotesting ($p16^{INK4a}$) in the primary screening programme. Due to the incorporation HPV cotesting, there is a beneficiary effect on both the patient and less costly to the health care system.

"Low cost – point of care screening test for the general population and a Government subsidized global vaccination programme" if followed, it is entirely conceivable that women will no longer die from cervical cancer in the near future.

LIST OF ABBREVIATIONS

1. PBCR – Population Based Cancer Registry
2. HBCR – Hospital Based Cancer Registry
3. HPV- Human Papilloma Virus
4. SCJ- Squamo-Columnar Junction
5. TNHSP- Tamil Nadu Health System Project
6. VIA- Visual Inspection with Acetic acid
7. VILI – Visual Inspection with Lugol's Iodine
8. CIN – Cervical Intraepithelial Neoplasia
9. H&E – Haematoxylin and Eosin
10. IHC – ImmunoHistoChemistry
11. TMCH – Thanjavur Medical College and Hospital
12. CNSC – Chronic Non Specific Cervicitis.
13. LSIL – Low-grade Squamous Intraepithelial Lesion
14. HSIL – High-grade Squamous Intraepithelial Lesion
15. WHO – World Health Organisation
16. SCC – Squamous Cell Carcinoma
17. LCKSCC – Large Cell Keratinizing Squamous Cell Carcinoma
18. LCNKSCC – Large Cell Non Keratinizing Squamous Cell Carcinoma

19. SCNKSCC – Small Cell Non Keratinizing Squamous Cell Carcinoma

20. AC – Adeno Carcinoma

21. HPF – High Per Field

22. HR-HPV – High Risk - Human Papilloma Virus

23. DNA – Deoxyribo Nucleic Acid

24. AJCC – American Joint Committee on Cancer

25. CA – Carcinoma

26. CK – Cytokeratin

27. EMA – Epithelial Membrane Antigen

28. pRB – Retinoblastoma protein

29. N:C – Nucleo: Cytoplasmic

30. ER – Estrogen Receptor.

31. PR - Progesterone Receptor

32. CEA – Carcino Embryonic Antigen

33. CAM 5.2 – Cytokeratin 8,18, Low Molecular Weight

34. ACS – American Cancer Society,

35. ASCP – American Society for Clinical Pathology.

ANNEXURES

ANNEXURE-I

Form IV

Government of Tamil Nadu
Tamil Nadu Health Systems Project

Pilot Project for Screening of Cervical Cancer
Thanjavur/Theni District

Government Hospital _____

HPE - Requisition Form

Name & Address : _____

_____

_____

Age :

*PSP Code No :

OP/IP No :

Result of Screening : VIA / VILI

Coloposcopic directed biopsy ECC done and Specimen from Cervix sent for HPE

Date: Signature of M.O. :

Seal:

To
The Department of Pathology

_____ Hospital

_____ District

* PSP - Pilot Screening Programme

ANNEXURE-II

HAEMATOXYLIN AND EOSIN STAIN

PREPARATION OF SOLUTION:

HARRIS HAEMATOXYLIN

Distilled water: 1000ml

Ammonium alum: 100g

Absolute ethyl alcohol: 50ml

Mercuric oxide: 2.5g

- 100g of ammonium alum dissolved in 1000ml of distilled water by heatingand shaking at 60°C.
- Add solution of 5g of haematoxylin in 50ml of ethyl alcohol and bring rapidly to boil.
- When it begins to boil, remove from flame.
- Add 2.5 g of mercuric oxide.
- Mix by swirling gently.

PREPARATION OF EOSIN STAIN

Eosin Y: 1 g.

Distilled water: 20ml

95% ethanol: 80ml

Glacial acetic acid: 0.2ml

- Dissolve 1 gm of eosin Y in 20ml of water,and then add 80 ml of 95% ethyl alcohol and 0.2 ml of glacial acetic acid.

