ALL INDIA INSTITUTE OF MEDICAL SCIENCES, JODHPUR

अखिल भारतीय आयुर्विज्ञान संस्थान, जोधपुर

DEPARTMENT OF PATHOLOGY & LAB. MEDICINE

विकृति विज्ञान विभाग

संदर्भ :- AIIMS/JDR/PATH/5558 /2022 दिनांक: 14/02/2022

To,

The Dean (Academics)
All India Institute of Medical Sciences
Jodhpur

Subject: Submission of M.D thesis.

Respected Sir,

This is to submit that the M.D thesis by the Academic Junior Resident (July, 2019 Batch) of our Department has been duly completed and signed and is ready for submission. Please accept the same.

Details of ~~his~~ her thesis are attached herein:

Name of candidate	Thesis topic
Dr. Anju G	EXPRESSION OF PROGRAMMED CELL DEATH LIGAND 1 (PD-L1) AND MISMATCH REPAIR STATUS IN SQUAMOUS CELL CARCINOMAS OF CERVIX

धन्यवाद,

भवदीया

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आचार्य एवं विभागाध्यक्ष
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EXPRESSION OF PROGRAMMED CELL DEATH LIGAND 1 (PD-L1) AND MISMATCH REPAIR STATUS IN SQUAMOUS CELL CARCINOMAS OF CERVIX

THESIS

Submitted to

All India Institute of Medical Sciences, Jodhpur

In partial fulfillment of the requirement for the degree of

DOCTOR OF MEDICINE (MD)

PATHOLOGY

JULY, 2022 **Dr. ANJU G**

AIIMS, JODHPUR

DECLARATION

I hereby declare that the thesis titled **"EXPRESSION OF PROGRAMMED CELL DEATH LIGAND 1 (PD-L1) AND MISMATCH REPAIR STATUS IN SQUAMOUS CELL CARCINOMAS OF CERVIX"** embodies the original work carried out by the undersigned in All India Institute of Medical Sciences, Jodhpur.

Dr. ANJU G

Department of Pathology and Lab Medicine

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CERTIFICATE

This is to certify that the thesis titled "**EXPRESSION OF PROGRAMMED CELL DEATH LIGAND 1 (PD-L1) AND MISMATCH REPAIR STATUS IN SQUAMOUS CELL CARCINOMAS OF CERVIX**" is the bonafide work of **Dr. ANJU G** carried out under our guidance and supervision, in the Department of Pathology and Lab Medicine, All India Institute of Medical Sciences, Jodhpur.

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ACKNOWLEDGMENTS

"IF EVERYONE IS MOVING FORWARD TOGETHER, THEN SUCCESS TAKES CARE OF ITSELF"

HENRY FORD

I feel immensely thankful to the Almighty for bringing me to the verge of the most prestigious achievement of my career.

I would like to express my gratitude and sincere thanks to my supervisor, mentor, and teacher **Dr. Meenakshi Rao**, Associate Professor in the Department of Pathology and Lab Medicine, All India Institute of Medical Sciences, Jodhpur. She has always been guiding me, pointing out minute details, being supportive and trusting my abilities and more so believing in me more than I ever did in myself. She will always be my inspiration. I wish to thank her for devoting her valuable time to this project.

I express my sincere gratitude to **Dr. Poonam Abhay Elhence,** Professor and Head, Department of Pathology and Lab Medicine, AIIMS, Jodhpur, who has always encouraged me, inspired me and steered me through the difficult times.

I am extremely thankful to **Dr. Aasma Nalwa**, my MD thesis co-Guide and Associate Professor, Department of Pathology and Lab Medicine, All India Institute of Medical Sciences, Jodhpur for her keen interest, valuable suggestions towards this work.

Dr. Pratibha Singh, Professor and Head, Department of Obstetrics and Gynaecology, AIIMS, Jodhpur for her support, encouragement and guidance throughout my work.

I am grateful for the constant encouragement and enthusiasm of all the consultants in my department for ensuring that no patient matching my inclusion criteria is missed, helping me finish my thesis in time.

Also, I would thank my seniors, juniors and colleagues Dr. Ismetara Begum and Dr. Kartik Jain without whose help, this research work would not have been possible.

I am also extremely thankful to all the technical staff members, especially Mrs. Meenakshi Upadhyay, Miss. Ankita Ghelot, Mr. Shravan and Mr. Prem Shankar for helping me in the practical aspect of the thesis.

I owe my parents Mr. Shaji V K and Mrs. Geetha G, my husband Dr. Arun Mukesh R and my younger brother Balu S who have given me the strength to endure all my hardships and steered me through my failures, making me who I am today.

My friends have been the strongest pillars of my support throughout my journey, as a resident, I cannot thank them enough.

Last but not least, I would take this opportunity to thank the patients who agreed to participate in my study and contributed to this study.

Dr. Anju G

INDEX

SYNOPSIS

Background: Cervical cancer is the fourth most frequent cancer in women globally and the second most common cancer in India. [1] The co-regulatory molecule programmed death-ligand-1 (PD-L1), which is expressed on cancer cells and immune cells, reduces local tumor immunity, allowing tumor cells to proliferate and metastasis. Mismatch repair deficiency has been linked to a variety of cancers which has been shown to influence response to anti-PD-L1 targeted therapy.

Objectives: To assess mismatch repair (MMR) status and programmed cell death-ligand 1 (PD-L1) expression in squamous cell carcinomas of the cervix and their correlation with clinicopathologic parameters.

Materials and methods: Expression of PD-L1 and mismatch repair status (MSH2, MSH6, MLH1 and PMS2) was assessed by immunohistochemistry on 50 cases of SCCs of the cervix.

Results: PD-L1 expression was seen in 80% of tumors cells (40 out of 50 cases) and 42 out of 50 cases (84%) showed PD-L1 expression in tumor-infiltrating lymphocytes (TILs). 40 out of 50 cases (80%) had a combined positive score > 1 (PD-L1 positive cases) whereas 10 out of 50 cases (20%) had a score of <1 (PD-L1 negative cases). Out of 50 cases, 47 cases (94%) showed retained MMR proteins (MMR stable) while 3 cases (6%) showed MMR deficiency. A statistically significant association was noted between PD-L1 expression in the tumor and the grade of the tumor ($p=0.022$).

Conclusion: This study underscores the need for evaluation of the mismatch repair status of these malignancies as a routine practice, as it will provide valuable information for management and prognostication of the patients. Among the SCCs of the cervix, 80% of the cases showed PD-L1 expression in the tumor and 6% of the cases were mismatch repair deficient. Our study found no significant connection between mismatch repair status and PD-L1 expression in tumors, however, this could be due to the small sample size. Additional studies with a larger sample size are needed to enable the selection of patients who are likely to benefit from targeted therapy.

LIST OF ABBREVIATIONS

AJCC	American Joint Committee on Cancer
ATP	Adenosine Triphosphate
CCRT	Combined Chemoradiotherapy
CDK	Cyclin Dependent Kinase
COPD	Chronic Obstructive Pulmonary Disease
dMMR	Deficient Mismatch Repair
DNA	Deoxyribonucleic Acid
FDA	Food and Drug Administration
FIGO	Federation Internationale de Gynecologie et d 'Obstetrique
FOX	Fork Head Box Protein
H and E	Hematoxylin and Eosin
hMSH2	Hypermethylated MutS Homolog2
HPV	Human Papillomavirus
HRP	Horseradish Peroxidase
IDO	Indoleamine 2,3- dioxygenase
IFN	Interferon
IHC	Immunohistochemistry
IRF 9	Interferon Regulatory Factor 9
kDa	Kilo Dalton
LVI	Lymphovascular Invasion
MHC	Major Histocompatibility Complexes
MLH-1	MutL Homolog 6
MMR	Mismatch Repair
MSH-2	MutS Homolog 2

MSH-6	MutS homolog 6
MSI	Microsatellite Instability
NAC	Neoadjuvant Chemotherapy
NCI	National Cancer Institute
PCR	Polymerise chain reaction
PD-1	Programmed Death-1
PDCD-1/PD-1	Programmed Cell Death-1
PD-L1	Programmed Death Ligand-1
PD-L2	Programmed Death Ligand-2
pMMR	Proficient Mismatch Repair
pTNM	Pathological Tumour Nodes and Metastasis
Rb	Retinoblastoma
SD	Standard Deviation
TCGA	The Cancer Genome Atlas
TERT	Telomerase reverse transcriptase
TILs	Tumour Infiltrating Lymphocytes
WHO	World Health Organisation

LIST OF TABLES

LIST OF FIGURES

LIST OF PHOTOMICROGRAPHS


INTRODUCTION

INTRODUCTION

Cervical cancer is the fourth most frequent cancer in women globally and the second most common cancer in India[1]. Approximately 90% of cervical cancers occur in low and middle income countries that lack systematic screening and immunization programs against the Human Papilloma Virus (HPV).[2] China and India together contributed more than a third of the global cervical cancer burden.[1] The treatment for cervical cancer depends on disease extent at diagnosis and locally available resources.

The rate of new cases of cervical cancer was 7.5 per 100,000 women per year. The death rate was 2.2 per 100,000 women per year.[3]

An important factor in the development of precancerous lesions such as LSIL, HSIL and cervical cancer is the presence of high-risk HPVs. It is well known that cytologic cancer screening has dramatically reduced cervical cancer mortality as well as vaccination against high-risk oncogenic HPV is another important aspect of cervical cancer prevention.[4]

The studies on DNA repair genetics and immune checkpoint mechanism plays an important role in suppressing tumor specific immune responses within the tumor microenvironment.[5] Recent studies have provided a breakthrough in the understanding of tumor pathology. The immune checkpoint proteins programmed cell death 1 (PDCD-1/PD-1) and programmed cell death-ligand 1 (CD274/PD-L1) are expressed on both tumor cells and immune cells, which have been reported to suppress anti-tumor T cell-mediated immune responses.[5]

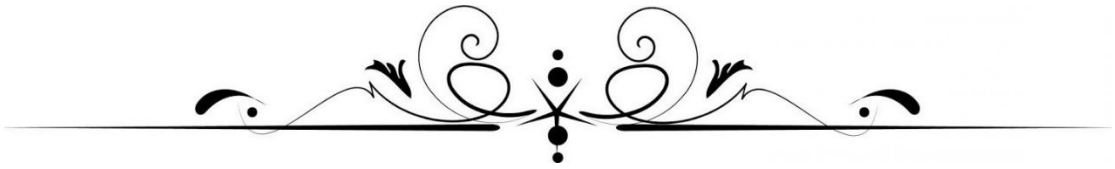
PD-1/PD-L1, including PD-1 in lymphocyte and PD-L1 in the tumor cell, dendritic lymphocyte and placenta cell also called the tumor immune checkpoint, play critical roles in human immune regulation pathways and preventing effective antitumor immunity.[6] PD-1/PD-L1 represents an adaptive immune resistance mechanism by providing inhibitory signals, such as T cell inactivation and inhibition.[7]

Immunotherapy options like PD-1/PD-L1 antibody drugs such as pembrolizumab have been started for cervical cancer and therefore PD-1/PD-L1 antibody drugs are regarded as a potential strategy for cervical cancer patients.[8],[9] Recent clinical findings indicate that deficient DNA mismatch repair (dMMR) can improve the anticancer effects of the PD-1/PD-L1 pathway, implying that dMMR may act as a prognostic predictor of PD-1/PD-L1 antibody therapies.[7]

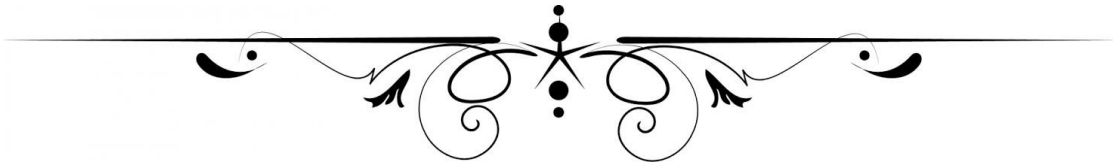
The DNA mismatch repair (MMR) system coded by MMR genes, has a series of specific proteins. This system can keep DNA replication fidelity by maintaining genetic integrity and stability and avoiding replication mutations.[10],[11] MMR consists of six core proteins, MSH2, MLH1, MSH3, MSH6, PMSH1 and PMSH2. Deficient MMR (dMMR) is the condition when MMR proteins are deficient. Another MMR subgroup is proficient MMR (pMMR), a counterpart phenomenon in which the patient has normal and biologically normal MMR expression. These functions were analyzed by polymerase chain reaction (PCR). [5]

Higher PD-L1 in cancer cells can be observed when patients are under dMMR status, rather than under pMMR status, suggesting that cervical cancer patients with dMMR may achieve higher therapeutic effects with PD-1/PD-L1 antibody drugs.[7] For patients with microsatellite instability (MSI)- dMMR metastatic colorectal cancer who are refractory to standard chemotherapy combinations, PD-1 blockade has emerged as a highly successful therapeutic target.[13],[14]

This is a study evaluating the expression of the DNA mismatch repair (MMR) and programmed cell death-ligand 1 (PD-L1) in Human papillomavirus (HPV) associated and HPV independent squamous cell carcinoma of the cervix.



REVIEW OF LITERATURE



REVIEW OF LITERATURE

Cervical cancer is the world's fourth most prevalent cancer in women.[1] The majority of cervical cancers are invasive squamous cell carcinomas and are caused by high-risk papillomaviruses such as HPV types 16, 18, 45, or 56 and types 6, 11, 42, 43 and 44 are designated low-risk viral types. HPV infection and its neoplastic implications dominate cervical epithelial pathology, which has long been thought to be crucial for the development of cervical cancer.[1]

The current WHO tumor classification varies from earlier editions in that it divides epithelial tumors and their precursors based on their association (or lack thereof) with HPV infection. [1] The distinction between HPV associated and HPV independent SCCs cannot be made accurately based on morphological parameters alone; p16 immunostaining or HPV testing is required.

Since many HPV infections do not progress to morphologically identifiable lesions, the presence of HPV DNA is necessary but not sufficient for the formation of squamous intraepithelial lesions (SIL). Low grade squamous intraepithelial lesion (LSIL) develops when HPV infection becomes productive in cells that have begun maturation, whereas High grade squamous intraepithelial lesion (HSIL) is caused by virally driven clonal growth of cells throughout the epithelium.[15] As there is no evidence of HPV independent precursor lesion, all squamous intraepithelial lesions such as low grade intraepithelial lesions and high grade intraepithelial lesions are classified into HPV related categories.[16] [17]

Hypermethylation of CpG islands in the promoter regions of tumor suppressor genes is common in HSIL but rare in LSIL. It appears to be important for malignant progression. HSILs are thought to arise from a specialized type of squamocolumnar junctional cell that is present between the transformation zone and the columnar epithelium and has a distinct immunohistochemical signature. [18]

Oncogenic proteins like E6, E7 which are produced by high risk HPV strains, inactivate tumor suppressors, activate cyclins, prevent apoptosis and resist cellular senescence, resulting in oncogenesis. The E6 protein interacts and degrades p53, as well as stimulates the expression of telomerase reverse transcriptase (TERT). E7 binds to the RB protein and

displaces the E2F transcription factors normally sequestered by RB, promoting cell cycle progression. E7 also inactivates the CDK inhibitors p21 and p27 resulting in oncogenesis.[4]

As the preinvasive lesion progresses into malignancy, the immune system also comes into play. The PD-1/PD-L1 axis is a well-known immune check point system that has a mechanism of immune evasion for cancer cells and hence inhibits the immune response in numerous types of solid tumors. [6]

MMR (mismatch repair) genes code for several particular proteins that make up the DNA mismatch repair (MMR) mechanism. When MMR proteins are deficient or biologically malfunctioning, the condition is described as deficient MMR (dMMR). Another MMR subgroup is proficient MMR (pMMR), which is a counter-phenomenon in which the patient's MMR expression is normal and biologically functional. By preserving genetic integrity and stability, as well as preventing replication mutations, this mechanism can maintain DNA replication fidelity.[7]

Effective treatment for patients with recurrent, persistent, or metastatic cervical cancer is palliative therapy or chemotherapy. Inhibitors of programmed cell death-1/programmed cell death-ligand 1 (PD-1/PD-L1) might be a novel option for improving these patients clinical results.

Although the percentage of HPV independent SCCs in the cervix is very low, there is currently no difference in treatment between HPV associated and HPV independent tumors.

However, the type of cervical SCCs (HPV associated or HPV independent) has to be documented on the pathology report.[1]

A morphological diagnosis without distinguishing between the two categories is an acceptable option in cases where the facilities required to make this distinction are unavailable. There is no evidence of HPV independent precursor lesion, squamous intraepithelial lesions being grouped into a single HPV related category.[1]

The world health organization categories of cervical neoplasm are as follows,[1]

WHO Classification of tumors of the uterine cervix

Squamous epithelial tumors

Squamous metaplasia

Atrophy

Condyloma acuminatum

Low-grade squamous intraepithelial lesion

Cervical intraepithelial neoplasia, grade 1

High-grade squamous intraepithelial lesion

Cervical intraepithelial neoplasia, grade 2

Cervical intraepithelial neoplasia, grade 3

Squamous cell carcinoma, HPV-associated

Squamous cell carcinoma, HPV-independent

Squamous cell carcinoma NOS

Glandular tumors and precursors

Endocervical polyp

Mullerian papilloma

Nabothian cyst

Tunnel clusters

Microglandular hyperplasia

Lobular endocervical glandular hyperplasia

Diffuse laminar endocervical hyperplasia

Mesonephric remnants and hyperplasia

Arias-Stella reaction

Endocervicosis

Tuboendometrioid metaplasia

Ectopic prostate tissue

Adenocarcinoma in situ NOS

Adenocarcinoma in situ, HPV-associated

Adenocarcinoma in situ, HPV-independent

Adenocarcinoma NOS

Adenocarcinoma, HPV-associated

Adenocarcinoma, HPV-independent, gastric type

Adenocarcinoma, HPV-independent, clear cell type

Adenocarcinoma, HPV-independent, mesonephric type

Adenocarcinoma, HPV-independent, NOS

Endometrioid adenocarcinoma NOS

Carcinosarcoma NOS

Adenosquamous carcinoma

Mucoepidermoid carcinoma

Adenoid basal carcinoma

Carcinoma, undifferentiated, NOS

Mixed epithelial and mesenchymal tumors

Adenomyoma NOS

Mesonephric-type adenomyoma

Endocervical-type adenomyoma

Adenosarcoma

Germ cell tumors

Germ cell tumor NOS

Mature teratoma NOS

Dermoid cyst NOS

Endodermal sinus tumor

Yolk sac tumor NOS

Choriocarcinoma NOS

TNM and FIGO classification of carcinomas of the uterine cervix (1)

TNM Categories	FIGO Stage	Definition
TX		Primary tumor cannot be assessed
TO		No evidence of primary tumor
Tis		Carcinoma in situ (preinvasive carcinoma)
T1	I	Tumor confined to the cervix
T1a	IA	Invasive carcinoma is diagnosed only by microscopy. Stromal invasion with a maximal depth of 5.0 mm measured from the base of the

		epithelium and a horizontal spread of 7.0 mm or less
T1a1	IA1	Measured stromal invasion 3.0 mm or less in depth and 7.0 mm or less in a horizontal spread
T1a2	IA2	Measured stromal invasion more than 3.0 mm and not more than 5.0 mm with a horizontal spread of 7.0 mm or less
T1b	IB	Clinically visible lesion confined to the cervix or microscopic lesion greater than T1a/IA2
T1b1	IB2	Clinically visible lesion 4.0 cm or less in greatest dimension
T1b2	IB1	Clinically visible lesion more than 4.0 cm in greatest dimension
T2	II	Tumor invades beyond uterus but not to the pelvic wall or lower third of the vagina
T2a	IIA	Tumor without parametrial invasion
T2a1	IIA1	Clinically visible lesion 4.0 cm or less in greatest dimension
T2a2	IIA2	Clinically visible lesion more than 4.0 cm in greatest dimension
T2b	IIB	Tumor with parametrial invasion
T3	III	Tumor involves the lower third of the vagina, extends to the pelvic wall, or causes hydronephrosis or non-functioning kidney
T3a	IIIA	A tumor involves the lower third of the vagina
T3b	IIIB	Tumor extends to the pelvic wall or causes hydronephrosis or non-functioning kidney
T4	IVA	Tumor invades mucosa of the bladder or rectum or extends beyond the true pelvis

Table 1: T – Definition of Primary Tumor

N	Regional Lymph Nodes
NX	Regional lymph nodes cannot be assessed
No	No regional lymph node metastasis
N1	Regional lymph node metastasis

Table 2: Definition of Regional Lymph Node (N)

M	Distant Metastasis
Mo	No distant metastasis
M1	Distant metastasis (includes inguinal lymph nodes and intraperitoneal disease). It excludes metastasis to the vagina, pelvic serosa and adnexa.

Table 3: Definition of Distant Metastasis (M)

pTNM Pathological Classification

The pT and pN categories correspond to the T and N categories.

pNo	Histological examination of a pelvic lymphadenectomy specimen will ordinarily include 10 or more lymph nodes. If the lymph nodes are negative, but the number ordinarily examined is not met, classify as pNo.
pM	Distant Metastasis
pM1	Distant metastasis microscopically confirmed

Table 4: pTNM Pathological Classification

pMo and pMX are not valid categories

The TNM and FIGO staging is based on the size and extent of the tumor, whereas WHO classification is based upon histomorphology along with the association of human papillomavirus infection.(1)

Pathogenesis of Human Papillomavirus (HPV)

Human papillomavirus (HPV) infection and its neoplastic implications dominate cervical epithelial pathology and HPV infection has generally been thought to be required for the development of cervical cancer. [(1)] It can lead to a variety of disease processes, including genital warts, dysplastic lesions and invasive malignancies of the anus, penis, vulva, vagina, cervix and oropharyngeal cancers. HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59 are carcinogenic in the uterine cervix, according to the International Agency for Research on Cancer.[(17)]

HPV is a 55-nm icosahedral, nonenveloped, 8000-base-pair, double-stranded DNA virus. An early (E) gene area, a late (L) gene region and a noncoding section with regulatory elements make up the HPV genome. Early in the development cycle, the E1, E2, E5, E6 and E7 proteins are expressed and are needed for viral replication and cellular transformation. Malignant transformation is caused by the E6 and E7 proteins, which target the human cell-cycle regulators p53 and Rb (retinoblastoma protein) degradation. [(6)]

HPV targets basal keratinocytes after microtrauma resulting in exposure of these cells to the virus and the virus completes the replication cycle in these cells. CD4+ T cell regulation is particularly important in controlling HPV infections.

The viral oncogenes E6 and E7 interfere significantly with apoptosis and cell cycle control in transforming HPV infection. It is known that the E6 protein produced by high-risk HPV types 16 and 18 can combine with the p53 protein and cause the same functional consequence as a p53 gene mutation. The E7 protein of HPV-16 is also shown to bind to the Rb protein encoded by the retinoblastoma gene (Rb1). The interaction between E7 and Rb1 is responsible for the significant elevation of p16 protein expression in high-risk human papillomavirus (HR-HPV) infected lesions. The absence of block-type p16 immunopositivity in lesions infected with low-risk human papillomavirus (LR-HPV) types is explained by the fact that LR-HPV E7 proteins do not trigger p16 overexpression.[(1)] The p53 and Rb proteins participate in the activity at the G1-S cell cycle checkpoint that normally causes cells with DNA damage to undergo either cellular arrest at G1 or apoptosis. [(5)]

The cellular tumor suppressor protein p16INK4a (p16) has been identified as a biomarker for transforming HPV infections.[(19)] Affected cells overexpress p16 to compensate for the irregular cell cycle activation; however, because E2F is produced via E7 rather than CDK4/6,

p16 expression does not affect cell cycle activation. [9] Literature shows that high T-cell response to E2 protein is linked to a lack of cervical disease development in women with HPV type 16 infection. [6]

HPV-independent cervical carcinomas are often more aggressive than HPV-associated carcinomas in other anatomical locations such as the oropharynx and the vulva, a feature that is becoming clinically important. Persistent HPV infection is the most significant risk factor for cervical cancer. Controlling the development of HPV infection is enhanced by a cell-mediated immune response.

Screening tests such as Papanicolaou (Pap test), HPV DNA test and biopsy for histopathologic and immunohistochemistry study can help detect cervical cancer and precancerous cells and the latter is the gold standard for diagnosis. Since the introduction of the Papanicolaou (Pap-test) cytological screening for cervical precancerous lesions in the 1940s, the incidence and death from cervical cancer have decreased significantly. [7]

The introduction of human papillomavirus (HPV) DNA testing into clinical practice raised hopes for improved primary screening, triage and post-treatment monitoring.

The discovery of HPV as an etiological factor in HPV related cancers opens up the possibility of controlling these cancers by vaccines and other targeted therapies.

Programmed death -1/ Programmed death ligand- 1 (PD-1/PD-L1)

The T cell-based immune system has been developed to recognize and eliminate abnormal cells, such as pathogen-infected cells and cancer cells. The binding of the T cell receptor (TCR) on T cells to peptide-major histocompatibility complexes (MHC) on target cells results in the detection of such aberrant cells. These checkpoint pathways play an important role in preventing tissue damage and maintaining self-tolerance by controlling the amount and functional activity of antigen-specific T lymphocytes.[22] Among all immune checkpoints, the PD-L1/PD-1 pathway has stood out because of its proven value as a therapeutic target in a large number of malignancies.

Overview of the PD-1/PD-L1 pathway

Programmed death ligand 1 (PD-L1)

Programmed death ligand 1 (PD-L1), otherwise known as B7-H1 or CD274, is the first functionally characterized ligand of the coinhibitory programmed death receptor 1 (PD-1). PD-L1 is encoded by the PDCDL1 gene and is found on chromosome 9 in humans at position p24.1.[23] The biological actions of PD-L1 are dependent on its ability to bind to PD-1.

PD-L1 is usually expressed by macrophages, some activated T cells and B cells, dendritic cells and some epithelial cells, particularly under inflammatory conditions and also expressed by tumor cells as an "adaptive immune mechanism" to escape anti-tumor responses.[24] By binding to its receptors and activating proliferative and survival signaling pathways, PD-L1 acts as a pro-tumorogenic factor in cancer cells.

By negative selection of autoreactive lymphocytes and establishment of immunological tolerance in secondary lymphoid organs, the PD-1/PD-L1 pathway plays a key role in central and peripheral tolerance. In tumor-infiltrating lymphocytes, persistent up-regulation of PD-1 is very common. PD-L1 expresses on the surface of cervical tumor cells, APCs and TILs, while the PD-1 positive cells were mostly identified as T cells in the stroma of cervical tumors. [12]

PD-1/PD-L1 axis can be modulated by various signals in cancer cells. These are mainly PI3K/AKT signaling pathway, MAPK signaling pathway, JAK-STAT signaling pathway, WNT signaling pathway and Hedgehog signaling pathway.[26]

PD-L1 has been researched in various cancers besides cervical cancer. For example, PD-L1 expression has been linked to local recruitment of PD-L1-positive CD8+ T cells in invasive lobular and ductal breast cancer. [3]

PD-L1 expression is common to 70% of epithelial ovarian cancer. It has an inverse relationship with CD8+ T cells, which suppresses the antitumor cytotoxic response and is a well-known prognostic factor in epithelial ovarian cancer.[27]

In colorectal cancer, tumor expression of PD-L1 is infrequent (5%) and strongly associated with PD-1-positive lymphocytic infiltrate and the mismatch-repair deficiency (MMR-d).[28] PD-L1 expression has been associated with poor clinical outcomes. PD-L1 expression is

regulated by several inflammatory cytokines and PD-1/PD-L1 binding can trigger active T-cell death and interleukin-10 (IL-10) expression as a negative feedback mechanism.[29]

According to recent clinical observations, deficient DNA mismatch repair (dMMR) is capable of improving antitumor effects of the PD-1/PD-L1 pathway, suggesting that PD-L1 expression is higher in, deficient DNA mismatch repair (dMMR) patients than in proficient MMR (pMMR) patients, suggesting that PD-1/PD-L1 antibody therapies may be effective in dMMR cervical cancer patients and may act as a prognostic indicator of PD-1/PD-L1 antibody drugs. [3] Anti-PD-1 checkpoint inhibitor immunotherapy has enhanced tumor response and survival. Pembrolizumab was demonstrated in phase Ib KEYNOTE-012 and single-arm phase II KEYNOTE-055 studies to have an 18% response rate and a median overall survival of 6 to 8 months in treated, recurrent and metastatic patients.[30]

Programmed cell death protein 1 (PD-1)

PD-1, also known as CD279, was identified in 1992 in IL-3-deprived LyD9 (murine hematopoietic progenitor) and 2B4-11 (murine T-cell hybridoma) cell lines.[31] PD-1 is a 55-kDa transmembrane protein of 288 amino acids that includes an extracellular N-terminal domain (IgV-Like), a membrane-permeating region and a cytoplasmic tail with two tyrosine bases at the N and C end.

PD-1 is an inhibitor of both adaptive and innate immune responses and is found on activated T, NK and B lymphocytes, macrophages, dendritic cells (DCs) and monocytes. It is overexpressed in tumor-specific T cells.[32]

Transcription factors such as a nuclear factor of activated T cells, NOTCH, Fork head box protein (FOX) O1and interferon (IFN) regulatory factor 9 (IRF9) may be involved in PD-1 transcription. [4]

PD-1 is produced in exhausted T cells (CD8) during persistent infections and the FOXO1 transcription factor attaches to the PD-1 promoter to boost its expression. Leakage from cancer cells increases the expression of the c-FOS component, which increases the expression of PD-1. So PD-1 plays two opposing roles, as it can be both beneficial as well as harmful.[26]

The Mismatch Repair Status

Microsatellites are short, tandemly repeated (usually 10–60 times) sequences of mononucleotide, dinucleotide, or higher-order nucleotide repeats. These sites are prone to DNA replication errors. It is estimated that the replicative DNA polymerases make errors approximately once for every 10^4 and 10^5 nucleotides that they polymerize. [(6)] Thus, each time a cell divides, polymerase errors occur, which must be corrected through the combined actions of proofreading activity of polymerase enzyme. However, some errors always escape proofreading, which is effectively corrected through the mismatch repair (MMR) system. [(7)]

MLH1, MutS protein homolog 2 (MSH2), MutS homolog 6 (MSH6) and PMS1 homolog 2 (PMS2) are the main proteins involved in this MMR system. These interact as heterodimers: MSH2 couples with either MSH6 or MSH3 and MLH1 couples with PMS2 or MLH3. MSH2 and MSH6 proteins create a heterodimeric complex (mutS) that aids in the recognition of mismatched nucleotides and thereby initiates DNA repair. When it binds to the mismatched nucleotides, it undergoes an ATP-dependent conformational shift, which recruits mutL, a heterodimeric MLH1 and PMS2 complex.

