"EXPRESSION OF CLASPIN IN SQUAMOUS CELL CARCINOMA OF CERVIX AND ITS ASSOCIATION WITH P16 EXPRESSION AND CLINICOPATHOLOGIC PARAMETERS"

BY

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DISSERTATION SUBMITTED TO
SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION & RESEARCH
TAMAKA, KOLAR, KARNATAKA
IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF MEDICINE

IN

PATHOLOGY

UNDER THE GUIDANCE OF

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PROFESSOR AND FORMER HOD, DEPARTMENT OF PATHOLOGY

DEPARTMENT OF PATHOLOGY
SRI DEVARAJ URS MEDICAL COLLEGE,
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The Institutional Ethics Committee of Sri Devaraj Urs Medical College, Tamaka, Kolar has examined and unanimously approved the synopsis entitled **"Expression of claspin in squamous cell carcinoma of cervix and it's association with P16 expression and clinicopathologic parameters"** being investigated by **Dr.Haneena Mariyam Kukkamgai**, Dr.Kalyani R & Dr.Rathnamma P[1] in the Departments of Pathology & OBG[1] at Sri Devaraj Urs Medical College, Tamaka, Kolar. **Permission is granted by the Ethics Committee to start the study.**

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ABSTRACT

Background:

Aimof the study

Methods:

Results

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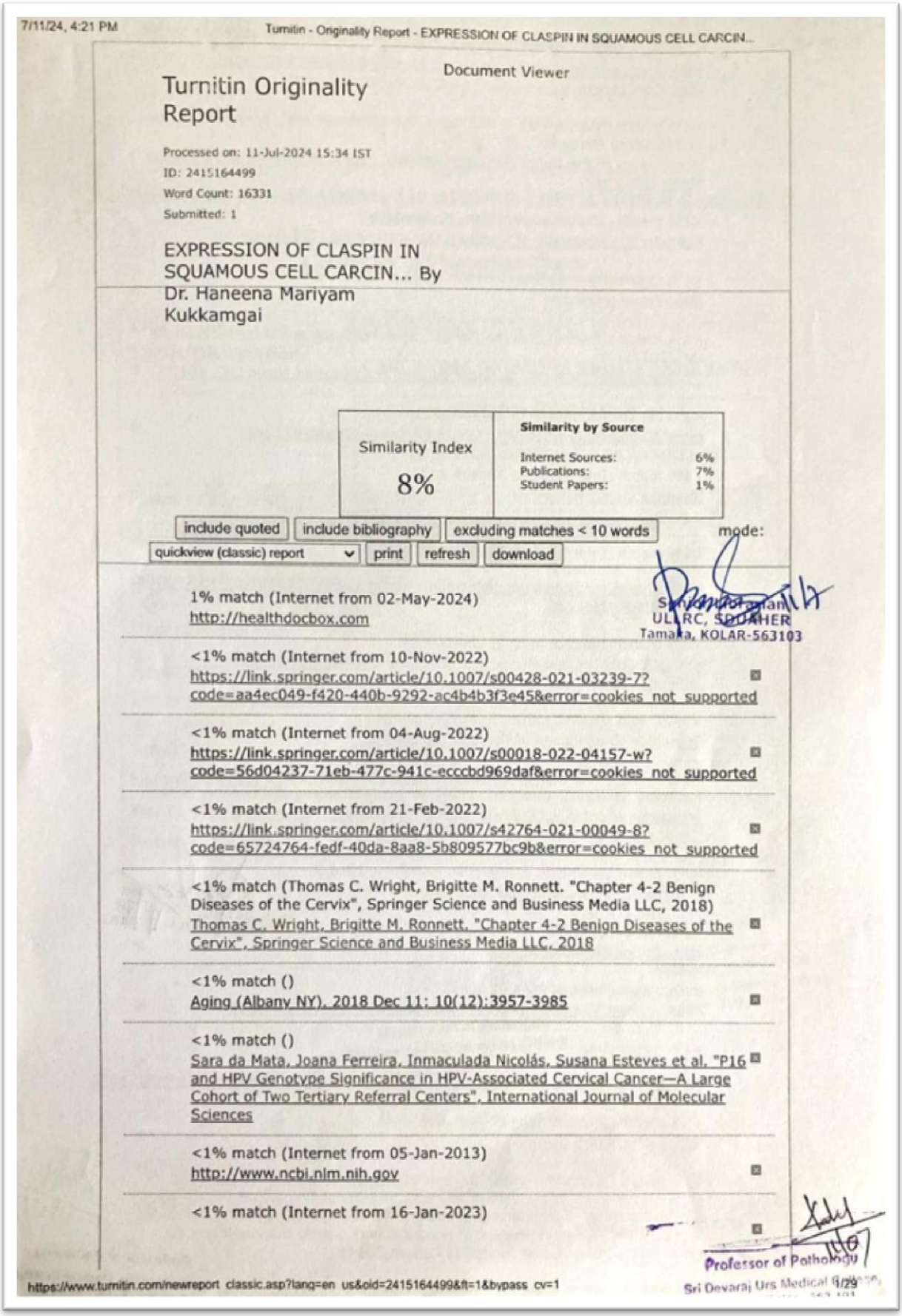

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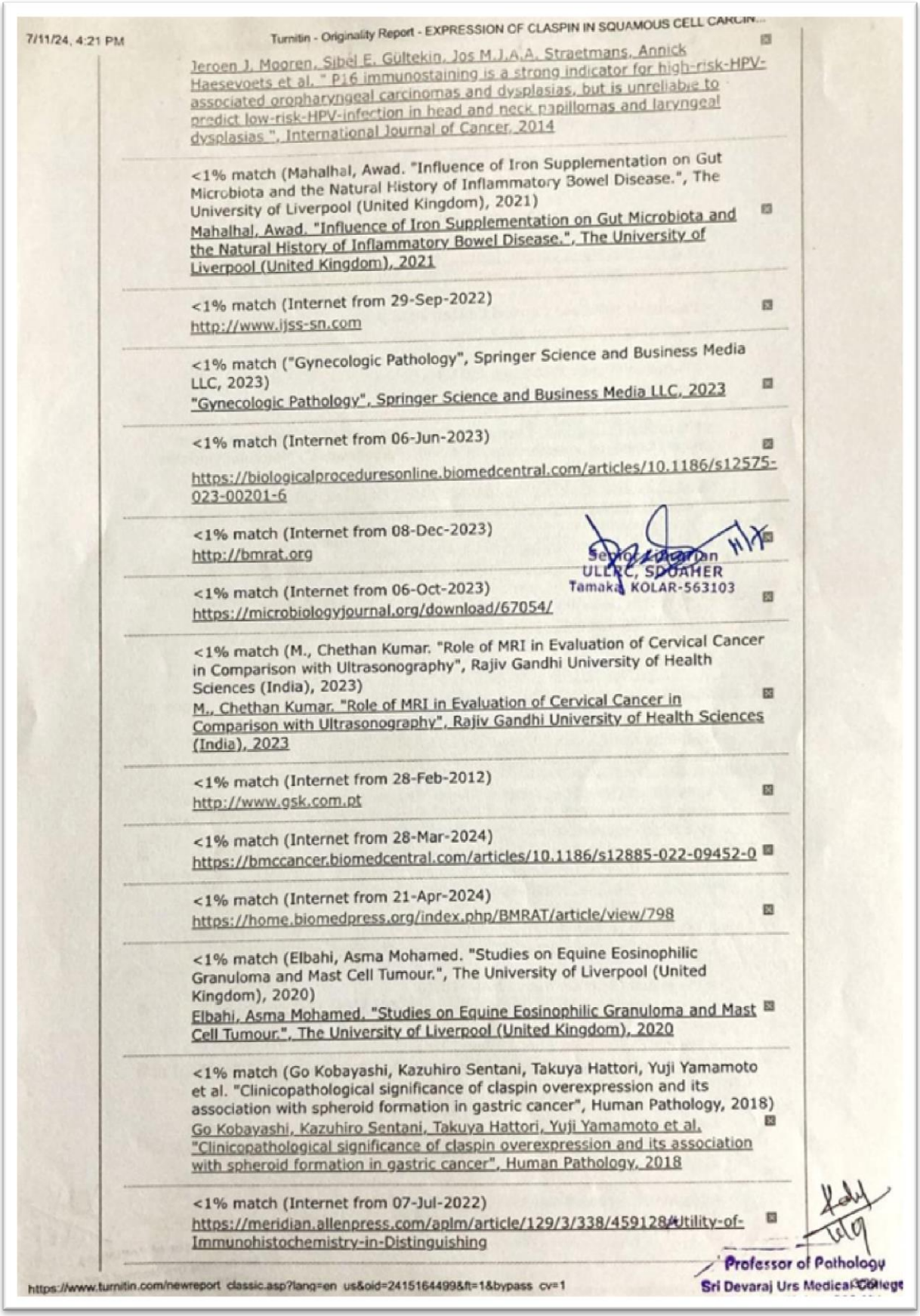

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ABSTRACT

Background:

Cervical cancer is a preventable gynecological malignancy, yet it remains a significant health burden in developing countries. While screening and vaccination must be our primary objective to fight against cervical cancer, the ground reality that the diagnosis is still made most frequently at an advanced stage cannot be forgotten. Hence biomarkers identified in cervical cancer has significance in understanding the prognosis and reducing cervical cancer related deaths. Claspin is a protein with key role in checkpoint signaling, genome duplication and consecutively increased replication. Elevated levels of Claspin have been seen in multiple solid tumors.

Aim of the study:

To associate of Claspin expression with clinicopathological parameters and P16 expression in Squamous Cell Carcinoma (SCC) of Cervix.

Methods:

The study was conducted in Department of Pathology in Collaboration with Department of Obstetrics and Gynecology (OBG), Sri Devaraj Urs Medical College attached to R.L.Jalappa Hospital and Research center, Tamaka, Kolar during the period of July 2022 to December 2023.The study included 80 cases of primary diagnosis of SCC cervix along with 10 cases of High grade squamous intraepithelial lesion (HSIL) and 10 cases of cervical tissue with normal histological features. Immunohistochemistry (IHC) was performed using antibodies against P16 and Claspin. Expression of Claspin and P16 was analyzed and interpreted. Claspin expression was associated with P16 and

clinicopathological parameters of carcinoma cervix. Statistical analysis was performed using Chi-square test. A p value of less than 0.05 was considered substantially significant.

Results:

The average age of cervical carcinoma diagnosis in this study was 55 years, with a significant majority (73.75%) being post-menopausal. All of the subjects had symptomatic presentation, most commonly bleeding per vagina (52.5%) and on per speculum examination 70% cases had an ulceroproliferative growth. On radiological examination majority of the cases had tumor size more than 4cm (68.5%) but lymph node involvement was seen in only (31.5%) cases. Maximum number of cases were diagnosed in Stage III highlighting the late presentation of the rural population in India. On histopathological examination most cases were well- differentiated (63.75%) and TILs were seen markedly increased in 56.92% cases. The study also found block positivity for P16 in 83.75% of cases, indicating HPV-associated SCC. Claspin is a potential biomarker, highly expressed in SCC cervix (88.75%, $p<0.005$), significantly higher than HSIL (50%) and normal tissue (0%). Increased Claspin expression was linked to P16 positivity but was not statistically significant. Neither Claspin nor P16 showed any significant association with the various clinicopathological parameters such as age, postmenopausal status, parity, clinical findings, lymph node status, size of tumor, stage, grade and TILs.

Conclusion:

Claspin is a potential biomarker for cervical cancer, particularly in HPV-associated cases. This study has shown the increased expression of Claspin and P16 in cervical cancer.

Drugs targeting Claspin has the potential to increase sensitivity to chemotherapeutic drugs like Cisplatin. Further research is needed to explore its prognostic significance and relationship with clinicopathological parameters. This study contributes to the ongoing quest for effective biomarkers, ultimately aiming to improve patient outcomes and reduce mortality rates in cervical cancer.

Keywords: SCC of Cervix, Claspin expression in cervical cancer, P16 expression in cervical cancer.

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SERIAL NO.	ABBREVIATION	EXPANSION
1.	SCC	Squamous Cell Carcinoma
2.	CIN	Cervical intraepithelial neoplasia
3.	LSIL	Low grade squamous intraepithelial lesion
4.	HSIL	High grade squamous intraepithelial lesion
5.	TILs	Tumor Infiltrating Lymphocytes
6.	HPV	Human Papilloma Virus
7.	DNA	Deoxyribonucleic acid
8.	IHC	Immunohistochemistry
9.	WD	Well Differentiated
10.	MD	Moderately Differentiated
11.	PD	Poorly Differentiated
12.	AIS	Adenocarcinoma in situ
13.	FIGO	Federation of International Gynaecologists and Obstetricians
14.	OBG	Obstetrics and Gynaecology
15.	MRI	Magnetic Resonance Imaging
16.	H&E	Hematoxylin and Eosin

17.	TILs	Tumor-infiltrating lymphocytes
18.	CLSPN	Claspin gene
19.	Chk	Checkpoint kinase
20.	ATR	Ataxia telangiectasia mutated and Rad3-related protein
21.	PD-1	Programmed death 1
22.	PD-L1	Programmed death ligand 1
23.	OBG	Obstetrics and Gynaecology
24.	SOP	Standard operating procedure
25.	DPX	Dibutylphthalate Polystyrene Xylene
26.	BSA	Bovine Serum Albumin
27.	TBS	TRIS buffer solution
28.	DAB	Di-aminobenzidine
29.	HPF	High Power fields
30.	BP	Block positive
31.	HP	High positive
32.	MP	Moderate positive
33.	LP	Low positive
34.	UPG	Ulceroproliferative growth

35.	WHO	World Health Organisation
36.	LVI	Lymphovascular invasion

INTRODUCTION

INTRODUCTION

Cervical cancer is the 4th most common cancer in females in 2020 in the world. Approximately 6,04,000 cases were newly diagnosed with cervical cancer and 3,42,000 died from the disease in 2020.[1] In India, cervical cancer is the 2nd most common cancer in women. 9.4% of all cancers and 18.3% of newly diagnosed cases in 2020 were of cervical cancer.[2] The incidence rate of cervical cancer in the Cancer registry in Bengaluru is 15.3.[3] The prevalence of Cervical Cancer in Kolar was reported as 17.55% of total female cancers and is the 2nd most common site of cancer in females. Majority of the cases are Squamous cell carcinoma (SCC) of cervix.[4]

Human papillomavirus (HPV) has been identified in approximately 95% of cervical cancers. P16 is considered to be a good surrogate for HPV infection due to the strong association between P16 overexpression and HPV status.[5] Significant P16 expression was observed in early stage of the disease.[6] An important factor for better survival of cervical carcinoma patients is early detection.[3] A prognostic biomarker that can be used for detection of cervical carcinoma with HPV association in early stage of the disease can be extremely useful and the way forward in the management of cervical cancer.

Claspin is a scaffold protein with key role in checkpoint signaling and genome duplication.[7,8,9] Elevated levels of Claspin has been seen in tumors of stomach, kidney, prostate, breast, lung and so on.[9,10,11,12,13] Overexpression of Claspin protected the cancer cells from chronic replication stress and gave them a proliferative advantage.[9] Claspin has the potential to be a new therapeutic target in cancer cells.[8,9,11,12,13]

As far as our knowledge goes, there has not been many studies conducted on the expression of Claspin in Cervical Carcinoma in English literature. Considering the potential of Claspin to be a new therapeutic target, it is imperative to have a better understanding of the expression of Claspin, hence this study was taken up.

AIMS & OBJECTIVES

AIM AND OBJECTIVES OF THE STUDY:

AIM:

Association of Claspin expression with clinicopathological parameters and P16 expression in SCC Cervix.

OBJECTIVES:

1.To determine the expression of Claspin biomarker in SCC Cervix.

2.To associate Claspin biomarker expression with P16 expression in SCC Cervix.

3.To associate the Claspin marker expression with clinicopathological parameters.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

GROSS ANATOMY

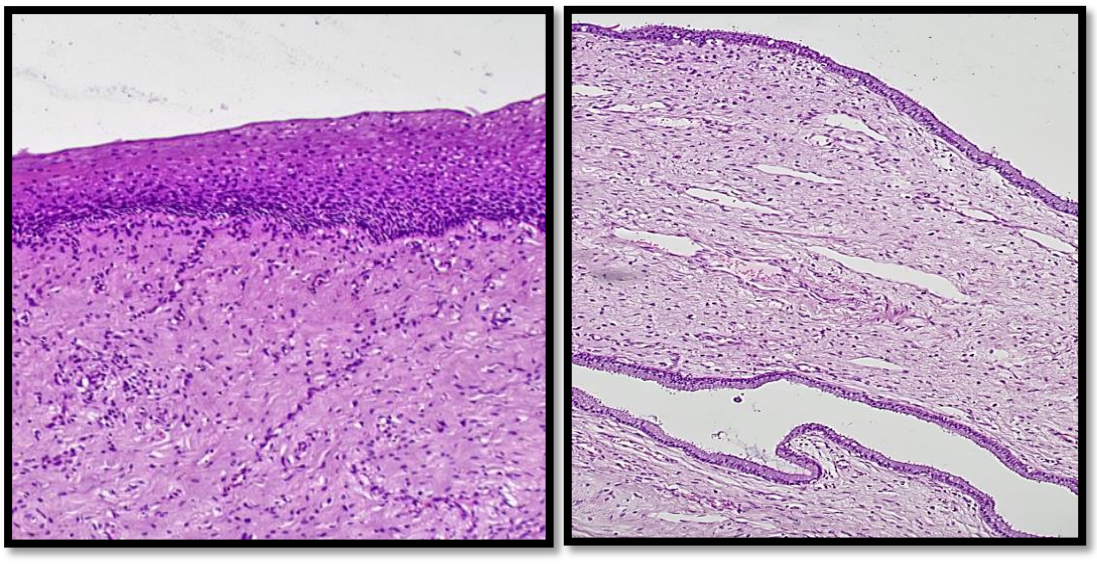
Cervix is the inferior most portion of the uterus. It measures about 3 cm in an adult nulligravida woman and has two parts; the ectocervix and the endocervix. The endocervix is the upper part of cervix that transitions from the lower segment of the uterus or the isthmus of the uterus. While the ectocervix is the outer portion of the cervix that protrudes into the vagina.[14]

HISTOLOGY

The ectocervix is lined by stratified squamous epithelium which has three zones (Fig 1A). The lowermost being the metabolically active basal/parabasal zone, above which is the midzone of stratum spinosum and the superficial zone of most differentiated squamous cells.[14]These cells are represented in Pap smear which is the foundation of cervical cancer screening as parabasal cells, intermediate squamous cells and superficial squamous cells respectively.[15,16]

The endocervical epithelium and the subepithelial glands are lined by a single layer of mucin producing columnar cells (Fig 1B).[14]

Figure 1: Histological representation of A) Ectocervical lining and B) Endocervical lining



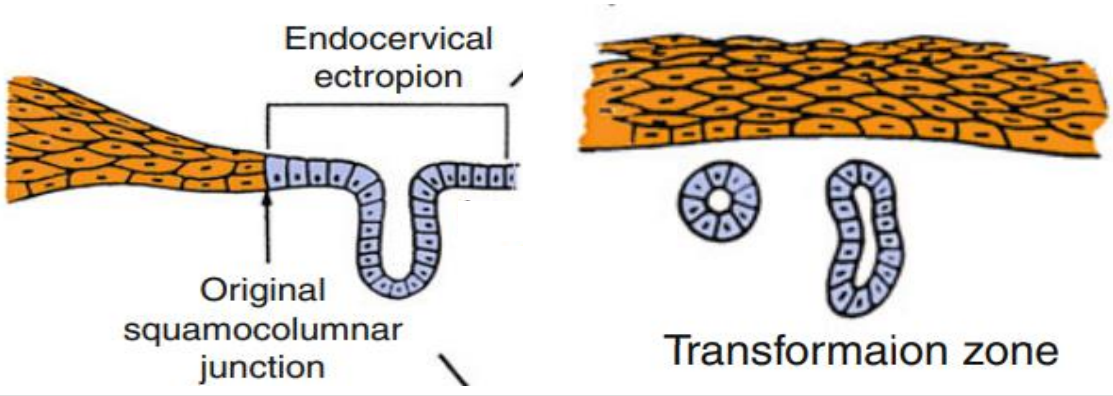
EMBRYOLOGY AND DEVELOPMENT

The cervix develops from paramesonephric ducts, similar to uterus and vagina. At birth, the majority of uterus is made up of cervix which is fibrous, while the uterine cervix is muscular. The junction of ectocervix (squamous epithelium) and endocervix (columnar epithelium) is the original squamocolumnar junction. The position of this junction is different in each individual. As a woman grows, the squamocolumnar junction moves into the endocervical canal under the influence of age and hormones. This causes replacement of endocervical epithelium by advancing ectocervical epithelium producing squamous metaplasia.[14,17]

TRANSFORMATION ZONE

The original squamocolumnar junction is the abrupt junction between endocervical and ectocervical epithelium at birth. While the functional squamocolumnar junction is the newly formed junction of endocervix and ectocervix by the migration of squamous epithelium upwards into the columnar epithelium in a post pubertal female. The transformation zone is the area between the original and functional squamocolumnar junction and is the part of cervix where both the squamous epithelium and columnar epithelium are present.[14,17] Microscopically, it is characterized by squamous metaplastic epithelium. This is the location where most cervical cancer and its precursors begins.[14]

Figure 2: Pictorial representation of original squamocolumnar junction and transformation zone[14]



IMMUNITY IN CERVIX

Cervix has cellular immune system composed of dendritic cells and lymphocytes in the epithelium and sub epithelium. This mucosal immunity is part of the defense mechanism of the host against viral and bacterial pathogens.[14]

BENIGN DISEASES OF CERVIX

Benign pathology of cervix can be cysts, benign tumors and inflammatory conditions.

INFLAMMATORY

Cervicitis: It can be acute or chronic. Acute cervicitis is histologically characterized by neutrophils in the epithelium and stroma along with stromal edema and vascular congestion. Chronic cervicitis is characterized by an inflammatory infiltrate of predominantly lymphocytes, plasma cells and histiocytes along with granulation tissue and stromal fibrosis.[14]

Noninfectious cervicitis: It is caused by chemical irritation like douching or local trauma due to insertion of tampons, pessaries or contraceptive devices.[14]

Infectious cervicitis: The presence of lactobacilli in the cervix maintains a low PH that doesn't allow other organisms to grow.[17] Bacterial, viral, fungal and parasitic organisms as listed below can all affect the cervix.

Bacterial organisms: Chlamydia trachomatis, Neisseria gonorrhoeae, Mycoplasma hominis, Group B Streptococcus, Ureaplasma ureolyticum, Gardnerella vaginalis, Actinomyces israelii, Mycobacterium tuberculosis, Treponema pallidum

Viral organisms: Herpes simplex virus, Human papillomavirus (HPV)

Fungal organisms: Candida, Aspergillus

Protozoa and parasites: Trichomonas vaginalis, Ameba Schistosomes[14]

The most crucial organism infecting cervix is the HPV which is an oncogenic virus.[14]

HPV

HPV has been identified as a causative agent in approximately 95% of cervical cancer.[5] The 5th edition of WHO has classified the epithelial neoplastic lesions and their precursors into HPV associated and HPV independent. For this categorization of tumors, they have recommended P16 IHC or HPV DNA testing. While this categorization does not change the treatment, it has been observed that HPV independent tumors are more aggressive than HPV associated.[18]

HPV is an oncogenic DNA virus that has been implicated in SCCs arising from cervix, anogenital region and head and neck regions.[17] While more than 200 types of HPV has been identified, about 20 of these can cause cervical cancer. Among these high-risk HPV genotypes the most prevalent are HPV16 and 18.[5] The oncogenic potential of these viruses is due to the viral oncoproteins E6 and E7.[10,17] The E6 and E7 protein bind to the p53 and RB gene respectively inhibiting their activity. This in turn will cause immortalization of the cells.[17]

Cervical carcinogenesis can be explained in 3 steps[14]

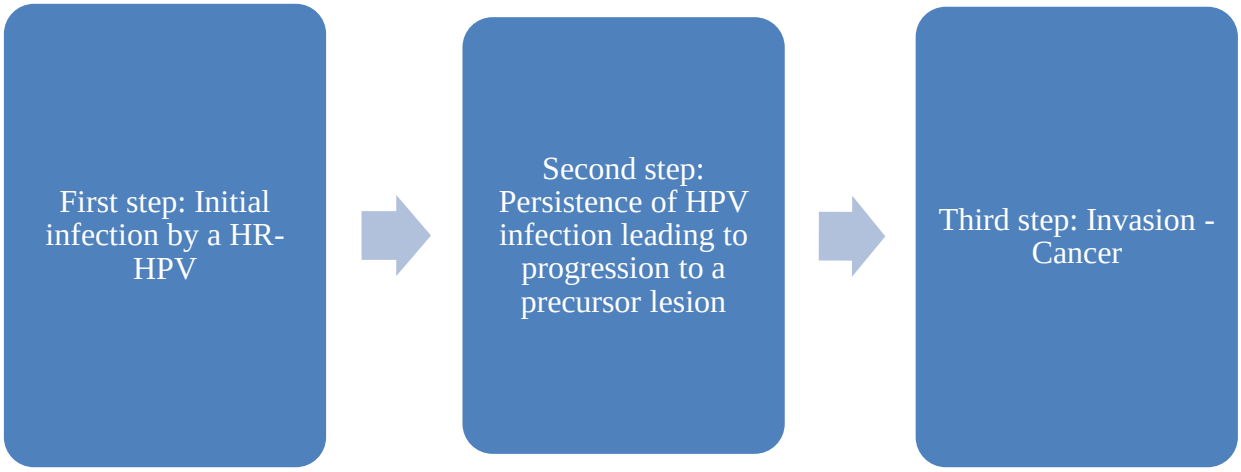
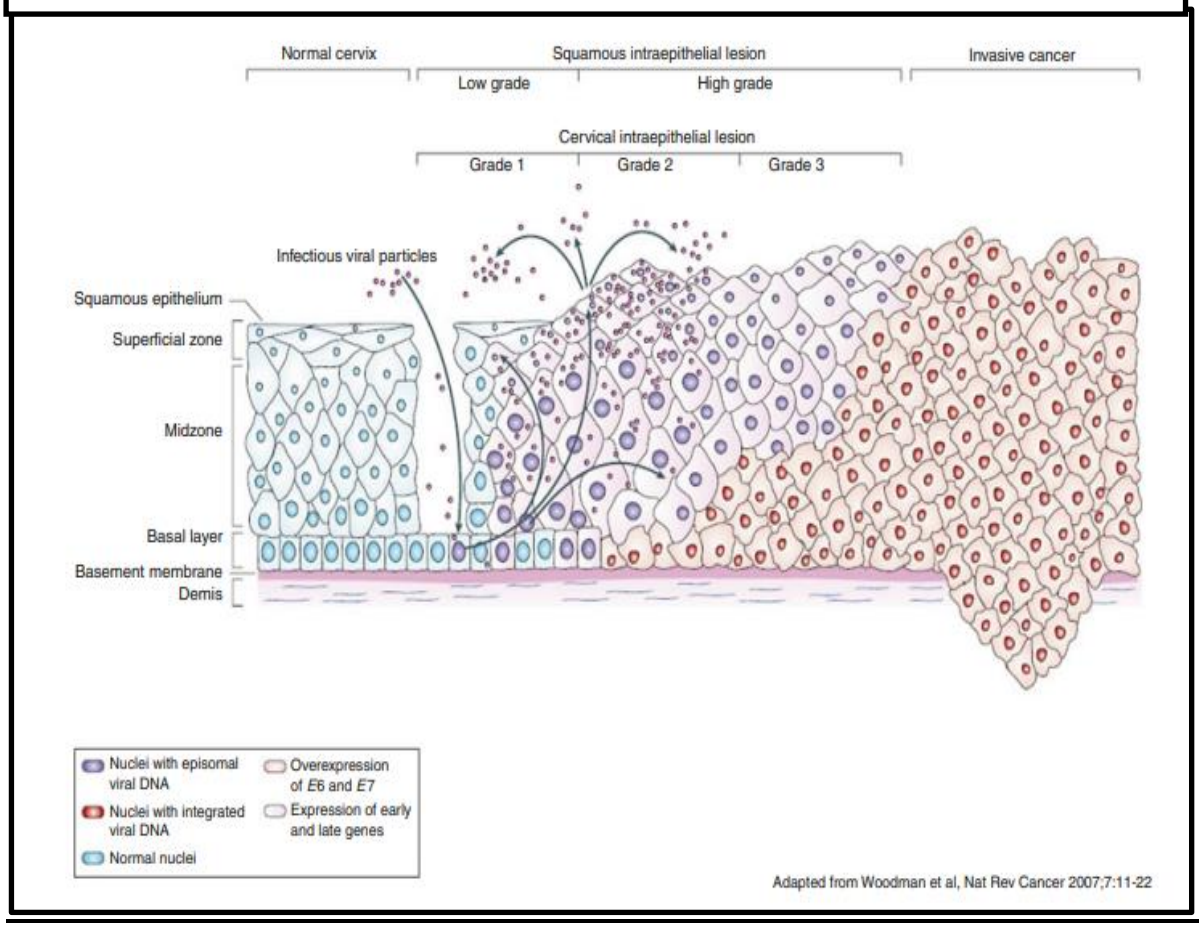


Figure 3: Pictorial representation of cervical carcinogenesis[14]



CYSTS

The most common cyst seen in cervix is the Nabothian cyst. These develop in the transformation zone. Other cysts seen in cervix can be tunnel clusters or inclusion cysts.

