“SIGNIFICANCE OF PROGRAMMED DEATH LIGAND-1 IN INVASIVE SQUAMOUS CELL CARCINOMA OF UTERINE CERVIX”

BY

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MBBS

DISSERTATION SUBMITTED TO
SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION & RESEARCH
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IN

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UNDER THE GUIDANCE OF

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DEPARTMENT OF PATHOLOGY
SRI DEVARAJ URS MEDICAL COLLEGE, KOLAR
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LIST OF ABBREVATIONS

1. HPV –Human Papilloma Virus
2. DNA –Deoxyribonucleic acid
3. PD-L1 -Programmed Death Ligand-1
4. PD-1 -Programmed Cell death protein-1
5. UTR -Untranslated region
6. TZ -Transformation zone
7. SCJ -Squamocolumnar Junction
8. IUCD -Intrauterine contraceptive devices
9. CIN -Cervical intraepithelial neoplasia
10. LSIL -Low grade squamous intraepithelial lesion
11. HSIL -High grade squamous intraepithelial lesion
12. LAST - Lower anogenital squamous terminology
13. DES -Diethylstilbestrol
14. OCP -Oral contraceptives
15. SCC -Squamous cell carcinoma
16. SCC NOS -Squamous cell carcinoma Not otherwise specified
17. FIGO - Federation of International Gynecologists and Obstetricians
18. SHP-1 -Src homology region domain-containing phosphatase-1
19. SHP-2 -Src homology region domain-containing phosphatase-2
20. ITSM -Immunoreceptor Tyrosine- Based Switch Motif
21. TNM -Tumor ,Node ,Metastasis
22. ORR -Overall Response Rate
23. HCT -Hematopoietic Cell Transplantation

24. irAES -Immune related Adverse effects

25. FDA -Food and Drug Adminstration

26. AIHA -Autoimmune Hemolytic anemia

27. OBG –Obstetrics and Gynaecology

28. USG -Ultrasonography

29. MRI -Magnetic Resonance Imaging

30. CT -Computed Tomography

31. DWI -Diffusion weighted imaging

32. ADC -Apparent diffusion coefficient

33. T1-FS -T1 Fat Saturation

34. HIER - Heat Induced epitope Retrieval

35. TRIS EDTA- Tris Ethylenediamine Tetraacetic Acid

36. TBS - TRIS buffer solution

37. DAB -Di-aminobenzidine

38. IHC -Immunohistochemistry

39. H& E –Hematoxylin and Eosin

ABSTRACT

Background :

Cervical cancer is the most common gynecological malignancy worldwide. Integration of viral (HPV) genome into the host genome with acquisition of other genetic abnormalities results in malignant transformation. PD-L1 is a trans-membrane protein assumed to play a major role in suppressing the adaptive arm of immune system. In carcinoma patients, PD-L1 is primary ligand of PD-1 and is expressed on tumor cells. The binding of PD-L1 to PD-1 leads to an immunosuppressive effect allowing the tumor to escape immune destruction. Normal cervical epithelium do not express PD-L1. PD-L1 expression noted in cervicaltumour cells suggest that the PD-1/PD-L1 pathway can be a potential immunotherapy targetin patients with cervical cancer. Information on the clinical importance of PD-L1 expression in cervical cancer is largely lacking.

Aim of the study:

To determine the expression of PD-L1 in Invasive Squamous cell carcinoma of uterine cervix and its correlation with clinico-pathological parameters.

Methods:

The study was conducted in Department of Pathology in Collaboration with Department of Obstetrics and Gynaecology, Sri Devaraj Urs Medical College attached to R.L Jalappa Hospital and Research centre, Tamaka, Kolar during the period of July 2016 to June 2018. The study included 90 cases of Cervical carcinoma diagnosed by histopathology.Immunohistochemistry was performed using antibodies against PD-L1. Expression of PD-L1

was documented and analyzed. PD-L1 expression was correlated with clinic-pathological parameters of carcinoma cervix. Statistical analysis was performed using **Chi-square test or Fischer's exact test.** A p value of less than 0.05 was considered substantially significant.

Results:

The average age of presentation of cervical carcinoma cases in current study was 52 years. Maximum number of Cervical Carcinoma cases were observed in the age group of 40-49 years (31.1%) followed by 50-59 years (24.4%) and 60-69 years (22.22%). As the age increases PD-L1 positive expression increases. 83.3% (10 out of 12 cases in 70-79 year age group) showed PD-L1 positive expression with **p value of 0.037**. Maximum number of cases were of moderately differentiated SCC around [47 cases (52.2%)] followed by poorly differentiated SCC [28 cases (31.1%)] and well differentiated SCC [15 cases(16.7%)]. Positive expression of PD-L1 was observed maximum in moderately differentiated SCC [40 cases (85.1%) out of 47]. In well differentiated SCC 8 cases (53.3%) out of 15 showed PD-L1 positive expression followed by poorly differentiated carcinoma in which 12 cases out of

28 cases(42.9%) showed PD-L1 positive expression with **p value <0.001**, there was statistical significant difference found between histological grading and PD-L1 expression. No statistical significant correlation was seen between PD-L1 expression and various other clinico-pathological parameters such as parity, clinical findings, lymph node status, staging, size of lesion and overall survival.

Conclusion:

This study observed 66.6% expression of PD-L1 in cervical squamous cell carcinoma. The findings in present study further support the role for investigation of anti-PD-L1/PD-1 immunotherapies for the treatment of PD-L1-positive cervical tumors. Future clinical trials

with larger population size and multi-centric studies can prove the role of PD-L1 in treatment of cervical carcinoma.

Keywords: Cervical cancer, PD-L1, Human papilloma Virus

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INTRODUCTION

INTRODUCTION

Cervical cancer is the commonest gynecological malignancy worldwide. Cervical cancer has the second highest mortality and morbidity rates among all tumors of female genital tract.[1] Worldwide cervical cancer is the fourth-most common cause of cancer and fourth most common cause of deaths in women. Worldwide 570,000 cases of cervical cancer wereestimated to have occurred with over 300,000 deaths. New cases of cervical cancer detectedin India were 96,922 in year 2018 and deaths due to cervical cancer in India reported were 60,078.In Karnataka alone 5,000 new cases are identified each year. In Bangalore, cervical cancer is the second most frequent cancer among women with 850 cases being diagnosed every year.[2] In a study done in Kolar, 17% cases were diagnosed of cervical carcinoma among all the cancers of females.[3] Human Papilloma virus (HPV) is a DNA (Deoxyribonucleic acid) Virus. HPV-16 and HPV-18 are the high risk genotypes for cervical carcinoma. Integration of viral genome into the Host genome with acquisition of othergenetic abnormalities results in malignant transformation.[4]

Cervical infection and over expression of HPV16 E7 in the carcinoma cervix increases PD-L1(Programmed Death Ligand-1) protein expression. Cervical carcinoma with HPV infection have high PD-L1 expression. Cervical carcinoma without HPV infection do not have high PD-L1 expression. High PD-L1 expression create an immunosuppressive state in uterine cervix increasing the time of HPV clearance. High HPV-16 L1 gene methylation and multipleHPV infection prolongs the time to clean HPV infection. HPV genome integrated into the PD-L1 gene locus induces increased PD-L1 expression by 3' untranslated region (UTR) disruption of PD-L1 gene a possible mechanism for high PD-L1 expression in premalignant cervical lesion with HPV infection. Anti PD-L1 Can be a potential effective way of treatmentof HPV infection in premalignant cervical lesion.[5]

Information on the clinical importance of PD-L1 expression in cervical cancer is largely lacking.[6] A few studies are published from the developed countries. There is paucity of studies from the developing countries on programmed death ligand-1 (PD-L1) with respect tocervical cancer.[7]

AIMS & OBJECTIVES

AIM AND OBJECTIVES OF THE STUDY:

To determine the expression of PD-L1 in Invasive Squamous cell carcinoma of uterine cervix and its correlation with clinico-pathological parameters.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

ANATOMY:

Uterus is divided into three parts anatomically: Corpus, isthmus and cervix. The protruding part of cervix into vagina is called as external os. Endocervix opens internally into uterus and externally opens into vagina.[8]

HISTOLOGY:

Endocervix:

Lining consists of monolayered mucin secreting columnar epithelium. Endocervical glands and endocervical canal is also lined by monolayered columnar epithelium.[8]

Ectocervix:

Lined by mature non-keratinizing stratified squamous epithelium. Epithelial layer is divided into three layers :Germinal/parabasal cell layer, intermediate cell layer and superficial cell layer. Germinal cell layer helps in continuous epithelial regeneration. Major part of epithelial lining is formed by midzone. Most Mature cell is observed in superficial layer.

TRANSFORMATION ZONE:

Squamo-columnar junction of cervix is defined as the junction between ectocervical stratified squamous epithelium and endocervical columnar epithelium. Two types of squamocolumnar junction; original Squmaocolumnar junction which is present at birth and physiological Squamoucolumnar junction which develops during menarche, The area between original Squamoucolumnar junction(SCJ) and physiological SCJ is called as the Transformation zone.(TZ). All Cervical cancers and precursor lesions of cervix arise from transformation zone (TZ).[8]

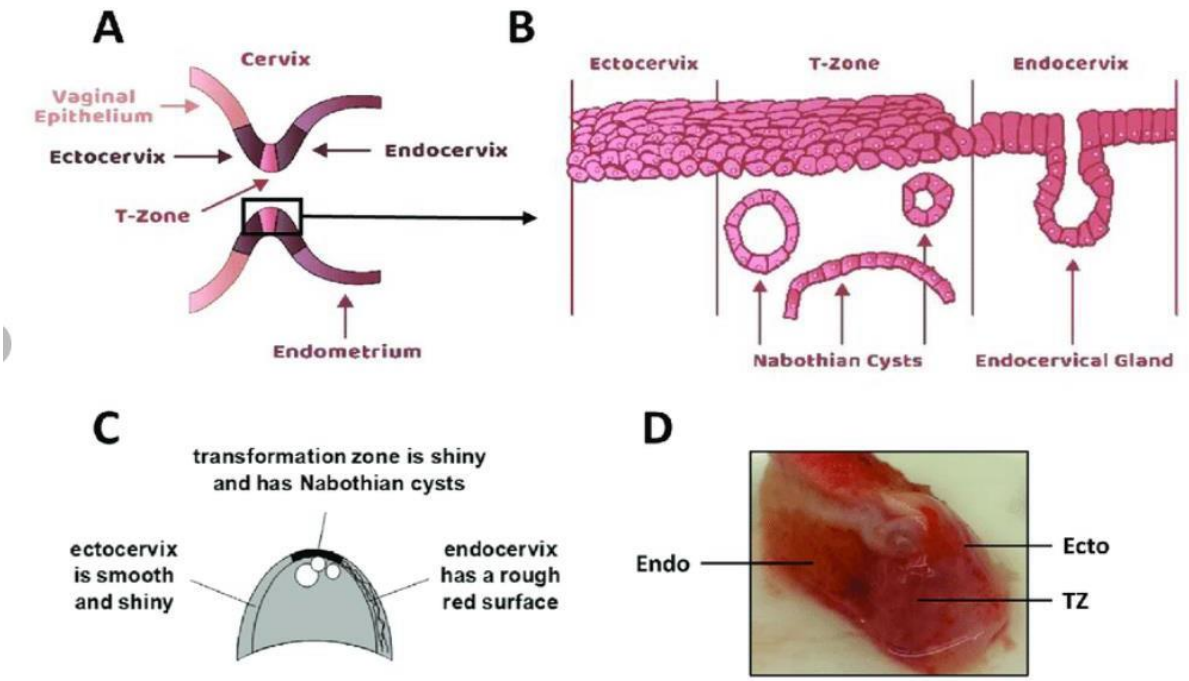

Figure 1

Structure and Histology of Transformation zone of cervix

A. Schematic diagram demonstrating the TZ between ectocervix and endocervix.

B. Histology of the cervical TZ depiciting stratified squamous epithelium and Nabothian cysts.

C. Diagram representing the surface features of ectocervix, endocervix and TZ that aid in tissue dissection.

The ectocervix surface is smooth, white and shiny without mucous.

The endocervix surface is red in color, rough surface and covered with mucous.

The TZ consists of Nabothian cysts.

D. Photograph of a cervical specimen demonstrating different region. [9]

CERVIX PATHOLOGY:

Inflammatory diseases

CERVICITIS: is inflammation of the cervical tissue. Based on the etiology , cervicitis is further subdivided into two subgroups : i.e non-infectious cervicitis and infectious cervicitis.[10]

NON-INFECTIOUS CERVICITIS: It is defined as non-specific inflammatory condition attributed to chemical and mechanical trauma. Common etiological factors are : Pessaries , Diaphragms , Tampons and IUCD (Intrauterine contraceptive devices). Iatrogenic cause such as instrumentation can also lead to non-infectious cervicitis. Other causes are chemicalirritation which occurs secondarily to douching.[10]

Histo-morphology of Non-infectious cervicitis: shows epithelium infiltrated by neutrophils ,stroma also shows neutrophilic infiltration and stromal edema , dilated and congested blood vessels. These features are suggestive of acute cervicitis. [10]

Chronic cervicitis:

Microscopically: shows epithelial infiltration by lymphocytes and plasma cells. Stromal also shows lymphoplasmacytic infiltration. Occasionally fibrosis of stroma , histiocytes and granulation tissue can be observed.[10]

INFECTIOUS CERVICITIS:

Etiological factors: A)Bacteria : Mycobacterium tuberculosis, Chlamydia trachomatis, Niesseria gonorrhea.

B) **Virus:** Herpes simplex virus , Human papilloma virus

C) Fungus: Candida, Aspergillus

D) Parasites : Amoeba, Schistostomes[8]

PRECANCEROUS CONDITIONS :

All precursor lesion of squamous cell carcinoma of cervix is represented as a single disease process termed as Cervical intraepithelial neoplasia(CIN).

CIN is divided into three subgroups:

CIN1: Mild dysplasia

CIN2: Moderate dysplasia

CIN 3: Carcinoma in situ (CIS) or Severe dysplasia

In cytology Betheseda system defines low grade squamous intraepithelial lesion (LSIL)for CIN1 and High grade squamous intraepithelial lesion (HSIL) for CIN 2 and CIN 3. This system is widely utilized for reporting of cytological pap smears. [11]

College of American Pathologists along with American society for colposcopy and Pathology suggested utilization of two tier system LSIL and HSIL in accordance with LAST 2012 (i.e lower anogenital squamous terminology) which is used for Histopathological reporting of precancerous lesions of squamous cell carcinoma of cervix.[12]

LSIL : features consists of koilocytosis, epithelial hyperplasia , nuclear atypia. Thesefeatures are associated with HPV (Human Papilloma virus) and it is restricted to lower one third of the squamous cell layer. Koilocytes are cells with perinuclear halo and thickened cytoplasmic membrane. 80 % of LSIL cases are self limiting and do not progress to High grade lesions and carcinoma.[13]

HSIL: CIN 2 and CIN3 features comprises of atypia in squamous epithelial layers affecting intermediate and superficial layers. Atypia is more significantly observed in basal and parabasal cells alongwith presence of mitotic figures. Additional features include anisonucleosis, nuclear pleomorphism and loss of Polarity with cellular crowding. Around 8% of HSIL cases develop into cervical carcinoma particularly in women with age more than 30 years.[13]

CERVICAL CANCER:

Peak age range for cervical cancer reported is 55 -59 years with average age of presentation around 52 years. The risk factors for carcinoma cervix are Human Pailloma virus(HPV) , sexually transmitted infections –Coitus before 18 years of age , multiparity , Poor personal hygiene , poor socioeconomic status, smoking ,immunosuppressed individuals, women on OCP(oral contraceptives). Other risk factors are: Progesterone therapy for a long period of time predisposes to adenocarcinoma of cervix and in utero exposure to Diethylstilbestrol (DES).[14]

WHO CLASSIFICATION OF UTERINE CERVICAL TUMOURS :(2020)[15]

I) Squamous epithelial tumors:

A) Mimics of squamous precursor lesions

Squamous metaplasia

Atrophy of the uterine cervix

B) Squamous cell tumors and precursors

Condyloma acuminatum

Squamous intraepithelial lesions of the uterine cervix

Squamous cell carcinoma, HPV associated, of the uterine cervix

Squamous cell carcinoma, HPV independent, of the uterine cervix

Squamous cell carcinoma, NOS of the uterine cervix

II) Glandular tumors and precursors

A) Benign glandular lesions

Endocervical polyp

Mullerian papilloma of the uterine cervix

Nabothian cyst

Tunnel clusters

Microglandular hyperplasia

Lobular endocervical glandular hyperplasia

Diffuse laminar endocervical hyperplasia

Mesonephric remnants and hyperplasia

Arias Stella reaction of the uterine cervix

Endocervicosis of the uterine cervix

Tuboendometrioid metaplasia

B) Adenocarcinomas

Adenocarcinoma in situ, HPV associated, of the uterine cervix

Adenocarcinoma, HPV associated, of the uterine cervix

Adenocarcinoma in situ, HPV independent, of the uterine cervix

Adenocarcinoma, HPV independent, gastric type, of the uterine cervix

Adenocarcinoma, HPV independent, clear cell type, of the uterine cervix

Adenocarcinoma, HPV independent, mesonephric type, of the uterine cervix

Other adenocarcinomas of the uterine cervix

C) Other epithelial tumors

Carcinosarcoma of the uterine cervix

Adenosquamous and mucoepidermoid carcinomas of the uterine cervix

Adenoid basal carcinoma of the uterine cervix

Carcinoma of the uterine cervix, unclassifiable

D)Mixed epithelial and mesenchymal tumors

Adenomyoma of the uterine cervix

Adenosarcoma of the uterine cervix

E) Germ cell tumors:

Germ cell tumors of the uterine cervix.

