High serum levels of soluble PD-1 and PD-L1 are associated with advanced clinical stages in patients with cervical cancer

SILVIA ELIZABETH PÉREZ-PICAZO[1], PATRICIA MARTÍNEZ-MORALES[2], ILEANA CONDE-RODRÍGUEZ[1], JULIO REYES-LEYVA[3] and VERÓNICA VALLEJO-RUIZ[1]

[1]Eastern Biomedical Research Center, Mexican Institute of Social Security, Atlixco, Puebla 72760, Mexico; [2]CONAHCYT-Faculty of Biological Sciences, Autonomous University of Puebla, Puebla 72570, Mexico; [3]Faculty of Chemical Sciences, Autonomous University of Puebla, Puebla 72570, Mexico

Received December 11, 2024; Accepted February 10, 2025

DOI: 10.3892/br.2025.1948

Abstract. The binding of programmed cell death receptor-ligand 1 (PD-L1) to programmed cell death protein 1 (PD-1) inhibits T-cell activation, playing a negative role in the anticancer immune response. The soluble forms of these proteins, found in blood circulation, have recently received increasing attention and their function in the cancer immune response remains unclear. The present study evaluated the serum levels of soluble (s)PD-1 and sPD-L1 in patients with cervical cancer and healthy controls, and their associations with clinicopathological characteristics and clinical outcomes. The serum concentrations of both soluble proteins were determined via ELISA. The concentrations of sPD-1 and sPD-L1 were higher in patients with cervical cancer and advanced clinical stages. The evaluation of sPD-1 and clinical outcome revealed higher levels in deceased patients than in total remission patients. sPD-1 and sPD-L1 concentrations were moderately positively correlated; however, in patients with clinical stage IV disease, a very strong correlation was observed. sPD-1 and sPD-L1 could be used as potential diagnostic biomarkers for patients with cervical cancer. Considering the higher levels in advanced clinical stages, their role in cervical cancer progression or treatment response must be explored.

Introduction

Cervical cancer is a public health problem and is the fourth most prevalent cancer in women worldwide (1). Most women diagnosed in middle- and low-income countries are in advanced stages. The treatment and outcome of cervical cancer are determined by the clinical stage of the patient, which is classified according to the criteria of the International Federation of Gynecology and Obstetrics (FIGO) (2). Early stages are commonly treated with surgery, while for locally advanced cancer the treatment is concurrent chemoradiation (3). Patients with locally advanced cervical cancer that respond to concurrent chemoradiation therapy have increased numbers of tumor-infiltrating lymphocytes and those with no response present increased immune reactivity to programmed cell death receptor-ligand 1 (PD-L1) (4).

For patients with cervical cancer with metastasis and recurrence, the alternative treatment is immunotherapy. Anti-programmed cell death receptor 1 (PD-1) is the most used treatment and the clinical response to anti-PD-1 varies among different monoclonal anti-PD-1 antibodies. Other options have been evaluated as combination therapies and improved the treatment response (5). Patients who received anti-PD1 and anti-cytotoxic T-lymphocyte associated protein 4 bispecific antibodies and expressed PD-L1 in tumors had an improved objective response rate compared with those who were negative for PD-L1 (5,6).

PD-1 and PD-L1 are transmembrane proteins: PD-1 is a receptor, and its ligand is PD-L1. PD-1 is a negative regulator of T cells and is involved in the maintenance of immune tolerance through interaction with its ligand PD-L1 (7). Tumor cells overexpress PD-L1 to escape immune surveillance (8), therefore the binding of PD-L1 to PD-1 inhibits T-cell activation, playing a negative role in the anticancer immune response (9). It has been reported that genetic aberration modifies PD-L1 expression in cancer cells. PD-L1 maps to chromosome 9p24.1 and amplification of this locus has been reported in nodular sclerosing Hodgkin lymphoma. In primary mediastinal large B-cell lymphomas, renal cell carcinoma, breast cancer and non-small cell lung cancer, among others, this amplification has been associated with increased PD-L1 expression (10-14).

PD-1 and PD-L1 were initially identified as membrane proteins but also found in blood circulation. Soluble immune checkpoints (sICs) are relevant in cancer. Soluble (s)PD-1 and sPD-L1 can be released into the circulation because of alternative splicing, which generates protein variants that lack the

Correspondence to: Dr Verónica Vallejo-Ruiz, Eastern Biomedical Research Center, Mexican Institute of Social Security, Km 4.5 Carretera Federal Atlixco-Metepec, Atlixco, Puebla 72760, Mexico
E-mail: veronica.vallejor@imss.gob.mx

Key words: cervical cancer, soluble programmed cell death protein 1, soluble programmed cell death receptor-ligand 1, soluble immune checkpoints, serum biomarkers

transmembrane domain, or by proteolytic cleavage in the cell membrane (15-17).

PD-1 is primarily expressed by T-cells, but also by B cells, natural killer cells, dendritic cells and monocytes. On the other hand, PD-L1 can be expressed by tumor cells, cancer-associated fibroblasts and less frequently by epithelial and endothelial cells, immune cells such as antigen-presenting cells, activated T and B cells, dendritic cells, monocytes and macrophage lineage cells (16,18-21). Therefore, the origin of these soluble proteins in circulation could be of different cell types and the changes in the levels of these soluble proteins could be a consequence of changes in their expression or their release from the cells that express them. sPD-1 has been related to higher expression of the splice variant that encodes the soluble form (17). For PD-L1, it has been proposed that increased expression of the metalloproteases that mediate its proteolytic cleavage of the membrane could increase the levels of the sPD-L1. In relation to this, increased levels of sPD-L1 have been associated with higher expression of these metalloproteases (15,22). But the exact origin of the soluble forms is not enough documented.

sPD-1 and sPD-L1 have been evaluated in some cancer types, and the results revealed that, in most cancers, high sPD-1 and sPD-L1 levels are related to poor prognosis. In Hodgkin lymphoma, lower levels of sPD-L1 are associated with decreased progression-free survival (23). In patients with bladder cancer, higher levels of sPDL-1 were associated with poorer prognosis, independent of the treatment method, whether chemotherapy or immune checkpoint inhibitors (24). In pancreatic adenocarcinoma and gastrointestinal stromal tumors, higher levels of both sPD-L1 and sPD-L1 correlate with poor survival (25,26). For patients with hepatocellular carcinoma, sPD-L1 and sPD-1 are, respectively, a negative and a positive prognostic factor, showing different behaviors, as has been reported in other cancer types (27).