BENIGN TUMORS

The differential diagnosis of benign tumors of cervix are Polyp, Squamous papilloma, Micro glandular endocervical hyperplasia, Condyloma acuminatum, Papillary adenofibroma, Leiomyoma, Adenomyoma, and Fibroadenoma. The most commonly seen benign growth among these in the clinical setting are the endocervical polyp.[14]

Figure 4: WHO CLASSIFICATION OF UTERINE CERVICAL TUMOURS (2020):[18]

I) SQUAMOUS EPITHELIAL TUMORS:	II) OTHER EPITHELIAL TUMORS:
A) MIMICS OF SQUAMOUS PRECURSOR LESIONS **Squamous metaplasia**: Endocervical epithelium of single columnar cell layer replaced by the ectocervical epithelium **Atrophy of the uterine cervix:**Estrogen deficiency causing arrested maturation of squamous cells	Carcinosarcoma of the uterine cervix Adenosquamous and mucoepidermoid carcinomas of the uterine cervix Adenoid basal carcinoma of the uterine cervix Carcinoma of the uterine cervix, unclassifiable
B) SQUAMOUS CELL TUMORS AND PRECURSORS **Condylomaacuminatum** **Squamous intraepithelial lesions of the uterine cervix**: Dysplasia caused by HPV in the squamous cells of ectocervixlimited to the epithelium: **Squamous cell carcinoma, HPV associated, of the uterine cervix** **Squamous cell carcinoma, HPV independent, of the uterine cervix** **Squamous cell carcinoma, NOS of the uterine cervix**	**III)MIXED EPITHELIAL AND MESENCHYMAL TUMORS:** Adenomyoma of the uterine cervix Adenosarcoma of the uterine cervix **IV) GERM CELL TUMORS:** Germ cell tumors of the uterine cervix

V) GLANDULAR TUMORS AND PRECURSORS	
A) BENIGN GLANDULAR LESIONS **Endocervical polyp:** Exophytic growth covered by columnar endocervical cells with fibrovascularstroma **Mullerian papilloma of the uterine cervix:** Benign tumor of mullerian epithelium **Nabothian cyst:** Cyst filled with mucus and lined by endocervical columnar cells **Tunnel clusters:**Endocervical epithelium lined glands closely arranged in the cervical wall **Microglandular hyperplasia:**Hyperplastic lesion of small glands covered by mucinous epithelium that are closely packed **Lobular endocervical glandular hyperplasia**: Glands lined by endocervical columnar cells increased in a lobular pattern **Diffuse laminar endocervical hyperplasia**: Diffuse increase of irregular endocervical epithelium lined glands in a laminar pattern **Mesonephric remnants and hyperplasia**: Proliferation of the remnants of mesonephric duct **Arias Stella reaction of the uterine cervix**: Pregnancy and progestin associated changes in the columnar cells of endocervical gland **Endocervicosis:**Glands lined by mucinous columnar cells at the outer cervical wall **Tuboendometrioid metaplasia:** Change of endocervical glands to tubal type or endometrioid type, or both **Ectopic prostate tissue:** Epithelial cells in cervix that look like prostatic epithelium	**B) ADENOCARCINOMAS** **Adenocarcinoma in situ, HPV associated, of the uterine cervix** **Adenocarcinoma, HPV associated, of the uterine cervix** **Adenocarcinoma in situ, HPV independent, of the uterine cervix** **Adenocarcinoma, HPV independent, gastric type, of the uterine cervix** **Adenocarcinoma, HPV independent, clear cell type, of the uterine cervix** **Adenocarcinoma, HPV independent, mesonephric type, of the uterine cervix** **Other adenocarcinomas of the uterine cervix**

MALIGNANT TUMORS OF CERVIX:

Amongst all of the tumors in cervix, squamous epithelial tumors account for 80-90% of cervical cancers and adenocarcinomas account for 5%, but an increase in this distribution of adenocarcinoma to 10-25% is being observed in developed countries. Adenosquamous and mucoepidermoid carcinomas of cervix accounts for 5-6% of tumors. The poorly differentiated adenosquamous tumor is called Glassy cell carcinoma. Adenosarcoma of the uterine cervix is seen in about 0.16% of all cervical cancers. The other tumor subtypes are seen rarely arising primarily in cervix.[18]

RISK FACTORS

HPV is the most important etiological factor in cervical carcinogenesis hence risky sexual behaviors like multiple sexual partners and early sexual activity which could lead to acquiring an HPV infection is the most important risk factor in cervical cancer. Other factors such as prolonged use of oral contraceptive pills and smoking have also been observed to increase the risk of cervical cancer. Cigarette smoking is associated with SCC but a similar trend is not seen in the case of adenocarcinoma. Immunosuppression is another important risk factor as it determines the response of the immune system to HPV infection. This could also explain the association between HIV and cervical neoplasia. [14]

PRECANCEROUS LESIONS OF SQUAMOUS EPITHELIAL TUMORS–CERVIX

The precursor lesions of cervix include the Cervical Intraepithelial neoplasia (CIN) 1,2,3 which is the terminology used in WHO. Bethesda uses the terminology Squamous intra epithelial lesion (SIL) –Low Grade (LSIL) and High Grade (HSIL).[14] LSIL includes CIN1 which was previously termed mild dysplasia. These lesions represent a productive HPV infection with high viral replication but only mild changes are seen in the epithelium at the basal level. Approximately 10% of LSIL cases progresses to HSIL, 90% of these cases regress within 1 year.[18]HSIL includes CIN 2 and 3, which was previously termed moderate and severe dysplasia respectively. CIN 2 is represented by a progressive atypia and dysplastic cells are seen above the lower third of the epithelium. CIN 3 shows full thickness dysplasia of the cervical epithelium.[14,17] HSIL is caused by dysregulation of the cell cycle by HPV that lead to irreversible changes and eventually malignancy.[14]The time between first detection of HSIL and its transformation to invasive malignancy is long with an average of 20 years. If left untreated, 30% of HSIL will progress to malignancy.[21]

INVASIVE SQUAMOUS CELL CARCINOMA OF CERVIX

SCC of cervix is characterized infiltration of the malignant squamous cells into the stroma. The progression of HSIL into SCC requires additional genetic and epigenetic mutation. Factors like multiparity, smoking, oral contraceptive pill use and immune suppression increases the risk of progression to carcinoma.[18]

Figure 5: Classification of SCC into different grades and histological patterns.[19]

Grade	Histological Patterns:
➢ Well-differentiated (WD) ➢ Moderately-differentiated (MD) ➢ Poorly-differentiated (PD)	- Keratinizing SCC - Non-keratinizing SCC - Basaloid SCC - Warty (condylomatous) SCC - Papillary SCC - Verrucous SCC - Lymphoepithelioma-like SCC - Squamotransitional carcinoma

Depth of stromal invasion, lymphovascular invasion, tumor volume and positive resected margins are all factors that are considered to have an increased risk for recurrence and nodal metastasis.[14]The histological pattern, grading and HPV type do not affect the prognosis. The factors that have maximum prognostic implication in SCC cervix is the lymph node status and FIGO stage.[18]

PRECANCEROUS LESIONS OF GLANDULAR TUMORS –CERVIX

Adenocarcinoma in situ (AIS), which is a precursor to adenocarcinoma is seen much less commonly than the SCC precursors (SILs). AIS is seen in the endocervical glands, in which the lining epithelium becomes dysplastic showing hyperchromasia and pseudo stratification but no invasion is seen. These lesions are commonly HR-HPV dependent and only rare cases have been seen in the absence of HPV infections.[14,18] AIS is harder to pick up on cervical screening than SIL. This is leading to an increase in the incidence of glandular tumors in the screened populations.

ADENOCARCINOMA–CERVIX

Adenocarcinoma is the malignant counterpart in the endocervix involving the glands. It can be both HPV dependent and independent. Majority of these tumors are associated with infection by HR- HPV but with the increase in HPV detection and vaccination, there is a significant increase in the number of the HPV independent type. Morphologically, HPV dependent adenocarcinoma can be the usual type which mimics serous carcinoma or the mucinous type with the latter having intracytoplasmic mucin in at least 50% of the cells or more than that. The HPV independent adenocarcinoma show variable histology like the gastric type, clear cell type and mesonephric type.[18]

CARCINOSARCOMA

Carcinosarcoma is characterized by malignant component of both epithelium and mesenchyme. Microscopic examination will show an admixture of carcinomatous component (SCC, adenocarcinoma, neuroendocrine carcinoma, adenoid basal carcinoma) and sarcomatous component (fibrosarcoma, endometrial stromal sarcoma, rhabdomyosarcoma, osteosarcoma).[18]

ADENOSQUAMOUS AND MUCOEPIDERMOID CARCINOMA

Adenosquamous and mucoepidermoid carcinomas are epithelial malignancies with both glandular and squamous differentiation. There will be an admixture of both the components that can be appreciated under microscopy without the need of special stains.[18]

ADENOID BASAL CARCINOMA

Adenoid basal carcinoma is not visible grossly and is characterized histologically by rounded nests of small, monomorphic basaloid cells.

ADENOMYOMA AND ADENOSARCOMA

These are mixed tumors with both mesenchymal and epithelial component. In adenomyoma there is a benign proliferation of both endocervical glands and the stroma. The malignant counterpart of adenosarcoma shows a benign glandular component with a sarcomatous component that is generally low grade.

GERM CELL TUMORS OF THE UTERINE CERVIX

Germ cell tumors like mature teratoma, yolk sac tumor, and non-gestational choriocarcinoma can occur primarily in the cervix. Histologically they are similar to the ovarian counterpart and metastasis has to be excluded before the diagnosis.

Figure 6: FIGO STAGING 2018[20]

2018 International Federation of Gynecology and Obstetrics staging classification of cervical cancer

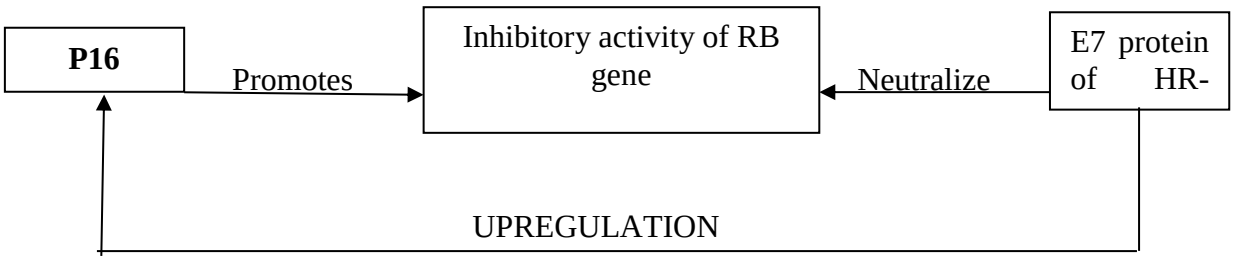
Stage	Description
I	Carcinoma is strictly confined to the cervix (extension to uterine corpus should be disregarded)
IA	Invasive carcinoma that can be diagnosed only by microscopy with a maximum depth of invasion <5 mm
IA1	Measured stromal invasion ≤3 mm in depth
IA2	Measured stromal invasion >3 and ≤5 mm in depth
IB	Invasive carcinoma with measured deepest invasion >5 mm; lesion limited to the cervix uteri with size measured by maximum tumor diameter
IB1	Invasive carcinoma >5 mm depth of stromal invasion and ≤2 cm in greatest dimension
IB2	Invasive carcinoma >2 and ≤ 4 cm in greatest dimension
IB3	Invasive carcinoma >4 cm in greatest dimension
II	The carcinoma invades beyond the uterus, but has not extended into the lower third of the vagina or to the pelvic wall
IIA	Involvement limited to the upper two-thirds of the vagina without parametrial involvement
IIA1	Invasive carcinoma ≤4 cm in greatest dimension
IIA2	Invasive carcinoma >4 cm in greatest dimension
IIB	With parametrial involvement but not up to the pelvic wall
III	The carcinoma involves the lower third of the vagina and/or extends to the pelvic wall and/or causes hydronephrosis or nonfunctioning kidney and/or involves pelvic and/or para-aortic lymph nodes
IIIA	The carcinoma involves the lower third of the vagina, with no extension to the pelvic wall
IIIB	Extension to the pelvic wall and/or hydronephrosis or nonfunctioning kidney (unless known to be due to another cause)
IIIC	Involvement of pelvic and/or para-aortic lymph nodes (including micrometastases), irrespective or tumor size and extent
IIIC1	Pelvic lymph node metastasis only
IIIC2	Para-aortic lymph node metastasis
IV	The carcinoma has extended beyond the true pelvis or has involved (biopsy proven) the mucosa of the bladder or rectum.
IVA	Spread to adjacent pelvic organs
IVB	Spread to distant organs

MRI IN CERVICAL CANCER

The latest FIGO stage allows high accuracy of locoregional staging of cervical carcinoma on MRI. MRI can be used to measure the tumor size and determine the extent of the disease. This has therapeutic implications as tumor size is the determining factor on the type of surgery performed in Stages I and IIA. Similarly in stages IIB, III and IV where chemoradiation is the primary modality of treatment, MRI images can help in deciding the radiation field. Hence MRI plays a vital role cervical cancer staging and treatment.[20]

P16 IN CERVICAL CANCER

P16 is a cyclin-dependent protein kinase inhibitor that gets upregulated due to inhibition of RB gene by the E7 oncoprotein of HR-HPV.[14,18] All cases of SCC, near all cases of HSIL and some cases of LSIL shows overexpression of P16.[18] Hence it is a surrogate biomarker for in situ and advanced SCC Cervix.[5,6,18] Most of the HPV associated tumors show a diffuse and strong immunostaining of P16 in IHC.[5] P16 gene is amplified in HR-HPV infection and integration of viral genome with host genome.[6]Hence, it is an indicator of the same. The P16 negative tumors have a more aggressive behavior when compared to the P16 positive tumors, indicating the strong association between HPV association and positive P16 immunostaining.[5]



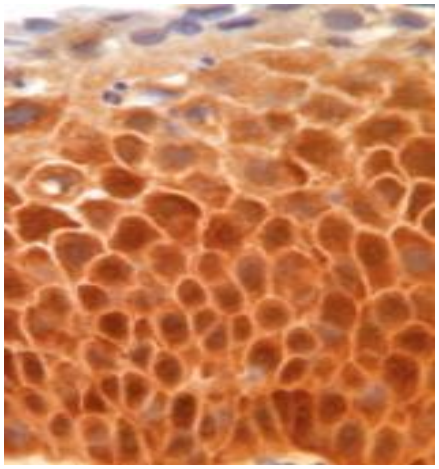


Figure 7: Diffuse block staining by P16 immunohistochemistry in cases of HR-HPV due to the upregulation of P16[17]

TUMOR-INFILTRATING LYMPHOCYTES (TILs) IN CERVICAL CANCER

The immune system plays a crucial role in cervical cancer as immune suppression can cause progression of LSIL and HSIL. HPV infection mounts a cell mediated immune response consisting of T helper lymphocytes that are associated with regression of lesion. The tumor microenvironment can have both immunoactive and immune suppressive cells. CD4+ Th1 cell can have antitumor tumor function but the CD4+ Th2 and Treg cells can cause immunosuppression and tumor progression. CD 8+ cytotoxic T cells play a major role in killing the tumor cells. Similarly, while NK cells and Dendritic cells are said to have anti-tumor effect, B cells are observed to be associated with tumor progression in cervical cancer. Knowledge on the tumor microenvironment can help in the upcoming immune therapies against cancer.[21] A study by Gultekin et al showed that on multivariate analysis, a low percentage of stromal TILs was associated decreased overall survival and disease-free survival. It was also associated with a higher rate of metastasis and had better predictive value than intraepithelial TILs.[22]

CLASPIN

Claspin is a multifunctional protein that plays a crucial role in Deoxyribonucleic Acid (DNA) replication and response to DNA damage.[7,8]

As an intrinsic component of the replisome, Claspin has an inherent affinity for branched DNA.[7,8]

During DNA replication, replication forks can stall due to replication stress, DNA damage, or DNA synthesis inhibition.[7,8] In response, a multiprotein complex forms on the single-stranded DNA at these stalled replication forks and sites of DNA damage. Claspin, an adaptor molecule within this complex, facilitates the activation of checkpoint kinase 1 (Chk1) by

ataxia telangiectasia mutated and Rad3-related protein (ATR). Once activated Chk1 is released from the complex, disperses throughout the nucleus, and triggers checkpoint activation by phosphorylating multiple substrates.[7]

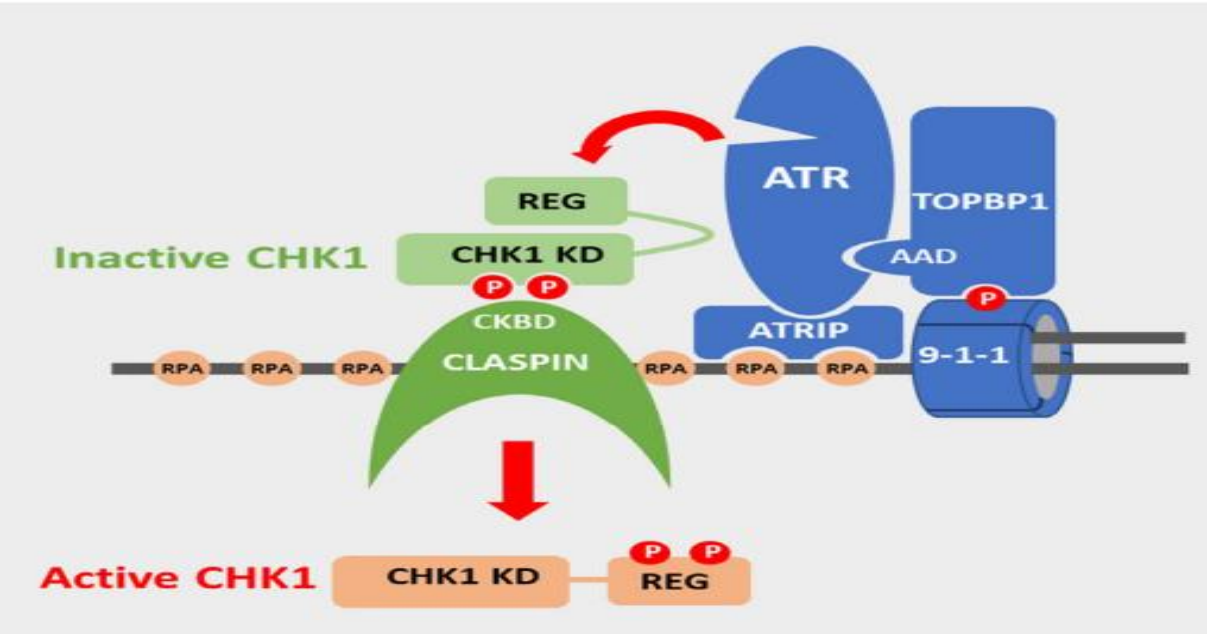


Figure 8: Pictorial representation of role of Claspin on DNA replication[7]

Hence, Claspin is vital for successful replication checkpoint activation in response to replication stress.[7,8]It maintains the stability of replication fork by being a mediator between ATR and Chk1 and protects the genome.[8]

Claspin has a dual role in DNA replication, extending beyond its checkpoint function. In addition to facilitating checkpoint activation, Claspin also has a structural role at replication forks, independent of its checkpoint function. As an integral component of the replisome progression complex, Claspin is essential for normal fork progression. Loss of Claspin can reduce the rate of replication in cells.[8]

An increased level of Claspin expression is seen in tumor cells when compared to normal cells. Since tumor cells generally replicate faster than surrounding normal tissue, higher levels of Claspin in cancer cell lines or tumor tissues can be reflecting their higher

proliferative index. It was found that the relative increase in Claspin expression observed in epithelial tumor tissue versus normal was considerably greater than for Ki67, suggesting that proliferative index may not be the only reason for increased expression of Claspin in cancer.[7]

Cancer cells have been found to rely more heavily on the ATR-CHK1 pathway, rather than the ataxia telangiectasia mutated (ATM)–checkpoint kinase 2 (Chk2) pathway, to survive after DNA damage.[13] Claspin, a critical protein in the ATR-CHK1 pathway, plays a vital role in this process.[7,8]Research has shown that Chk1 activation is more dependent on Claspin during the S phase of the cell cycle, while in the G1 phase, Claspin's role is less essential.[23]

A comprehensive analysis of 9,125 tumor samples across 33 cancer types, using the TIMER and Glex databases, revealed significantly higher expression of Claspin in tumor cells.[24] This highlights the importance of Claspin in cancer cell survival and potential as a target for cancer therapy.

CLASPIN AND CERVICAL CANCER

Analysis of Claspin expression in human cervical tissue revealed a steady and significant increase in Claspin positivity as cervical carcinogenesis progressed from normal tissue to SCC Cervix. The study showed that Claspin positivity increased gradually from mild dysplasia to severe dysplasia and carcinoma. Specifically, moderate to high Claspin positivity was observed in 15.8% of CIN1 lesions, 76.2% of CIN2 lesions, 87.5% of CIN3 lesions, and 93.3% of SCC cervix lesions.[10] This suggests a strong association between Claspin expression and cervical cancer development, highlighting Claspin's potential as a biomarker for cervical cancer diagnosis and prognosis.

CLASPIN AND HIGH-RISK HUMAN PAPILLOMA VIRUS

Research by Benevelo et al. revealed a strong correlation (97.8%) between moderate/high Claspin positivity and HR-HPV infection, regardless of the specific HR-HPV genotype. This suggests that Claspin expression is closely linked to infection by HR-HPV, which is the chief risk factor for development of cervical cancer.[10]

CLASPIN AS A BIOMARKER IN CANCER

Claspin overexpression has been detected in Immunohistochemistry (IHC) studies done on samples of Renal cell carcinoma (RCC), gastric cancer and prostate cancer. A significant association was demonstrated between the increased expression of Claspin and the tumor grade, nuclear grade, Tumor stage, venous invasion, lymphatic invasion and perineural invasion. Kobayashi G et al analyzed the expression and distribution of Claspin in Urothelial cancer and observed a negative association with progression-free survival and cancer-specific survival.[25] Claspin overexpression was associated with poor prognosis and has the potential to be a marker for tumor progression.[11,12,13,25]

CLASPIN AND TUMOR-INFILTRATING LYMPHOCYTES (TILs) IN CANCERS

A comprehensive multi-omics pan-cancer analysis used StromalScore, ImmuneScore and ESTIMATEScore to reveal that CLSPN (Claspin gene) was associated with TILs in cancers. The same study used immunofluorescence staining in lung adenocarcinoma to show that Claspin was negatively associated with CD8 + T cell infiltration and immune checkpoints including PD-1 and PD-L1.[24]

CLASPIN AS A THERAPEUTIC TARGET

Overexpression of Claspin protect cancer cells from endogenous replication stress and help in the maintenance of replication fork integrity.[9] Inhibition of Claspin in combination with added replication stress can suppress cancer cell growth.[8]

A study by Babasaki T et al done on the role of Claspin in docetaxel resistance in prostate cancer demonstrated that inhibition of Claspin increased docetaxel sensitivity.[13]

Inhibition of Claspin by CLSPN small interfering RNA treatment resulted in significant reduction in cell proliferative ability in RCC and decreased gastric cancer cell proliferation and invasion when compared with negative control cells.[12]

Yamada s et al established that Claspin had a role in cisplatin resistance in urothelial carcinoma and CLSPN peptide-specific immunotherapy could increase cisplatin sensitivity.[26] It was noted that the cell survival in response to cisplatin in urothelial carcinoma was considered to be due to phosphorylation of Claspin.[27]

MATERIAL & METHODS

MATERIALS AND METHODS

STUDY DESIGN – Cross sectional observational study.

PLACE OF STUDY – Department of Pathology, SDUMC, Tamaka, Kolar.

SOURCE OF DATA: Primary SCC of Cervix, HSIL and normal cervical specimens will be collected from Department of Pathology and Department of OBG from R.L. Jalappa Hospital and Research Centre attached to Sri Devaraj Urs Medical College, Tamaka, and Kolar.

DURATION OF STUDY– Cases will be collected for a period of 18 months (July 2022 to December 2023).

Inclusion Criteria: All fresh cases of primary SCC of cervix and HSIL diagnosed by cervical Biopsy or Hysterectomy.

Exclusion Criteria: Post-Chemotherapy, Post-Radiotherapy cases, recurrent cases, secondary metastasis in cervix or any other cancer in the patient.

SAMPLE SIZE: The Estimated Sample Size is 100.

Sample size for present cross-sectional study is estimated based on 93.3% positivity for Claspin in SCC cervix as reported in study[10], considering an absolute error of 5% with 95% confidence interval, the estimated sample size for the study is 97.

$$n = \frac{Z\alpha^2 PQ}{(d^2)}$$

P : Positivity rate

Q: 100-P

Z: Standard Normal Variate at 95 % .C I = 1.96

D: Absolute Error.

METHODS:

All freshly diagnosed primary SCC of Cervix and HSIL cases, diagnosed by incisional biopsy of cervix and Hysterectomy was included.

Information sheets were given to all the participants and Informed consent was obtained from them after explaining the details of the study.

Parameters like Age, Parity and Clinical Features was collected by interacting with the patient and from the case files. Stage, Size of tumor and Lymph node status was collected from relevant radiological investigations. Grade, lymphovascular invasion and TILs were determined by microscopic examination of the histopathological specimen.

The cervical tissue was grossed as per the standard operating procedure (SOP) of the lab and representative bits were given. The tissue bits were processed as per the protocol of the lab. Tissue sections were stained with Haematoxylin and Eosin (H & E) stains. The tissue sections were screened and analysed for microscopic features like histopathological type, grade of the tumor, TILs and lymphovascular invasion. The stage of the tumor, tumor size and lymph node status as per radiological investigation were noted. Tissue sections was subjected to Claspin and P16 Immunohistochemistry.

Scoring Of TILs:

TILs were scored in the stroma on H & E slides by visual inspection. A four-tier system was used.[28]It was calculated in the tumor stromal area without direct contact with the tumor cells

as the percentage of area occupied by mononuclear inflammatory cells.[29] Hotspot areas with good amount of stroma having immune cell infiltration was selected for evaluation.

Figure 9: Scoring of TILs

SCORE	PERCENTAGE	DESCRIPTION
0	0%	Absence of TILs
1+	<30%	Low TILs
2+	30%-60%	Moderate TILs
3+	>60%	Marked increase in the lymphocytic infiltrate

ANALYSIS OF IHC:

CLASPIN:-

IHC staining was done on 10% formalin-fixed paraffin-embedded tissue. Sections of approximately 3-4 micrometres was cut and floated on to positive charged slides and incubated at 37^0C for one day and further incubated at 58^0C overnight.

De-paraffinization of tissue section by xylene for two times, 15 minutes each. Rehydrating the section by absolute alcohol for two times, 1 minute each, 90% alcohol once for 1 minute and 70% alcohol once for 1 minute.

Wash with tap water for 10 minutes and rinse with distilled water for 5 minutes.

Microwaved at power 10 for 6 minutes in citrate buffer PH 6.0 for Antigen retrieval.

Washed with Tris-buffered saline (TBS) 2 times for 5 minutes each.

Incubated the sample for 5-10 minutes in 3% H_2O_2 to block the endogenous peroxidase activity.

Washed with TBS buffer 3 times for 5 minutes each.

Added 5% Bovine Serum Albumin (BSA) blocking buffer to the sample and incubated at room temperature for 20 minutes.

Drained and covered sections with Primary Antibody (Rabbit Polyclonal Anti-Claspin Antibody) and incubated at room temperature for 60 mins.

Washed with TBS buffer 3 times for 5 minutes each.

Added Secondary antibody to the tissue section and incubate at room temperature for 30 minutes.

Washed with TBS buffer 3 times for 5 minutes each.

Stained the tissue section with DAB (3,3'-Diaminobenzidine) Chromogenic kit.

Washed with TBS buffer 3 times for 5 minutes each and then with tap water for 5 minutes.

Slightly counterstained the tissue section with Haematoxylin.

Washed the excess stain with tap water for 5 minutes.

Dehydration by 90% Alcohol for 2 minutes and Absolute Alcohol for 2 minutes.

Clearing by Alcohol: Xylene (1:1) for 2 minutes and Xylene for 2 minutes.

Mounting done with Dibutyl phthalate Polystyrene Xylene (DPX).

The tissue section was ready for observation under microscope.

Figure 10: Claspin antibody

Antigen	Clone	Species	Producer	Dilution	Control	Stain
Claspin Ab	Polyclonal	Rabbit	Affinity Biosciences	Ready to use	Human gastric tissue	Nuclear

Staining of gastric glands were used as a positive control and staining without the antibody was used as negative control.

Immunostaining of Claspin was done according to criteria used in the study[10] by Benevolo M et al.

Hotspot regions, characterized by increased staining and absence of necrosis and hemorrhage, will be identified using a low-power light microscope at a magnification of 100X.

Positive staining will be determined by nuclear staining, irrespective of its intensity. We shall not include the squamous epithelium's basal layer, where proliferating cells are normally found.

With a light microscope, we will count the positive nuclei in five randomly chosen High Power fields (HPF, x400) that correspond to the lesion for each sample.

200 nuclei/HPF at most were counted. The mean number of nuclei which were immunoreactive were tallied in all selected fields for each sample, and the nuclei/HPF value was reported as a single value (Figure 9).

Figure 11: Claspin expression:

On the basis of the nuclei/HPF value, claspin expression was classified into:	
Negative	0 or less than 1 immunoreactive nuclei/HPF
Low-positive	1 to 20 immunoreactive nuclei/HPF
Moderate-positive	1 to 20 immunoreactive nuclei/HPF
High-positive	more than 80 immunoreactive nuclei/HPF

P16:-

IHC staining was done on 10% formalin-fixed paraffin-embedded tissue. Sections of approximately 3-4 micrometres was cut and floated on to positive charged slides and incubated at 37^0C for one day and further incubated at 58^0C overnight.

De-paraffinization of tissue section by xylene for two times, 15 minutes each. Rehydrating the section by absolute alcohol for two times, 1 minute each, 90% alcohol once for 1 minute and 70% alcohol once for 1 minute.

Wash with tap water for 10 minutes and rinse with distilled water for 5 minutes.

Microwaved at power 10 for 6 minutes in citrate buffer PH 6.0 for Antigen retrieval.

Washed with Tris-buffered saline (TBS) 2 times for 5 minutes each.

Incubated the sample for 5-10 minutes in 3% H_2O_2 to block the endogenous peroxidase activity.

Washed with TBS buffer 3 times for 5 minutes each.

Added 5% Bovine Serum Albumin (BSA) blocking buffer to the sample and incubated at room temperature for 20 minutes.

Drained and covered sections with Primary Antibody (Mouse monoclonal P16 Antibody) and incubated at room temperature for 60 mins.

Washed with TBS buffer 3 times for 5 minutes each.

Added Secondary antibody to the tissue section and incubate at room temperature for 30 minutes.

Washed with TBS buffer 3 times for 5 minutes each.

Stained the tissue section with DAB (3,3'-Diaminobenzidine) Chromogenic kit.

Washed with TBS buffer 3 times for 5 minutes each and then with tap water for 5 minutes .

Slightly counterstained the tissue section with Haematoxylin.

Washed the excess stain with tap water for 5 minutes.

Dehydration by 90% Alcohol for 2 minutes and Absolute Alcohol for 2 minutes.

Clearing by Alcohol: Xylene (1:1) for 2 minutes and Xylene for 2 minutes.

Mounting done with Dibutyl phthalate Polystyrene Xylene (DPX).

The tissue section was ready for observation under microscope.