SQUAMOUS CELL CARCINOMA:

MICROINVASIVE SCC:

Diagnosed only by microscopy not on macroscopy. Maximum depth of invasion is 5 mm and horizontally does not extend beyond 7 mm. Microinvasive SCC corresponds to Stage IA of FIGO Staging.[16]

Patients are usually asymptomatic , on gross examination cervix is normal or may manifest with erosion or chronic cervicitis. On colposcopy acetowhite areas are noted similar to HSIL and haphazard branching vessels may be observed. On microscopy the basement membraneis infiltrated by malignant tumor cells and also the cervical stroma. Rest of the cervical epithelium shows features of SIL (squamous intraepithelial lesion) . Ragged shape/contourof the invading margin is considered the most specific criteria for microinvasion.[17]

INVASIVE SCC:

CLINICAL FINDINGS:

Clinical manifestation of patients of invasive carcinoma cervix depends on the stage of lesion and size of the lesion. Most common manifestation is abnormal vaginal bleeding. Other most common significant manifestation is postcoital bleeding. Other clinical findings include :frank hemorrhage, Serosanguinous discharge and intermittent spotting. In locally advanced cervical cancer patients presents with pallor , fatigue , pedal edema , weight loss , dysuria and hematuria.[18]

MACROSCOPY OF INVASIVE SCC:

Exophytic or endophytic lesion on visual inspection. On palpation induration can be noted. Cervical cancers frequently presents as exophytic , polypoidal or fungating growth. Focal ulceration , focal induration , raised granular that bleeds on touch is noted in early lesions of cervical cancers.[18]

Endophytic cervical carcinoma are ulcerative or nodular. Endophytic carcinoma grows within the endocervical canal resulting in large sized barrel shaped cervix. Endophytic cervical carcinomas are noted in advanced stages of the disease because they have clinically occult manifestation and in these cases sampling is not feasible because of the late clinical presentation.[18]

MICROSCOPY OF INVASIVE SCC:

Invasive SCC has variable patterns of growth, different cell type and degree ofdifferentiation. All variants of SCC of cervix have HPV as a common etiological factor.

Microscopically neoplastic cells infiltrates the stroma in form of irregular ragged cords. Tumour cells are polygonal in shape with eosinophilic cytoplasm with a well defined cell membranes. Intercellular bridges may also be noted. Nuclear pleomorphism is observed with coarse chromatin and mitotic figures can be seen occasionally.

SCC is graded utilizing modified broder's system on the basis of extent of keratinization and degree of keratinization, cellular atypia and mitosis into three subgroups:

Well differentiated SCC

Moderately differentiated SCC

Poorly differentiated SCC.[18]

OTHER VARIANTS OF SCC:

1) **BASALOID SCC:** Shows tumor cells arranged in nest. Basal cell type with increased mitotic figures. Geographic necrosis is a frequent finding.[18]

2) **VERRUCOUS SCC**: Clinically similar to condyloma. Slow growing cancer. Common characteristic findings are hyperkeratotic , warty surface with pushing borders. Cells do not have atypia, koilocytotic change. Mitotic figures are not seen.

   Inflammatory cell infiltrates are noted at the junction of epithelium and stroma.

   Wide local excision is the most preferred treatment. Increased incidence of recurrence is noted but rarely these tumors metastasize.[18]

3) **WARTY SCC:** Also known as condylomatous carcinoma . Features are warty surface resembles to condyloma on low power. Tumor cell shows nuclear changes that is similar to koilocytotic atypia and cell have vacuolated cytoplasm. Less aggressive variant.[18]

4) **PAPILLARY SCC:** On Microscopy Atypical cells arranged in papillary pattern. Cells are basaloid in nature similar to that of HSIL. Cells have hyperchromatic ovoid nuclei with scant cytoplasm. Mitotic figures are frequently encountered. Focal area of squamous differentiation may be noted.[18]

5) **ADENOCARCINOMA:** Constitutes of about 10-25% of cervical carcinoma. It is seen in association with OCP(oral contraceptive use) . High Risk HPV is observed in 94% of Cervical adenocarcinoma cases. Most common High risk HPV is HPV 18. Most

common manifestation is Abnormal uterine bleeding observed in around 75% of patients. Few patients present with vaginal discharge.

MACROSCOPY : In 50 % of the patients exophytic polypoidal or fungating growth is seen. Other 50% cases shows nodular or diffuse infiltrative growth. Grossly lesion may not be seen in 15% of cases.

MICROSCOPY:

ENDCOERVICAL ADENOCARCINOMA USUAL TYPE:

Most common variant observed in 90% of Cases. Tumor cells show moderate to well differentiated cells with complex glandular pattern arrangement. Cells are round to ovoid in shape without mucin formation and shows characteristic pseudostratification. Nucleus is elongated and hyperchromatic with prominent nucleoli. Mitotic figures are seen.[18]

6) **VILLOGLANDULAR CARCINOMA:** characterized by villous and papillary folds. Lining is by endocervical columnar cells demonstrating mild moderate atypia. Mitotic figures are frequently seen.[18]

7) **ENDOMETROID CARCINOMA:** Consists of tumor cells similar to those of primary adenocarcinoma of uterus . Cells show stratification consisting of round to ovoid shaped nuclei . These tumor cell do not have mucin and comprises of scant amount of cytoplasm as compared to endocervical carcinoma of usual type.It can be differentiated from endometrial carcinoma bu utilizing p16 marker.[18]

8) CLEAR CELL CARCINOMA:

It comprises of 4% cases of Cervical carcinoma. It has association with in-utero DES exposure. It can also occur in women not exposed to DES.

Microscopy : Three patterns: Solid,papillary and tubulocystic. Tumor cells have abundnant clear to granular eosinophilic cytoplasm. Clearing of cytoplasm is due to glycogen accumulation. Nucleus shows high pleomorphism, hyperchromatic nucleus projecting into the lumen giving a Hobnail appearance. [18]

9) SEROUS CARCINOMA:

Rare variant of adenocarcinoma . It is a diagnosis of exclusion. Cells are arranged in papillary pattern depicting nuclear atypia. Psammoma bodies can also be noted**.**[18]

10) ADENOCARCINOMA ADMIXED WITH NEUROENDOCRINE CARCINOMA:

Rare Variant shows cervical adenocarcinoma demonstrating neuroendocrine differentiation.[18]

OTHER EPITHELIAL TUMOURS:

ADENOSQUAMOUS CARCINOMA:

Malignant epithelial tumor comprising of glandular cells and squamous cells. Occurs in young as well as old women. Squamous cell shows well differentiated squamous cells along-with keratin pearls with individual cell keratinization. Suffice glandular component differentiation of adenocarcinoma component should be noted for establishing diagnosis of adenosquamous carcinoma.[18]

GLASSY CELL CARCINOMA:

Poorly differentiated adenosquamous carcinoma accounting for 1% cases of cervical carcinoma. Cells are uniform , large and polygonal with ground glass type of chromatin. Cells have a well -defined cell membrane and prominent nucleoli. Dense lymphoplasmacytic infiltrate cells are seen in the stroma .[18]

UNDIFFERNTIATED CARCINOMA:

Tumor cells arranged in sheets . Lacks squamous and glandular formation.[18]

PD-L1 Marker:

PD-L1 is a transmembrane protein assumed to play a major role in suppressing theadaptive arm of immune system. Normally the adaptive immune system reactsto antigens that are associated with Immune system activation by exogenous or endogenous danger signals. In turn, clonal expansion of antigen-specific CD8+Tcells and/or CD4+ helper cells is Propagated. The binding of PD-L1 to the inhibitory checkpoint molecule PD-1 (Programmed cell death protein-1) transmits an inhibitorysignal based on interaction with phosphatases Src Homology region domain-containing phosphatase-1 &2 (SHP-1 or SHP-2) via Immunoreceptor Tyrosine-Based Switch Motif (ITSM) motif. This reduces the proliferation of antigen-specific-T-cells and simultaneously reducing apoptosis in regulatory T cells(Suppressive T-cells). [19]

PD-L1(programmed death ligand -1) act as immune checkpoint inhibitors and is vital to maintain tolerance against autoimmunity in physiological conditions. Binding of PD-1 and PD-L1 has a role in regulating the immune system's response to the cells of the human body by down-regulating immune system and promoting self tolerance by

suppressing T-cell inflammatory activity. This prevents auto-immune diseases , but it can also prevent the immune system from killing cancer cells.The binding of PD-L1 to PD-1 leads to an immunosuppressive effect and this allows the tumor to evade immune destruction. In carcinoma patients , PD-L1 is primary ligand of PD-1,is expressed ontumor cells. [19] Normal cervical epithelium do not express PD-L1. Benign cervical tissuesalso do not express PD-L1. Significant expression of PD-L1 is reported in 34.4 % of squamous cell carcinoma of cervix.PD-L1 highest expression appears in squamous cell carcinoma. While positive PD-L1 expression in adenosquamous carcinomas and endocervical carcinomas are lower than squamous cell carcinoma i.e 29% and 17% respectively. The expression Of PD-L1 is seen in 32% of cervical carcinomas and Increased PD-L1 expression is significantly associated with higher TNM (Tumor,node,metastasis) stage and worse prognosis in cervical carcinomas.[20]

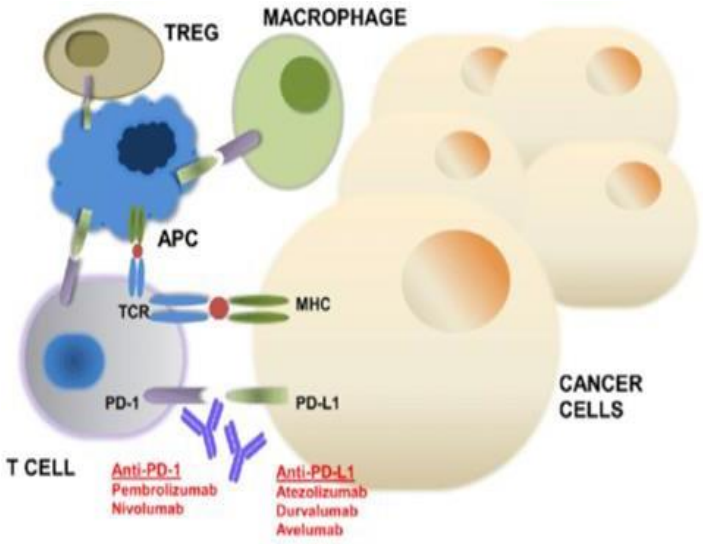

Figure 2: Mechanism of action of PD-1 and PD-L1 inhibitors. The programmed cell death1 (PD-1) receptor is expressed on activated T cells, Binding of PD-1 to its B7 family of ligands, programmed death ligand 1 (PD-L1) or PD-L2 results in suppression of proliferation and immune response of T cells. Activation of PD-1/PD-L1 signaling serves as a principal mechanism by which tumors evade antigen-specific T-cell immunologic responses. [21]

Role of PD-L1 in Cervical Cancers :

Patients with recurrent cervical carcinoma and metastatic cervical carcinoma have paucity of treatment options and receive palliative platinum based chemotherapy with insignificant survival benefit . Recent studies support usage of immunotherapy (immune checkpoint inhibitors) in advanced Cervical Cancers. [22] Around 17% of Cervical adenocarcinoma and 35% of squamous cell carcinoma cases expressed PD-L1.[23]

D-L1 expression is rarely observed in normal cervical tissue whereas PD-L1 expression is observed in 50% of cervical cancer cells, PD-L1 has been identified as a prognostic factor and also as a treatment target for cervical carcinoma.[24] PD-L1 is present on tumor cells and PD-1 receptor is present on T-cells. PD-L1 and PD-1 interaction permits tolerance of tumor antigens evading the anti-tumor immune response. Blockade of PD-L1 and PD-1 interactionis a considered as potential therapeutic option. Pembrolizumab is a selective, human monoclonal antibody that attaches to PD-1 and inhibits the PD-L1 pathway.[25] According to Schellens JHM et al[26] 24 patients with advanced cervical cancer showed PD-L1 expression of ≥1% in the tumor tissue. These patients did not respond to prior systemic therapy and patients who had previously received two or more systemic drugs did not respond to therapy whereas patients who were administered pembrolizumab monotherapy at 10 mg/kg once every 2 weeks for 2 years had an overall response rate (ORR) of 12.5%. In subsequent phase II study patients with advanced cervical cancer were treated with pembrolizumab at 200 mg every 3 weeks, regardless of PD-L1 status. The ORR was 12.2% and for patients with larger duration of follow-up (at least 6.5 months) ORR increased to 27%.[26]

Utility of PD-L1 inhibitors and PD-1 inhibitors in other Cancers:

PD-L1 inhibitors are :Atezolizumab ,Avelumab and Durvalumab

PD-1 inhibitors are : Nivolumab, Pembrolizumab

Urothelial Cancer : Atezolizumab binds to PD-L1 and blocks its interactions with PD-1 Receptors. FDA approved Atezolizumab in May 2016 for locally advanced and metastatic Urothelial cancer. It is the first new treatment to be approved for urothelial carcinomas in past three decades.[27]

Lung Cancer : For Metastatic Non-small cell Lung cancer FDA approved PD-L1 inhibitors are atezolizumab, Avelumab and Durvalumab. Median Overall survival reported is more in lung cancer treated with PD-1 inhibitors. With usage of Nivolumab overall survival increases to 9.2 months whereas with chemotherapy drug (docetaxel) overall survival was 6.0 months. Nivolumab is approved as second line of treatment for both squamous and Non-squmaous Non Small cell Lung cancer.[27]

Classical Hodgkin's Lymphoma : PD -1 exhibits increased expression and is a T-cell exhaustion marker. Upto 97% of patients with Hodgkin's lymphoma will show alterations in the PD-L1 gene loci which reflects correlation with PD-L1 protein expression. The FDA approved Nivolumab 3mg/kg every two weeks for the treatment of Classical Hodgkin's Lymphoma after failure of Autologous HCT(Hematopoietic Cell Transpanatation) .[27]

Head and Neck Squamous Cell Carcinoma: PD-1 antibodies are also actively seen in Head and neck squamous cell cancers. Pembrolizumab is a monoclonal type of antibody that attaches to PD-1 receptor and inhibits it interaction. Pembrolizumab is FDA(Food and Drug administration) approved for the previously treated advanced Head and Neck squamous cell cancers.[27]

Melanoma : Nivolumab is a Human immunoglobulin G4 (Ig4) monoclonal antibody that binds to PD-1 receptor and inhibits its interaction with PD-L1. Nivolumab is FDA approvedas first line treatment in previously treated melanoma cases.[27]

Adverse effects of PD-L1 inhibitors: Increase in immune activation by PD-1 and PD-L1 inhibitors is responsible for immune related adverse effects(irAEs). Pneumonitis is the most frequent pulmonary adverse event. **Cutaneous** adverse effects are Rash, pruritis and psoriasis. **Nephrologic** adverse effects are rare that includes Acute interstitial nephritis .Gastrointestinal adverse effect; most common is pancreatitis**. Neurological** side effects includes myasthenia gravis, Other iRAEs are pericardial effusion, Myocarditis with right Heart Failure , agranulocytosis and Autoimmune Hemolytic Anemia (AIHA).[27]

Table 1 : FIGO STAGING OF CERVICAL CARCINOMA [2018] [28]

FIGO Staging	2018 FIGO Definition
I	Confined to the cervix
IA	≤5 mm depth
IA1	≤3 mm depth
IA2	>3 mm and ≤5 mm depth
IB	>5 mm depth
IB1	≤2 cm maximum diameter
IB2	>2 cm and ≤4 cm maximum diameter
IB3	>4 cm maximum diameter
II	Extension beyond the uterus but lower one-third of the vagina is not involved.
IIA1	Involvement of upper two-thirds of the vagina
IIA2	Upper two-thirds of the vagina and ≤4 cm
IIB	Invasion of parametrium
III	Involvement of lower vagina, ureters, lymph nodes
IIIA	Inferior one-third of the vagina
IIIB	Pelvic sidewall
IIIC	Involvement of para-aortic and pelvic lymph nodes.
IIIC1	Pelvic lymph node involvement
IIC2	Para-aortic lymph node involvement
IV	Adjacent and distant organs
IVA	Rectal or bladder involvement
IVB	Outside the pelvis involving distal organs

ROLE OF MRI IN CERVICAL CANCER:

Magnetic resonance imaging (MRI) demonstrates morphological details of the female pelvis and it is helpful for assessing benign as well as malignant cervical masses. Clinical evaluation of cervical cancer is vital in defining the best therapeutic modality but clinical staging has it own limitations. Clinical staging, as defined by FIGO (International Federation of Gynecologic Oncology), has following parameters: physical examination findings, Biopsy from the representative area of lesion , cystoscopy, these parameters results can be false depending on the stage of the disease. The cervical cancer prognosis is assessed not only by stage alone other parameters such as nodal status, tumor volume and depth of invasion whichare not included in the FIGO guidelines. MRI has been characterized as the most precise, non-invasive imaging option for staging of cervical carcinoma. [29] MRI is the favored imagingmodality because of its accuracy in evaluation of soft tissue and it allows proper assessmentof stromal and parametrial invasion in comparison with computed tomography (CT). MRI depicts more accurately shape, volume and direction of the primary lesion; local extent of the disease, nodal status assisting the clinician in planning treatment. Tumor response to chemo- radiation is better assessed with MRI.[30] MRI is a useful tool in staging of cervical cancer due its precision in identifying the exact extent of tumor owing to its fine contrast resolution.MRI is a advanced multiplanar multiparametric diagnostic option which aid in staging and subsequent management relatively easier thus overshadowing its expensive cost.[31]

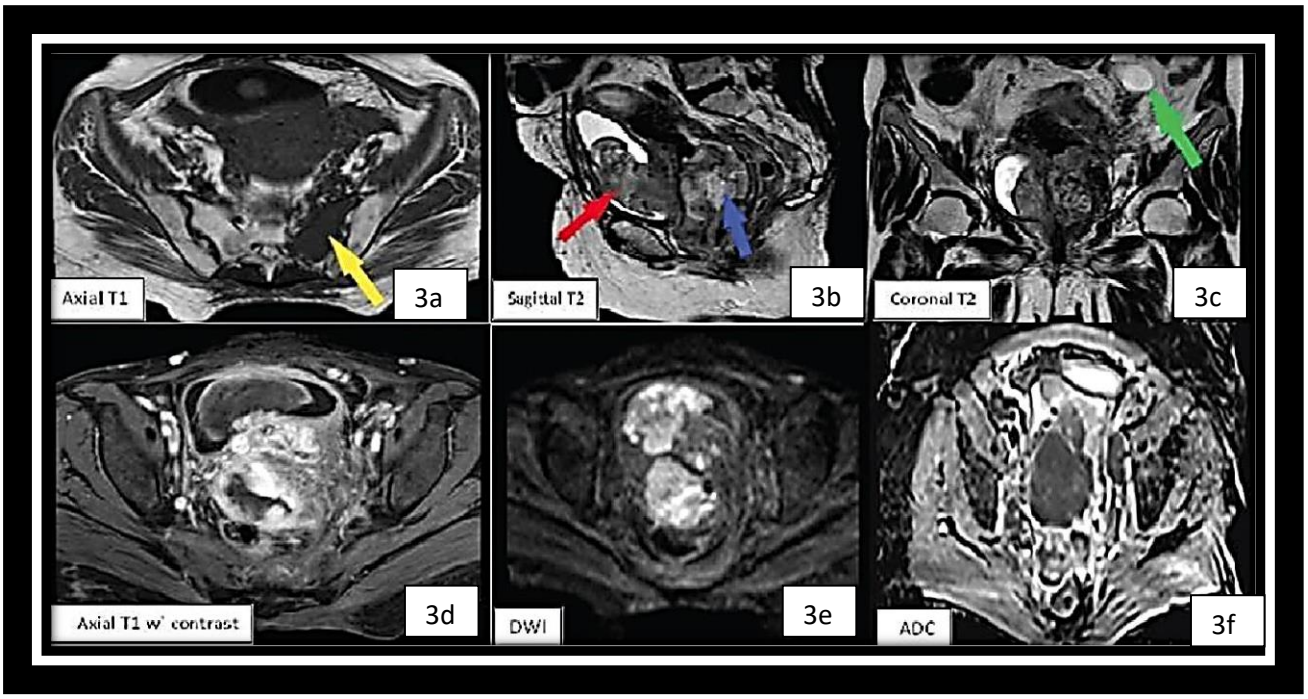

Figure 3 : A case of Carcinoma Cervix stage IVB in different views and sequences of MRI.

Figure 3a :Axial T1 weighted imaging showing hypointense signal changes within the left sacral ala, suggestive of Bone marrow infiltration(indicated by yellow arrow).

Figure 3b: Sagittal T2 weighted imaging showing a heterogeneous lesion in the cervix suggestive of Carcinoma cervix(Indicated by Blue arrow). The lesion is extending into urinary bladder anteriorly(Indicated by red arrow)

Figure 3c:Coronal T2 weighted image showing a left external iliac lymph node(Indicated by green arrow)

Figure 3d: Axial T1-FS(Fat saturation) post contrast imaging showing heterogeneous enhancement of the vertical lesion.

Figure 3e and f: On DWI (Diffusion weighted imaging) and corresponding ADC(Apparent Diffusion coefficient) images, The lesion demonstrates restriction of diffusion.[32]

MATERIAL & METHODS

MATERIALS AND METHODS

STUDY DESIGN: Laboratory based observation study

DURATION OF STUDY :

Samples were collected from July 2016 to June 2018.

Patient were followed up for three years (July 2018 to June 2021) to assess overall survival of the cases.

STUDY PLACE : Department of Pathology in Collaboration with Department of Obstetrics and Gynaecology, Sri Devaraj Urs Medical College attached to R.L Jalappa Hospital and Research centre, Tamaka, Kolar.

SAMPLE SIZE:

Sample size estimated by based on expression of PD-L1 expression in Squamous cell carcinoma cervix as 35 % in a study by Feng M et al[20] in 2018 with 95% confidence interval and an absolute error of 10%, the sample size was 90.[20] Formula used :

$$n = \frac{Z^2\ 1-\alpha/2\ p(1-p)}{d^2}$$

p : Expected proportion

d : Absolute Precision

α/2 : Desired Confidence level

INCLUSION CRITERIA :

All the cases of Invasive Squamous cell carcinoma of cervix diagnosed with Histopathology was considered for the study.

EXCLUSION CRITERIA:

Recurrent cases and Cases with metastatic deposits in cervix are excluded from the study.

Cases that underwent chemotherapy and radiotherapy before biopsy were excluded from the study.

METHOD:

1. Ninety cases were considered for the study. The study was done in Department of Pathology and OBG (Obstetrics and Gynaecology), Sri Devaraj Urs Medical college , Kolar. Affiliated to Sri Devaraj Urs Academy of Higher education and research. Cases were collected from archives of Department of Pathology.

2. Age, Parity, Staging with clinical findings (Bleeding per vagina/white discharge per vagina) of each case was noted from Hospital record section. Radiologic findings were evaluated with help of MRI for size of lesion and lymph node involvement.

3. Haematoxylin & Eosin stain tissue section slides were reviewed. Squamous cell carcinoma was classified into Well differentiated, moderately differentiated and poorly Differentiated.

4. Tissue section was taken from paraffin embedded tissue blocks and were subjected to immunohistochemistry for expression of markers of PD-L1 (PD-L1 Antibody Biogenex AN921-M) according to standard protocol using positive and negative controls.

Table 2: Antibody utilized for Immunohistochemistry:

Antigen	Clone	Species	Producer	Dilution	Control	Stain
Anti –PD L1	Monoclonal	Rabbit	Biogenex	Ready to Use	Tonsil	Membranous Staining

IHC (Immunohistochemistry)Procedure :

The procedure of IHC as per the manufacturer's protocol was followed:

1. Sections were cut approximately 3-4 um and floated on to positive charged slides and incubated at 37 degree Celsius for one day. Further incubated at 38 degree Celsius over night. Sections were not allowed to be dry at any stage of the staining procedure.
2. Deparaffinization was done using xylene-I And Xylene –II using both for 15 minutes each.
3. Dexylinisation was done using Absolute Alcohol-I and Absolute Alcohol –II using each for 1 minute .
4. Dealcoholisation was done using 90% Alcohol and 70% Alcohol for 1 minute each.
5. Distilled water rinsing was done for 5 min.
6. Antigen retrieval was done utilizing Heat Induced epitope Retrieval (HIER) technique. Sections were microwaved at power 10 for 6 minutes in TRIS EDTA buffer at pH 9.0 for 4 cycles.
7. Sections were then washed with TRIS buffer solution (TBS) at pH 7.4 for 3 cycles of 5 minutes each.
8. Peroxidase Block was done for 30 minutes in dark to block the endogenous peroxidase enzyme.
9. TBS Washing was done for 3 cycles of 5 minutes each
10. Power block was used on the sections for 10 minutes. The sections were not washed by TBS at this step.

11.Sections were covered with primary antibody for 60 minutes.

12. TBS buffer washing was done for 3 cycles of 5 minutes each.

13.Sectioned were covered with super sensitive poly horse radish peroxidase(Secondary antibody) for 30 minutes.

14. TBS buffer washing was done for 3 cycles of 5 minutes each

15. Superenhancer was utilized on the sections for 30 minutes.

16.Colour development was done utilizing colour development solution Di-Amino benzidine (DAB) For 15 minutes.

17.Distilled water washing was done for 3 cycles of 5 minutes each

18. The sections were counter stained with Harris Hematoxylin for one 1 minute

19. The sections were dehydrated, cleared and mounted with DPX.

IMMUNOHISTOCHEMICAL ANALYSIS:

Sections were first examined at low magnification then at 40 x magnification with help of Olympus Cx 21i microscope to identify areas of highest positivity(Hotspot). Areas of Hotspots were utilized for interpretation of Immunohistochemical staining.

IMMUNOHISTOCHEMICAL INTERPRETATION :

The membranous positivity was considered as positive for PD-L1.

Expression of PD-L1 was evaluated in tumour cells in five Hotspot areas in high power fields. [19]

The expression was analyzed as:

1. Score 0=< 5 % of positive cells.
2. Score 1 = 5-29%,
3. Score 2= 30—59%,
4. Score 3= >60% .[19]

The intensity of staining on the cell membranes was scored as:

Intensity 0 : There is no reaction on the cell membranes.

Intensity 1 : The reaction is visible barely or partially circumferential.

Intensity 2 : The reaction is clearly visible and is completely circumferential.

Intensity 3: The reaction is intense and fully circumferential.[19]

Average of score was taken and was multiplied by intensity to obtain the Grading.Grading :

Score1-3 as Grade I

Score 4-6 as Grade II

Score 7-9 as Grade III. [19]

STATISTICAL ANALYSIS:

Data was entered into Microsoft excel data sheet and was analyzed using SPSS 22 version software. Categorical data was represented in the form of Frequencies and proportions. **Chi-square test or Fischer's exact test** (for 2x2 tables only) was used as test of significance for qualitative data.

Graphical representation of data: MS Excel and MS word was used to obtain various types of graphs.

p value (Probability that the result is true) of <0.05 was considered as statistically significant after considering all the rules of statistical tests.

Statistical software: MS Excel, SPSS version 22 (IBM SPSS Statistics, Somers NY, USA) was used to analyze data.[20]

RESULTS

RESULTS

Ninety cases of cervical carcinoma were assessed during the period of July 2016 to June 2018 in the Department of Pathology in Sri Devaraj Urs Medical College and R.L Jalappa Hospital and Research Centre , Tamaka,Kolar.

Immunohistochemistry was done in all 90 cases for PD-L1. The following data was evaluated:

1. Age distribution
2. Parity
3. Clinical findings
4. Histological Grading
5. FIGO Staging
6. Size of lesion(Evaluated By MRI)
7. Overall survival
8. Radiological Evaluation (MRI) for lymphadenopathy
9. PD-L1 Expression
10. PD-L1 expression correlation with Age
11. PD-L1 expression correlation with Parity
12. PD-L1 expression correlation with Clinical findings
13. PD-L1 expression correlation with Histological Grading
14. PD-L1 expression correlation with FIGO Staging
15. PD-L1 expression correlation with size of lesion
16. PD-L1 expression with overall survival

Table 3 : Showing PD-L1 expression based on proportion of tumour cells

PD-L1 Proportion score	No of Cases	Percentage of cases
0 (0 to 5 % of cells)	35	38.88%
1 (5 to 29% of cells)	20	22.22%
2(30 to 59% of cells)	22	24.44%
3>60 % of cells	13	14.44%
Total	90	100%

IHC PD-L1 scoring based on proportion : Proportion of tumour cells showing PD-L1positive expression was analyzed. In present study score 0 (0 to 5 % of cells demonstrating PD-L1 activity 35 cases were noted (38.8%) followed by Score 2 (30 -59 % of cells) 22 cases (24.44%) were observed. Score 1 (5-29% of cells) 20 cases (22.22%) were noted and score >3 PD-L1 positive expression was observed in 13 cases(14.44%).

Table 4 :Showing PD-L1 expression based on Intensity of cell membrane staining the tumor

PD-L1 Intensity Score	No of cases	Percentage of cases
0	35	38.8%
1	15	16.6%
2	14	15.5%
3	26	28.8%
Total	90 cases	

IHC PD-L1 scoring based on Intensity on cell membrane:

Intensity 0: 35 cases (38.8%) cases were observed followed by cases which showed Intensity 3; 26 cases(28.8%) , Intensity 1 :15 cases(16.6%) and Intensity 2 : 14 cases(15.5%).

Table 5: Showing Distribution of subjects according to Age group

Age	Number of Cases
30-39 years	8 cases.(8.8%)
40-49 years	**28 cases.(31.1%)**
50-59 years	22 cases.(24.4%)
60-69 years	20 cases(22.22%)
70-79 years	12 cases.(13.3%)

More number of Cervical Carcinoma cases were noted in the age group of 40-49 years (31.1%) followed by 50-59 years (24.4%) and 60-69 years (22.22%). In present study average age of presentation in cervical carcinoma cases was around 52 years.

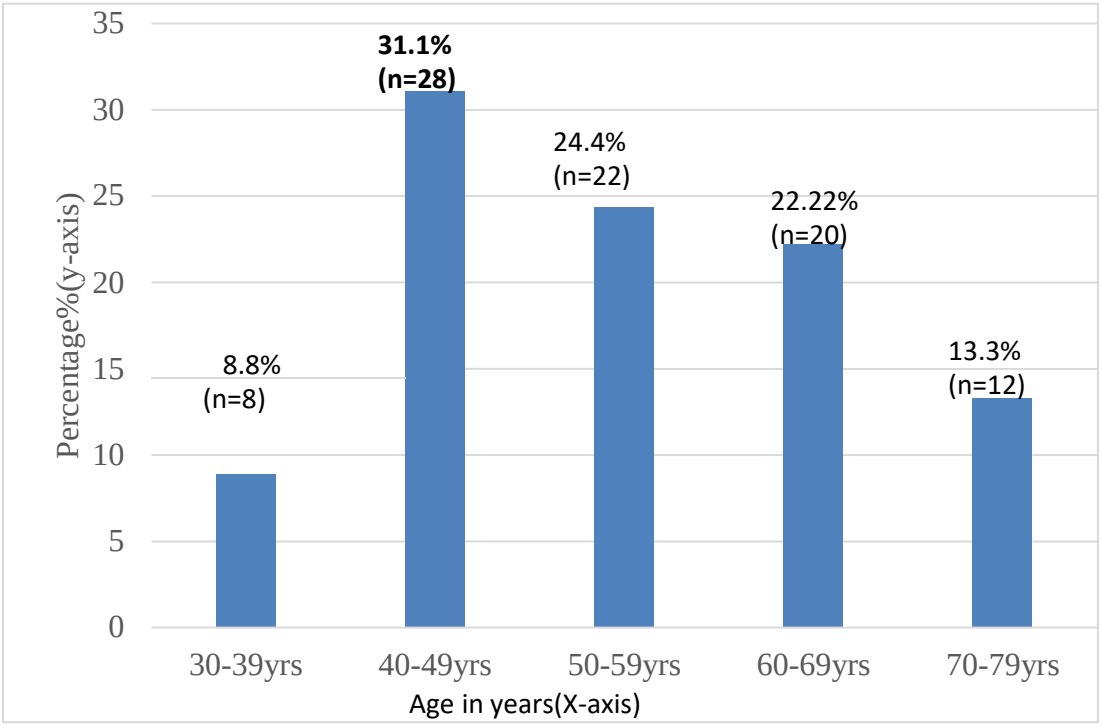

Chart 1: Graph showing Distribution of subjects according to Age group (n=number of cases)

Table 6: **Distribution of subjects according to parity**

Parity	Frequency(n)	Percentage
1-3	73	**81.1%**
4-6	16	17.8%
>6	1	1.1%

More number of Cervical carcinoma cases were noted in parity (1-3) n= 73 cases (81.1%) followed by parity (4-6) n=16 cases(17.8%) and >6 only 1 case(1.1%) was noted.