In patients with locally advanced cervical cancer, the effect of concurrent chemoradiotherapy on the serum levels of sPD-1 and sPD-1 was evaluated, and the results revealed that treatment increased the levels of sPD-1 and sPD-L1, but there is no information on the possible role of these changes in the immune response (28). At present, the relationships between the serum levels of sPD-1 and sPD-L1 and clinicopathological characteristics and the clinical outcomes of cancer remain unclear. Therefore, the current study provides information about the potential use of sPD-1 and sPD-L1 as prognostic biomarkers of cervical cancer and their relationships with clinical outcomes.

Materials and methods

Patients. The present investigation was a prospective and observational study performed on a cohort of 194 patients diagnosed with cervical cancer at the Radiotherapy Service of the National Health Centre, Manuel Avila Camacho, from the Mexican Institute of Social Security (IMSS; Puebla, Mexico) from November 2017 to October 2023. Patients included in the study were diagnosed with cervical cancer by a pathologist and were staged according to the FIGO system (Stage I, II, III and IV) (2). Patients had not received any previous treatment. The exclusion criteria included previous or concurrent cancer, pregnancy, autoimmune sickness or the presence of acute infection.

The tumor histological types included in the study were squamous cell carcinoma (SCC), adenocarcinoma (AC) and adenosquamous cell carcinoma (ASC). The treatment received by the patients during the study was chemo-radiotherapy, which was determined according to their clinical characteristics. No alternative treatment, such as anti-PD-L1, was applied to the patients.

The control group included 40 women whose cytology reports were negative for intraepithelial lesions or malignancy. The exclusion criteria were pregnancy, autoimmune sickness or the presence of acute infection.

The mean age of patients with cervical cancer was 53.24 years (range, 26-87 years). The mean age of the control group was 31.7 years (range, 21-60 years).

The present study was conducted in accordance with the Declaration of Helsinki and the protocol was approved (approval no. R-2023-2106-004) by the Local Ethics Committee of the IMSS (Puebla, Mexico). All the women included in the study signed a consent form before sample collection.

Sample collection. Serum was obtained through phlebotomy, centrifuged at 4,000 x g for 10 min at 4˚C and stored in aliquots at -20˚C until use. Demographic data, pathological diagnosis, histological type, tumor differentiation grade, clinical stage and clinical outcome (total remission of disease or death) were collected from the clinical records.

Serum sPD-1 and sPD-L1 ELISA. To determine the sPD-1 serum concentration, the Quantikine ELISA Human PD1 Kit (cat. no. DPD10; R&D Systems, Inc.) was used. Serum PD-L1 levels were determined via a PD-L1/B7-H1 Quantikine ELISA kit (cat. no. DB7H10; R&D Systems, Inc.). No serum dilution was performed and each sample was analyzed in duplicate. The absorbance of the plates was read in a BioTek Synergy-4 plate reader (Agilent Technologies, Inc.) at 450 nm and the absorbance at 570 nm was subtracted. The average of the duplicates of each sample was calculated and the sPD-L1 and sPD-L1 concentrations were calculated via a standard curve provided with the kit.

Statistical analysis. The Mann-Whitney test was performed to compare the serum levels of sPD-1 and sPD-L1 in the control and cervical cancer groups, between the keratinizing types and between the clinical outcomes. The Kruskal-Wallis test followed by Dunn's test was performed to compare the serum levels of sPD-1 and sPD-L1 between the different clinicopathological characteristics, such as histological type, differentiation grade and clinical stage. Data are presented as the medians and ranges. $P \leq 0.05$ was considered to indicate a statistically significant difference.

Additionally, Spearman's correlation analysis was used to analyze the correlation between sPD-1 and sPD-L1 concentrations. Statistical analyses were performed using GraphPad Prism version 10.2.3 (Dotmatics) for Windows.

Receiver operating characteristic (ROC) curve analysis was performed to determine the diagnostic value of sPD-1 and sPD-L1. In this ROC curve, the point with the maximum sensitivity and specificity was selected as the cut-off value.

Table I. Clinicopathological characteristics of patients with cervical cancer.

Characteristics	Number			
Histological type	SCC	AC	ASC	
	156	28	10	
Keratinizing type	Keratinizing		Non-Keratinizing	
	77		30	
Differentiation grade	G1	G2	G3	
	11	101	47	
Clinical stage	I	II	III	IV
	17	68	68	24
Clinical outcome	Total remission		Deceased	
	15		7	

SCC, squamous cell carcinoma; AC, adenocarcinoma; ASC, adenosquamous cell carcinoma; G1, well differentiated; G2, moderately differentiated; G3, poorly differentiated and undifferentiated.

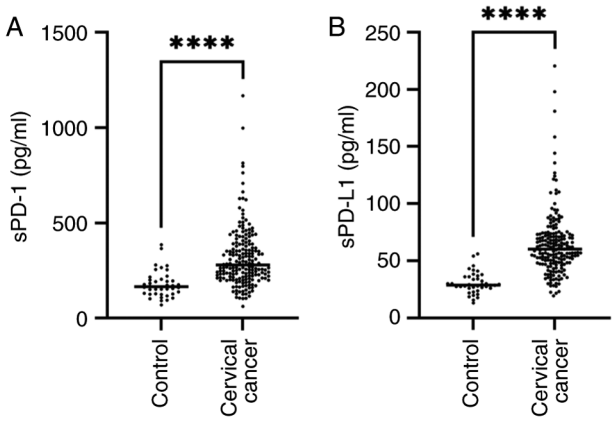

Figure 1. Serum levels of sPD-1 and sPD-L1 in the control and cervical cancer groups. (A) Serum concentration of sPD-1 was increased in the cervical cancer group (n=192) compared with the control group (n=40). (B) The serum concentration of sPD-L1 was increased in the cervical cancer group (n=194) compared with the control group (n=40). Mann-Whitney test, ****P<0.0001. s, soluble; PD-L1, programmed cell death receptor-ligand 1; PD-1, programmed cell death protein 1.