Figure12: P16 antibody

Antigen	Clone	Species	Producer	Dilution	Control	Stain
Purified recombinant prokaryotic full length human P16 INK4 protein	Monoclonal	Mouse	Diagnostic Bio Systems	Ready to use	SCC cervix	Nuclear and Cytoplasmic

Staining of HPV associated SCC cervix was used as a positive control and staining without the antibody was used as negative control.

P16 was considered positive when nuclei was stained with or without cytoplasmic staining, the staining of only cytoplasm was considered negative.

P16 expression was classified into block positive, ambiguous staining, or negative as per the LAST criteria 2012.[30]

Figure 13: P16 staining based on LAST criteria[30]

P16 LAST criteria		
Negative	**Absent staining**	- Total absence of staining - Weak, focal, and discontinuous staining - Only cytoplasmic staining (without nuclear staining)
	Ambiguous staining	- Strong, basal, diffuse, and continuous staining (limited to lower 1/3rd of epithelium) - Weak, diffuse, and discontinuous staining (involving at least 2/3rd of epithelium) - Strong, focal, and discontinuous staining (at any epithelial level)
Positive	**Block positive**	**Strong and diffuse block staining for p16** - Continuous strong nuclear or nuclear plus cytoplasmic staining of the basal cell layer with extension upwards involving at least 1/3 of the epithelial thickness

STATISTICAL ANALYSIS:

All the data was entered in Microsoft Excel Sheet and statistical analysis was done by using SPSS Software version -2024.

The quantitative data like Age, Parity, Size of tumor, Lymph node status and Lymphovascular invasion was presented by using mean and standard deviation.

Categorical data like Clinical Features, Grade and Stage was presented by frequency and percentage. Association between Claspin expression and P16 expression was tested by Chi squared test.

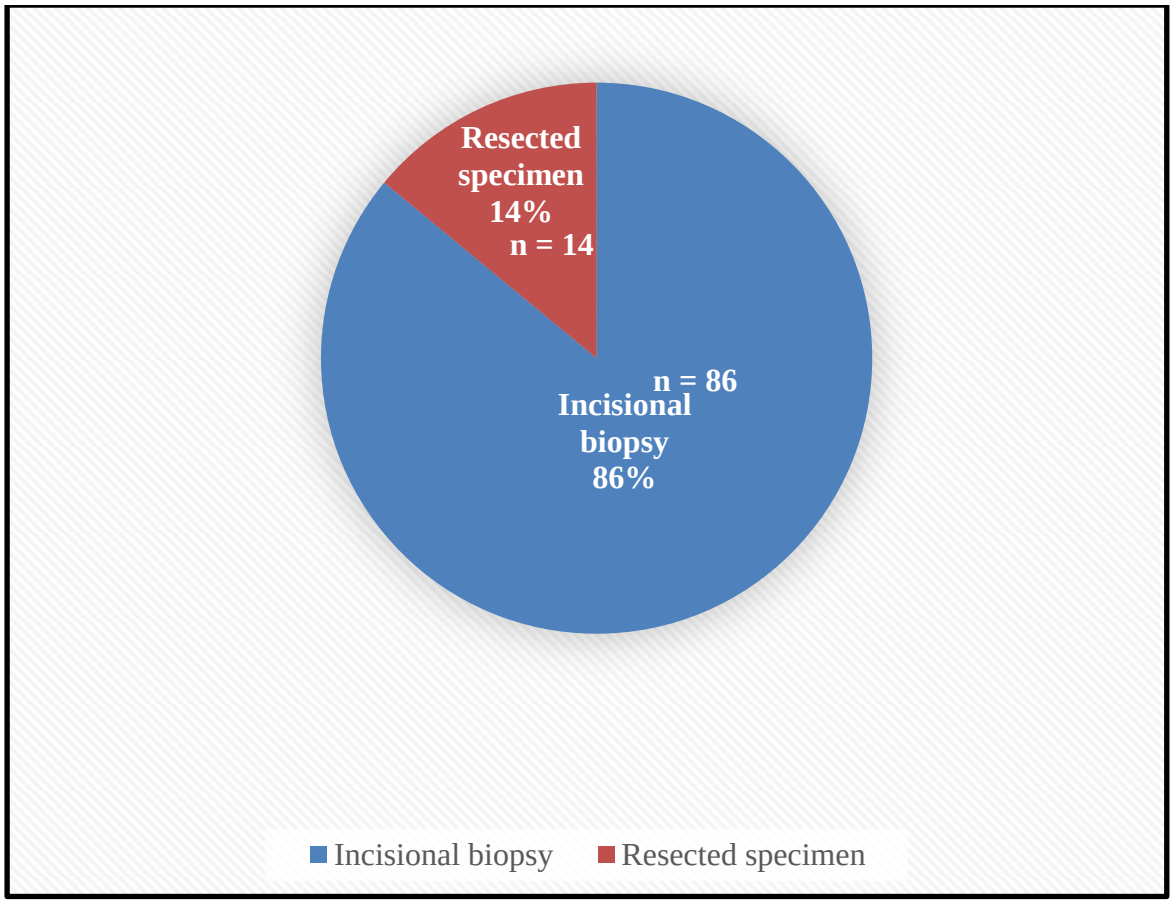
Results were statistically significant when p value was calculated to be less than 0.05.

RESULTS

RESULTS

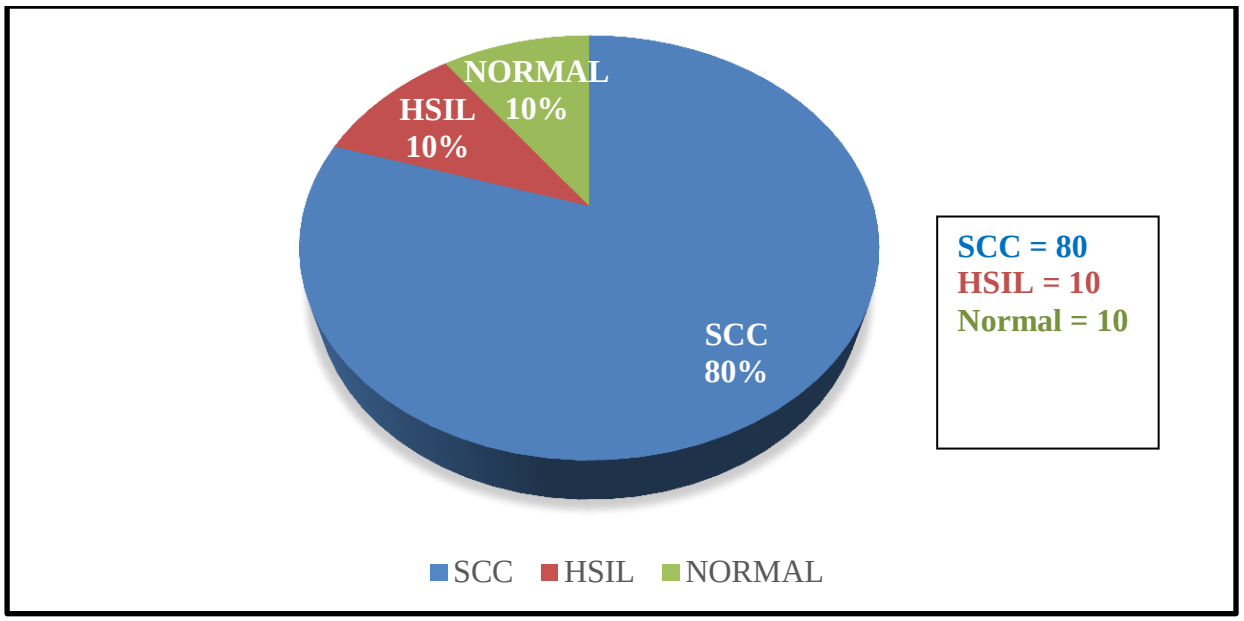
A total of 100 cases of squamous cell carcinoma cervix was collected, among which 86 cases were incisional biopsies and 14 cases were from surgically resected cervix (Figure 14).

Figure 14: Type of specimens



Of the 100 cases, 80 cases had a primary diagnosis of cervical cancer, 10 cases had a histological diagnosis of HSIL and 10 cases had normal histologic features (Figure 15).

Figure 15: Primary diagnosis of the 100 cases



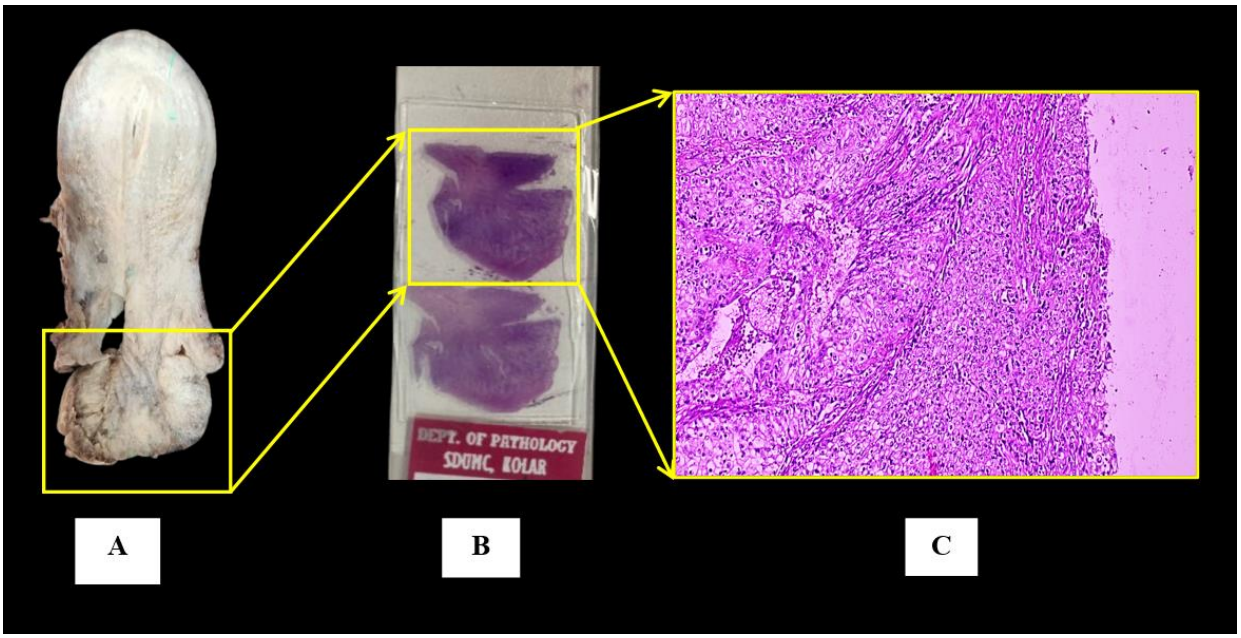


Figure 16: Resected hysterectomy specimen showing UPG arising from cervix (A), with the corresponding whole mount view of the growth on slide (B) and the microscopic finding of SCC of the same (H & E, x100) (C)

The following data was recorded for the 80 SCC cases:

1. **Patient demographics**

a) Age

b) Reproductive status

c) Parity

2. **Clinical findings**

a) Symptom

b) Per speculum finding

3. **Radiological findings**

a) Tumor size

b) Lymph node involvement

c) FIGO stage

4. **Microscopic findings**

a) Tumor grade

b) TILs

5. **Immunohistochemical studies**

a) P16 expression

b) Claspin expression

PATIENT DEMOGRAPHICS

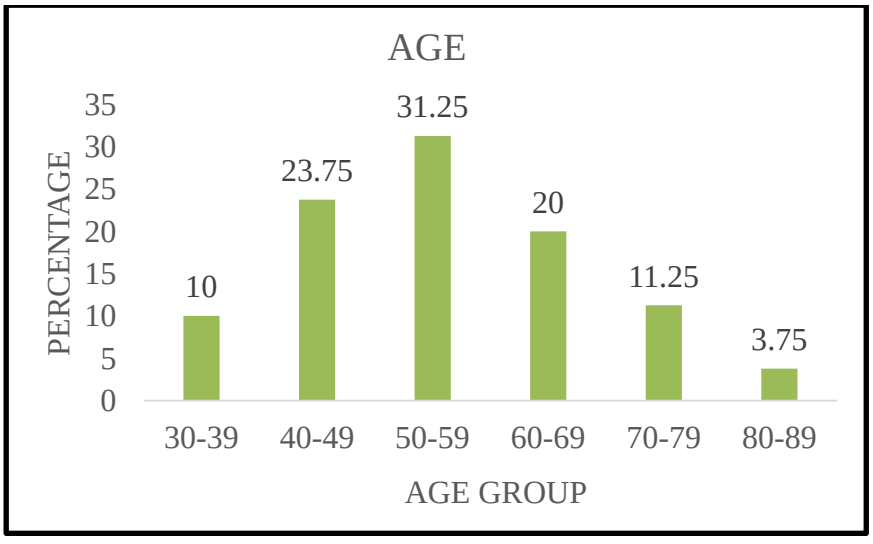
AGE

The age of the subjects with SCC cervix ranges from 33 to 87 years of age.

Table 1: Distribution of cervical cancer cases in various age groups:

Age group	Frequency	Percentage
30-39	8	10
40-49	19	23.75
50-59	25	31.25
60-69	16	20
70-79	9	11.25
80-89	3	3.75
Grand Total	80	100

Figure 17: Distribution of cervical cancer cases in various age groups:



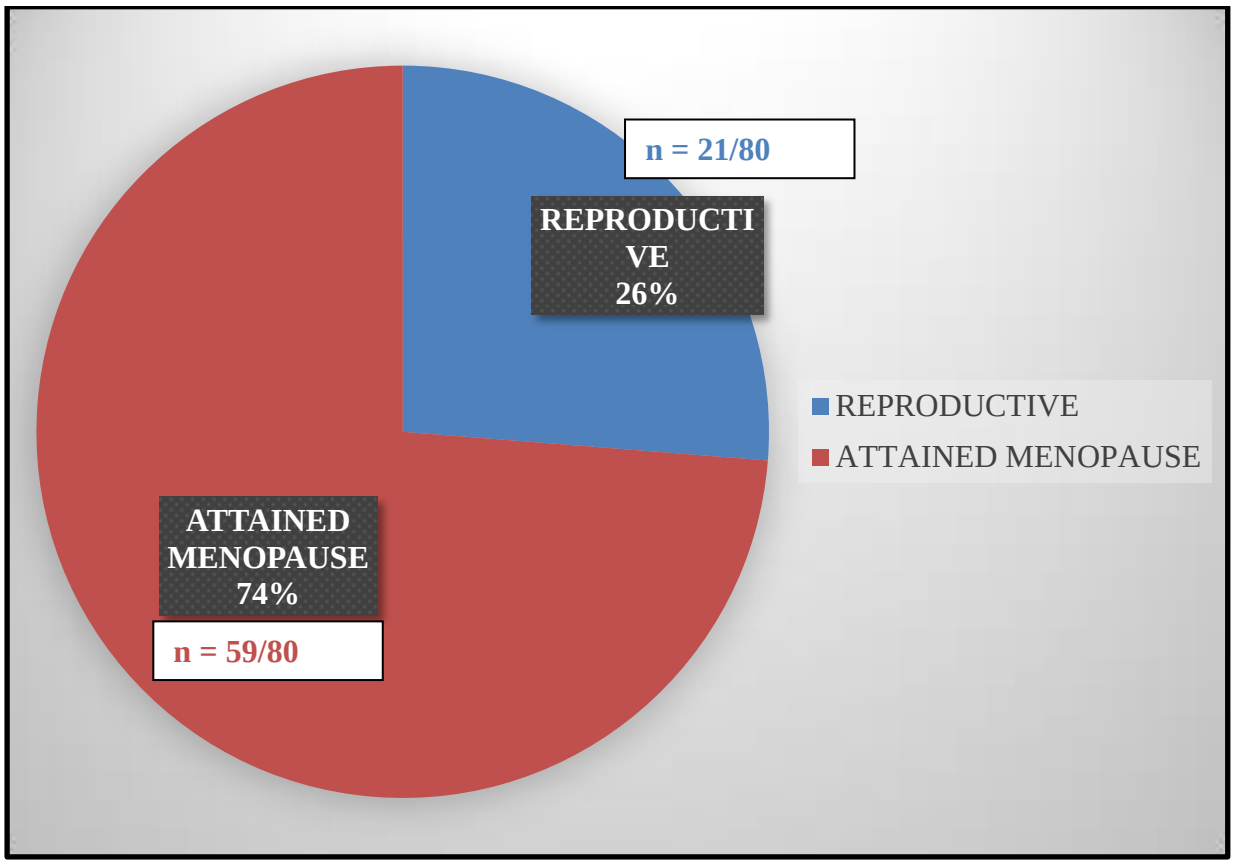
Among the cases of SCC of cervix, it was observed that maximum number of the patients were between the ages of 50 and 59 (31.25%), followed by ages between 40 and 49 (23.75%) and between 60 and 69 years (20%). The mean age was found to be 55 (Table 1 and figure 17).

REPRODUCTIVE STATUS

Table 2: Reproductive status of the SCC cases

Menstrual Status	Frequency	Percentage
Post-menopausal	59	73.75
Pre-menopausal	21	26.25
Grand Total	80	100

Figure 18: Distribution of the reproductive status of the cases



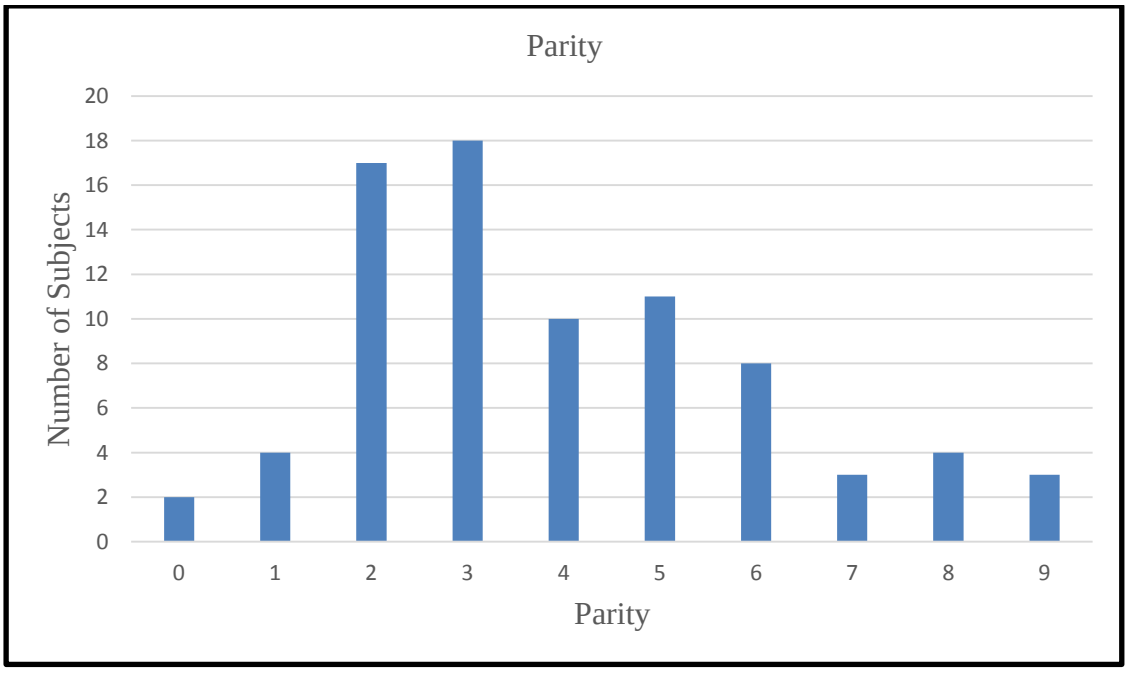
The reproductive status of these cases were taken into consideration and it was observed that substantial percentage of the patients (74%) had attained menopause (Table 2 and figure 18).

PARITY

Table 3: Distribution based on parity

PARITY	Frequency	Percentage
0	2	2.5
1	4	5
2	17	21.25
3	18	22.5
4	10	12.5
5	11	13.75
6	8	10
7	3	3.75
8	4	5
9	3	3.75
Grand Total	80	100

Figure 19: Distribution based on parity



The parity of the subjects were noted and all the cases having 3 or more were taken as high parity. It was observed that predominantly patients were in P3 category (22.5%) followed by P2 category (21%) (Table 3 and figure 19). It was noted that majority (71%) of the patients had more than 3 childbirths (Table 4).

Table 4: Distribution based on high parity and low parity

Parity	Frequency	Percentage
High parity (P3-P9)	57	71.25
Low parity (P0-P2)	23	28.75
Grand Total	80	100

CLINICAL PRESENTATION AND EXAMINATION FINDING

Most of the subjects presented clinically with bleeding per vagina (52.5%) including post-menopausal bleeding (40%) and intermenstrual bleeding (12.5%) followed by white discharge per vagina (26.25%). Other symptoms that the subjects presented with was pain abdomen, mass per vagina and back pain (Table 5 and figure 20). On examination of these patients with a per speculum, it was observed that majority of the cases had an ulceroproliferative growth (70%) followed by a smaller portion of the subjects having hyper trophied cervix (21.25%) and cervical erosions (8.75%) (Table 6 and figure 21).

Table 5: Clinical presentation of the subjects

Clinical Features	Frequency	Percentage
Post menopausal bleeding	32	40%
Intermenstrual bleeding	10	12.50%
White discharge per vagina	21	26.25%
Pain abdomen	13	16.25%
Mass per vagina	3	3.75%
Back pain	1	1.25%
Total	80	100%

Figure 20: Clinical presentation of the subjects

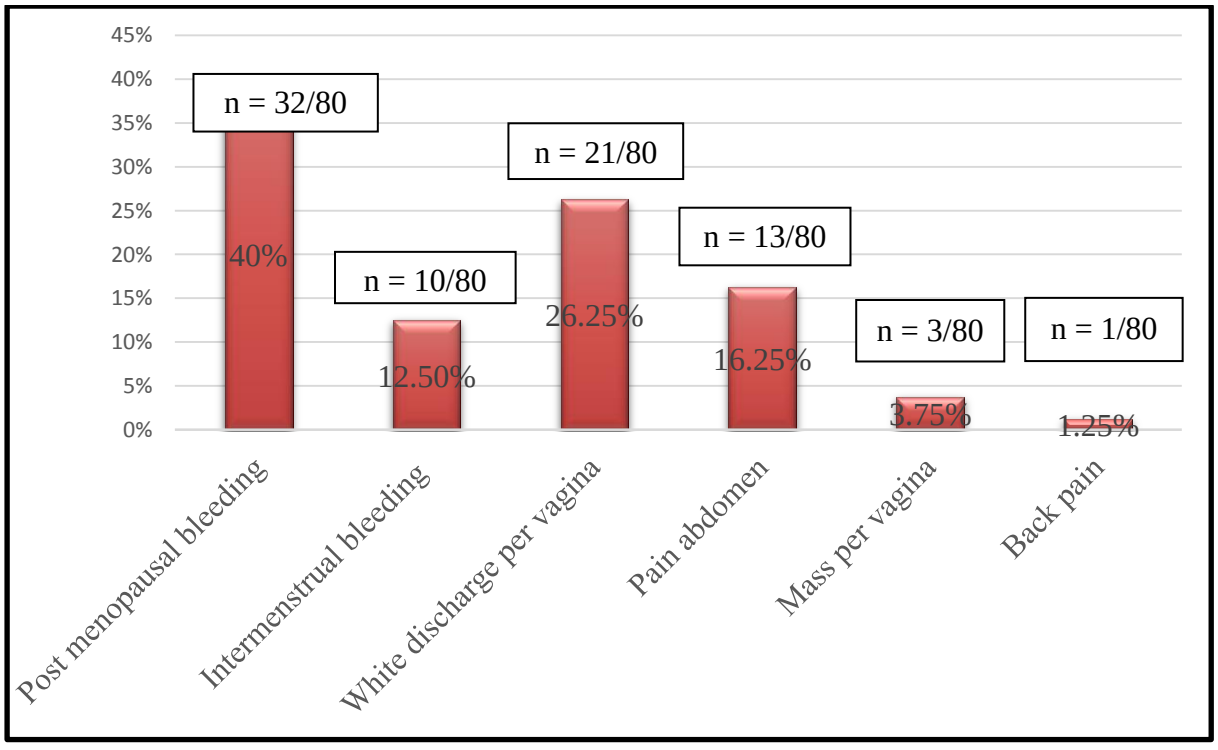
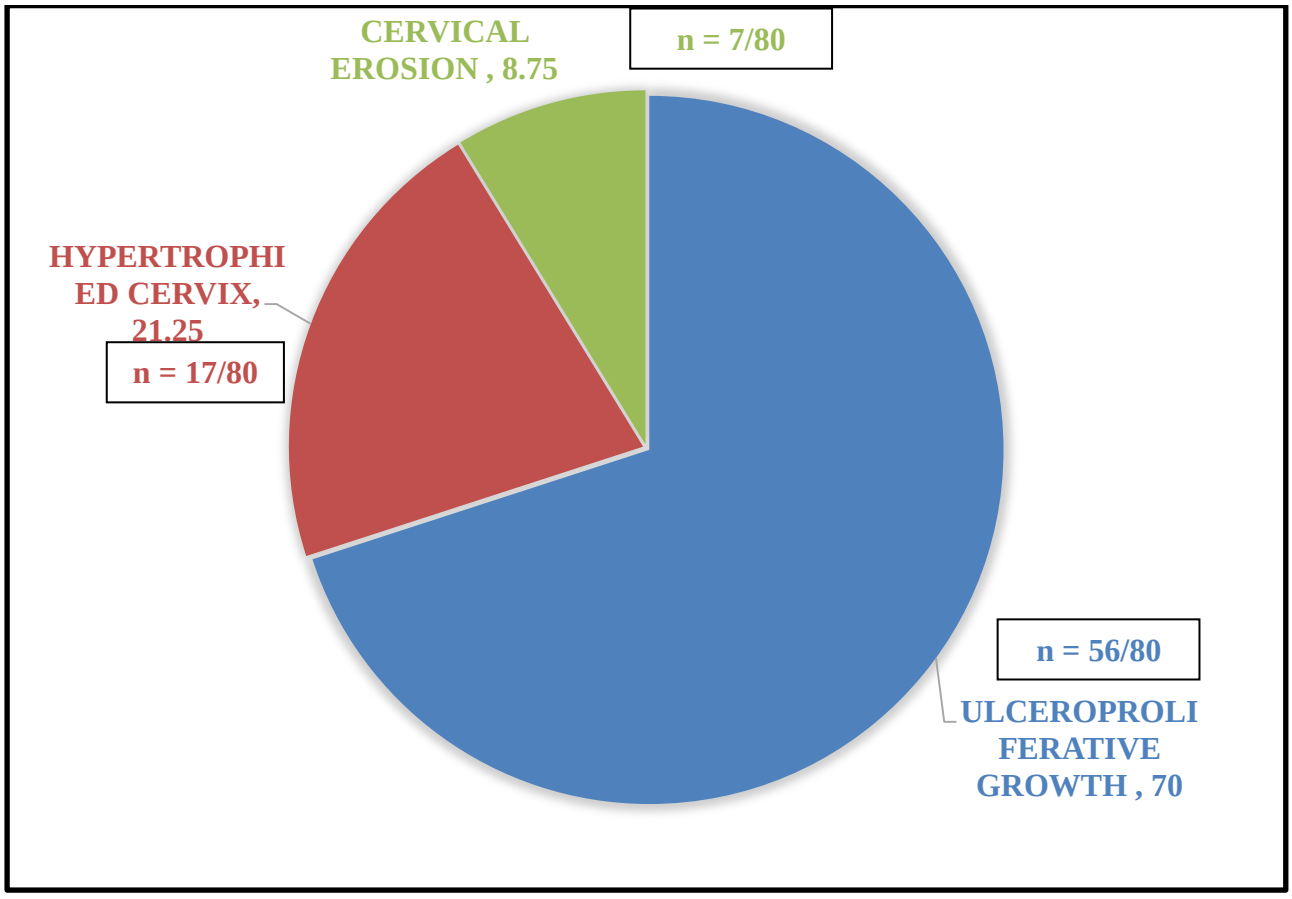


Table 6: Per speculum findings in the 80 cases

Per Speculum	Frequency	Percentage
ULCEROPROLIFERATIVE GROWTH	56	70%
HYPERTROPHIED	17	21.25%
EROSION	7	8.75%
Total	80	100%

Figure 21: Per speculum findings in the 80 cases



RADIOLOGICAL FINDINGS

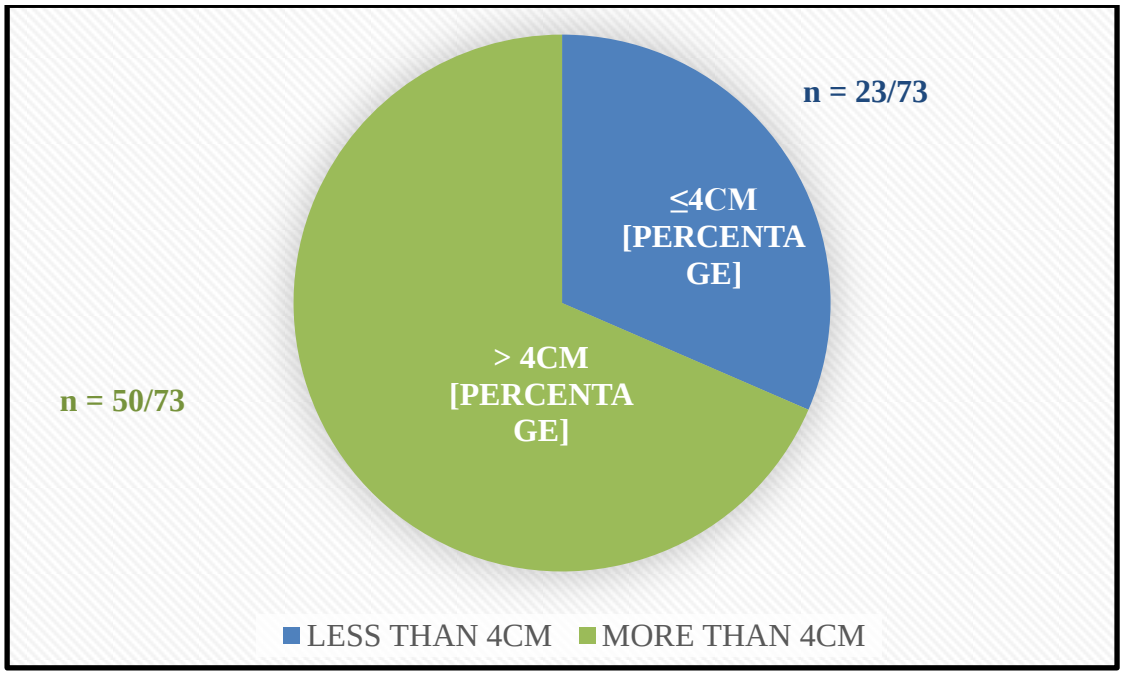
73 of the 80 subjects with diagnosis of SCC cervix had MRI pelvis done and findings such as size of tumor, lymph node involvement and FIGO stage were noted.