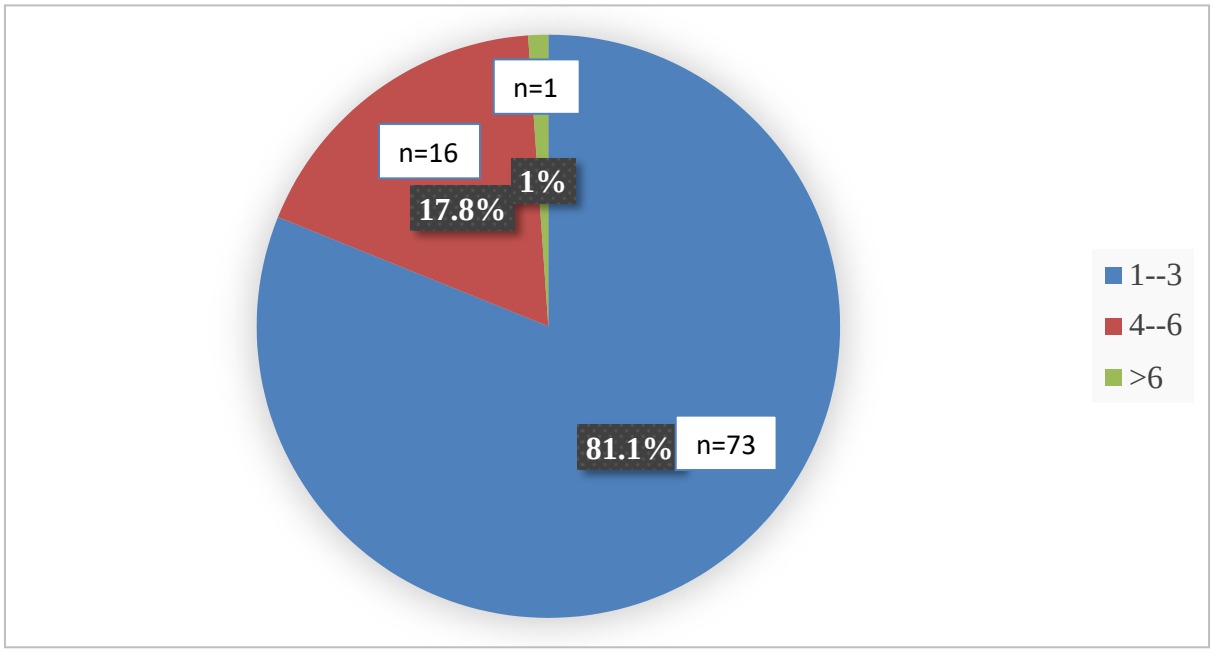

Chart 2: Graph showing Distribution of subjects according to parity. (n=number of cases)

Table 7 : **Distribution of subjects according to Individual parity**

Parity	Cases	Percentage
Parity 1	9	10%
Parity 2	**56**	**62%**
Parity 3	11	12%
Parity 4	7	8%
Parity 5	6	6%
Parity 8	1	2%

Maximum number of cases of cervical carcinoma were noted in Parity 2 around 56 cases(62%) followed by parity 3 around 11 cases (12%) and Parity 1 around 9 cases (10%).

Table 8 : Distribution of subjects according to Clinical findings

Clinical findings	**Frequency**	**Percentage**%
Bleeding per vagina	65	72.2%
White discharge per vagina	25	27.8%

Most frequent clinical finding noted in carcinoma cervix was bleeding per vagina: 65 cases (72.2%) followed by white discharge per vagina around 25 cases (27.8%).

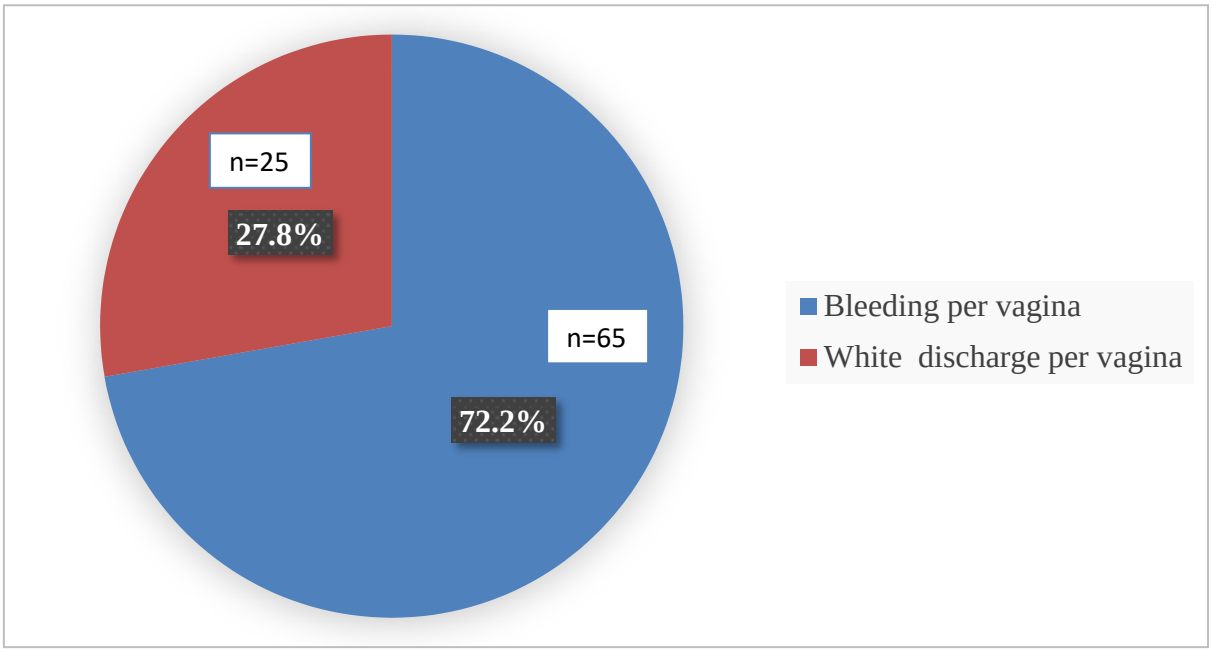

Chart 3 :- Graph showing Distribution of subjects according to Clinical findings

(n=number of cases)

Table 9:- Distribution of subjects according to Histological Grade

Histological grading	Frequency	Percentage
Well differentiated	15	16.7%
Moderately differentiated	47	**52.2%**
Poorly differentiated	28	31.1%
Total	90	100.0%

Maximum number of cases of cervical carcinoma according to histological grade were moderately differentiated cases around 47 cases (52.2%) followed by poorly differentiated cervical carcinoma cases around 28 cases(31.1%) and well differentiated cervical carcinoma 15cases(16.7%).

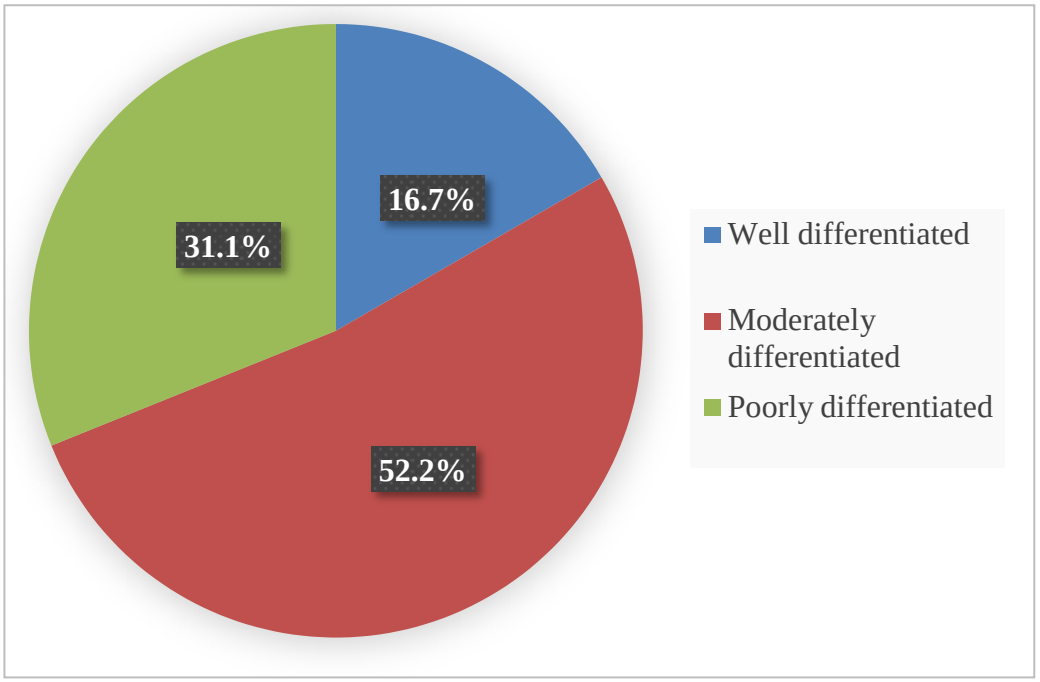

Chart 4:- Graph showing Distribution of subjects according to histological grading. **(**n=number of cases)

Table 10: Distribution of subjects according to Stage **(FIGO STAGING)**

Staging	Frequency(n)	Percentage
Stage II A	5	5.6%
Stage II B	36	**40.0%**
Stage III A	3	3.3%
Stage III B	41	**45.6%**
Stage IV A	5	5.6%

In Carcinoma cervix cases, most common stage encountered was stage III B n=41(45.6%cases) followed by stage II B n= 36 cases (40%). Stage II A And Stage IV A showed equal number of cases: n=5 cases (5.6%). Least number of cases were noted in Stage III A n=3 cases(3.3%).

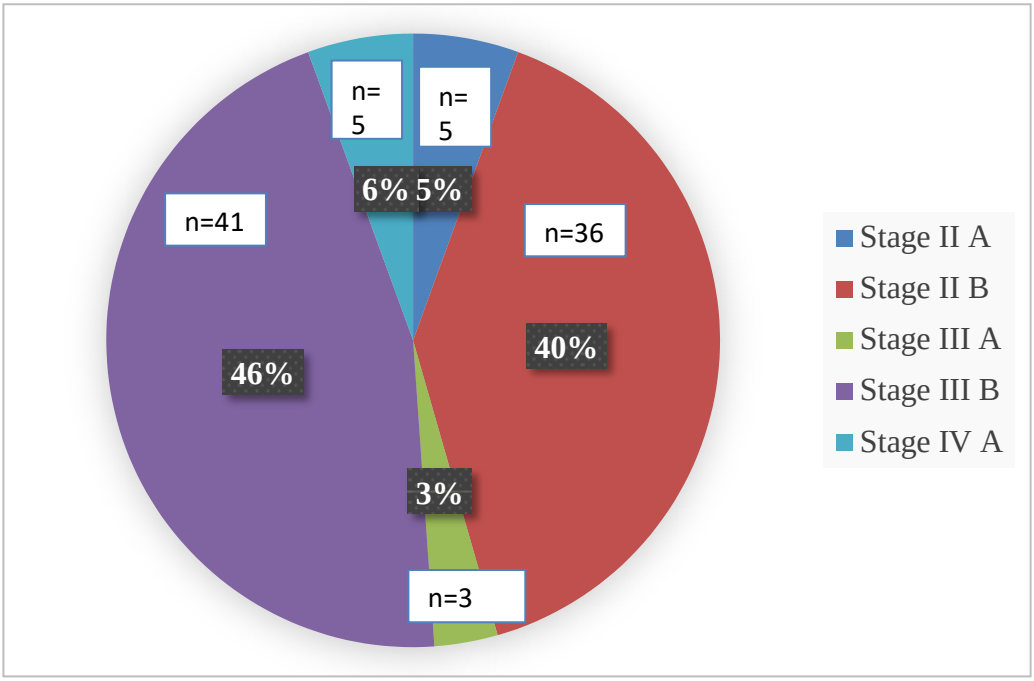

Chart 5:- Graph showing Distribution of subjects according to stage.

(n=Number of cases). In Carcinoma cervix cases, most common cases were noted in stage IIIB n=41(45.6%cases) followed by stage II B n= 36 cases (40%).

Table 11: **Distribution of subjects according to Overall survival**

Overall Survival (Three years Follow up)	Frequency	Percentage%
Survived	84	93.3%
Died	6	6.66%
Total	90	100.0%

Out of 90 cases of carcinoma cervix 84 cases had survived (93.3% cases) and 6 cases unfortunately succumbed to death (6.6% of cases). Out of 6 cases four cases were in stage IIIB (66.6% cases) and one case in stage IVA (16.6% cases) and one case was noted in stage IIB(16.6% cases).

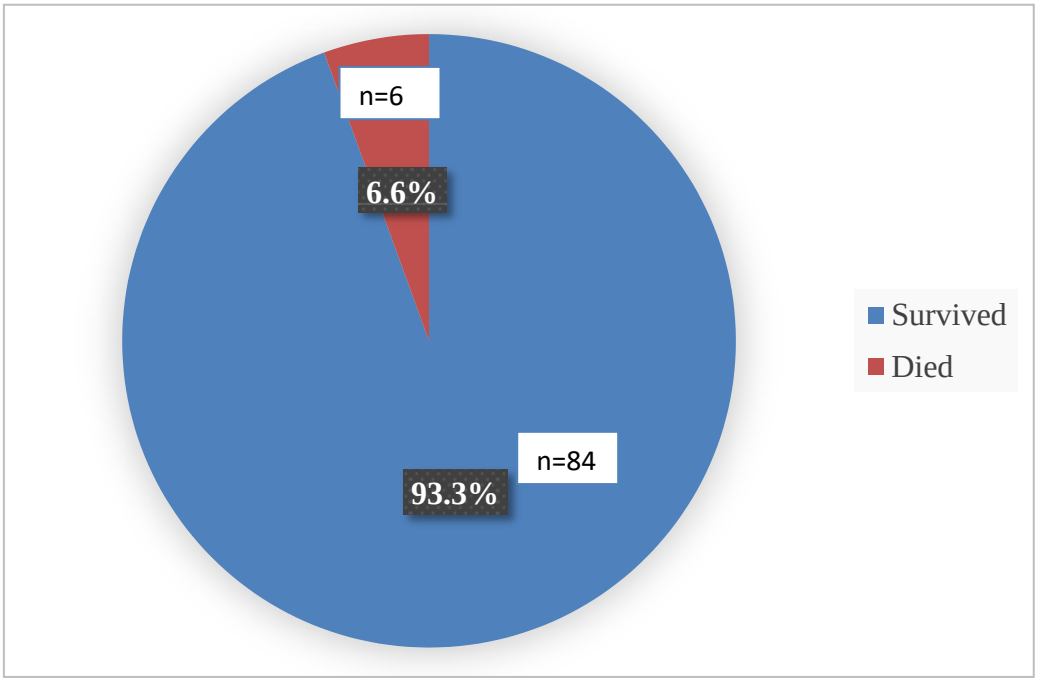

Chart 6 : Graph showing Distribution of subjects according to Overall survival

Out of 90 cases of carcinoma cervix 84 subjects had survived (93.3% cases) and 6 subjects died (6.6% of cases). (n=number of cases)

Table 12: Distribution of subjects according to Lymphadenopathy assessed by Radiological Investigation MRI)

MRI Evaluation for Lymphadenopathy	Frequency	Percent
Lymphadenopathy noted	46	51.1%
No significant lymphadenopathy	44	48.9%

On Assessment by MRI (Radiological evaluation) Lymphadenopathy was noted in 46 cases of cervical carcinoma (51.1%) and no significant lymphadenopathy was observed in 44 cases (48.9%).

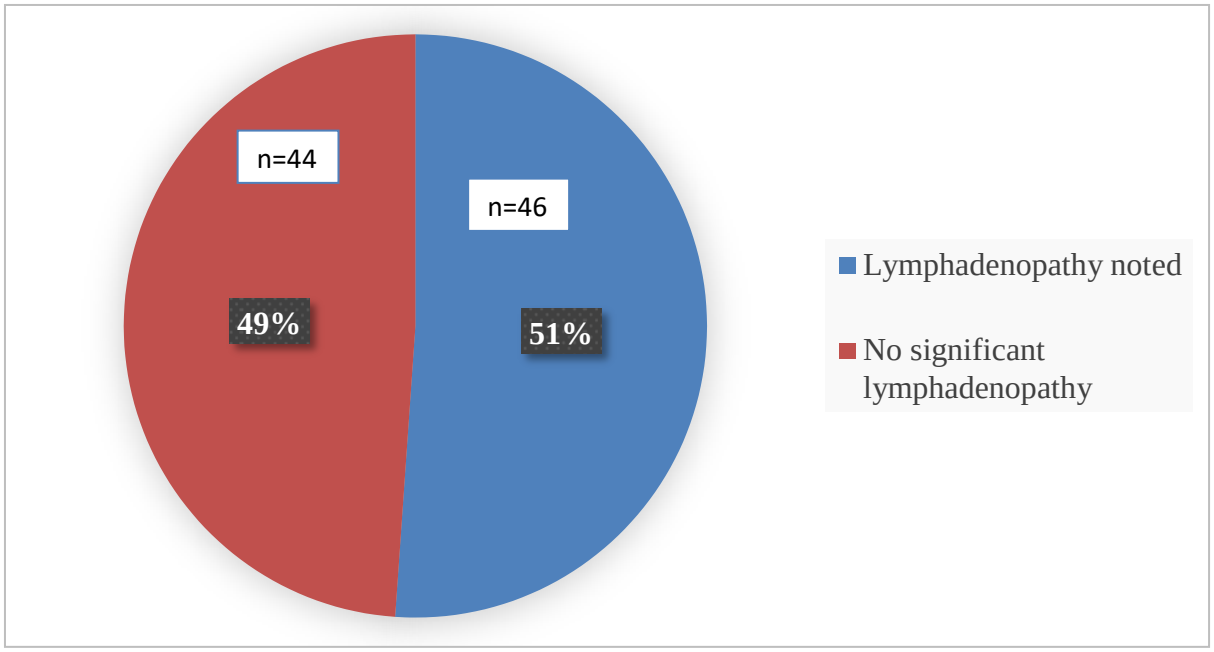

Chart 7: Graph showing Distribution of subjects according to Lymphadenopathy. (n=number of cases.)