ROC curve analysis was performed with the method of DeLong *et al* (29) using MedCalc software version 22.030 (MedCalc software, Ltd.).

Results

Patient characteristics. The clinicopathological characteristics considered in the present study were histological type, keratinizing, differentiation grade, clinical stage and clinical outcome (total remission and death). The numbers of patients included in the different clinicopathological groups are shown in Table I.

sPD-1 and sPD-L1 concentrations are increased in patients with cervical cancer. The sPD-1 and sPD-L1 concentrations increased significantly in the patients with cervical cancer (Fig. 1A and B). The median concentration of sPD-1 in the control group was 165.3 pg/ml (range, 69.96-385 pg/ml) while it was 279.4 pg/ml (range, 62.48-1,168.35 pg/ml) in the cervical cancer group. The median concentration of sPD-L1 in the control group was 28.88 pg/ml (range, 13.25-56.07 pg/ml), while it was 60.38 pg/ml (range, 19.39-220.4 pg/ml) in the cervical cancer group. The results showed a significantly increased concentration of sPD-1 and sPD-L1 in the cervical cancer group P<0.0001.

ROC curve analysis. To determine the diagnostic value of sPD-1 and sPD-L1, ROC curve analysis was performed. The specificity, sensitivity, area under the curve (AUC) and cut-off values are shown in Fig. 2A and B. sPD-1 was a favorable discriminator for patients with cervical cancer with a sensitivity of 0.79 and a specificity of 0.775; however, sPD-L1 proved superior, demonstrating a higher sensitivity of 0.80 and a higher specificity of 0.95. These results support the fact that important changes in sPD-1 and sPD-L1 levels occur during the development of cervical cancer, which allows them to be distinguished from healthy women.

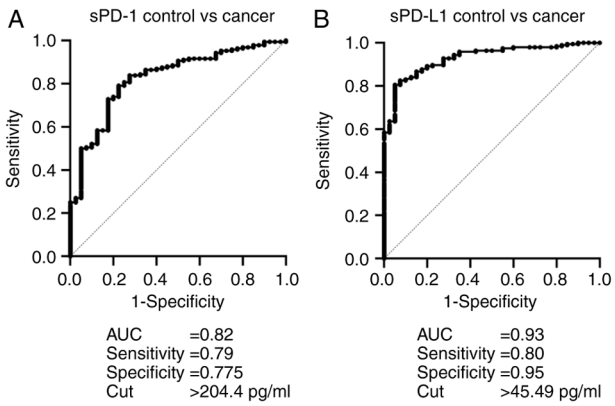

Figure 2. ROC analysis of sPD-1 and sPD-L1 in the control vs. cervical cancer groups. (A) ROC curve of sPD1 of the control group (n=40) compared with the cervical cancer group (n=192); the AUC, sensitivity, specificity, the Youden index and cut-off value are shown. (B) ROC curve of sPD-L1 of the control group (n=40) compared with the cervical cancer group (n=194); the AUC, sensitivity, specificity, the Youden index and cut-off value are shown. ROC, receiver operating characteristic; AUC, area under the curve; s, soluble; PD-L1, programmed cell death receptor-ligand 1; PD-1, programmed cell death protein 1.

Relationships of sPD-1 and sPD-L1 concentrations with clinicopathological parameters. sPD-1 and sPD-L1 concentrations were not associated with histological type, keratinizing type or differentiation grade. The median concentration of sPD-1 was 269 pg/ml for patients with SCC, 281.2 pg/ml for patients with AC and 289.83 pg/ml for patients with ASC (P=0.9143). The median concentration of sPD-L1 was 59.82 pg/ml for patients with SCC, 62.5 pg/ml for patients with AC and 59.22 pg/ml for patients with ASC (P=0.84497). For the keratinizing type, the median sPD-1 concentration was 284.2 pg/ml while 278.4 pg/ml for the non-keratinizing type (P=0.9533). For sPD-L1, the median for the keratinizing type was 56.96 pg/ml while for the non-keratinizing type was 61.23 pg/ml (P=0.4919). Concerning differentiation grade, a slight increase was observed in the concentration