SIZE OF TUMOR

Table 7: Size of tumor

Tumor Size	Frequency	Percentage
> 4 CM	50	68.5%
≤ 4 CM	23	31.5%
Total	73	100%

Figure 22: Size of tumor



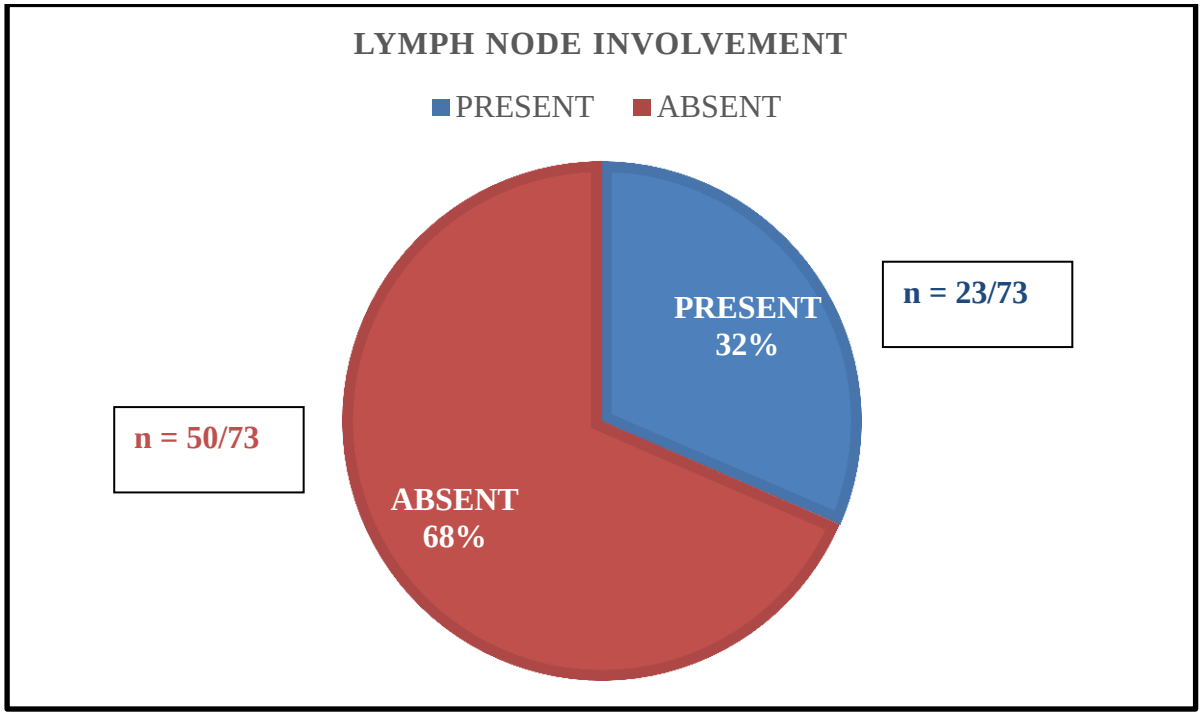
Based on the size of tumor on the MRI, the cases were classified into tumors with the largest dimension being more than 4cm or less than/ equal to 4cm (Table 7 and figure 22). It was observed that majority of the tumors were more than 4cm at the time of diagnosis (68%). This implies that most of the cases are already at higher stages when diagnosed.

LYMPH NODE INVOLVEMENT

Table 8: Lymph node involvement distribution

Lymph node	Frequency	Percentage
Absent	50	68.50%
Present	23	31.5%
Total	73	100%

Figure 23: Lymph node involvement



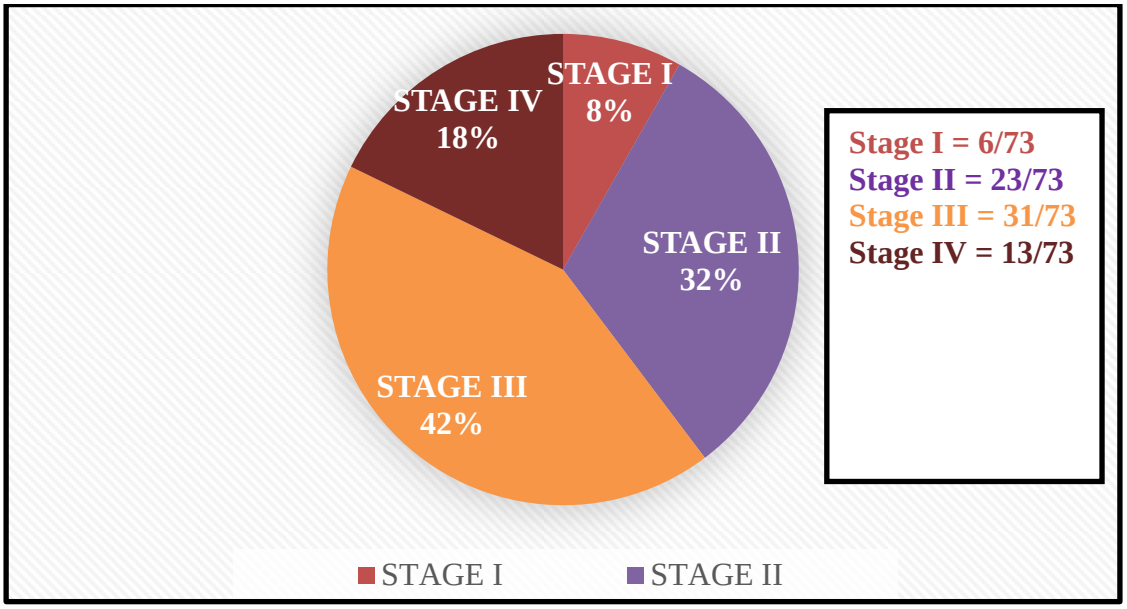
Lymph node involvement when noted among the 80 cases, showed that only 32% of cases had lymph node metastasis at the time of presentation (Table 8 and figure 23).

FIGO STAGE

Table 9: FIGO stage of the cases

Stage	Frequency	Percentage
Stage I	6	8.21
Stage II	23	31.50
Stage III	31	42.46
Stage IV	13	17.80
Total	73	100

Figure 24: FIGO stage of the cases

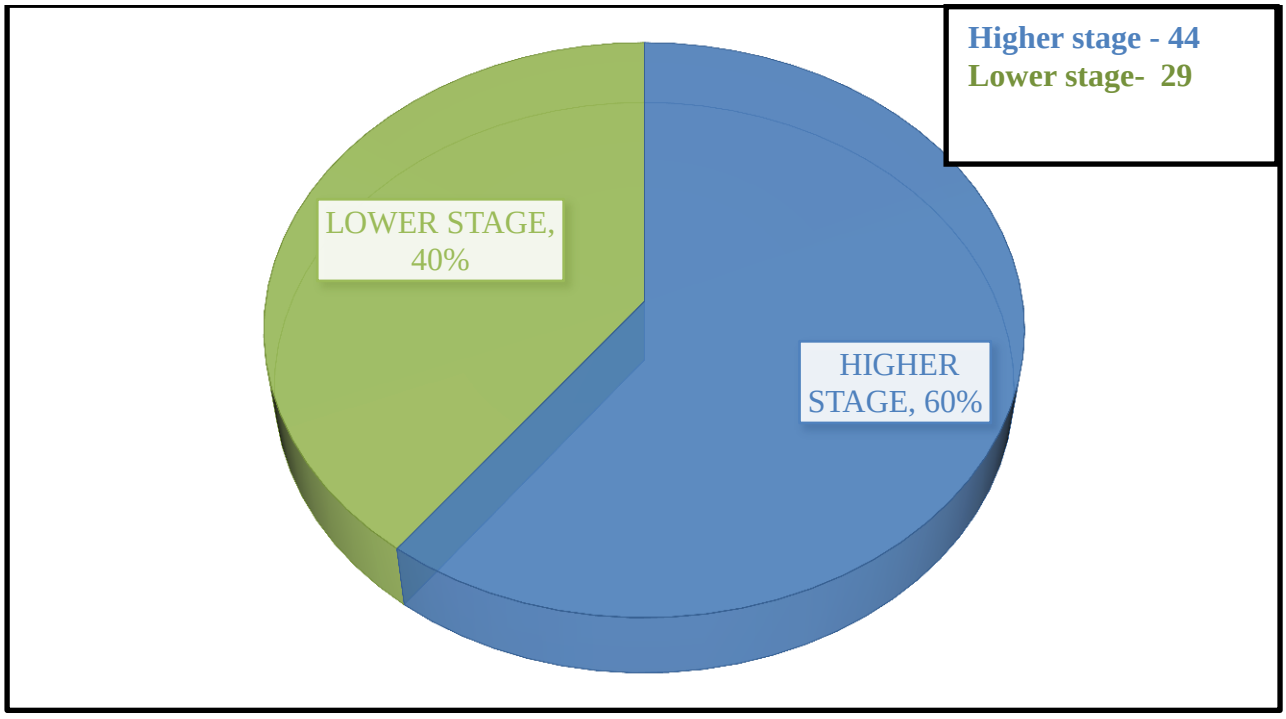


FIGO stage of the cases from MRI was taken and it was observed that majority of cases were diagnosed at stage III (42%) followed by stage II (32%), stage IV (18%) and the least number of cases were diagnosed at stage I (8%) (Table 9 and figure 24). The stages were categorized into lower (stages I and II) and higher (stages III and IV) stages, it was noted that majority of the cases were diagnosed at advanced stage (59%) (Table 10 and figure 25).

Table 10: Distribution of cases into higher and lower stages

Stage	Frequency	Percentage
Lower stage	29	39.72
Higher stage	44	60.27
Total	73	100

Figure 25: Distribution of cases into higher and lower stages



ASSOCIATION OF STAGE WITH DEMOGRAPHIC AND CLINICAL PARAMETERS

Table 11: Association of stage with clinicopathologic parameters

Parameters	STAGE		p value
	Lower Stage % (n)	Higher Stage % (n)	
Age Group			0.18
30-39	8 (6)	2(1)	
40-49	11 (8)	12 (9)	
50-59	8 (6)	**23 (17)**	
60-69	8 (6)	11(8)	
70-79	3 (2)	9(7)	
80-89	2(1)	3 (2)	
TOTAL	40 (29)	60 (44)	100% (73)
Parity			0.529
High parity(P3-P9)	27(19)	**43(32)**	
Low parity(P0-P2)	13(10)	17(12)	
TOTAL	40 (29)	60 (44)	100% (73)
Reproductive status			0.033
Post-menopausal	24(18)	**51(37)**	
Pre-menopausal	16(11)	9(7)	
TOTAL	40 (29)	60 (44)	100% (73)
Clinical features			0.028
Postmenopausal bleeding	17 (12)	24(18)	
Intermenstrual bleeding	6(5)	4 (3)	
White discharge per vagina	13(10)	12(9)	
Pain abdomen	0 (0)	17 (12)	
Mass per vagina	2(1)	3 (2)	
Back pain	2(1)	0 (0)	
TOTAL	40 (29)	60 (44)	100% (73)
Per speculum			0.544
Growth	27(19)	47(34)	
Erosion	4 (3)	4 (3)	
Hypertrophied	9(7)	9(7)	
TOTAL	40 (29)	60 (44)	100% (73)

The stage of cervical cancer was associated with clinical parameters and showed significant association with menopausal status and clinical presentation with post-menopausal bleeding (Table 11 and figure 26).

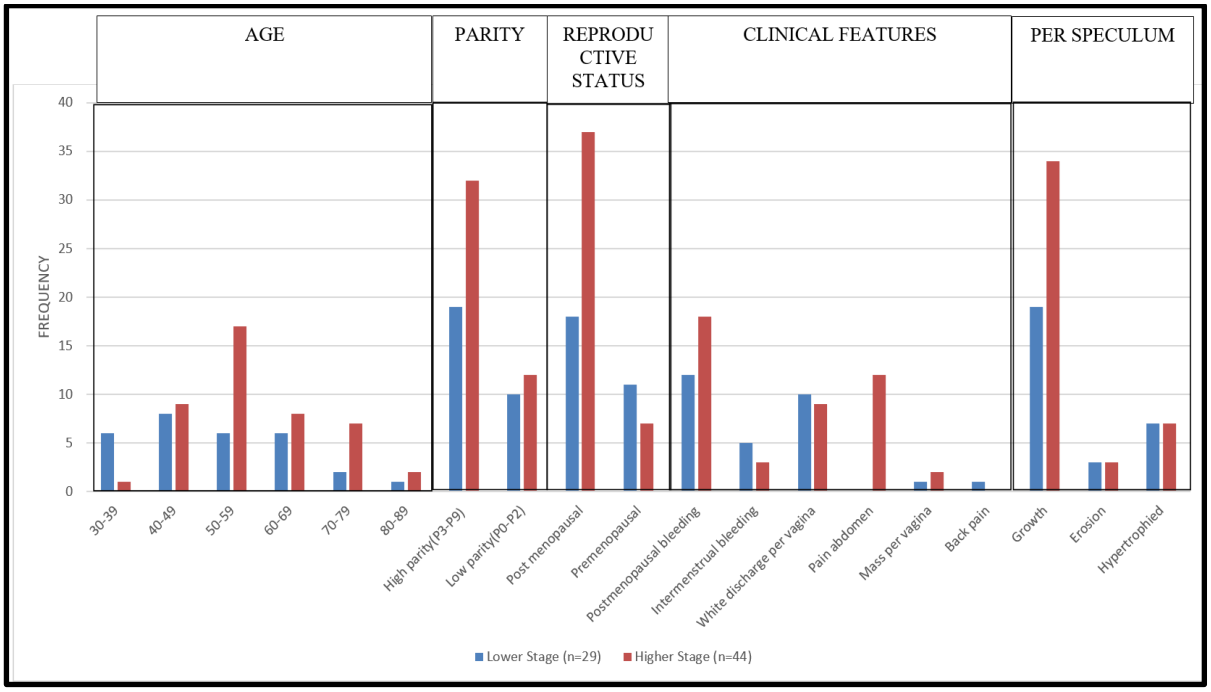


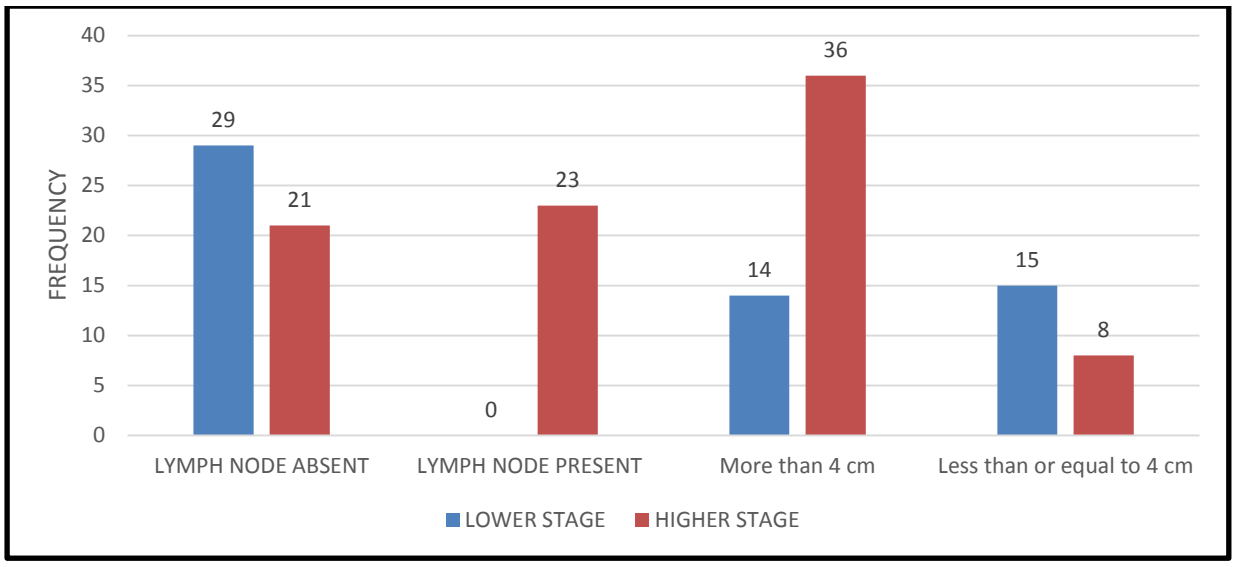
Figure 26: Association of stage with demographic and clinical parameters

ASSOCIATION OF STAGE WITH RADIOLOGICAL PARAMETERS

Table 12: Associationof stage with size of tumor and lymph node involvement:

		STAGE		p value
		Lower stage % (n)	Higher stage % (n)	
LN INVOLVEMENT	Present	0 (0)	**31 (23)**	**< 0.001**
	Absent	40 (29)	29 (21)	
TOTAL		40 (29)	60 (44)	100% (73)
SIZE	> 4 cm	19 (14)	**49 (36)**	**0.003**
	≤ 4 cm	21 (15)	11 (8)	
TOTAL		40 (29)	60 (44)	100% (73)

Figure 27: Associationof stage with lymph node involvement and size of tumor



The stage of disease showed significant association with lymph node involvement and size of the tumor (Table 12 and figure 27).

PATHOLOGICAL FINDINGS

H and E stained sections of cervix that were diagnosed as SCC was further graded according to Broder's classification and was also evaluated for TILs.

GRADING

Table 13: Histological grading of the 80 SCC cases

GRADE	Frequency	Percentage
Well Differentiated (WD)	51	63.75%
Moderately Differentiated (MD)	27	33.75%
Poorly Differentiated (PD)	2	2.50%
Total	80	100%

The SCC cases when classified using Broder's classification, majority of cases were Well differentiated (64%) followed by Moderately differentiated (34%) and Poorly differentiated (2%) (Table 13 and figure 27).

The grades were associated with parameters like age, parity, reproductive status, clinical presentation and per speculum finding and showed no significant association was found (Table 14 and figure 28).

Figure 28: Histological grading of the 80 SCC cases

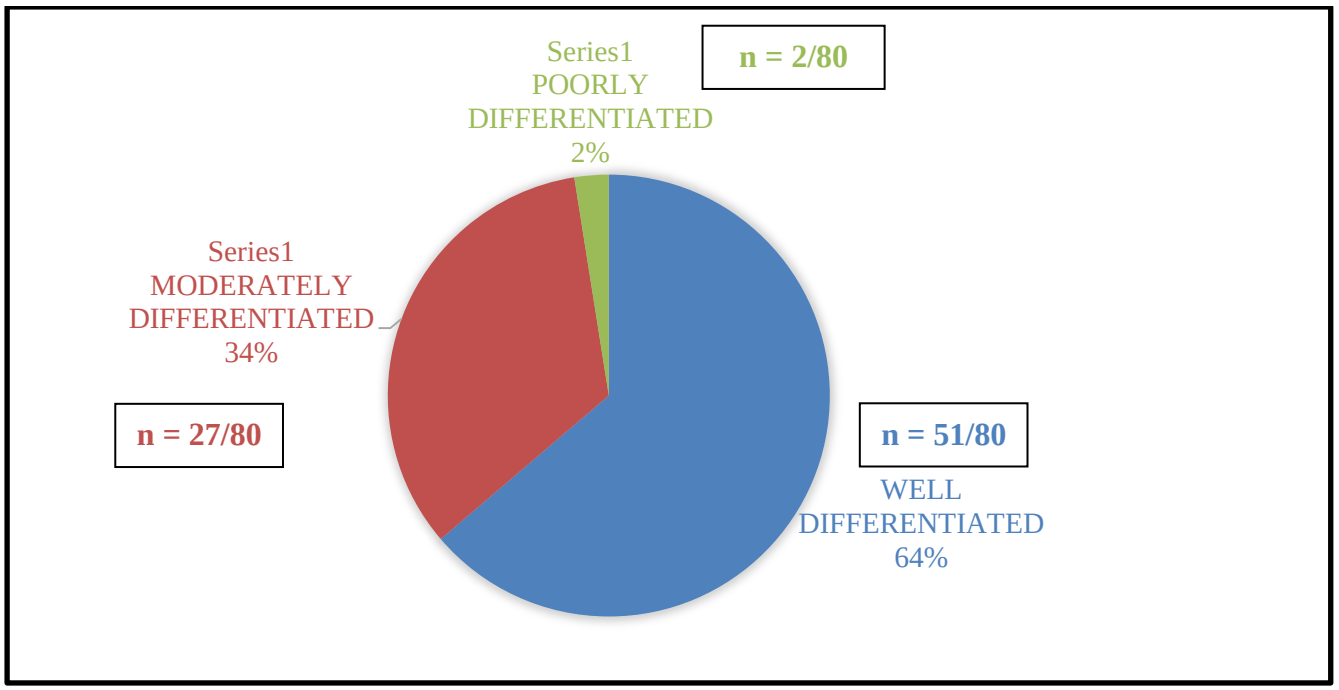
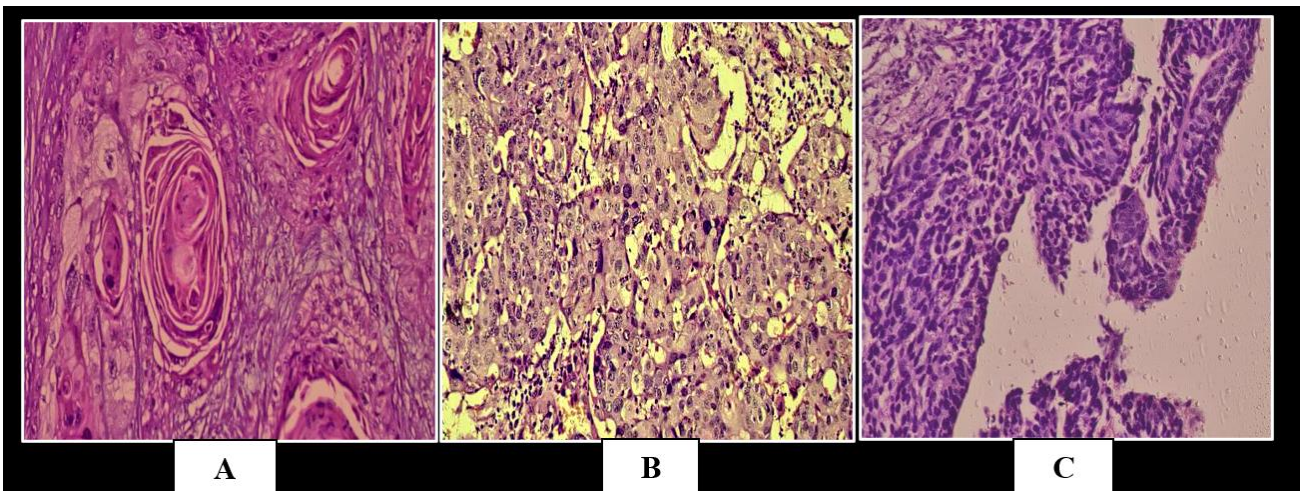


Figure 29: Histopathological grade of tumor. A) Well differentiated tumor cells with keratin pearl formation, B) Moderately differentiated tumor cells and C) Poorly differentiated tumor cells (H & E. x200)



ASSOCIATION OF GRADE WITH DEMOGRAPHIC AND CLINICAL PARAMETERS

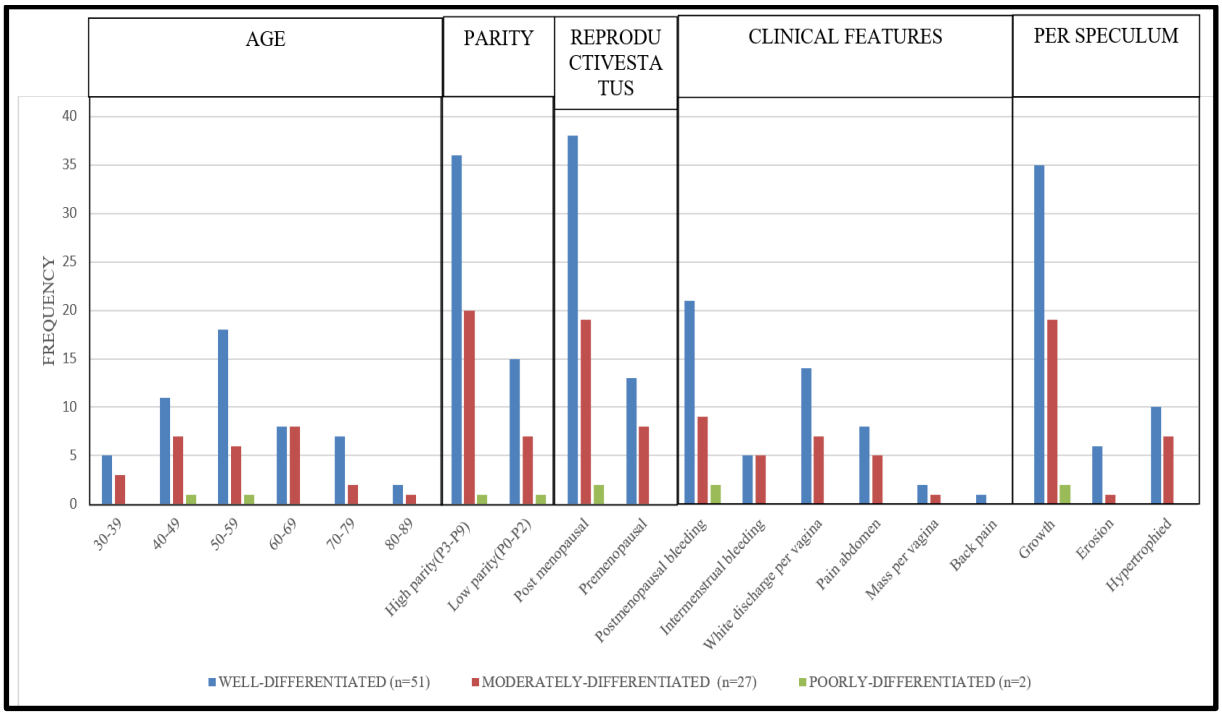
Table 14: Association of histological grade

Parameters	GRADE			p value
	WD % (n)	MD % (n)	PD % (n)	
Age Group				
30-39	6 (5)	4 (3)	0 (0)	0.875
40-49	14 (11)	9(7)	1.5 (1)	
50-59	22.5 (18)	7 (6)	1.5 (1)	
60-69	10 (8)	10 (8)	0 (0)	
70-79	9 (7)	2.5 (2)	0 (0)	
80-89	2.5 (2)	1.5 (1)	0 (0)	
TOTAL	64 (51)	34 (27)	3 (2)	100% (80)
Parity				
High parity(P3-P9)	45 (36)	25 (20)	1.5 (1)	0.875
Low parity(P0-P2)	19 (15)	9 (7)	1.5 (1)	
TOTAL	64 (51)	34 (27)	3 (2)	100% (80)
Reproductive status				
Post-menopausal	48 (38)	24 (19)	3 (2)	0.642
Pre-menopausal	16 (13)	10 (8)	0 (0)	
TOTAL	64 (51)	34 (27)	3 (2)	100% (80)

Clinical features				0.887
Postmenopausal bleeding	26 (21)	11.5 (9)	3(2)	
Intermenstrual bleeding	6 (5)	6 (5)	0 (0)	
White discharge per vagina	17 (14)	9 (7)	0 (0)	
Pain abdomen	10 (8)	6 (5)	0 (0)	
Mass per vagina	3 (2)	1.5 (1)	0 (0)	
Back pain	1.5 (1)	0 (0)	0 (0)	
TOTAL	64 (51)	34 (27)	3 (2)	100% (80)
Per speculum examination				0.639
Growth	44 (35)	23.5 (19)	3 (2)	
Erosion	8 (6)	1.5 (1)	0 (0)	
Hypertrophied	12 (10)	9 (7)	0 (0)	
TOTAL	64 (51)	34 (27)	3 (2)	100% (80)

The grade of tumor had no significant association with parameters like age, parity, menopausal status, clinical presentation and per speculum examination findings (Table 14 and figure 30).

Figure 30: Association of histological grade with clinical parameters



ASSOCIATION OF GRADE WITH STAGE

The association of stage of the tumor with grade of tumor was done and the p-value was calculated which was 0.204. The p value showed that there is no significant association between the grade and the stage of the tumor.

Table 15: Association of stage and grade

GRADE	STAGE		p value
	Lower stage % (n)	Higher stage % (n)	0.204
WD	23 (17)	42 (31)	
MD	17 (12)	15 (11)	
PD	0 (0)	3 (2)	
Total	40 (29)	60 (44)	100% (73)

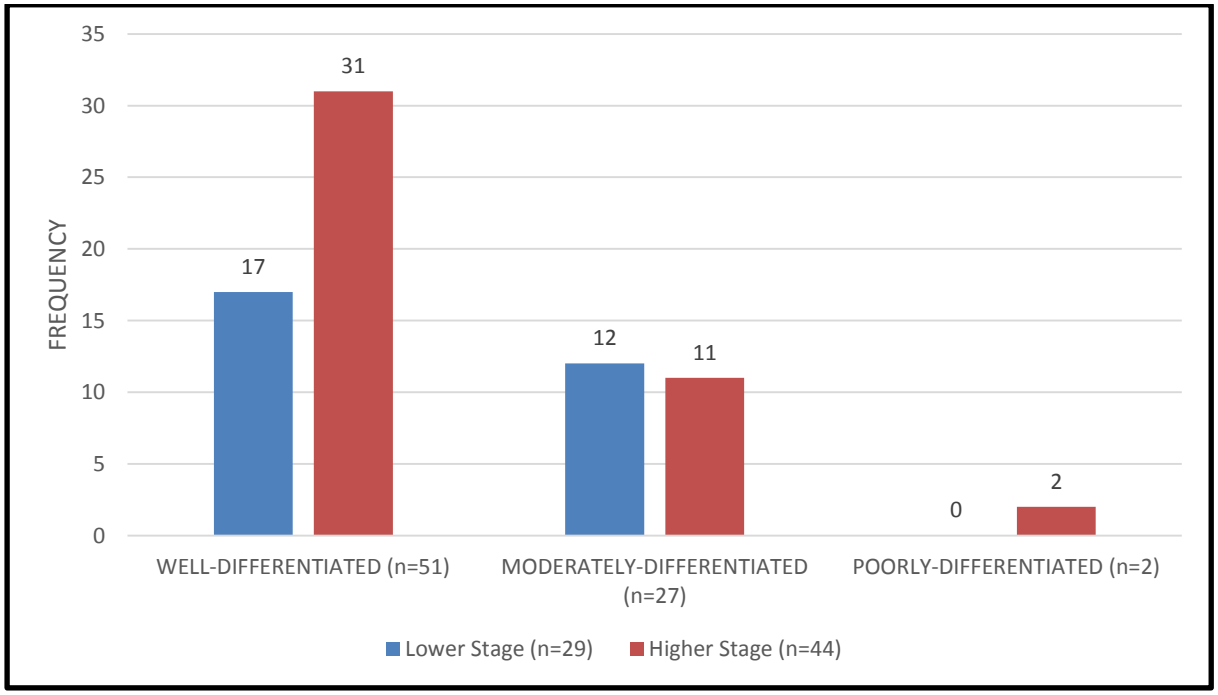


Figure 31: Association of stage and grade

TILs

TILs were calculated for 65 cases. 15 cases did not have enough stroma in the biopsy for an accurate scoring of TILs on microscopy.