Table 13: **Distribution of subjects according to PD-L1 Expression**

PD-L1 Expression	Frequency	Percentage%
Positive	60	66.7%
Negative	30	33.3%

PD-L1 positive expression was observed in 60 cases of cervical carcinoma (66.7% of cases) and PD-L1 negative expression was noted in 30 cases of cervical carcinoma(33.3%)

PD-L1 expression and it's correlation with clinic-pathological parameters

Table 14: Distribution of subjects according to PD-L1 Expression and Age group.

Age group	PD-L1		Total
	Positive	Negative	
30-39yrs	8	0	8
	100.0%	.0%	100.0%
40-49yrs	14	14	28
	50.0%	50.0%	100.0%
50-59yrs	13	9	22
	59.1%	40.9%	100.0%
60-69yrs	15	5	20
	75.0%	25.0%	100.0%
70-79yrs	10	2	12
	83.3%	16.7%	100.0%
Total	60	30	90
	66.7%	**33.3%**	100.0%

In our study as the age increases PD-L1 positive expression increases. 83.3% (10 out of 12 cases in 70-79 age group) showed PD-L1 positive expression in age group of 70-79 years with **p value of 0.037**, there was statistical significant difference found between age and PD- L1 expression in current study.

Table 15: **Distribution of subjects according to PD-L1 Expression and parity.**

Parity	PD-L1 Expression		Total
	Positive	Negative	
1-3	50	24	74
	67.6%	32.4%	100.0%
>3	10	6	16
	62.5%	37.5%	100.0%

In our study women with more than three children showed **decreased** expression of PD-L1 around 10 cases (62.5%) whereas women with parity 1-3 50 cases(67.6%) out of 74 showed PD-L1 positive expression and it was not statistically significant with p value of 0.776, there was no statistical significant difference found between parity and PD-L1.

Table 16: **Distribution of subjects according to PD-L1 Expression and clinical findings.**

Clinical Findings	PD-L1 Expression		Total
	Positive	Negative	
Bleeding	47	18	65
	72.3%	27.7%	100.0%
White discharge	13	12	25
	52.0%	48.0%	100.0%

PD-L1 Positive expression was more in patients with bleeding per vagina. 47 cases (72.3%) out of 65 cases with clinical complaint of bleeding per vagina showed PD-L1 positive expression and 18 cases (27.7%) showed PD-L1 negative expression. In women with clinical complaint of white discharge 13 cases(52%) out of 25 cases showed PD-L1 positive expression whereas 12 cases(48%) showed PD-L1 negative expression and p value was 0.083, there was no statistical significant difference found between clinical findings and PD- L1 expression.

Table 17: **Distribution of subjects according to PD-L1 and Histological Grading**

Histological Grading	PD-L1 Expression		Total
	Positive	Negative	
Well differentiated	8	7	15
	53.3%	46.7%	100.0%
Moderately Differentiated	40	7	47
	85.1%	14.9%	100.0%
Poorly differentiated	12	16	28
	42.9%	57.1%	100%
Total	60	30	90
	66.7%	33.3%	100.0%

PD-L1 positive expression was observed more in moderately differentiated Squamous cell carcinoma of Cervix around 40 cases (85.1%) out of 47 showed PD-L1 positive expression, In well differentiated cervical cancer 8 cases (53.3%) out of 15 showed PD-L1 positive expression followed by poorly differentiated carcinoma in which 12 cases out of 28 cases(42.9%) showed PD-L1 positive expression with **p value <0.001**, there was statistical significant difference found between histological grading and PD-L1.

Table 18: **Distribution of subjects according to PD-L1 expression and FIGO staging**

Staging	PD-L1 expression		Total
	Positive	Negative	
Stage II A	2	3	5
	40.0%	**60.0%**	100.0%
Stage II B	24	12	36
	66.7%	33.3%	100.0%
Stage III A	3	0	3
	100.0%	0%	100.0%
Stage III B	28	13	41
	68.3%	31.7%	100.0%
Stage IV A	3	2	5
	60.0%	40.0%	100.0%

PD-L1 Positive expression was observed more in Stage IIIA 3 cases(100%) out of 3 followed by Stage IIIB 28(68.3%) out of 41 cases showed PD-L1 positivity , Stage IIB 24 out(66.7%) of 36 cases showed PD-L1 positive expression, Stage IV A 3(60%) cases out of 5showed PD-L1 positivity. Least PD-L1 positive expression was noted in Stage II A 2 out of 5cases(40%) with p value 0.662 and there was no statistical significant difference found between stage and PD-L1.

Table 19 : **Distribution of subjects according to PD-L1 Expression and size of lesion**

Size of lesion	PD-L1		Total
	Positive	Negative	
1-3cms	7	3	10
	70.0%	30.0%	100.0%
3-6cms	38	21	59
	64.4%	35.6%	100.0%
>6cms	15	6	21
	71.4%	28.6%	100.0%

PD-L1 positive expression was noted in 38 cases(64.4 %) out of 59 with size 3-6 cm and lesion with size > 6cm 15 cases(71.4%) out of 21 showed PD-L1 Positive expression (75.4%of cases) with p value of 0.819, there was no statistical significant difference found between size and PD-L1. PD-L1 positive expression increased with size of lesion but was not of substantial statistical significance.

Table 20: **Distribution of subjects according to PD-L1 expression and lymph node status (Evaluated by MRI)**

MRI LYMPH NODE STATUS	PD-L1 Expression		Total
	Positive	Negative	
Lymphadenopathy noted	27	19	46
	58.7%	41.3%	100.0%
No significant lymphadenopathy	33	11	44
	75.0%	25.0%	100.0%

More number of cases which showed PD-L1 positive expression was noted in patients with no significant lymphadenopathy;33 cases(75%) out of 44 and in cases with lymphadenopathy 27 cases(58.7%) out of 46 cases showed PD-L1 positvity with p value of 0.121, there was no statistical significant difference found between lymph node status and PD-L1. PD-L1 expression was not affected by lymph node status.

Table 21: Distribution of subjects according to PD-L1 expressionand Overall survival

Overall survival	PD-L1 Expression		Total
	Positive	Negative	
Survived	58	27	84
	69%	31.8%	100.0%
Died	3	3	6
	50.0%	50.0%	100.0%

Out of 90 cases six subjects expired. More number of cases around 58 cases(69%) out of 85 showed PD-L1 Positive expression who have survived. Patients who had died showed equal proportion of PD-L1 positive and PD-L1 negative expression. Out of 6 cases three cases showed positive expression and three cases showed negative expression with p value 0.329, there was no statistical significant difference found between Overall survival and PD-L1 in our study.

FIGURES

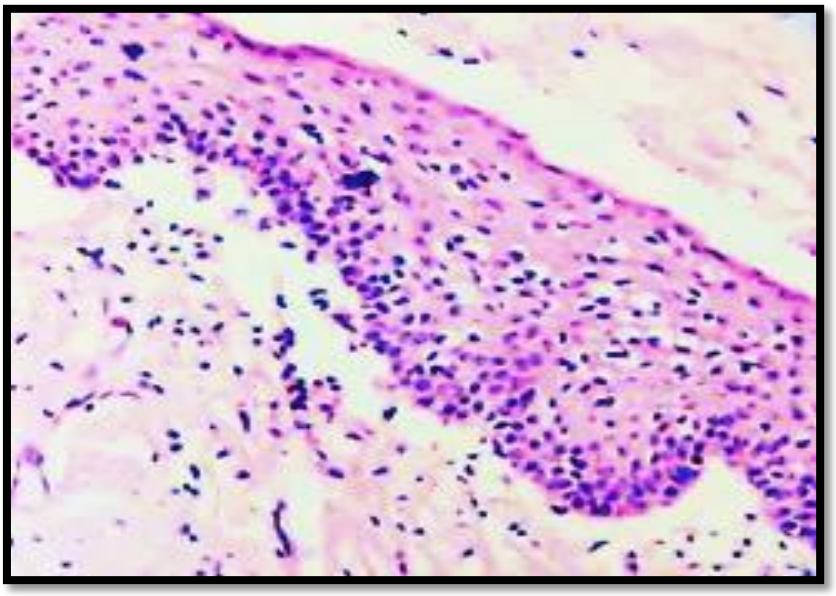

Figure 3 CIN I: showing atypia in basal one third of the epithelium with Koilocytotic changes in cells of superficial layers(H & E 40 X)[4]

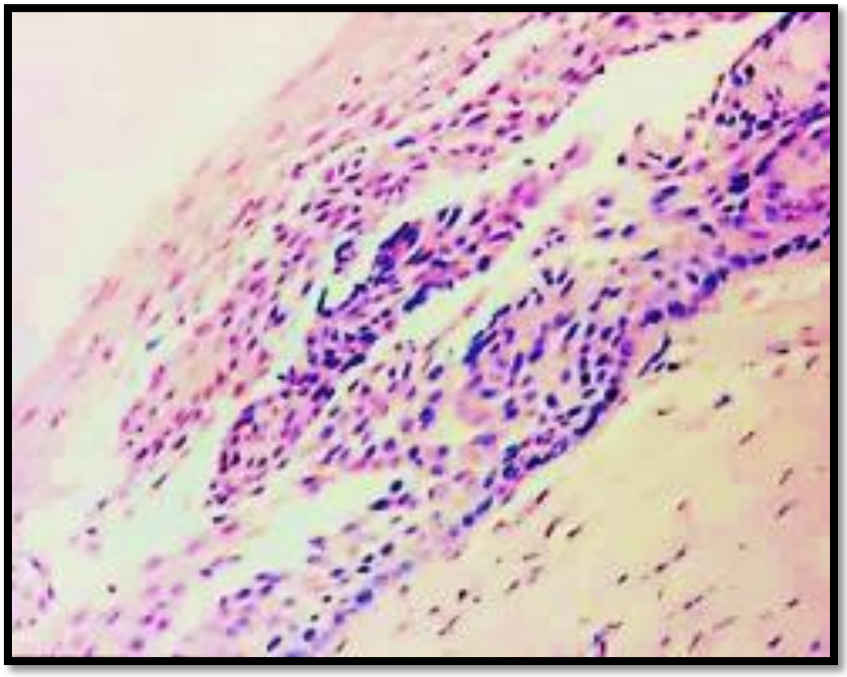

Figure 5 CIN II: shows cellular atypia and mitotic activity in lower two third of the epithelium. Upper third epithelium is normal.(H& E 40x)[4]

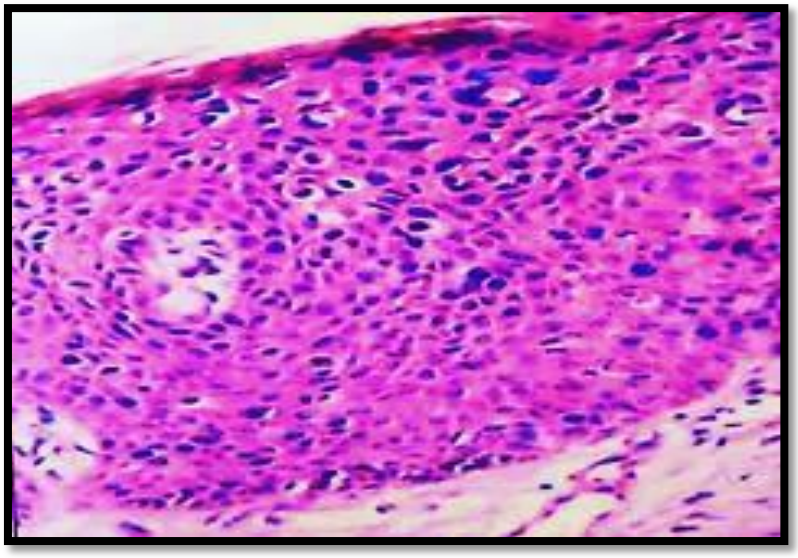

Figure 6 CIN III: shows pleomorphic cells with atypical mitotic figures in the entire thickness of the epithelium(H & E , 40 x)[4]

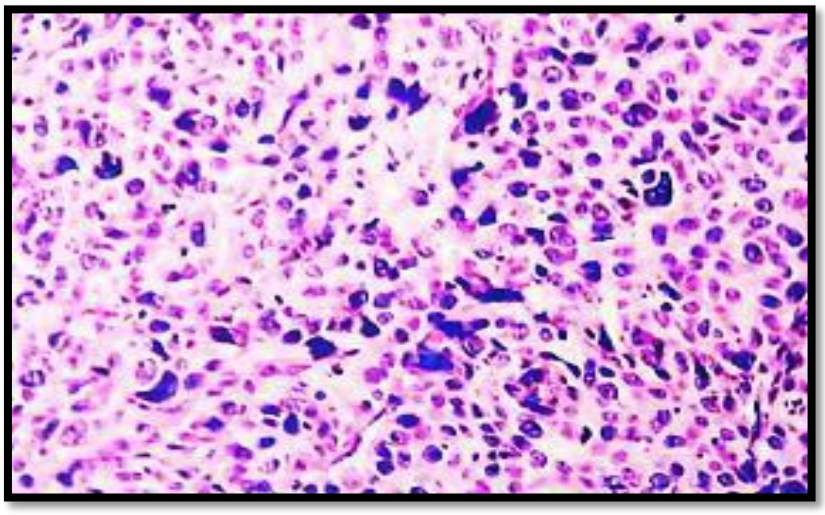

Figure 7 Squamous cell carcinoma(SCC) :Highly pleomorphic polygonal cells arranged in nests with highly pleomorphic nuclei and increased mitotic figures(H&E 40x)[4]

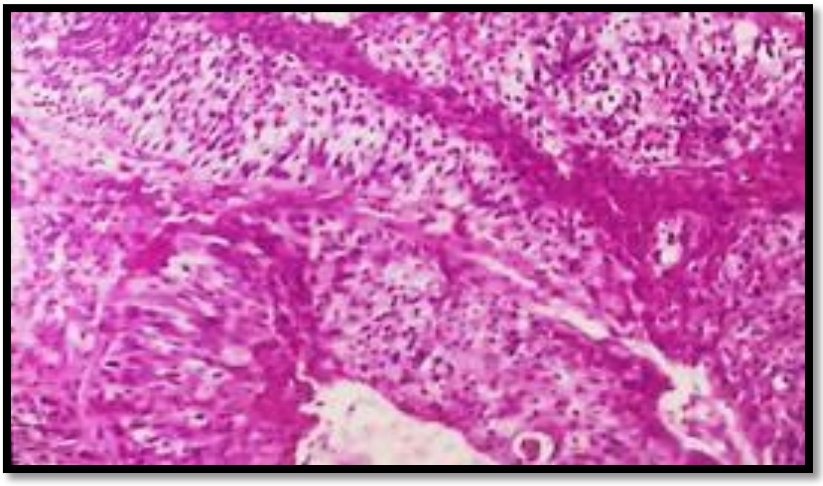

Figure 8 : Papillary SCC : Showing pleomorphic ovoid to spindle cells arranged in layers with flattening of cells towards surface. Fibrovascular core noted(H & E 40x)[4]

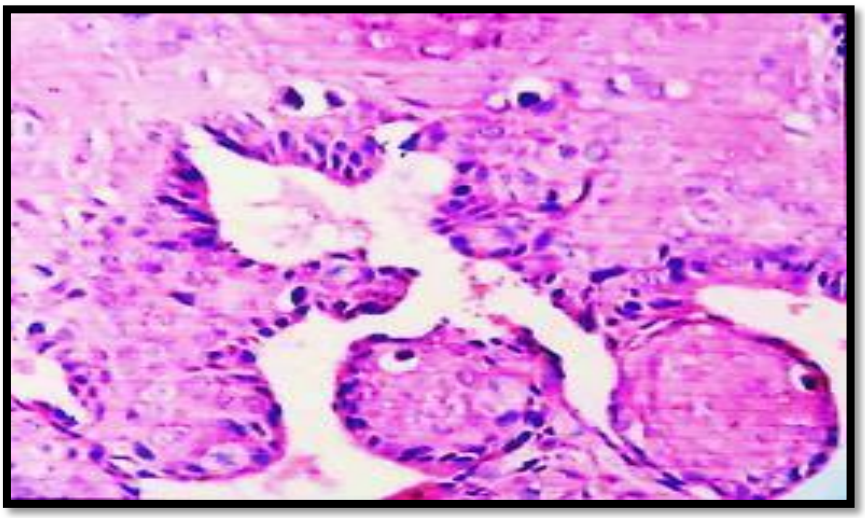

Figure 9 : Warty SCC : Moderately pleomorphic cells arranged in sheets and nests. Many cells are showing koilocytotic atypia.(H & E 40 X)[4]