The repair complexes make sure that the newly synthesized strand of DNA is the one that has to be repaired. Microsatellite instability is a trait that occurs when the MMR system develops a functional error or defect (MSI). Patients with a defect in any of these components or, in a gene upstream of MSH2 that encodes the epithelial cell adhesion molecule (EPCAM), will develop a "mutator phenotype" with numerous frameshift mutations. This results in the microsatellite instability-high (MSI-H) phenotype, closely related to carcinogenicity of hereditary and sporadic tumors.

Various methods are available for identifying MMR proteins. Immunohistochemistry analysis of MMR proteins (MLH1, MSH2, MSH6and PMS2) is commonly used as an alternative to MSI to detect MMR deficiency in clinical practice. The absence of PMS2 expression alone indicates a defect in the PMS2 gene. However, the loss of both PMS2 and MLH1 suggests that the defect is in MLH1, as MLH1 is responsible for PMS2 stability. MSH6 and MSH2 are in a comparable situation, with loss of MSH6 exclusively indicating defective MSH6, whereas lack of expression of both proteins indicates the issue is inside MSH2.[(35)]

Feng et al conducted a study in 2018 had collected sixty-six patient samples of squamous cell carcinoma and data of their clinical characteristics were gathered. Based on these samples, the expression levels of MLH1, MSH2 and PD-L1 in cancer cells were tested by

immunohistochemical assay (IHC). According to the expression of MLH1, MSH2 and the MSI test, all 66 cases were divided into deficient DNA mismatch repair (dMMR) or proficient DNA mismatch repair (pMMR) groups. In this study, results show 25.8% were associated with deficient MMR. PD-L1 in cancer cells, PD-L1 in tumor-infiltrating cell (TILs) and PD-1 in TILs took up 59.1%, 47.0% and 60.6%, respectively. When compared to pMMR patients, dMMR patients have higher PD-L1 expression, suggesting that PD-1/PD-L1 antibody therapies could be beneficial in dMMR cervical cancer patients. Furthermore, dMMR may be a molecular detection target for clinical use of PD-1/PD-L1 antibody therapies in patients with reproductive age groups.[7]

Z Chinn et al. conducted a study in 2018, with a sample size of sixty-five cases of cervical and vulvar intraepithelial neoplasia and SCCs was diagnosed during 2014 to 2017 were assessed for Indoleamine 2,3- dioxygenase (IDO) and PD-L1 expression. Using the combined positive score (CPS) threshold of 1 to account for both tumoral and immune staining. This study suggests that combination immunotherapy may have a function in a subset of cervical and vulvar squamous cell carcinoma than intraepithelial lesions.[36]

Chung et al conducted a study in 2019, on 98 patients who were positive for PD-L1 and advanced cervical cancer were treated with single lined drug (pembrolizumab) from January 27, 2016, to August 18, 2016. They concluded that pembrolizumab has durable antitumor activity and manageable safety in patients with advanced cervical cancer. Based on these findings, the US Food and Drug Administration gave pembrolizumab accelerated approval for patients with advanced PD-L1 positive cervical cancer who progressed during or after chemotherapy. [18]

E K Enwere et al conducted a study that included 120 women with locally advanced cervical cancer (International Federation of Gynecology and Obstetrics stages IB to IVA) between 1999 and 2008. They found that the combination of robust PD-L1 expression, extensive T-cell infiltration, altered immune function from human papillomavirus effect and/or a high degree of somatic mutations which indicate that cervical cancers may be excellent candidates for PD-1/PD-L1 blocking immunotherapies.[38]

W. Yang et al. studied 20 SCCs patients with or without metastasis. They performed immunohistochemistry to detect PD-L1 expression in tumor cells and PD-1 expression in tumor-related macrophages and tumor-infiltrating lymphocytes, along with P16INK4a

expression and interferon- levels in cervical tissues. According to their findings, an increase in PD-L1 and PD-1 expression was associated with HPV positivity and overexpression of the PD-1/PD-L1 pathway was linked to lower levels of the proinflammatory cytokine interferon- and higher levels of P16INK4a.[40]

Reddy et al. conducted a study in 2017, based on immunohistochemical staining for PD-L1 expression performed on a tissue microarray of 101 normal and neoplastic cervical tissues. The expression of PD-L1 was graded using a scoring system that took into account the percentage and degree of the positive score and concluded as significant expression of PD-L1 in 34.4% of cervical carcinomas and no expression of PD-L1 in benign cervical tissues. They concluded that anti-PD-L1/PD-1 immunotherapies may have a role in the treatment of PD-L1-positive cervical cancers. [42]

This study aims to evaluate DNA mismatch repair status (MMR) and programmed cell death-ligand 1 (PD-L1) expression in Human papillomavirus (HPV) associated and independent squamous cell carcinomas of the cervix.

AIM & OBJECTIVES

AIM AND OBJECTIVES

Aim:

To study mismatch repair deficiency status and the expression of programmed cell death ligand 1 (PD-L1) receptor status in histopathologically diagnosed cases of squamous cell carcinoma of the cervix.

Objectives:

Primary objectives:

1. To study the expression of programmed cell death ligand 1 in squamous cell carcinoma of the cervix

2. To study the mismatch repair deficiency in squamous cell carcinoma of the cervix

Secondary objective:

To study the relation between PD-L1 expression and mismatch repair deficiency if any.

MATERIAL & METHODS

MATERIALS AND METHODS

Type of study

The study was an ambispective type of observational study.

Data collection

The study included small biopsy specimens of cervical carcinoma in the Department of Pathology and Lab Medicine at AIIMS, Jodhpur from the year January 2018 to July 2021. The study was started after receiving approval from the institutional review board.

All the slides diagnosed for squamous cell carcinomas cervix were reviewed, antibodies for programmed cell death ligand 1 (PD-L1) and mismatch repair deficiency by MSH2, MLH1, MSH6 and PMS2 were applied on the tumor containing representative section.

Duration of study

January 2018 to July 2021

Ethical clearance

The study was approved by the institutional ethics committee on 01/01/2020.

- Certificate No.: AIIMS/IEC/2019-20/949

Inclusion criteria:

50 biopsies of squamous cell carcinoma of the cervix received in the Department of Pathology and Lab Medicine at AIIMS Jodhpur from January 2018 to 2021 were included in this study.

Exclusion criteria:

- Inadequate samples
- Any previous chemotherapy or radiotherapy given

Sample size calculation

We enrolled all patients with squamous cell carcinoma of the cervix coming to AIIMS Jodhpur in the study from January 2018 to 2021. Data was entered in an excel sheet. Considering the level of significance of 5%, the sample size determination is as follows.

Formula used for sample size determination:

$n = Z^2 \times P(1-P) / d^2$

Where n = sample size

Z (statistic for a level of confidence of 95%) = 1.96

P = Prevalence

d (assumed) = Precision = 10%

Considering the references of Feng et al, the prevalence of squamous cell carcinoma of the cervix is 59%. Substituting these values in the above equation and based on the study, the sample size was 50 cases of squamous cells carcinomas of cervix.[66]

Sample processing

After approval from the Institutional ethics committee, the study was started. Informed consent was obtained from the patients.

Grossing of cervical small biopsy specimen:

10% formalin-fixed cervical biopsy specimens were measured and processed.

Paraffin blocks were prepared using routine histopathological techniques. Thin sections (4-5 μm) were stained with routine Hematoxylin and Eosin (H&E). Light microscopy results and histopathological gradings were recorded. The appropriate representative blocks were subjected to immunohistochemistry (IHC).

1) Steps of block preparation and section cutting

After the representative sections were taken, tissue was processed as follows:

1. Dehydration was carried out by passing the sections through a series of ascending grades of ethyl alcohol, from 50%, 70%, 95% to absolute alcohol.

2. The clearing was done by passing the tissue through two changes of xylene.

3. Impregnation was done in molten paraffin wax which had a melting point of 54 – 62˚C.

4. Embedding: Embedding station (Leica EG 1150 H) was used through which a small amount of liquid paraffin was layered into aluminum molds. Properly oriented tissues were placed inside the molds, which were then filled with liquid paraffin 60 – 62˚C and allowed to cool and harden. The lower portion of the cassette with an identification number was used as the final block.

5. Microtomy: Microtome (Leica-RM2255) was used and thin ribbons (4-5 μm) were cut and floated in warm water (~56˚C) for expansion of the curled sections. These sections were then collected on frosted glass slides and kept for drying.

2) Staining of sections: (for H and E stain)

1. Deparaffinization – The glass slides containing the tissue sections were kept over the hot plate at 60 ˚C for 10 minutes, followed by two changes in xylene (Xylene I & Xylene II), 10 minutes each.

2. Hydration – Through graded alcohol (100%, 95%, 70%, 50%) to water, 10 minutes respectively.

3. Hematoxylin – The sections were kept in Harris's Hematoxylin for 5 minutes.

4. Washing – The sections were washed well in water for 2 minutes.

5. Differentiation – Done in 1% acid alcohol (1% HCl in 70% alcohol) for 10 seconds.

6. Washing – Done under running tap water (usually for 15 – 20 minutes) until the sections 'blue'.

7. Eosin – Stained in 1% Eosin Y for 10 seconds.

8. Washing – Done in running tap water for 2 minutes.

9. Dehydration – Through graded alcohol (50%, 70%, 95%, 100%), 10 minutes each.

10. Clearing –Through xylene (Xylene II & Xylene I), 2 minutes each.

11. Mounting – The sections were mounted in DPX with a coverslip.

3) Immunohistochemistry

Antibodies used:

Primary antibody:

Ready to use.

For identifying programmed death-ligand status PD-L1 antibody was used.

- PD-L1 (Programmed death-ligand 1: Prediluted, Clone: CAL10, Company: Biocare Medical)

For identifying mismatch repair status PMS-2, MLH-1, MSH-2 and MSH-6 were used

- PMS-2 (postmeiotic segregation 2, Preparation: Prediluted, Clone: A16-4, Company: Biocare Medical)
- MSH-2 (mutS homologue 2, Mouse monoclonal antibody, Preparation: Ready to use, Clone: DBM15.82, Company: Diagnostic BioSystems)
- MSH-6 (mutS homologue 6, Mouse monoclonal antibody, Preparation: Ready to use, Clone: 44, Company: Diagnostic BioSystems)
-MLH-1 (mutL homologue 1, Mouse monoclonal antibody, Preparation: Ready to use, Clone: G168-15, Company: Diagnostic BioSystems)

Secondary Antibody: Bond Polymer Refine Detection, Leica

- Peroxide block, 3-4%(v/v)
- Post Primary, Rabbit anti-mouse IgG in 10% (v/v) animal serum in tris-buffered saline
- Polymer, Anti-rabbit Poly-HRP-IgG containing 10% (v/v) animal serum in tris-buffered saline
- DAB Part 1, in stabilizer solution
- DAB Part B ≤0.1% (V/V) Hydrogen peroxide in stabilizer solution
- DAB Part B ≤0.1% (V/V) Hydrogen peroxide in stabilizer solution
- Hematoxylin, 0.1%

Steps of IHC staining:

A. Preparation of Buffer–Two types of buffers were used.

1. Wash Buffer
2. Antigen Retrieval Buffer (ARB)

Wash buffer preparation: 6 gm powdered TRIS buffer salt was dissolved into 1 liter of distilled water and pH was set at 7.4.

ARB preparation: 6.05 gm TRIS salt and 0.744 gm EDTA salt were dissolved in 1 liter of distilled water, pH was set at 9.0.

Note:

- To increase the pH, NaOH solution was added drop by drop and pH was titrated.
- To decrease the pH, HCl was added drop by drop and pH was titrated.

B. Preparation of Poly-L-Lysine Solution (PLL Solution):

1 ml of PLL was diluted with 9 ml of distilled water (1 in 10 dilutions).

C. Slide Coating Procedure:

Step 1: Diluted PLL solution was taken in a clean container/Coplin jar

Step 2: Both sides of the glass slides were cleaned with tissue paper

Step 3: The clean slides were immersed in a PLL solution for 5 minutes

Step 4: After 5 minutes, the coated slides were removed and kept overnight for air drying. The coated slides were kept at room temperature. Tissue sections of 4 µ thickness were obtained on the PLL coated slides.

Baking: The slides were kept at 60˚C for 1 hour and then cooled to room temperature.

IHC staining procedure

Step 1: Deparaffinization – The slides were kept in Xylene I (10 minutes), followed by Xylene II (10 minutes).

Step 2: Rehydration – The slides were kept in 100%, 70%and 50% alcohol for 5 minutes each followed by running tap water for 5 minutes.

Step 3: Antigen retrieval – by pressure cooker method [38]. 200 ml of clean tap water was taken in the empty pressure cooker and heated up to the steam formation. The slides were

placed in a rack. 300 ml of ARB was put in the container and the rack with slides was placed inside the container. Then the container containing the rack with slides, was placed inside the pressure cooker and the lid was closed. After two whistles the pressure was released by lifting the air vent and allowed to cool till it reached room temperature.

Step 4: Wash – Slides were washed in Wash Buffer (pH7.4) thrice at a 1-minute interval.

Step 5: Peroxide blocking – Blocking reagent was added to the sections and incubated for 10 minutes in the Humidity chamber at room temperature. This step prevents unwanted, non-specific background staining.

Step 6: The peroxide was decanted and not washed with buffer.

Step 7: Primary antibody – PD-L1, MSH-2, MSH-6, MLH-1and PMS-2 was added to the sections and incubated in the Humidity chamber for one hour.

Step 8: Wash – After that slides were washed in Wash Buffer (pH 7.4) thrice at a 1-minute interval.

Step 9: Amplifier – Amplifier was added over the sections and incubated for 30 minutes in the Humidity chamber at room temperature.

Step 10: Wash – The slides were washed in Wash Buffer (pH 7.4) thrice at a 1-minute interval.

Step 11: HRP label – The HRP was added and incubated for 30 minutes in the Humidity chamber at room temperature.

Step 12: Wash – The slides were washed in Wash Buffer (pH 7.4) thrice at a 1-minute interval.

Step 13: DAB – The DAB chromogen was applied to the sections and incubated in the Humidity chamber for 10 minutes, avoiding light exposure as much as possible.

Step 14: Wash – The sections were washed in distilled water twice at a 1-minute interval.

Step 15: Counterstain – Slides were counterstained using Harris Hematoxylin for 2-3 minutes.

Step 16: Wash – The slides were washed in running tap water for 5 minutes.

Step 17: Dehydration – was done in graded alcohol (50%, 70%, 95%, 100%), 1 minute each.

Step 18: Mounting – Slides are air-dried, mounted with DPX and examined under the microscope.

Interpretation of immunohistochemical stains:

Scoring

Programmed Death- Ligand 1 (PD-L1)

Expression of PD-L1 in the tumor was quantified manually and classified as positive when staining (PD-L1: membranous) was present in ≥1% of tumor cells. Staining extent was further characterized in the following subcategories:
1–5%, 6–10%, 11–25%, 26–50%and >50%. The 1% threshold for positivity was selected based on data demonstrating clinical response to PD-L1 inhibition. Immune microenvironment staining was scored positive, when ≥1% of peritumoral and intratumoral immune cells showed reactivity. It was subdivided as 1–10%, 11–25%, 26–50% and > 50%.[36]

Percentage (PD-L1 expression by CPS)	Interpretation
<1%	Negative
1-5%	Positive
6-10%	Positive
11-25%	Positive
26-50%	Positive
>50%	Positive

Table 5: Interpretation of PD-L1 Immunohistochemistry (IHC)

Percentage (Tumor-infiltrating lymphocytes (TILs)	Interpretation
<1%	Negative
1-10%	Positive
11-25%	Positive
26-50%	Positive
>50%	Positive

Table 6: Interpretation of Tumor-infiltrating lymphocytes (TILs)

Combined positive score (CPS)

The combined positive score was determined manually and was based on the equation described previously for gastric and gastroesophageal junction cancers.

CPS = [(number of PD-L1-positive tumor cells and mononuclear inflammatory cells)/(total number of tumor cells)].

In the CPS system, immune cell scoring is based on PD-L1-positive lymphocytes and macrophages (‘mononuclear inflammatory cells’) identified in association with a tumoral immune response.

This includes both intratumoral immune cells and peritumoral immune stromal cells, but not immune cells in stroma distant from the tumor. [36]

Control of IHC

Mismatch Repair (MMR) panel

Positive control

• Nuclei of stromal cells

• Nuclei of lymphocytes

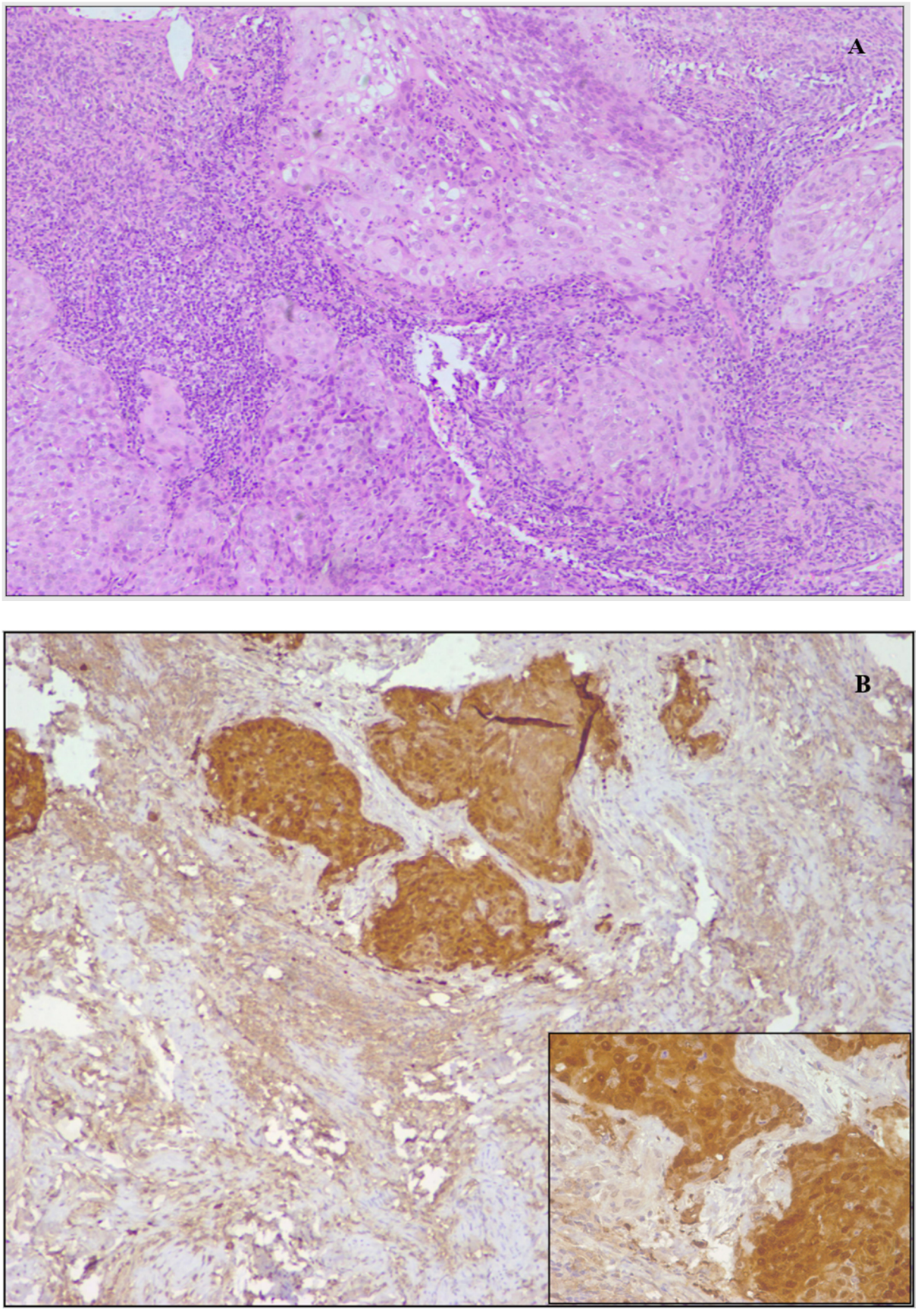
Interpretation of IHC

Positive: Any nuclear staining within the tumor cells

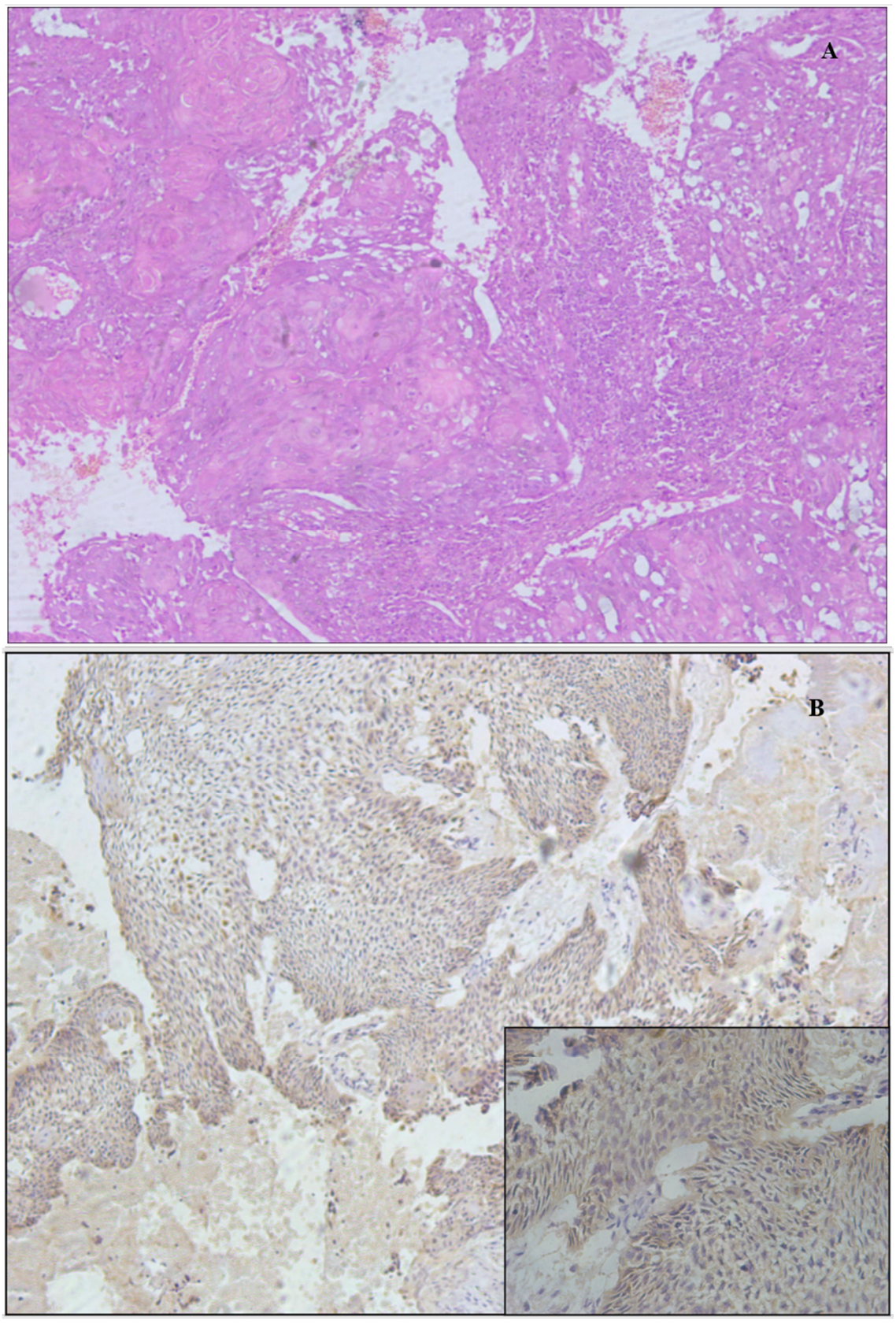
Negative: Complete absence of nuclear staining within the tumor cells with concurrent internal control positive

When all four antibodies showed positive nuclear staining of the tumor cells, the cases were classified as MMR proficient (pMMR) and when one or more antibodies showed no nuclear staining of the tumor cells it was classified as MMR deficient (dMMR). (43)

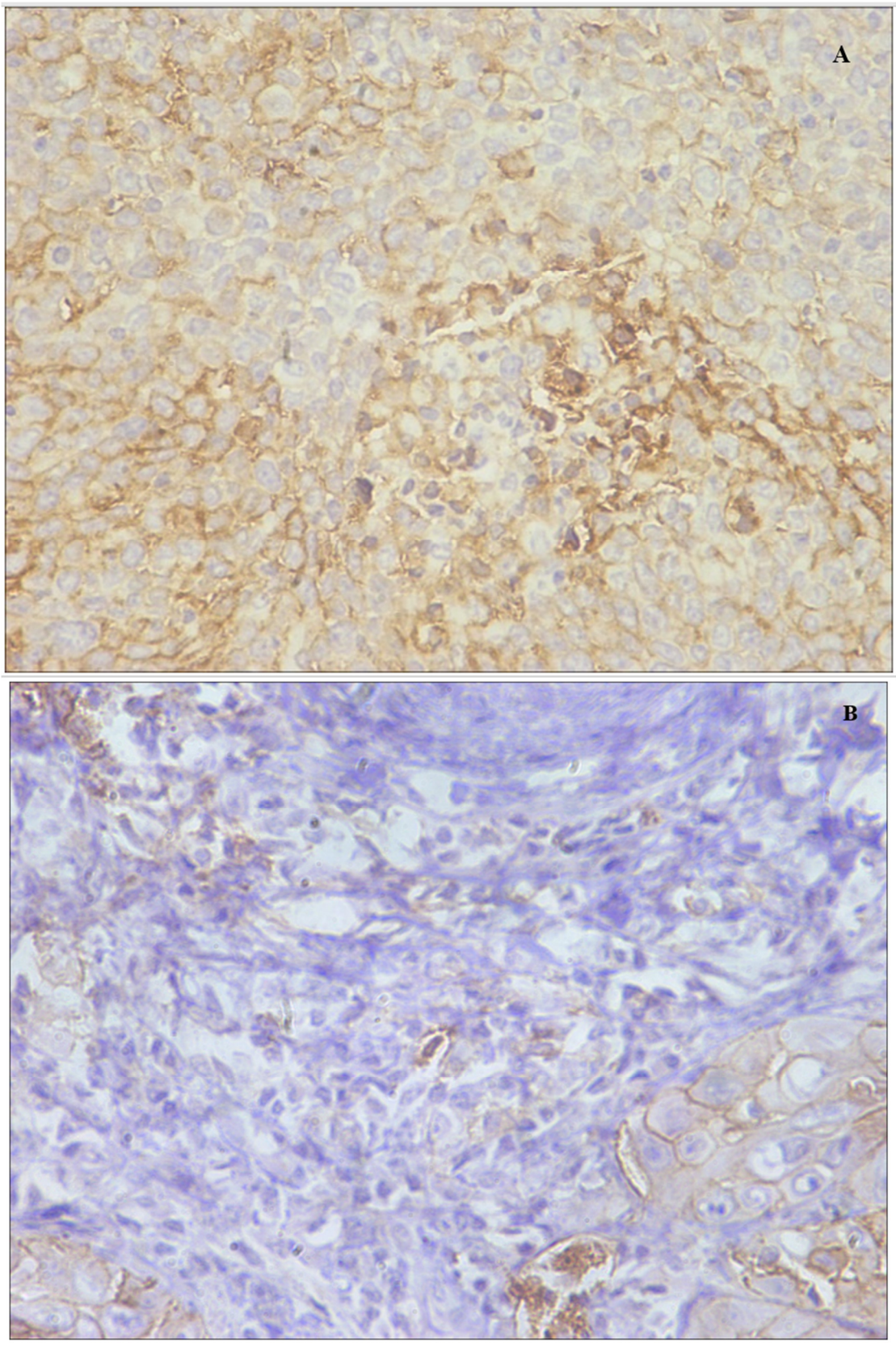
Photomicrograph 1: HPV-associated SCC of the cervix, A: H&E image, Block type positivity in the tumor cells. B:10X, Inset: B 40X



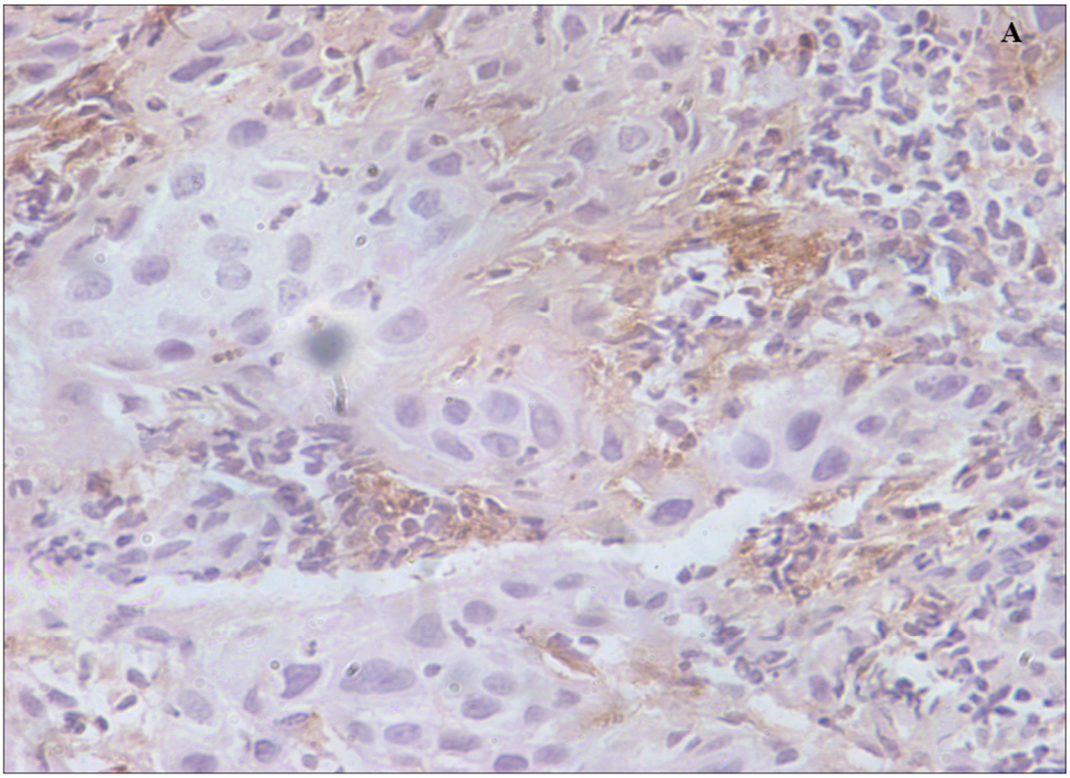
Photomicrograph 2: HPV independent SCC of the cervix, A: H&E image, with the absence of block type positivity in the tumor cells. B:10X, Inset: B, 40X

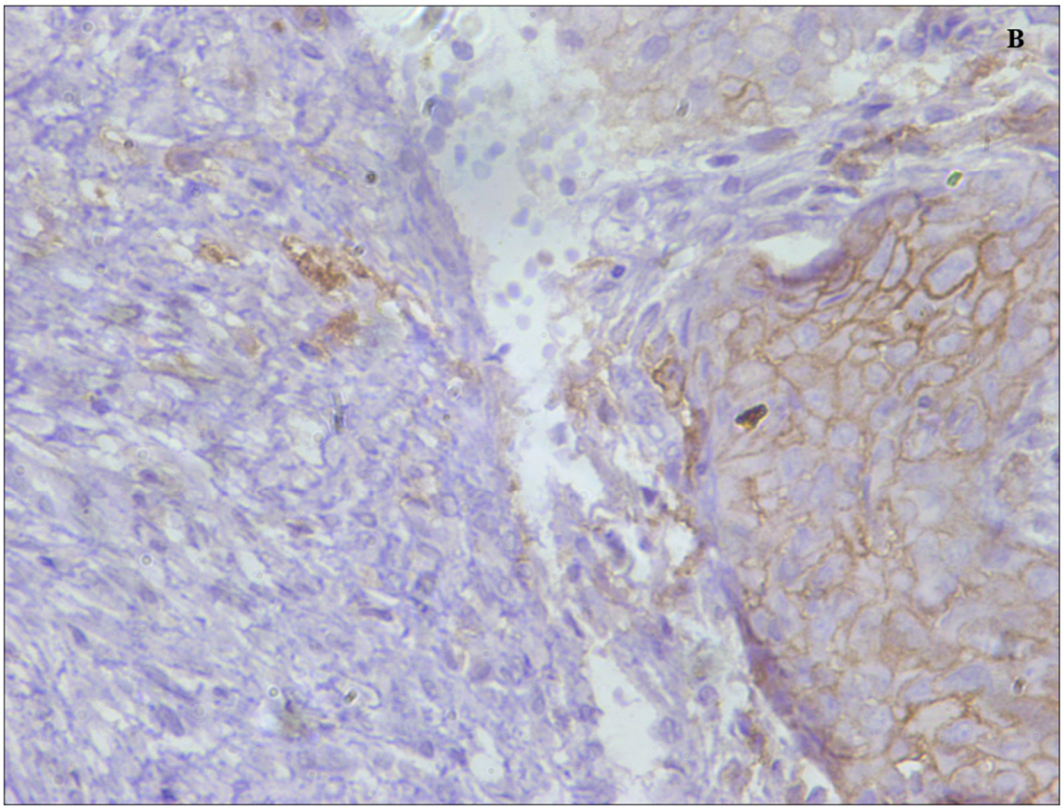


Photomicrograph 3: A case of Well differentiated Keratinising Squamous Cell Carcinoma of the cervix with the presence of PD-L1 expression in tumor cells (A) and PD-L1 in TILs (B).