Table 16: Scoring and distribution of TILs

SCORE	PERCENTAGE	DESCRIPTION	NUMBER	PERCENTAGE (%)
0	0%	Absence of TILs	0	0
1+	<30%	Low TILs	12	18.46
2+	30%-60%	Moderate TILs	16	24.61
3+	>60%	Marked increase in the lymphocytic infiltrate	37	56.92
TOTAL			65	100

Figure 32: Representative microphotograph of TILs (H&E, x200)

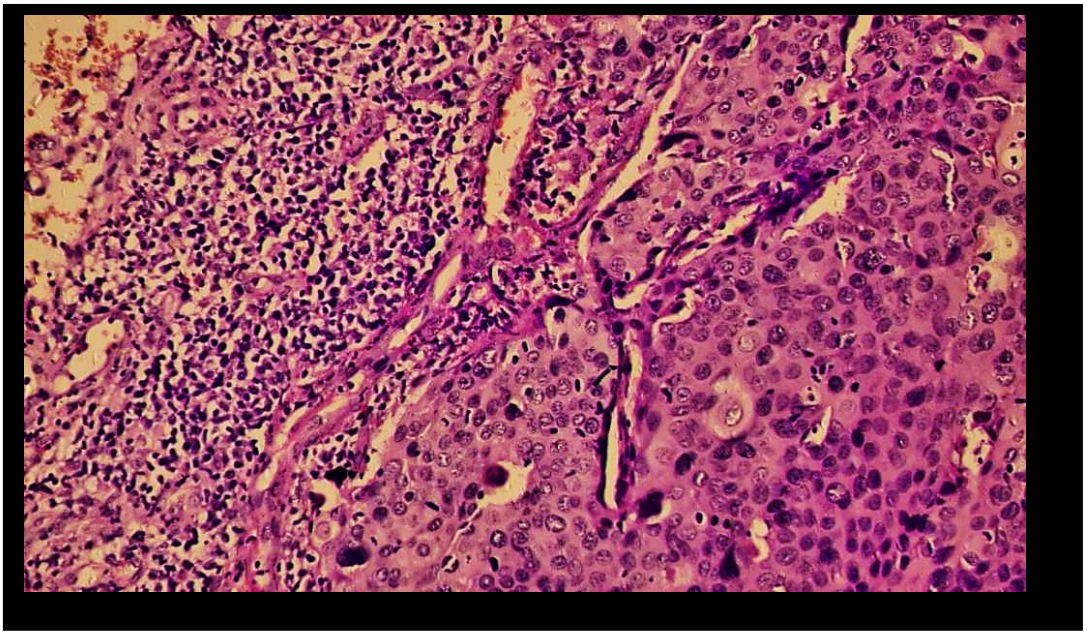
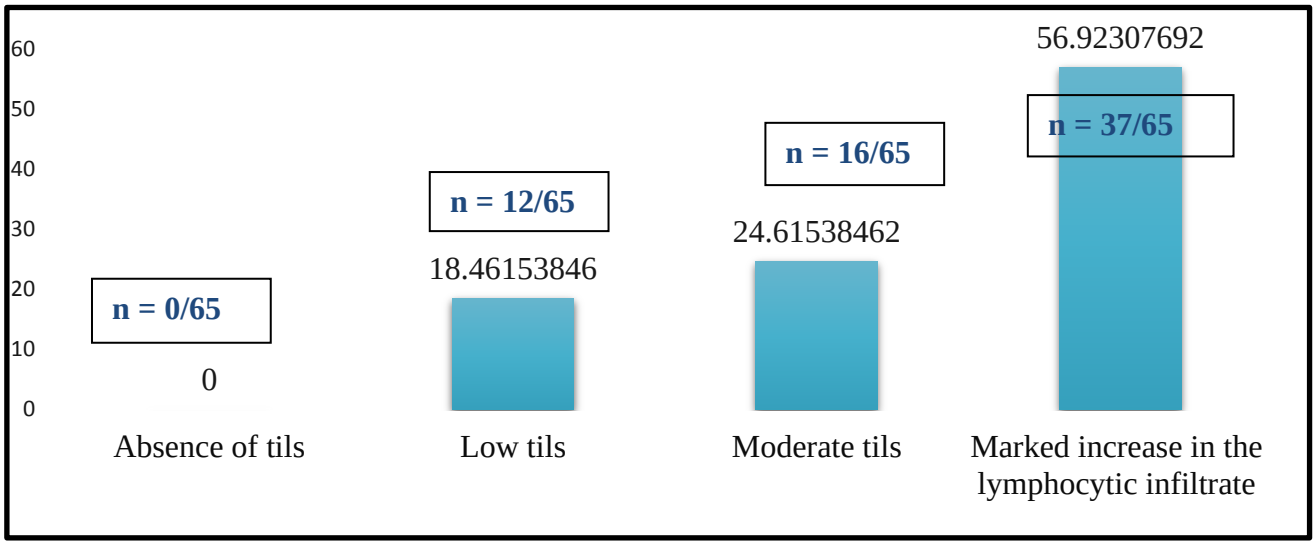


Figure 33: Scoring and distribution of TILs



Majority of the cases showed a marked increase in the lymphocytic infiltrates (57%) (Table 16 and Figure 33). The cases showing marked increase in lymphocytes was considered to have high TILs. TILs showed no significant association with the clinicopathologic parameters (Table 17,18 and Figure 34).

ASSOCIATION OF TILs WITH GRADE WITH DEMOGRAPHIC AND CLINICAL PARAMETERS

Table 17: Association of TILs with demographic and clinical parameters

Parameters	TILs		p value
	High TILs % (n)	Low and moderate TILs % (n)	
Age Group			0.059
30-39	2 (1)	9 (6)	
40-49	12 (8)	11 (7)	
50-59	20 (13)	9 (6)	
60-69	11 (7)	9 (6)	
70-79	9 (6)	3 (2)	
80-89	3 (2)	2 (1)	
TOTAL	57 (37)	43 (28)	100% (65)
Parity			0.452
High parity(P3-P9)	42 (27)	28 (18)	
Low parity(P0-P2)	15 (10)	15 (10)	
TOTAL	57 (37)	43 (28)	100% (65)
Reproductive status			0.069
Post-menopausal	46 (30)	26 (17)	
Pre-menopausal	11 (7)	17 (11)	

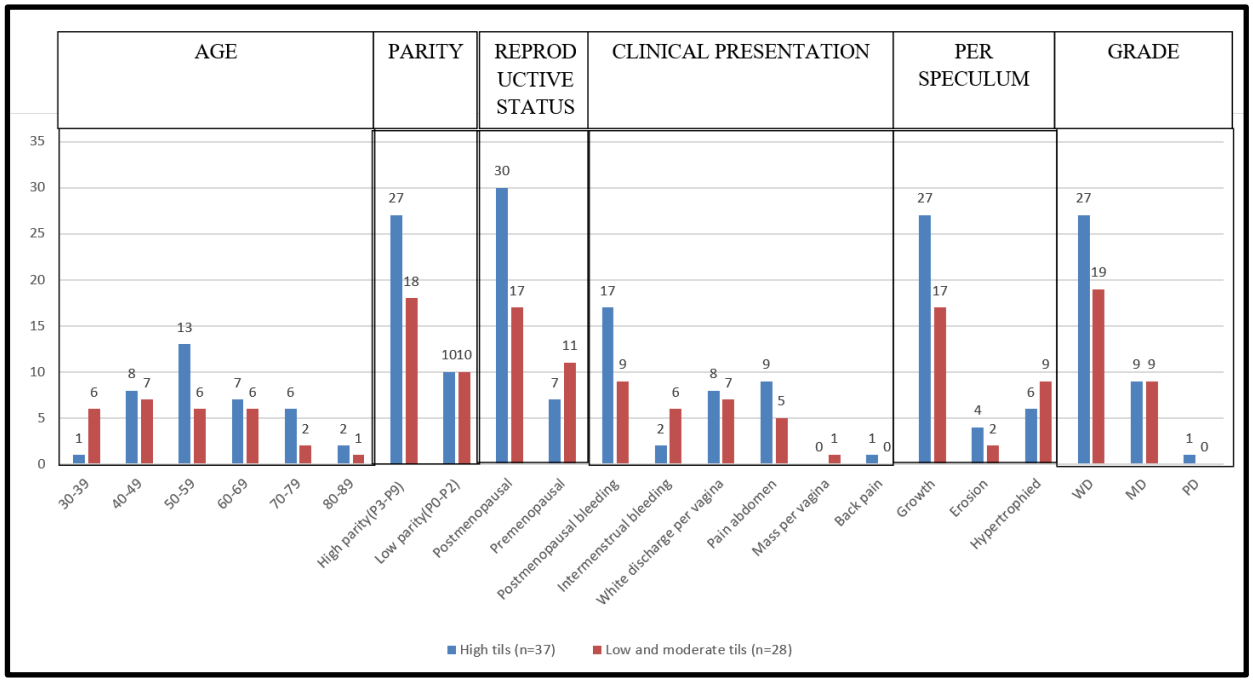
TOTAL	57 (37)	43 (28)	100% (65)
Clinical features			
Postmenopausal bleeding	26 (17)	14 (9)	
Intermenstrual bleeding	3 (2)	9 (6)	
White discharge per vagina	12 (8)	11 (7)	
Pain abdomen	14 (9)	7 (5)	0.053
Mass per vagina	0 (0)	2 (1)	
Back pain	2 (1)	0 (0)	
TOTAL	57 (37)	43 (28)	100% (65)
Per Speculum			
Growth	42 (27)	26 (17)	
Erosion	6 (4)	3 (2)	0.3107
Hypertrophied	9 (6)	14 (9)	
TOTAL	57 (37)	43 (28)	100% (65)

ASSOCIATION OF TILs WITH GRADE

Table18: Association of TILs with grade

		TILs		p Value
		High TILs % (n)	Low and moderate TILs % (n)	
GRADE	WD	41 (27)	29 (19)	0.558
	MD	14 (9)	14 (9)	
	PD	2 (1)	0/65	
	TOTAL	57 (37)	43 (28)	100% (65)

Figure 34: Association of tils with age, parity, menopausal status, clinical presentation, examination findings and grade of tumor



LYMPHOVASCULAR INVASION (LVI)

LVI was observed in 4 cases of SCC cervix among the total 80 cases. However, it's important to note that only incisional biopsies were used for 75 of the collected samples, which may not have provided a complete picture of the tumor. In the 5 resected specimens of SCC cervix, where the entire tumor was visualized under microscopy, LVI was observed in only 1 case, the other 4 cases showed no LVI.

Table 19: LVI in the resected and incisional biopsy specimens

	Type of specimen		TOTAL
LVI	INCISIONAL BIOPSY	RESECTED SPECIMEN	
PRESENT	4 (3)	1 (1)	5(4)
ABSENT	-	5 (4)	5(4)
CANNOT BE DETERMINED	90 (72)	-	90 (72)
TOTAL	94 (75)	6 (5)	100 (80)

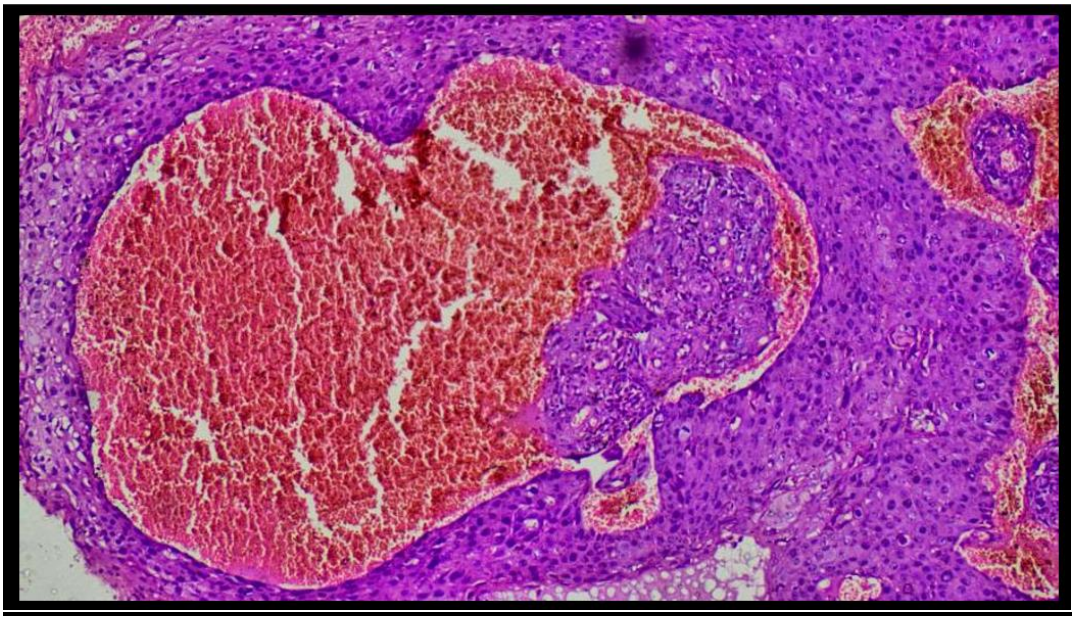


Figure 35: Representative microphotograph of LVI. (H&E, x200)

IMMUNOHISTOCHEMICAL STUDIES

IHC was done for P16 and Claspin and the results were tabulated.

P16 expression

Table 20: P16 IHC

P16 STATUS		Frequency	Percentage	
POSITIVE (Block positive)		67	83.75%	
NEGATIVE	Ambiguous	7	9%	16.25%
	Negative	6	7%	
Total		80	100%	

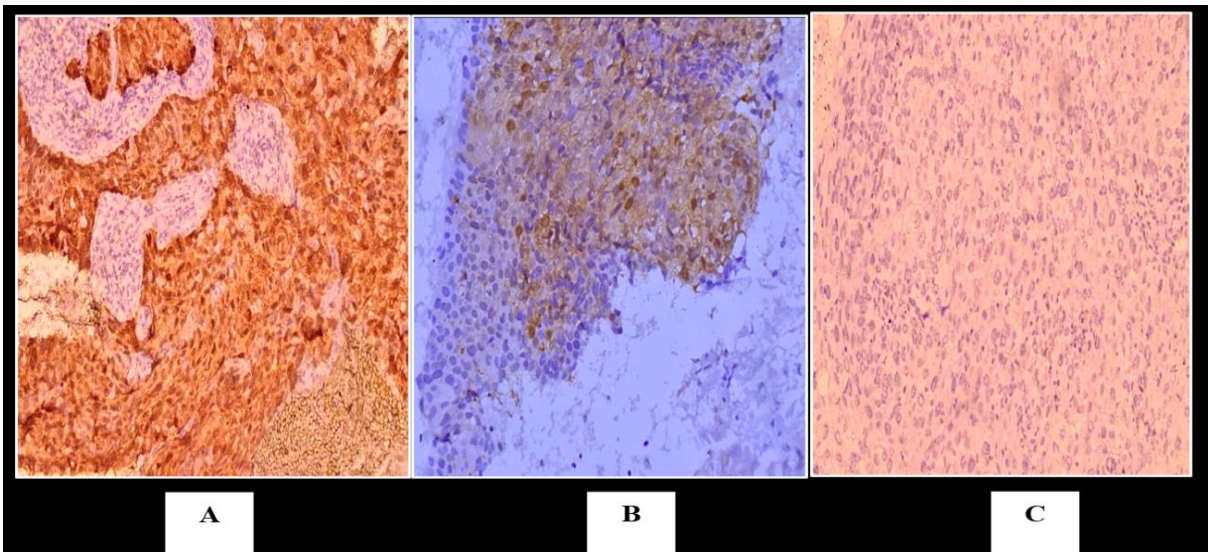


Figure 36: Immunohistochemistry staining with P16 antibody. A) Block positivity B) Ambiguous staining C) Negative staining (P16 IHC, x200)

Figure 37: Distribution of cases bases on P16 expression

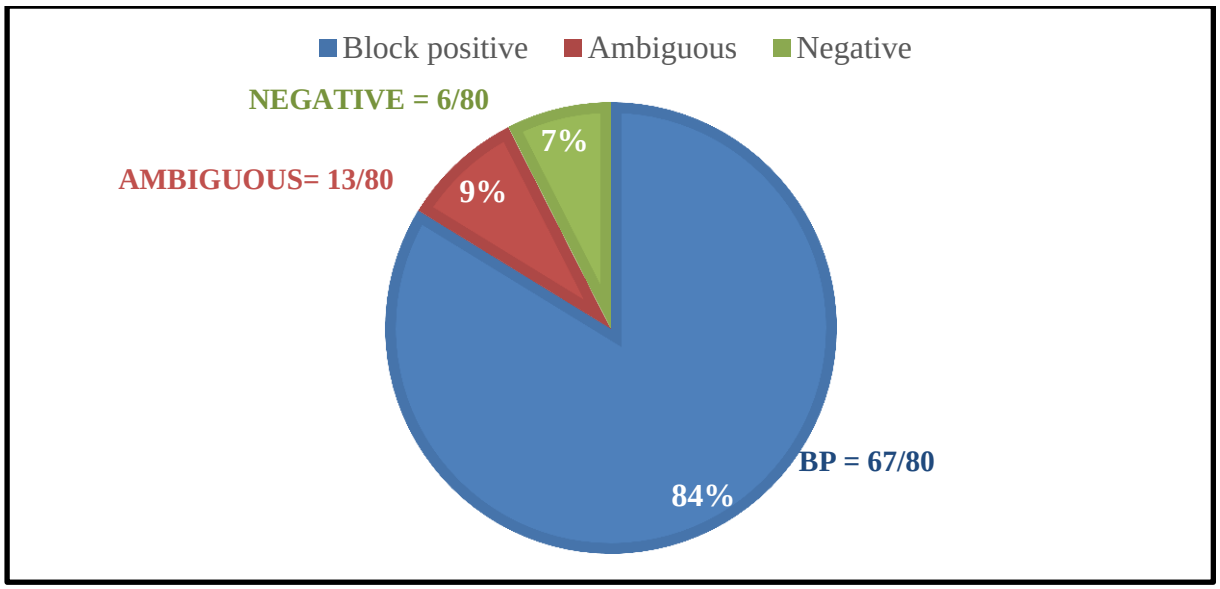
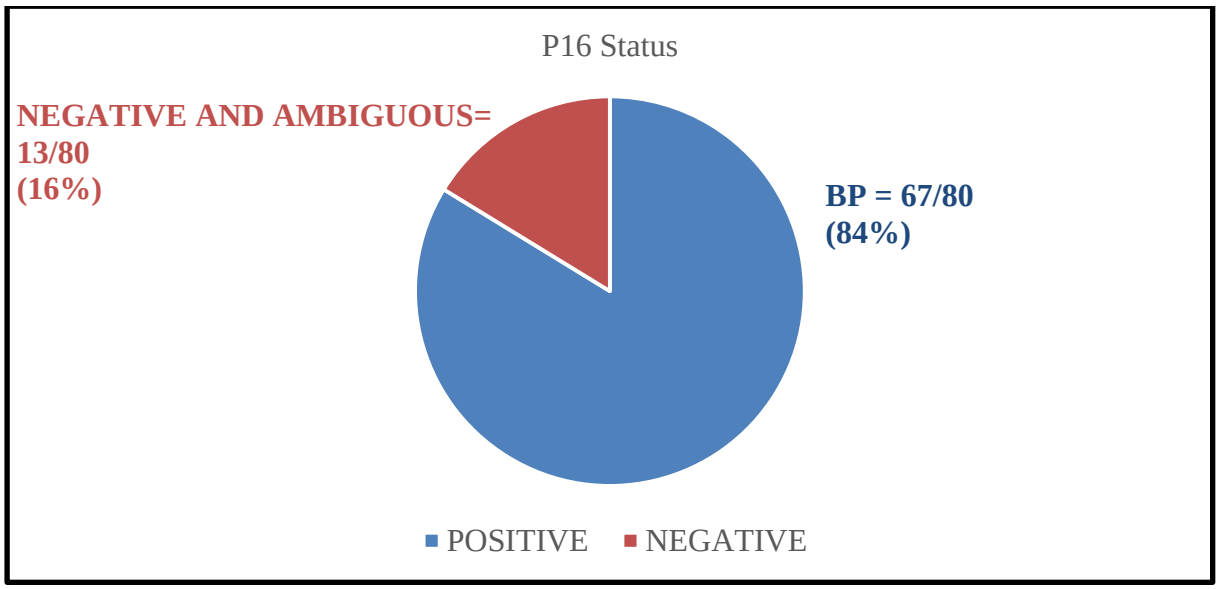


Figure 38: Distribution of cases bases on P16 expression considering only block positive as positive



The P16 IHC studies showed that 67 cases were block positive and 7 cases showed ambiguous staining and 6 cases showed negative staining (Table 20 and figure 37). For this study, only block positive P16 was considered positive (Figure 38).

ASSOCIATION OF P16 WITH DEMOGRAPHIC AND CLINICAL PARAMETERS

Table 21: Association of P16 with clinical parameters

Parameter	P16 STATUS		p value
	POSITIVE % (n)	NEGATIVE % (n)	
Age Group			
30-39	9 (7)	1 (1)	0.951
40-49	20 (16)	4 (3)	
50-59	26 (21)	5 (4)	
60-69	18 (14)	2 (2)	
70-79	9 (7)	2 (2)	
80-89	2 (2)	1 (1)	
Total	84 (67)	16 (13)	100% (80)
Parity			
High parity(P3-P9)	60 (48)	11 (9)	0.86
Low parity(P0-P2)	24 (19)	5 (4)	
Total	84 (67)	16 (13)	100% (80)
Reproductive status			
Post-menopausal	59 (47)	15 (12)	0.097
Pre-menopausal	25 (20)	1 (1)	
Total	84 (67)	16 (13)	100% (80)
Clinical features			
Post menopausal bleeding	35 (28)	5 (4)	0.619
Intermenstrual cycles	11 (9)	1 (1)	
White discharge per vagina	22 (17)	5 (4)	
Pain abdomen	11 (9)	5 (4)	
Mass per vagina	4 (3)	0 (0)	
Back pain	1 (1)	0 (0)	
Total	84 (67)	16 (13)	100% (80)
Per speculum examination			0.456
Growth	57 (46)	12 (10)	
Erosion	9 (7)	0 (0)	
Hypertrophied	18 (14)	4 (3)	
Total	84 (67)	16 (13)	100% (80)

The association of P16 status with age, parity, reproductive status, clinical presentation, per speculum finding and tumor grade showed no significant association (Table 21 and figure

39). P16 showed no significant association with stage of tumor, size of tumor and lymph node involvement but 50%, 59%, and 28% cases showed P16 positivity with higher stage, tumor size >4 cm and lymph node involvement (Table 22 and Figure 40).

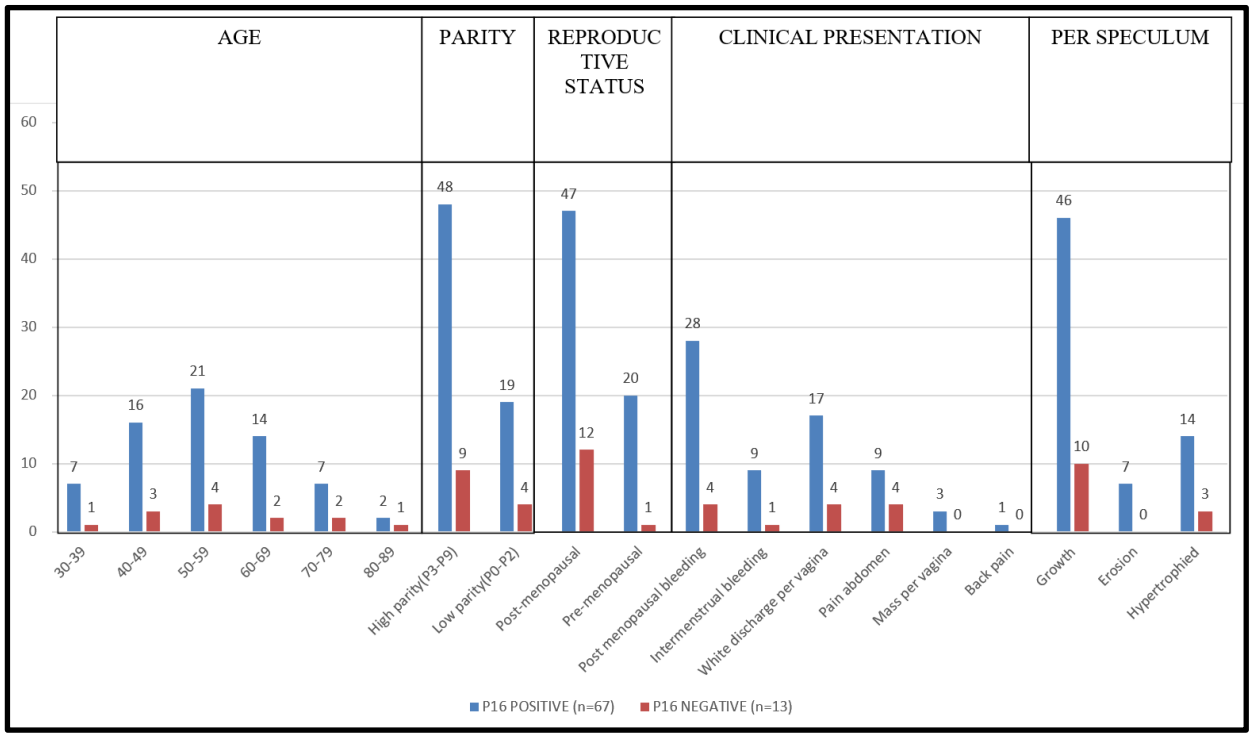


Figure 39: Association of P16 with clinical parameters

ASSOCIATION OF P16 WITH HISTOLOGIC AND RADIOLOGICAL PARAMETERS

Table 22: P16 with histological and radiological findings

Parameter	P16 STATUS		p value
	POSITIVE % (n)	NEGATIVE % (n)	
GRADE			
WD	**55 (44)**	9 (7)	0.513
MD	26 (21)	7 (6)	
PD	3 (2)	0 (0)	
Total	84 (67)	16 (13)	100% (80)
TILs			0.412
Low	35 (23)	8 (5)	
High	**51 (33)**	6 (4)	
Total	86 (56)	14 (9)	100% (65)
STAGE			0.885
Lower stage	34 (25)	5 (4)	
Higher stage	**50 (36)**	11 (8)	
Total	84 (61)	16 (12)	100% (73)
LYMPH NODE INVOLVEMENT			0.716
ABSENT	56 (41)	12 (9)	
PRESENT	**28 (20)**	4 (3)	
Total	84 (61)	16 (12)	100% (73)
TUMOR SIZE			0.585
> 4 cm	**59 (43)**	9 (7)	
≤4 cm	25 (18)	7 (5)	
Total	84 (61)	16 (12)	100% (73)

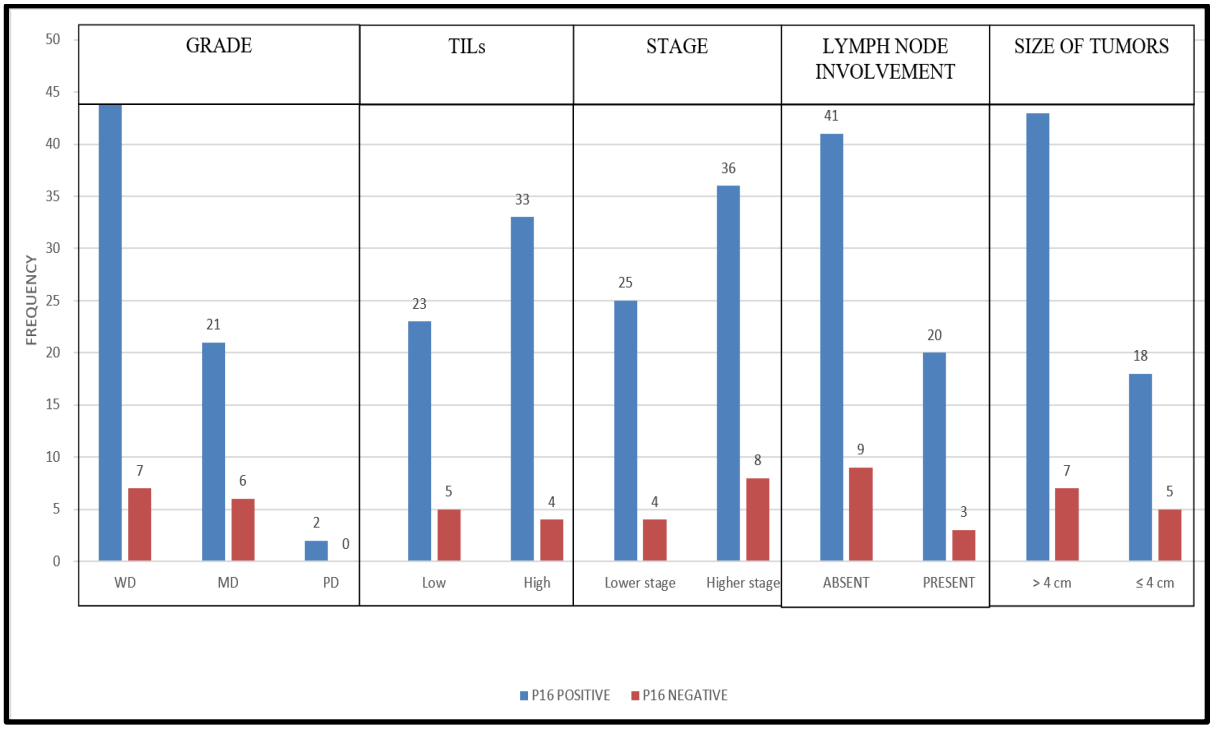


Figure 40: P16 with histological and radiological findings

CLASPIN EXPRESSION

Table 23: Claspin expression in SCC cervix

CLASPIN STATUS	Frequency	Percentage
POSITIVE (High and moderate positive)	71	88.75
NEGATIVE (Low positive and negative)	9	11.25
Total	80	100

Positive expression of Claspin was seen in 89% of cases (Table23 and figure 41).