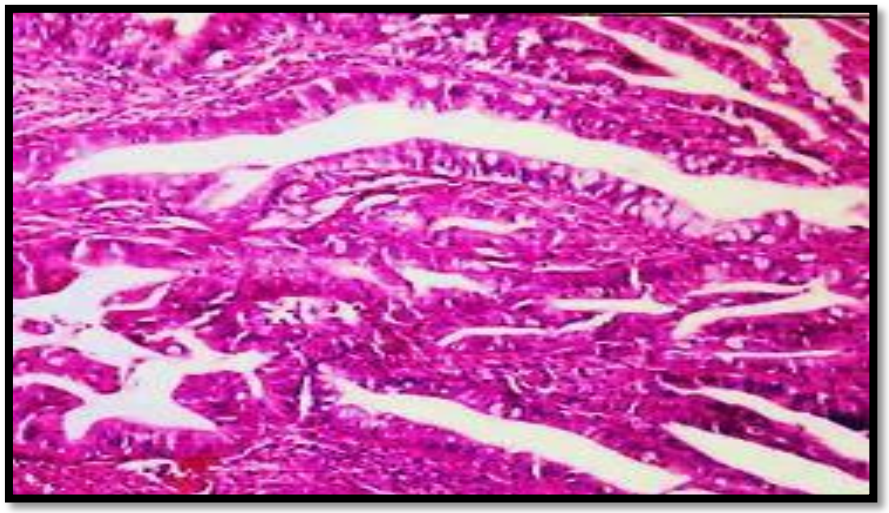

Figure 10 : Adenocarcinoma Endocervical variant : Atypical tall columnar cells with abundant intracytoplasmic mucin. Fibrovascular core is seen.(H & E 40x)[4]

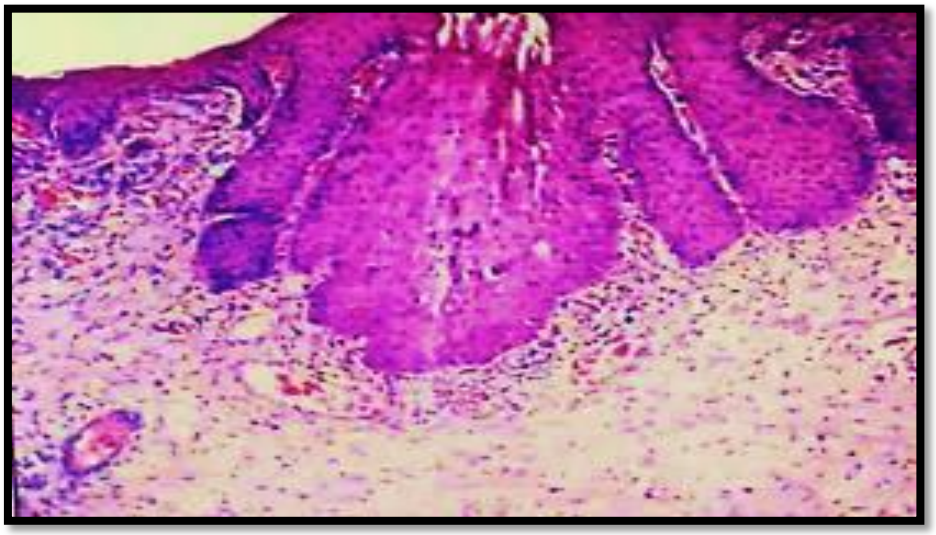

Figure 11 :Microinvasive SCC : invasion of tumor island with <3mm depth surrounded by inflammatory cell infiltrate and desmoplastic stroma.(H & E 40x)[4]

IMMUNOHISTOCHEMISTRY FIGURES

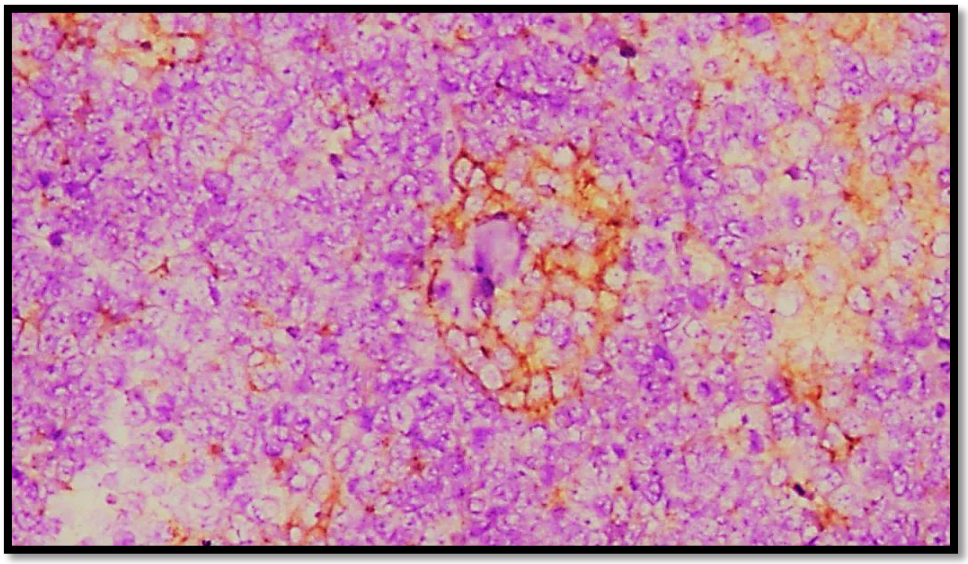

Figure 12: (IHC) PD-L1 expression Score 1 depicting 5-29% PD-L1 positive tumour cells with partially circumferential intensity on cell membrane of tumor cells.(40x)

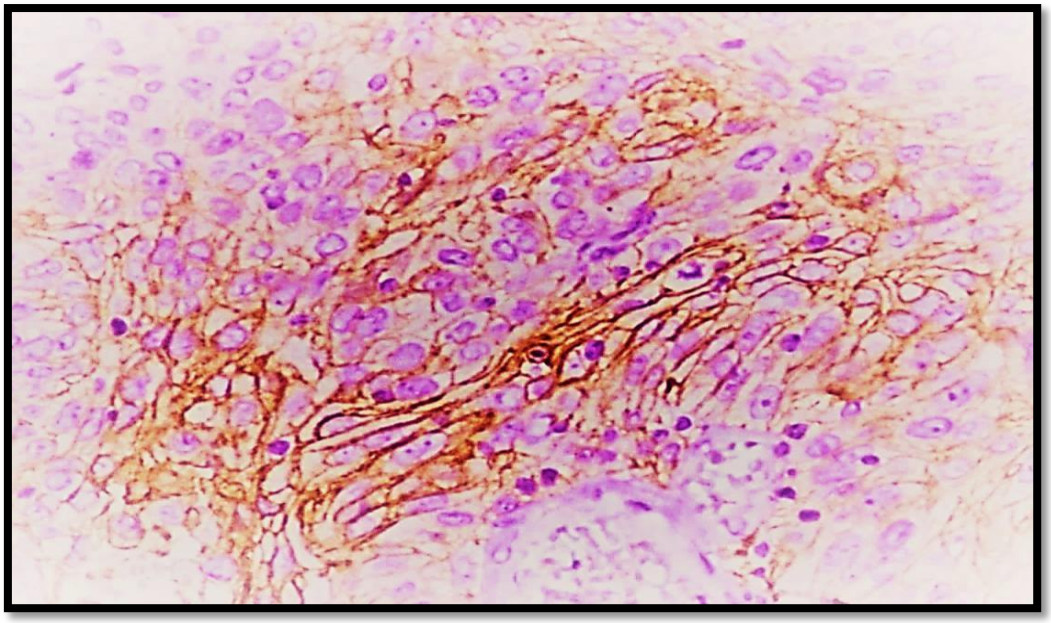

Figure 13: PD-L1 expression IHC Score 2: demonstrarting 30-59% PD-L1 positive tumour cells with clearly visible and circumferential intensity on cell membranes of tumour cells.(40X)

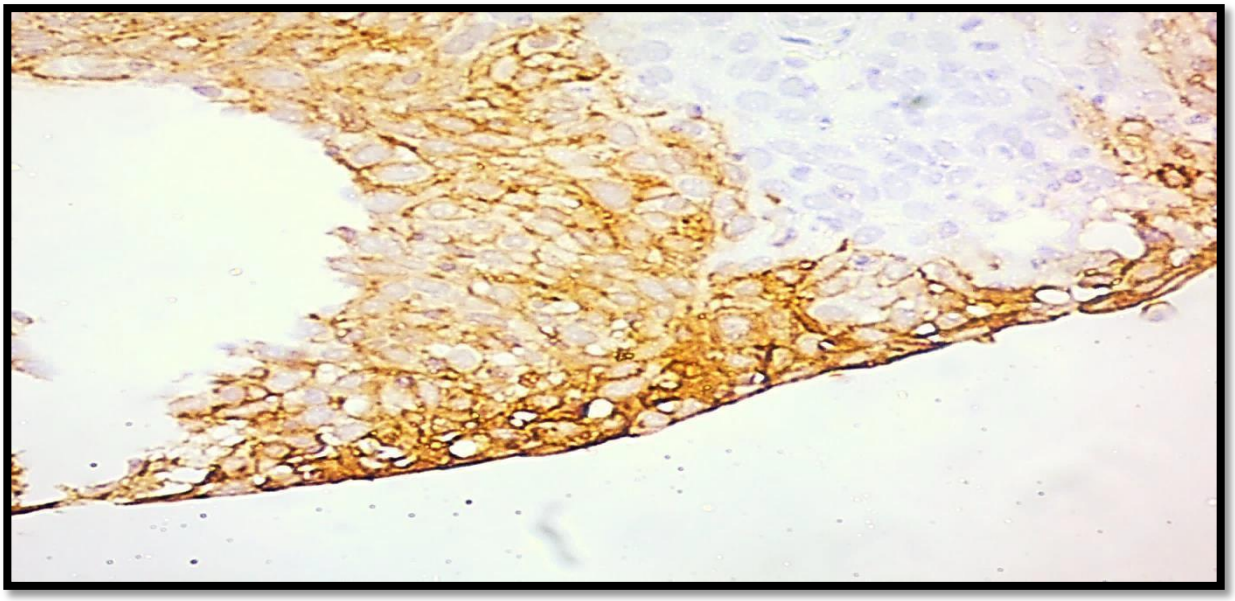

Figure 14: IHC Score 3: PD-L1 expression IHC Score 3: demonstrarting 60% PD-L1 positive tumour cells with intense and fully circumferential intensity on cell membranes of tumour cells.(40X)

DISCUSSION

DISCUSSION

Immunotherapy can probably be a noteworthy therapeutic option in cervical cancer as it involves HPV (human papilloma virus infection) as one of the etiologic factor. [33] Clinical trials are on-going to assess the effectiveness of PD-1/PD-L1 blockers in cervical cancer.[34] PD-L1 expression by (IHC) immunohistochemistry has been evaluated as a biomarker for assessing therapeutic response to anti PD-1/PD-L1 therapy with contrasting findings published in the literature as in patients where tumor did not show PD-L1 expression had clinically benefited from anti PD-L1 treatment.[35] Major challenges involved in interpretation of PD-L1 immunostaining are heterogeneity of tumor, transient expression of PD-L1 and lack of standardized PD-L1 readings/score[36]. Cervical SCC(squamous cell carcinoma) samples express higher PD-L1 levels in comparison to adenocarcinoma and adeno-squamous carcinoma of the cervix and PD-L1 expression also varies according to histologic grading.[37]In our study, analysis of PD-L1 expression in the neoplastic tissue for cervical carcinoma wascarried out. The tissue sections were interpreted after subjected to IHC for PD-L1. PD-L1 marker shows membranous positivity. In the present study a scoring system was assigned for PD-L1 scoring.[19] Earlier researchers have identified tumor-stroma interface staining pattern which is associated with better prognosis in comparison to diffuse PD-L1 expression in subjects of Cervical carcinoma.[37] PD-L1 expression can occur as a dynamic or slow process and previous radiation treatment can also alter the PD-L1 expression.[38] so cases that underwent chemotherapy and radiotherapy before biopsy were excluded from the present study.

CLINICOPATHOLOGICAL PARAMETERS:

AGE DISTRIBUTION :

Table 22: Age distribution of Cervical Squamous cell carcinoma cases along with PD-L1 expression and Comparison with other studies.

Parameter	**Liang Y et al(2020)**	**O Saglam et al(2019)**	**Present Study**
Age distribution according to PD-L1 positive expression	20-30 years	40-50 years	70-79 years

Present study showed as the age increases PD-L1 positive expression also increases. In current study 83.3% cases showed PD-L1 Positivity in age group 70-79 years with p value of **0.037** indicating substantial statistical significance difference between age and PD-L1 positive expression which was in contrast to study done by Liang Y et al[39]; as in their study increased PD-L1 expression was more commonly observed in younger patients (20-30 years of age). Study done by O Saglam et al[22] also showed higher PD-L1 positive expression in younger patients (p value=0.028).

Among the clinic-pathologic parameters in our study , elderly age was associated with high PD-L1 expression in Cervical squamous cell carcinoma which is contrast to other studies. Possible explanation that why young patients are affected more than elderly is due to Immune-senescence. With aging, thymic involution occurs and decreased T-cell progenitors from bone marrow leads to paucity of naive T-cell production. Aging is associated with decreased expression of CD28 on the surface of CD8+ T cells affecting immune activation.[40,41,42]. CD57, senescence marker is considered to be augmented on the surface of

cytotoxic T cells of elderly ultimately leading to reduced anti-tumoral immunogenic response.[43,44] Levels of perforin and granzyme which are vital for T cell's cytotoxic activity are lower in elderly population as compared to younger population.[45] In present study PD- L1 expression was positive in 10 out of 12 cases(83.3%) for the age group 70-79 years as the sample size in that age group is very small further research is recommended in a larger subset of elderly patients for drawing any conclusive inference for the correlation betweenage and PD-L1 positive expression.

Table 23: Correlation of Parity with PD-L1 expression in Cervical SCC cases:

Parity	PD-L1 Expression	
	Positive	Negative
1-3	50(67.6%)	24(32.4%)
>3	10(**62.5%**)	6(37.5%)

Parity correlation with PD-L1 positive expression : In our study women with more than three children showed decreased expression of PD-L1 and it was not statistically significant with p value of 0.772 comparison was not feasible with other studies as no studies are published with regards to correlation between PD-L1 expression and parity.

Correlation of Clinical findings of Cervical carcinoma cases with PD-L1 positive expression :

PD-L1 Positive expression was noted more in patients with bleeding per vagina as compared to patients with white discharge per vagina. In our study p value was 0.083, there was no substantial significant difference found between clinical finding and PD-L1 which is comparable to study done by Liang Y et al[39] which also showed no association between high PD-L1 expression and clinical features.

Table 24: PD-L1 expression correlation with histological Grades in Cervical SCC cases and Comparison with other studies.

Parameter Histological grading	**PD-L1 positive expression**		
	Chen Z et al (2016) Cases	**Feng M et al (2018) Cases**	**Present study**
Well differentiated	11/14**(78.5%)**	22/25 (**88%**)	8/ 15 (53.3%)
Moderately differentiated	28/36**(77.7%)**	22/25(**88%**)	40/47 (**85.1%**)
Poorly differentiated	39/50**(78%)**	49/169(**28.9%)**	12/28 (42.9%)

Study done by Chen Z et al[1]; maximum PD-L1 expression was noted more in well differentiated carcinoma cases 11 out of 14 cases (78.5%) followed by poorly differentiated carcinoma 39 out of 50 cases (78%) and least in moderately differentiated carcinoma Cervical SCC 28 out of 36 cases(77.7%). In their study 22 cases demonstrated PD-L1 negative expression. In Current study PD-L1 positive expression was noted more in moderately differentiated Squamous cell carcinoma of Cervix 40;(85.1% cases) out of 47 demonstrated PD-L1 expression which was comparable to study done by Feng M et al[20] in which 22 out of 25 cases(88%) demonstrated maximum PD-L1 positive expression in moderately differentiated carcinoma Cervical SCC cases.

In present study PD-L1 negative expression was more noted in poorly differentiated carcinoma 16 out of 28 cases (57.1%) followed by 7 out of 15 cases(46.7%) in well differentiated carcinoma and 7 out of 47 cases(14.9%) in moderately differentiated carcinoma. In Study by Feng M et al[20] 126/219 (57.5%) cases showed PD-L1 negative expression which was not categorized according to tumor differentiation in their study. In present study **p value was <0.001** and there was statistical significant difference found

between histological grading and PD-L1 positive expression which was comparable to study done by Chen Z et al[1] which also showed substantial statistical association(p value <0.005) of PD-L1 positive expression and histological grading. Whereas study done by Feng M et al[20] showed no statistical association between histological grading and PD-L1 positive expression.

Table 25: PD-L1 Positive expression and FIGO Staging in Cervical SCC cases and comparison with other studies

Parameter FIGO Staging (2018)	**PD-L1 positive expression**		
	Grochot RM et al (2018)	**Chen Z et al (2016)**	**Present study**
Stage I	10.1% (6/59)	79.4%(31/39)	**0%**
Stage II	30.5%(18/59)	79%(34/43)	63.4% (26/41)
Stage III	**40.7**%(24/59)	80%(8/10)	**70.4%** (31/44)
Stage IV A	13.6%(8/59)	**83.3**%(5/6)	60% (3/5)
Stage IV B	5.1%(3/59)	0/2(0%)	0%

In present study PD-L1 positive expression was noted more in Stage III around 31 out of 44 cases (70.4%) which was comparable to study done by Grochot RM et al[46] which also showed maximum PD-L1 positive expression in stage III 24 out of 59 cases (40.7%).