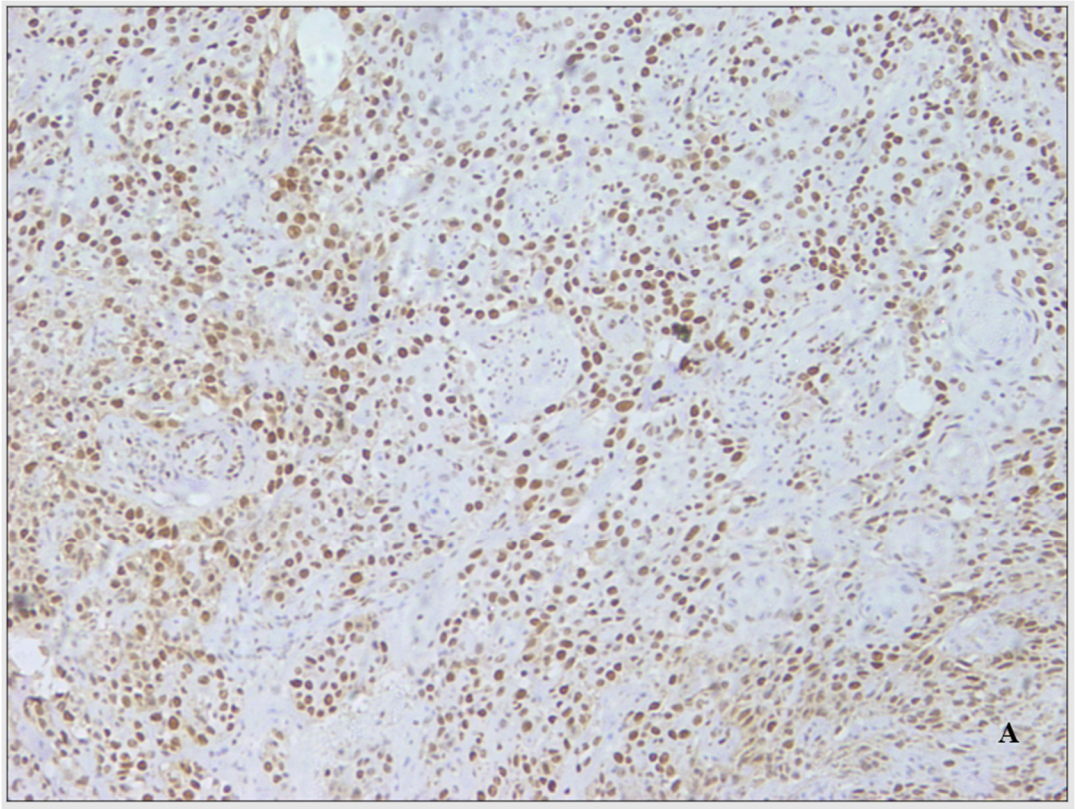


Photomicrograph 4: A case of Moderately differentiated Keratinising Squamous Cell Carcinoma of the cervix with the presence of PD-L1 expression in tumor cells (A) and PD-L1 in TILs (B).

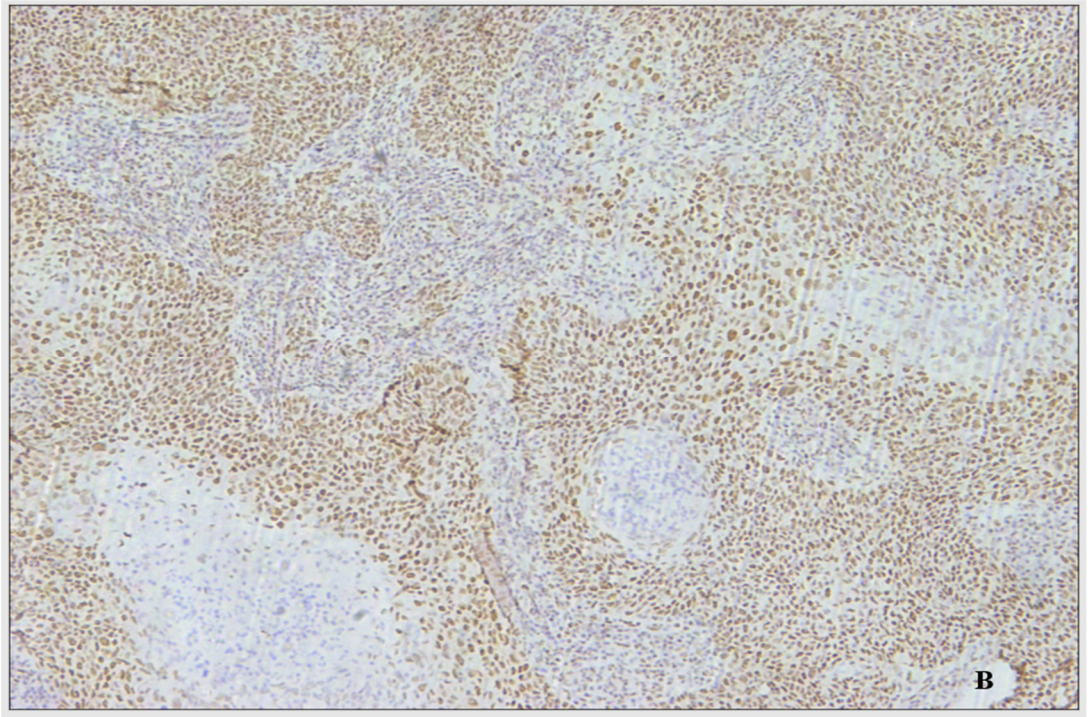




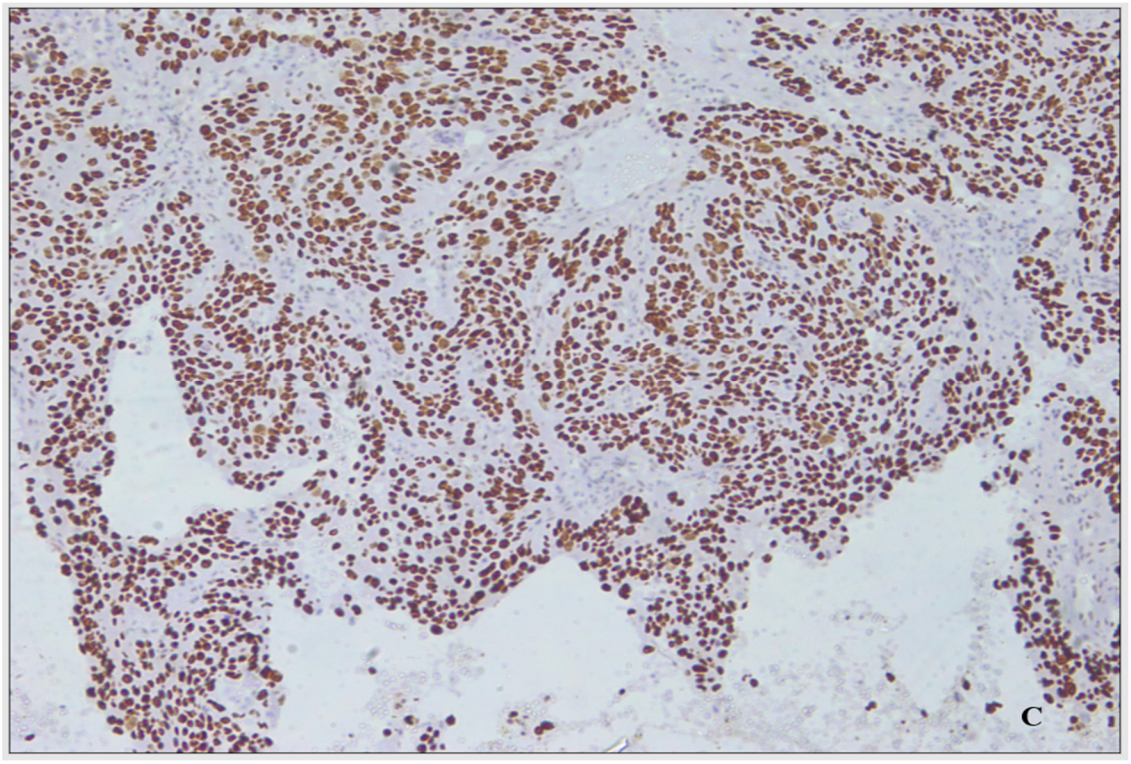
Photomicrograph 5: Squamous Cell Carcinoma of the cervix. The tumor is mismatch repair proficient and shows retained MSH-2 protein (A).



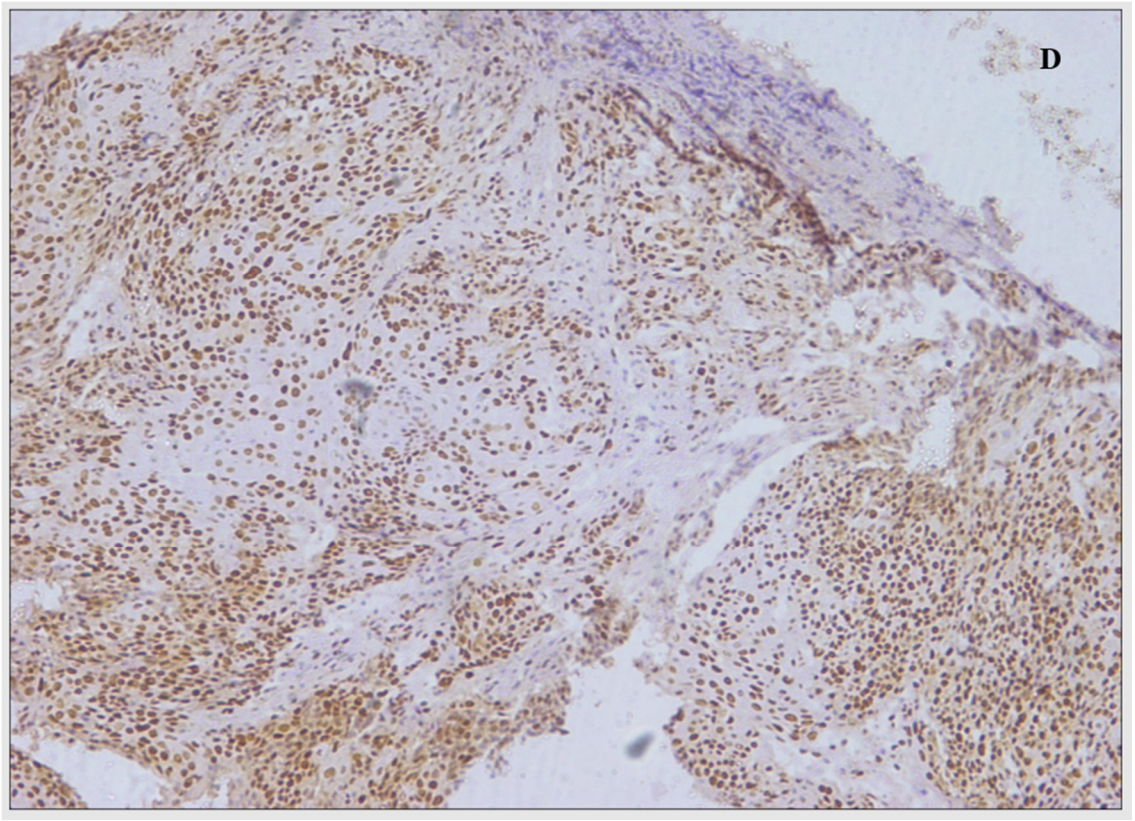
Photomicrograph 6: Squamous Cell Carcinoma of the cervix. The tumor is mismatch repair proficient and shows retained MSH-6 protein (B).

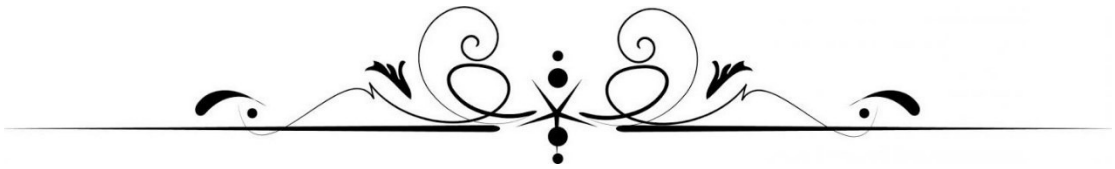


Photomicrograph 7: Squamous Cell Carcinoma of the cervix. The tumor is mismatch repair proficient and shows retained MLH-1 protein (C).

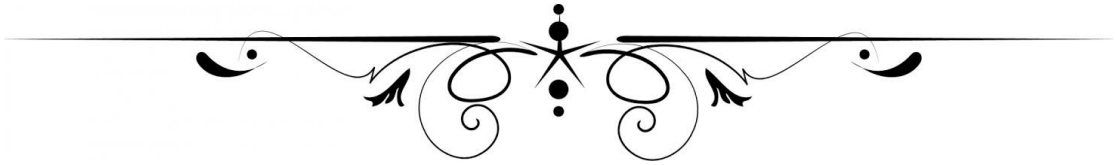


Photomicrograph 8: Squamous Cell Carcinoma of the cervix. The tumor is mismatch repair proficient and shows retained PMS-2 protein (D).





OBSERVATIONS & RESULTS



OBSERVATIONS AND RESULTS

This study was an ambispective, observational hospital-based study conducted on 50 cases of squamous cell carcinomas (SCCs) of the cervix (from January 2018 to July 2021) at the All India Institute of Medical Sciences (AIIMS), Jodhpur in the Department of Pathology and Lab Medicine.

Age distribution

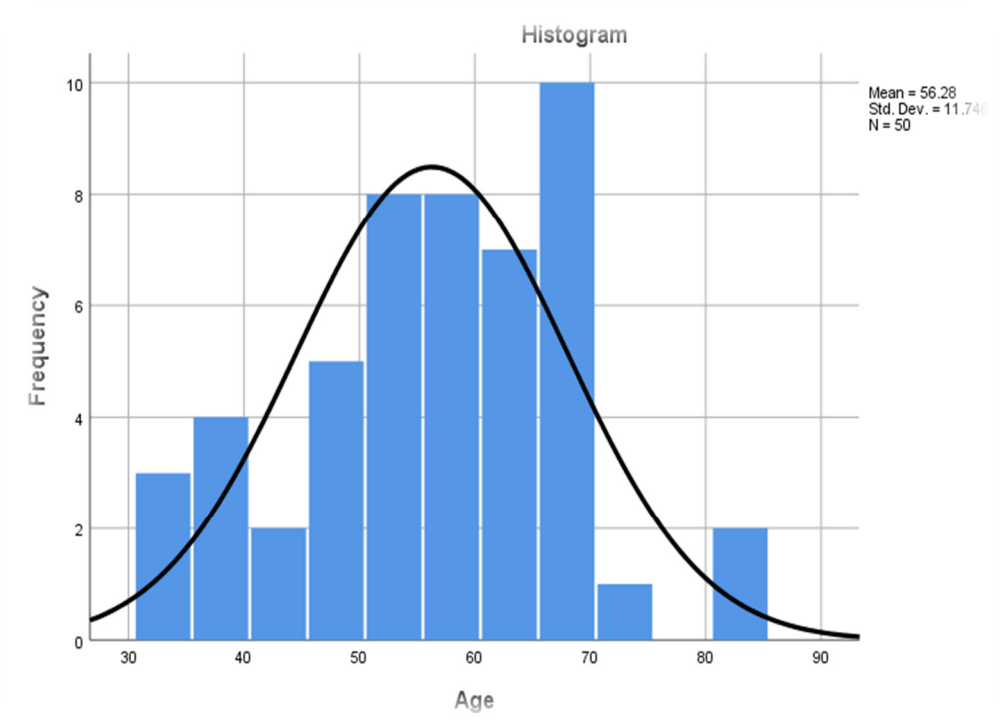


Figure 1: Age distribution of squamous cell carcinomas of the cervix with mean and standard deviation (SD)

Age (years)	Frequency n=50	Percentage
30-40	7	14.0
41-50	7	14.0
51-60	16	32.0
61-70	17	34.0
71-80	1	2.0
81-90	2	4.0

Table 7: Age distribution in SCCs of the cervix

The age of the patients in the study ranged from 33 years to 81 years, with a mean age of 56.28 years with a standard deviation of 11.74 and a median age of 58.00 years. Maximum number of patients was between 60-70 years of age group (17 out of 50, 34%).

Tumor grade

Tumor grade	Frequency (percentage) n=50
Well-differentiated squamous cell carcinoma	2 (4%)
Moderately differentiated squamous cell carcinoma	40 (80%)
Poorly differentiated squamous cell carcinoma	8 (16%)

Table 8: Tumor grade in SCCs of the cervix

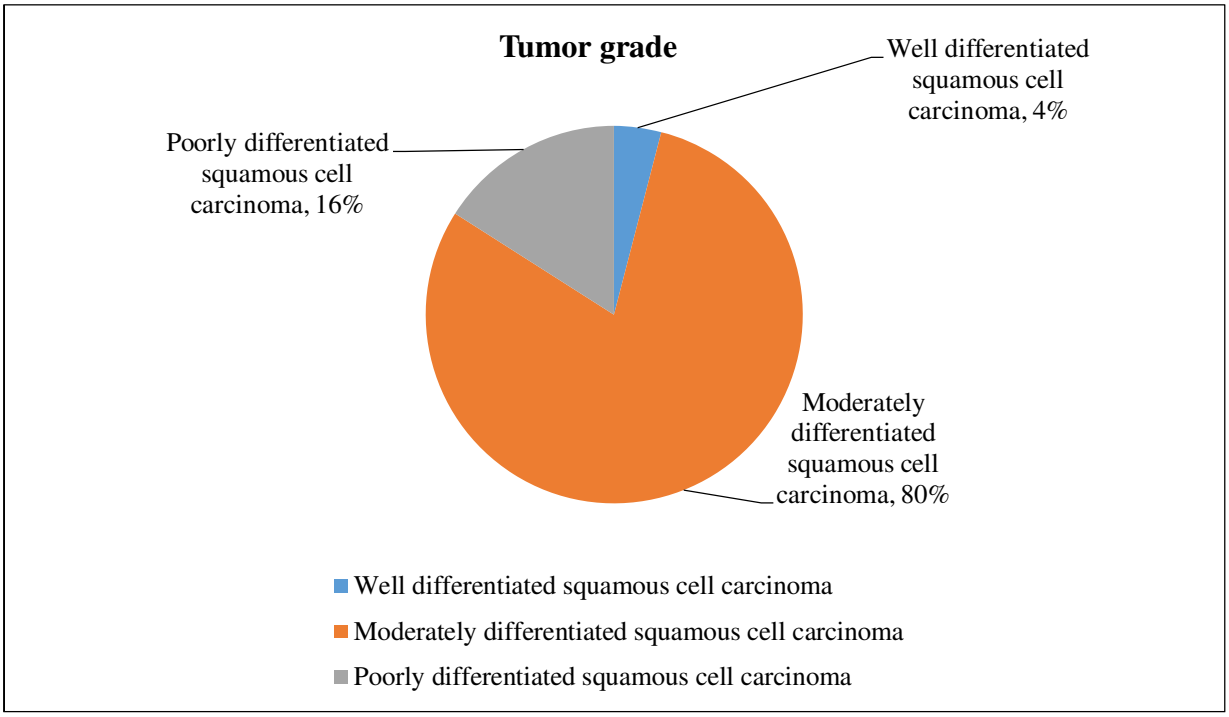


Figure 2: Tumor grade in SCCs cervix

Type of cancer (Keratinising/Nonkeratinising)	Frequency (Percentage) n=50
Keratinising squamous cell carcinoma	45 (90%)
Non-keratinising squamous cell carcinoma	5 (10%)

Table 9: Type of cancer in SCCs of the cervix

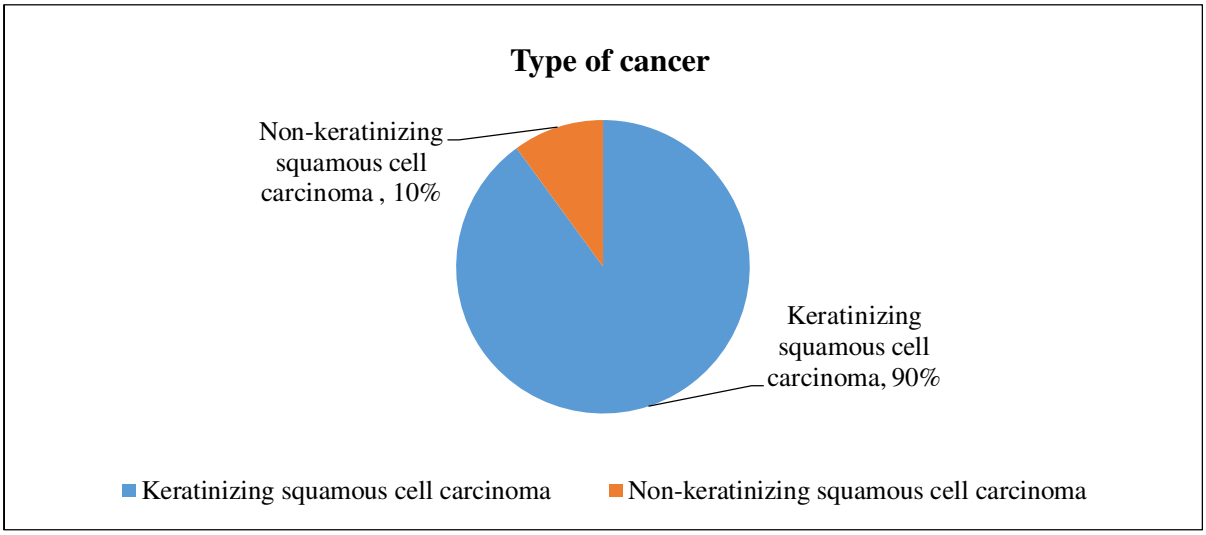


Figure 3: Type of cancer in SCCs cervix

In this study, 40 out of 50 cases (80%) were diagnosed with moderately differentiated squamous cell carcinomas, 8 out of 50 cases (16%) were poorly differentiated squamous cell carcinomas and only 2 out of 50 cases (4%) were well-differentiated squamous cell carcinomas.

Among the 50 patients, 45 (90%) had keratinising squamous cell carcinomas, whereas 5 (10%) had non-Keratinising squamous cell carcinomas.

Even though the current WHO classification does not advocate the grading of SCCs, it was performed in the study.[1]

Tumor necrosis

Tumor necrosis	Frequency (Percentage) n=50
Present	15 (30%)
Absent	35 (70%)

Table 10: Tumor necrosis in SCCs of the cervix

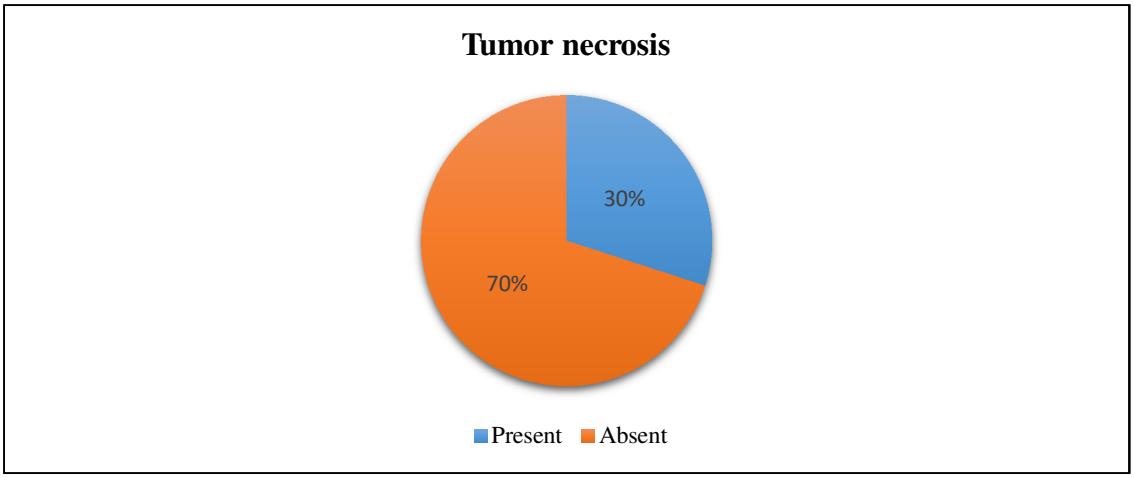


Figure 4: Tumor necrosis in SCCs of the cervix

Fifteen out of 50 cases (30%) showed tumor necrosis whereas 35 out of 50 cases (70%), did not show tumor necrosis.

Dysplasia

Dysplasia	Frequency (Percentage) n=50
Present	23 (46%)
Absent	27 (54%)

Table 11: Dysplasia in SCCs of the cervix

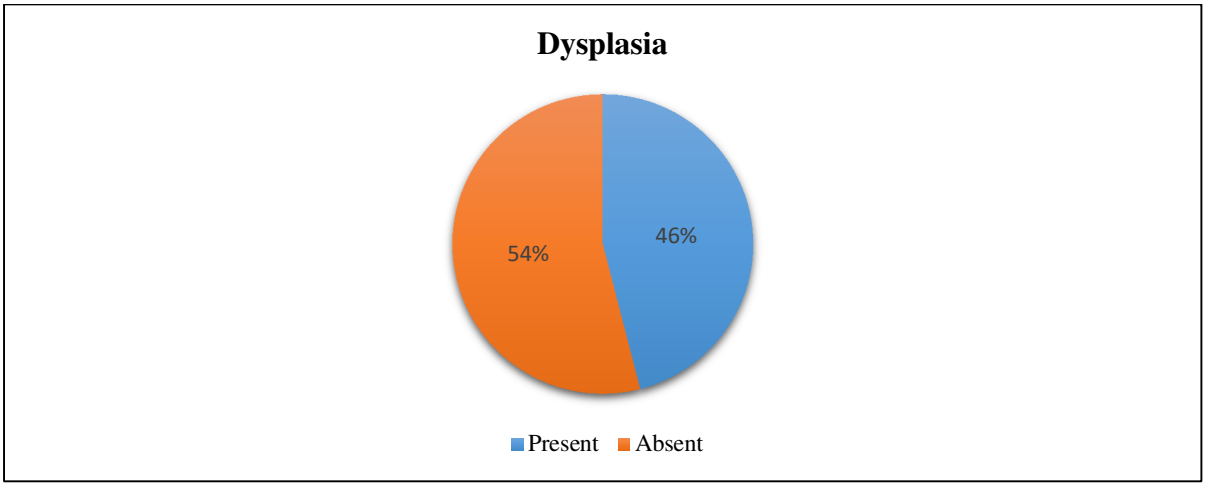


Figure 5: Dysplasia in SCCs of the cervix

Twenty three out of 50 patients (46%) in the study, showed high-grade dysplasia/high grade squamous intraepithelial lesion (HSIL) in the adjacent stratified squamous epithelial lining. Twenty seven out of 50 cases (54%) did not show dysplasia. However, this could be sampling bias as the study was on biopsy samples and not on the resection specimens.