Procedure:

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

ANNEXURE-III

IMMUNOHISTOCHEMISTRY

PREPARATION OF SOLUTIONS:

Citrate buffer solution- antigen retrieval solution

Trisodium citrate: 2.94 gm

1 N Hydrochloric acid: 5ml

Distilled water: 1000 ml

Required pH is 6.0, which are obtained by titration with 1N HCl.

Tris Buffer Saline (TBS) - wash buffer

Sodium chloride: 8gms

Tris (hydroxymethylamine): 0.605gms

1 N Hydrochloric acid: 4 ml

Distilled water: 1 litre

Required pH is 7.6, which are obtained by titration with 1N HCl.

Preparation of chrom alum coated slides:

Potassium dichromate: 50 mgs

Gelatin: 300 mgs

Distilled water: 100 ml

Potassium dichromate is added to distilled water and then boiled to 60◦C. Gelatin is then added slowly to it. Glass slides are then dipped in this solution and dried overnight.

After taking the required sections onto the coated slides, it is baked overnight at 45°C in the autoclave. The next day, the slides are taken for the procedure.

ANTIGEN RETRIEVAL:

The slides are arranged in a metal racket and placed in citrate buffer inside the pressure cooker, and allowed to boil up to three whistles.

Procedure:

1.Dewax the section in xylene(15 minutes each, 2 changes) and then in decreasing grades of alcohol then finally bring the sections to running tap water followed by distilled water.
2. Antigen retrieval using TBS by pressure cooker method
3. Cool to room temperature in running tap water for 20 minutes.
4. Wash in TBS -2 changes for 5 minutes each.
5. Drain and cover the sections with peroxidise block (endogenous peroxidise blocking agent) for 15 minutes.
6. Wash in TBS -2 changes for 5 minutes each.
7. Drain and cover the tissue sections with power block for 15 minutes
8. Drain and blot the excess power block.
9. Cover the sections with the respective primary antibody for 90 minutes.
10. Wash in TBS -2 changes for 5 minutes each.
11. Drain and cover the sections with super enhancer for 30 minutes.
12. Wash in TBS -2 changes for 5 minutes each.
13. Drain and cover the tissue sections with secondary antibody (HRP-horse raddish peroxidise) for 30 minutes.
14. Wash in TBS -2 changes for 5 minutes each.
15. Drain and cover the tissue sections with **DAB** (DiaminoBenzidine) substrate buffer for 5-10 minutes (depending on the time suggested in the supplied kit)
16. Wash in distilled water, counter stained with haematoxylin, clear in xylene and mount with DPX.

ANNEXURE-IV

HISTOLOGICAL CLASSIFICATION OF TUMORS OF UTERINE CERVIX-WHO

Epithelial tumour

Squamous tumours and precursors

Squamous cell carcinoma, not otherwise specified

- Keratinizing
- Non-keratinizing
- Basaloid
- Verrucous
- Warty
- Papillary
- Lymphoepithelioma-like
- Squamotransitional

Early invasive (micro invasive) squamous cell carcinoma

Squamous intraepithelial neoplasia

- Cervical intraepithelial neoplasia (CIN) 3

Squamous cell carcinoma in situ

Benign squamous cell lesions

- Condyloma accuminatum
- Squamous papilloma
- Fibro epithelial polyp

Glandular tumours and precursors

- Adenocarcinoma
- Mucinous adenocarcinoma
  - Endocervical
  - Intestinal
  - Signet-ring cell
  - Minimal deviation
  - Villoglandular
- Endometrioid adenocarcinoma
- Clear cell adenocarcinoma

