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Overexpression of PDL-1 is a Risk Factor for Lymph Node Metastasis in Cervical Cancer

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Abstract---*Cervical cancer is the fourth most prevalent malignancy in women globally and the 2nd in Indonesia. In 2008, the Indonesian Cancer Foundation projected that 52 million Indonesian women are at risk for cervical cancer and that 36% of all cancer patients are cervical cancer patients. Increasing by 0.6% per year. There are 9,496 cervical cancer-related deaths in Indonesia, compared to an estimated 24,400 worldwide. The crude incidence rate of cervical cancer in Indonesia is 17 per 100.000 women. The majority of individuals with cervical cancer who passed away have received chemotherapy and radiation therapy. In recent years, tremendous progress has been made in immune therapy studies focused on preventing cancers from evading the immune response of the tumor. Among them is PD-I research. The association between PD-1 and its PDL-1 ligand suppresses cytotoxic T cells in the immune response to tumors. PDL-1 overexpression was also associated with a poor prognosis in a few other carcinomas. This study intends to determine whether PDL I overexpression is a risk factor for cervical cell carcinoma metastasis. Metastasis is indicative of a poor prognosis in cancer. According to the study's findings, there is a correlation between PDL-1 and lymph node metastases (p-value = 0.025). Samples with PDL-1 expression demonstrated a 6,000-fold increased risk for lymph node and distant metastases (PR= 6,000). According to prior research, the binding of PD-1/PDL-1 to T lymphocytes detaches tumor cells from the immune system, allowing them to disseminate and metastasis with ease.*
Keywords---*cervical cancer, metastasis, PDL-1, risk factor.*

Introduction

The incidence of cervical cancer in Indonesia is 23.4% per 100,000 population (Bruni et al., 2017; Ferlay et al., 2021). Cervical carcinoma mortality is still high, at around 90%, and most patients with cervical carcinoma die from metastases. Therefore, determining the prognostic factors associated with metastases is very important because this will determine further management (Endo et al., 2015; Haie-Meder et al., 2010; Huang et al., 2016; Kurman et al., 2010; Kumar et al., 2014). Conventional treatments for cancer, such as chemotherapy and radiation, are far from ideal and remain suboptimal, and is accompanied by severe side effects. The capacity of cervical cancer cells to evade the immune response of the tumor is a contributing factor to the ineffectiveness of this treatment. In consequence, cancer cells in the cervix continue to multiply and have the potential to disseminate (Wherry & Kurachi, 2015). There has been rapid advancement in the last five years toward the aim of using immunotherapy to strengthen the body's natural defenses against cancer. The use of programmed death ligand 1 (PDL-I) as a

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therapeutic target in immunotherapy has increased rapidly in recent years. Using the binding of the PD-1/PD-L1 pathway, tumor cells can evade antitumor immunity when they express PD-L1. The administration of anti-PD-L1 became an option in the immunotherapy treatment against cancer. Until now, in Indonesia and Bali, there are no empirical data pertaining to the expression of PD-L1 in cervical cancer and its distinctive characteristics. This study will investigate the association between PDL-1 expression and lymph node metastases (Waggoner, 2003; Buskwofie et al., 2020; Peters et al., 2010; Biewenga et al., 2008).

This study aimed to examine the association between PDL-1 overexpression and cervical cancer metastasis. Cervical cancer remains the second most prevalent form of cancer in Indonesia and Bali. The majority (90%) of cervical cancer patients who develop metastases while receiving treatment die. Determining if cervical carcinoma has metastasized to the inguinal lymph nodes is crucial because this form of cancer will metastasis through the lymphatics to the inguinal lymph nodes before spreading to other organs. Considering the adverse effects of this procedure, lymph node excision is not typically performed in cases with cervical cancer with an early stage but aggressive character. Determining prognostic indicators based on PDL-1 expression in cervical cancer is now one of the most promising parameters in immunotherapy (Strauss et al., 2020; Bilgin et al., 2017; Droeser et al., 2013; Yokoyama et al., 2016). Important prognostic markers for cervical squamous cell carcinoma include lymph node metastases and distant metastases, although there have been no studies directly connecting PDL-1 overexpression with these outcomes. The prognosis for cervical cancer is poor in Indonesia and Bali, despite the widespread disapproval of PDL-1s as adjuvant therapy. Furthermore, this study will also describe the potential efficacy of anti-PDL-1 as part of immunotherapy for cervical cancer in Indonesia, with a focus on Bali.

