Original Article

Programmed death ligand (PD-L1) expression in invasive squamous cell carcinoma of the uterine cervix: A cross-sectional observational study

ABSTRACT

Background: Normal cervical epithelium does not express programmed death ligand-1 (PD-L1) protein. Expression of PD-L1 has been reported in 50% cells of cervical carcinoma. Approximately 17% of adenocarcinomas and 35% of squamous cell carcinomas (SCC) of the cervix express PD-L1.

Objectives: To determine the expression of PD-L1 in invasive SCC of the cervix and to correlate the expression with various clinicopathological parameters.

Materials and Methods: This was a cross-sectional study conducted between July 2016 and June 2021 in the Department of Pathology, in collaboration with the Department of Obstetrics and Gynecology at the R. L. Jalappa Hospital and Research Center, Kolar, in South India. proportion of PD-L1 expression was evaluated on the biopsy specimens by immunohistochemistry (PD-L1 Antibody Biogenex AN921-M) and based on the PD-L1 positive cells, was scored as 0 (<5%), 1 (5–29%), 2 (30–59%), and 3 (>60%). We evaluated the association of PD-L1 expression with the clinicopathological parameters.

Results: We included 90 cases of SCC of the cervix. The median age was 52.5 years (range, 30–74), with the majority of patients between the ages of 40–49 years. Overall, PD-L1 expression was noted in 66.7% cases of SCC of the cervix. PD-L1 marker expression increased with age, from 40–49 years (n = 14/28, 50%) to 70–79 years (n = 10/12, 83.3%); $P = 0.037$. PD-L1 expression was maximum (n = 40/47, 85.1%) in moderately differentiated SCC; there was a significant correlation between histological grade and PD-L1 expression; $P < 0.001$. There were no significant correlations between the PD-L1 expression and other clinicopathological parameters such as parity, clinical findings, disease stage, size of lesion, lymph node status, and overall survival.

Conclusion: Two-thirds of cervical SCC cases express PD-L1. The PD-L1 expression is significantly associated with the patient's age and the grade of the disease. Evaluation of PD-L1 expression in SCC of the cervix is important as anti-PD-L1/PD-1 immunotherapies can be used in PD-L1 positive cervical cancer.

Keywords: Cervical cancer, human papilloma virus, immunotherapy, carcinoma cervix, PD-L1

INTRODUCTION

Carcinoma of the cervix is the commonest gynecological malignancy across the globe. It ranks second in mortality and morbidity among the tumors of the female genital tract.[1] Worldwide, approximately 570,000 cases of carcinoma cervix are reported with 300,000 deaths, annually. In 2018 in India, the reported incidence of carcinoma cervix was 96,922 cases, with 60,078 deaths.[2] In Karnataka, approximately 5,000 new cases are reported every year.[3] In Bangalore city in Karnataka, cervical cancer is the second most common cancer in women,

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PUTTING IN PERSPECTIVE

Central question

- What is the expression of PD-L1 in invasive SCC of uterine cervix, and does the PD-L1 expression correlate with various clinicopathological parameters?

Key findings

- About 66.6% cases of SCC of cervix showed PD-L1 expression
- PD-L1 expression significantly correlated with the age distribution and grade of the disease
- Stage of the disease, parity, clinical presentation, size of the lesion, lymphadenopathy and overall survival did not show association with PD-L1 expression

Impact

- As 66.6% cases showed positive expression of PD-L1, immunotherapy can be considered a noteworthy therapeutic option in PD-L1 positive SCC of cervix cases in Indian population

with approximately 850 cases reported every year.[3] In a study done at Kolar, in South India, cervical cancer caused 17% of oncological cases among women.[3]

The peak age at which cervical cancer is reported is 55–59 years, with a mean age of presentation of approximately 52 years. The risk factors for carcinoma cervix include human papillomavirus (HPV) infection, sexually transmitted diseases, early age at marriage, poor personal hygiene, multiparity, smoking, poor socioeconomic status, immunosuppression, and the use of oral contraceptive pills.[4]

Programmed death ligand-1 (PD-L1) is a transmembrane protein, which inhibits the adaptive arm of the immune system. PD-L1 act as an immune checkpoint to prevent autoimmunity in healthy persons. Binding of PD-1 to PD-L1 regulates the immune system by suppressing immunity, enhancing self-tolerance, and decreasing the T cell inflammatory activity. This mechanism protects against autoimmune diseases, but it also prevents the killing of cancer cells by the immune system.[5]

