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Research Article

Correlation between PD-L1 Expression, Clinicopathological Factors, and Metastasis Risk in Colorectal Cancer Patients

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This study investigated the correlation between PD-L1 expression, metastasis, and survival in colorectal cancer (CRC) patients. PD-L1 expression was not significantly associated with overall survival, disease-free survival, or mortality rate. However, a significant difference was observed between PD-L1 positive and negative patients regarding the presence of metastasis, which was higher in the PD-L1 positive group. These findings suggest that PD-L1 expression may impact metastasis in CRC patients but not overall survival.

1. Introduction

Colorectal carcinoma (CRC), among diagnosed malignancies, has the third most prevalent ranking and the second main death reason for cancer-related mortality in the world as the primary health concern [1]. CRC mortality and incidence are increasing annually, and more than 1.1 million mortality cases and 2.2 million new patients are expected in 2030 [2]. The 5-year survival rate following the CRC diagnosis is 65% [3]. The diagnosis stage is closely correlated with survival rates; main subjects at later stages are diagnosed, and 5-year survival rate subjects with distant metastases are 13% [4].

Regardless of the enhancement of overall survival because of novel therapies and medications in CRC, distant metastasis and local and regional recurrence are also leading management failure causes. Approximately 25 percent of cases present metastasis when diagnosed, and 50 percent of CRC cases treated during their lifetime will develop metastasis [5]. It is recognized that immune escape and suppression are essential in the metastasis, recurrence, and progression of tumor. In these pathways, programmed death ligand 1 (PD-L1) signaling stimulation processes were considered a considerable tumor immune evasion mechanism via CD8 cytotoxic immune response suppression and T-cell proliferation inhibition [6].

PD-L1 is on immune cell surfaces like T cells, B cells, macrophages, and dendritic cells. Additionally, on tumor cells, PD-L1 is expressed and acts as a survival signaling pathway and proliferative induction, and on immune cells, by ligation to PD-1, it is a protumorigenic ligand in tumor cells [7]. It has been found that PD-L1 high concentration expression in various solid human malignancies and many cancers is correlated with disease outcomes [8, 9]. Dismal overall survival is associated with a high level of expression of PD-L1, in CRC cases [10]. Hence, this study purposed to evaluate the association between PD-L1 and metastasis and survival rate in CRC cases.

2. Methods and Materials

This Cross-sectional study was conducted between August 2021 and August 2022 at Tabriz Imam Reza Hospital. All patients with a confirmed diagnosis of CRC were included in the study. Tabriz University of Medical Sciences Medical Ethics Committee approved the study protocol (Registration Code: IR.TBZMED.REC.1400.640) and informed consent was obtained from all participants. Inclusion criteria included cases with CRC confirmed by a pathologist. Exclusion criteria included lack of consent to participate in the study, having any other type of malignancy, and the incompleteness of the information collected from the patient.

A checklist collected all patients' demographic information, stage, grade, number, and location of involved lymph nodes, metastasis, local extension, and overall survival data. Overall survival was determined by calculating the time interval from the CRC diagnosis to the last follow-up (for surviving patients) or the date of death (for deceased patients).

The pathology slides (positively charged microscopic slides with catalog number 71873-02) of each patient were assessed for expression of PD-L1 utilizing the immunohistochemistry method by anti PD-L1 kits derived from rabbits (Máster Diagnóstica S.L. company with specific catalog number for the equipment used in our experiments is MAD-004070R/D). The PD-L1 protein percentage expression was reported, and a 1% or higher cut-off point was used for PD-L1 positivity.

After preparing and assessing the histological slides under a light microscope (Olympus CX23), the cell number and stained cells were counted to calculate the percentage of positively-stained cells. Normal colon tissue was used as an internal control for PD-L1 expression. PD-L1 expression was determined using the combined positive score method by calculating the ratio of positively-stained cells to total tumor cells and multiplying by 100. A 1% or higher cut-off point defined PD-L1 positivity in tumor cells.

Based on the study design and using reference articles with a significance level of 0.05 and a power of 85% in Stata software, the minimum sample size required was 80 cases. All participants met the inclusion criteria and had none of the exclusion criteria. Patients were divided into two groups, including the positive and negative PD-L1, and they compared these two groups with each other.

By using the SPSS version 23 software, in this study, statistical analysis was performed. The quantitative data were displayed as the median + interquartile range (IQR) or mean ± standard deviation, while the qualitative data were presented as frequency and percentage. The Kolmogorov–Smirnov test was used to evaluate distribution normality. A Mann–Whitney U test or sample T-test was utilized to compare the quantitative parameters between the groups, as well as for comparing the qualitative parameters between the groups; the chi-square test was used. Pearson or Spearman correlation test was used to determine the relationship between variables. The study's primary outcome was overall survival, which was assessed using the Kaplan–Meier method.

Furthermore, to examine the association between the mortality risk and all variables, Cox regression analysis was used. The hazard ratio was assessed following adjusting for all confounding parameters, with a 95% confidence interval reported. A p value less than 0.05 was considered statistically significant.