Serous adenocarcinoma

Mesonephric adenocarcinoma

Early invasive adenocarcinoma

Adenocarcinoma in situ

Glandular dysplasia

Benign glandular lesions

Müllerian papilloma

Endocervical polyp

Other epithelial tumours

Adenosquamous carcinoma

Glassy cell carcinoma

Adenoid cystic carcinoma

Adenoid basal carcinoma

Neuroendocrine tumours

Carcinoid

Atypical carcinoid

Small cell carcinoma

Large cell neuroendocrine carcinoma

Undifferentiated carcinoma

Mesenchymal tumours and tumour-like conditions

Leiomyosarcoma

Endometrioid stromal sarcoma, low grade

Undifferentiated endocervical sarcoma

Sarcoma botryoides

Alveolar soft part sarcoma

Angiosarcoma

Malignant peripheral nerve sheath tumour

Leiomyoma

Genital rhabdomyoma

Postoperative spindle cell nodule

Mixed epithelial and mesenchymal tumours

Carcinosarcoma (malignant müllerian mixed tumour;

Metaplastic carcinoma)

Adenosarcoma

Wilms tumour

Adenofibroma

Adenomyoma

Melanocytic tumours

Malignant melanoma

Blue naevus

Miscellaneous tumours

Tumours of germ cell type

Yolk sac tumour

Dermoid cyst

Mature cystic teratoma

Lymphoid and haematopoietic tumour

Malignant lymphoma (specify type)

Leukaemia (specify type)

Secondary tumours

ANNEXURE-V

TNM and FIGO classification of carcinoma of the uterine cervix

Cervical cancer is the only gynaecological cancer that is clinically staged by physical examination, chest X-ray, intravenous pyelogram, cystoscopy and proctoscopy. The staging of cervical tumours is by the TNM/FIGO classification

TNM classification

T – Primary Tumour		
TNM	FIGO	
Categories	Stages	
TX		Primary tumour cannot be assessed
T0		No evidence of primary tumour
Tis	0	Carcinoma in situ (preinvasive carcinoma)
T1	I	Cervical carcinoma confined to uterus (extension to corpus should be disregarded)
T1a	IA	Invasive carcinoma diagnosed only by **microscopy**
T1a1	IA1	Stromal invasion no greater than **3.0 mm in depth** and **7.0 mm or less in horizontal spread**
T1a2	IA2	Stromal invasion **more than 3.0 mm and not more than 5.0 mm** with **a horizontal spread 7.0 mm or less**
T1b	IB	**Clinically visible lesion** confined to the cervix or microscopic lesion greater than T1a2/IA2
T1b1	IB1	Clinically visible lesion **4.0 cm or less** in greatest dimension
T1b2	IB2	Clinically visible lesion **more than 4 cm** in greatest Dimension

T2	II	Tumour invades **beyond uterus but not to pelvic wall** or to lower third of the vagina
T2a	IIA	**Without** parametrial invasion
T2b	IIB	**With** parametrial invasion
T3	III	Tumour extends to pelvic wall, involves lower third of vagina, or causes hydronephrosis or non-functioning kidney
T3a	IIIA	Tumour involves **lower third of vagina**, no extension to pelvic wall
T3b	IIIB	Tumour extends **to pelvic wall or causes hydronephrosis or non-functioning kidney**
T4	IVA	Tumour invades **mucosa of bladder or rectum or extends beyond true pelvis**
Note: The presence of bullous oedema is not sufficient to classify a tumour as T4.		
M1	IVB	**Distant metastasis**

N – Regional Lymph Nodes

NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Regional lymph node metastasis

M – Distant Metastasis

MX	Distant metastasis cannot be assessed
M0	No distant metastasis
M1	Distant metastasis

Stage Grouping

Stage 0	Tis	N0	M0
Stage IA	T1a	N0	M0
Stage IA1	T1a1	N0	M0
Stage IA2	T1a2	N0	M0
Stage IB	T1b	N0	M0
Stage IB1	T1b1	N0	M0
Stage IB2	T1b2	N0	M0
Stage IIA	T2a	N0	M0
Stage IIB	T2b	N0	M0
Stage IIIA	T3a	N0	M0
Stage IIIB	T1,T2,T3a T3b	N1 Any N	M0 M0
Stage IVA	T4	Any N	M0
Stage IVB	Any T	Any N	M1

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