Method

Research design
Research plan

This study used a case-control methodology. Figure 1 depicts the link between PDL-1 overexpression and tumor grading and the occurrence of lymph node metastases in cervical cancer which will be investigated in this investigation.

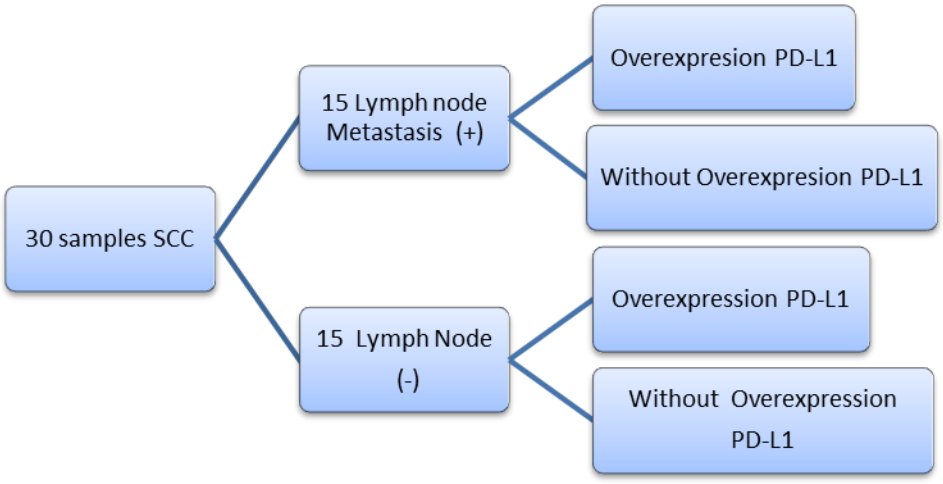

Figure 1. Research design chart

The research was conducted for six months in 2022 at the Biomolecular Laboratory of the Warmadewa University Faculty of Medicine and Health Sciences in Denpasar, Indonesia.

Population and sample

The research population consists of all cervical cancer samples collected at Balimed Denpasar Hospital between 2019 and 2021. After calculating the sample, thirty individuals were obtained as the sample. In this investigation,

there were 15 samples from individuals with lymph node metastases and 15 samples from individuals with non-metastatic lymph nodes. All cervical cancer preparation samples with complete data.

Immuno histochimia of PDL-1

PD-L1 overexpression refers to the high level of PD-L1 expression, which inhibits T lymphocyte cells in tumor immunity. The evaluation begins by picking 10 fields of view at 400X magnification at random. The immunohistochemical results were then analyzed using a semiquantitative method, namely a Histoscore based on the intensity of the predominant staining. Formula: [1 x (% cells 1+) +2 x (% cells 2+) +3 (% cells 3+)] = 0 if tumor cell membrane and/or cytoplasm are unstained, 1+ if stained faintly, 2+ if stained moderately, and 3+ if stained strongly. The final grade ranges from 0 to 300 points. In addition, PD-L1 expression will be classified as [16]: 1 = Negative if 0 to 99 is the result, 2 = Positive/strongly stated for results between 100 and 300. The investigator and two Anatomic Pathologists examined and reevaluated histological slides stained with Hematoxylin-Eosin and immunohistochemistry. Lymph node metastasis, meanwhile, is the spread of tumor cells beyond the initial tumor, specifically in regional and extra-pelvic lymph node tissue (KGB) (Kumar et al., 2014). The link between PD-L1 overexpression and lymph node metastases in cervical cancer was evaluated descriptively using the chi-square statistic test. The significance test yielded a p-value of less than 0.05. 95% Confidence Interval (CI) is used to determine the precision of data (Mustika et al., 2017; Pamela Kusumadewi Putri Thaib & Anny Setijo Rahaju, 2021).