Figure 41: Claspin expression in SCC cervix

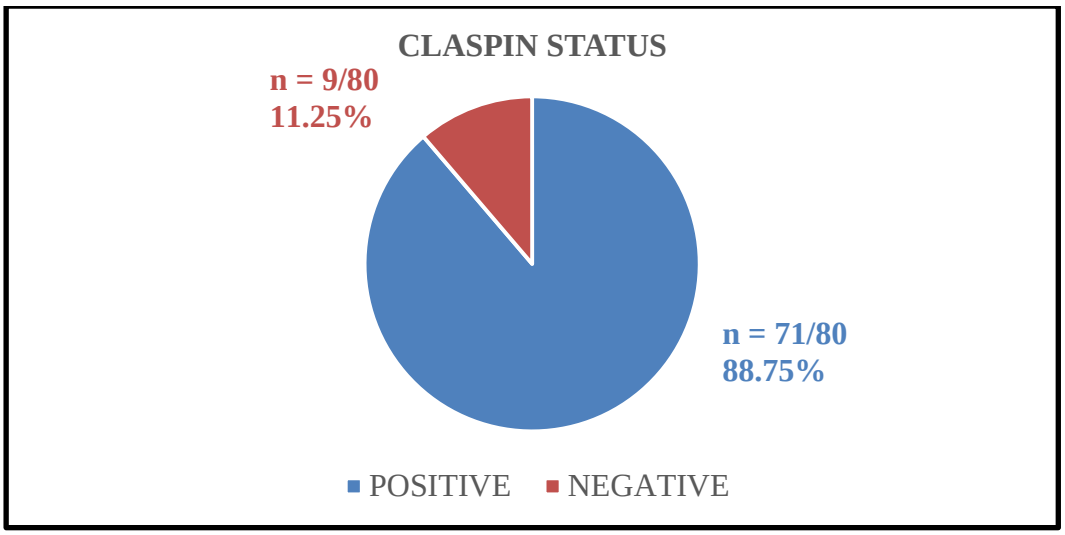
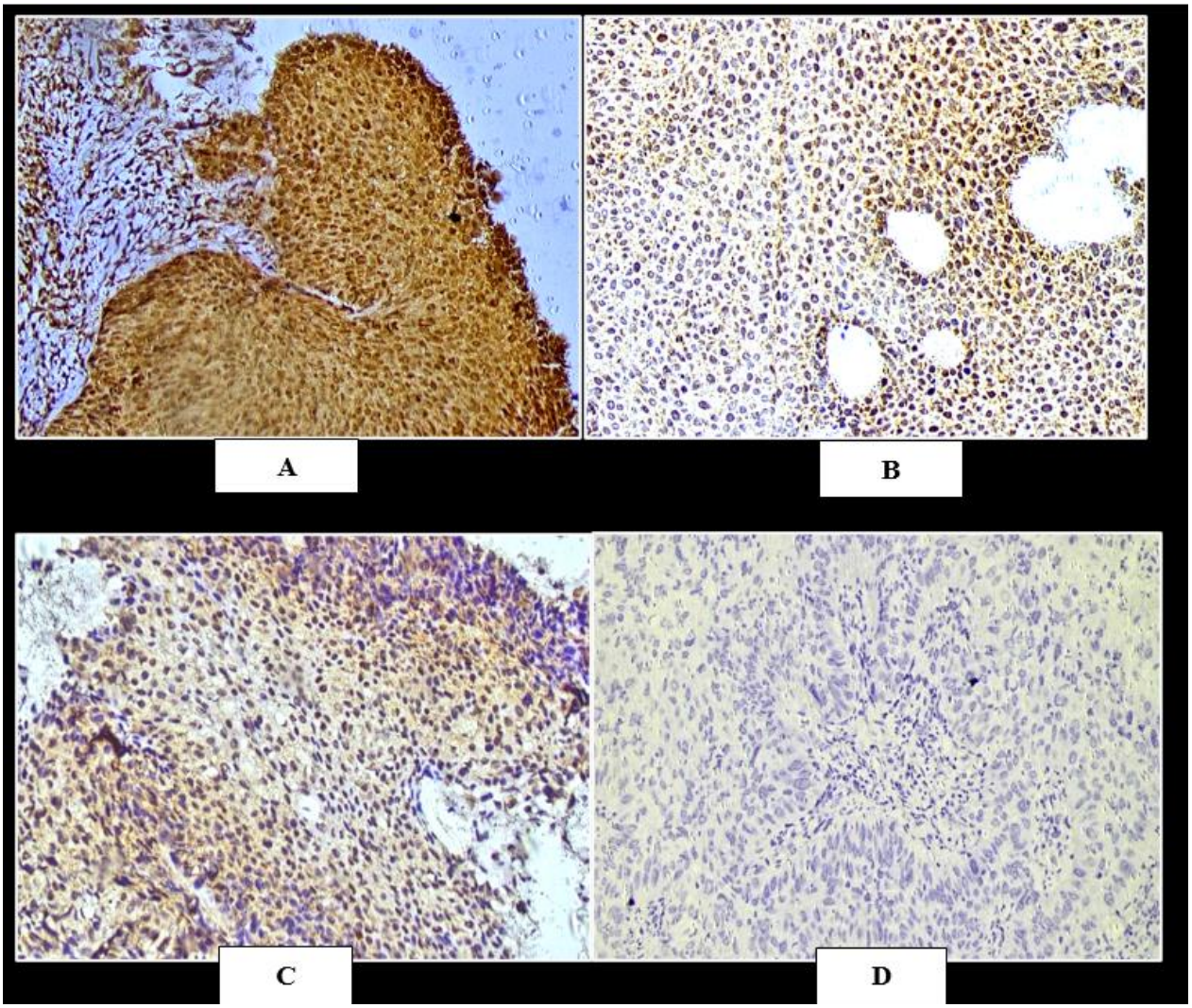


Figure 42: Immunohistochemistry staining with Claspin antibody. A) High positivity B) Moderate positivity C) Low positivity D) Negative staining (Claspin IHC, x200)



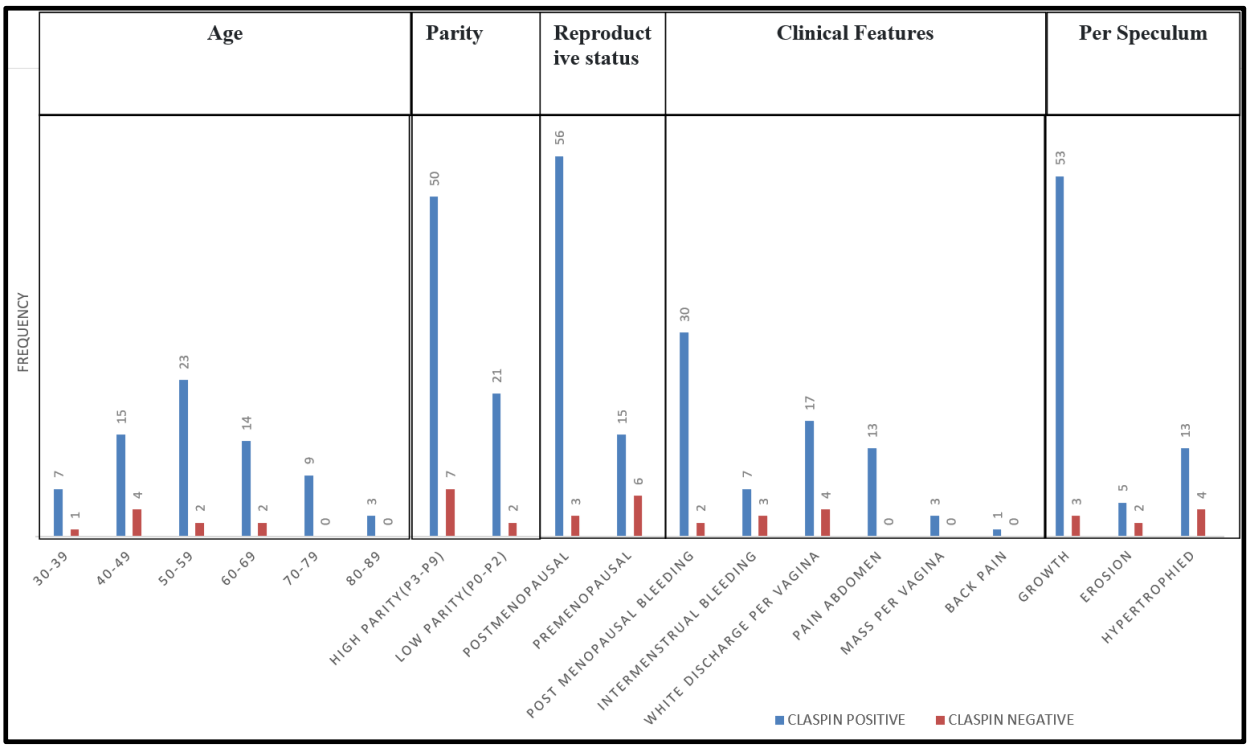
ASSOCIATION OF CLASPIN WITH DEMOGRAPHIC AND CLINICAL PARAMETERS

Table24: Claspin with clinical parameters

Age Group	CLASPIN STATUS		p value
	POSITIVE % (n)	NEGATIVE % (n)	
30-39	9 (7/80)	1 (1/80)	0.601
40-49	19 (15/80)	5 (4/80)	
50-59	**29 (23/80)**	2.5 (2/80)	
60-69	17 (14/80)	2.5 (2/80)	
70-79	11 (9/80)	0 (0/80)	
80-89	4 (3/80)	0 (0/80)	
TOTAL	89 (71/80)	11 (9/80)	100% (80)
Parity			
High parity(P3-P9)	**63 (50)**	9 (7)	0.646
Low parity(P0-P2)	26 (21)	2 (2)	
TOTAL	89 (71)	11 (9)	100% (80)
Reproductive status			
Post-menopausal	**70 (56)**	4 (3)	**0.003**
Pre-menopausal	19 (15)	7 (6)	
TOTAL	89 (71)	11 (9)	100% (80)
Clinical Features			
Post menopausal bleeding	**38 (30)**	2 (2)	0.17
Intermenstrual bleeding	9 (7/80)	4 (3)	
White discharge per vagina	21 (17)	5 (4)	

Pain abdomen	16 (13)	0 (0)	
Mass per vagina	4 (3)	0 (0)	
Back pain	1 (1)	0 (0)	
TOTAL	89 (71)	11 (9)	100% (80)
Per Speculum			
Growth	**67 (53)**	4 (3)	
Erosion	6 (5)	2 (2)	**0.037**
Hypertrophied	16 (13)	5 (4)	
TOTAL	89 (71)	11 (9)	100% (80)

Figure 43: Association of Claspin with clinical parameters



Association of Claspin with clinical parameters showed significant association with post-menopausal status (p=0.003) and with a finding of ulceroproliferative growth on per speculum examination(p=0.037) (Table 24 and figure 43).

ASSOCIATION OF CLASPIN WITH HISTOLOGIC AND RADIOLOGICAL PARAMETERS

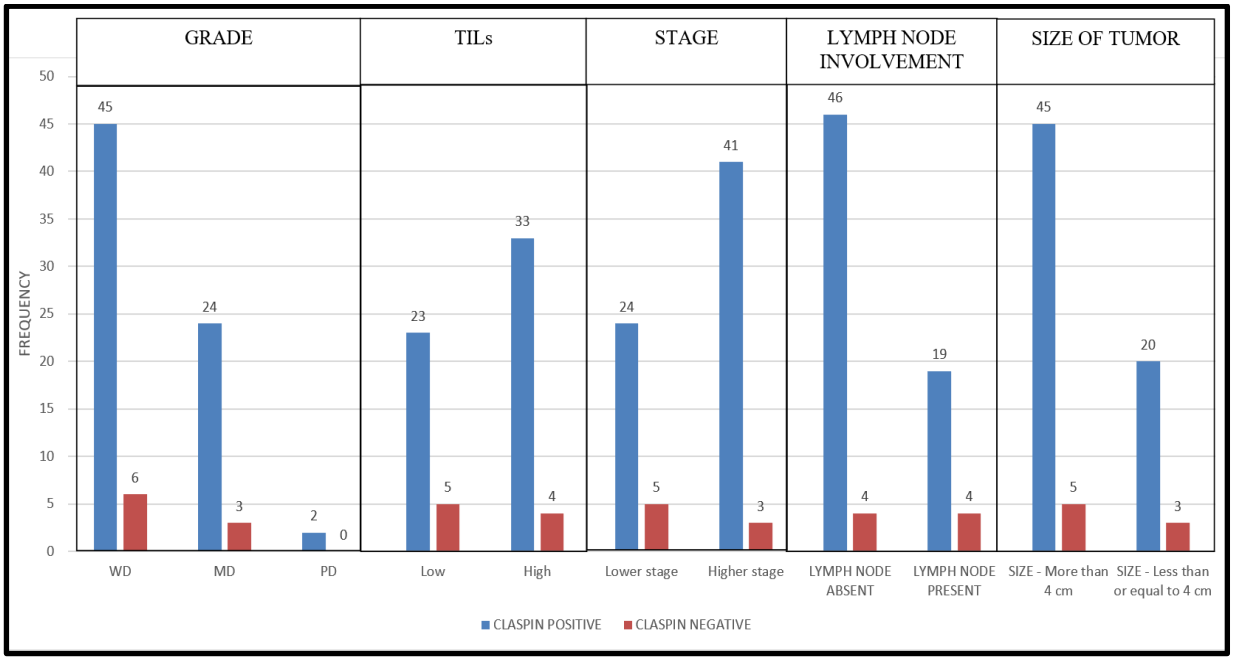
Table 25: Claspin with histological and radiological findings

Parameter	CLASPIN STATUS		p value
	POSITIVE % (n)	NEGATIVE % (n)	
Grade			0.27
WD	**56 (45)**	7 (6)	
MD	30 (24)	4 (3)	
PD	3 (2)	0 (0)	
TOTAL	89 (71)	11 (9)	100% (80)
TILs			0.762
Low	35 (23)	8 (5)	
High	**51 (33)**	6 (4)	
Total	86 (56)	14 (9)	100% (65)
Stage			0.442
Lower stage	33 (24)	7 (5)	
Higher stage	**56 (41)**	4 (3)	
Total	89 (65)	11 (8)	100% (73)
Lymph Node Involvement			0.225
ABSENT	63 (46)	5.5 (4)	
PRESENT	26 (19)	5.5 (4)	
Total	89 (65)	11 (8)	100% (73)
Size Of Tumor			0.478
> 4 cm	**62 (45/73)**	7 (5/73)	
≤ 4 cm	27 (20/73)	4 (3/73)	
Total	89 (65/73)	11 (8/73)	100% (73)

Association of Claspin with radiological stage of tumor, lymph node involvement and size of tumor showed no significant association (Table 25 and figure 44). But it was observed that that 56% and 62% of cases that showed high expression of Claspin were in higher stage and

had tumor size more than 4 cm, respectively. Association of Claspin with histological grade showed maximum cases (56%) having positive Claspin expression and well differentiated tumor grade. When associated with TILs, majority cases (51%) had high TILs and positive expression of Claspin. These associations were not significant (Table 25 and figure 44)

Figure 44: Association of Claspin with histological and radiological findings



COMPARISON OF CLASPIN EXPRESSION AND P16 EXPRESSION IN SCC OF CERVIX

Table 26: Association of Claspin and P16 expression

P16 STATUS	CLASPIN STATUS		p value
	POSITIVE % (n)	NEGATIVE % (n)	
POSITIVE	73 (58)	11 (9)	0.161
NEGATIVE	16 (13)	0 (0)	
Total	89 (71)	11 (9)	100% (80)

Chi^2 test was performed between Claspin expression and P16 expression. There was no statistically significant relationship between Claspin and P16 status, as the calculated p-value was 0.161 which was above the defined significance level of 0.05.

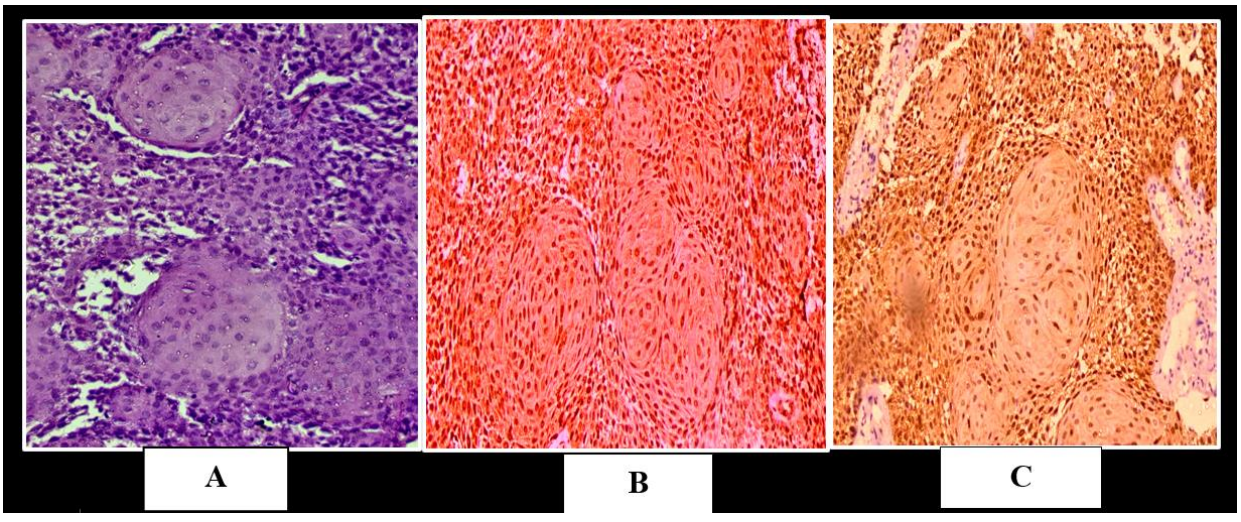


Figure 45: Case of well differentiated squamous cell carcinoma. A) Microphotograph (H&E, x200) B) Claspin immunohistochemistry showing high positivity (Claspin IHC, x200) C) P16 immunohistochemistry showing block positivity (P16 IHC, x200)

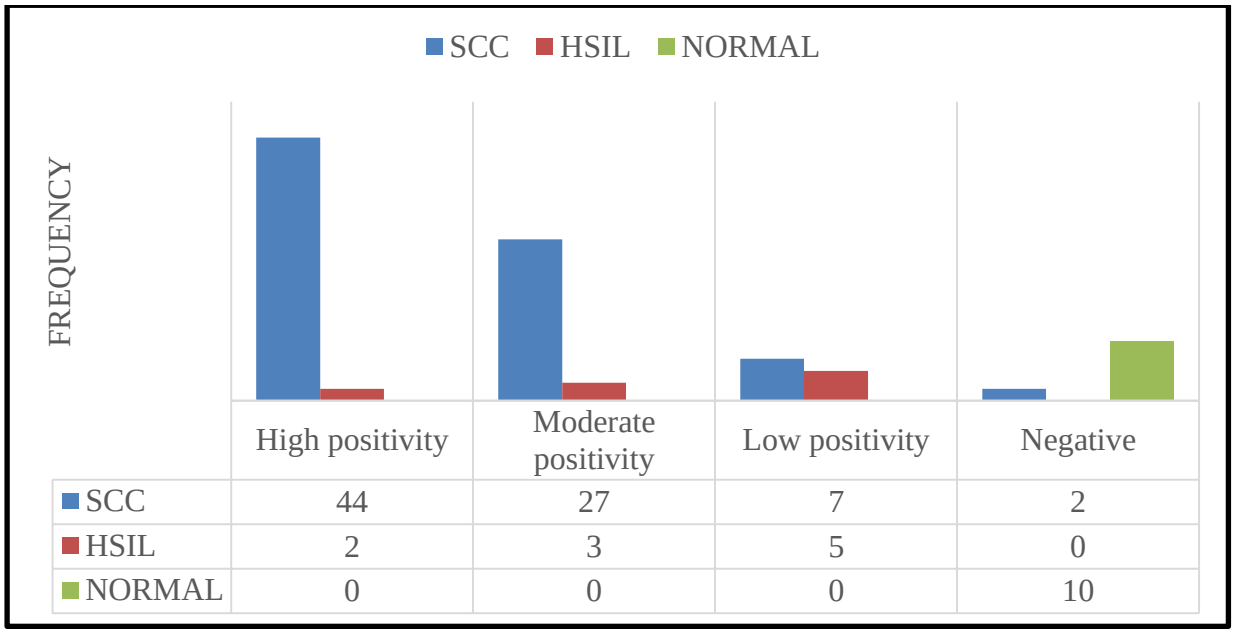
COMPARISON OF CLASPIN EXPRESSION IN SCC, HSIL AND NORMAL BIOPSY OF CERVIX

Claspin expression in SCC, HSIL and normal cases were compared and a statistically significant association between the histopathological diagnosis and Claspin expression level were observed. This means the differences observed in how these categories are distributed across each other are not due to random chance, indicating a meaningful relationship between them. Hence, a strong association between positive expression of Claspin and histological diagnosis of SCC is derived (p=<0.01) (Table 27 and Figure 44).

Table 27: Association of Claspin expression in SCC, HSIL and Normal biopsy

		CLASPIN expression					P Value
		High Positivity	Medium Positivity	Low Positivity	Negative	Total	
Histology	SCC	44	27	7	2	80	**<.001**
	HSIL	2	3	5	0	10	
	NORMAL	0	0	0	10	10	
	Total	46	30	12	12	100	

Figure 46: Association of Claspin expression in SCC, HSIL and Normal biopsy



P16 EXPRESSION IN SCC, HSIL AND NORMAL BIOPSY OF CERVIX

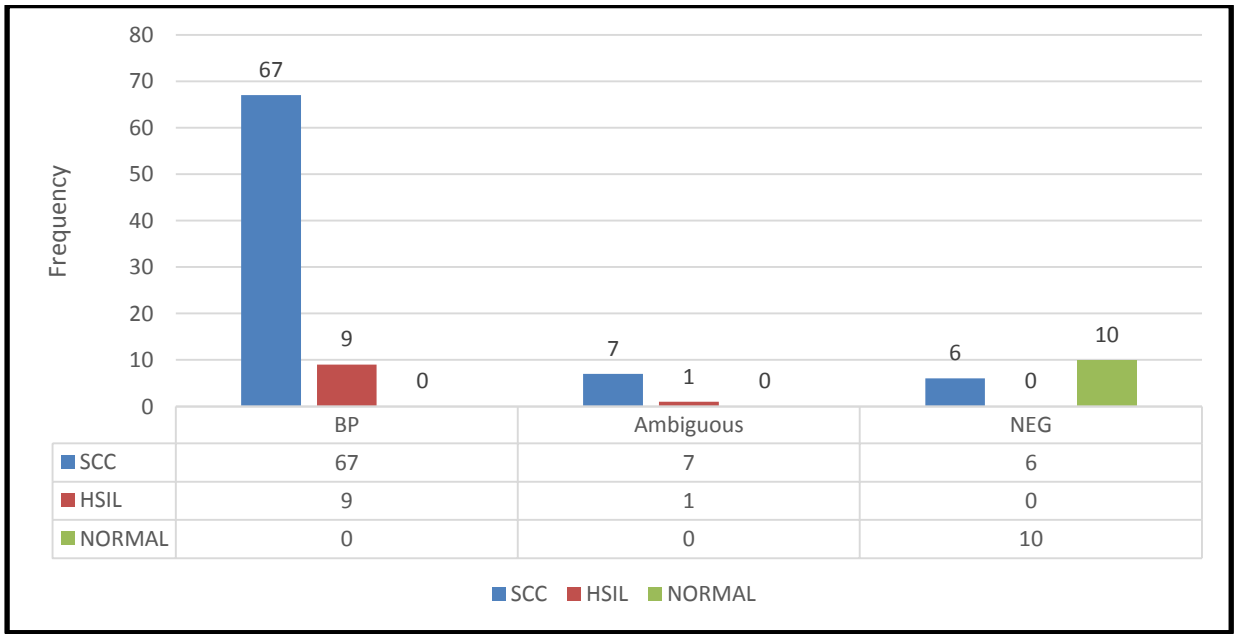
The HSIL cases showed block positivity in 9 cases and ambiguous staining in 1 case.

The normal cervical tissue showed negative P16 staining in all cases with basal layer positivity in 5 cases of the total 10 (Table 28 and Figure 44). P16 showed significantly higher expression in HSIL and SCC cases.

Table 28: Association of P16 expression in SCC, HSIL and Normal biopsy

		P16 expression				p value
		Block Positive	Ambiguous	Negative	Total	
Histology	SCC	67	7	6	80	p < **0.000001**
	HSIL	9	1	0	10	
	NORMAL	0	0	10	10	
	Total	76	8	16	100	

Figure 47: P16 positivity in SCC, HSIL and normal biopsy



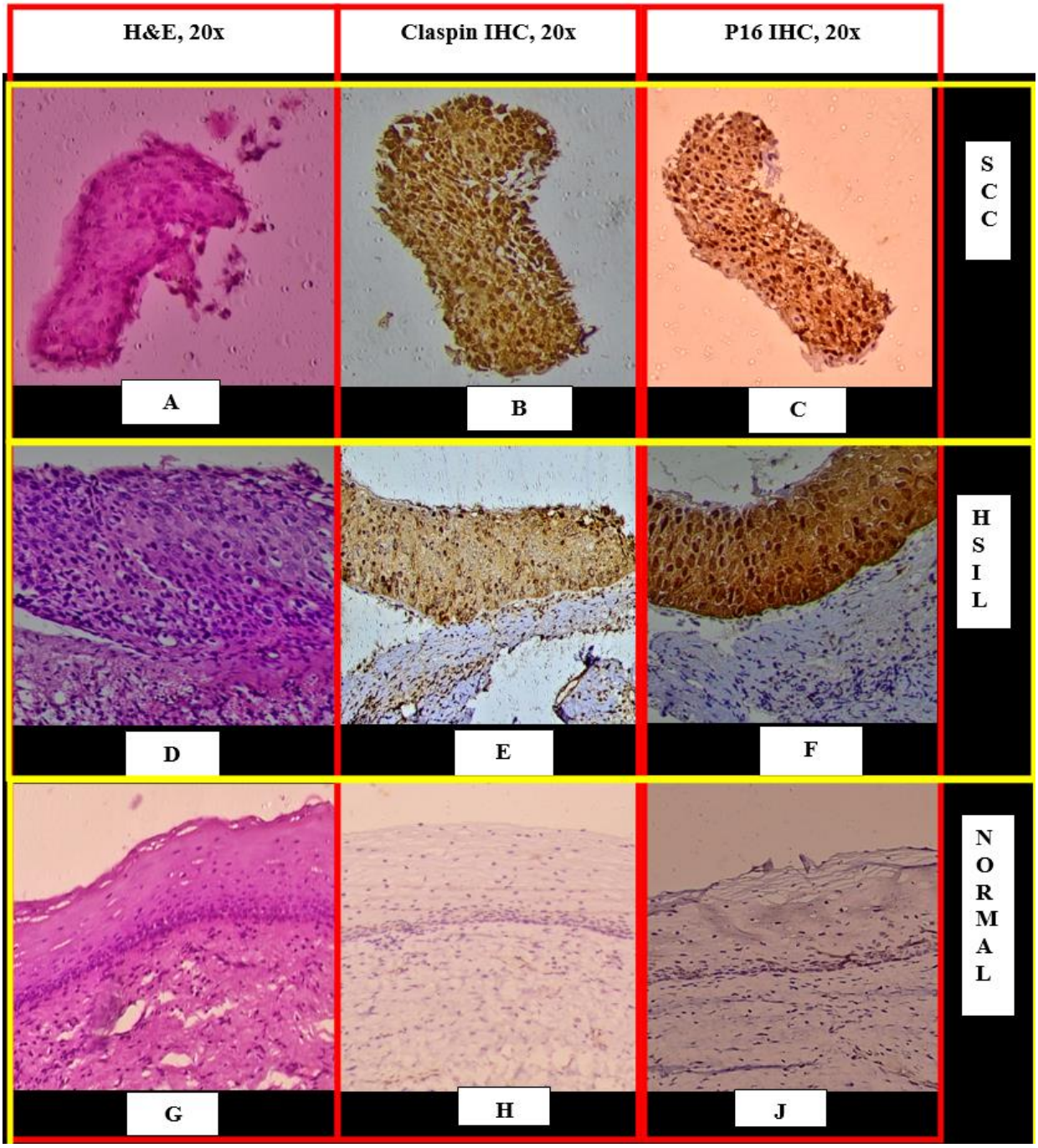


Figure 48: Representative images of SCC, HSIL & Normal cervix on H&E, 200x (A,D,E respectively) with corresponding claspin IHC showing high positivity, low positivity & Negative staining (B,E,H respectively) and P16 IHC showing block positivity, block positivity & Negative staining (C,F,J respectively)

DISCUSSION

DISCUSSION

In the contemporary world of development and medical advances, cancer has become a great adversary to pathologists and oncologists worldwide and considerable resources are spent to understand the pathogenesis and treatment of these diseases. Cervical cancer is one of the few malignancies with an accepted viral etiopathogenesis that has made its prevention a possibility. This is substantiated by the steady decline in the incidence of cervical cancer in countries that have implemented HPV vaccination and screening programs.[31]

Cervical cancer is currently 4th in the world in both incidence and mortality among women. It is also a cancer which is disproportionately high in the developing countries when compared to the developed countries.[1]The proportion of cases reported from Asia was 58% and from North America was 2.5% which highlights the asymmetry of cervical cancer distribution. It is noteworthy that India was responsible for 21% of the 58% cases reported in Asia.[32] Among females in India it is the 2nd most common cancer to be reported.[33]

An overall decrease in the incidence and mortality of cervical cancer has been noted in most parts of the world including India which can be attributed predominantly to screening and vaccination along with other factors like better genital hygiene and decreased parity.[1] Cancer registries in India has shown a decrease albeit a small one in the incidence of cervical cancer. Karnataka has the 2nd lowest change in incidence from 21.61 per 100,000 women in 1999 to 19.83 per 100,000 women in 2019. In data published by Singh M et al on the incidence of cervical cancer in various states, it was observed that Karnataka ranked 2nd highest.[33]

At the country level, a disparity is again noted between the urban and rural areas. In the rural areas' marriage at a younger age, poor genital hygiene and high parity is still prevalent, which along with lack of screening can be the cause of higher incidence.[32] The setting of the current study is in Kolar which is a rural region in Karnataka. It was observed that in Kolar,

cervical cancer is more common than breast cancer which is contradictory to the Indian data where breast cancer is the commonest cancer in women followed by cervical cancer.[4]

Table 29: Incidence of cervical cancer

REGION	INCIDENCE	YEAR	REFERENCE
Global	14.1%	2022	Bray F et al[1]
India	20.5%	2020	Singh D et al[32]
Kolar	17.5%	2010	Kalyani R et al[4]

AGE DISTRIBUTION

Cervical cancer incidence increases with age until menopause following which they may remain the same or decrease in incidence. This relationship between cervical cancer and age can be attributed to the underlying HPV infection and its progression to carcinoma.[34]

A systematic review conducted by palmer et al showed that HPV infection with normal cytology is seen in younger age group and HPV positive cases with cervical cancer was predominantly seen in older age group.[35]Another study showed that while HPV infection was above 30% in patients below 30 years, it was 6% in patients above 60 years. Similarly squamous intraepithelial lesion was diagnosed at an earlier age than SCC.[36] In present study also, the average age of diagnosis of HSIL is younger than the average age at which SCC was diagnosed. This was comparative to other studies where similar findings were reported.[36,37,38,39]

Table 30: Comparison of age at diagnosis of HSIL and SCC in various studies

STUDY	AGE	
	Mean age at diagnosis of HSIL	**Mean age at diagnosis of SCC**
Lu et al (2021)[36]	42.8	50.9
Wang et al (2024)[37]	51.89	52.33
Chen et al (2020)[38]	41.71	49.87
Hajra et al (2023)[39]	47.0	50.4
PRESENT STUDY	48.5	55.09

In developed countries where most of the population is screened systematically from an early age, the diagnosis of cervical cancer is showing shift in the age of diagnosis to a younger age group.[40,41]Singh M et al in their study compared countries with organised screening programs and countries with low screening coverage like India and it was observed that in the first category, the incidence increased till 35 years and then plateaued while in the second category there was a directly proportionate increase in the incidence with age and majority of cases were diagnosed at 55-64 years.[32] This is reflected in present study where the maximum number of patients were diagnosed within the age group of 50-59 years (31.25%) and is corroborated in other studies from India and China.[42,43,6]

Table 31: Comparison of age of patients with SCC in various studies

STUDY	Guo et al (2018)[41]	Ramesh et al (2023)[42]	Kalyani et al (2020)[6]	PRESENT STUDY
Place of study	China	Chennai	Kolar	Kolar
Mean age	51.32 ± 9.72	53.93 ± 10.24	54.3 ± 12	55.09 ± 12.31
Range	25-72	37-74	30-80	33-87

REPRODUCTIVE STATUS

Most of the patients (73.75%) in the present study had already attained menopause when they were diagnosed with SCC. This could indicate the late presentation of patients to the hospital for their symptoms. This is similar to the data from another study in the same institute but it is a higher proportion of patients diagnosed in post-menopausal state than in comparative studies from other regions.[6,38,44] This could be attributed to the rural population of Kolar having limited awareness along with lack of organized screening programs in the area. Meanwhile two studies have shown more patients in the pre-menopausal age group.[37,45] Chen et al studied the role of menopause in progression of CIN to SCC and hypothesized that females who are in the reproductive age group have higher sexual activity which can result in persistent HPV infection and they have more estrogen that can result in integration of the HPV, hence increased progression to SCC in women before menopause.[38] Similarly when, Ding et al. analyzed the significance of menopause in progression of LSIL to HSIL, they observed that post-menopausal women were significantly associated with regression (34.6%) and persistence (41.7%) and very few post-menopausal women showed progression (6.3%).[46] A study by Renata et al on the other hand showed that invasion into stroma is a common feature in post-menopausal women.[47] Thereby the role of menopause in cervical carcinogenesis is yet to be fully understood.