In study by Chen Z et al[1] maximum PD-L1 positive expression in stage IVA 5 out of 6 cases(83.3%). In both the studies done by and Chen Z et al[1] and Grochot RM et al[46] there was no statistical association found between FIGO staging and PD-L1 positive expression. In

present study also there was no statistical significant difference(p value of 0.662) found between stage and PD-L1 positive expression.

In our study we analyzed PD-L1 expression in each stage as follows :

PD-L1 positive expression:

In Stage IIIA maximum positivity was noted 3 out 3 cases showed PD-L1 positivity followed by Stage IIIB 28 out 41 (68.3%) cases showed PD-L1 positive expression. In Stage II B 24 out of 36 (66.7%) demonstrated PD-L1 Expression followed by Stage IVA 3 out of 5 (60%) cases showed PD-L1 Positive expression. In stage II A 2 out 5 cases (40%) showed PD-L1 positive expression. Study done by Meng Y et al[47] showed similar findings as in their study 90% of cases showed PD-L1 positive expression in stage IV as compared to 62.3% in stage II. This indicates PD-L1 positivity increases with advanced stage of cancer but in present study we had a very small sample size in Stage III (only 3 cases) to draw any significant conclusion.

PD-L1 negative expression:

In stage IIA 3 out of 5 cases (60%) showed PD-L1 negativity. 12 out of 36 cases (33.7%) showed PD-L1 negative expression in Stage IIB. In stage IIIA all 3 cases out of 3 showed PD-L1 positive expression. PD-L1 negative expression was not observed in stage IIIA.

Table 26: Correlation of PD-L1 Positive expression and Size of lesion (Evaluation By MRI) in Cervical SCC cases and comparison with other studies:

Parameter Size of lesion	**PD-L1 positive expression**			
	Heeren AM et al(2016)	**Chen Z et al(2016)**	**Feng M et al (2018)**	**Present study**
<4cm	27/38(71.5%)	43/55(78.1%)	24/63 (38%)	7/10(70%)
>4cm	68/97(70.1%)	35/45(77.7%)	47/156(30.1%)	53/80(66.25%)

In Present study PD-L1 positivity was noted in 7 out of 10 cases(70%) with tumour size less than 4cm and tumour size more than 4 cm;53 out of 80 cases (66.25% cases) showed PD-L1 positive expression which was comparable to the study done by Heeren AM et al[6] which demonstrated PD-L1 positive expression in 27 out of 38 cases(71.5%) with tumour size less than 4cm and 68 out of 97 cases (70.1%) showed PD-L1 positivity with tumour size >4cm.[6] Study done Feng M et al [20] showed contrasting findings;24 cases out of 63(38%) cases demonstrated PD-L1 positivity with tumour size <4cm and with tumor size >4cm;47 out of 156 cases(30.1%) showed PD-L1 positivity. Study done by Chen Z et al[1] also showed similar findings to our study in tumour <4 cm; 43 out of 55 cases (78.1%) cases showed PD- L1 positivity and in cases with tumor size >4cm 35 out 45 cases showed PD-L1 positive expression(77.1%). In current study p value was 0.819, there was no statistically significant difference found between size of lesion and PD-L1 positive expression. In study done by, Chen Z et al[1], Heeren AM et al[6] ,Feng M et al[20] also there was no statistical significance noted between size of lesion and PD-L1 expression. In Study done by Xu G et al[48] and Lathika AS et al[49] also no statistical association was found between PD-L1 expression and tumor size but in their studies no size range was defined.

Correlation of PD-L1 Positive expression and Lymph node status (evaluated by MRI) in Cervical SCC cases and comparison with other studies:

PD-L1 expression was not affected by lymph node status as in current study cases with no significant lymphadenopathy;33 out of 44 cases (75%) showed PD-L1 positive expression and cases with significant lymphadenopathy 27 out of 46 cases (58.7%) cases showed PD-L1 positivity with p value of 0.121, there was no statistically significant difference found between lymph node status and PD-L1 positivity. Results of the present study were comparable to the study done by Lathika AS et al[49], Liang Y et al[50] and Hui Z et al[51] where no statistical associations were found between Lymph node status and PD-L1 positive expression.

Correlation of PD-L1 positive expression and overall survival in Cervical SCC cases and comparison with other studies :

In present study ; Out of 90 cases six subjects expired. PD-L1 Positive expression was noted in 58 out of 85 cases (69%) in the patients who had survived and 3 out of 6 cases (50%) showed PD-L1 positive expression in patients who did not survive with p value 0.329, there was no statistical significant difference found between Overall survival and PD-L1 positive expression in our study. There was no independent association with PD-L1 positivity and overall survival in study done by Grither W et al.[52] and Enwere EK et al.[53] ·In Our study the survival status of each patient was traced telephonically until 1st October 2021(For threeyears from October 2019 to October 2021). Study done Chen J et al[54] also showed no impact of PD-L1 positive expression on Overall survival of the patient.

Table 27: PD-L1 Positive expression in Cervical SCC cases. Comparison with other studies

S.No	Studies	PD-L1 expression
1.	**Meng Y et al(2018)[47]**	68/97**(70.1%)**
2.	Reddy OL et a(2017)[19]	56/148(37.8)%
3.	Feng M et al[20](2018)	71/219(32.4%)
4.	Grochot RM et al (2019)[46]	(19/59)32%
5.	Grither W et al(2020)[52]	58/64(90.6%)
6.	**Chen J et al(2020)[54]**	61/95(64.2%)
7.	Present study	60/90**(66.6%)**

In our study PD-L1 positivity was noted in 60 out of 90 cases(66.6%) of Cervical squamous cell carcinoma these findings were comparable to the study done by Meng Y et al[47] in which 68 out of 97 cases (70.1%) showed PD-L1 positivity. Study done by Chen J et al[54] also showed similar findings in which 61 out of 95 cases (64.2%) showed PD-L1 positive expression.Whereas study done by Reddy OL et al[19] , Feng M et al[20],Grochot RM et al[46] showed contrasting findings. In study done by Reddy OL et al[19] 56 out of 148 (37.8%) cases showed PD-L1 positive expression. In study done by Feng M et al[20] 71 out 219(32.4%) cases demonstrated PD-L1 positivity and study done by Grochot RM et al[46] 19 out of 59 cases (32%) showed PD-L1 positive expression. Maximum PD-L1 positivity was observed in studydone Grither W et al[52] in which 58 out of 64 cases(90.6%) showed PD-L1 positive expression. The possible reason for different PD-L1 expression in Cervical SCC in various studies can be attributed to different sample size of different geographical region, different procedure used for evaluation, no standardized reporting format for PD-L1 expression and different antibody kits used for IHC.

To summarize, In current study PD-L1 expression was assessed in cervical cancer cells(Particularly in Squamous cell carcinoma of Cervix)and also correlation of PD-L1 expression with Clinico-pathological were studied. The finding of our study demonstrated the PD-L1 positive expression in most of the cervical cancer tissues (66.6%). On correlation of PD-L1 expression with Clinico-pathological parameters only age and histological grading showed statistical significant association with PD-L1 expression whereas no significant statistical association was found with FIGO staging, size of lesion , lymphadenopathy and overall survival.

Our findings suggest that targeting the PD-1/PD-L1 pathway can be a promising immunotherapy approach in patients with cervical cancer, as PD-L1 was expressed in 66.6% of the squamous cell carcinoma in our patient population. Recent research has shown that even patients with PD-L1-negative expression in primary tumors(lung carcinoma, colorectal carcinoma , bladder cancer and melanoma shows response to anti-PD-L1 treatment.[55,56] The reason for this might be due to heterogeneous and discordant PD-L1 tumor cell staining between primary tumor cells and metastatic tumor cells and in some instances PD-L1-positive metastases arising from PD-L1-negative primary tumors.[57,58] PD-L1 expression in tumours has been evaluated in various solid malignancies with varying percentages ranging from 24.2%-46.1%.[59,60,61,62.]

The overall rate of PD-L1 expression in cervical cancer tumors(Both adenocarcinoma and Squamous cell carcinoma) in study done by Kim M et al[63] noted was 44.4% which was similar to that was noted in other malignancies. In our study, PD-L1 expression was detected only in tumors that were confirmed to exhibit SCC histology as adenocarcinoma cases were not included in present study and the frequency of PD-L1 expression was **66.6%** in SCC

cases which was similar to Kim M et al[63] which showed **66.7%** PD-L1 positive expression in Cervical SCC cases. Mezache et al[64] also showed notable PD-L1 expression in 18 out of 37 (51%) of cervical SCC cases. Adding to these significant rates of PD-L1 expression in tumour cells, our findings could suggest that the PD-1/PD-L1 pathway can be a potential immunotherapy target in patients with cervical cancer who exhibits SCC histology. Further research is needed on a larger population to investigate whether inhibiting the PD-L1/PD-1 pathway can unlock the potency of T cell-mediated immunity in patients with cervical carcinoma.

CONCLUSION

CONCLUSION

This study has found a significant expression of PD-L1 in cervical squamous cell carcinoma cases around 66.6% of cases. The findings in this study further support a role for the investigation of anti-PD-L1/PD-1 immunotherapies for the treatment of PD-L1-positive cervical tumors. In addition, our adopted scoring system can help to identify different responders to the immunotherapy as correlation was analyzed with degree of PD-L1 expression. In present study significant correlation of PD-L1 expression was noted between age and histological grading and no significant correlation was found between PD-L1 expression and various clinico-pathological parameters. The latest FIGO system advices the utilization of imaging as an adjunct to clinical assessment. MRI is the imaging modality of choice for staging and follow-up of cervical cancers. Future clinical trials with larger population size and multi-centric studies can prove the role of PD-L1 in treatment of cervical carcinoma.

LIMITATIONS OF THE STUDY:

1.Small Sample size.

2.Single centre study.

3.Only Squamous cell carcinoma cases were considered for the study.

SUMMARY

SUMMARY

Retrospective study done to correlate the Immunohistochemical expression of PD-L1 in squamous cell carcinoma of cervix. The samples were collected from July 2016 to June 2018. Ninety cases were considered for the study.

Following salient features are noted:

1. The average age of presentation in current study was 52 years.
2. Most cases of cervical carcinoma were noted in women having two children (parity 2) around 56% of cases.
3. Most common chief complaint finding noted was bleeding per vagina(72.2% cases)
4. According to Histological grade most common grade of cervical carcinoma noted was moderately differentiated carcinoma(52.2% cases).
5. Most common FIGO staging of carcinoma cervix cases was stage IIIB.
6. In Overall survival assessment: Out of 90 cases of carcinoma cervix 84 cases had survived (93.3% cases) and 6 cases unfortunately succumbed to death (6.6% ofcases).
7. On Assessment by MRI (Radiological evaluation) lymphadenopathy was noted in 46 cases of cervical carcinoma (51.1%) and no significant lymphadenopathy was observed in 44 cases (48.9%).
8. PD-L1 positive expression was observed in 60 cases of cervical carcinoma (66.7% of cases) and PD-L1 negative expression was noted in 30 cases of cervical carcinoma(33.3%)
9. Substantial statistical correlation was noted of PD-L1 Expression with age and histologic grade.

10. No statistical correlation was seen between PD-L1 expression and various otherclinico-pathological parameters such as Parity, Clinical findings, Staging ,overallsurvival , lymph node status, size of lesion.

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ANNEXURE

ANNEXURE-

INFORMED CONSENT FORM

TITLE: SIGNIFICANCE OF PROGRAMMED DEATH LIGAND-1 IN INVASIVE SQUAMOUS CELL CARCINOMA OF UTERINE CERVIX

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

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

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

Name and signature / thumb impression Date:

(Subject) Place:

Name and signature / thumb impression Date:

Place:

(Witness/Parent/ Guardian/ Husband)

ANNEXURE-77

TITLE: SIGNIFICANCE OF PROGRAMMED DEATH LIGAND-1 IN INVASIVE SQUAMOUS CELL CARCINOMA OF UTERINE CERVIX

PATIENT INFORMATION SHEET :

STUDY TITLE: Significance of Programmed Death Ligand-1 in invasive squamous cell carcinoma of uterine cervix

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

The main aim of the study is to check for the presence of PD-L1 expression in tumor cells in cervical carcinoma and its correlation with clinico-pathological parameters.

You are requested to participate in a study conducted by the department of pathology as a part of dissertation. This study will be done on Cervical carcinoma specimens of the patients. The specimens will be collected from the Department of pathology, Sri Devaraj Urs medical college, Kolar.

The information collected will be used only for dissertation and publication. There is no compulsion to agree to participate. You are requested to sign / provide thumb impressiononly if you voluntarily agree to participate in the study. All information collected from you will be kept confidential and will not be disclosed to any outsider. Your identity will not be revealed. You will not receive any monetary benefits to participate in this research.

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

For any clarification you are free to contact the investigator.

PRINCIPAL INVESTIGATOR : Dr. Nikhil

Phone number : 9354086199

ANNEXURE-III

TITLE: SIGNIFICANCE OF PROGRAMMED DEATH LIGAND-1 IN INVASIVE SQUAMOUS CELL CARCINOMA OF UTERINE CERVIX

PATIENT PROFORMA

Name :
Age:
Parity:
Hospital Number:
Biopsy No:
Nature of specimen:

CHIEF COMPLAINTS:

HISTORY OF PRESENTING ILLNESS**:**

PAST HISTORY:

FAMILY HISTORY:

RADIOLOGICAL FINDINGS (Evaluated by MRI)**:**

SIZE OF LESION:

LYMPH NODE INVOLEVEMENT:

MICROSCOPY:

HISTOPATHOLOGICAL DIAGNOSIS **:**

GRADE OF SQUAMOUS CELL CARCINOMA

FIGO Staging:

IMMUNOHISTOCHEMICAL FINDING:

PD-L1 EXPRESSION:

FINAL IMPRESSION:

ANNEXURE-IV

KEYS TO MASTER CHART

WDPV	White Discharge per Vagina
BPV	Bleeding Per Vagina
MRI	Magnetic Resonance Imaging
PD-L1	Programmed Death Ligand-1
P	Positive
N	Negative

MASTER CHART

S.NO	UHID Number	Age	Parity	Clinical findings	Histological grading	Staging	Size of lesion(MRI evaluation)	Lymph node status(MRI Evaluation)	PD-L1 expression	Overall survival	Biopsy Number
1	358898	50	Parity (P2)	WDPV	Well differentiated Squamous cell carcinoma Cervix	Stage III B	4.4 x 3.2 x 4.2 cm	Lymphadenopathy noted	N	Survived	B-2896-16
2	313380	45	parity (P3)	BPV	Well differentiated Squamous cell carcinoma Cervix	Stage III B	4.7 X 4.3 X 4.1 cm	No significant lymphadenopathy	N	Survived	B-1983-2016
3	321794	55	Parity (P5)	WDPV	Well differentiated Squamous cell carcinoma Cervix	Stage III B	5.3 x 5.7 x 5.3 cm	Lymphadenopathy noted	N	Survived	B-2333-16
4	323335	70	Parity (P2)	BPV	Well differentiated Squamous cell carcinoma Cervix	Stage IV A	3.5 X 2.4 x 3 cm	Lymphadenopathy noted	N	Expired	B-2332-16
5	323929	47	Parity (P4)	BPV	Well differentiated Squamous cell carcinoma Cervix	Stage III B	2.2 x 1.7 x 1.9 cm	Lymphadenopathy noted	N	Survived	B-2249-2016
6	370720	45	Parity (P2)	WDPV	Well differentiated Squamous cell carcinoma Cervix	Stage II B	3.0 X 2.8 x 2.5 cm	No significant lymphadenopathy	N	Survived	B-3161-2016
7	303408	50	Parity (P2)	BPV	Poorly differentiated squamous cell carcinoma	Stage III B	4.5 X 3.3 X 4.1 cm	Lymphadenopathy noted	N	Survived	B-1559-2018
8	247495	43	Parity (P2)	WDPV	Poorly diffferentiated squamous cell carcinoma	Stage II B	3.0X2.9X1.8 cm	No significant lymphadenopathy	N	Survived	B-205-2016
9	526692	45	Parity (P1)	WDPV	Moderately differentiated squamous cell carcinoma	Stage III B	3.2 X 2.9 x 2.4 cm	No significant lymphadenopathy	N	Survived	B-2755-2017
10	516355	40	Parity (P3)	WDPV	Well differentiated Squamous cell carcinoma Cervix	Stage III B	4.2 x 5.2 x 6.4 cm	No significant lymphadenopathy	P	Survived	B-2674-2017
11	492189	55	Parity (P2)	BPV	Moderately differentiated squamous cell carcinoma	Stage II B	3.8 x 2.3 x 3.5 cm	No significant lymphadenopathy	P	Survived	B-2238-2017
12	475273	45	Parity (P2)	WDPV	Well differentiated Squamous cell carcinoma Cervix	Stage II B	4.5 x 4 x 3.7 cm	Lymphadenopathy noted	P	Survived	B-1949-2017
13	461060	45	Parity (P2)	BPV	Well differentiated Squamous cell carcinoma Cervix	Stage III B	5.4 x 4.3 x 3.9 cm	Lymphadenopathy noted	P	Survived	B-2801-2017
14	605701	55	Parity (P4)	WDPV	Well differntiated Squamous cell carcinoma Cervix	Stage III B	8.5 x 4.7 x 3.1 cm	No significant lymphadenopathy	P	Survived	B-1703-2018
15	608877	45	Parity (P2)	WDPV	Poorly differentiated squamous cell carcinoma	Stage III B	4.3 x 2.9 x 5.5 cm	Lymphadenopathy noted	N	Expired	B-291-2019
16	484935	30	Parity (P2)	WDPV	Moderately differentiated Squamous cell carcinoma	Stage II A	2.5 x 1.8 x 2.8 cm	No significant lymphadenopathy	P	Survived	B-2111-2017
17	443723	47	Parity (P4)	BPV	Poorly differentiated squamous cell carcinoma	Stage II B	9 X 7.3 X 6cm	Lymphadenopathy noted	N	Survived	B-1377-2017
18	495253	50	Parity (P3)	BPV	Poorly differentiated squamous cell carcinoma	Stage II B	4.2 x 4.2 x 2.6 cm	No significant Pelvic lymphadenopathy	N	Survived	B-1378-2016
19	491600	45	Parity (P5)	BPV	Poorly differentiated squamous cell carcinoma	Stage II B	9.7 x 2.7 x 4.9 cm	Lymphadenopathy noted	N	Survived	B-2219-17
20	256640	49	Parity (P2)	BPV	Poorly differentiated squamous cell carcinoma	Stage II A	3.5 x 2.8 x 3 cm	No significant lymphadenopathy	N	Survived	B-481-2016
21	394601	50	Parity (P2)	BPV	Poorly differntiated squamous cell carcinoma cervix	Stage II B	2.5 x 2.1 x 2.0 cm	No significant lymphadenopathy	P	Survived	B-1278-2018
22	562317	66	Parity (P4)	BPV	Well differentiated Squamous cell carcinoma Cervix	Stage III B	8.0 x 4.0 x 2.9 cm	No significant lymphadenopathy	P	Survived	B-655-2018
23	566511	60	Parity (P2)	BPV	Poorly differntiated carcinoma cervix	Stage III B	5.9 x 5.0 x 3.9 cm	Lymphadenopathy noted	N	Expired	B-766-2018
24	620230	62	Parity (P2)	BPV	Moderately differentiated squamous cell carcinoma	Stage II B	5.1 x 4.9 x 2.4 cm	Lymphadenopathy noted	P	Survived	B-2077-2018
25	621627	35	Parity (P2)	BPV	Moderately differentiated squamous cell carcinoma	Stage II B	3.6 x 1.6 x 2.2 cm	No significant lymphadenopathy	P	Expired	B-2091-2018
26	632255	70	Parity (P2)	BPV	Well differentiated Squamous cell carcinoma Cervix	Stage III B	5.8 x 4.6 x 5.5 cm	Lymphadenopathy noted	P	Survived	B-2324-2018
27	634967	61	Parity (P2)	BPV	Poorly differentiated squamous cell carcinoma	Stage II A	4.7 x 4.3 x 3.9 cm	Lymphadenopathy noted	N	Survived	B-2372-2018