Results

The distribution of cervical cancer research samples showed that the majority of cases occurred at the age of 45 years and over 19 people (63.3%). Cervical carcinoma is not among the top three cancers among women younger than 45, contrary to reports from the International Agency for Research on Cancer (IARC). Twenty-five patients (83.3%) had tumors larger than 4 centimeters in diameter at the time of surgery.

Table 1
Research sample characteristics

Characteristics	n(%)
Age (years)	
<45	11 (36,7%)
>/=45	19 (63,3%)
Tumor size	
<4	5(16,7%)
>/=4	25 (83,3%)
Lymph node metastasis	
I. Positve	15 (50%)
II. Negative	15 (50%)

The results of this study demonstrated that PDL-1 overexpression was not correlated with patient age or tumor size (p = 0.279). This is consistent with prior research that showed PDL-1 expression did not change with age or aging.

Table 2
Correlation between PDL-1 overexpression with age and tumor size (n= 30)

Characteristic	n(%)	PDL-1 Overexpression (n)	P value
Age (year)			
<45	11 (36.3%)	8	0.279
>=45	19 (63.7%)	10	
Tumor size			
<4	5(30%)	2	0.317
>=4	25 (70%)	16	

Table 2. shows that the presence of PDL-1 overexpression was unrelated to tumor size ($p<0.05$). This contradicts the results of several other studies that have linked PDL-1 expression to the carcinogenic hallmarks of tumor proliferative activity and tumor development, such as invasion and metastasis.

Table 3
Correlation of PDL-1 overexpression with lymph node metastasis status

Metastasis	n (%)	Overekspresi PDL-1 (n)	P value
Negative	15 (36.3%)	6	0.025
Positive	15 (63.7%)	12	

There is a correlation between PDL-1 expression and lymph node metastases (p-value = 0.025) as seen in Table 3. Expression of PDL-1 was associated with a 6,000-fold increased risk of lymph node and distant metastases (PR = 6,000). There is a correlation ($p > 0.05$) between PD-L1 expression and the incidence of distant and lymph node metastases.

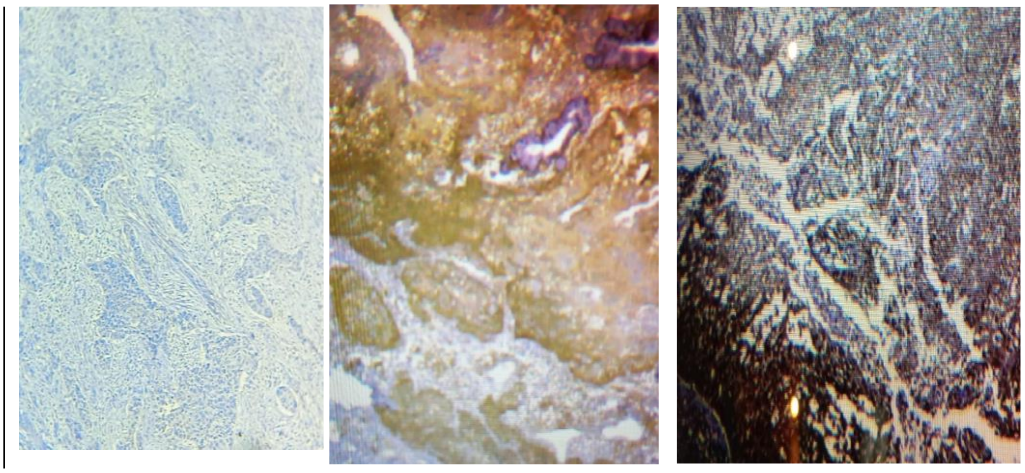

Figure 2. A. Immunostain is negative PDL-1 expression in cervical cancer. Magnification 100x. B Immunostain is positive for PDL-1, brown in the membrane of tumor cell, magnification 100 x C. Immunosatain is positive for PDL-1, Brown in the membrane of tumor cell. Magnification 400x