HPV is a deoxyribonucleic acid (DNA) virus associated with cervical cancer. The common high-risk HPV strains are HPV-16 and HPV-18 which cause carcinoma cervix. Integration of the viral genome into the host genome along with acquisition of other genetic abnormalities results in malignant transformation.[6] The HPV genome integrates at the *PD-L1* gene locus resulting in increased *PD-L1* protein expression by disrupting the PD-L1 gene at the 3' untranslated region (UTR) especially in premalignant lesions of the cervix, subsequently giving rise to cervical cancer. Thus, anti-PD-L1 therapy could theoretically be efficacious in HPV-induced premalignant lesions of the cervix.[7,8]

Information regarding the level of PD-L1 expression and correlation with clinicopathological parameters in patients with cervical cancer is sparse. There is particularly a paucity of studies from developing countries on PD-L1 expression in cervical cancer.[8] Schellens *et al*.[9] reported 50% PD-L1 expression in cervical cancer cells and stated that patients with advanced cervical cancer showed PD-L1 expression of ≥1% in tumor cells (41 cases out of 47). Balar *et al*.[10] have reported 17% and 35% positive PD-L1 expression in cervical adenocarcinoma and squamous cell carcinoma (SCC), respectively. Given the paucity of data on PD-L1 in cervical carcinoma, we therefore set out to study this in our cohort of patients.

MATERIALS AND METHODS

General study details

This was a cross-sectional laboratory observational study conducted from July 2016 to June 2021 in the Department of Pathology, in collaboration with the Department of Obstetrics and Gynecology at R. L. Jalappa Hospital and Research Center, a tertiary healthcare center attached to Sri Devaraj Urs Medical College, Sri Devaraj Urs Academy of Higher Education and Research, Kolar in South India. Patients were followed up for three years from July 2018 to June 2021 to assess their overall survival. The Institutional Ethics Committee approved the study on Oct 11, 2019 (approval number IEC/109/2019-20); study protocol available as Supplementary Appendix 1. This study was not registered in any public clinical trials registry, as it was not an interventional trial. Written informed consent was obtained from all patients. The study was conducted according to the standard ethical guidelines including Good Clinical Practice Guidelines, the Declaration of Helsinki and those published by the Indian Council of Medical Research. The cost of the immunohistochemistry (IHC) kits was borne by the authors.

Participants

We included patients with invasive SCC of the cervix who had been diagnosed by tissue biopsy and had clinical details available in the case files, including radiological (Magnetic Resonance Imaging [MRI]) findings. We excluded recurrent cases, cases with metastatic deposits in the cervix, malignancies other than SCC of the cervix, and patients who had received chemotherapy and radiotherapy before biopsy [Figure 1].

Aims/objectives

Our primary objective was to evaluate the PD-L1 expression in invasive SCC of the cervix. Our secondary objective was to study its association with various clinicopathological parameters, including age, parity, clinical findings, grade and stage of the disease, size of the lesion, lymph node involvement, and overall survival. Cases were grouped as PD-L1 positive and negative (primary outcome) and correlated with clinicopathological parameters (secondary outcome).

Study methodology

Sociodemographic and clinical details of the cases such as age, parity, clinical presentation and overall survival of every case were captured from the records section of the hospital. Radiological findings were extracted from the MRI reports including the size of the lesion, lymph node involvement and disease stage. The International Federation of Gynecology and Obstetrics (FIGO) staging system was used for this study.[11] The hematoxylin and eosin-stained tissue section slides were reviewed, and the carcinoma was graded on histopathologic differentiation as well differentiated, moderately differentiated, or poorly differentiated.

Tissue sections were taken from the paraffin tissue blocks and stained with PD-L1 marker (PD-L1 Antibody Biogenex AN921-M) by IHC. The procedure for IHC was as per the manufacturer's instructions using negative and positive controls. Tissue sections were first examined at low magnification, and then at 40x magnification to identify the areas of highest positivity (hot spots). Areas of hot spots were utilized for the evaluation of IHC staining.

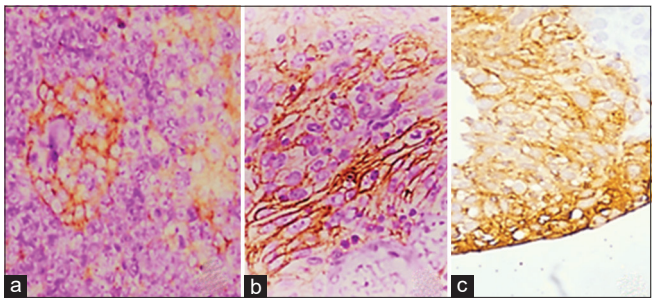

Figure 1: (a) PD-L1 expression of Score 1 (5-29% PD-L1 positive tumor cells) with partial circumferential staining on the cell membrane of tumor cells. (b) PD-L1 expression of Score 2 (30-59% PD-L1 positive tumor cells) with clearly visible and circumferential staining on cell membranes of tumor cells. (c) PD-L1 expression of Score 3 (approximately 60% PD-L1 positive tumor cells) with intense and fully circumferential staining on cell membranes of tumor cells. (Immunohistochemistry [IHC] PD-L1 40X)