Lymphovascular invasion and perineural invasion

None of the cases showed lymphovascular invasion or perineural invasion. However, this could be a sampling bias as the study was done on biopsy samples, not on the resection specimens.

Tumor infiltrating lymphocytes (TILs)

Tumor infiltrating lymphocytes	Frequency (percentage) n=50
Present	50 (100%)
Absent	0 (0%)

Table 12a: Tumor infiltrating lymphocytes (TILs) in SCCs of the cervix

Tumor infiltrating lymphocytes grading	Frequency (percentage) n=50
<1%	0
1-10%	11 (22%)
11-25%	16 (32%)
25-50%	13 (26%)
>50%	10 (20%)

Table 12b: Tumor infiltrating lymphocytes (TILs) in SCCs of the cervix

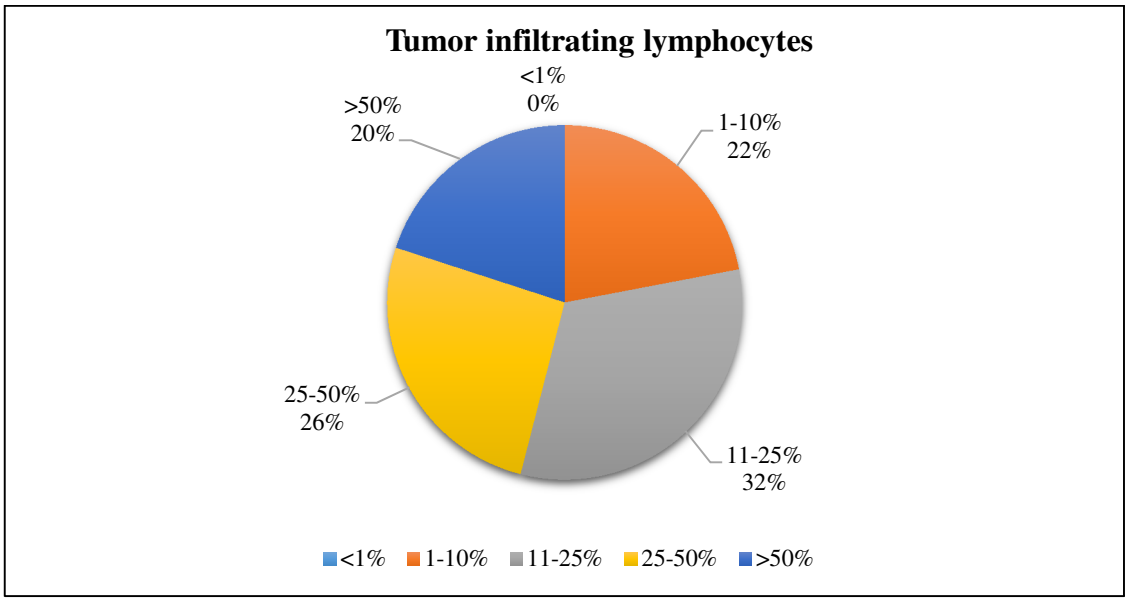


Figure 6: Tumor infiltrating lymphocytes (TILs) in SCCs of the cervix

All the cases in the study showed tumor infiltrating lymphocytes (TILs). Maximum cases had between 11-25% TILs (16/50 cases, 32%). 10 cases (20%) had >50% TILs.

Human papillomavirus (HPV) associated and HPV independent squamous cell carcinomas

Squamous cell carcinomas	Frequency (Percentage) n=50
HPV associated	47 (94%)
HPV independent	3 (6%)

Table 13: Human papillomavirus (HPV) associated and HPV independent squamous cell carcinomas (SCCs) of the cervix

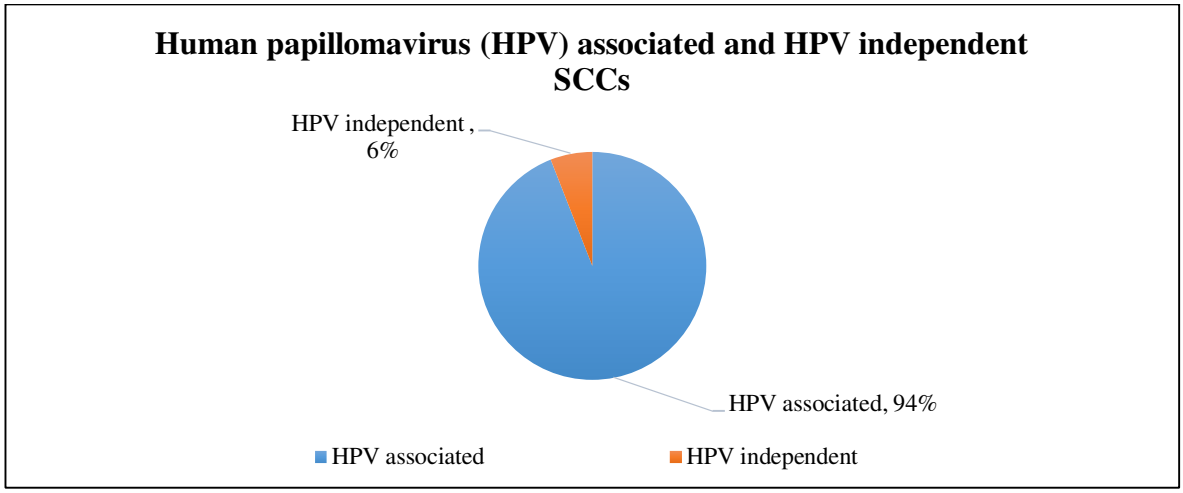


Figure 7: Human papillomavirus (HPV) associated and HPV independent squamous cell carcinomas of the cervix

Most of the cases of cervical squamous cell carcinomas (47/50, 94%) in the current study, were HPV-associated. Only 3 cases (6%) were HPV independent.

PD-L1 expression in tumor cells

PD-L1 expression in tumor cells	Frequency (percentage) n=50
Present	10 (20%)
Absent	40 (80%)

Table 14a: PD-L1 expression in tumor cells in SCCs of the cervix

PD-L1 expression in tumor cells	Frequency (percentage) n=50
<1%	10 (20%)
1-5%	12 (24%)
6-10%	3 (6%)
11-25%	6 (12%)
26-50%	9 (18%)
>50%	10 (20%)

Table 14b: PD-L1 expression in tumor cells in SCCs of the cervix

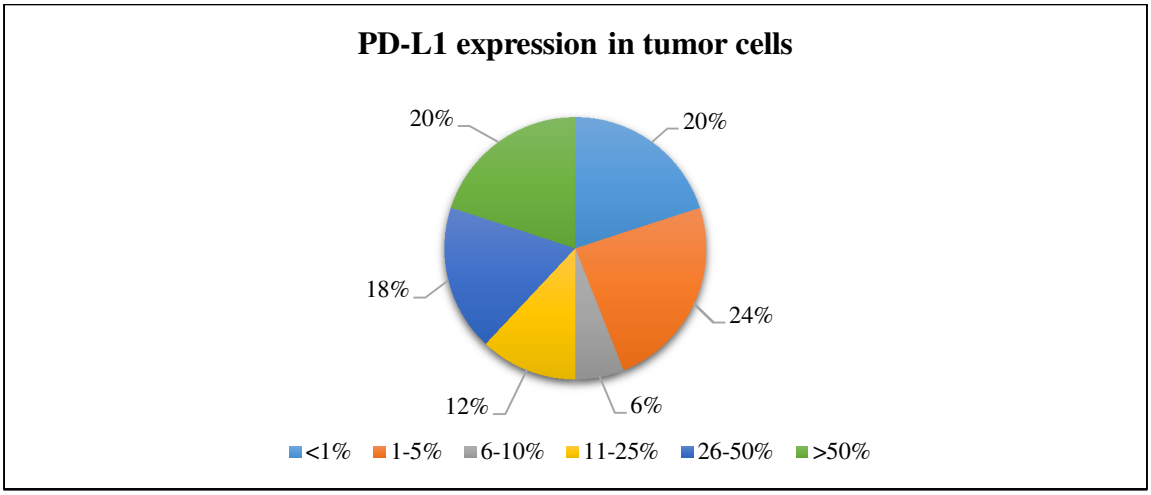


Figure 8: PD-L1 expression in tumor cells

Most of the cases expressed PD-L1 in tumor cells (40 out of 50 cases, 80%). Maximum number of cases,12 out of 50 cases (24 %), had PD-L1 expression in 1-5% of the tumor cells. 10 cases (20%) showed PD-L1 expression in >50% of the tumor cells.

PD-L1 expression in tumor infiltrating lymphocytes (TILs)

PD-L1 expression in tumor infiltrating lymphocytes (TILs)	Frequency (percentage) n=50
<1%	8 (16%)
1-10%	18 (36%)
11-25%	10 (20%)
25-50%	10 (20%)
>50%	4 (8%)

Table 15: PD-L1 expression in tumor infiltrating lymphocytes (TILs) in SCCs of the cervix

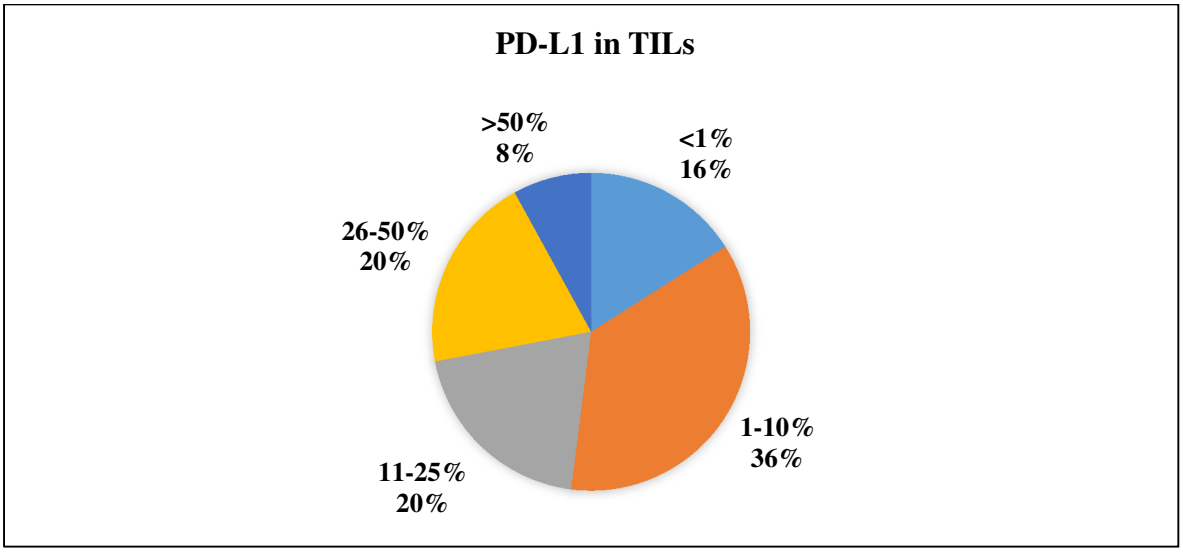


Figure 9: PD-L1 expression in tumor infiltrating lymphocytes (TILs)

Majority of the cases, 42 out of 50 cases (84%) showed PD-L1 expression in tumor infiltrating lymphocytes (TILs). The maximum number of cases had PD-L1 expression in 1-10% of TILs (18/50 cases, 36%). Four cases out of 50 (8 %), showed PD-L1 expression in >50% of the TILs.

Combined Positive Score (CPS)

Combined Positive Score (CPS)	Frequency (percentage) n=50
<1 (negative)	10 (20%)
>1 (positive)	40 (80%)

Table 16a: Combined Positive Score (CPS) in SCCs of the cervix

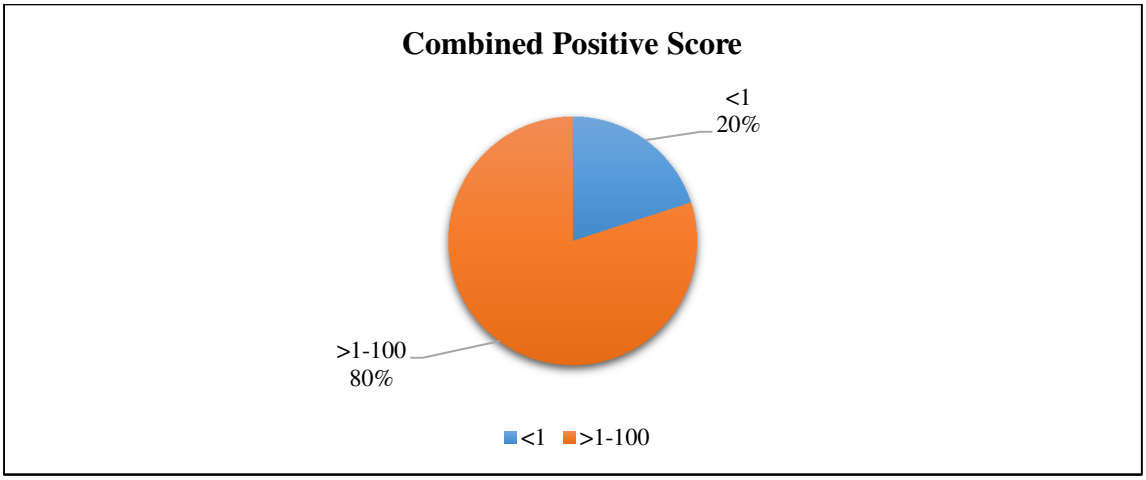


Figure 10: Combined Positive Score (CPS)

In this study, 40 out of 50 cases (80%), had a combined positive score > 1 (PD-L1 positive cases) whereas 10 out of 50 cases (20%) had a score of <1 (PD-L1 negative cases).

Combined Positive Score

Combined Positive Score	Frequency (percentage) n=50
<1	10 (20%)
1-5	9 (18%)
6-10	5 (10%)
11-25	9 (18%)
26-50	11 (22%)
>50	6 (12%)

Table 16b: Combined Positive Score (CPS) in SCCs of the cervix

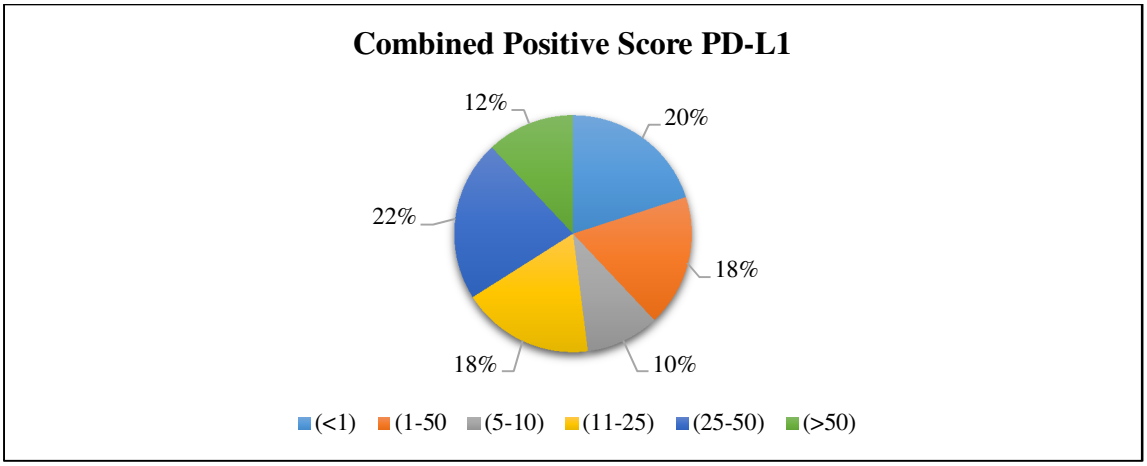


Figure 11: Combined Positive Score PD-L1

Overall, 10 of the 50 cases (20%) showed the absence of PD-L1 in the tumor, as calculated by CPS. Six of the 50 cases (12%) had a CPS of >50 and a maximum number of the PD-L1 positive cases had a CPS of 26-50 (11 out of 50, 22%).

Mismatch repair status (MMR)

MMR status	**Frequency n=50**
Deficient	3 (6%)
Proficient	47 (94%)

Table 17: Mismatch repair status (MMR) in SCCs of the cervix

MMR Proteins	**Frequency n=50**	**Percentage**
Combined loss of PMS2 and MLH1	0	-
Combined loss of MSH2 and MSH6	0	-
Isolated loss of MLH-1	2	(33.34%)
Isolated loss of PMS-2	1	(66.66%)
Isolated loss of MSH-2	0	-
Isolated loss of MSH-6	0	-

Table 18: MMR loss pattern on IHC in SCCs of the cervix

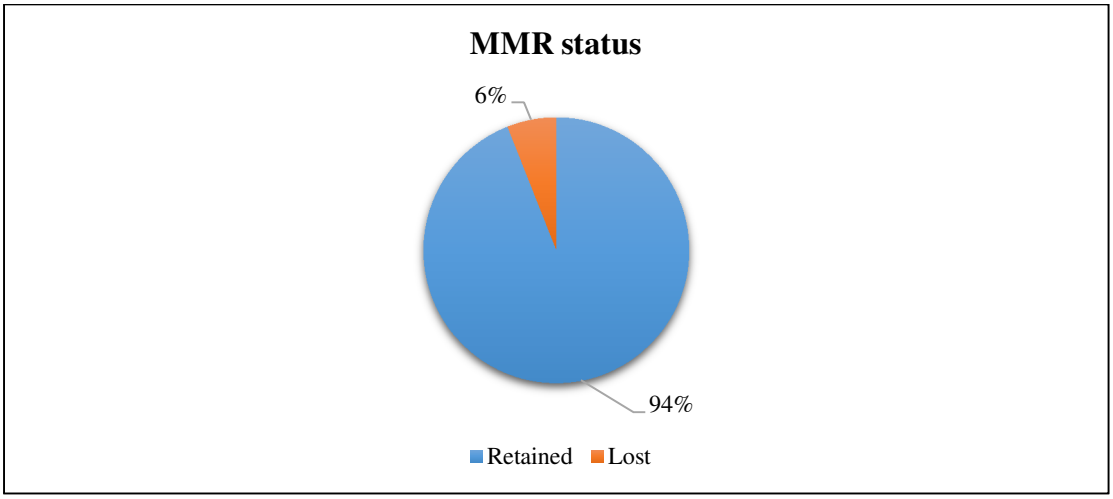


Figure 12: Mismatch repair status (MMR)

Out of 50 cases, 47 cases (94%) showed retained MMR protein (MMR stable) while only 3 cases (6%) showed MMR deficiency. One case (33.34%) among the three MMR deficient cases showed isolated PMS2 loss while isolated MLH1 loss was seen in 2 cases (66.66%). None of the cases show a combined loss of PMS2 and MLH1 or MSH2 and MSH6.

Relation between Human papillomavirus (HPV) associated and HPV independent squamous cell carcinoma of the cervix with clinicopathological parameters

Relation between Human papillomavirus (HPV) associated and HPV independent squamous cell carcinomas of the cervix with the age.

Age (years)	HPV independent cases n=3	HPV Associated cases n=47	Total	p-value
30-40	1 (33%)	6 (6.47%)	7	0.914 (chi-square test)
41-50	0	7 (14.89%)	7	
51-60	1 (33%)	15 (31.91%)	16	
61-70	1 (33%)	16 (34.04%)	17	
71-80	0	1(2.12%)	1	
81-90	0	2 (4.25%)	2	
Total	3	47	50	

Table 19: Relation between Human papillomavirus (HPV) associated and HPV independent squamous cell carcinomas of the cervix with age of the patient

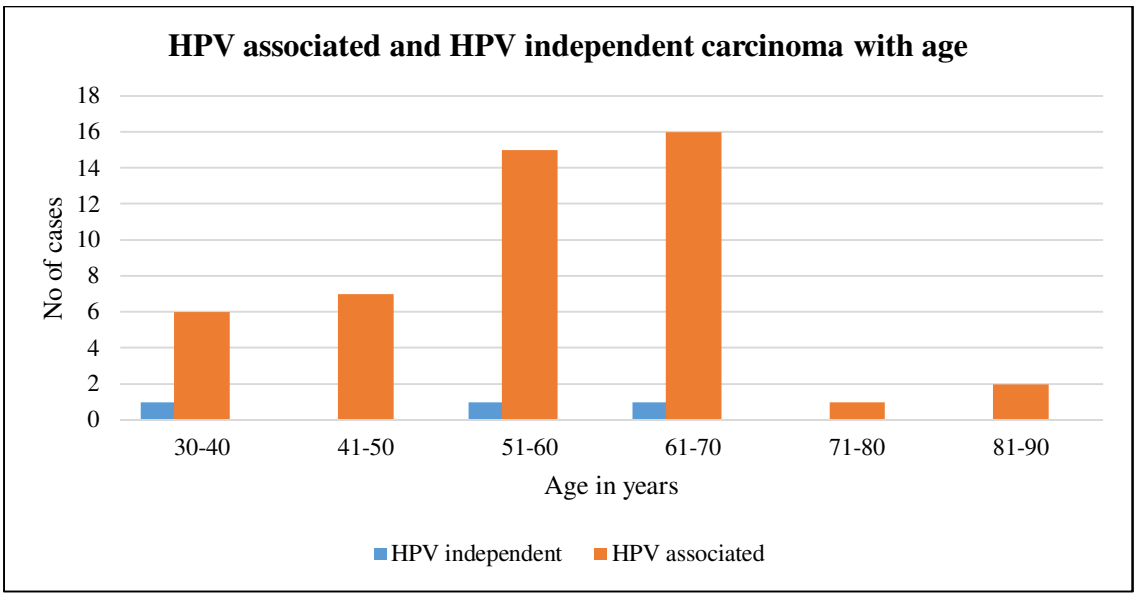


Figure 13: Relation between Human papillomavirus (HPV) associated and HPV independent squamous cell carcinomas of the cervix with age of the patient

All the HPV independent cases were seen in < 70 years of age group. Three patients of 50 in the study (6%) who were > 70 years of age and all had associated HPV SCCs.

However, no statistically significant association was seen between HPV association of cervical squamous cell carcinomas and the age of the patients.

Relation between Human papillomavirus (HPV) associated and HPV independent squamous cell carcinomas of the cervix with the type of cancer (Keratinising/ Nonkeratinising)

Type of cancer (Keratinising/Nonkeratinising)	HPV independent cases n=3	HPV associated cases, n=47	Total	p-value
Keratinising squamous cell carcinoma	3 (100%)	42 (89.36%)	45	1.000 (chi-square test)
Nonkeratinising squamous cell carcinoma	0	5 (10.63%)	5	
Total	3	47	50	

Table 20: Relation between Human papillomavirus (HPV) associated and HPV independent squamous cell carcinomas of the cervix with the type of cancer (Keratinising/Nonkeratinising)

All the nonkeratinising SCCs of the cervix were HPV associated. There was no statistically significant association between tumor grade and HPV association of the tumor.

Tumor grade	HPV independent cases, n=3	HPV associated cases, n=47	Total	p-value
Well-differentiated squamous cell carcinoma	0	2 (4.25%)	2	0.671 (chi-square test)
Moderately differentiated squamous cell carcinoma	3 (6%)	37 (78.72%)	40	
Poorly differentiated squamous cell carcinoma	0	8 (17.02%)	8	
Total	3	47	50	

Table 21: Relation between Human papillomavirus (HPV) associated and independent carcinomas with tumor grade

All the poorly differentiated SCCs were HPV associated. No statistically significant association was seen between tumor grade and HPV associated and HPV independent tumors.

Relation between Human papillomavirus (HPV) associated and HPV independent carcinomas with tumor necrosis

Tumor necrosis	HPV Independent cases, n=3	HPV Associated cases, n=47	Total	p-value
Absent	3 (100%)	32 (68.08%)	35	0.545 Chi-square test (Fisher exact test)
Present	0	15 (31.91%)	15	
Total	3	47		

Table 22: Relation between Human papillomavirus (HPV) associated and independent carcinomas with tumor necrosis

None of the HPV independent SCCs of the cervix showed tumor necrosis in the biopsies sampled. However, no statistically significant association between tumor necrosis and HPV associated and HPV independent tumors.

Relation between Human papillomavirus (HPV) associated and HPV independent squamous cell carcinomas with tumor infiltrating lymphocytes (TILs)

TILS	HPV independent cases, n=3	HPV associated cases, n=47	Total	p-value
<1%	0	0	0	0.170 Chi-square test
1-10%	2 (66.66%)	9 (19.14%)	11	
11-25%	0	16 (34.04%)	16	
26-50%	0	13 (27.65%)	13	
>50%	1 (33.33%)	9 (19.14%)	10	
Total	3	47	50	

Table 23: Relation between Human papillomavirus (HPV) associated and HPV independent squamous cell carcinomas with tumor infiltrating lymphocytes (TILs)

There was no statistically significant association between HPV associated and HPV independent carcinomas with tumor-infiltrating lymphocytes (TILs).

Relation between Human papillomavirus (HPV) associated and HPV independent cases with PD-L1 expression in tumor

PD-L1 expression	HPV independent cases, n=3	HPV associated cases, n=47	Total	p-value
<1	0	10 (21.27%)	10	0.372 (chi-square test)
>1	3 (100%)	37 (78.72%)	40	
Total	3	47	50	

Table 24: Relation between Human papillomavirus (HPV) associated and HPV independent cases with PD-L1 expression in tumor

Relation between Human papillomavirus (HPV) associated and HPV independent cases with PD-L1 expression

PD-L1 expression	HPV independent cases, n=3	HPV associated, n=47	Total	p-value
<1	0	10 (21.27%)	10	0.389 (chi-square test)
1-5	0	9 (19.14%)	9	
6-10	0	5 (10.63%)	5	
11-25	1 (33.33%)	8 (17.02%)	9	
26-50	2 (66.66%)	9 (19.14%)	11	
>50	0	6 (12.76%)	6	
Total	3	47	50	

Table 25: Relation between Human papillomavirus (HPV) associated and HPV independent cases with PD-L1 expression

All the HPV independent SCCs of the cervix showed PD-L1 expression. But, none of the HPV independent cases had a PD-L1 CPS of >50.

No statistically significant association was seen between HPV association of tumor and PD-L1 expression in the tumor.

Relation between Human papillomavirus (HPV) associated and HPV independent carcinomas with MMR status of the tumor.

MMR status	HPV Independent cases	HPV associated cases	Total	p-value
Deficient	0	3 (6.38%)	3	0.652 (chi-square test)
Proficient	3(100%)	44 (93.61%)	47	
Total	3	47	50	

Table 26: Relation between Human papillomavirus (HPV) associated and HPV independent carcinomas with MMR status of the tumor

None of the HPV independent SCCs showed mismatch repair deficiency. However, no statistically significant association was seen between HPV association of tumor and MMR status of the tumor.

Relation between tumor infiltrating lymphocytes (TILs) with clinicopathological parameters

Relation between tumor infiltrating lymphocytes (TILs) with age

Age (years)	<1 %	1-10%	11-25%	26-50%	>50%	Total	p-value
30-40	0	3 (42.85%)	2 (28.57%)	1 (14.28%)	1 (14.28%)	7	0.568 (chi-square test)
41-50	0	1 (14.28%)	1 (14.28%)	3 (42.85%)	2 (28.57%)	7	
51-60	0	1 (6.25%)	7 (43.75%)	4 (25%)	4 (28.57%)	16	
61-70	0	5 (29.41%)	5 (29.41%)	5 (29.41%)	2 (11.76%)	17	
71-80	0	0	0	0	1 (100%)	1	
81-90	0	1 (50 %)	1 (50%)	0	0	2	
Total	0	11	16	13	10	50	

Table 27: Relation between tumor infiltrating lymphocytes (TILs) with age

Maximum number of cases in the age group 41-60 years showed >50 TILs (28.5%). Lesser TILs were seen in patients <40 years of age. However, there was no statistically significant correlation between tumor infiltrating lymphocytes (TILs) and the age of the patient.

Relation between tumor infiltrating lymphocytes (TILs) with HPV association of SCCs.

PD-L1 IN TILs	HPV Independent SCCs	HPV associated SCCs	Total	p-value
<1%	0	8 (17.02%)	8	0.855 Chi-square test
1-10%	1 (33.33%)	17 (36.17%)	18	
11-25%	1 (33.33%)	9 (19.14%)	10	
26-50%	1 (33.33%)	9 (19.14%)	10	
>50%	0	4 (8.51%)	4	
Total	3	47	50	

Table 28: Relation between tumor infiltrating lymphocytes (TILs) with HPV association of SCCs

No statistically significant association was seen between HPV association of cervical SCC TILs.

Relation between tumor infiltrating lymphocytes (TILs) with type of cancer (Keratinising/Nonkeratinising)

Type of cancer (Keratinising/ Nonkeratinising)	<1 %	1 -10%	11 -25%	26 -50%	>50%	Total	p-value
Keratinising squamous cell carcinomas	0	10 (22.22%)	15 (33.33%)	11 (24.44%)	9 (20%)	45	0.679 (chi-square test)
Nonkeratinising squamous cell carcinomas	0	1 (20%)	1 (20%)	2 (40%)	1 (20%)	5	
Total	0	11	16	13	10	50	

Table 29: Relation between tumor infiltrating lymphocytes (TILs) with type of cancer (Keratinising/Nonkeratinising)

This study showed a higher number of TILs in non keratinising squamous cell carcinomas compared to keratinising squamous cell carcinoma. However, the finding was not statistically significant.

Tumor grade	<1%	1 -10%	11 -25%	26 -50%	>50%	Total	p-value
Well-differentiated squamous cell carcinomas	0	1 (50%)	0	1 (50%)	0	2	0.842 (chi-square test)
Moderately differentiated squamous cell carcinomas	0	9 (22.5%)	13 (32.5%)	10 (25%)	8 (20%)	40	
Poorly differentiated squamous cell carcinomas	0	1(12.50 %)	3 (37.5%)	2 (25%)	2 (25%)	8	
Total	0	11	16	13	10	50	50

Table 30: Relation between Tumor infiltrating lymphocytes (TILs) with tumor grade

This study showed a higher number of TILs in moderately squamous cell carcinoma cases. More TILs were seen in poorly differentiated tumors. However, there was no statistical significance identified between TILs and the grade of the tumor.