Discussion

According to the distribution of cervical cancer research samples depicted in the table, the majority of cases (63.3%) affect those aged 45 and older than 190. This is in contrast to the literature from the International Agency for Cancer Research (IARC), which states that cervical carcinoma is one of the top three cancers among women aged 45. This is due to the delay in detecting cervical cancer cases. It was determined that 25 patients (83.3%) had a tumor size of T2 or greater than 4 cm. This is also consistent with epidemiological reports indicating that in developing nations, such as Indonesia, the incidence of cervical cancer is still higher at an advanced stage (80%), where the tumor is larger than 4 centimeters and has caused severe clinical symptoms such as heavy bleeding and spread to surrounding tissues such as the vagina or bladder. The high rate of cervical cancer in women over the age of 45 is also attributable to the 10- to the 20-year period between HPV infection and cervical cancer (Endo et al., 2015; Haie-Meder et al., 2010).

The fact that there is no relationship between PDL-1 and age is the link between PDL-1 overexpression and patient clinical data. This is because the expression of PDL-1 is not changed by the aging process. In addition, there was no correlation between the amount of PDL-1 expression and the size of the tumor. The expression of PDL-1 is not the only factor that affects the rapid proliferation of cancer cells, which in turn causes an increase in the size of the tumor (Lorusso et al., 2014; Berman et al., 1984; Goncalves et al., 2008). The growth factor group and the components of the proliferative signaling pathway, which are frequently exploited as therapeutic targets, are among the factors that have a greater impact on the disease. PDL-1 has a greater impact on suppressing apoptosis, which

occurs when cancer cells are not identified by T lymphocyte cells and hence does not go through the process of apoptosis (Azuma et al., 2014; Erdogdu, 2019; Šmahel, 2017; Ferns et al., 2016; Sahasrabudhe et al., 2019).