Membranous positivity was considered as positive for PD-L1. PD-L1 marker expression was evaluated in five hot spot areas in high power fields. The expression was scored as follows: score 0: <5% of positive cells, score 1: 5–29%, score 2: 30–59%, and score 3: >60%. The intensity of PD-L1 marker staining was scored as follows: score 0: no expression observed, score 1: expression is visible partially or barely circumferential, score 2: expression is clearly visible/completely circumferential, and score 3: expression is intense/fully circumferential [Figure 1a–c]. The average score of five hot spot areas was taken and multiplied by the intensity to obtain the grading. Grading was done as follows; score 1 to 3 = grade I, score 4 to 6 = grade II, and score 7 to 9 = grade III.[12]

Statistics

The sample size of 90 was estimated based on the study by Feng *et al*.,[13] who reported that the PD-L1 expression in SCC cervix was 35%. The power was 0.95, the confidence interval was 95% with a 10% absolute error.

The data were entered in a Microsoft Excel data sheet. The data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 22 software (IBM SPSS Statistics, Somers NY, USA). Continuous variables were expressed as frequencies, percentages, mean and standard deviation. Student 't' test and Analysis of Variance (ANOVA) was utilized to derive the test of significance to evaluate the mean difference between the groups for quantitative data. Categorical data were represented as frequencies and proportions. Fisher's exact or Chi-square test was utilized to evaluate the test of significance for qualitative data. A *P* value of <0.05 was considered statistically significant.

RESULTS

PD-L1 expression

We included 90 cases of SCC of cervix, of which 60 (66.7%) showed PD-L1 expression [Figure 2]. Among the 60 PD-L1 positive cases, 22 (36.7%) had grade I PD-L1 expression, 26 (43.3%) had grade II, and 12 (20%) had grade III.

Clinicopathological features and correlation

Age: The majority of patients were between the ages of 40 and 49 years (n = 28, 31.1%), followed by 50–59 years (n = 22, 24.4%). Patients aged between 30 to 39 years had the highest level of PD-L1 expression (n = 8, 100%) followed

by those aged 70–79 years (n = 10 of 12 patients, 83.3%). The level of PD-L1 expression gradually increased from ages 40 to 79 years. There was a significant association of PD-L1 expression with the age distribution ($P = 0.037$) [Table 1].

Parity: There was no significant difference in the PD-L1 expression based on parity. The PD-L1 expression in patients with a parity of 1 to 3 (n = 50 of 74, 67.6%) was similar to that in those with a parity of more than 3 (n = 10 of 16, 62.5%); $P = 0.776$.

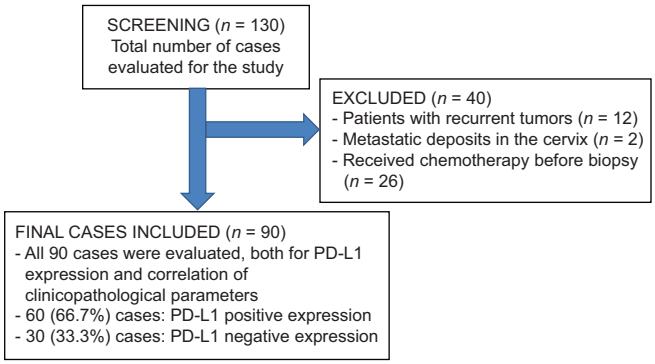

Figure 2: The scheme of patient enrollment and analysis

Clinical presentation: There was also no significant difference noted in the PD-L1 expression based on the presenting symptoms. The expression of PD-L1 was similar in patients who presented with bleeding per vagina (n = 47 of 65, 72.3%) compared to that in those who presented with white discharge per vagina (n = 13 of 25, 52%); $P = 0.083$.

Tumor differentiation: Expression of PD-L1 was maximum in moderately differentiated SCC (n = 40 of 47, 85.1%), followed by well differentiated SCC (n = 8 of 15, 53.3%), and then poorly differentiated SCC (n = 12 of 28, 42.9%); $P < 0.001$ [Table 1].

Disease stage: PD-L1 expression was similar among patients with stage IIIA disease (n = 3 of 3, 100%) and those with stage IIIB SCC (n = 28 of 41, 68.3%); $P = 0.662$ [Table 1].