Relation between tumor infiltrating lymphocytes (TILs) with tumor necrosis

Tumor necrosis	<1%	1 -10%	11 -25%	26 -50%	>50%	Total	p-value
Absent	0	9(28.12%)	9(28.12%)	9(28.12%)	8(25%)	35	0.448 (chi-square test)
Present	0	2(13.33%)	7(46.67%)	4(26.67%)	2(13.33%)	15	
Total	0	11	16	13	10	50	

Table 31: Relation between tumor infiltrating lymphocytes (TILs) with tumor necrosis

This study showed TILs were more in patients with less tumor necrosis. However, this finding was not statistically significant.

Relation between tumor infiltrating lymphocytes (TILs) with PD-L1 score in tumor

Tumor-Infiltrating Lymphocytes (TILs) / PD-L1 score in tumor	<1%	1 -10%	11 -25%	26 -50%	>50%	Total	p-value
<1%	0	2(18.18%)	3(18.75%)	1(7.69%)	4(40%)	10	0.448 (chi-square test)
1-5%	0	2(18.18%)	3(18.75%)	3(23.07%)	1(10%)	9	
6-10%	0	1(9.09%)	1(6.25%)	3(23.07%)	0	5	
11-25%	0	3(27.27%)	4(25%)	2(15.38%)	0	9	
26-50%	0	2(18.18%)	2(12.5%)	3(23.07%)	4(40%)	11	
>50%	0	1(9.09%)	3(18.75%)	1(7.69%)	1(10%)	6	
Total		11	16	13	10	50	

Table 32a: Relation between tumor infiltrating lymphocytes (TILs) with PD-L1 score in tumor

Tumor-Infiltrating Lymphocytes (TILs) / PD-L1 score in tumor	<1%	1 -10%	11 -25%	26 -50%	>50%	Total	p-value
<1	0	2 (20%)	3 (30%)	1(10%)	4 (40%)	10	0.287 (chi-square test)
>1-100	0	9 (22.5%)	13 (32.5%)	12(30%)	6(15%)	40	
Total	0	11	16	13	10	50	

Table 32b: Relation between tumor infiltrating lymphocytes (TILs) with PD-L1 score in tumor

PD-L1 expressing tumor had more TILs. However, no statistically significant relation was seen between PD-L1 expression in tumors and TILs.

Relation between tumor infiltrating lymphocytes (TILs) with MMR status

Tumor-infiltrating lymphocytes (TILs) / MMR status	<1%	1 -10%	11 -25%	26 -50%	>50%	Total	p-value
Deficient	0	0	1 (33.33%)	2(66.66%)	0	3	0.338 (chi-square test)
Proficient	0	11(23.40%)	15(31.47%)	11 (23.40%)	10(21.27%)	47	
Total	0	11	16	13	10	50	

Table 33: Relation between tumor infiltrating lymphocytes (TILs) with MMR status

None of the MMR deficient cases had <10% of TILs. However, 23.40% (11 out of 47 cases) of MMR stable cases had <10% TILs. However, there was no statistically significant association between the MMR status of tumors and TILs.

Relation between Programmed death ligand-1(PD-L1) by combined prognostic score (CPS) in tumor with clinicopathological parameters

Relation between PD-L1 expression in tumor and age

PD-L1 expression / Age	Absent	Present	Total	p-value
30-40	0	7 (100%)	7	0.162(chi-square test)
41-50	2 (28.57%)	5 (71.42%)	7	
51-60	2 (11.76%)	12 (70.58%)	17	
61-70	4 (21.05%)	15 (78.94%)	19	
71-80	1 (10%)	0	1	
81-90	1(50%)	1 (50%)	2	
Total	10	40	50	

Table 34a: Relation between PD-L1 expression in tumor and age

PD-L1 expression %	<1% (20%)	1-5% (18%)	6-10% (10%)	11-25% (18%)	26-50% (22%)	>50% (12%)	Total	p-value
Age								0.595 (chi-square test)
30-40	0	2 (28.57%)	0	1 (14.28%)	4 (57.14%)	0	7	
41-50	2 (28.57%)	0	1 (14.28%)	2 (28.57%)	1 (14.28%)	1 (14.28%)	7	
51-60	2 (12.50%)	2 (12.50%)	2 (25.00%)	4 (44.44%)	2 (12.50%)	2 (12.50%)	16	
61-70	4 (23.52%)	5 (29.41%)	2 (11.76%)	2 (11.76%)	4 (23.52%)	2 (11.76%)	17	
71-80	1 (100%)	0	0	0	0	0	1	
81-90	1 (50%)	0	0	0	0	1 (50%)	2	
Total	10	9	5	9	11	6	50	

Table 34b: Relation between PD-L1 expression in tumor and age

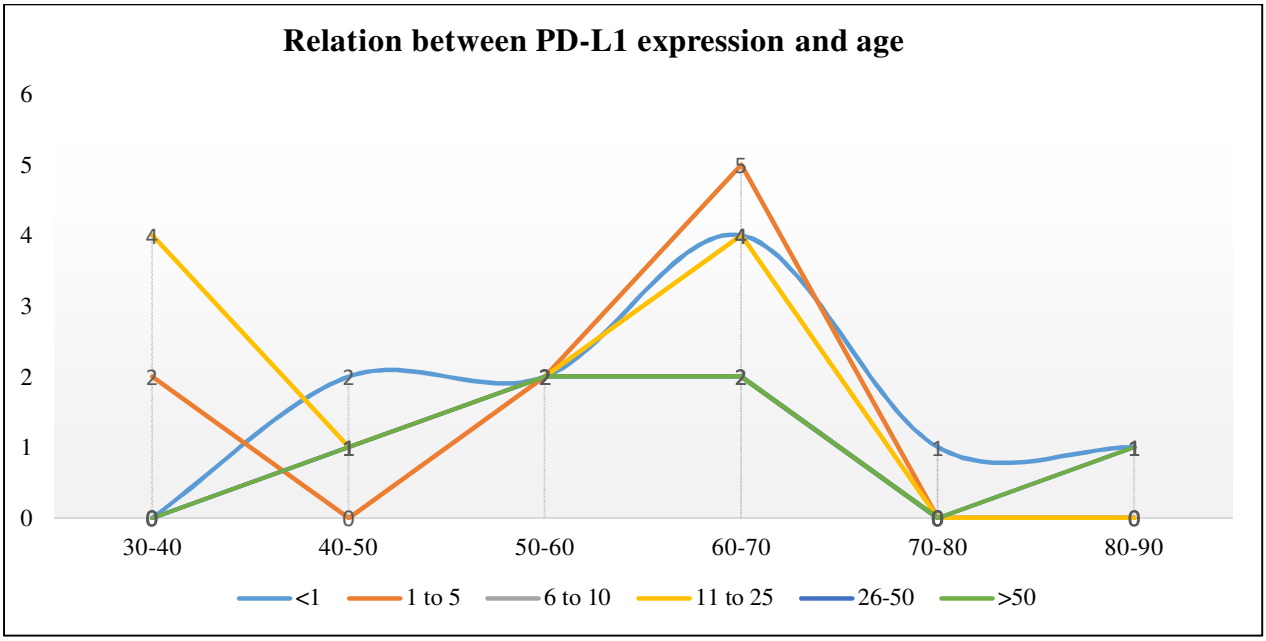


Figure 14: Relation between PD-L1 expression in tumor and age

PD-L1 expression was seen in all the tumors in the youngest age group (7/7 cases, 100%). However, no statistically significant association was seen between the age of the patient and PD-L1 expression.

Relation between PD-L1 expression and type of cancer (Keratinising/Nonkeratinising)

PD-L1 expression	Absent	Present	Total	p-value
Type of cancer (Keratinising/Nonkeratinising)				0.689 (chi-square test)
Keratinising squamous cell carcinomas	9 (20%)	36 (80%)	45	
Non-Keratinising squamous cell carcinomas	1 (20%)	4 (80%)	5	
Total	10	40	50	

Table 35a: Relation between PD-L1 expression and type of cancer (Keratinising/Nonkeratinising)

No statistically significant association was seen between PD-L1 expression and tumor grade.

PD-L1 expression	<1 (20%)	1-5 (18%)	6-10 (10%)	11-25 (18%)	26-50 (22%)	>50 (12%)	Total	p-value
Grade								0.939 (chi-square test)
Keratinising squamous cell carcinomas	9 (20%)	8 (17.77%)	4 (8.88%)	8 (17.77%)	10 (22.22%)	6 (13.33%)	45	
Non-Keratinising squamous cell carcinomas	1 (20%)	1 (20%)	1(20%)	1 (20%)	1 (20%)	0	5	
Total	10	9	5	9	11	6	50	

Table 35b: Relation between PD-L1 expression and type of cancer (Keratinising/Nonkeratinising)

None of the nonkeratinising SCCs had a CPS score of >50 and no statistically significant association was seen between PD-L1 expression and the grade of the tumor.

Relation between PD-L1 expression and tumor grade

PD-L1 expression	Absent	Present	Total	p-value
Grade				0.732 (chi-square test)
Well-differentiated SCCs, G1	0	2 (5%)	2	
Moderately-differentiated SCCs, G2	8 (80%)	32 (80%)	40	
Poorly-differentiated SCCs, G3	2 (20%)	6 (15%)	8	
Total	10	40	50	

Table 36a: Relation between PD-L1 expression and tumor grade

PD-L1 expression	<1 (20%)	1-5 (18%)	6-10% (10%)	11-25% (18%)	26-50% (22%)	>50% (12%)	Total	p-value
Tumor grade								0.022 (chi-square test)
Well-differentiated SCCs, G1	0	0	2 (100%)	0	0	0	2	
Moderately-differentiated SCCs, G2	8 (20%)	8 (20%)	3 (7.50%)	8 (20%)	9 (22.5%)	4 (10%)	40	
Poorly-differentiated SCCs, G3	2 (25%)	1 (12.5%)	0	1 (12.5%)	2 (25%)	2 (25%)	8	
Total	10	9	5	9	11	6	50	

Table 36b: Relation between PD-L1 expression and tumor grade

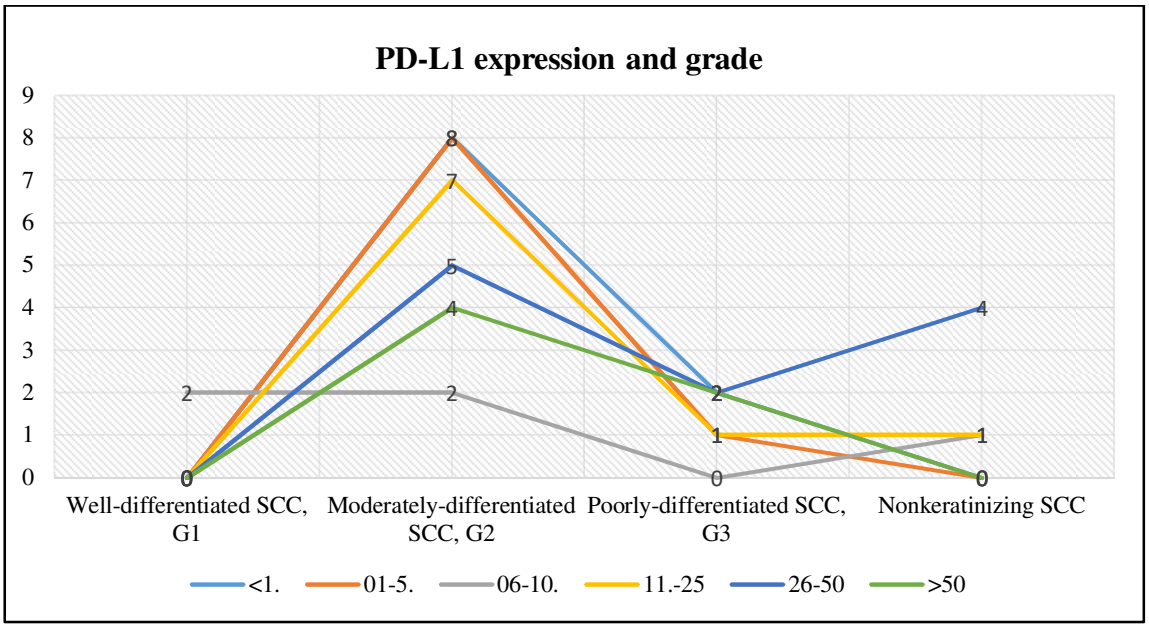


Figure 15: Relation between PD-L1 expression and tumor grade

A statistically significant association was noted between PD-L1 expression in the tumor and the grade of the tumor. Poorly differentiated SCCs showed more PD-L1 expression than well differentiated and moderately differentiated tumors.

Relation between PD-L1 expression and necrosis

PD-L1	Absent	Present	Total	p-value
Necrosis				0.440 (chi-square test)
Absent	8 (22.85%)	27 (67.5%)	35	
Present	2 (13.33%)	13 (86.66%)	15	
Total	10	40	50	

Table 37a: Relation between PD-L1 expression and tumor necrosis

PD-L1%	<1% (20%)	1-5% (18%)	6-10% (10%)	11-25% (18%)	26-50% (22%)	>50% (12%)	Total	p-value
Tumor Necrosis								0.190 (chi-square test)
Absent	8 (22.85%)	4 (11.42%)	5 (14.28%)	7 (20%)	6 (17.14%)	5 (14.28%)	35	
Present	2 (13.33%)	5 (33.33%)	0	2 (13.33%)	5 (33.33%)	1 (6.66%)	15	
Total	10	9	5	9	11	6	50	

Table 37b: Relation between PD-L1 expression and tumor necrosis

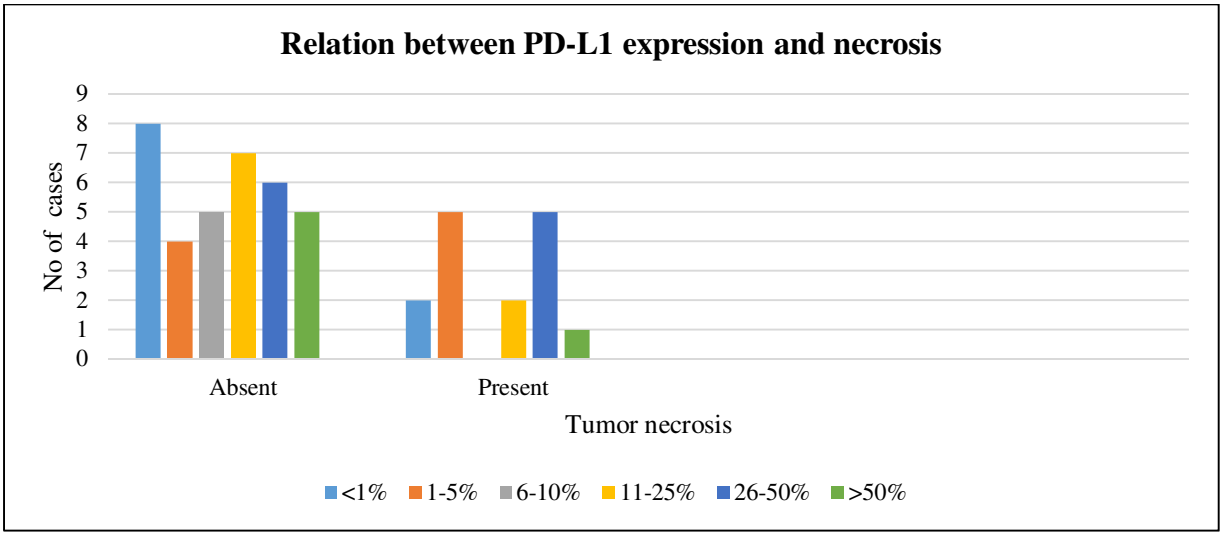


Figure 16: Relation between PD-L1 expression and tumor necrosis

Necrosis was seen in 2 out of 15 (13.33%) of PD-L1 positive cases and 13 out of 15 (86.66%) of PD-L1 negative status. However, no statistically significant association was seen between tumor necrosis and PD-L1 expression.

Relation between Mismatch repair status with clinicopathological parameters

Relation between MMR status and age

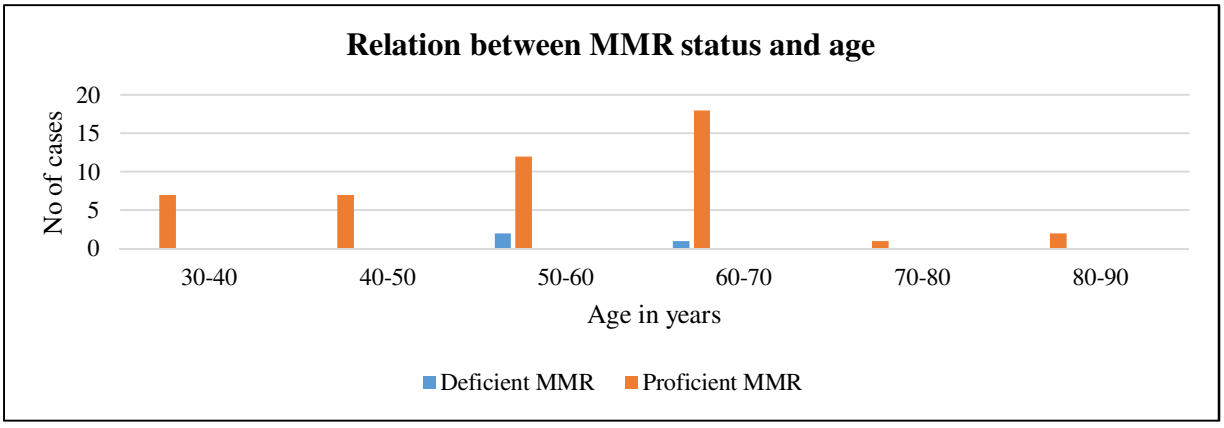


Figure 17: Relation between MMR status and age

Age	Deficient MMR	Proficient MMR	Total	p-value
	n=3	N=47		0.809
30-40	0	7 (14.89%) (100%)	7	(chi-square test)
41-50	0	7 (14.89%) (100%)	7	
51-60	2 (66.66%) (12.5%)	14 (29.78%) (87.5%)	16	
61-70	1 (33.33%) (5.88%)	16 (34.04%) (94.11%)	17	
71-80	0	1 (2.12%) (100%)	1	
81-90	0	2 (4.25%) (100%)	2	
Total	3	47		

Table 38: Relation between MMR status and age

All the dMMR cases were >50 years of age. None of the 14 patients (< 50 years of age) showed dMMR. Also, none of the patients >70 years of age showed dMMR. All the dMMR patients were in the 50-70years age group. There was no statistically significant relation seen between MMR status and the age of the patient.

Relation between MMR status and type of cancer (Keratinising/Nonkeratinising)

Type of cancer (Keratinising/Nonkeratinising)	Deficient MMR	Proficient MMR	Total	p-value
	n=3	N=47		
Keartinizing squamous cell carcinoma	3 (100%) (6.66%)	42 (89.36%) (93.33%)	45	0.724 (chi-square test)
Nonkeratinising squamous cell carcinoma	0	5 (10.63%) (100%)	5	
Total	3	47	50	

Table 39: Relation between MMR status and Type of cancer (Keratinising/Nonkeratinising)

All the nonkeratinising SCCs were mismatch repair proficient. All the mismatch repair-deficient tumors were keratinising SCCs. However, no statistically significant association was seen between tumor grade and MMR status.

Tumor Grade	Deficient MMR	Proficient MMR	Total	p-value
	n=3	N=47		
Well-differentiated squamous cell carcinoma	0	2 (100%) (4.2%)	2	0.671 (chi-square test)
Moderately differentiated squamous cell carcinoma	3 (100%)	37 (92.5%)(78.72%)	40	
Poorly differentiated squamous cell carcinoma	0	8 (20%)(17.02%)	8	
Total	3	47	50	

Table 40: Relation between MMR status and tumor grade

All the well-differentiated SCCs and poorly differentiated squamous cell carcinomas were MMR stable. MMR deficiency was seen only in moderately differentiated SCC. However, no statistically significant association was seen between tumor grade and MMR status.

Relation between MMR status and tumor necrosis

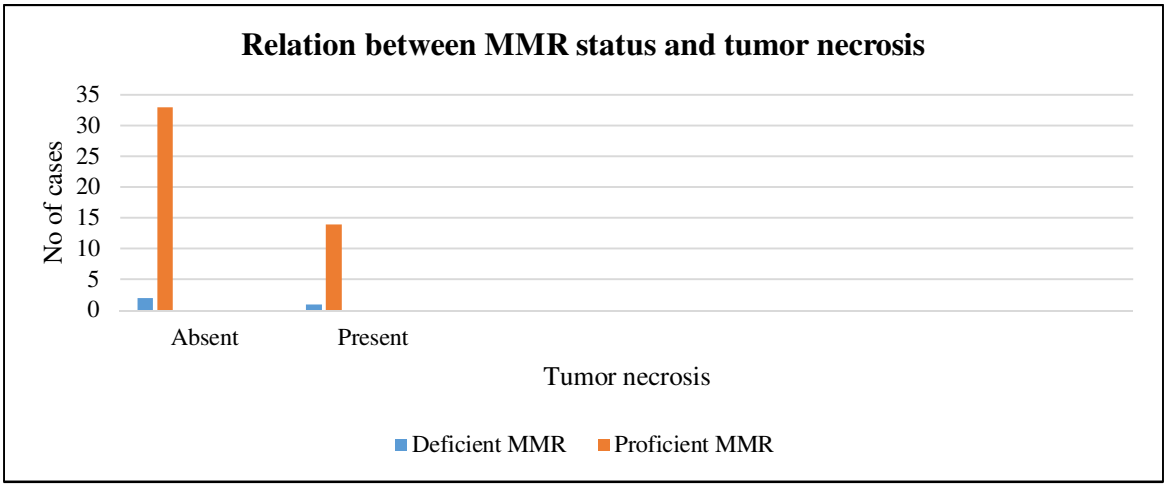


Figure 18: Relation between MMR status and tumor necrosis

Tumor necrosis	Deficient MMR	Proficient MMR	Total	p-value
	n=3	N=47		1.000
Absent	2 (66.66%) (5.72%)	33 (70.21%) (94.2%)	35	(chi-square test)
Present	1 (33.33%) (6.66%)	14 (29.78%) (93.33)	15	
Total	3	47	50	

Table 41: Relation between MMR status and tumor necrosis

Most of the MMR stable cases found an absence of tumor necrosis (33/47, 70.21%). Also, two-thirds of the MMR deficient cases did not show necrosis. However, no statistically significant association was seen between MMR status and tumor necrosis.

Relation between MMR status and TILs

TILs	Deficient MMR	Proficient MMR	Total	p-value
	n=3	N=47		0.564
<1%	1 (33.33%) (12.5%)	7 (14.89%) (87.5%)	8	(chi-square test)
1-10%	2 (66.66%) (11.11%)	16 (34.04%) (88.88%)	18	
11-25%	0	10 (21.27%) (100%)	10	
26-50%	0	10 (21.27%) (100%)	10	
>50%	0	4 (8.5%) (100%)	4	
Total	3	47	50	

Table 42: Relation between MMR status and TILs

There was no statistically significant association between MMR status and TILs in the study.

Relation between PD-L1 expression and MMR status

PD-L1 expression	Absent	Present	Total	p-value
MMR				0.496
dMMR	1 (10%) (33.33)	2 (5%) (66.66%)	3	(Chi-square test) Fisher exact test
pMMR	9 (90%) (19.14%)	38 (80.85%) (95%)	47	
Total	10	40	50	

Table 43a: Relation between PD-L1 expression and MMR status

PD-L1 expression	<1 (20%)	1-5 (18%)	6-10 (10%)	11-25 (18%)	26-50 (22%)	>50 (12%)	Total	p-value
MMR								0.264 (chi-square test)
dMMR	1 (33.34%)	2 (66.66%)	0	0	0	0	3	
pMMR	9 (19.14%)	7 (14.89%)	5 (10.63%)	9 (19.14%)	11 (23.40%)	6 (12.76%)	47	
Total	10	9	5	9	11	6	50	

. Table 43b: Relation between PD-L1 expression and MMR status

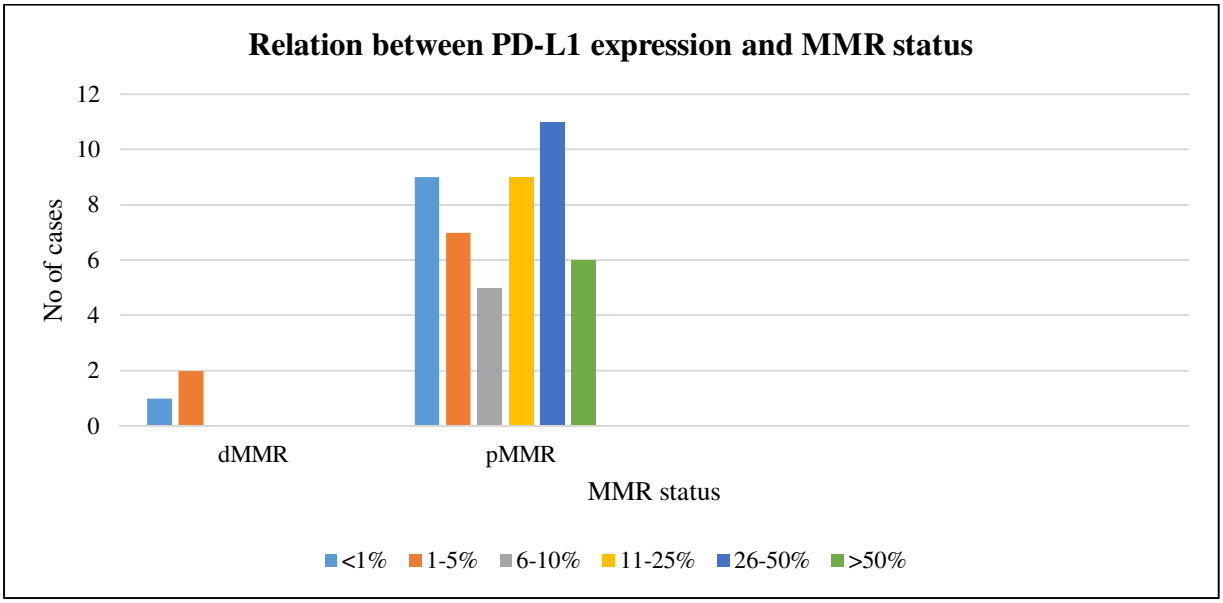


Figure 19: Relation between PD-L1 expression and MMR status

All the dMMR SCCs of the cervix showed a CPS of <5 and one-third of the deficient MMR cases showed the absence of PD-L1 in the tumor. However, no statistically significant association was seen between MMR status and PD-L1 expression in the tumors.

In this study, among 50 cases of squamous cell carcinoma of the cervix, the majority of the patients were between 60-70 years of age. Considering the histological grade of the tumor, the majority of cases were keratinising and moderately differentiated squamous cell

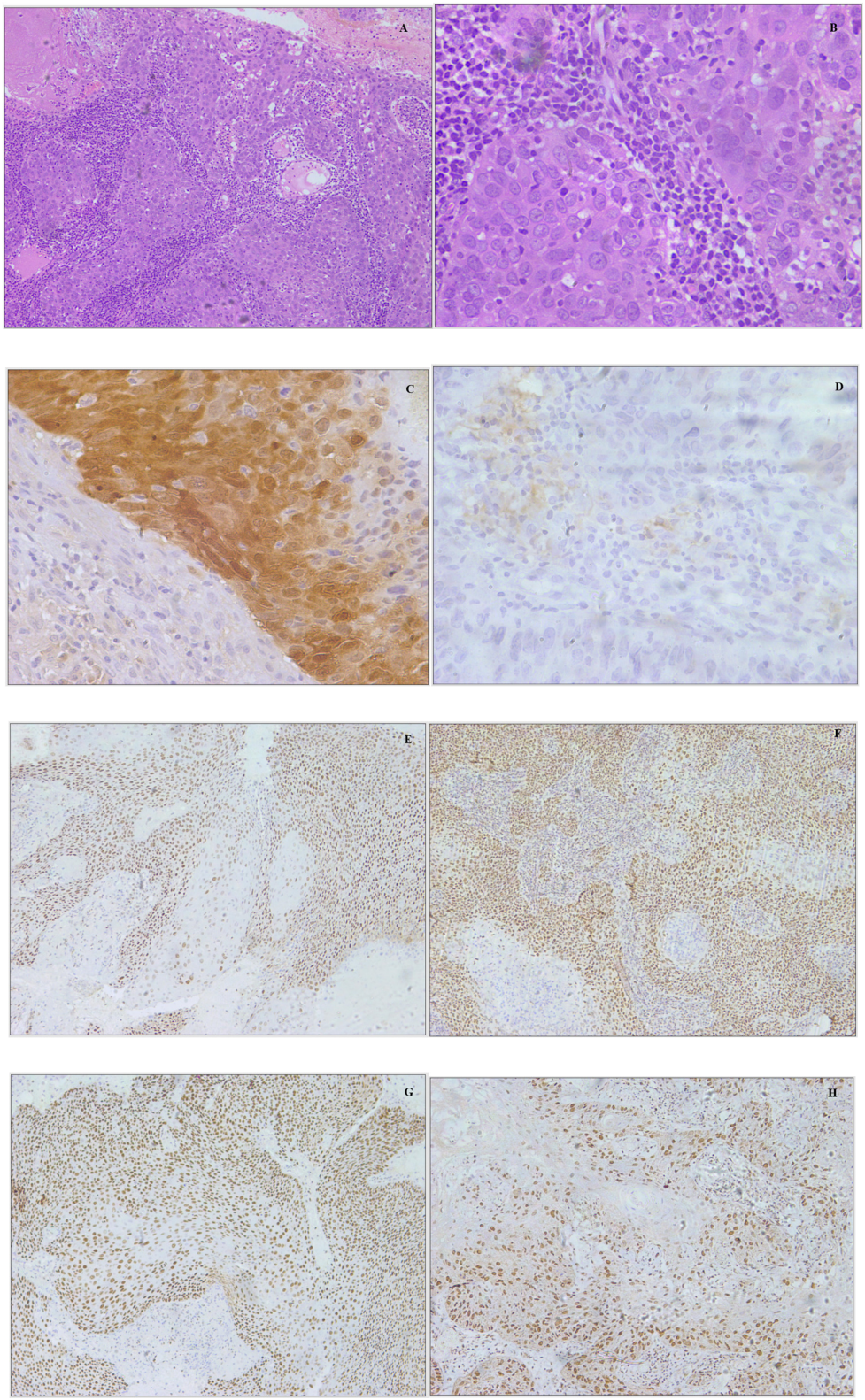
carcinoma. 30 % of the cases showed necrosis and 46% of the cases showed dysplasia. All the cases of SCCs cervix had an incidence of TILs (100%). 94% of cases showed HPV association. PD-L1 expression was absent in 10 cases and 40 cases showed PD-L1 expression. MMR deficient cases were 3 and proficient cases were 47.