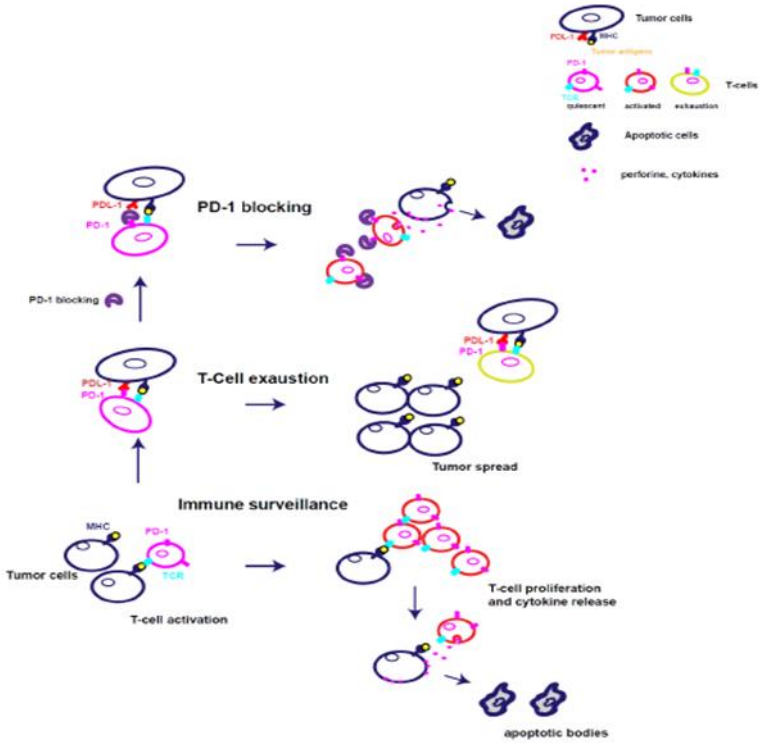

Figure 3. Cell reaction

Figure 3. PD-1 is an inhibitor of T cells so T cells cannot work to kill cancer cells (1) Antigens are presented to T cells through the MHC arms present in cancer cells. These T lymphocytes then proliferate and differentiate into effector cells and memory cells. Effector cells will recognize cancer cells based on antigens expressed by cancer cells and then kill cancer cells by releasing inflammatory cytokines and cytotoxic granules that cause cancer cell apoptosis. PD-1 is a checkpoint inhibitor that inhibits T-cell activation when inflammation subsides. (2) after activation, PD-1 increases in T cells and binds to its PDL-1 ligand, and inhibits the function of T lymphocyte cells so that they are unable to kill cancer cells which causes cancer cells to easily spread and metastasize. (3) Blocking PD-1 receptors with monoclonal antibodies will restore the function of T lymphocyte cells to proliferate and differentiate into effector or memory cells that function to kill cancer cells (Erdogdu, 2019; Šmahel, 2017; Ferns et al., 2016; Sahasrabudhe et al., 2019).

Lymph node metastases are linked to PDL-1 overexpression, and there is a correlation between distant lymph node metastases and PDL-1 overexpression ($p = 0.025$). PDL-1-positive samples had a 6,000-fold increased risk of lymph node and distant metastases (PR value = 6,000). This is consistent with prior research showing that tumor cells become immune-dormant after PD-1/PDL-1 attachment to T lymphocytes, allowing for more efficient metastasis and spread. The Pdcd 1 gene, located on human chromosome 2q37, codes for the 288-amino-acid protein known as programmed death 1 (PD-1, CD279). Meanwhile, the CD 274 gene on human chromosome 9 codes for a transmembrane protein known as Programmed death ligand 1 (PD-L1). Antigen-presenting cells contain a glycoprotein called programmed death ligand 1 (PD-L1). Tumor cells were shown to express PD-L1 immunoreactivity. Overexpression of PD-L1 is linked to a poor prognosis in several cancers, including cervical carcinoma. Lymph node metastases, deep infiltration, and a large tumor all contribute to a dismal prognosis for cancer (Ferns et al., 2016; Sahasrabudhe et al., 2019).

PDL-1 expression was shown to be present in both groups of research samples, those with lymph node metastases and those without, but at significantly lower levels in the group without metastases. Over-expression of PDL-1 is correlated to cervical carcinoma carcinogenesis because it facilitates tumor isolation from the tumor immune system, which allows cancer cells to evade apoptosis. One of the hallmarks of carcinogenesis is that cancer cells avoid

apoptosis. This is evident for both metastatic and non-metastatic tumors. (Zerdes et al., 2018; Mezache et al., 2015; Liu et al., 2019; Wu et al., 2006). Anti-PD-L1 medication plays a crucial role in cancer immunotherapy. Several other cancers responded similarly to anti-PDL-1 treatment. When a patient's PD-L1 expression test findings are positive, the FDA has authorized the use of immunotherapy medicines for both advanced cervical cancer and cervical cancer that is unresponsive/progressive following chemotherapy (Eto et al., 2016; Hodi et al., 2010; Massard et al., 2016).

Conclusion

Based on the results and discussion above, it can be concluded that there is a correlation between overexpression of PDL-1 and distant lymph node metastases (p = 0.025). Samples with PDL-1 expression demonstrated a 6,000-fold increased risk for lymph node and distant metastases (PR = 6,000). According to prior research, the binding of PD-1/PDL-1 to T lymphocytes detaches tumor cells from the immune system, allowing them to disseminate and metastasis with ease. Further study is required to establish the strength of the connection between the primary factors in metastasis. Studies are needed to determine whether anti-PDL-1 is effective against cervical cancer alone or in conjunction with other therapies.

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