Tumor size: Expression of PD-L1 was similar between tumors larger than 6 cm (n = 15 of 21, 71.4%) and those between 1 and 3 cm in size (n = 7 of 10, 70%); $P = 0.819$ [Table 1].

Lymphadenopathy: Expression of PD-L1 was similar in cases without lymphadenopathy (n = 33 of 44, 75%) compared

Table 1: Distribution of subjects according to age group, histological grade, FIGO Stage, Size of the lesion, Lymphadenopathy, 3-year overall survival and PD-L1 expression

Clinicopathological parameters	Frequency in the overall cohort; *n*=90 (%)	PD-L1 positive cohort, in *n* (%) (*n*=60)	PD-L1 negative cohort, in *n* (%) (*n*=30)	*P*
Age of patients				
30-39 years	8 (8.8)	8 (100)	0 (0)	0.037
40-49 years	28 (31.1)	14 (50)	14 (50)	
50-59 years	22 (24.4)	13 (59.1)	9 (40.9)	
60-69 years	20 (22.2)	15 (75)	5 (25)	
70-79 years	12 (13.3)	10 (83.3)	2 (16.7)	
Histological grading				
Well differentiated	15 (16.7)	8 (53.3)	7 (46.7)	<0.001
Moderately differentiated	47 (52.2)	40 (85.1)	7 (14.9)	
Poorly differentiated	28 (31.1)	12 (42.9)	16 (57.1)	
FIGO Stage				
Stage IIA	5 (5.6)	2 (40.0)	3 (60.0)	0.662
Stage IIB	36 (40.0)	24 (66.7)	12 (33.3)	
Stage IIIA	3 (3.3)	3 (100)	0 (0)	
Stage IIIB	41 (45.6)	28 (68.3)	13 (31.7)	
Stage IVA	5 (5.6)	3 (60)	2 (40.0)	
Size of the lesion				
1-3 cm	10 (11.1)	7 (70)	3 (30)	0.819
3-6 cm	59 (65.5)	38 (64.4)	21 (35.6)	
>6 cm	21 (23.4)	15 (71.4)	6 (28.6)	
Lymphadenopathy				
Present	46 (51.1)	27 (58.7)	19 (41.3%)	0.121
Absent	44 (48.9)	33 (75.0)	11 (25.0)	
Survival status at 3 years				
Alive	85 (94.4)	58 (68.2)	27 (31.8)	0.329
Died	5 (5.6)	2 (40.0)	3 (60.0)	

FIGO: International Federation of Gynecology and Obstetrics

to those with lymphadenopathy (n = 27 of 46, 58.7%); $P = 0.121$ [Table 1].

3-year Overall Survival: Expression of PD-L1 was similar in patients who were alive (n = 58 of 85, 68.2%) compared to that in those who had died (n = 2 of 5, 40%); $P = 0.329$ [Table 1].

DISCUSSION

We found PD-L1 positivity in 66.6% cases of SCC of the uterine cervix. This finding was similar to that reported by Meng *et al*.[14] and Chen *et al*.,[15] who noted PD-L1 expression in 68 out of 97 cases (70.1%) and 61 out of 95 cases (64.2%) cases, respectively. Reddy *et al*.[12] (56 out of 148 cases, 37.8%), Feng *et al*.[13] (71 out of 219 cases, 32.4%) and Grochot *et al*.[16] (19 out of 59 cases, 32.0%) reported a lower expression of PD-L1 in their studies [Table 2]. The possible reason for the differing expression of PD-L1 in cervical SCC in various studies can be attributed to the different geographical regions the studies were conducted in, varying antibody kits used for IHC, different procedures used for evaluation, the lack of a standardized reporting format for PD-L1 expression, and varying sample sizes. Studies have reported that SCC of the cervix has higher PD-L1 expression compared to adenocarcinoma and adeno-squamous histologies.[17] In the present study, we included only patients with SCC of the cervix, which may have been a reason for the relatively higher PD-L1 expression.