The salient features are summarized in the following table:

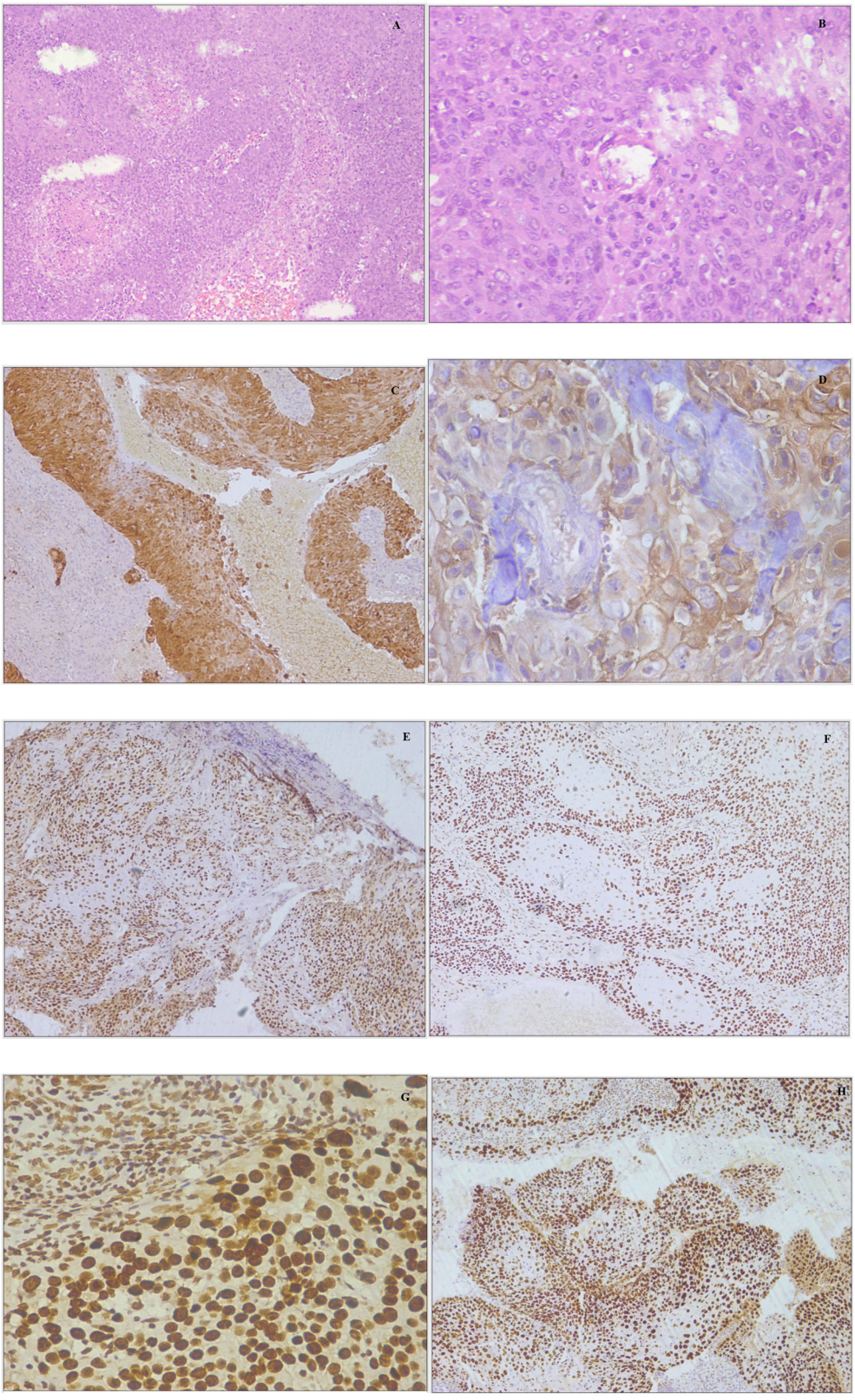
Features	Characteristics	Frequency, Total number of cases=50	Percentage (%)
Age (in years)	30-40	7	14.0
	41-50	7	14.0
	51-60	16	32.0
	61-70	17	34.0
	71-80	1	2.0
	81-90	2	4.0
Tumor grade	Well-differentiated	2	4.0
	Moderately differentiated	40	80.0
	Poorly differentiated	8	16.0
HPV association	Associated	47	94.0
	Independent	3	6.0
Necrosis	Not identified	35	70.0
	Present	15	30.0
Dysplasia	Absent	27	54.0
	Present	23	46.0
PD-L1expression in tumor	Absent	10	20.0
	Present	40	80.0
TILs	Absent	0	0.0
	Present	50	100
MMR	Deficient	3	6.0
	Proficient	47	94.0

Table 44: Clinical and pathological parameters in SCCs of the cervix

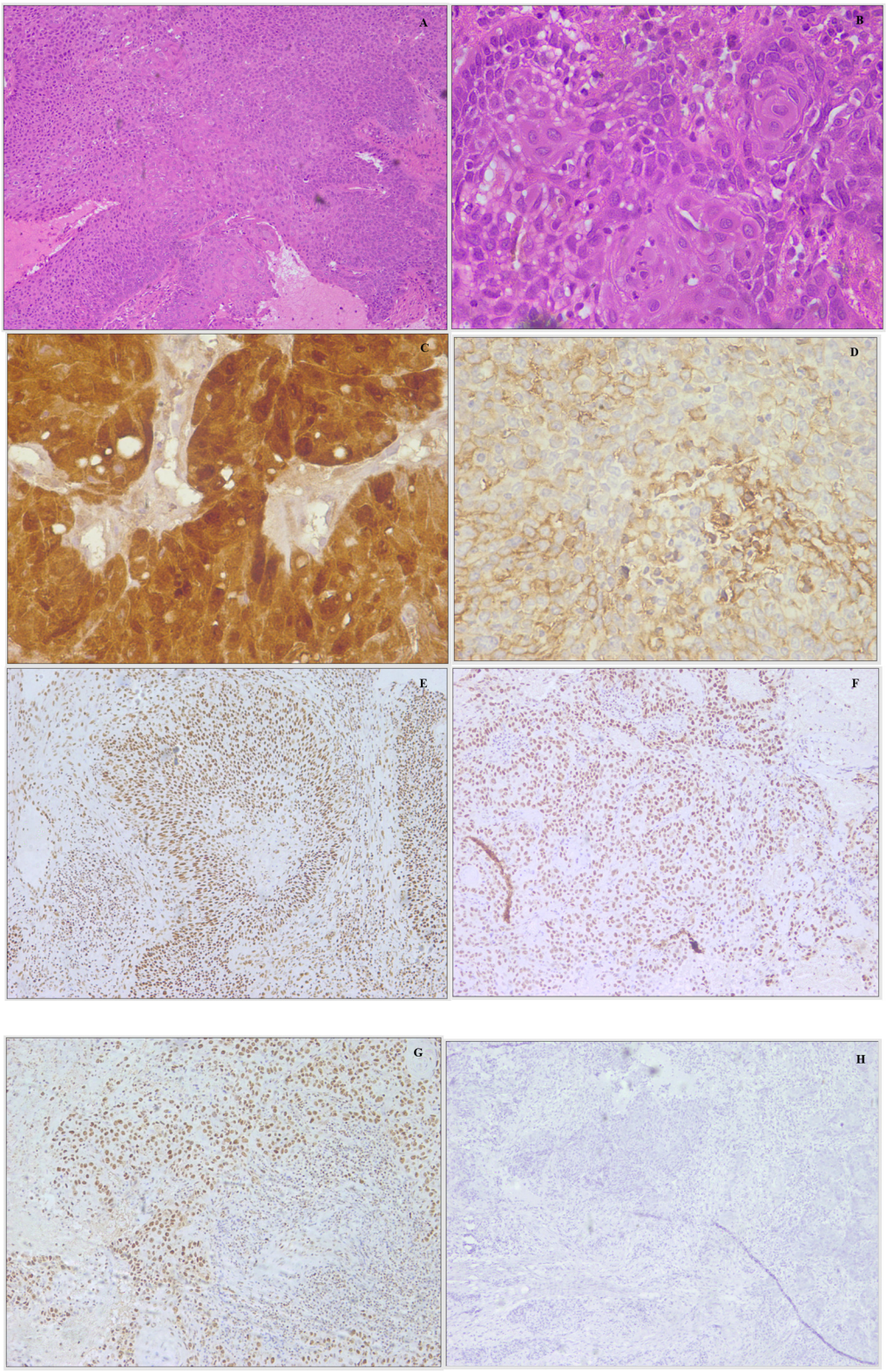
Photomicrograph 9: A case of squamous cell carcinoma of the cervix (H&E: Panel A, B) HPV association (Panel C). The PD-L1 expression in tumor cells and TILs (Panel D). The tumor is mismatch repair proficient shows retained MSH-2 protein (Panel E), MLH-1 protein (Panel F), MSH-6 protein (Panel G) and retained PMS-2 protein (Panel H).



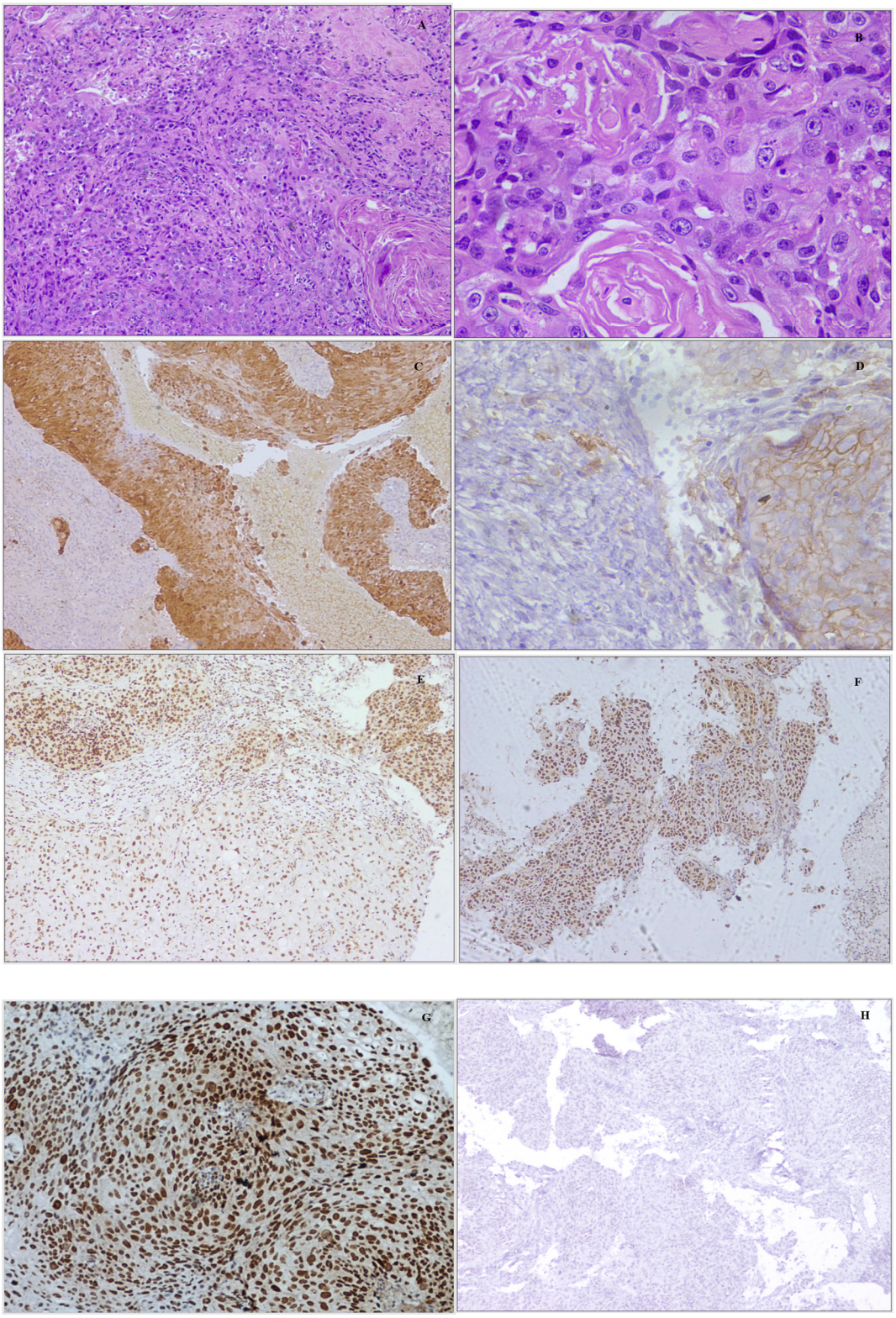
Photomicrograph 10: A case of squamous cell carcinoma of the cervix (H&E: Panel A, B) HPV associated (Panel C). The PD-L1 expression in tumor cells (Panel D). The tumor is mismatch repair proficient shows retained MSH-2 protein (Panel E), MLH-1 protein (Panel F), MSH-6 protein (Panel G) and retained PMS-2 protein (Panel H).



Photomicrograph 11: A case of squamous cell carcinoma of the cervix (H&E: Panel A, B) HPV associated (Panel C). The PD-L1 expression in tumor cells and TILs (Panel D). The tumor is mismatch repair deficient shows retained MSH-2 protein (Panel E), PMS-2 protein (Panel F), MSH-6 protein (Panel G) and loss of MLH-1 protein (Panel H).



Photomicrograph 12: A case of squamous cell carcinoma of the cervix (H&E: Panel A, B) HPV associated (Panel C). The PD-L1 expression in tumor cells and TILs (Panel D). The tumor is mismatch repair deficient shows retained MSH-2 protein (Panel E), PMS-2 protein (Panel F) MSH-6 protein (Panel G) and loss of MLH-1 protein (Panel H).



DISCUSSION

DISCUSSION

This study was an ambispective, observational hospital-based study conducted on 50 cases of squamous cell carcinomas (SCCs) of the cervix (from January 2018 to July 2021) at the All India Institute of Medical Sciences (AIIMS), Jodhpur in the Department of Pathology and Lab Medicine.

In this study, PDL1 expression and mismatch repair (MMR) status of SCCs, of the cervix were assessed.

Squamous cell carcinomas of the cervix

Cervical cancer was the fourth most common cancer in women worldwide (after breast, colorectal and lung cancers) and also the second most common cancer among women aged 15 to 44. It is one of the top three cancers in women aged < 45 years in 145 countries.[1]

The majority of cervical cancers are invasive squamous-cell carcinomas and are associated with human papillomavirus (HPV).[1] According to The Cancer Genome Atlas (TCGA) a joint effort of the National Cancer Institute (NCI) the estimated cases in 2021 were 14,480 which is 0.8% of all new cancer cases. The rate of new cases of cervical cancer was 7.5 per 100,000 women per year. The death rate was 2.2 per 100,000 women per year.[3]

The cancer treatment includes surgery, chemotherapy, radiotherapy and immunotherapy. Treatment options vary mainly with the stage of the tumor and the age of the patient. For Fédération Internationale de Gynécologie et d'Obstétrique (FIGO) early-stage (stage IA1) lesions, treatment by conization were an option for women who want to preserve their fertility, and simple extra fascial hysterectomy, if fertility is not sought.[16] For stage IA2, the standard treatment has been radical hysterectomy and bilateral pelvic lymphadenectomy. For FIGO stage IB1 cervical tumors and in high-stage of tumors, radical hysterectomy with pelvic and para-aortic lymphadenectomy is the preferred treatment option.[16]

In young females, neoadjuvant chemotherapy can be used to downsize stage IB1 lesions, allowing for subsequent fertility-sparing surgery. Given the high risk of recurrence, radical hysterectomy and pelvic lymphadenectomy were utilized to treat stage IB2 cancer, followed by adjuvant radiation. Long-term morbidity is associated with combined radical hysterectomy and adjuvant pelvic irradiation. Some oncologists advocate for primary combined

chemoradiotherapy (CCRT)-alone or CCRT followed by simple extra fascial hysterectomy IB2 lesions.[18] When compared to stages IA and IB, locally advanced cervical cancer (stages IIB–IVA) has a worse prognosis.[44]

Treatment for stages IIB to IVA is non-surgical, but local access to radiotherapy facilities is required.[16]

Immune checkpoint inhibitors have shown promising efficacy in multiple cancer types. The recent Food and Drug Administration (FDA) approval of PD-1 inhibitors for mismatch repair (MMR)-deficient tumors have extended the use of these treatments to all cancer types. Programmed death-ligand 1 (PD-L1) positivity in tumor tissue has also been shown to predict susceptibility to immunotherapy.[45] Pembrolizumab inhibits the immune checkpoint protein programmed cell death-1 (PD-1) and has been approved by the US Food and Drug Administration for use in advanced cervical cancer with the progressive disease either during or after chemotherapy, based on the results of the KEYNOTE-158 (NCT02628067) trial. [46] [47] [37]

Clinicopathological parameters

Age

The age of the patients in the study ranged from 33 years to 81 years, with a mean age of 56.28 years with a standard deviation of 11.74 and a median age of 58.00 years. Maximum number of patients was between 60-70 years of age group (17 out of 50, 34%).

Lars-Christian Horn et al. conducted a study in 2019 with a sample size of 233 cases of surgically treated SCCs of the cervix. They studied in the age group of 23–67 years with a mean of 40.7 years and standard deviation of 10.4 and a median of 39 years.[48]

Chung et al. did a study in 2019 on 98 patients with SCCs cervix in the age group of 24 to 75 years with a median age of 46.0 years.[47]

Liang et al. conducted a study in 2020, that included 142 patients with squamous cell carcinomas of the cervix and found that the median age was 45 among the age group of the patient ranging from 24 to 67 years old.[49]

Pannayanapalya Suresh Shruthi et al. conducted a study in 2014, in 109 cases of histopathologically diagnosed SCCs cervix. They found that the patient's age ranged from 20 to 80 years.[50]

Min Feng et al. studied 219 cervical squamous cell tumors and 30 healthy controls in 2018. They found that patients were in the age group of 26 to 75 years with a mean age of 46.7 and a median of 49 years.[51]

A. Ciavattini et al. examined the hMSH2 and hMLH1 status by immunohistochemistry, in 28 cases of aggressive squamous cervical carcinomas in 2005. They found that patients were in the age group of 38- 86 years with a mean age of 63.9.[52]

C.M. Ho et al. studied 197 patients who underwent radical hysterectomy and pelvic lymphadenectomy in 2003 for early-stage invasive cervical carcinomas. They found that 116 cases were 50 years old and 81 cases out of 197 were over 50 years old, with a median age of 47.36.[53]

W. Yang et al. conducted a study in 2017, on 20 SCCs patients with or without metastases. They found that patients age ranged from 39 to 63 years old, with a mean age of 51 years and a standard deviation of 6.28 years.[54]

In 2021, Mori et al. conducted a study on 75 patients with uterine cervical squamous cell cancer. They found that the patients were between the ages of 32 and 87 years, with a median age of 62.[55]

In 2002, Y.F. Wong et al. conducted a study in 2003 on 93 SCCs samples that were tested for MSI status. They found that 10 patients of the 93 cases were under the age of 40, 39 were between the ages of 40 and 60 and 44 cases were over the age of 60 years.[56]

In 2005, Agnaldo L. Silva-Filho et al. conducted a study on 71 patients with stage IB (FIGO) cervical squamous cell carcinomas. They found that the patient's age ranged between 27 to 76 years old, with a mean age of 48.1 years and a standard deviation of 11.5 years.[57]

Heeren et al. conducted a study in 2016 on 156 cases of SCCs of the cervix. They included patients with the age group of 22 to 87 years and a mean age of 48 years.[58]

Tahmineh Haidary et al. conducted a study in 2019, on 60 cervical SCCs to evaluate PD-L1 expression and clinical significance. They found that 60% were between the ages of 30 and 55 and 40% were above the age of 55.[59]

Ying Meng et al. conducted a study in 2018, on 97 cervix SCCs patients and 30 normal cervix samples to assess PD-L1and HPV expression in cervical cancer and normal cervix using immunohistochemistry staining. They recorded that patients < 55 years of age were 56.70% (55 out of 97 cases) and 42 out of 97 patients were over the age of 55 (43.30 %).[60]

E R Nijhuis et al. conducted a study in 2015, on 135 cases of SCCs cervix were evaluated for MMR status. They included patients ranging from 35 to 53 years of age, with a median age of 42 years.[61]

Tumor Grade

In this study,40 out of 50 cases (80%) were diagnosed with moderately differentiated squamous cell carcinomas, 8 out of 50 cases (16%), were poorly differentiated squamous cell carcinomas and only 2 out of 50 cases (4%), were well-differentiated squamous cell carcinomas.

Among the 50 patients, 45 (90%), had keratinising squamous cell carcinomas, whereas 5 (10%) had nonkeratinising squamous cell carcinomas.

Even though the current WHO classification does not advocate the grading of SCCs, the grading was performed in the study.[1]

Lars-Christian Horn et al. conducted a study in 2019 with a sample size of 233 cases of surgically treated SCCs of the cervix. They categorized individuals using a WHO-based grading system and found that 105 out of 233 cases (45.1 %) of the tumors were well-differentiated (G1), 68 out of 233 cases (29.2 %) were moderately differentiated (G2) and 60 out of 233 cases (25.8 %) were poorly differentiated (G3) SCCs.[48]

Liang et al. conducted a study in 2020 that included 142 patients and found that all cases were histologically diagnosed squamous cell carcinomas, with 115 cases being well-differentiated or moderately differentiated and 27 cases being poorly differentiated SCC.[49]

T.K.H. Chung et al. conducted a study in 2001 with 50 cases of invasive SCCs of the cervix. They found that out of 50 patients, 4 cases (8%) were well-differentiated carcinomas, 30 cases (60%) moderately differentiated squamous cell carcinomas and 16 cases (32%) were poorly differentiated carcinomas.[62]

Pannayanapalya Suresh Shruthi et al, conducted a study in 2014, in 195 cases of histopathologically diagnosed SCCs cervix. They found that out of 195 patients, the majority

were moderately differentiated squamous cell carcinomas (109 cases out of 195, 55.89%) followed by poorly differentiated (51/195, 26.15%) and well-differentiated carcinomas (35/195, 17.94%).[50]

Min Feng et al. studied 219 cervical squamous cell tumors and 30 healthy controls in 2018.

They found that 169 cases out of 219 (77.16%) were poorly differentiated SCCs, 50 cases out of 219 (22.83%) were moderate & well-differentiated SCCs.[51]

A. Ciavattini et al. studied the immunohistochemistry status of hMSH2 and hMLH1 in 28 cases of aggressive squamous cervical carcinomas in 2005. They found that 4 cases out of 28 (14.3%) were well-differentiated -histologic Grade I, 15 cases out of 28 (53.6%) were moderately differentiated -histologic Grade II and 9 cases out of 28 (32.1%) were poorly differentiated -histologic Grade III SCCs of the cervix.[52]

In 2017, W. Yang et al. conducted a study on 20 SCCs patients with or without metastases. They found that 8 cases (40 %) of the 20 patients had well-differentiated carcinomas, 7 cases (35 %) had moderately differentiated squamous cell carcinomas and 5 cases (25 %) had poorly differentiated carcinomas.[54]

In 2002, Y.-F. Wong et al. conducted a study in 2003 on 93 SCCs samples that were tested for MSI. They found that 14 (15.05%) of the 93 patients had well-differentiated carcinomas, 53 (56.98 %) had moderately differentiated squamous cell carcinomas and 26 (27.95 %) patients had poorly differentiated carcinomas. [56]

In 2005, Agnaldo L. Silva-Filho et al. conducted a study on 71 patients with stage IB (FIGO) cervical cancer. The tumor was well-differentiated (G1) in 8 (11.3%) patients, moderately differentiated (G2) in 40 (56.3%) cases and poorly differentiated (G2) in 23 (32.4%) cases.[57]

Ying Meng et al. conducted a study in 2018, on 97 SCCs of the cervix patients and 30 cases of the normal cervix, to examine PD-L1 and HPV expression in cervical cancer and normal cervix,by immunohistochemistry staining. Among the 97 SCCs patients, 12 (12.37 %) had poorly differentiated SCCs cervix, 40 (41.24%) had moderately differentiated SCCs cervix and 45 (46.39 %) had well-differentiated SCCs cervix.[60]

Tahmineh Haidary et al. conducted a study in 2019 on 60 cervical SCCs to evaluate PD-L1 expression and clinical significance. They found that 33 patients had poorly differentiated, 20 moderately differentiated and 7 well-differentiated SCCs.[59]

Tumor Necrosis

Fifteen out of 50 cases (30%) showed tumor necrosis whereas 35 out of 50 cases (70%) did not show tumor necrosis.

W. Yang et al. conducted a study in 2013, in which 20 patients had primary invasive cervical SCCs. In this study, they found that 6 cases out of 20 patients (30%) had an incidence of tumor necrosis.[54]

Lymphovascular invasion and perineural invasion

None of the cases in the study, showed lymphovascular invasion or perineural invasion. However, this could be a sampling bias as the study was done on biopsy samples, not on the resection specimens.

Lars-Christian Horn et al conducted a study in 2019 with a sample size of 233 cases of surgically treated SCCs of the cervix. They found that 109 out of 233 cases (46.8%) showed lymphovascular invasion and 124 out of 233 (53.2%) cases not showed lymphovascular invasion.[48]

C.-M. Ho et al. studied 197 patients who underwent radical hysterectomy and pelvic lymphadenectomy in 2003 for early-stage invasive SCCs of the cervix. They found that lymphovascular invasion was present in 76 of the 197 patients (38.6 %) and was absent in 121 of the 197 cases (61.4%).[53]

In 2005, Agnaldo L. Silva-Filho et al. conducted a study on 71 patients with stage IB (FIGO) cervical cancer. LVI was not found in 57 (80.3 %) of the patients and was found in 14 patients (19.7 %). LVI was related to pelvic lymph node metastases, as well as vaginal and parametrial involvement, also found in that investigation.[57]

G. Van de Putte et al. conducted a study on 212 patients who underwent radical hysterectomy and bilateral lymphadenectomy for stage IB SCCs in 2003. They found that lymphovascular invasion was absent in 122 cases out of 212 (57.5%) and present in 99 cases out of 212 (46.6%).[63]

Tahmineh Haidary et al. conducted a study in 2019 on 60 cervical SCCs patients to evaluate PD-L1 expression and clinical significance. 33 patients out of 60 (55%) had poorly

differentiated SCCs, 20 patients out of 60 (33.33%) had moderately differentiated SCCs and 7 patients out of 60 (11.66%) had well-differentiated tumors.[59]

Tumor-infiltrating lymphocytes (TILs)

All the cases in the study showed tumor-infiltrating lymphocytes (TILs). Maximum cases had between 11-25% TILs (16/50 cases, 32%). 10 cases (20% of the cases) had >50% TILs.

Min Feng et al. studied 219 cervical squamous cell carcinomas cases and 30 healthy controls in 2018. They found that 128 cases (58.4 %) had more significant lymphocytic infiltrate (scores 2+ and 3+), while 91 (41.6 %) had low TILs (scores 0 and 1+). They found that tumors with a higher accumulation of TILs have a higher survival rate in cervical cancer, suggesting that local immunity plays an important role in restricting tumor growth. [51]

In 2021, Mori et al conducted a study on 75 patients with uterine cervical squamous cell cancer. They studied the alteration of T cell infiltration before and after radiotherapy treatment in patients with cervical squamous cell carcinomas. They found that a high density of TILs may contribute to better outcomes than in cases with a low density of TILs before therapy. [55]

In 2020, Nicoletta D'Alessandris et al. conducted a study on 38 cervical SCCs patients to assess PD-L 1 expression and TILs. They found that all of the cases had stromal TILs. Twenty-nine cases (77%) had stromal TILs more than 10%, 23 cases had moderate TILs (10–40%)and 6 cases had high TILs (> 40%). [64]

Human Papillomavirus

The present study found that most of the cases of cervical squamous cell carcinomas (47/50, 94%), were HPV-associated (showed P16 immunoreactivity). Only 3 cases (6%), were HPV independent.

T.K.H. Chung et al. conducted a study in 2001 with 50 cases of invasive SCCs of the cervix. HPV infection was tested with a DNA study and found present in 38 cases (76 %) and absent in 12 cases (24 %).[62]

W. Yang et al. conducted a study in 20 SCCs patients with or without metastasis in 2017. They found that all 20 patients showed positive results for HPV by immunohistochemical staining (anti-P16INK4). [40]

LIU et al. conducted a study on 40 patients with SCCs cervix in 2016. They studied HPV16E7 using RNA interference, found that HPV was related to 33 of 40 cases (82.5 %) and that 7 of 40 cases (17.5 %) were HPV independent.[65]

G. Van de Putte et al. conducted a study on 212 patients who underwent radical hysterectomy and bilateral lymphadenectomy for stage IB SCCs in 2003. They found that 211 individuals (99.5 %) were positive for P16 immunopositivity (HPV associated) and 1 patient (5 %) was HPV independent.[63]

PD-L1 expression

The current study showed most of the cases expressed PD-L1 in tumor cells (40 out of 50 cases, 80%). Maximum number of cases,12 out of 50 cases (24 %), had PD-L1 expression in 1-5% of the tumor cells. 10 cases (20%) showed PD-L1 expression in >50% of the tumor cells.

In the majority of the cases, 42 out of 50 cases (84%) showed PD-L1 expression in tumor-infiltrating lymphocytes (TILs). The maximum number of cases had PD-L1 expression in 1-10% of TILs seen (18/50 cases, 36%). 4 cases out of 50 (8 %), showed PD-L1 expression in >50% of the TILs.