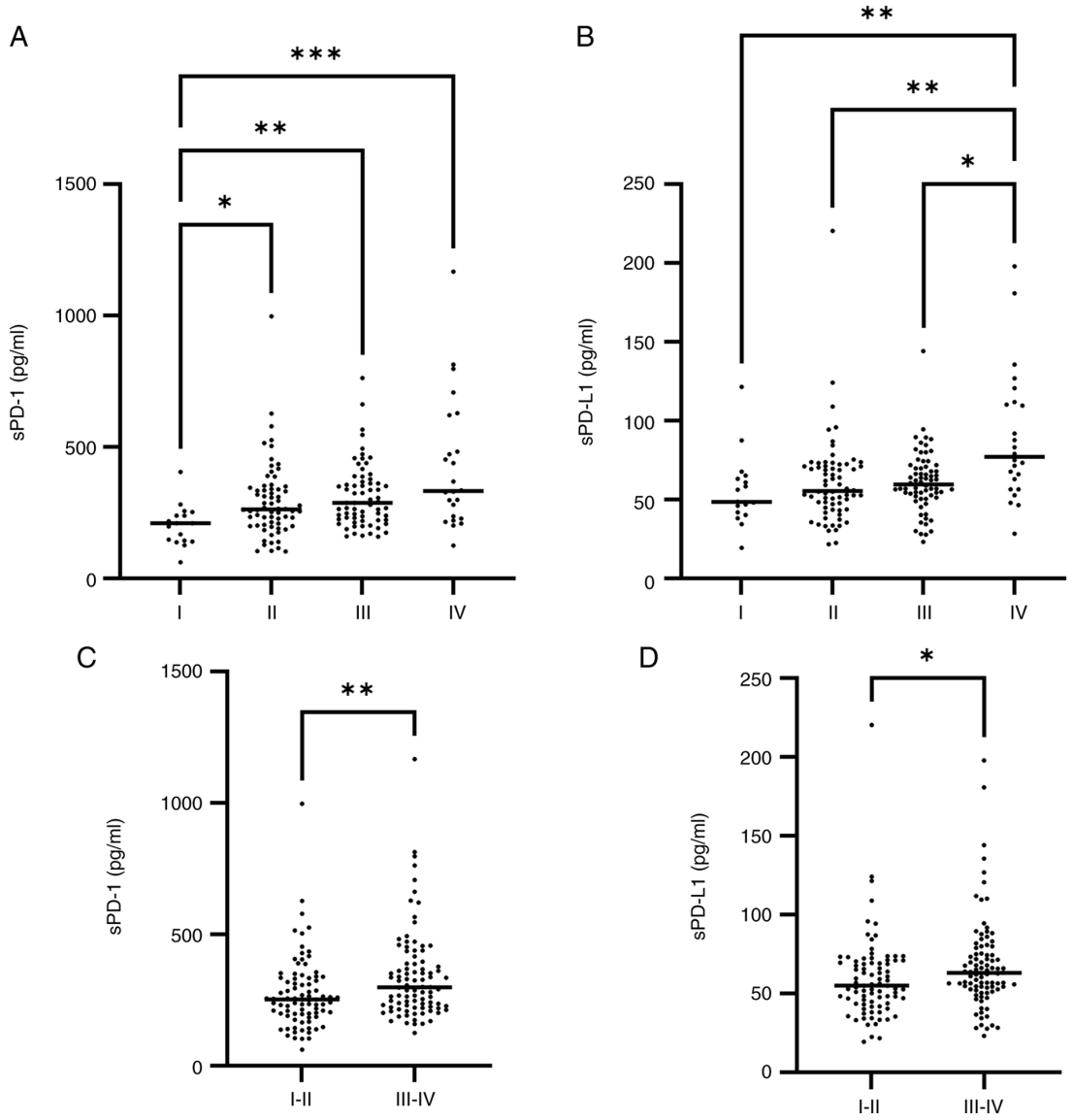

Figure 3. Serum sPD-1 and sPD-L1 levels are increased in advanced clinical stages. (A) sPD-1 concentration for the clinical stages I (n=17), II (n=68), III (n=67) and IV (n=24). (B) sPD-L1 concentration for the clinical stages I (n=17), II (n=68), III (n=68) and IV (n=24). (C) sPD-1 concentration between early clinical stages I-II (n=85) and advanced clinical stages III-IV (n=91). (D) sPD-L1 concentration between early clinical stages I-II (n=85) and advanced clinical stages III-IV (n=92). Kruskal-Wallis test, *P<0.05, **P<0.01 and ***P<0.001. s, soluble; PD-L1, programmed cell death receptor 1 ligand; PD-1, programmed cell death protein 1.

of both soluble proteins as tumor differentiation decreased, although it did not reach significance. The median sPD-1 level was 264.4 pg/ml in the well differentiated (G1) group, 267.9 pg/ml in the moderately differentiated (G2) group and 326.4 pg/ml in the poorly differentiated and undifferentiated (G3) group (P=0.3749). The median sPD-L1 concentration was 57 pg/ml in the G1 subgroup, 58.92 pg/ml in the G2 subgroup and 63.79 pg/ml in the G3 subgroup (P=0.5576).

The analysis of sPD-1 concentrations for clinical stage revealed significantly higher concentrations in patients with clinical stage II, III and IV than in those with clinical stage I (Fig. 3A) (P=0.0317, P=0.0025 and P=0.0001, respectively; Kruskal-Wallis test and Dunn's multiple comparisons test). The sPD-L1 concentration was also significantly higher in patients with clinical stage IV disease than in those with stages I, II and III (Fig. 3B) (P=0.0026, P=0.0021 and P=0.0135, respectively). Analysis of sPD-1 and sPD-L1 in advanced clinical

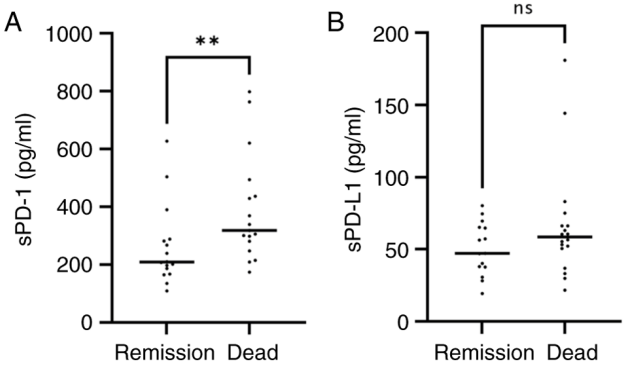

Figure 4. Serum sPD-1 and sPD-L1 levels in patients with total remission vs. deceased patients. (A) sPD-1 concentration analysis of the total remission group (n=16) vs. the deceased group (n=8). (B) sPD-L1 concentration analysis of the total remission group (n=15) vs. the deceased group (n=7). Mann-Whitney test, **P<0.01. s, soluble; PD-L1, programmed cell death receptor-ligand 1; PD-1, programmed cell death protein 1; ns not, significant.