The binding of PD-L1 to PD-1 results in an immunosuppressive effect whereby the tumor evades immune destruction. In patients with cancer, PD-L1 is the primary ligand of PD-1 and is expressed on malignant cells. Increased expression of PD-L1 on various solid tumors including cervical carcinoma may result in evasion of the tumor from the immune system. The PD-L1 component of B7/cluster differentiation (CD) 28 co-stimulatory proteins may be induced in cervical cancers and can evade the immune responses to the tumor.[13,19] Expression of PD-L1 is rarely seen in normal cervical tissue, but is reported in 50% cases of cervical carcinoma.[1]

Table 2: PD-L1 expression in cervical squamous cell carcinoma cases noted in various studies in the literature

Study	PD-L1 expression, in number positive/total cases (%)
Reddy *et al*. (2017)[12]	56/148 (37.8)
Meng *et al*. (2018)[14]	68/97 (70.1)
Feng *et al*. (2018)[13]	71/219 (32.4)
Grochot *et al*. (2019)[16]	19/59 (32)
Grither *et al*. (2020)[18]	58/64 (90.6)
Chen *et al*. (2020)[15]	61/95 (64.2)
Present study	60/90 (66.6)

Approximately 17% of adenocarcinoma and 35% of SCC cases of cervix express PD-L1.[13] Recent studies support the use of immune checkpoint inhibitors in patients with advanced cervical cancer as adjuvant targeted immunotherapy.[20] In the present study, 66.7% cases of SCC showed PD-L1 expression, which indicates that two-thirds of our patients with advanced cervical SCC may be considered for immunotherapy.

In our study, 31% of the cases were in the age group of 40–49 years, followed by 24% in the 50–59 years age category. We found that 100% of the patients aged 30 to 39 years demonstrated PD-L1 positivity (100%), compared to 83% of those between 70 to 79 years. PD-L1 expression significantly increased with increasing age, starting from 40 years. Liang *et al*.[21] reported an increased expression of PD-L1 in younger patients (20–30 years of age). A similar observation was reported by Saglam *et al*.[22] who reported a significant proportion of PD-L1 expression in patients aged 40–50 years of age; $P = 0.028$. A possible explanation as to why younger patients have a higher PD-L1 expression than older patients is immune senescence that occurs with advancing age. Ageing results in thymic involution and reduced numbers of T-cell progenitors in the bone marrow, which leads to a paucity of naive cells and production of mature T-cells.[23-25]

We did not find any significant correlation between parity and the expression of PD-L1; to the best of our knowledge, ours is the first study to have evaluated this. There was also no significant difference between the clinical presentation and PD-L1 expression in our study. Similarly, Liang *et al*.[21] reported no association between PD-L1 marker expression and clinical presentation.

We found a statistically significant difference between the histological grade of the tumor and PD-L1 expression [Table 1]. The PD-L1 expression was maximum (85%) in moderately differentiated SCC cases; $P < 0.001$. A similar observation was also reported in the study by Chen *et al*.[1] in which 77.7% of the cases with moderately differentiated SCC showed PD-L1 marker expression; $P < 0.005$. In a study by Feng *et al*.,[13] 88% of cases demonstrated maximum PD-L1 positive expression in moderately differentiated squamous cell carcinoma (MDSCC); $P = 0.06$.

In the current study, the highest level of PD-L1 expression was noted in patients with stage IIIA and IIIB SCC (100% and 68.3%, respectively) followed by those with stage IIB (66.7%). This finding was similar to that reported by Grochot *et al*.[16] who also found that the expression of PD-L1 was maximum in stage III cases (40.7%). Contrarily, in a study by Chen *et al*.,[1] the maximum positive PD-L1 expression was noted

in patients with stage IVA disease (83.3%). Of note, these differences reported from the various other studies as well as in our own study were merely numerical differences, and did not attain statistical significance. Thus, there was no significant correlation noted between the FIGO staging and PD-L1 expression.[1,16]

In our study, there was no significant difference between the size of lesion and PD-L1 expression; PD-L1 positivity was noted in 70% cases with tumors smaller than 4 cm, and in 66.3% of those with tumors larger than 4 cm. These findings were similar to those reported by Heeren *et al*.[7] in which PD-L1 expression was noted in 71.5% cases with tumor sizes less than 4 cm and 70.1% cases with tumor sizes larger than 4 cm. Feng *et al*.[13] also reported that 38% cases demonstrated PD-L1 positivity in tumors smaller than 4 cm, and in 30.1% cases in tumors larger than 4 cm; these differences were not statistically significant, $P = 0.604$. Xu *et al*.[26] and Lathika *et al*.[27] also reported no significant association between the PD-L1 expression and tumor size.