In this study, 40 out of 50 cases (80%), had a combined positive score > 1 (PD-L1 positive cases) whereas 10 out of 50 cases (20%) of cases, had a score of <1 (PD-L1 negative cases).

Overall, 10 of the 50 cases (20%) showed the absence of PD-L1 in the tumor, as calculated by CPS. Six of the 50 cases (12%) had a CPS of >50and a maximum number of the PD-L1 positive cases had a CPS of 26-50 (11 out of 50, 22%).

Feng et al conducted a study in 66 tissue samples of squamous cell carcinomas of the cervix in 2018. They found that strong positive, positive and negative PD-L1 expression was detected in cervical cancer cells in 21 cases (31.8 %), 18 cases (27.3 %)and 27 cases (40.9 %), respectively.[66]

Min Feng et al. studied the expression of PD-L1 in 219 cervical squamous cell tumors and 30 healthy controls in 2018. They found that PD-L1 expression positive was found in 71/219 (32.4%) of cervical squamous cell carcinomas and negative in 148 out of 219 cases (67.5%).[51]

In 2016, LIU et al. conducted a study on SCCs cervical carcinomas in 40 patients. They found that 38 out of 40 patients (95 %) had PD-L1 expression, whereas 2 out of 40 cases (5 %) were PD-L1 negative.[65]

Emeka K Enwere et al. studied in 2017, on 120 women with locally advanced cervical cancer (International Federation of Gynecology and Obstetrics stages IB to IVA). They found that 96% of patient samples were PD-L1-positive by the 1% cut-off for positivity, while only 12 percent of cases were negative.[38]

In 2021, Mori et al conducted a study on 75 consecutive patients with uterine cervical squamous cell carcinomas. They found that 4 patients (5%) were positive for PD-L1 expression and 71 patients (95%) were negative. [55]

Liang et al conducted a study in 2020 on 142 SCCs specimens. They evaluated PD-L1 expression in tumor cells as a patchy, marginal, or diffuse staining pattern. Using a 1% threshold, tumor PD-L1 expression was found in 124 (87.3%) patients, with 46 (32.4%) having strong PD-L1 staining (> 50%).[49]

Z Chinn et al. evaluated, indoleamine dioxygenase 2,3 (IDO) and PD-L1 expression in 16 cervical SCCs by the combined positive score in 2017. They found positive PD-L1 expression in tumors in 13 of 16 cases (81 %) of cervical SCCs and PD-L1 expression in CPS in 14 of 16 cases (88 %).[36]

Heeren et al. conducted a study in 2016 on 156 cases of SCCs cervix. They found that PD-L1 positivity was present in 84 cases out of 156 (54%) of the squamous cell carcinomas > 5% (used as a cut-off) of the tumor cells.[58]

Reddy et al. studied the expression of PD-L1 in 74 cervix squamous cell carcinomas (SCCs) in cervical cancers in 2017. They found that 28 (32 %) of the tumors tested positive for PD-L1 expression, while 46 (62 %) tested negative.[42]

Tahmineh Haidary et al. conducted a study in 2019 on 60 cervical SCCs to evaluate PD-L1 expression and clinical significance. They found that 93.3% of tumors had positive staining for PD-L1.[59]

In 2020, K Loharamtaweethong et al and N Puripatet al, conducted a study on 152 patients with cervical cancer and clinical parameters. They found that 84.9 % of patients were PD-L1-positive according to CPS >1.[67]

Nicoletta D'Alessandris et al. conducted a study in 2020, on 38 cervical SCC patients to assess PD-L 1 expression and TILs. They found that the great majority of neoplasms expressed PD-L1: 100% on immune cells and 92% on tumor cells. Membrane staining of malignant cells was found in 32 of 35 specimens (92 %). [64]

Ying Meng et al. conducted a study in 2018 on 97 SCCs of the cervix patients and 30 cases of the normal cervix, to assess PD-L1 and HPV expression in cervical cancer by immunohistochemistry staining. PD-L1 staining was found in 68 of 97 cervical cancer samples (70.10 %) and 29 out of 97 cases were PD-L1 negative 29.90%.[60]

Louisa Mezache et al. in 2015, researched 70 cervical cancer patients to assess the programmed death ligand-1 (PD-L1), as well as its therapeutic importance. They found that PD-L1 expression was present in 56 of 70 cervical squamous cell carcinomas (80 %) and not in 14 of 70 cases (20%).[68]

Rotman et al. conducted a study in 2020 on 44 patients, histologically diagnosed as SCCs cervix and were assessed for PD-L1/PD-L2 protein expression on tissue microarrays by immunohistochemistry (IHC). They found that 23 of 44 patients (52.27 %) were negative for PD-L1 expression and 21 cases (47.72 %) were positive for PD-L1 expression.[69]

Karim et al. conducted a study in 2020 on 88 patients with SCCs of the cervix who were immunohistochemically analyzed for PD-L1/PD-L1 protein expression (IHC). They found that PD-L1 was expressed in 20 out of 88 patients (23%) and negative in 68 out of 88 cases (77%).[70]

Mismatch Repair Status (MMR)

Out of 50 cases, 47 cases (94%) showed retained MMR protein (MMR stable) while only 3 cases (6%) showed MMR deficiency. One case (33.34%) among the three MMR deficient cases showed isolated PMS2 loss while isolated MLH1 loss was seen in 2 cases (66.66%). None of the cases shows a combined loss of PMS2 and MLH1 or MSH2 and MSH6.

T.K.H. Chung et al. conducted a study in 2001 with 50 cases of invasive SCCs of the cervix. They found that 7 cases (14%) displayed high-frequency microsatellite instability (MSI-H) and 6 (12%) displayed low-frequency microsatellite instability (MSI-L) i.e. MSI positive. The remaining 37 cases (74%) were microsatellite stable.[62]

Y.-F. Wong et al. conducted a study on 93 SCCs samples, in 2002, that was evaluated for MSI. They found that 11 (11.8%) and 16 (17.2%) of 93 cervical carcinomas were MSI-H and MSI-L, respectively and 66 cases out of 93 (70%) were microsatellite stable. They also found no significant difference between microsatellite instability (MSI-L and MSI-H) or microsatellite stable cases in terms of patient age and tumor grade.[56]

Feng et al. conducted a study on 66 cases of SCCs cervix, in 2018. They found that in 66 patients, 49/66 (74.2 %) were both MSH2 and MLH1 positive, 15/66 (22.7 %) were absent in either MSH2 or MLH1and both MSH2 and MLH1 were absent in only two cases out of 66 (3.1 %).[66]

Ciavattini et al. examined the status of hMSH2 and hMLH in 28 cases of aggressive squamous cervical carcinomas in 2005. They found a significant association between hMLH1 and hMSH2 immunostaining in invasive lesions ($r < 0.462$; $p < 0.0125$). They also found that the immunoreactivity for hMLH1 protein in cases of invasive carcinoma was statistically lower than in non-neoplastic epithelial cells ($p < 0.0009$).[52]

E R Nijhuis et al. conducted a study in 2015, on 135 cases of SCCs cervix were evaluated for MMR status. The majority of tumors 94/135 (70%) had retained MSH2, whereas MSH2 expression was absent in 43/135 (30%) tumors were found as MSI.[61]

Human papillomavirus (HPV) associated and HPV independent squamous cell carcinoma of the cervix with clinicopathological parameters

This study found that all the HPV independent cases were seen in <70 years of age group. The 3 patients out of a total of 50 cases in our study (6%) who were > 70 years of age, all had associated HPV SCCs. However, no statistically significant association was seen between HPV association of cervical squamous cell carcinomas and the age of the patients.

All the Nonkeratinising SCCs of the cervix were HPV-associated. All the poorly differentiated SCCs were HPV-associated.

None of the HPV independent SCCs of the cervix showed tumor necrosis in the biopsies sampled. However, no statistically significant association between tumor grade, tumor necrosis, tumor-infiltrating lymphocytes (TILs)and HPV-associated and HPV-independent tumors.

All the HPV independent SCC of the cervix showed PD-L1 expression. But, none of the HPV independent cases had a PD-L1 CPS of >50. None of the HPV independent SCCs showed mismatch repair deficiency. However, no statistically significant association was seen between HPV association with PD-L1 expression in tumor and MMR status of the tumor.

T.K.H. Chung et al. conducted a study in 2001 with 50 cases of invasive SCCs of the cervix. They investigated the relationship between MSI and HPV infection tested with DNA study and other clinicopathological characteristics. They found that 7 cases (14%) displayed MSI-H and 6 (12%) displayed MSI-L. The remaining 37 cases (74%) were microsatellite stable (MSI-L and MSI-H cases were grouped as MSI-positive). The statistical analysis of HPV infection, tumor grade, clinical stage and clinical status failed to reveal differences between MSI-positive and MSI-negative cases ($p > 0.05$).[62]

W. Yang et al. conducted a study in 20 SCCs patients with or without metastasis in 2017. They found that all 20 patients tested positive for HPV by immunohistochemical staining(anti-P16INK4). P16INK4a was found in cancer cells at a diffuse but high level. The levels of expression in the SCCs metastasis (+) group were significantly higher than in the metastasis (-) group ($p= 0.05$). P16INK4a expression in lymphatic tumor emboli and lymph node metastases was significantly higher than in the primary lesion. [40]

LIU et al. conducted a study on 40 individuals with SCCs cervical carcinomas in 2016 on PD-L1 expression and HPV association by immunohistochemical staining and reverse transcription-polymerase chain reaction. The statistical analysis of these data revealed that the expression of HPV16E7 and PD-L1 proteins in cervical cancer tissues was positively associated ($r=0.531$) and reached statistical significance ($p=0.043$). In cervical cancer tissues, the expression of HPV16E7 and PD-L1 showed a favorable connection.[65]

G. Van de Putte et al. conducted a study on 212 patients who underwent radical hysterectomy and bilateral lymphadenectomy for stage IB SCCs in 2003. They found that P16 immunoreactivity is found in the majority of cervical cancer samples and that low p16

expression was strongly related to decreased overall (p=0.036) but not disease-free survival (p=0.103).[63]

TILs and clinicopathological parameters

This study showed that tumor-infiltrating lymphocytes were found in all the cases of SCCs cervix, of which maximum cases were in subcategories 11-25%, 16 (32%) out of 50 cases. This study also concluded that all the cases were having TILs of <50%.

The maximum number of cases in the age group 41-60 years showed >50 TILs (28.5%). Lesser TILs were seen in patients <40 years of age. However, there was no statistically significant correlation between Tumor-infiltrating lymphocytes (TILs) and the age of the patient. TILs were more common in HPV-associated cases than in HPV-independent cases, maximum at 1-10% of TILs 17 (36.17 %) out of 47. The majority of the cases were seen in the age group between 61-70 years had TILs with 17 out of 50 cases (34%) and predominantly had TILs <50%.

This study showed a higher number of TILs in nonkeratinising squamous cell carcinomas compared to keratinising squamous cell carcinoma. This study showed a higher incidence of TILs in moderately squamous cell carcinoma cases. More TILs were seen in poorly differentiated tumors. This study showed TILs were more in patients with less tumor necrosis.

No significant correlation was found between PD-L1 expression in TILs and clinicopathological parameters like age, grade, necrosis, dysplasia, lymphovascular and perineural invasion. TILs noted in cases that had an absence of tumor necrosis. More TILs percentages were seen in cases with PD-L1 expression. Cases of SCCs cervix with deficient MMR had TILs <50% were 3 out of 50 cases (6%). Maximum number of cases had proficient MMR status with a high number of TILs seen between 11-25% (15 out of 50 cases 31.47%). None of the MMR deficient cases had <10% of TILs. However, 23.40% (11 out of 47 cases) of MMR stable had <10% TILs.

Min Feng et al. studied 219 cervical squamous cell tumors and 30 healthy controls in 2018. TILs were significantly associated with higher PD-L1 levels (p = 0.033), but not with age, tumor size, vascular invasion, lymph node positivity, TNM stage or histologic grade.

Furthermore, the presence of substantial lymphocytic infiltrates was related to a definite tendency toward longer survival (p = 0.048).[51]

In 2021, Mori et al conducted a study on 75 consecutive patients with uterine cervical squamous cell cancer. They studied the alteration of T cell infiltration following radiotherapy in patients with cervical squamous cell carcinomas who received either definitive chemoradiotherapy or radiotherapy. They found that 68% (21 out of 31 patients) of the increased TILs density group also exhibited an increase in PD-L1 expression, whereas (27 of 44) of the decreased TILs density group exhibited no increase in PD-L1 expression but no statistical association was noted between the two. [55]

PD-L1 expression with clinicopathological parameters

In this study, PD-L1 expression was seen in all the cases of tumors in the youngest age group (7 /7 cases, 100%). No statistically significant association was seen between PD-L1 expression and the age of the patients. There is a significant correlation is seen between the grade of the tumor and the expression of PD-L1 in tumor cells (p-value= 0.022.) Twenty percent of keratinising SCCs showed the absence of PD-L1 expression and 1 out of 5 nonkeratinising SCCs (20%) showed the absence of PD-L1 expression. Necrosis was seen in 2 out of 15 (13.33%) of PD-L1 positive cases and 13 out of 15 (86.66%) of PD-L1 negative cases. However, there is no correlation seen with PD-L1 in tumors and clinicopathological parameters such as necrosis, lymphovascular invasion, perineural invasion and TILs.

The present study concluded that there is no statistical correlation between PD-L1 by CPS and MMR status & HPV associated and independent cases of SCCs cervix. However, our study did not include DNA methylation analysis.

Min Feng et al. studied 219 cervical squamous cell tumors and 30 healthy controls in 2018. Cases had substantially higher PD-L1 levels with elevated TILs (p= 0.033), but not with age, tumor size, vascular invasion, lymph node-positive, TNM stage, or histologic grade.[51]

Feng et al conducted a study in 66 tissue samples of squamous cell carcinoma cases in 2018. They found that women between the ages of 35 and 55 years with cervical cancer, had the highest PD-L1 expression in tumor cells, while patients under the age of 35 years had a lower number of PD-L1 expressions. They also found that dMMR patients have more PD-L1

expression than pMMR patients, implying that PD-1/PD-L1 antibody medicines may be effective in dMMR cervical cancer patients.[66]

LIU et al. conducted a study on SCCs cervical carcinomas in 2016 (40 patients). They concluded that PD L1 overexpression induced by HPV16E7 may be responsible for lymphocyte malfunction. Furthermore, by blocking the PD-L1/PD-1 signaling pathway, soluble PD-1 may restore the function of tumor-infiltrating lymphocytes. These findings could lead to new immunotherapeutic methods for the treatment of cervical cancer. [65]

In 2017, Emeka K Enwere et al. studied 120 women with locally advanced cervical cancer (International Federation of Gynecology and Obstetrics stages IB to IVA). They found that there was a trend towards worse progression-free survival in patients whose tumors expressed PD-L1 but lacked tumor-infiltrating lymphocytes (p=0.053). Nevertheless, the high percentage of cervical cancer tumor samples expressing PD-L1 suggests that anti-PD-L1 or anti-PD-1 therapies are potential treatment options for this patient population.[38]

In 2021, Mori et al conducted a study on 75 patients with uterine cervical squamous cell carcinomas. The PD-L1+ rate increased significantly from 5% (4/75) before radiotherapy to 52% (39/75) following radiotherapy (p=0.01). Because radiotherapy increased PD L1 expression in cervical cancer specimens, immune checkpoint medications may be helpful in patients who have received radiotherapy. They also found no statistically significant relationship between changes in TILs density and PD-L1 expression and prognosis. [55]

Liang et al. investigated 142 paired SCCs specimens recovered from cervical cancer patients before and after platinum-based neoadjuvant chemotherapy (NAC) in 2020. They concluded that chemotherapy based on cisplatin can increase PD-L1 expression in cervical carcinoma. The enhanced PD-L1 expression and lymphocyte-predominant microenvironment following chemotherapy provide a rationale for using a PD-1/PD-L1 axis inhibitor in the neoadjuvant setting.[49]

L. Reiniger et al. investigated 268 lung adenocarcinoma patients in 2019 and correlated the data such as smoking, COPD, tumor stage, necrosis, lepidic growth pattern, vascular invasion, stromal immune cells density and EGFR/KRAS status of the tumors with PD-L1 expression. They found that PD-L1 expression of tumor cells with necrosis and tumor grade were significantly correlated ($p < .001$).[71]

A. M. Heeren et al. conducted a study in 2016 on two cohorts of 156 cases of SCCs of the cervix.

They found that 122 cases out of 156 (78.20%) had stromal immune cells with PD-L1 expression while 34 cases out of 156 (21.8%) had low stromal immune cells with PD-L1 expression. [58]

Reddy et al. conducted a study on 74 cervix squamous cell carcinomas (SCCs) in 2017. There were 7 well-differentiated tumors with patients age ranging from 37 to 68 years, with a median of 46 years and among them, 3 (42.9 %) of the cases had positive PD-L1 expression. There were 52 moderately differentiated cases out of 74, with patients age ranging from 30 to 67, with a median of 44.5 years and 24 (46.2 %) of the cases had positive PD-L1 expression. The poorly differentiated tumor included 15 individuals ranging in age from 30 to 59 years, with a median of 39 years and only one (6.7%) case had positive PD-L1 expression.[42]

Nicoletta D'Alessandris et al. conducted a study in 2020, on 38 cervical SCCs patients to evaluate PD-L1 expression and TILs. They found that the percentage of PD-L1+ neoplastic cells was connected with a high percentage of TILs ($p = 0.0073$) and increased PD-L1 expression on inflammatory cells ($p = 0.0297$). They concluded that cervical cancer might be a potential immunotherapy target.[64]

Ying Meng et al. conducted a study in 2018 on 97 SCCs of the cervix patients and 30 cases of the normal cervix to assess PD-L1 expression and HPV status in cervical cancer by immunohistochemistry staining. 78 cervical cancer patients had HPV infection and 19 cases who had not. The finding showed that PD-L1 was more frequently found in cervical cancer tissues than in normal tissues, particularly those with high levels of HPV staining.[60]

Rotman et al conducted a study in 2020 on 44 cases that were histologically diagnosed as SCCs cervix and were assessed for PD-L1/PD-L2 protein expression by immunohistochemistry (IHC) on tissue microarrays. They found that stromal field (tumor-infiltrating lymphocytes) cells were PD-L1 positive in 83% of the cases. They also found that PD-L1 expression on tumor cells was strongly correlated ($p < 0.001$). PD-L1 and a strong correlation on cells in stromal fields ($p < 0.001$) were also noted.[69]

Karim et al. conducted a study in 2020 on 88 patients with SCCs of the cervix who were immunohistochemically analyzed for PD-L1/PD-L1 protein expression (IHC).

They found that PD-L1 was expressed in 23% of the cases (20 cases out of 88 patients) and negative in 68 out of 88 cases (77%). They found that PD-L1 is expressed by a large number of invading TILs, implying that inhibiting PD-L1 could be beneficial in cervical cancer patients.[70]

Brooke E. Howitt et al. evaluated 48 cervical SCCs for immunohistochemistry (IHC) expression of PD-L1 on formalin-fixed, paraffin-embedded (FFPE) biopsy specimens in 2016. They found that immunohistochemical labeling with a PD-L1 antibody exhibited high protein expression in 95% of the cancer cells with a predominantly membranous staining pattern.

They concluded that a high proportion of individuals with these tumors are rational candidates for clinical trials of PD-1 inhibition based on genetic analysis of the same sample along with PD-L1 expression.[72] [37]

Tahmineh Haidary et al. conducted a study in 2019 on 60 cervical SCCs to evaluate PD-L1 expression and clinical significance. Patients aged 30 to 55 years had a greater rate of PD-L1 expression and a significant correlation between age and PD-L1 expression on tumor cells was found ($p = 0.047$).[59]

Mismatch Repair status and clinicopathological parameters

According to the results published in the literature, microsatellite instability has been reported in invasive cervical carcinomas as well as pre-invasive lesions.[52]

In the present study out of 50 cases, 47 (94%) showed retained MMR status while only 3 (6%) showed MMR deficiency. One (33.34%) among the 3 MMR deficiency cases showed isolated PMS2 loss while isolated MLH1 loss was seen in 2 (66.66%) cases. All the dMMR cases were >50 years of age. None among the 14 patients (< 50 years of age) showed dMMR. Also, none of the patients >70 years of age showed dMMR. All the dMMR patients were in the 50-70 years age group. All the nonkeratinising SCCs were mismatch proficient. All the mismatch repair-deficient tumors were keratinising SCCs. However, no statistically significant association was seen between tumor grade and MMR status.

All the well-differentiated SCCs and poorly differentiated squamous cell carcinomas were MMR stable. MMR deficiency was seen only in moderately differentiated SCC. However, no statistically significant association was seen in age, tumor grade, TILs and MMR status. Most

of the MMR stable cases found an absence of tumor necrosis (33/47, 70.21%) Also two-thirds of the MMR deficient cases did not show necrosis. These findings showed a statistically significant association between tumor necrosis and MMR status (p=0.017).

T.K.H. Chung et al. conducted a study in 2001 with 50 cases of invasive SCCs of the cervix. They found that 7 cases (14%) displayed high-frequency microsatellite instability (MSI-H) and 6 (12%) displayed low-frequency microsatellite instability (MSI-L) i.e. MSI positive. The remaining 37 cases (74%) were microsatellite stable. From this study, they concluded that MSI is present in cervical squamous cell carcinomas and defects resulting in MSI may be related to tumor progression and possibly poor prognosis. However, they found that while statistical analysis of HPV infection, tumor grade, clinical stage and clinical status failed to reveal differences between MSI-positive and MSI-negative cases.[62]

In 2002, Y.-F. Wong et al. conducted a study on 93 SCCs samples that were evaluated for MSI. MSI-H and MSI-L were found in 11 (11.8%) and 16 (17.2%) of 93 cervical carcinomas, respectively, while microsatellite stable (MSS) was found in 66 (71%) cervical carcinomas. They found that the overall survival of MSI-positive patients was significantly lower than that of microsatellite stable patients (p=0.02). Although the rate of MSI-H increased with the disease stage (p=0.035).[56]

In 2018, Feng et al. conducted a study on 66 cases of SCCs cervix. They found that in 66 patients, 49 (74.2 %) were showed retained MSH2 and MLH1, 15 (22.7 %) were showed loss of either MSH2 or MLH1 and both MSH2 and MLH1 were lost in only two cases (3.1 %). They also found that the dMMR cases were 25.8%.[66]

A. Ciavattini et al. examined the immunohistochemistry expression of hMSH2 and hMLH1 in 28 cases of aggressive squamous cervical carcinomas in 2005. They found neoplastic stromal invasiveness is related to a significant loss of hMLH1 function. There was also a significant association between hMLH1 and hMSH2 immunostaining in invasive lesions ($r < 0.462$; $p < 0.0125$).[52]

Relation between PD -L1 and MMR status

In this study, all the dMMR SCCs of the cervix showed a CPS of <5 and one-third of the deficient MMR cases showed the absence of PD-L1 in the tumor. However, a statistically significant relation between MMR status and PD-L1 expression was not seen.

In 2018, Feng et al. conducted a study on 66 cases of squamous cell carcinomas tissue samples. They found that dMMR patients have more PD-L1 expression than pMMR patients, implying that PD-1/PD-L1 antibody therapies may be effective in dMMR cervical cancer patients. High PD-L1 expression in cancer cells contributes to loss of balance when patients have dMMR status, revealing no correlations between PD-L1 in cancer cells. Meanwhile, other immune molecules, such as immune cytokines, may help in the repair of the imbalance and the tumor microenvironment should achieve a fragile balance. PD-1/PD-L1 antibody therapies are capable of disrupting the precarious balance, resulting in good therapeutic outcomes. [66]

Tahmineh Haidary et al. conducted a study in 2019 on 60 cervical SCCs to evaluate PD-L1 expression and MMR status with clinical significance. Patients aged 30-55 years had a greater rate of PD-L1 expression and a significant correlation between age and PD-L1 expression on tumor cells was found ($p = 0.047$). They also found that the majority of people (91.7 %) have intact MMR. However, no statistical association was noted between MMR status and PD-L1 expression.[59]

JI et al. conducted a study on 20 cases of cervical cancer patients in 2021. The study was about the expression of programmed cell death protein 1 ligand 1(PD-L1), poly ADP-ribose polymerase-1(PARP1) and MMR status. The positive rates for PD-L1 were 70%. Microsatellite instability (MSI) was observed in 6 (30%) cases. The expression status of PD-L1 in the MSI subgroup was identical ($p = 0.004$). They concluded that immune checkpoint therapy was helpful in patients with PD-L1 positivity and MMR loss.[73]

Authors	Study	Study Results
Feng et al, in 2018	PDL1 expression and mismatch repair (MMR) status of SCCs, of the cervix, were assessed in 66 tissue samples of squamous cell carcinomas	• Strong positive, positive and negative PD-L1 expression was detected in 21 cases (31.8 %), 18 cases (27.3 %) and 27 cases (40.9 %) cases respectively. • 49 (74.2 %) were both MSH2 and MLH1 stable, 15 (22.7 %) were showed loss of either MSH2 or MLH1and both MSH2 and MLH1 were lost in 2 cases (3.1%) • dMMR cases were was 25.8 % (17 out of 66

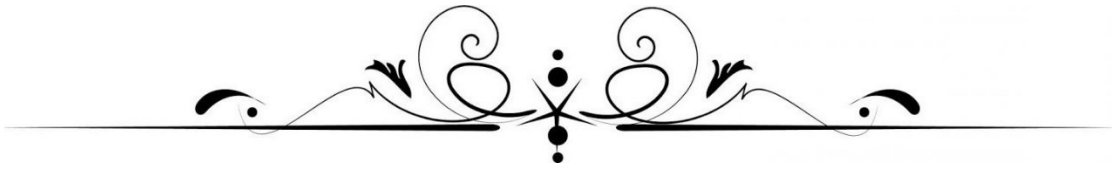
		cases). • dMMR patients have more PD-L1 expression than pMMR patients.
Tahmineh Haidary et al, 2019	• PD-L1 expression and MMR status with clinical significance.	• Patients aged 30-55 had a greater rate of PD-L1 expression and a significant correlation between age and PD-L1 expression in the tumor was found (p = 0.047). • Majority of people (91.7 %) had intact MMR. • No statistical association was noted between MMR status and PD-L1 expression.
LIU et al, 2016	PDL1 expression and HPV association assessed in cervical carcinoma (40 patients) and normal cervical tissue (8 persons)	• HPV16E7 and PD-L1 in cervical cancer were positively associated (r=0.531) and reached statistical significance (P=0.043). • PD L1 overexpression induced by HPV16E7 may be responsible for lymphocyte malfunction
Mori et al, 2021	PD-L1 expression studied in 75 uterine cervical carcinoma patients	• The PD L1 expressed in SCCs cervical cancer patients. • No statistically significant relationship between changes in TIL density and PD L1 expression was noted.
Liang et al, 2020	142 paired SCCs specimens of the cervix were studied for PD-L1 expression and lymphocyte microenvironment.	• The enhanced PD-L1 expression and lymphocyte-predominant microenvironment provide a rationale for using a PD-1/PD-L1 axis inhibitor.
A. Ciavattini, 2005	Expression of hMSH2 and hMLH1 in 28 cases of	• Neoplastic stromal invasiveness was related to a loss of hMLH1 function. • Significant association between hMLH1 and

	aggressive squamous cervical carcinomas	hMSH2 was seen in invasive lesions ($r < 0.462$; $p < 0.0125$).
Y.-F. Wong et al, 2002	93 cases of SCCs of cervix samples were studied for microsatellite instability status	• • MSI-H and MSI-L were seen in 11 (11.8%) and 16 (17.2%) of 93 cervical carcinomas, respectively, while microsatellite stability (MSS) was seen in 66 (71%) cervical carcinomas. • The MSI-H increased with the disease stage (P 0.035).
Z Chinn et al, 2017	65 cases of cervical SCCs assessed for PD-L1 expression with CPS.	• 14 of 16 cases (88 %) showed PD-L1 expression in CPS.
EK Enwere et al, 2017	120 patients of SCCs of the cervix were assessed for PD-L1	• The high percentage of cervical cancer tumor samples expressing PD-L1 suggests that anti-PD-L1 therapies are potential treatment options for this patient population.
Min Feng et al, 2018	219 cervical squamous cell tumors and 30 healthy controls, assessed for PD-L1 and TILs other clinicopathological parameters.	• PD-L1 expression was found in 32.4 percent (71/219) of cervical carcinomas and 10.0 percent (22/219) of partial TILs. • Normal cervical epithelium did not express PD-L1. • The number of TILs was shown to be strongly related to PD-L1 expression in TILs. • Cases had substantially higher PD-L1 levels with elevated TILs ($P = 0.033$), but not with age, tumor size, vascular invasion, lymph node-positive, TNM stage, or histologic grade.
T.K.H. Chung et al, 2001	50 subjects, with invasive squamous cell carcinomas of	• 7 cases (14%) displayed high-frequency microsatellite instability (MSI-H) and 6 (12%) displayed low-frequency

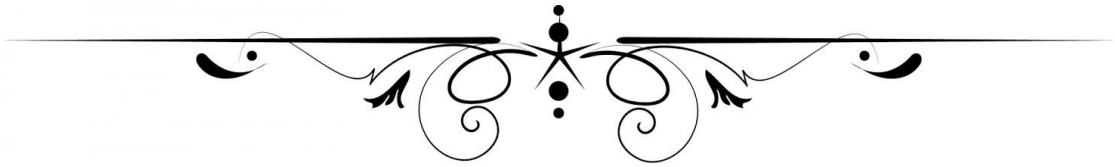
	the cervix, examined for MSI status	microsatellite instability (MSI-L). • 37 cases (74%) were microsatellite stable. • No statistical association was seen between MSI, HPV infection and tumor grade.
JI et al. 2021	The expression of programmed cell death protein 1 ligand 1(PD-L1) and MMR status in 20 cases of cervical cancer	• The PD-L1 was expressed in 70% of cervical cancer. Microsatellite instability (MSI) was observed in six (30%) of the cases. • The expression status of PD-L1 in the MSI subgroup was significant (p = 0.004).
Ying Meng et al, 2018	97 SCCs of the cervix patients and 30 cases of normal cervix assessed for PD-L1 expression and HPV status	• 78 cervical cancer patients were HPV associated and 19 cases were HPV independent. • PD-L1 was more frequently found in cervical cancer tissues than in normal tissues, particularly those with HPV association.
The current study	PDL1 expression and mismatch repair (MMR) status of SCCs, of the cervix, were assessed	• 40 out of 50 cases (80%), had a combined positive score > 1 (PD-L1 positive cases) whereas 10 out of 50 cases (20%), of cases, had a score of <1 (PD-L1 negative cases). • Out of 50 cases, 47 cases (94%) showed retained MMR protein (MMR stable) while only 3 cases (6%) showed MMR deficiency. • A statistically significant association was noted between PD-L1 expression in the tumor and the grade of the tumor. (p=0.022). • All the HPV independent SCC of the cervix showed PD-L1 expression. But, none of the HPV independent cases had a PD-L1 CPS of >50. • None of the HPV independent SCCs showed mismatch repair deficiency.