S.NO	UHID Number	Age	Parity	Clinical findings	Histological grading	Staging	Size of lesion(MRI evaluation)	Lymph node status(MRI Evaluation)	PD-L1 expression	Overall survival	Biopsy Number
28	638088	60	Parity (P2)	BPV	Poorly differentiated squamous cell carcinoma	Stage II A	5.3 x 4.2 x 3.5 cm	Lymphadenopathy noted	N	Survived	B-2451-2018
29	643523	70	Parity (P2)	BPV	Poorly differentiated squamous cell carcinoma	Stage III B	6.2 x 5.2 x 4.8 cm	No significant lymphadenopathy	P	Survived	B-2568-2018
30	283318	55	Parity (4)	BPV	Moderately differentiated squamous cell carcinoma	Stage II B	6.3 X 5.3 X 5.0 cm	No significant lymphadenopathy	N	Survived	B-1292-16
31	370447	45	Parity (1)	BPV	Poorly differentiated squamous cell carcinoma	StageIII B	6.4 x 2.9 x 4.7 cm	Lymphadenopathy noted	N	Survived	B-3142-16
32	375284	50	Parity (1)	WDPV	Poorly differentiated squamous cell carcinoma	Stage IV A	6.7 x 5.5 x 5.8 cm	Lymphadenopathy noted	N	Survived	B-3250-16
33	331886	50	Parity (2)	BPV	Moderately differntiated Squamous cell carcinoma	Stage III B	3.3 x 2.9 x 3.0 cm	No significant lymphadenopathy	N	Survived	B-2043-16
34	175993	45	Parity (2)	WDPV	Poorly differentiated squamous cell carcinoma	Stage II B	4.9 x 4.4 x 3.6 cm	Lymphadenopathy noted	N	Survived	B-2046-16
35	460944	60	Parity (3)	BPV	Moderately differentiated squamous cell carcinoma	Stage II B	5.1 x 3.4 x 5.4 cm	Lymphadenopathy noted	P	Survived	B-1793-17
36	322038	45	Parity (3)	BPV	Moderately differentiated squamous cell carcinoma	stage II B	7.8 x 6.2 x 4.7 cm	No significant lymphadenopathy	N	Survived	B-2246-16
37	281312	50	Parity (1)	BPV	Poorly differentiated squamous cell carcinoma	Stage III B	5.9 x 4.5 x 5.0 cm	No significant lymphadenopathy	P	Survived	B-1469-17
38	501609	60	Parity (4)	WDPV	Poorly differentiated squamous cell carcinoma	Stage III B	4.5 x 4.4 x 2.4 cm	No significant lymphadenopathy	P	Survived	B-2435-17
39	369434	51	Parity (2)	BPV	Poorly differentiated squamous cell carcinoma	Stage III B	5.8 X 5.4 x 3.9 cm	No significant lymphadenopathy	N	Survived	B-3112-16
40	436116	45	Parity (8)	BPV	Poorly differentiated squamous cell carcinoma	Stage III B	3.7 x 5.4 x 2.3 cm	No significant lymphadenopathy	P	Survived	B-1196-17
41	446789	45	Parity (5)	WDPV	Poorly differentiated squamous cell carcinoma	Stage III B	4.6 X3.4 x 4.2cm	Lymphadenopathy noted	N	Survived	B-1365-16
42	435198	60	Parity (2)	BPV	Moderately differntiated Squamous cell carcinoma	Stage III B	4.2 x 3.6 x 3.1 cm	Lymphadenopathy noted	P	Survived	B-1185-17
43	390916	55	Parity (3)	WDPV	Poorly differentiated squamous cell carcinoma	Stage III	4.8 x 3.9 x 4.9 cm	Lymphadenopathy noted	P	Survived	B-3345-16
44	428994	50	Parity (5)	WDPV	Moderately differntiated Squamous cell carcinoma	Stage II B	2.6 x 2.2 x 2.0 cm	No significant lymphadenopathy	P	Survived	B-1336-17
45	427804	62	Parity (5)	WDPV	Moderately differentiated squamous cell carcinoma	Stage II B	4.4 x 3.3 x 3.2 cm	No significant lymphadenopathy	P	Survived	B-836-17
46	424939	40	Parity (2)	WDPV	Well differntiated Squamous cell carcinoma	Stage II B	5.9 X 4.8 X 5.0 cm	Lymphadenopathy noted	N	Survived	B-1714-17
47	418399	52	Parity (2)	BPV	Poorly differentiated squamous cell carcinoma	Stage II B	5.4 x 4.5 x 5.3 cm	No significant lymphadenopathy	P	Survived	B-3324-16
48	360706	40	Parity (3)	BPV	Moderately differentiated Squamous cell carcinoma	Stage II B	5.4 x 2.6 x 5.0 cm	No significant lymphadenopathy	P	Survived	B-2290-17
49	374099	35	Parity (2)	BPV	Poorly differentiated squamous cell carcinoma	Stage II B	5.4 x 4.5 x 5.3 cm	No significant lymphadenopathy	P	Survived	B-3224-16
50	410264	44	Parity (3)	BPV	Moderately differntiated Squamous cell carcinoma	Stage III B	4.7 x 3.8 x 6.4 cm	Lymphadenopathy noted	P	Survived	B-582-17
51	268535	60	Parity (5)	BPV	Moderately differntiated Squamous cell carcinoma	Stage II B	4.5 x 2.5 x 4.6 cm	No significant lymphadenopathy	P	Survived	B-787-16
52	616252	50	Parity (P6)	BPV	Moderately differentiated squamous cell carcinoma	Stage III B	4.4X3.1X4.1 cm	No significant lymphadenopathy	P	Survived	B-1979-2018
53	508851	64	Parity (P2)	WDPV	Moderately differntiated Squamous cell carcinoma	Stage II B	4.4 x 3.2 x 4.2 cm	Left internal iliac lymph node,pararectal	P	Survived	B-2579-2017
54	493423	60	Parity (P2)	BPV	Moderately differentiated squamous cell carcinoma	Stage III B	4.7 X 4.3 X 4.1 cm	Lymphadenopathy noted	P	Survived	B-2362-2017

S.NO	UHID Number	Age	Parity	Clinical findings	Histological grading	Staging	Size of lesion(MRI evaluation)	Lymph node status(MRI Evaluation)	PD-L1 expression	Overall survival	Biopsy Number
55	561416	45	Parity (P3)	BPV	Poorly differentiated squamous cell carcinoma	Stage IV A	5.3 x 5.7 x 5.3 cm	Lymphadenopathy noted	P	Survived	B-727-2018
56	580449	45	Parity (P5)	BPV	Moderately differentiated squmaous cell carcinoma	Stage II B	3.5 X 2.4 x 3 cm	Lymphadenopathy noted	P	Survived	B-1227-2018
57	587201	35	Parity (P3)	BPV	Moderately differntiated Squamous cell carcinoma	Stage II B	2.2 x 1.7 x 1.9 cm	Lymphadenopathy noted	P	Survived	B-1285-2018
58	592899	70	Parity (P3)	BPV	Moderately differentiated squamous cell carcinoma	Stage III B	3.0 X 2.8 x 2.5 cm	Lymphadenopathy noted	P	Survived	B-1416-2018
59	594165	65	Parity (P1)	WDPV	Poorly differentiated squamous cell carcinoma	Stage II B	4.5 X 3.3 X 4.1 cm	Lymphadenopathy noted	N	Survived	B-1439-2018
60	594578	74	Parity (P2)	WDPV	Well differentiated Squamous cell carcinoma Cervix	Stage II B	4.4X3.1X4.1 cm	No significant lymphadenopathy	P	Survived	B-1559-2018
61	600124	70	Parity (P5)	WDPV	Moderately differntiated Squamous cell carcinoma	Stage III B	3.2 X 2.9 x 2.4 cm	No significant lymphadenopathy	P	Survived	B-1585-2018
62	328549	60	Parity (P2)	BPV	Moderately differntiated Squamous cell carcinoma	Stage III B	4.2 x 5.2 x 6.4 cm	No significant lymphadenopathy	P	Survived	B-2348-2016
63	306940	70	Parity (P2)	BPV	Moderately differntiated Squamous cell carcinoma	Stage III A	3.8 x 2.3 x 3.5 cm	No significant lymphadenopathy	P	Survived	B-1922-2016
64	264667	40	Parity (P2)	WDPV	Moderately differntiated Squamous cell carcinoma	Stage II B	4.5 x 4 x 3.7 cm	Lymphadenopathy noted	P	Survived	B-676-2016
65	474249	42	Parity (P2)	BPV	Moderately differntiated Squamous cell carcinoma	Stage II B	5.4 x 4.3 x 3.9 cm	Lymphadenopathy noted	P	Survived	B-1935-2017
66	424939	40	Parity (P2)	BPV	Moderately differntiated Squamous cell carcinoma	Stage III B	8.5 x 4.7 x 3.1 cm	No significant lymphadenopathy	P	Expired	B-1122-2018
67	562211	40	Parity (P2)	BPV	Moderately differentiated squamous cell carcinoma	Stage II A	4.3 x 2.9 x 5.5 cm	Lymphadenopathy noted	P	Survived	B-653-2018
68	573697	35	Parity (P3)	BPV	Moderately differntiated Squamous cell carcinoma	Stage III B	2.5 x 1.8 x 2.8 cm	Lymphadenopathy noted	P	Survived	B-968-2018
69	592979	65	Parity (P1)	BPV	Moderately differentiated squamous cell carcinoma	Stage II B	9 X 7.3 X 6cm	Lymphadenopathy noted	P	Survived	B-1424-2018
70	595616	70	Parity (P1)	BPV	Moderately differentiated squamous cell carcinoma	Stage II B	4.2 x 4.2 x 2.6 cm	No significant lymphadenopathy	P	Survived	B-1465-2018
71	598421	60	Parity (P1)	BPV	Poorly differntiated carcinoma cervix	Stage III B	9.7 x 2.7 x 4.9 cm	Lymphadenopathy noted	P	Survived	B-1527-2018
72	665342	38	Parity (P1)	BPV	Moderately differentitaed squamous cell carcinoma	Stage III B	3.5 x 2.8 x 3 cm	No significant lymphadenopathy	P	Survived	B-1704-2018
73	639583	49	Parity (P2)	BPV	Moderately differntiated Squamous cell carcinoma	Stage III B	2.5 x 2.1 x 2.0 cm	No significant lymphadenopathy	P	Survived	B-2488-2018
74	651263	65	Parity (P2)	BPV	Moderately differntiated Squamous cell carcinoma	Stage III B	8.0 x 4.0 x 2.9 cm	No significant lymphadenopathy	P	Survived	B-2735-2018
75	652085	50	Parity (P2)	BPV	Moderately differentiated squmaous cell carcinoma	Stage III B	5.9 x 5.0 x 3.9 cm	Lymphadenopathy noted	N	Survived	B-2761-2018
76	256640	47	Parity (2)	BPV	Moderately differntiated Squamous cell carcinoma	Stage III A	5.1 x 4.9 x 2.4 cm	Lymphadenopathy noted	P	Survived	B-481-16
77	323335	70	Parity (2)	BPV	Moderately differntiated Squamous cell carcinoma	Stage IV A	3.6 x 1.6 x 2.2 cm	No significant lymphadenopathy	P	Survived	B-2243-16
78	284941	64	Parity (3)	WDPV	Moderately diiferentiated squamous cel carcinoma	Stage II B	5.8 x 4.6 x 5.5 cm	Lymphadenopathy noted	N	Survived	B-2579-17
79	422195	50	Parity (4)	WDPV	Moderately differntiated Squamous cell carcinoma	Stage III B	4.7 x 4.3 x 3.9 cm	Lymphadenopathy noted	P	Survived	B-820-17
80	382537	50	Parity (1)	BPV	Moderately differntiated Squamous cell carcinoma	Stage III B	5.3 x 4.2 x 3.5 cm	No significant lymphadenopathy	P	Survived	B-3401-16
81	541261	38	Parity (1)	BPV	Moderately differntiated Squamous cell carcinoma	Stage IV A	6.2 x 5.2 x 4.8 cm	No significant lymphadenopathy	P	Survived	B-2743-18

S.NO	UHID Number	Age	Parity	Clinical findings	Histological grading	Staging	Size of lesion(MRI evaluation)	Lymph node status(MRI Evaluation)	PD-L1 expression	Overall survival	Biopsy Number
82	243861	50	Parity (2)	BPV	Moderately differntiated Squamous cell carcinoma	Stage III B	6.3 X 5.3 X 5.0 cm	No significant lymphadenopathy	P	Expired	B-121-16
83	544289	65	Parity (2)	BPV	Moderately differntiated Squamous cell carcinoma	Stage III B	6.4 x 2.9 x 4.7 cm	Lymphadenopathy noted	P	Survived	B-299-18
84	560608	78	Parity (2)	BPV	Poorly differentiated squamous cell carcinoma	Stage III B	6.7 x 5.5 x 5.8 cm	Lymphadenopathy noted	P	Survived	B-647-18
85	571055	55	Parity (2)	BPV	Well differntiated Squamous cell carcinoma Cervix	Stage III B	3.3 x 2.9 x 3.0 cm	Lymphadenopathy noted	P	Survived	B-887-18
86	577987	55	Parity (2)	BPV	Poorly differentiated squamous cell carcinoma	Stage II B	4.9 x 4.4 x 3.6 cm	Lymphadenopathy noted	P	Survived	B-1120-18
87	584508	38	Parity (2)	BPV	Moderately differntiated Squamous cell carcinoma	Stage II B	5.1 x 3.4 x 5.4 cm	Lymphadenopathy noted	P	Survived	B-1265-18
88	591499	75	Parity (2)	BPV	Moderately differentiated squamous cell carcinoma	Stage II B	7.8 x 6.2 x 4.7 cm	No significant lymphadenopathy	P	Survived	B-1376-18
89	592897	60	Parity (2)	BPV	Moderately differentiated squamous cell carcinoma	Stage II B	5.9 x 4.5 x 5.0 cm	No significant lymphadenopathy	P	Survived	B-1425-18
90	595556	70	Parity (2)	BPV	Moderately differentiated squamous cell carcinoma	Stage II B	4.5 x 4.4 x 2.4 cm	No significant lymphadenopathy	N	Survived	B-1464-18

MRI	Cranioadual X Transverse X Anterioposterior	dimensions
C	Chemotherapy	
R	Radiotherapy	
S	Surgery	