In the current study, there were 46 cases with significant lymphadenopathy of which 27 cases (58.6%) showed PD-L1 expression. Among the 44 cases with no significant lymphadenopathy, 33 (75%) showed positive PD-L1 expression. There was no statistically significant correlation between the presence of lymphadenopathy and the expression of PD-L1. Similar observations were reported in studies by Liang *et al*.[28] and Hui *et al*.[29] We also found no significant correlation between OS and the expression of PD-L1 in our study cohort. Similar findings were reported in studies by Grither *et al*.,[18] Enwere *et al*.,[30] and Chen *et al*.[15]

The limitation of the current study was the small sample size. Additionally, we did have any details regarding the therapy received, therefore we were unable to correlate the PD-L1 expression level with responses to therapy. Immunotherapy can be used in PD-L1 positive SCC of cervix, as PD-L1 has a role in the pathogenesis of HPV-infection which is the most common etiologic factor for carcinoma cervix.[31,32] Ongoing clinical trials are assessing the effectiveness of PD-1/PD-L1 blockers in carcinoma cervix.[33] Pembrolizumab is already Food and Drug Administration approved in patients with metastatic cervical cancer in the first- and second-line settings.[20,32] Future multicentric studies with larger sample size may be helpful to establish the utility of PD-L1 as a biomarker in the management of carcinoma of the cervix.

CONCLUSION

PD-L1 is expressed in 66.6% cases of SCC of the cervix. Only age and tumor grade correlate with PD-L1 expression, while parity, clinical presentation, disease stage, tumor size, lymphadenopathy and OS have no correlation with the PD-L1 expression. The high PD-L1 positivity in our study suggests that anti-PD-L1/PD-1 immunotherapies may be a useful addition to the therapeutic armamentarium of our patients with PD-L1-positive SCC of the cervix.

Author contributions

Conception or design of the work: KR; Data collection, Data analysis and interpretation, Drafting the article: NC; Data analysis and interpretation: KR; Critical revision of the article; SSR, AKS, MGN; Data collection, final approval of the version to be published: All authors.

Data sharing statement

Individual deidentified participant data will be shared by the corresponding author, if required, in the form of deidentified participants' details in an excel sheet including other related documents like the statistical analysis plan and ethical clearance certificate issued by the institutional ethics committee. The data will be available for five years.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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SUPPLEMENTARY APPENDIX 1: STUDY PROTOCOL

Title of Topic: Significance of Programmed death ligand-1 in Invasive squamous cell carcinoma of uterine cervix

Brief Resume of Intended Work

Need For the Study

Cervical cancer is the most common 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. 570,000 cases of cervical cancer were estimated to have occurred, with over 300,000 deaths. New cases of cervical cancer detected in **India** is 96,922 in year 2018.[2] Deaths due to cervical cancer in India reported is 60,078. In Karnataka alone 5,000 new cases are identified each year. In Bangalore, **cervical cancer** is the second most common **cancer** among females with 850 cases being diagnosed every year.[3] In a study done in Kolar, 17% cases were diagnosed of cervical carcinoma among all the cancers of females.[4] HPV is a DNA 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 other genetic abnormalities results in malignant transformation.[5] Productive infection of the cervix and over expression of HPV16 E7 in the cervical epithelial carcinoma increases PD-L1 protein expression. Cervical carcinoma with HPV infection has 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 multiple HPV infection prolongs the time to clean HPV infection. HPV genome integrated into the PD-L1 gene locus induces increased PD-L1 expression by 3'UTR disruption of PD-L1 gene a possible mechanism for high PD-L1 expression in premalignant cervical lesion with Human papilloma virus infection. Anti PD-L1 Can be a potential effective way of treatment of HPV infection in premalignant cervical lesion.[6] Information on the clinical importance of PDL-1 expression in cervical cancer is largely lacking.[7] A few studies done are from the developed countries.[8] There is paucity of studies from the developing and least developed countries on programmed death ligand (PD-L1) with respect to cervical cancer.[8]

Review of Literature

The most common age group—Mean age for cervical cancer 52.2 years. The risk factors for carcinoma cervix are Human Papilloma virus, sexually transmitted infections—Coitus before 18 years of age, multiparity, Poor personal hygiene, poor socioeconomic status, smoking, immunosuppressed individuals, women on oral contraceptives, Progesterone therapy for a long period of time predisposed to adenocarcinoma of cervix, in utero exposure to Diethylstilbestrol (DES).[9]

PD-L1 is a transmembrane protein assumed to play a major role in suppressing the Adaptive arm of immune system. Normally the adaptive immune system reacts to antigens that are associated with Immune system activation by exogenous or endogenous danger signals. In turn, clonal expansion of antigen-specific CD8 + T cells and/or CD4 + helper cells is Propagated. The binding of PD-L1 to the inhibitory checkpoint molecule PD-1 transmits an inhibitory signal based on interaction with phosphatases (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).[10]

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 on tumor cells.[10] Normal cervical epithelium do not express PD-L1. No expression of PD-L1 noted in benign cervical tissues. 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.[11] The expression of PD-L1 seen is 32% of cervical carcinomas and 10% tumor infiltrating lymphocytes in cervical cancer. Increased PD-L1 expression significantly associated with High TNM stage, reduced number of Tumor infiltrating lymphocytes and worse prognosis in cervical carcinomas.[12]