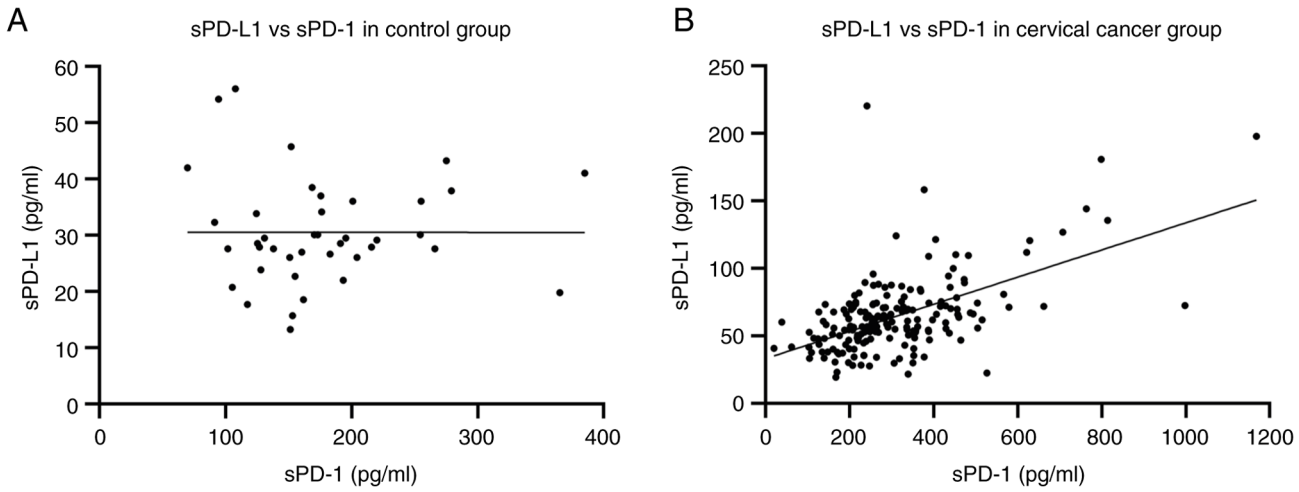

Figure 5. Spearman's correlation of sPD-1 and sPD-L1 levels in the control and cervical cancer groups. (A) Spearman's correlation of sPD-1 and sPD-L1 in the control group (n=40); r=0.066, P=0.6875. (B) Spearman's correlation of sPD-1 and sPD-L1 in the cervical cancer group (n=192); r=0.4660, P<0.0001. s, soluble; PD-L1, programmed cell death receptor-ligand 1; PD-1, programmed cell death protein 1.

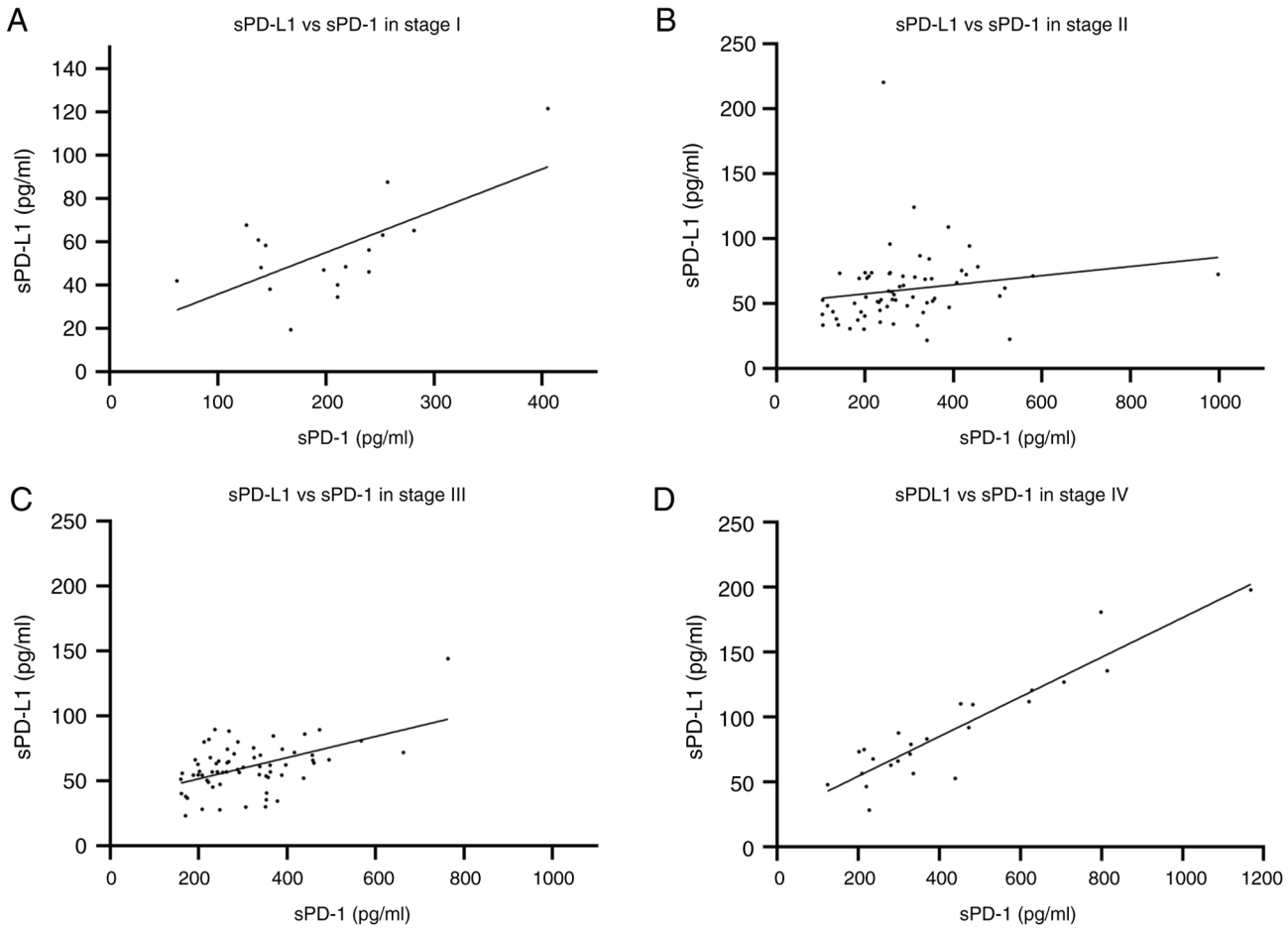

Figure 6. Spearman's correlation of sPD-1 and sPD-L1 levels in the different clinical stage groups. (A) Spearman's correlation of sPD-1 and sPD-L1 in the clinical stage I (n=17); r=0.3676, P=01471. (B) Spearman's correlation of sPD-1 and sPD-L1 in patients with clinical stage II disease (n=68); r=0.3665, P=0.0023. (C) Spearman's correlation of sPD-1 and sPD-L1 in patients with clinical stage III disease (n=67); r=0.3784, P=0.017. (D) Spearman's correlation of sPD-1 and sPD-L1 in patients with clinical stage IV disease (n=24); r=0.8183, P<0.0001. s, soluble; PD-L1, programmed cell death receptor-ligand 1; PD-1, programmed cell death protein 1.