Table 32: Comparison of reproductive status of women with SCC in various studies

Study	Berraho et al (2017)[44]	Wang et al (2024)[37]	Chen et al (2020)[38]	Nasreen et al (2023)[45]	Kalyani et al (2020)[6]	Present study
Place of Study	Morocco	China	China	Kashmir, Northern India	Kolar, Southern India	Kolar, Southern India
Pre-menopausal	53.5%	57.14%	43.3%	32.6%	25.3%	26.25
Post-Menopausal	46.5%	42.86%	56.7%	67.4%	74.7	73.75

PARITY

In this study the parity of the parity of patients ranged from 0 to 9 with maximum number of subjects having 3 child birth (22.5%). Parity of ≥3 was significantly associated with progression of HSIL but it did not have a significant role in progression of LSIL to HSIL.[48,46] In current study, we have taken ≥3 as cutoff for high parity and observed that 71.25% of patients were highly parous. Increased parity has been shown to have a direct risk for development of SCC.[44,49]This trend has been explained by the increased level of estrogen in highly parous women which is considered to have a role in HPV carcinogenesis.[49]Repeated injury to cervix during multiple child birth also contributes to the increased risk associated with multiparous women.[50] A higher proportion of highly parous women are associated with cervical carcinoma in present study and other parallel studies.[44,48,51,52]

Table 33: Comparison of parity of women with SCC in various studies

STUDY	Paul et al (2023)[51]	Shruthi PS et al (2014)[52]	Berraho et al (2017)[44]	Misra et al.[48]	PRESENT STUDY
Cut Off for High Parity	≥5	≥4	≥4	≥3	≥3
High parity	64.5%	57.8%	71.6%	76.5%	71.25%
Low parity	35.5%	42.2%	28.4%	23.5%	28.75%

CLINICAL PRESENTATION AND PER SPECULUM FINDING

A significant proportion of patients in current study presented with postmenopausal bleeding (40%) followed by white discharge per vagina (26.25%). This is consistent with other studies were bleeding per vagina and white discharge per vagina are the predominant symptoms.[52,53]

The World Health Organization (WHO) recommends screening and symptomatic pathways for diagnosis of cervical cancer.[54] Ideally, screening enables early detection of lesions, leading to

improved outcomes. However, in our study population, no patients were diagnosed incidentally through screening, and all presented with symptoms, highlighting a gap in screening implementation and emphasizing the need for enhanced screening efforts to achieve early detection and better patient outcomes.

Moreover, a significant proportion (70%) of females in our study presented with ulceroproliferative growth at initial hospital visit, substantiating the notion that rural populations often delay seeking medical attention until symptoms have advanced, leading to late-stage diagnoses. This finding is consistent with a study conducted in low-resource settings in Kenya, which suggested that limited autonomy among women, financial dependence on men, and stigma surrounding cervical malignancies may contribute to delayed healthcare seeking behaviors, ultimately resulting in advanced disease stages at diagnosis.[53]

Table 34: Comparison of presenting symptom of women with SCC in various studies

	Bleeding per vagina	Vaginal discharge
Shaffi AF et al (2024)[53]	79%	54.2%
Kalyani R et al (2020)[6]	80%	68%
Shruthi PS et al (2014)[52]	63.3%	60.3%
PRESENT STUDY	66.25%	12.50%

Table 35: Comparison of proportion of women with ulceroproliferative growth (UPG) as per speculum finding in women with SCC in various studies

STUDY	Misra JS et al[48]	Kalyani R et al (2020)[6]	PRESENT STUDY
UPG	51.3%	65.3%	70%

SIZE OF TUMOR

Size of tumor plays a decisive role in staging of the tumor. A tumor size greater than 4 cm has been independently determined to affect the survival rate of cervical cancer patients.[55] In the current study, a notable proportion of patients (68.5%) of patients exhibited tumor dimensions exceeding 4 cm on MRI, diverging from the findings of previous studies where the majority of patients presented with tumors smaller than 4 cm in largest dimension.[37,55,56,57,58,59] This discrepancy may be attributable to the delayed treatment-seeking behavior exhibited by the current study population, which potentially contributed to the divergent findings. Ruengkhachorn I et al identified large tumor size (more than 4cm) as a significant risk factor for recurrence, whereas Rao et al failed to replicate these findings, suggesting discordant results across studies.[57,58]

Table 36: Comparison of size of tumor in women with SCC in various studies

Study	Ruengkhachorn I et al (2015)[57]	Wang L et al (2024)[37]	Zhang Y et al (2024)[59]	Shigeta S et al (2023)[55]	Rao Q et al (2023)[58]	Fang et al (2024)[56]	PRESENT STUDY
≥ 4cm	8.5%	38.1%	32.05%	36.9%	20.4%	45%	68.5%
< 4cm	91.5%	61.9%	67.95%	62.3%	79.6%	55%	31.5%

LYMPH NODE INVOLVEMENT

Involvement of lymph node upstages SCC cervix as status of lymph node is integrated to FIGO staging. Present study revealed that 31.5% of patients presented with lymph node involvement at initial diagnosis, which is consistent with existing literature, yet suggests a slightly higher propensity for lymphatic metastasis in current study population.[42,57,58,60,61,62,63] This finding aligns with the overall trend in the current study, where patients exhibited more severe disease manifestations. Lymph node involvement is a well-established independent predictor of poor prognosis and decreased overall survival.[59,60] A study by Guo et al showed that lymph node metastasis significantly worsens progression free survival but does not change the overall survival.[42] Lymph node metastasis also increases the chance of recurrence in post operative cervical cancer patients.[56,61] Furthermore, Shigeta et al found that metastasis to multiple pelvic lymph nodes is associated with a worse prognosis compared to single lymph node involvement.[56]

Table 37: Comparison of lymph node involvement in women with SCC in various studies

Study	Ruengkhachorn I et al (2015)[57]	Deng et al (2023)[63]	Bizzarri et al (2023)[62]	Jiang et al (2019)[61]	Rao et al (2023)[58]	Cao et al (2019)[60]	Guo et al (2023)[42]	PRESENT STUDY
ABSENT	90%	81.27%	80.6%	79.1%	78%	76.7%	70%	68.5%
PRESENT	10%	18.65%	19.4%	20.9%	22%	23.3%	30%	31.5%

STAGE OF DISEASE

The current study employed the MRI-based 2018 FIGO staging system, which has a reported accuracy of 89.3%.[64] his updated system incorporates high-risk factors such as parametrial involvement and lymph node metastasis to the pelvic or paraaortic regions, enhancing its

predictive accuracy.[56,65] It has an impact on overall survival and plays a decisive role in treatment considerations.[42,66] The present study population consisted of a majority of patients in stage III (42%) and stage II (32%), consistent with findings in similar population groups.[6,52,53,56,67] Interestingly, a study in Latin America observed that while developing countries often diagnose cervical cancer in stages II and III, developed countries like the United States typically diagnose it in stage I.[68]

Table 38: Comparison of Stage of tumor in women with SCC in various studies

Stage	Fang et al (2024)[56]	Shruthi PS et al (2014)[52]	Shaffi AF et al (2024)[53]	Lin MY et al (2023)[67]	Kalyani R et al (2020)[6]	PRESENT STUDY
I	16%	2.51%	18.2%	22%	8%	8.2%
II	44%	14.57%	26.3%	23%	32%	31.5%
III	36%	75.88%	44.6%	53%	40%	42.5%
IV	4%	7.04%	10.8%	2%	20%	17.8%

A comparison of the clinicopathological characteristics with higher stages (Stage III and IV) revealed significant associations with postmenopausal status and the presentation with post-menopausal bleeding. This is likely due to the atrophic changes in the cervix that occur during post menopause, which allow cancer to invade and spread more easily and lead to higher stages at diagnosis.[47] Similarly, a statistically significant association between increased tumor size, lymph node involvement, and higher disease stages was observed. This association is attributable to the incorporation of these factors in the disease staging system. Notably, tumor size, lymph node involvement, and advanced stage are all established adverse prognostic factors, highlighting the importance of considering these factors in patient assessment and treatment planning.

GRADE

Histological grading of the study population revealed that the majority of patients (63.75%) had low-grade/well-differentiated tumors. However, comparison with other studies shows variability in the proportion of tumor grades, which can be attributed to interobserver variability among pathologists.[6,52,61,69,70,71] The prognostic significance of tumor grade in SCC cervix is a topic of debate, with conflicting results reported in the literature. For instance, Jiang et al. found no significant association between histological grade and poor outcome, whereas Wu et al. observed a significant association between grade and recurrence.[61,69] These discrepancies highlight the need for standardized grading criteria and further research to determine the clinical utility of tumor grade in predicting SCC outcomes. In the present study there was no significant association between the grade of tumor and higher stage, it was also not associated with other clinicopathologic parameters.

Table 39: Comparison of grade of tumor in women with SCC in various studies

	Huang et al (2019)[71]	Jiang et al (2019)[61]	Shruthi et al (2014)[52]	Kalyani R et al (2020)[6]	Wu et al (2024)[69]	Zheng et al (2016)[70]	PRESENT STUDY
Well-differentiated	11%	9.3%	18%	61.8%	17.3%	16.8%	63.75%
Moderately differentiated	43%	34.9%	55.9%	23.5%	69.8%	52.2%	33.75%
Poorly differentiated	46%	55.8%	26.1%	14.7%	12.9%	31%	2.5%

TILs

In the present study, we categorized tumor-infiltrating lymphocytes (TILs) into three tiers: high (56.92%), moderate (24.61%), and low (18.46%). Our classification approach differs from that of Larre et al., who used the median value (20%) to dichotomize TILs into high (49.2%) and low (50.8%) groups in their analysis of H&E-stained carcinoma cervix specimens. The disparity in median TILs values (70% in our study vs. 20% in Larre et al.) may be attributed to differences in study design, as our study included small biopsies from all stages of cervical cancer, whereas Larre et al. examined resected specimens from early-stage cervical carcinoma. Interobserver variability may also contribute to this discrepancy. Notably, our study found no significant association between TILs and clinicopathologic parameters like tumor grade, contrary to Larre et al.'s findings.[29] The prognostic value of TILs in cervical cancer is well recognized, particularly when considering the specific lymphocyte types.[72] Future studies utilizing immunohistochemical (IHC) analysis to quantify TILs may provide even greater prognostic and therapeutic value.

Table 40: Comparison of TILs calculated by H & E

<table>
<tr><td></td><td colspan="3">TILs</td></tr>
<tr><td rowspan="2">Larre et al (2019)[29]</td><td colspan="2">High (greater than 20%)</td><td>Low (Less than 20%)</td></tr>
<tr><td colspan="2">49.2%</td><td>50.8%</td></tr>
<tr><td rowspan="2">Present study</td><td>High (greater than 60%)</td><td>Moderate (30-60%)</td><td>Low (Less than 30%)</td></tr>
<tr><td>56.92%</td><td>24.61%</td><td>18.64%</td></tr>
</table>

P16 EXPRESSION

The current study employed P16 immunohistochemistry as a surrogate marker for human papillomavirus (HPV) infection, a well-established biomarker. Only samples with block positivity for P16 were considered HPV-positive squamous cell carcinoma (SCC). Our results showed a high P16 positivity rate of 83.75%, which is consistent with the rates reported in other studies.[6,39,73,74,75]This suggests that the majority of SCC cases in our cohort were HPV-associated which is in keeping with the low incidence of HPV independent tumor in cervix.[74]

Table 41: Comparison of P16 positivity of cervical cancer in various studies

Study	Kulhan et al (2023)[74]	Singh et al (2023)[75]	Lu et al (2021)[31]	Kalyani R et al (2020)[6]	Nicolás et al (2020)[73]	PRESENT STUDY
P16 positivity in SCC	84.5%	95.2	92.3	89.3	96%	83.75%

We investigated the association between P16 expression and various clinicopathological factors, including age, parity, reproductive status, clinical presentation, per speculum findings, tumor size, lymph node status, stage, grade, and TILs. Notably, no statistically significant associations were observed. This finding is consistent with Wispelaere et al.'s study, which reported no significant association between P16 expression and tumor prognosis.[76] In cervical cancer however, Nicolas et al. found that P16-negative, HPV-positive cases had a worse prognosis, while Kulhan et al. did not observe such an association.[73,74]Literature suggests that P16and HPV negativity is associated with more aggressive disease.[5] Stolnicu et al demonstrated that HPV independent SCC is rare and

associated with higher stage and older patients.[77] Our study showed a similar trend, although the results did not reach statistical significance.

Table 42: Comparison of age and stage with HPV status and P16 expression

	MEDIAN AGE (Years)		STAGE III & IV (%)	
Stolnicu et al (2023)[77]	HPV Independent SCC	HPV associated SCC	HPV Independent SCC	HPV associated SCC
	72	49	40%	18%
PRESENT STUDY	P16 negative SCC	P16 positive SCC	P16 negative SCC	P16 positive SCC
	57	52	67% (8/12)	59% (36/61)

P16 expression is also significantly elevated in HSILs, and block positivity for P16 can reliably distinguish HSILs from normal tissue.[75,78] In our study, 9 out of 10 HSIL cases exhibited block positivity for P16, while 1 case showed ambiguous positivity, consistent with findings from other studies.[75,79,80] Notably, P16 positivity is strongly associated with the progression of CIN, underscoring its crucial role in the management and early diagnosis of CIN.[81] The high sensitivity of P16 block positivity in detecting HSILs highlights its potential as a valuable biomarker for triaging patients with abnormal cytology or HPV positivity, enabling targeted interventions and improved patient outcomes.

Table 43: Comparison of P16 positivity in HSIL

Study	Singh et al (2023)[75]	Banet et al (2023)[79]	Yıldız et al (2007)[80]	PRESENT STUDY
P16 positivity in HSIL	80%	85%	100	90%

CLASPIN

Claspin has emerged as a promising biomarker in various solid tumors, with studies primarily focusing on CLSPN gene expression. However, various investigators have utilized immunohistochemistry (IHC) to examine Claspin protein expression in primary cancers, including those of the cervix, urinary bladder, prostate and kidney.[10,11,13,25]

Our study aimed to evaluate the potential of Claspin as a biomarker in SCC cervix. We analyzed 80 cases of primary SCC cervix, along with 10 cases of normal cervical tissue and 10 cases of HSILs to investigate the progressive increase in Claspin expression with dysplasia. Our results showed a significant upregulation of Claspin expression ($p<0.001$) from normal tissue to dysplasia to carcinoma, suggesting its potential as a diagnostic biomarker. This finding is consistent with the results of a similar study conducted by Benevelo et al., further solidifying the role of Claspin as a valuable biomarker in cervical cancer.[10]

Table 44: Comparison of the progressive expression of Claspin in the current study and the study by Benevelo et al

PRESENT STUDY	WNL (10)	HSIL (10)	SCC (80)
CLASPIN Negative/Low Positivity	10 (100%)	5 (50%)	9 (11.25%)
CLASPIN Moderate Positivity /High Positivity	0	5 (50%)	71 (88.75%)
BENEVELO ET AL	WNL (9)	CIN 2/3 (37)	SCC (15)
CLASPIN Negative/Low Positivity	9 (100%)	7 (91%)	1 (6.7%)
CLASPIN Moderate Positivity /High Positivity	0	30 (81.08%)	14 (93.3%)

CLASPIN EXPRESSION

In the present study, we observed elevated Claspin expression in 88.75% of cervical cancer samples, which is higher than reported in other tumor types, but consistent with another study focused on cervical cancer.[10]Previous investigations in gastric, renal, prostatic, and urothelial cancers have shown significant association of Claspin expression with adverse prognostic factors.[11,12,13,25]

While this study did not find similar associations between Claspin expression and various clinical parameters. There were some notable trends and observations. We found a significant association between high Claspin expression and post-menopausal status, as well as ulceroproliferative growth, indicating an association with late presentation. Furthermore, a substantial proportion of cases with high Claspin expression were associated with a higher stage (63%; 41/65) and large tumor size (69%; 45/65) suggesting a potential link with advanced disease. Additionally, when analyzed with TILs 51% of cases had high TILs and positive Claspin expression, suggesting a relationship between Claspin and immune response. Although these associations were not statistically significant, they may still be important and warrant further investigation to fully understand the role of Claspin in cervical cancer.

Table 45: Comparison of the increased expression of Claspin in various tumors

STUDY	TISSUE	INCREASED CLASPIN EXPRESSION (%)
Kobayashi G et al (2019)[12]	Gastric	47% (94/203)
Babasaki T et al (2021)[13]	Prostate	35% (31/89)
Kobayashi G et al(2020)[11]	Renal	47% (45/95)
Kobayashi G et al (2022)[25]	Bladder	42% (58/138)
Benevolo et al (2012)[10]	Cervix	93.3% (14/15)
PRESENT STUDY	Cervix	88.75% (71/80)

CLASPIN, P16 AND HPV

To explore the relationship between Claspin and HPV status, we employed P16 immunostaining as a surrogate marker for HPV positivity. Our results showed that 72.5% of cases were both P16 and Claspin positive, although this association did not reach statistical significance. In contrast, Benevelo et al.'s study, which exclusively examined HPV-positive SCC cases, found a significant association between moderate/high Claspin expression and HR-HPV positivity.[10] These findings suggest a potential link between Claspin and HPV status, warranting further investigation to elucidate the nature of this association.

Table 46: Comparison of the Claspin expression and P16/HPV status

		PRESENT STUDY	**BENEVELO ET AL[10]**
CLASPIN	**P16/HPV**	SCC (n = 80)	SCC (n = 15)
CLASPIN Negative/Low Positivity	P16/HPV POSITIVE	9 (11.25%)	1 (6.7%)
	P16/HPV NEGATIVE	0	0
CLASPIN Moderate Positivity /High Positivity	P16/HPV POSITIVE	58 (72.5%)	14 (93.3%)
	P16/HPV NEGATIVE	13 (16.25%)	0

CLASPIN AND CERVICAL CANCER

The exceptionally high levels of Claspin expression in cervical cancer, compared to other tumors, may be linked to the association between HPV and Claspin expression. During cervical carcinogenesis, HPV infection leads to the binding of E7 oncoproteins to the RB complex, releasing E2F and triggering increased cellular replicatory activity. Claspin, a crucial protein for DNA checkpoint activation, plays a vital role in preventing DNA damage and cell death during heightened replication states.[82] Consequently, it can be hypothesized that the upregulation of Claspin protein in response to HPV-induced DNA damage can cause its increased expression in cervical cancer.

CLASPIN AND THERAPY

Cervical cancer treatment encompasses a range of modalities, including surgery, chemotherapy, and radiotherapy, with the 2018 FIGO stage serving as a critical determinant of treatment strategy.[83] In stages II and III, the most commonly presented stages in our study and other comparative studies, concurrent chemotherapy (CCRT) is the standard treatment approach.[84] Cisplatin-based CCRT has been shown to enhance overall and disease-free survival.[85] Interestingly, investigations on Claspin have revealed its involvement in chemotherapy resistance to docetaxel in prostatic cancer and, more notably, cisplatin resistance in urothelial carcinoma.[13,26]Yamada et al stated that knockdown of Claspin mRNA in cisplatin-resistant bladder cancer cell lines resulted in higher sensitivity to cisplatin. Given the pivotal role of cisplatin in cervical cancer treatment and the high expression of Claspin in cervical cancer, immunotherapy against Claspin in adjunct to cisplatin can increase the sensitivity to chemotherapy in cervical cancer cases. A thorough investigation into the potential role of Claspin in cisplatin resistance specific to cervical cancer is imperative for future research.

SUMMARY

SUMMARY

The primary objective of the current study was to evaluate the expression of Claspin in squamous cell carcinoma cases of cervix. For this purpose, 80 cases of primary diagnosis of SCC cervix were taken along with 10 cases of cervical tissue with normal histological features as control and 10 cases of HSIL cases to look for a progressive increase in the expression of Claspin was also taken. The samples were collected from July 2022 to December 2023.

Following salient features are noted:

1. The average age of presentation in current study was 55 years.
2. Most cases of cervical carcinoma were noted in women having three children (parity 3) around 22.5% of cases.
3. Most common chief complaint noted was bleeding per vagina (52.5%cases)
4. On assessment by MRI (Radiological evaluation) large size (>4cm) was noted in 68.5% case and lymph node involvement was noted in 31.5% cases of cervical carcinoma.
5. Most common FIGO staging of carcinoma cervix cases were stage III.
6. Stage was significantly associated with post-menopausal status and post-menopausal bleeding.
7. According to Histological grade most common grade of cervical carcinoma noted was Well differentiated carcinoma (63.75%cases).
8. TILs were markedly increased (>60%) in 56.92% cases.
9. Grade and TILs showed no significant association with clinicopathologic parameters.
10. P16 block positivity was seen in 83.75% cases.
11. No statistical association was seen between P16 staining and other clinicopathologic parameters.

12. Claspin positive expression was observed in 88.75% cases of cervical carcinoma.
13. Claspin expression increased significantly from normal to HSIL to SCC cases ($p<0.001$)
14. 72.5% cases showed both high expression of Claspin and P16 block positivity.
15. Claspin expression was significantly associated with post-menopausal status and presence of ulceroproliferative growth on per speculum examination.
16. No statistical association was seen between Claspin expression and various other clinicopathological parameters such as age, parity, clinical findings, staging, lymph node status and size of lesion.

CONCLUSION

CONCLUSION

This study demonstrated that Claspin is a promising biomarker for SCC cervix, with significantly increased expression (88.75%, P<0.001) and a potential link to P16 positivity (72.5%). Notably, Claspin expression did not associate with other clinicopathologic parameters in the present study. Future research directions include investigating the relationship between Claspin expression and overall survival in cervical carcinoma, which may further clarify its role in prognosis and treatment outcomes.

Although our study did not find statistically significant associations between P16 expression and clinicopathological factors, it reinforces the notion that P16 expression is a prevalent feature of cervical cancer, with 83.75% of cases showing block positivity. However, the lack of association between P16 expression and prognosis in our study suggests that other molecular mechanisms may be involved in determining the aggressive behavior of cervical cancer, and further research is needed to elucidate these factors.

This study contributes to the ongoing quest for effective biomarkers to combat cervical cancer, a critical step towards improving patient outcomes and reducing mortality rates. Claspin's overexpression in cervical cancer cells may make it a promising target for adjunct therapy to enhance the effectiveness of chemotherapy. By targeting Claspin, researchers may be able to develop strategies to increase cancer cells' sensitivity to chemotherapy, leading to improved treatment outcomes. This is a potential avenue for future research and development in the field of cervical cancer treatment.

LIMITATIONS

LIMITATIONS OF THE STUDY:

1. Most of the cases collected are from incisional biopsies of cervix. This is because resection is only indicated in stage and in our study population most cases were diagnosed at advanced stages, about 60% of cases were in stages III and IV at the time of diagnosis.
2. Lots of hemorrhage and exudates in the small biopsies made it difficult to accurately assess TILs in H & E sections. The use of IHC for TILs assessment would have made it more accurate.
3. LVI could not be assessed adequately in small biopsies.
4. The only histological type of cervical cancer considered in this study was squamous cell carcinoma of cervix.

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ANNEXURE

ANNEXURE-I

INFORMED CONSENT FORM

STUDY TITLE: EXPRESSION OF CLASPIN IN SQUAMOUS CELL CARCINOMA OF CERVIX AND ITS ASSOCIATION WITH P16 EXPRESSION AND CLINICOPATHOLOGIC PARAMETERS

I, _____have read or have been read to me the patient information sheet and understand the purpose of the study, the procedure that will be used, the risk and benefits associated with my involvement in the study and the nature of information will be collected and disclosed during the study.

I have had my opportunity to ask my questions regarding various aspects of the study and my questions are answered to my satisfaction.

I, the undersigned, agree to participate in this study and authorize the collection and disclosure of my personal information for the dissertation.

Name and signature / thumb impression (subject)

Date:
Place:

Name and signature / thumb impression

Date:
Place:

(Witness/Parent/ Guardian/ Husband)

PRINCIPAL INVESTIGATOR: Dr. HANEENA MARIYAM KUKKAMGAI

PHONE NO. : 7907262273

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION & RESEARCH

SRI DEVARAJ URS MEDICAL COLLEGE

Tamaka, Kolar

DEPARTMENT OF PATHOLOGY

ಒಪ್ಪಿಗೆ ಪತ್ರ

ಅಧ್ಯಯನ ಶೀರ್ಷಿಕೆ :

ಮುಖ್ಯ ಸಂಶೋಧಕರ ಹೆಸರು : ಡಾ|| ಹನೀನಾ.ಎಂ.ಕೆ, ಸ್ನಾತಕೋತ್ತರ ವಿದ್ಯಾರ್ಥಿನಿ, ಶ್ರೀ ದೇವರಾಜ್ ಅರಸು ವೈದ್ಯಕೀಯ ಮಹಾವಿದ್ಯಲಯ, ಟಮಕ, ಕೋಲಾರ.

ಅಧ್ಯಯನದ ಸ್ಥಳ : ಶ್ರೀ ದೇವರಾಜ್ ಅರಸು ವೈದ್ಯಕೀಯ ಮಹಾವಿದ್ಯಲಯ, ಟಮಕ, ಕೋಲಾರ.

ಭಾಗವಹಿಸುವವರ ಹೆಸರು _____, ಈ ಅಧ್ಯಯನದ ಪೂರ್ತಿ ವಿವರವನ್ನು ಹಾಗೂ ಅದರ ಉದ್ದೇಶವನದನು ನನಗೆ ಅರ್ಥ ಆಗುವ ಹಾಗೆ ತಿಳಿಸಿಕೊಟಿದ್ದಾರೆ. ಈ ಅಧ್ಯಯನದ ಬಗ್ಗೆ ಹಲವಾರು ಪ್ರಶ್ನೆ ಕೇಳಲು ಅವಕಾಶ ದೊರೆತಿದೆ ಹಾಗೇ ನನ್ನ ಪ್ರಶ್ನೆಗಳಿಗೆ ನನಗೆ ತೃಪ್ತಿಕರವಾದ ಉತ್ತರಗಳು ದೊರೆತಿದೆ.

ನನ್ನಿನಿಂದ ಓದಲ್ಪಟ್ಟ ಅಥವಾ ನನಗೆ ಓದಿದ ಅಧ್ಯಯನದ ಉದ್ದೇಶ ನನಗೆ ಅರ್ಥವಾಗಿದ್ದು ನನ್ನಿಂದ ಸಂಗ್ರಹಿಸಲ್ಪಟ್ಟ ಮಾಹಿತಿಯನ್ನು ಕೇವಲ ಅಧ್ಯಯನಕ್ಕಾಗಿ ಬಳಸಲಾಗುವುದು ಹಾಗೂ ಮಾಹಿತಿಯನ್ನು ಗೌಪ್ಯವಾಗಿ ಇರಿಸಲಾಗುವುದು ನನ್ನ ಗುರುತನ್ನು ಬಹಿರಂಗ ಪಡಿಸುವುದಿಲ್ಲ. ನನಗೆ ಅಧ್ಯಯನದಲ್ಲಿ ಯಾವುದೇ ವೆಚ್ಚ ತಗಲುವುದಿಲ್ಲ ಎಂಬ ಅರಿವು ನನಗೆ ಆಗಿದೆ.

ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಅಧಿಕೃತವಾಗಿ ಮಾಹಿತಿ ಸಂಗ್ರಹಿಸಲು ಮತ್ತು ನನ್ನ ಸ್ವಂತ ಮಾಹಿತಿಯನ್ನು ಗೌಪ್ಯವಾಗಿರಲು ನನ್ನ ಇಚ್ಛೆಯಿಂದ ಒಪ್ಪಿ ಸಹಿ ಹಾಕಿರುತ್ತೇನೆ.

ಭಾಗವಹಿಸುವವರ ಹೆಸರು ಮತ್ತು ಸಹಿ :

ಸಾಕ್ಷಿದಾರರ ಹೆಸರು ಮತ್ತು ಸಹಿ :

ಮೂಖ್ಯ ಸಂಶೋಧಕರು : ಡಾ|| ಹನೀನಾ.ಎಂ.ಕೆ, ಸ್ನಾತಕೋತ್ತರ ವಿದ್ಯಾರ್ಥಿನಿ,
ಶ್ರೀ ದೇವರಾಜ್ ಅರಸು ವೈದ್ಯಕೀಯ ಮಹಾವಿದ್ಯಲಯ, ಟಮಕ, ಕೋಲಾರ.

ANNEXURE-II

PATIENT INFORMATION SHEET:

STUDY TITLE: EXPRESSION OF CLASPIN IN SQUAMOUS CELL CARCINOMA OF CERVIX AND ITS ASSOCIATION WITH P16 EXPRESSION AND CLINICOPATHOLOGIC PARAMETERS

PLACE OF STUDY: Department of Pathology, Sri Devaraj Urs Medical College, Kolar.

Cervical Cancer is the 2nd most common cancer in females in Kolar. The main aim of the study is to determine the proportion and intensity of immunohistochemical expression of CLASPIN in SQUAMOUS CELL CARCINOMA OF CERVIX and to evaluate its association with P16 and clinicopathologic parameters.

You are requested to participate in a study conducted by the department of Pathology as a part of dissertation. This study will be done on histopathologically diagnosed cases of SCC of Cervix in the biopsy specimens. The specimens will be collected from the department of Pathology, SDUMC, Kolar. For this study no extra tissue will be collected from you. This study is approved by the institutional ethical committee. The information collected will be used only for dissertation and publication. There is no compulsion to agree to participate. You are requested to sign / provide thumb impression only if you voluntarily agree to participate in the study. All information collected from you will be kept confidential and will not be disclosed to any outsider. Your identity will not be revealed. You will not receive any monetary benefits to participate in this research.

This informed consent document is intended to give you a general background of study. Please read the information carefully and discuss with your family members. You can ask your queries related to study at any time during the study. If you are willing to participate in the study you will be asked to sign an informed consent form by which you are acknowledging that you wish to participate in the study and entire procedure will be explained to you by the study doctor. You are free to withdraw your consent to participate in the study any time without explanation and this will not change your future care.

For any clarification you are free to contact the investigator.

PRINCIPAL INVESTIGATOR:Dr. HANEENA MARIYAM KUKKAMGAI

PHONE NO. : 7907262273

ಮಾಹಿತಿ ಪತ್ರ

ಅಧ್ಯಯನದ ಸ್ಥಳ : ಶ್ರೀ ದೇವರಾಜ್ ಅರಸು ವೈದ್ಯಕೀಯ ಮಹಾವಿದ್ಯಲಯ, ಟಮಕ, ಕೋಲಾರ.

ನಾನು ಶ್ರೀ ದೇವರಾಜ್ ಅರಸು ವೈದ್ಯಕೀಯ ಮಹಾವಿದ್ಯಲಯನಿಂದ ಬಂದಿದ್ದೇನೆ. ನಮ್ಮ ಕಾಲೇಜು ವತಿಯಿಂದ ನಾನು ಕೋಲಾರ ನಗರದಲ್ಲಿ ಪರಿಸರದ ವಾಯು ಮಾಲಿನ್ಯದ ಬಗ್ಗೆ ಅಧ್ಯಯನ ಮಾಡುತ್ತಿದ್ದೇನೆ.

ಅಧ್ಯಯನ ಶೀರ್ಷಿಕೆ : ಗರ್ಭಕಂಠದ ಸ್ಕ್ಯಾಮಸ್ ಸೆಲ್ ಕಾಸಿ ನೋಮದಲ್ಲಿ ಕ್ಲಾಸ್ಟಿನ್ನ ಅಭಿವ್ಯಕ್ತಿ ಮತ್ತು ಪಿ16 ಅಭಿವ್ಯಕ್ತಿ ಮತ್ತು ಕ್ಲಿನಿಕೋಪೆಥೋಲಾಜಿಕಲ್‌ನ ನಿಯತಾಂಕಗಳೊಂದಿಗೆ ಅದರ ಸಂಯೋಜನೆ

ಡಾ|| ಹನೀನಾ.ಎಂ.ಕೆ, ಮೊದಲನೆಯ ವರ್ಷದ ಸ್ನಾತಕೋತ್ತರ ವಿದ್ಯಾರ್ಥಿನಿಯಾಗಿ ಒದುತ್ತಿದ್ದೇನೆ. ಈ ಅಧ್ಯಯನದ ಪ್ರಮುಖ ಸಂಶೋಧಕಿ ನಿಮ್ಮನ್ನು ಈ ಮೇಲ್ಕಂಡ ಅಧ್ಯಾಯನಕ್ಕೆ ಸ್ವಾಗತಿಸುತ್ತೇನೆ.

ಕೋಲಾರ ನಗರದಲ್ಲಿ ಗರ್ಭಕಂಠದ ಸ್ಕ್ಯಾಮಸ್ ಸೆಲ್ ಕಾಸಿ ನೋಮದಲ್ಲಿ ಕ್ಲಾಸ್ಟಿನ್ನ ಅಭಿವ್ಯಕ್ತಿ ಮತ್ತು ಪಿ16 ಅಭಿವ್ಯಕ್ತಿ ಮತ್ತು ಕ್ಲಿನಿಕೋಪೆಥೋಲಾಜಿಕಲ್‌ನ ನಿಯತಾಂಕಗಳೊಂದಿಗೆ ಅದರ ಸಂಯೋಜನೆ.

ಈ ಮೇಲಿನ ಅಧ್ಯಾಯನದ ಬಗ್ಗೆ ಅರಿವು ಮೂಡಿಸಲು ನಿಮ್ಮನ್ನನು ಸ್ವಾಗಿಸಿಸುತ್ತೇನೆ ಮತ್ತು ಈ ಮೇಲಿನ ಅಧ್ಯಾಯನದ ಬಗ್ಗೆ ಅರಿವಿನ ಸಲುವಾಗಿ ಮತ್ತು ಅದರ ವತರ್ನೆ ಹಾಗೂ ಅಭ್ಯಾಸದ ಬಗ್ಗೆ ಕೆಲವು ಪ್ರಶ್ನೆಗಳನ್ನು ನಾನು ಕೇಳಲು ಇಚ್ಚಿಸುತ್ತೇನೆ.

ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಬೇಕೆಂಬ ಕಡ್ಡಾಯವೇನು ಇಲ್ಲ ಇದು ನಿಮ್ಮ ಸ್ವಇಚ್ಚೆಯಿಂದ ಭಾಗವಹಿಸಲು ಯಾವುದೇ ವೆಚ್ಚ ತಗಲುವುದಿಲ್ಲ. ನಾವು ನಿಮ್ಮಲ್ಲಿ ಯಾವುದೇ ಪರೀಕ್ಷೆ ಮಾಡುವುದಿಲ್ಲ. ನೀವು ಈಗ ಒಪ್ಪಿಕೊಂಡು ನಂತರ ಬೇಕಿದ್ದಲ್ಲಿ ನಿರಾಕರಿಸಬಹುದು. ನಾವು ನಿಮಗೆ ಕೇಳುವ ಪ್ರಶ್ನೆಗಳಲ್ಲಿ ಯಾವುದನ್ನಾದರೂ ನೀವು ಉತ್ತರಿಸಲು ನಿರಾಕರಿಸಬಹುದು. ಹೀಗೆ ಮಾಡುವುದರಿಂದ ನಿಮಗೆ ಯಾವುದೇ ತೊಂದರೆ ಇರುವುದಿಲ್ಲ ಮತ್ತೇನಾದರು ಸಂಶಯವಿದ್ದರೆ ಸಂಶೋಧಕರನ್ನು ಸಂಪರ್ಕಿಸಿ.

ಸಂಶೋಧಕರ ಹೆಸರು : ಡಾ|| ಹನೀನಾ.ಎಂ.ಕೆ, ಮೊದಲನೆಯ ವರ್ಷದ ಸ್ನಾತಕೋತ್ತರ ವೈದ್ಯಕೀಯ ವಿದ್ಯಾರ್ಥಿನಿ,
ಶ್ರೀ ದೇವರಾಜ್ ಅರಸು ವೈದ್ಯಕೀಯ ಮಹಾವಿದ್ಯಲಯ, ಟಮಕ, ಕೋಲಾರ.

ದೂರವಾಣಿ ಸಂಖ್ಯೆ : 7907262273

ANNEXURE-III

TITLE: EXPRESSION OF CLASPIN IN SQUAMOUS CELL CARCINOMA OF CERVIX AND ITS ASSOCIATION WITH P16 EXPRESSION AND CLINICOPATHOLOGIC PARAMETERS

PATIENT PROFORMA

Name : Age: Hospital Number:

Anonymised Sample No:

Chief complaint :

History of presenting illness :

Past history :

Personal history :

Parity :

Local examination:

Biopsy Number:

Microscopy :

Histopathological diagnosis :

Grading:

Lymphovascular Invasion :

Immunohistochemical Scoring (P16):

Negative-

Ambiguous-

Block positive -

Immunohistochemical Scoring (Claspin):

Negative-

Low-positive-

Moderate-positive –

High-positive -

<u>Radiological Investigation:</u>

TumorSize :

Metastatic Lymph Nodes :

TumorStage :

ANNEXURE-IV

KEYS TO MASTERCHART

WDPV	White Discharge per Vagina
BPV	BleedingPerVagina
PMB	Post Menopausal Bleeding
MPV	Mass Per Vagina
PAIN ABD	Pain abdomen
P/S	Per Speculum
UVD	UteroVaginal Descent
UPG	Ulceroproliferative growth
P/V	Per vagina
B/L F INV	Bilateral fornices involved
B/L F FREE	Bilateral fornices free
ANT F INV	Anterior fornix involved
L F INV	Left fornix involved
R F INV	Right fornix involved
WD	Well-differentiated
MD	Moderately-differentiated

PD	Poorly-differentiated
BP	Block positive
HP	High positive
MP	Moderate positive
LP	Low positive
NA	Not applicable

MASTER

CHART

S.NO	UHID	AGE	PARITY	PostmenopausalENPAUSAL	C/F	P/S	P/V	SIZE	LN	BIOPSY NO	HPE	GRADE	LVI	STAGE	TIL	P16	CLASPIN
1	71362	65	4	Postmenopausal	PMB	UPG	B/L F FREE	6X5.3X2.6	Absent	592/22	SCC	WD	NA	IIB	1+	BP	HP
2	74235	70	5	Postmenopausal	PMB	UPG	ANT F INV	1.6X1.3X0.3	Absent	701/22	SCC	WD	NA	IB1	1+	BP	HP
3	79230	43	6	PrePostmenopausalenopausal	MPV	UPG	B/L F INV	6X5.6X2.5	Absent	872/22	SCC	MD	NA	IIA2	2+	BP	HP
4	84443	70	5	Postmenopausal	PMB	UPG	B/L P INV	5.3X4.5X2.4	Present	1070/22	SCC	WD	NA	IVA	2+	BP	HP
5	87829	80	5	Postmenopausal	PAIN ABD	UPG	B/L F INV	5X4.3X4.1	Present	1131/22	SCC	MD	NA	IVA	3+	BP	MP
6	98751	64	2	Postmenopausal	WDPV	UPG	B/L F INV	4.6X2.4X1.8	Absent	1364/22	SCC	MD	NA	IIIB	3+	BP	HP
7	91505	45	2	Postmenopausal	PMB	UPG	ANT F INV	4.2X3.4X4.6	Present	1412/22	SCC	PD	NA	IIIC1	3+	BP	HP
8	74876	48	3	Postmenopausal	PMB	UPG	B/L F INV	5X3.8X4.2	Present	1426/22	SCC	MD	NA	IVA	1+	BP	MP
9	102530	65	2	Postmenopausal	WDPV	EROSION	R F INV	2.5X2.3X2.1	Absent	1443/22	SCC	WD	NA	IIIB	1+	BP	HP
10	104576	54	9	Postmenopausal	PMB	UPG	ANT & R F INV	3.5X3X0.8	Absent	1473/22	SCC	WD	NA	IIB	3+	BP	MP
11	105344	45	2	Postmenopausal	WDPV	UPG	R F INV	6.8X5.9X4.9	Absent	1474/22	SCC	WD	NA	IIIB	1+	BP	HP
12	109400	36	2	PrePostmenopausalenopausal	BACK PAIN	HYPERTROPHIED	B/L F FREE	5.4X3.9X2.6	Absent	1558/22	SCC	WD	NA	IIB	3+	BP	MP
13	110820	65	8	Postmenopausal	PMB	HYPERTROPHIED	B/L F FREE	1.8X1.5X0.6	Absent	1577/22	SCC	MD	NA	IB1	1+	BP	MP
14	109985	79	9	Postmenopausal	PMB	UPG	ANT F INV	5.6X4.3X4.6	Present	1584/22	SCC	MD	NA	IVA	3+	BP	HP
15	111989	56	2	Postmenopausal	PMB	UPG	L F INV	4.3X2.2X3.5	Absent	1605/22	SCC	WD	NA	IIIB	3+	BP	HP
16	115170	49	7	PrePostmenopausalenopausal	MPV	UPG	B/L F INV	9.2X5.3X6.7	Present	1675/22	SCC	WD	NA	IIIC1	2+	BP	MP
17	117635	74	6	Postmenopausal	MPV	UPG	B/L F INV	3.3X4.6X10.5	Absent	1727/22	SCC	WD	NA	IVA	NA	BP	MP
18	111411	57	1	Postmenopausal	PAIN ABD	HYPERTROPHIED	R F INV	3X4.5X5.9	Present	1745/22	SCC	WD	NA	IIIC1	2+	NEG	HP
19	112915	48	3	PrePostmenopausalenopausal	WDPV	EROSION	B/L F FREE	3X2.5X2	Absent	1783/22	SCC	WD	Absent	IB2	3+	BP	NEG
20	131666	48	3	Postmenopausal	PAIN ABD	UPG	ANT F INV	7.6X62X5.9	Absent	2003/22	SCC	WD	NA	IIIB	3+	BP	MP
21	133660	35	3	PrePostmenopausalenopausal	WDPV	HYPERTROPHIED	B/L F INV	2.2X1.8X0.2	Present	2049/22	SCC	WD	NA	IIIC1	1+	BP	LP
22	151148	75	3	Postmenopausal	WDPV	UPG	B/L F INV	1.5X1X2	Absent	2580/22	SCC	WD	Present	IIIA	3+	AMB	HP
23	104398	42	4	PrePostmenopausalenopausal	PAIN ABD	HYPERTROPHIED	B/L F FREE	3.9X3.8X1.2	Absent	2785/22	SCC	WD	NA	IIIB	3+	BP	HP
24	161908	75	5	Postmenopausal	PAIN ABD	EROSION	B/L F INV	5.2X4.9X3.8	Absent	2922/22	SCC	WD	NA	IIIB	3+	BP	HP
25	165520	87	5	Postmenopausal	PAIN ABD	UPG	B/L F INV	6.1X3.3X5.9	Absent	3040/22	SCC	WD	NA	IIIB	3+	BP	HP
26	167121	42	1	PrePostmenopausalenopausal	WDPV	HYPERTROPHIED	B/L F INV	3.9X3.5X2.5	Absent	3065/22	SCC	WD	NA	IIB	3+	BP	LP
27	170226	75	3	Postmenopausal	PMB	EROSION	B/L F FREE	1X0.8X1.8	Absent	3159/22	SCC	WD	NA	IIB	3+	BP	MP
28	170736	50	3	Postmenopausal	WDPV	HYPERTROPHIED	B/L F FREE	6.5X3.3X4.2	Present	3189/22	SCC	WD	NA	IIIC1	3+	BP	MP
29	129918	49	3	Postmenopausal	WDPV	UPG	B/L F INV	6.2X4.3X3.8	Present	3273/22	SCC	WD	NA	IVA	2+	NEG	HP
30	96869	65	7	Postmenopausal	PMB	HYPERTROPHIED	B/L F FREE	4.2X4X3.8	Absent	3338/22	SCC	WD	NA	IIIB	2+	BP	HP
31	172865	50	5	Postmenopausal	PMB	UPG	B/L F FREE	5.6X3.5X4.8	Absent	3368/22	SCC	MD	NA	IIB	3+	BP	HP
32	180266	50	4	Postmenopausal	PMB	UPG	B/L F FREE	5.2X5X4.6	Absent	3463/22	SCC	WD	NA	IIB	2+	BP	HP
33	180761	46	2	PrePostmenopausalenopausal	WDPV	UPG	B/L F INV	5X4.8X3.4	Present	3472/22	SCC	WD	NA	IIIB	3+	BP	HP
34	183383	50	8	Postmenopausal	WDPV	UPG	ANT F INV	1.4X3.6X3.1	Absent	16/23	SCC	MD	NA	IIB	2+	BP	MP
35	185189	70	2	Postmenopausal	PAIN ABD	UPG	B/L F INV	4.6X2.3X4.2	Absent	83/23	SCC	MD	NA	IIIB	3+	NEG	HP
36	191707	66	4	Postmenopausal	PMB	UPG	ANT & R F INV	6.2X5X4.6	Absent	280/23	SCC	MD	NA	IIIB	3+	BP	HP
37	191468	38	2	PrePostmenopausalenopausal	WDPV	HYPERTROPHIED	B/L F FREE	3.8X2.5X3.3	Absent	281/23	SCC	MD	NA	IIB	1+	BP	HP
38	198168	65	7	Postmenopausal	PMB	UPG	B/L F FREE	6.8X5.3X3.7	Present	512/23	SCC	MD	Present	IVA	3+	BP	HP
39	207520	65	4	Postmenopausal	PMB	UPG	R F INV	4.2X2.5X3.0	Absent	806/23	SCC	WD	NA	IIA2	3+	BP	HP
40	209378	50	2	Postmenopausal	PMB	UPG	R F INV	6X4.5X4	Absent	858/23	SCC	WD	NA	IIB	3+	BP	HP
41	214070	55	5	Postmenopausal	PAIN ABD	UPG	B/L F FREE	5.3X4.2X4.6	Present	979/23	SCC	WD	NA	IIIC1	3+	BP	MP
42	214073	55	3	Postmenopausal	PMB	UPG	B/L F INV	6.3X3.3X4.2	Present	1033/23	SCC	WD	NA	IVB	1+	BP	HP

S.NO	UHID	AGE	PARITY	PostmenopausalENPAUSAL	C/F	P/S	P/V	SIZE	LN	BIOPSY NO	HPE	GRADE	LVI	STAGE	TIL	P16	CLASPIN
43	218923	60	3	Postmenopausal	WDPV	UPG	B/L F INV	4.6X4.2X3.6	Present	1137/23	SCC	MD	NA	IIB	3+	BP	LP
44	220475	50	3	Postmenopausal	PMB	EROSION	B/L F FREE	5.1X3.3X4.1	Present	1176/23	SCC	WD	NA	IVB	3+	BP	LP
45	222728	50	1	PrePostmenopausalenopausal	BPV	UPG	R F INV	6.5X6.3X5.7	Absent	1260/23	SCC	MD	NA	IIIB	NA	BP	HP
46	223857	55	6	Postmenopausal	PAIN ABD	UPG	ANT F INV	3.8X24X2.9	Absent	1280/23	SCC	WD	Present	IIIB	3+	BP	HP
47	226237	58	6	Postmenopausal	PMB	HYPERTROPHIED	B/L F FREE	2.3X1.6X1.4	Absent	1379/23	SCC	MD	NA	IIIB	NA	NEG	HP
48	226896	48	4	Postmenopausal	WDPV	UPG	L F INV	4.1X3X4.8	Absent	1400/23	SCC	WD	NA	IIB	NA	BP	HP
49	229778	58	5	Postmenopausal	PMB	UPG	B/L F FREE	6.7X4.5X5.2	Absent	1482/23	SCC	WD	NA	IIB	3+	BP	MP
50	230573	55	3	Postmenopausal	PAIN ABD	UPG	B/L F INV	6.2X5.5X4.9	Absent	1531/23	SCC	MD	NA	IIIB	1+	AMB	HP
51	234902	80	3	Postmenopausal	WDPV	UPG	B/L F FREE	2.3X2.1X1.9	Absent	1663/23	SCC	WD	NA	IIB	2+	AMB	MP
52	233346	44	3	PrePostmenopausalenopausal	BPV	HYPERTROPHIED	POST F INV	NA	NA	1664/23	SCC	MD	NA	NA	2+	BP	NEG
53	239401	60	0	Postmenopausal	WDPV	UPG	B/L F INV	5.1X4.3X3.4	Present	1802/23	SCC	WD	NA	IVA	2+	BP	MP
54	239482	68	6	Postmenopausal	PMB	UPG	ANT F INV	5.5X3.7X4.8	Absent	1803/23	SCC	MD	NA	IIB	2+	BP	LP
55	243619	70	4	Postmenopausal	PMB	UPG	B/L F FREE	3.6X3.2X2.8	Absent	1940/23	SCC	WD	NA	IIIB	3+	BP	MP
56	225378	34	2	PrePostmenopausalenopausal	BPV	HYPERTROPHIED	B/L F FREE	1.4X1.1X1.2	Absent	2003/23	SCC	WD	NA	IIB	2+	BP	HP
57	251182	45	2	PrePostmenopausalenopausal	BPV	UPG	B/L F INV	2.2X3.8X4.2	Absent	2148/23	SCC	WD	NA	IIB	2+	BP	LP
58	257010	45	5	PrePostmenopausalenopausal	WDPV	EROSION	B/L F FREE	NA	NA	2310/23	SCC	MD	NA	NA	NA	BP	HP
59	245207	34	2	PrePostmenopausalenopausal	BPV	EROSION	B/L F FREE	2.2X2X1.8	Absent	2439/23	SCC	WD	Absent	IB2	1+	BP	HP
60	261277	52	3	Postmenopausal	PMB	UPG	B/L F INV	5.2X2.1X4.8	Absent	2453/23	SCC	WD	NA	IIIB	NA	BP	HP
61	265437	50	4	PrePostmenopausalenopausal	BPV	HYPERTROPHIED	B/L F INV	7.6X6.5X4.	Present	2621/23	SCC	WD	NA	IVB	2+	BP	LP
62	268459	34	1	PrePostmenopausalenopausal	BPV	UPG	L F INV	NA	NA	2712/23	SCC	MD	NA	NA	NA	BP	HP
63	230106	45	4	Postmenopausal	PMB	UPG	B/L F INV	5.6X4.2X3.4	Absent	2762/23	SCC	MD	NA	IIB	NA	AMB	HP
64	260083	45	0	Postmenopausal	WDPV	UPG	B/L F FREE	4.5X3.5X2	Absent	2841/23	SCC	MD	Present	IB3	NA	NEG	MP
65	272593	48	2	PrePostmenopausalenopausal	BPV	UPG	B/L F INV	4.5X2X4.6	Present	2861/23	SCC	WD	NA	IIIC1	3+	BP	MP
66	272737	34	2	PrePostmenopausalenopausal	WDPV	UPG	B/L F FREE	3.2X2.7X3	Absent	2862/23	SCC	WD	NA	IB2	2+	BP	MP
67	278450	60	4	Postmenopausal	WDPV	UPG	B/L F FREE	3.7X2.8X3	Absent	3055/23	SCC	MD	NA	IIB	NA	BP	HP
68	12108	45	3	PrePostmenopausalenopausal	BPV	HYPERTROPHIED	B/L F FREE	3.2X3.1X3.9	Absent	583/22	SCC	MD	NA	IIB	3+	BP	HP
69	283075	50	6	Postmenopausal	PMB	UPG	B/L F INV	4.8X3.5X3	Absent	3201/23	SCC	WD	NA	IIIB	3+	BP	MP
70	281404	33	2	PrePostmenopausalenopausal	BPV	HYPERTROPHIED	B/L F FREE	3.2X2.1X0.3	Absent	3211/23	SCC	MD	NA	IIB	1+	NEG	HP
71	288253	50	3	Postmenopausal	PMB	UPG	R F INV	4.9X3.9X4.3	Present	3379/23	SCC	WD	NA	IIIC1	NA	BP	MP
72	299748	58	8	Postmenopausal	PMB	UPG	B/L F INV	5.6X4.9X3.1	Present	3821/23	SCC	PD	NA	IVA	NA	BP	MP
73	303436	65	6	Postmenopausal	PAIN ABD	UPG	B/L F INV	3.8X2.9X1.2	Absent	3906/23	SCC	MD	NA	IIIB	NA	BP	MP
74	307304	60	9	Postmenopausal	WDPV	UPG	B/L F INV	NA	NA	4007/23	SCC	WD	NA	NA	3+	BP	MP
75	308557	57	3	Postmenopausal	PMB	UPG	B/L F INV	4.2X3X4.1	Absent	4116/23	SCC	WD	NA	IIIB	3+	AMB	HP
76	322010	67	8	Postmenopausal	PMB	UPG	B/L F FREE	NA	NA	4453/23	SCC	WD	NA	NA	NA	AMB	MP
77	327488	53	2	Postmenopausal	PMB	UPG	B/L F INV	6.6X5.9X5.1	Present	4621/23	SCC	WD	NA	IVA	3+	BP	HP
78	267594	53	6	Postmenopausal	PAIN ABD	HYPERTROPHIED	B/L F FREE	NA	NA	4633/23	SCC	MD	NA	NA	NA	BP	MP
79	328783	58	5	Postmenopausal	PMB	HYPERTROPHIED	B/L F FREE	NA	NA	4647/23	SCC	WD	NA	NA	3+	BP	MP
80	325319	60	5	Postmenopausal	PAIN ABD	UPG	B/L F FREE	2.5X2X3.5	Present	4657/23	SCC	WD	NA	IIIC1	3+	AMB	HP
81	65951	55	2	Postmenopausal	PMB	EROSION	B/L F FREE	NA	NA	476/22	HSIL	NA	NA	NA	NA	BP	MP
82	76498	56	4	Postmenopausal	PAIN ABD	UVD	B/L F FREE	NA	NA	791/22	HSIL	NA	NA	NA	NA	BP	LP
83	77996	60	2	Postmenopausal	PMB	HYPERTROPHIED	B/L F FREE	NA	NA	845/22	HSIL	NA	NA	NA	NA	AMB	HP
84	97671	46	3	PrePostmenopausalenopausal	MPV	UVD	B/L F FREE	NA	NA	1402/22	HSIL	NA	NA	NA	NA	BP	MP

S.NO	UHID	AGE	PARITY	PostmenopausalENPAUSAL	C/F	P/S	P/V	SIZE	LN	BIOPSY NO	HPE	GRADE	LVI	STAGE	TIL	P16	CLASPIN
85	149680	42	2	PrePostmenopausalenopausal	PAIN ABD	HYPERTROPHIED	B/L F FREE	NA	NA	2863/22	HSIL	NA	NA	NA	NA	BP	LP
86	204252	55	3	Postmenopausal	PMB	UPG	B/L F FREE	NA	NA	732/23	HSIL	NA	NA	NA	NA	BP	HP
87	268152	40	1	PrePostmenopausalenopausal	MPV	HYPERTROPHIED	B/L F FREE	NA	NA	2904/23	HSIL	NA	NA	NA	NA	BP	LP
88	280725	40	4	PrePostmenopausalenopausal	BPV	EROSION	B/L F FREE	NA	NA	3126/23	HSIL	NA	NA	NA	NA	BP	LP
89	309698	45	2	PrePostmenopausalenopausal	PAIN ABD	HYPERTROPHIED	B/L F FREE	NA	NA	4333/23	HSIL	NA	NA	NA	NA	BP	MP
90	322385	46	3	PrePostmenopausalenopausal	WDPV	HYPERTROPHIED	B/L F FREE	NA	NA	4669/23	HSIL	NA	NA	NA	NA	BP	LP
91	198471	35	2	PrePostmenopausalenopausal	BPV	HYPERTROPHIED	B/L F FREE	NA	NA	580/23	NORMAL	NA	NA	NA	NA	NEG	NEG
92	198349	67	4	Postmenopausal	MPV	UVD	B/L F FREE	NA	NA	812/23	NORMAL	NA	NA	NA	NA	NEG	NEG
93	206023	45	2	Postmenopausal	MPV	UVD	B/L F FREE	NA	NA	881/23	NORMAL	NA	NA	NA	NA	NEG	NEG
94	183927	75	5	Postmenopausal	MPV	UVD	B/L F FREE	NA	NA	886/23	NORMAL	NA	NA	NA	NA	NEG	NEG
95	207749	55	5	Postmenopausal	MPV	UVD	B/L F FREE	NA	NA	887/23	NORMAL	NA	NA	NA	NA	NEG	NEG
96	260926	40	4	PrePostmenopausalenopausal	BPV	HEALTHY	B/L F FREE	NA	NA	2535/23	NORMAL	NA	NA	NA	NA	NEG	NEG
97	243470	55	3	Postmenopausal	PMB	HYPERTROPHIED	B/L F FREE	NA	NA	2612/23	NORMAL	NA	NA	NA	NA	NEG	NEG
98	218390	36	2	PrePostmenopausalenopausal	BPV	HEALTHY	B/L F FREE	NA	NA	3374/23	NORMAL	NA	NA	NA	NA	NEG	NEG
99	328071	53	3	Postmenopausal	PAIN ABD	HEALTHY	B/L F FREE	NA	NA	4649/23	NORMAL	NA	NA	NA	NA	NEG	NEG
100	291947	43	4	PrePostmenopausalenopausal	MPV	HYPERTROPHIED	B/L F FREE	NA	NA	4663/23	NORMAL	NA	NA	NA	NA	NEG	NEG