Role of PD-L1 in Cervical Cancers Patients with metastatic and recurrent cervical cancer have limited treatment options and receive palliative platinum-based chemotherapy with insignificant survival benefit. Recent studies provided support for usage

of immunotherapy (immune checkpoint inhibitors) in advanced Cervical Cancers. Around 17% of cervical adenocarcinoma and 35% of squamous cell carcinomas expressed PD- L1.[13]

Utility of PD-L1 in other Cancers

Melanoma: Nivolumab is a Human immunoglobulin G4 (Ig4) monoclonal antibody that binds to PD-1 receptor and blocks its interaction with PD-L1 releasing PD-1. Nivolumab is FDA approved for first line or previously treated melanoma. [14]

Lung Cancer: In the studies done median Overall survival was more with PD-1 inhibitors.[14] With (Nivolumab) overall survival was more 9.2 months than with chemotherapy drugs (docetaxel) 6.0 months. Nivolumab is approved as second line therapy for both squamous and Non-squamous non-small cell lung carcinoma.[14]

Urothelial Cancer: Atezolizumab is a monoclonal antibody that binds to PD-L1 and blocks its interactions with PD-1 Receptors. The FDA approved Atezolizumab in May 2016 in patients with locally advanced and metastatic Urothelial cancer following progression during the platinum based chemotherapy or after platinum based chemotherapy. It is the first new treatment to be approved for urothelial carcinomas in past three decades.[14]

Classical Hodgkin's Lymphoma: PD -1 is commonly overexpressed and is a T-cell exhaustion marker. Up to 97% of patients with Hodgkin's lymphoma will show alterations in the PD-L1 gene loci which are associated 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.[14]

Head and Neck Squamous Cell Carcinoma: PD-1 antibodies are also actively seen in Head and neck squamous cell cancers. Pembrolizumab is a monoclonal antibody that binds to PD-1 receptor and blocks its interaction. Pembrolizumab is FDA approved for the previously treated advanced Head and Neck squamous cell cancers.[14]

Adverse effects of PD-L1 inhibitors: Increase in immune activation by PD-1 and PD-L1 inhibitors is responsible for immune related side effects. Endocrine Most common organ specific iRAEs. Pneumonitis most frequent pulmonary adverse event. Other adverse effects of PD-L1 inhibitors include a)Cutaneous adverse effects : in the form of rash , pruritus and psoriasis. b) Nephrologic adverse effects: are rare but can occur in the form of Acute interstitial nephritis. Neurologic-Myasthenia gravis. Other iRAEs—Pericardial effusion, myocarditis with Right Heart Failure, Agranulocytosis, Autoimmune Hemolytic Anemia.[15] Many prognostic markers have been recognized, such as lymph node status, tumor size, and tumor stage. These prognostic markers lack sensitivity and specificity for accurate prediction. So, there is immediate need of identifying novel and possible prognostic markers for guiding personalized treatment and predicting survival outcome in cervical carcinoma patients.PD-L1 is a promising prognostic indicator for cervical carcinoma patients.[16] Poor overall survival is seen in cervical cancer patients with PD-L1 overexpression and in Asian patients PD-L1 overexpression is associated with poor progression free survival.[16] PD-L1 positivity is seen in 90% of Cervical cancer patients.PD-L1 expression in squamous cell carcinoma varies from 19% to 88%. PD-L1 expression is less in cervical adenocarcinoma cases. Patients with diffuse PD-L1 positivity has poor survival rate than in patients with marginal PD-L1 positivity because in marginal positivity there is localized released of cytokines from tumor infiltrating lymphocytes as compared to constitutive activation in Diffuse activity.[17] In various solid tumors including gastrointestinal carcinomas, renal cell carcinomas, non-small cell lung carcinomas and cervical carcinomas there is improvement in overall survival rate and significant response rates after usage of therapeutic agents targeting at PD-1/PD-L1 Pathway. Pembrolizumab has been approved for use as first line therapy for non-small lung carcinomas in advanced stage where tumor cells show greater than 50% PD-L1 expression as it leads to longer overall survival and progression free survival with less adverse effects in comparison to chemotherapy.[18] Increased PD-L1 in tumor cells is associated with better overall survival as compared to high PD-L1 in tumor immune cells.[19] Women who are in immunosuppressed state have increased incidence of Human papilloma virus infection, cervical intraepithelial neoplasia and increased chances of progression to invasive neoplasia.[20] As of now, for early to established cervical carcinoma, first line therapy is Surgery. Chemotherapy and Radiotherapy have been used to treat patients who are in locally advanced state of uterine cervical cancer. PD-1/PD-L1 ligand inhibitor is highly efficacious in solid tumors including cervical carcinoma and it has been approved by FDA. PD-L1 is expressed on surface of cervical cancer tumor cells. PD-L1 expression is seen in 34–96% of cervical cancer tissues. In Squamous cell carcinoma of Cervix PD-L1 expression is observed in 80% of cases. PD-L1 expressed extensively in cervical cancer stroma and tumor cells indicating a potential treatment target by PD-1/PD-L1 inhibitor. PD-L1 can be used as a biomarker to differentiate carcinoma *in situ* from microinvasive cancer.