stages (III and IV) vs. early clinical stages (I and II) indicated significantly higher levels in advanced clinical stages (Fig. 3C and D) (P=0.0025 and P=0.0146, respectively). It is important to highlight that a gradual increase was observed as the clinical stage progressed, although there were no significant differences between all groups.

sPD-1 levels are higher in the group of deceased patients. The clinical outcome (total remission or deceased) was analyzed in relation to the serum levels of sPD-1 or sPD-L1. The results revealed an increased concentration of both proteins in the group of deceased patients. The median sPD-1 level was 209.3 pg/ml (range, 109.5-627.52 pg/ml) for the total remission group, while it was 318.9 pg/ml (range, 174.19-798.72 pg/ml) for the deceased patients. For sPD-L1, the median concentration in the total remission group was 47.27 pg/ml (range, 19.39-80.2 pg/ml), while, in the deceased group, it was 58.51 pg/ml (range, 21.8-180.87 pg/ml); however, the statistical analysis only showed significant difference for sPD-1 (P=0.0077) (Fig. 4A and B).

Correlations between sPD-1 and sPD-L1 in the control and cervical cancer groups. To determine whether there was a correlation between levels of sPD-1 and sPD-L1 in the control and cervical cancer groups, a Spearman's correlation analysis was performed. The results revealed no correlation in the control group, with r=0.066 and P=0.6875 (Fig. 5A). In the cervical cancer group, the results indicated a strong positive correlation, with r=0.4660 and P<0.0001 (Fig. 5B). The correlation between these soluble proteins in the cancer group could be related to the role of immunosuppression which is not present in the control group.

Spearman's correlation of sPD-1 and sPD-L1 by clinical stage. Additionally, a Spearman's correlation by clinical stage was performed. The clinical stage I group showed a positive association (r=0.3676), but this was found non-significant (P=01471) (Fig. 6A). For patients at clinical stage II, a moderately positive correlation (r=0.3665) with P=0.0023 was calculated (Fig. 6B). For patients at clinical stage III, a moderately positive correlation was detected (r=0.3784) with P=0.017 (Fig. 6C). Clinical stage IV disease was strongly correlated (r=0.8183 and P<0.0001) (Fig. 6D). The correlation of concentration of sPD-1 and sPD-L1 increased with diseased progression suggesting a potential role in the disease progression related with greater immunosuppression.

Discussion

The present study evaluated the serum concentrations of sPD-1 and sPD-L1 in patients with cervical cancer to determine their diagnostic and prognostic value. The serum concentrations of sPD-1 and sPD-L1 were greater in the cervical cancer group than in the control group. In other cancer types, increased concentrations of these soluble proteins were reported in patients with cancer compared with healthy people. sPD-L1 levels are increased in patients with Hodgkin lymphoma (23). sPD-1 was evaluated in patients with associated liver diseases and hepatocellular carcinoma, with higher levels reported in the cancer group (30,31). In prostate and non-small cell lung cancer, both soluble proteins were increased (32,33). These results showed that increased levels of these sICs are related to cancer. The results of the ROC curve analysis indicated that both soluble proteins are favorable discriminators for patients with cervical cancer, with greater specificity and sensitivity for sPD-L1. These results support the potential use of these proteins as diagnostic markers and suggest a role in cancer development as they may participate in immune tolerance; however, these findings must be further explored.

Analysis of clinicopathological characteristics and the serum concentrations of sPD-1 and sPD-L1 revealed associations only with the clinical stage. sPD-1 levels were significantly higher in patients with clinical stage II, III and IV than in those with clinical stage I disease. On the other hand, sPD-L1 levels were significantly higher in patients with clinical stage IV than in those with clinical stage I, II and III. Additionally, when early stages I and II were grouped and compared with advanced stages III and IV, a significant increase was observed in the advanced clinical stages. In other cancer types, such as extranodal NK/T-cell lymphoma and gastric cancer, higher levels of sPD-L1 are related to advanced-stage disease; in non-small cell lung cancer, higher levels of sPD-1 and sPD-L1 are related to advanced clinical stage but not to other pathological characteristics, such as the histological type, as observed in the present study (34,35).

The clinical outcomes of patients with total remission of the disease and those who succumbed were compared and the levels of sPD-1 were significantly higher in the deceased group. sPD-1 has shown inconsistent survival predictive value; for example, in pancreatic cancer, higher levels of sPD-1 are associated with survival (25); a higher concentration of sPD-1 in hepatocellular carcinoma is a favorable independent prognostic factor and in renal cell carcinoma it is associated with longer progression-free survival (27,36). It has been suggested that sPD-1 can promote antitumor immunity by blocking PD-L1 in tumor cells (37); nonetheless, this result is not supported by the results obtained in the present study; thus, the role of sPD-1 in cervical cancer progression must be further explored. For sPD-L1, increased levels were observed in the deceased group, but the difference was not statistically significant; however, in different cancer types, such as hepatocellular carcinoma, gastric, prostate and renal cell carcinoma, higher levels were associated with improved survival (27,32,35,36). Additionally, in some cancer types like gastric cancer, the patients with tumors expressing PD-L1 showed low overall survival. On this basis, it can be hypothesized that in cervical cancer, sPD-L1 could be secreted by tumor cells and the serum levels be related to an immunosuppressive tumor microenvironment (38). However, a larger sample size could increase the accuracy of the present results.

In different types of cancer, analogous elevations in sPD-1 and sPD-L1 were reported; therefore, the present study determined the correlation of these soluble proteins in the control and cervical cancer groups. In the control group, no correlation was observed, while a favorable correlation was observed in the cervical cancer group. Additionally, the correlation in the clinical stage IV group was very strong. The authors of the present study prefer not to hypothesize on the clinical implications of these results; however, Hayasi *et al* (16) reported in 2024 that levels of sCIs such as sPD-1, sPD-L1 and CTL-4 are related to the exhaustion of antitumor immunity and the response to immunotherapy because higher levels of sPD-1 and PD-L1 were associated with non-responsiveness to PD-1/PD-L1 blockade therapy. An increase in sPD-L1 may strengthen T-cell inhibition, promoting cancer immune evasion; however, the roles of both soluble proteins must be studied in patients with cancer.