		• All the dMMR SCCs of the cervix showed a CPS of <5 and one-third of the deficient MMR cases showed the absence of PD-L1 in the tumor. • However, no statistically significant connection was noted between mismatch repair status and PD-L1 expression in tumors.

Table 45: Comparison of PD-L1 expression in tumor cells of the present study and other studies in squamous cell carcinomas of the cervix



SUMMARY & CONCLUSION



SUMMARY AND CONCLUSION

This was an ambispective observational study on 50 cases of squamous cell carcinoma of the cervix (SCC). Clinicopathological parameters, PD-L1 expression in tumor cells, tumor-infiltrating lymphocytes and mismatch repair deficiency status were studied. The salient findings are summarized below:

- The age of the patients ranged from 33 years to 81 years, with a mean age of 56.28 years and a standard deviation of 11.74. Maximum number of patients were between 60 to70 years of age group (17 out of 50, 34%).
- Forty out of 50 cases (80%) were moderately differentiated, 8 out of 50 cases (16%), were poorly differentiated and only 2 out of 50 cases (4%) were well-differentiated squamous cell carcinomas.
- Fifteen out of 50 cases (30%) showed tumor necrosis and 35 cases (70%) did not show tumor necrosis.
- All the cases showed tumor-infiltrating lymphocytes (TILs). Maximum cases had between 11-25% TILs (16/50 cases, 32%) and 10 cases (20% of the cases) had >50% TILs.
- Most of the cases (47/50, 94%), were HPV-associated SCC. Only 3 cases (6%), were HPV independent SCC.
- Most of the tumors expressed PD-L1, 40 out of 50 cases (80%). Maximum number of cases, 12 out of 50 cases (24 %), had PD-L1 expression in 1-5% of the tumor cells. 10 cases (20%) showed PD-L1 expression in >50% of the tumor cells.
- PD-L1 expression was noted in 84 % of tumor-infiltrating lymphocytes (PD-L1 expressing tumor had more TILs).
- Forty out of 50 cases (80%), had a combined positive score of > 1 (PD-L1 positive cases) whereas 10 out of 50 cases (20%), had a score of <1 (PD-L1 negative cases).
- All the HPV independent SCCs of the cervix showed PD-L1 expression. But, none of the HPV independent cases had a PD-L1, CPS of >50.
- Thirty-seven out of 47 HPV-associated SCCs cases showed PD-L1 expression and 6 (12.76%) HPV-associated cases had a PD-L1, CPS of >50. Ten out of 47 HPV-associated cases, (21.27%) had a score of <1 (PD-L1 negative cases).
- None of the nonkeratinising SCCs had a CPS score of >50.

- A statistically significant association was noted between PD-L1 expression in the tumor and the grade of the tumor. Poorly differentiated SCC showed more PD-L1 expression than well-differentiated and moderately differentiated tumors. (P=0.022).
- No statistically significant association was seen between age, PD-L1 expression in tumor, TILs, tumor necrosis, HPV association and PD-L1 expression.
- Mismatch repair deficiency was noted in 3 out of 50 cases (6%) of cases of squamous cell carcinomas of the cervix.
- All the dMMR cases were >50 years of age. None of the patients >70 years of age showed MMR deficiency. All the dMMR patients were in the 50 to 70 years age group.
- Most of the MMR stable cases showed an absence of tumor necrosis (33/47, 70.21%). Also, two-third of the MMR deficient cases did not show necrosis. However, this finding is not statistically significant.
- All the dMMR SCCs of the cervix showed a CPS of <5 and one-third of the deficient MMR cases showed the absence of PD-L1 in the tumor.
- All the MMR deficient cases (3 cases) had >10% of TILs. Eleven out of 47 MMR stable cases (23.40%) had <10% TILs. However, there was no statistically significant association between the MMR status of tumors and TILs.
- There was no statistically significant association between MMR status and age, tumor grade, tumor necrosis, TILs and HPV association of tumor.
- No statistically significant association was seen between PD-L1 expression in tumor and MMR status.

In conclusion, the majority of the cases were in the age group 61-70years. 80% were diagnosed with moderately differentiated squamous cell carcinomas and 94% of cases were HPV associated. All the cases showed the presence of tumor infiltrating lymphocytes (TILs) and maximum cases had between 11-25% TILs (32%). PD-L1 expression was seen in 80% of the tumors. 80%, had a combined positive score > 1 (PD-L1 positive cases) whereas 20%, of cases, had a score of <1 (PD-L1 negative cases). A statistically significant association was noted between PD-L1 expression in the tumor and the grade of the tumor (p=0.022). Forty-seven cases (94%) were mismatch repair stable while only 3 cases (6%) showed mismatch repair deficiency. Our study did not find any significant association between the mismatch repair status and PD-L1 expression in the tumors, however, this could be due to the limited sample size of our study. Additional studies with a larger sample size are required to enable the selection of patients who are likely to benefit from targeted therapy.

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ANNEXURES

ANNEXURES

ANNEXURE - 1

ETHICAL JUSTIFICATION

- Informed written consent was taken from all study subjects. No pressure or coercion was exerted on subjects for participation in the study.

- Confidentiality and privacy was maintained at all stages.

- Enrolment in the study did not pose any additional risk to the patient and did not increase the cost of the treatment. Informed written consent was taken from the patient/guardian of all the patients as per the attached proforma.

अखिल भारतीय आयुर्विज्ञान संस्थान, जोधपुर
All India Institute of Medical Sciences, Jodhpur
संस्थागत नैतिकता समिति
Institutional Ethics Committee

No. AIIMS/IEC/2020/207) **Date: 01/01/2020**

ETHICAL CLEARANCE CERTIFICATE

Certificate Reference Number: **AIIMS/IEC/2019-20/949**

Project title: **"Expression of programmed cell death ligand 1 (PD-L1) and mismatch repair status in squamous cell carcinoma of cervix"**

Nature of Project:	**Research Project**
Submitted as:	**M.D. Dissertation**
Student Name:	**Dr.Anju.G**
Guide:	**Dr.Meenakshi Rao**
Co-Guide:	**Dr.Poonam Elhence, Dr.Aasma Nalwa & Dr.Pratibha Singh**

This is to inform that members of Institutional Ethics Committee (Annexure attached) met on **23-12-2019** and after through consideration accorded its approval on above project. Further, should any other methodology be used, would require separate authorization.

The investigator may therefore commence the research from the date of this certificate, using the reference number indicated above.

Please note that the AIIMS IEC must be informed immediately of:

- Any material change in the conditions or undertakings mentioned in the document.
- Any material breaches of ethical undertakings or events that impact upon the ethical conduct of the research.

The Principal Investigator must report to the AIIMS IEC in the prescribed format, where applicable, bi-annually, and at the end of the project, in respect of ethical compliance.

AIIMS IEC retains the right to withdraw or amend this if:

- Any unethical principle or practices are revealed or suspected
- Relevant information has been withheld or misrepresented

AIIMS IEC shall have an access to any information or data at any time during the course or after completion of the project.

On behalf of Ethics Committee, I wish you success in your research.

Dr. Praveen Sharma
Member secretary
Institutional Ethics Committee
AIIMS,Jodhpur

Enclose:
1. Annexure 1

Basni Phase-2, Jodhpur, Rajasthan-342005, **Website:** www.aiimsjodhpur.edu.in, **Phone:** 0291-2740741 Extn. 3109
Email: ethicscommittee@aiimsjodhpur.edu.in

ANNEXURE - 2

All India Institute of Medical Sciences Jodhpur, Rajasthan

Informed consent form (English)

Title of the project: **Expression of Programmed cell Death Ligand 1 (PD-L1) and Mismatch Repair Status in Squamous cell carcinomas of the cervix.**

Name of the Principal Investigator : Dr. Anju.G Tel. No. 948680927

Patient/Volunteer Identification No. : _____

I, _____ S/o or D/o _____

R/o_____

give my full, free, voluntary consent to be a part of the study during 2018-2021, the procedure and nature of which has been explained to me in my own language to my full satisfaction. I confirm that I have had the opportunity to ask questions.

I understand that my participation is voluntary and am aware of my right to opt out of the study at any time without giving any reason.

I understand that the information collected about me and any of my medical records may be looked at by Doctors from AIIMS, Jodhpur. I give permission for these individuals to have access to my records.

Date: _____ _____

Place: _____ Signature/Left thumb impression

This to certify that the above consent has been obtained in my presence.

Date: _____ _____

Place: _____ Signature of Principal Investigator

Witness 1

_____

Signature/ Left thumb impression

Name: _____

Address: _____

Witness 2

_____

Signature/ Left thumb impression

Name: _____

Address: _____

ANNEXURE - 3

All India Institute of Medical Sciences (AIIMS)
Jodhpur, Rajasthan
Informed consent form (Hindi)

थीसिस/निबंध का शीर्षक :

पीजी छात्र का नाम: डॉ. अंजू टेलीफोन: 9486809267

रोगी/स्वयं सेवक पहचान संख्या: _____

मैं,_____एस/ओयाडी/ओ_____

_____आर/ओ_____

_____अध्ययन"_____" का एक भाग बनने के लिए मेरी पूर्ण, स्वतंत्र, स्वैच्छिक सहमति दें, जिसकी प्रक्रिया और प्रकृति मुझे अपनी पूरी संतुष्टि के लिए अपनी भाषा में समझाई गई है। मैं पुष्टि करता हूं कि मुझे प्रश्न पूछने का अवसर मिला है।

मैं समझता हूं कि मेरी भागीदारी स्वैच्छिक है और मुझे किसी भी कारण दिए बिना किसी भी समय अध्ययन से बाहर निकलने के मेरे अधिकार की जानकारी है।

मैं समझता हूं कि मेरे और मेरे मेडिकल रिकॉर्ड के बारे में एकत्रित की गई जानकारी

को_____(कंपनी नाम) या विनियामक प्राधिकरणों से जिम्मेदार व्यक्ति द्वारा देखा जा सकता है। मैं इन व्यक्तियों को अपने अभिलेखों तक पहुंच के लिए अनुमति देता हूं।

तारीख : _____

जगह: _____ हस्ताक्षर/बाएं अंगूठे का छाप_____

यह प्रमाणित करने के लिए कि मेरी उपस्थिति में उपरोक्त सहमति प्राप्त की गई है।

तारीख : _____

जगह: _____ पीजी छात्र के हस्ताक्षर_____

गवाह1: _____ हस्ताक्षर: _____ तारीख : _____	**गवाह 2: : _____** **हस्ताक्षर: _____** **तारीख : _____**

ANNEXURE - 4

Patient Information Sheet

1. Risks to the patients: No interventions or life-threatening procedures will be done.
2. Confidentiality: Your participation will be kept confidential. Your medical records will be treated with confidentiality and will be revealed only to doctors/ scientists involved in this study. The results of this study may be published in a scientific journal, but you will not be identified by name.
3. Provision of free treatment for research related injury - Not applicable.
4. Compensation of subjects for disability or death resulting from such injury - Not Applicable.
5. Freedom of the individual to participate and to withdraw from the research at any time without penalty or loss of benefits to which the subject would otherwise be entitled.
6. You have complete freedom to participate and to withdraw from the research at any time without penalty or loss of benefits to which you would otherwise be entitled.
7. Your participation in the study is optional and voluntary.
8. The copy of the results of the investigations performed will be provided to you for your record.
9. You can withdraw from the project at any time and this will not affect your subsequent medical treatment or relationship with the treating physician.
10. Any additional expense for the project, other than your regular expenses, will not be charged from you.

ANNEXURE - 5

रोगी सूचना पत्रक

1. रोगियों के लिए जोखिम: कोई हस्तक्षेप या जीवन-धमकी प्रक्रिया नहीं की जाएगी।
2. गोपनीयता: आपकी भागीदारी गोपनीय रखी जाएगी। आपके मेडिकल रिकॉर्ड का इलाज गोपनीयता के साथ किया जाएगा और इस अध्ययन में शामिल डॉक्टरों / वैज्ञानिकों के लिए ही खुलासा किया जाएगा। इस अध्ययन के परिणाम वैज्ञानिक पत्रिका में प्रकाशित किए जा सकते हैं, लेकिन आपको नाम से पहचाना नहीं जाएगा।
3. अनुसंधान से संबंधित चोट के लिए नि: शुल्क उपचार की व्यवस्था - लागू नहीं है।
4. ऐसी चोट से होने वाली विकलांगता या मृत्यु के लिए विषयों का मुआवजा - लागू नहीं है।
5. किसी भी समय जुर्माना या लाभ के नुकसान के बिना व्यक्तिगत रूप से भाग लेने और अनुसंधान से वापस लेने के लिए स्वतंत्रता की स्वतंत्रता, जिसके विषय में अन्यथा हकदार होगा।
6. आपके पास किसी भी समय जुर्माना या लाभ के नुकसान के बिना भाग लेने और अनुसंधान से वापस लेने की पूर्ण स्वतंत्रता है जिसके लिए आप अन्यथा हकदार होंगे।
7. अध्ययन में आपकी भागीदारी वैकल्पिक और स्वैच्छिक है।
8. किए गए जांच के परिणामों की प्रति आपके रिकॉर्ड के लिए आपको प्रदान की जाएगी।
9. आप किसी भी समय परियोजना से वापस ले सकते हैं, और यह आपके बाद के चिकित्सा उपचार या उपचार चिकित्सक के साथ संबंधों को प्रभावित नहीं करेगा।
10. आपके नियमित खर्चों के अलावा, परियोजना के लिए कोई भी अतिरिक्त खर्च आपसे शुल्क नहीं लिया जाएगा।

ANNEXURE - 6

All India Institute of Medical Sciences (AIIMS), Jodhpur
Department of Pathology and Lab Medicine
PROFORMA

Date:

Name

Age: Sex: I.D:

Address:

Relevant clinical History:

Family history of malignancy:

Histological diagnosis (with the histopathological stage if available):

Requested information for optimal patient care:

(1) Known/Previous malignancy: ☐ (2) Clinical Tumor staging information: ☐

(3) Immunocompromised: ☐ (4) Chemotherapy: ☐ (5) Radiotherapy: ☐

(6) IHC: PD-L1

(a) Intensity of stain

- No stain: 0 ☐
- Weak stain: 1+ ☐
- Moderate: 2+ ☐
- Intense: 3+ ☐

(b) Positive cell percentage

- 0 :< 5% cells ☐
- 1: 5% - 25% cells ☐
- 2: 26% -50% cells ☐
- 3: 51% - 75% cells ☐
- 4: >=75% ☐

(7) Mismatch repair deficiency:

IHC	RETAINED	LOST
MLH 1	☐	☐
MSH 2	☐	☐
MSH 6	☐	☐
PMS 2	☐	☐

Sl No	AIIMS ID	Histopathology Number	Name	Age	Specimen	Diagonosis	PD-L 1	PMS2	MLH1	MSH2	MLH6	MMR	P16	TILS in tu	PD-L1 in T	PD-L1 Combin	Necrosis	Lymphova	Perineural i	Dysplasia
1	AIIMS/JDH/2017/12/013549	H/0064/18	Gero Devi	58	Biopsy (cervi	keratinizing squamous cell carcinoma (moderately differentiated)	Expressed	Retained	Retained	Retained	Retained	Retained	Block positive	45	15	5	8 Absent	Absent	Absent	Present
2	AIIMS/JDH/2018/01/022288	H/0174/18	RAMI DEVI	47	Biopsy (cervi	moderately differentiated squamous cell carcinoma	Lost	Retained	Retained	Retained	Retained	Retained	Block positive	54	0	0	0 Absent	Absent	Absent	Absent
3	AIIMS/JDH/2018/02/00039	H/0620/18	Sayar Devi	62	Biopsy (cervi	Poorly differentiated squamous cell carcinoma.	Lost	Retained	Retained	Retained	Retained	Retained	Block positive	20	0	0	0 Absent	Absent	Absent	Absent
4	AIIMS/JDH/2018/02/000194	H/0621/2018	Bidami Devi	60	Biopsy (cervi	Moderately differentiated keratinizing squamous cell carcinoma.	Lost	Retained	Retained	Retained	Retained	Retained	Block positive	55	0	0	0 Absent	Absent	Absent	Present
5	AIIMS/JDH/2018/02/007307	H/0880/18	Parvati	61	Biopsy, cervi	Squamous cell carcinoma - Moderately Differentiated.	Expressed	Retained	Retained	Retained	Retained	Retained	Block positive	18	5	20	20 Absent	Absent	Absent	Present
6	AIIMS/JDH/2018/03/004694	H/1314/18	SHOBHA	46	Biopsy cervix	Keratinizing Squamous cell carcinoma (moderately differentiated).	Expressed	Retained	Retained	Retained	Retained	Retained	Block positive	35	50	30	40 Absent	Absent	Absent	Absent
7	AIIMS/JDH/2018/03/004730	H/1418/18	Shanti Devi	66	Biopsy cervix	moderately differentiated(keratinizing) squamous cell carcinoma.	Lost	Retained	Retained	Retained	Retained	Retained	Block positive	10	0	0	0 Absent	Absent	Absent	Absent
8	AIIMS/JDH/2018/04/001798	H/2019/18	Chunni Devi	56	Biopsy cervix	moderately differentiated keratinizing squamous cell carcinoma	Lost	Lost	Retained	Retained	Retained	Lost	Block positive	25	0	0	0 Absent	Absent	Absent	Present
9	AIIMS/JDH/2018/07/018427	H/4717/18	Sukhi	49	Cervical biop	Poorly differentiated Squamous cell carcinoma.	Expressed	Retained	Retained	Retained	Retained	Retained	Block positive	22	45	25	23 Absent	Absent	Absent	Absent
10	AIIMS/JDH/2018/07/015704	H/4824/18	Parvati	61	Biopsy, cervi	Keratinizing squamous cell carcinoma,Well differeentited	Expressed	Retained	Retained	Retained	Retained	Retained	Block positive	5	5	5	10 Absent	Absent	Absent	Absent
11	AIIMS/JDH/2018/08/017146	H/5297/18	Dhapu	75	Biopsy, Cervi	moderately differentiated keratinizing squamous cell carcinoma	Lost	Retained	Retained	Retained	Retained	Retained	Block positive	60	0	0	0 Absent	Absent	Absent	Absent
12	AIIMS/JDH/2018/08/018017	H/5603/18	Geeta Devi	37	Biopsy, cervi	Non-keratinizing squamous cell carcinoma, moderately differentiated	Expressed	Retained	Retained	Retained	Retained	Retained	Not associated	5	15	5	20 Absent	Absent	Absent	Absent
13	AIIMS/JDH/2018/12/001867	H/0335/2019	Champa	64	Cervical Biop	Moderately differentiated keratinizing squamous cell carcinoma.	Expressed	Retained	Retained	Retained	Retained	Retained	Not associated	5	50	15	36 Absent	Absent	Absent	Absent
14	AIIMS/JDH/2019/01/030463	H/0846/2019	Papu Devi	35	Cervical biop	Moderately differentiated keratinizing squamous cell carcinoma	Expressed	Retained	Retained	Retained	Retained	Retained	Block positive	10	4	4	3.2 Absent	Absent	Absent	Absent
15	AIIMS/JDH/2019/02/005294	H/2183/2019	Saku	53	Cervical biop	Moderately differentiated keratinizing squamous cell carcinoma.	Expressed	Retained	Lost	Retained	Retained	Lost	Block positive	45	2	3	1.4 Absent	Absent	Absent	Present
16	AIIMS/JDH/2019/04/013496	H/2974/2019	Mohan Kanwar	40	Cervical biop	Moderately differentiated keratinizing squamous cell carcinoma.	Expressed	Retained	Retained	Retained	Retained	Retained	Block positive	5	50	40	36 Present	Absent	Absent	Absent
17	AIIMS/JDH/2019/05/008031	H/3295 /2019	Champa Devi	46	Cervical biop	Poorly differentiated squamous cell carcinoma	Expressed	Retained	Retained	Retained	Retained	Retained	Block positive	56	0.5	0.5	0.5 Present	Absent	Absent	Absent
18	AIIMS/JDH/2019/05/020694	H/3858 /2019	Patu Devi	58	Cervical biop	Features are of large cell non keratinizing Squamous Cell Carcinoma	Expressed	Retained	Retained	Retained	Retained	Retained	Block positive	25	2	30	10 Absent	Absent	Absent	Absent
19	AIIMS/JDH/2019/06/004781	H/4083 /2019	Panni Devi	45	cervix	Poorly differentiated, Non keratinizing squamous cell carcinoma.	Expressed	Retained	Retained	Retained	Retained	Retained	Block positive	30	70	55	56 Present	Absent	Absent	Present
20	AIIMS/JDH/2019/06/004929	H/4145/2019	Jadav Kanwar	66	Cervical biop	Keratinizing squamous cell carcinoma ,Well differeentited	Expressed	Retained	Retained	Retained	Retained	Retained	Block positive	45	10	5	6 Absent	Absent	Absent	Absent
21	AIIMS/JDH/2019/05/019505	H/4339/2019	Magu Kanwar	55	Cervical biop	Non-keratinizing squamous cell carcinoma, with focally basaloid morphology,moderately differentiated	Expressed	Retained	Retained	Retained	Retained	Retained	Not associated	70	80	40	48 Absent	Absent	Absent	Present
22	AIIMS/JDH/2019/06/015465	H/4524/2019	Margo Devi	43	Cervical biop	Moderately differentiated keratinizing squamous cell carcinoma.	Expressed	Retained	Retained	Retained	Retained	Retained	Block positive	4	5	20	14 Absent	Absent	Absent	Present
23	AIIMS/JDH/2019/06/015465	H/4525 /2019	Margo Devi	55	Cervical biop	Morphology is of Squamous cell carcinoma. Moderatly differeentited	Expressed	Retained	Retained	Retained	Retained	Retained	Block positive	15	70	70	60 Absent	Absent	Absent	Present
24	AIIMS/JDH/2019/07/002323	H/4813/19	Jamku Devi	70	Cervical biop	Squamous cell Carcinoma,Moderatly differeentited	lost	Retained	Retained	Retained	Retained	Retained	Block positive	5	0	0	0 Absent	Absent	Absent	Present
25	AIIMS/JDH/2019/07/009544	H/5118/19	Choudhi Devi	69	Cervical biop	Moderately differentiated keratinizing squamous cell carcinoma	Expressed	Retained	Retained	Retained	Retained	Retained	Block positive	35	50	35	24 Absent	Absent	Absent	Present
26	AIIMS/JDH/2017/10/012945	H/5453/19	Banaphula	81	Cervical biop	Moderately differentiated keratinizing squamous cell carcinoma	Expressed	Retained	Retained	Retained	Retained	Retained	Block positive	5	80	75	62 Absent	Absent	Absent	Present
27	AIIMS/JDH/2019/07/018156	H/5483/19	Ratni Devi ..	66	Biopsy, cervi	Poorly differentiated non-keratinizing squamous cell carcinoma	Expressed	Retained	Retained	Retained	Retained	Retained	Block positive	65	75	25	40 Present	Absent	Absent	Absent
28	AIIMS/JDH/2019/07/021514	H/5653/19	Sugni Devi	81	Biopsy (cervi	Moderately Differentiated Keratinizing Squamous cell carcinoma	Expressed	Retained	Retained	Retained	Retained	Retained	Block positive	25	2	1	0.8 Present	Absent	Absent	Absent
29	AIIMS/JDH/2019/08/000157	H/5664/2019	Viju Devi	33	Biopsy (cervi	Moderately differentiated keratinizing squamous cell carcinoma	Expressed	Retained	Retained	Retained	Retained	Retained	Block positive	45	5	5	4 Present	Absent	Absent	Present
30	AIIMS/JDH/2019/01/030409	H/5754/19	Sundar Devi	66	Biopsy (cervi	Porly differentiated squamous cell carcinoma	Expressed	Retained	Retained	Retained	Retained	Retained	Block positive	35	75	45	48 Present	Absent	Absent	Absent
31	AIIMS/JDH/2019/08/005387	H/5914/19	Suwa Devi	52	Biopsy (cervi	Moderately differentiated Squamous cell carcinoma.	Expressed	Retained	Retained	Retained	Retained	Retained	Block positive	15	20	12	16 Present	Absent	Absent	Absent
32	AIIMS/JDH/2019/08/008146	H/6412/19	Nenudi	52	Cervix biopsy	Moderately differentiated keratinizing squamous cell carcinoma	Expressed	Retained	Retained	Retained	Retained	Retained	Block positive	12	25	25	20 Present	Absent	Absent	Present
33	AIIMS/JDH/2020/01/017801	H/0041/2020	MOHANIC DEV	67	Cervical biop	Moderately to poorly differentiated squamous cell carcinoma.	Expressed	Retained	Retained	Retained	Retained	Retained	Block positive	15	5	2	3.5 Present	Absent	Absent	Present
34	AIIMS/JDH/2020/01/021020	H/0261/20	Anasi Devi	62	Biopsy cervix	Keratinizing Squamous cell carcinoma. Moderatly differeentited	Expressed	Retained	Retained	Retained	Retained	Retained	Block positive	22	0	5	2 Present	Absent	Absent	Absent
35	AIIMS/JDH/2019/10/001571	H/0802 /20	Jasu Devi	70	Biopsy, cervi	Moderately differentiated non- keratinizing squamous cell carcinoma.	Expressed	Retained	Lost	Retained	Retained	Lost	Block positive	35	5	5	2 Present	Absent	Absent	Absent
36	AIIMS/JDH/2020/01/031246	H/0810 /2020	Kamla Devi	62	Biopsy - cervi	Moderately differentiated keratinizing squamous cell carcinoma	Expressed	Retained	Retained	Retained	Retained	Retained	Block positive	40	0	5	0.2 Absent	Absent	Absent	Present
37	AIIMS/JDH/2018/03/002412	H/0903 /2020	Geeta Devi.	66	Biopsy - cervi	Poorly differentiated Squamous cell carcinoma.	Expressed	Retained	Retained	Retained	Retained	Retained	Block positive	10	5	1	3 Present	Absent	Absent	Absent
38	AIIMS/JDH/2020/02/006259	H/1625 /20	Rani Devi	51	Biopsy, cervi	Moderately differentiated Squamous cell carcinoma	Expressed	Retained	Retained	Retained	Retained	Retained	Block positive	55	10	10	4 Absent	Absent	Absent	Absent
39	AIIMS/JDH/2020/02/015696	H/1696 /2020	Magi Devi	47	Cervical biop	moderately differentiated squamous cell carcinoma	Expressed	Retained	Retained	Retained	Retained	Retained	Block positive	30	10	5	6 Absent	Absent	Absent	Absent
40	AIIMS/JDH/2020/02/018262	H/1808 /2020	Chani Devi	60	Cervical biop	Poorly differentiated squamous cell carcinoma	Expressed	Retained	Retained	Retained	Retained	Retained	Block positive	25	80	70	64 Absent	Absent	Absent	Absent
41	AIIMS/JDH/2020/02/011649	H/1907/2020	Shanti	40	Cervical biop	Moderately differentiated squamous cell carcinoma	Expressed	Retained	Retained	Retained	Retained	Retained	Block positive	15	40	10	50 Present	Absent	Absent	Present
42	AIIMS/JDH/2020/05/001527	H/2523/20	Devi	51	Cervical biop	moderately differentiated keratinizing squamous cell carcinoma	Expressed	Retained	Retained	Retained	Retained	Retained	Block positive	25	90	40	60 Absent	Absent	Absent	Absent
43	AIIMS/JDH/2020/06/000811	H/2645 /20	Nani	56	Cervical biop	Moderately differentiated Squamous Cell Carcinoma	Expressed	Retained	Retained	Retained	Retained	Retained	Block positive	35	20	30	20 Absent	Absent	Absent	Present
44	AIIMS/JDH/2020/07/003325	H/3146/20	Prem Kanwar	58	Cervical biop	Keratinizing Squamous cell carcinoma.Moderatly differeentited	Expressed	Retained	Retained	Retained	Retained	Retained	Block positive	65	80	20	50 Absent	Absent	Absent	Present
45	Aiims/jdh/2020/07/006794	H/3223/20	Anjali	60	Cervical biop	moderately differentiated keratinizing Squamous cell carcinoma.	Expressed	Retained	Retained	Retained	Retained	Retained	Block positive	10	20	8	18 Absent	Absent	Absent	Absent
46	AIIMS/JDH/2020/08/004653	H/3724/20	Durga Devi	37	Cervical biop	Non keratinizing Squamous cell carcinoma,moderately differentiated	Expressed	Retained	Retained	Retained	Retained	Retained	Block positive	80	30	25	26 Absent	Absent	Absent	Absent
47	AIIMS/JDH/2020/09/001796	H/3843/20	Senki	65	Cervical biop	Moderately differentiated Squamous cell carcinoma.	Expressed	Retained	Retained	Retained	Retained	Retained	Block positive	15	5	5	4 Absent	Absent	Absent	Present
48	AIIMS/JDH/2020/10/009259	H/4847/20	Sua Devi	51	Cervical biop	Non-keratinizing squamous cell carcinoma,moderately differentiated	Expressed	Retained	Retained	Retained	Retained	Retained	Block positive	30	30	50	45 Absent	Absent	Absent	Present
49	AIIMS/JDH/2020/12/009019	H/24/2021	Indra	35	Cervical biop	Non-keratinizing squamous cell carcinoma,moderately differentiated	Expressed	Retained	Retained	Retained	Retained	Retained	Block positive	20	40	15	30 Present	Absent	Absent	Present
50	AIIMS/JDH/2020/12/009135	H/102/2021	Shanti	68	Cervical biop	Keratinizing squamous cell carcinoma.Moderatly differeentited	Expressed	Retained	Retained	Retained	Retained	Retained	Block positive	62	70	25	60 Absent	Absent	Absent	Present