Anti PD-L1 therapy may inhibit the progression to invasive stage.[21]

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.

Material and Methods:

Study Design: Laboratory based observation study

Duration of Study: Samples will be collected from July 2016 to June 2018. Patient will be followed up for three years (July 2018 to June 2021) to assess overall survival of the cases.

Inclusion Criteria: All the cases of invasive squamous cell carcinoma of cervix diagnosed with Histopathology will be considered for the study.

Exclusion Criteria: Recurrent cases and cases with metastatic deposits in cervix will be excluded from the study. Cases who underwent chemotherapy and radiotherapy before biopsy will be excluded from the study.

Source of Data:

Ninety cases will be considered for the study. The study will be done in Department of Pathology, Sri Devaraj Urs Medical college, Kolar. Affiliated to Sri Devaraj Urs Academy of Higher education and research. Cases will be collected from archives of Department of Pathology.

Sociodemographic data along with clinical findings as: present history, past history, personal history, family history, including physical examination as per vaginal examination, per speculum examination, per rectal examination of each case will be noted from hospital record section. Radiologic findings (USG, MRI findings) with respect to stage of disease, size of lesion, Lymph node involvement will be noted. Hematoxylin and eosin stain tissue sections will be reviewed. Squamous cell carcinoma will be classified into well differentiated, moderately differentiated and poorly differentiated. Staging of the lesion will be done as per FIGO staging System.[22] Tissue section will be taken from paraffin embedded tissue blocks subjected to immunohistochemistry for expression of markers of PD-L1 (PD-1 Antibody Biogenex AN921-M). The procedure of IHC as per the manufacturer's protocol will be as Follows; section is cut approximately 3–4 um, floated on to positive charged slides and incubated at 37°C for one day, and will be further incubated at 38°C over night. Sections will not be allowed to dry at any stage of the staining procedure. Stromal cells and histiocytes will be considered as controls for each antibody tested. Deparaffinization using xylene-I And Xylene—II using both for 15 minutes each. Desalination using absolute alcohol-I and absolute alcohol—II using each for 1 minute. Dealcoholisation for 1 minute. Distilled water 5 min-washing. Secondary Antibody for 30 minutes. Super enhancer. TBS buffer wash 5 min three times. Color development with working color development solution for 5-8 minutes. Distilled water wash for 5 minutes. Counterstain with Harris hematoxylin for one minute. Dehydration. Mount with DP. The membranous positivity will be considered as positive for PD-L1. Expression of PD-L1 will be evaluated in tumor cells in five Hotspot areas in high power fields. The expression will be analyzed as; Score 0=<5% of positive cells, Score 1 = 5–29%, Score 2 = 30–59%, Score 3=>60%.[11]

The intensity of staining on the cell membranes will be 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

Average of score will be taken and will be multiplied by intensity to obtain the Grading. Grading: Score 1–3 as Grade I, Score 4–6 as Grade II, Score 7–9 as Grade III.[11]

Statistical Analysis

The findings will be entered in MS excel sheet and statistical analysis will be done. The continuous data will be analyzed

for mean and standard deviation and categorical data will be analyzed by Chi-square method for frequency and proportion. *P* value <0.05 will be considered statistically significant.

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 *et al*. in 2018 with 95% confidence interval and an absolute error of 10%, the sample size will be 90.[12] Formula to be used:

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

p: Expected proportion

d: Absolute Precision

α/2: Desired Confidence level

Does the study require investigations or interventions to be conducted on patients/humans/animals? If so please describe.

No.

Has the ethical clearance been obtained from your institution in case of the above? Yes, ethical clearance has been obtained from the institution.

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PATIENT PROFORMA

Anonymized Sample No:

Chief complaint:

History of presenting illness:

Past history:

Personal history:

Local examination:

Biopsy Number:

Gross:

Microscopy:

Histopathological diagnosis:

Grading:

SUPPLEMENTARY APPENDIX 2: VISUAL ABSTRACT