Serum sPD-1 and sPD-L1 are potentially accessible biomarkers that could be used in the diagnosis and prognosis

of patients with cervical cancer and for monitoring patients receiving anti-PD-1 or anti-PD-L1 therapy, highlighting the importance of studying sICs in patients with cancer.

Acknowledgements

Not applicable.

Funding

The present study was supported by the Mexican Institute of Social Security (grant no. R-2023-2106-004).

Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

SPP and ICR conducted investigation and data collection. PMM and VVR analyzed data, and designed figures and table. VVR and JRL conceptualized the study and wrote the manuscript. ICR and SPP confirm the authenticity of all the raw data. All authors drafted the manuscript, and read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study was approved (approval no. R-2023-2106-004) by the Human Ethics and Local Committee of the Mexican Institute of Social Security (Puebla, Mexico). Written informed consent was obtained from all the participants in the present study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA and Jemal A: Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 68: 394-424, 2018.
2. Mohamud A, Høgdall C and Schnack T: Prognostic value of the 2018 FIGO staging system for cervical cancer. Gynecol Oncol 165: 506-513, 2022.
3. Hsu HC, Li X, Curtin JP, Goldberg JD and Schiff PB: Surveillance epidemiology and end results analysis demonstrates improvement in overall survival for cervical cancer patients treated in the era of concurrent chemoradiotherapy. Front Oncol 5: 81, 2015.
4. Rocha Martins P, Luciano Pereira Morais K, de Lima Galdino NA, Jacauna A, Paula SOC, Magalhães WCS, Zuccherato LW, Campos LS, Salles PGO and Gollob KJ: Linking tumor immune infiltrate and systemic immune mediators to treatment response and prognosis in advanced cervical cancer. Sci Rep 13: 22634, 2023.
5. Li C, Cang W, Gu Y, Chen L and Xiang Y: The anti-PD-1 era of cervical cancer: Achievement, opportunity, and challenge. Front Immunol 14: 1195476, 2023.
6. Lou H, Cai H, Huang X, Li G, Wang L, Liu F, Qin W, Liu T, Liu W, Wang ZM, *et al*: Cadonilimab combined with chemotherapy with or without bevacizumab as first-line treatment in recurrent or metastatic cervical cancer (COMPASSION-13): A phase 2 study. Clin Cancer Res 30: 1501-1508, 2024.
7. Sharpe AH, Wherry EJ, Ahmed R and Freeman GJ: The function of programmed cell death 1 and its ligands in regulating autoimmunity and infection. Nat Immunol 8: 239-245, 2007.
8. Ai L, Xu A and Xu J: Roles of PD-1/PD-L1 pathway: Signaling, cancer, and beyond. Adv Exp Med Biol 1248: 33-59, 2020.
9. Alsaab HO, Sau S, Alzhrani R, Tatiparti K, Bhise K, Kashaw SK and Iyer AK: PD-1 and PD-L1 checkpoint signaling inhibition for cancer immunotherapy: Mechanism, combinations, and clinical outcome. Front Pharmacol 8: 561, 2017.
10. Chen B, Hu J, Hu X, Chen H, Bao R, Zhou Y, Ye Y, Zhan M, Cai W, Li H and Li HB: DENR controls JAK2 translation to induce PD-L1 expression for tumor immune evasion. Nat Commun 13: 2059, 2022.
11. Twa DD, Chan FC, Ben-Neriah S, Woolcock BW, Mottok A, Tan KL, Slack GW, Gunawardana J, Lim RS, McPherson AW, *et al*: Genomic rearrangements involving programmed death ligands are recurrent in primary mediastinal large B-cell lymphoma. Blood 123: 2062-2065, 2014.
12. Gupta S, Cheville JC, Jungbluth AA, Zhang Y, Zhang L, Chen YB, Tickoo SK, Fine SW, Gopalan A, Al-Ahmadie HA, *et al*: JAK2/PD-L1/PD-L2 (9p24.1) amplifications in renal cell carcinomas with sarcomatoid transformation: Implications for clinical management. Mod Pathol 32: 1344-1358, 2019.
13. Gupta S, Vanderbilt CM, Cotzia P, Arias-Stella JA III, Chang JC, Zehir A, Benayed R, Nafa K, Razavi P, Hyman DM, *et al*: Next-generation sequencing-based assessment of JAK2, PD-L1, and PD-L2 copy number alterations at 9p24.1 in breast cancer: Potential implications for clinical management. J Mol Diagn 21: 307-317, 2019.
14. Ikeda S, Okamoto T, Okano S, Umemoto Y, Tagawa T, Morodomi Y, Kohno M, Shimamatsu S, Kitahara H, Suzuki Y, *et al*: PD-L1 is upregulated by simultaneous amplification of the PD-L1 and JAK2 genes in non-small cell lung cancer. J Thorac Oncol 11: 62-71, 2016.
15. Dezutter-Dambuyant C, Durand I, Alberti L, Bendriss-Vermare N, Valladeau-Guilemond J, Duc A, Magron A, Morel AP, Sisirak V, Rodriguez C, *et al*: A novel regulation of PD-1 ligands on mesenchymal stromal cells through MMP-mediated proteolytic cleavage. Oncoimmunology 5: e1091146, 2015.
16. Hayashi H, Chamoto K, Hatae R, Kurosaki T, Togashi Y, Fukuoka K, Goto M, Chiba Y, Tomida S and Ota T: Soluble immune checkpoint factors reflect exhaustion of antitumor immunity and response to PD-1 blockade. J Clin Invest 134: e168318, 2024.
17. Nielsen C, Ohm-Laursen L, Barington T, Husby S and Lillevang ST: Alternative splice variants of the human PD-1 gene. Cell Immunol 235: 109-116, 2005.
18. Sharpe AH and Pauken KE: The diverse functions of the PD1 inhibitory pathway. Nat Rev Immunol 18: 153-167, 2018.
19. Wang W and Zhang T: Expression and analysis of PD-L1 in peripheral blood circulating tumor cells of lung cancer. Future Oncol 17: 1625-1635, 2021.
20. Bailly C, Thuru X and Quesnel B: Soluble programmed death ligand-1 (sPD-L1): A pool of circulating proteins implicated in health and diseases. Cancers (Basel) 13: 3034, 2021.
21. Kawasaki K, Noma K, Kato T, Ohara T, Tanabe S, Takeda Y, Matsumoto H, Nishimura S, Kunitomo T, Akai M, *et al*: PD-L1-expressing cancer-associated fibroblasts induce tumor immunosuppression and contribute to poor clinical outcome in esophageal cancer. Cancer Immunol Immunother 72: 3787-3802, 2023.
22. Hira-Miyazawa M, Nakamura H, Hirai M, Kobayashi Y, Kitahara H, Bou-Gharios G and Kawashiri S: Regulation of programmed-death ligand in the human head and neck squamous cell carcinoma microenvironment is mediated through matrix metalloproteinase-mediated proteolytic cleavage. Int J Oncol 52: 379-388, 2018.
23. Guo X, Wang J, Jin J, Chen H, Zhen Z, Jiang W, Lin T, Huang H, Xia Z and Sun X: High serum level of soluble programmed death ligand 1 is associated with a poor prognosis in Hodgkin lymphoma. Transl Oncol 11: 779-785, 2018.
24. Krafft U, Olah C, Reis H, Kesch C, Darr C, Grünwald V, Tschirdewahn S, Hadaschik B, Horvath O, Kenessey I, *et al*: High serum PD-L1 levels are associated with poor survival in urothelial cancer patients treated with chemotherapy and immune checkpoint inhibitor therapy. Cancers (Basel) 13: 2548, 2021.

25. Bian B, Fanale D, Dusetti N, Roque J, Pastor S, Chretien AS, Incorvaia L, Russo A, Olive D and Iovanna J: Prognostic significance of circulating PD-1, PD-L1, pan-BTN3As, BTN3A1 and BTLA in patients with pancreatic adenocarcinoma. Oncoimmunology 8: e1561120, 2019.
26. Fanale D, Incorvaia L, Badalamenti G, De Luca I, Algeri L, Bonasera A, Corsini LR, Brando C, Russo A, Iovanna JL, *et al*: Prognostic role of plasma PD-1, PD-L1, pan-BTN3As and BTN3A1 in patients affected by metastatic gastrointestinal stromal tumors: Can immune checkpoints act as a sentinel for short-term survival? Cancers (Basel) 13: 2118, 2021.
27. Chang B, Huang T, Wei H, Shen L, Zhu D, He W, Chen Q, Zhang H, Li Y, Huang R, *et al*: The correlation and prognostic value of serum levels of soluble programmed death protein 1 (sPD-1) and soluble programmed death-ligand 1 (sPD-L1) in patients with hepatocellular carcinoma. Cancer Immunol Immunother 68: 353-363, 2019.
28. Liu C, Li X, Li A, Zou W, Huang R, Hu X, Yu J, Zhang X and Yue J: Concurrent chemoradiotherapy increases the levels of soluble immune checkpoint proteins in patients with locally advanced cervical cancer. J Immunol Res 2022: 9621466, 2022.
29. DeLong ER, DeLong DM and Clarke-Pearson DL: Comparing the areas under two or more correlated receiver operating characteristic curves: A nonparametric approach. Biometrics 44: 837-845, 1988.
30. Cheng HY, Kang PJ, Chuang YH, Wang YH, Jan MC, Wu CF, Lin CL, Liu CJ, Liaw YF, Lin SM, *et al*: Circulating programmed death-1 as a marker for sustained high hepatitis B viral load and risk of hepatocellular carcinoma. PLoS One 9: e95870, 2014.
31. Li N, Zhou Z, Li F, Sang J, Han Q, Lv Y, Zhao W, Li C and Liu Z: Circulating soluble programmed death-1 levels may differentiate immune-tolerant phase from other phases and hepatocellular carcinoma from other clinical diseases in chronic hepatitis B virus infection. Oncotarget 8: 46020-46033, 2017.
32. Zvirble M, Survila Z, Bosas P, Dobrovolskiene N, Mlynska A, Zaleskis G, Jursenaite J, Characiejus D and Pasukoniene V: Prognostic significance of soluble PD-L1 in prostate cancer. Front Immunol 15: 1401097, 2024.
33. Ancın B, Özercan MM, Yılmaz YM, Uysal S, Kumbasar U, Sarıbaş Z, Dikmen E, Doğan R and Demircin M: The correlation of serum sPD-1 and sPD-L1 levels with clinical, pathological characteristics and lymph node metastasis in nonsmall cell lung cancer patients. Turk J Med Sci 52: 1050-1057, 2022.
34. Li JW, Wei P, Guo Y, Shi D, Yu BH, Su YF, Li XQ and Zhou XY: Clinical significance of circulating exosomal PD-L1 and soluble PD-L1 in extranodal NK/T-cell lymphoma, nasal-type. Am J Cancer Res 10: 4498-4512, 2020.
35. Chivu-Economescu M, Herlea V, Dima S, Sorop A, Pechianu C, Procop A, Kitahara S, Necula L, Matei L, Dragu D, *et al*: Soluble PD-L1 as a diagnostic and prognostic biomarker in resectable gastric cancer patients. Gastric Cancer 26: 934-946, 2023.
36. Incorvaia L, Fanale D, Badalamenti G, Porta C, Olive D, De Luca I, Brando C, Rizzo M, Messina C, Rediti M, *et al*: Baseline plasma levels of soluble PD-1, PD-L1, and BTN3A1 predict response to nivolumab treatment in patients with metastatic renal cell carcinoma: A step toward a biomarker for therapeutic decisions. Oncoimmunology 9: 1832348, 2020.
37. Qin YE, Tang WF, Xu Y, Wan FR and Chen AH: Ultrasound-mediated co-delivery of miR-34a and sPD-1 complexed with microbubbles for synergistic cancer therapy. Cancer Manag Res 12: 2459-2469, 2020.
38. Hassen G, Kasar A, Jain N, Berry S, Dave J, Zouetr M, Priyanka Ganapathiraju VLN, Kurapati T, Oshai S, Saad M, *et al*: Programmed death-ligand 1 (PD-L1) positivity and factors associated with poor prognosis in patients with gastric cancer: An umbrella meta-analysis. Cureus 14: e23845, 2022.