3. Results

80 CRC subjects were registered in the study. Twenty cases were positive, and 60 cases were negative for PD-L1. At the diagnosis time, the patient's mean age was 58.5 years in the PD-L1 negative group while it was 56.9 years in the PD-L1 positive group. 27 (45%) females and 33(55%) males were in the PD-L1 negative group. In contrast, in the PD-L1 positive group, eight patients (40%) were male and 12 (60%) were female. The demographic characteristics of these patients are shown in Table 1.

Metastasis was the only factor significantly different between the two groups, and it was higher in the PD-L1 positive patients compared to PD-L1 negative patients ($p = 0.024$). The mean disease-free survival and overall survival in PD-L1 negative patients was 435 and 532.5 days; 447 and 543.5 days in PD-L1 positive patients, respectively ($p = 0.718$, $p = 0.885$). 35% of PD-L1 positive patients and 30% of PD-L1 negative patients had positive CEA ($p = 0.676$). The clinicopathological characteristics are displayed in Table 1.

The subjects' overall survival based on the Kaplan–Meier curve is illustrated in Figure 1. According to the Log-Rank test, there was no significant difference in overall survival between the PD-L1 negative and positive groups ($p = 0.613$). The relationship between various variables and the risk of mortality according to the Cox Regression model is presented in Table 2. No significant association existed between the variables used in this model and the survival of patients with colorectal malignancies ($p > 0.05$).

The Cox regression model was utilized to determine the correlation between numerous parameters and the mortality risk, as presented in Table 2. None of the parameters in the model revealed a significant correlation with the survival of patients with CRC ($p > 0.05$).

4. Discussion

The study has several limitations that have reduced its reliability. These limitations include a small sample size, high heterogeneity among the samples, and possibly incorrect selection of patients. These factors may have affected the generalizability of the findings and should be considered when interpreting the results.

CRC is one of the most frequent malignancies and a primary cause of death globally. CRC risk factors are active and passive smoking, low physical activity, red meat consumption, obesity, and a high salt diet [11]. It has been demonstrated that there is an association between the expression of PD-L1 and clinical and pathological features [12]. PD-L1 is a critical molecule in the tumor microenvironment that blocks the T-cell role and enhances tumor

Table 1: Patients' clinicopathological and demographic characteristics.

Parameters	PD-L1 positive ($n = 20$)	PD-L1 negative ($n = 60$)	p value
Gender			0.245
Male	8 (40%)	33 (55%)	
Female	12 (60%)	27 (45%)	
Age at disease onset, years	56.9 ± 13	58.5 ± 14.4	0.671
Survival period, days	543.5	532.5	0.885
Disease-free survival	447	435	
Cases outcome			0.573
Mortality	7 (35%)	17 (28.3%)	
Survival	13 (65%)	43 (71.7%)	
Causes of death			0.350
Cancer	6 (30%)	8 (13.3%)	
Intracerebral hemorrhage	0 (0%)	2 (3.3%)	
Surgery	1 (5%)	7 (11.7%)	
Stage			0.077
1	0 (0%)	3 (5%)	
2	9 (45%)	13 (21.7%)	
3	9 (45%)	30 (50%)	
4	2 (10%)	8 (13.3%)	
TNM			
T			
1	0 (0%)	3 (5%)	
2	9 (45%)	19 (31.7%)	
3	9 (45%)	30 (50%)	
4	2 (10%)	8 (13.3%)	
N			
0	3 (15%)	23 (38.3%)	
1	12 (60%)	28 (47.7%)	
2	5 (25%)	9 (15%)	
M	10 (50%)	14 (23.3%)	**0.024***
Tumor differentiation			
Poor	2 (10%)	3 (5%)	
Moderate	17 (85%)	41 (68.3%)	
Well	1 (5%)	16 (27.7%)	
CEA	7 (35%)	18 (30%)	0.676
Metastasis site			0.074
Bladder	1 (5%)	1 (1.7%)	
Bone	0 (0%)	1 (1.7%)	
Liver	4 (20%)	7 (11.7%)	
Lung	3 (15%)	4 (6.7%)	
Ovary	1 (5%)	0 (0%)	
Vertebrae	1 (5%)	0 (0%)	
Spleen	0 (0)	1 (1.7)	

CEA: carcinoembryonic antigen. Bold and * indicate a significance level of p value less than 0.05. Qualitative data are presented as frequencies (%), quantitative variables with non-normal distribution are presented as median and quantitative variables with normal distribution are presented as mean ± SD.

immune evasion. It is essential in regulating immune tolerance and is a crucial target for cancer immunotherapy. The PD-L1 pathway blocking enhances T cell function and induces tumor cell lysis, offering a promising avenue for cancer treatment [13].

Significant differences between PD-L1 negative cases and positive cases groups have been demonstrated in line with our study. Additionally, evaluating CRC cases' eligibility for immunotherapy PD-L1 expression may act as an independent factor, as its expression generally is more in CRC cases with metastatic compared to 68.2% in metastatic lymph nodes versus 40.9% in primary lymph nodes in CRC [14]. Furthermore, a meta-analysis that included ten investigations and 3481 subjects examined clinical and pathological features associated with the PD-L1 expression in colorectal tumor subjects [15]. The research demonstrated that increased PD-L1 expression is associated with the invasion of lymph nodes and the advanced disease stage, which is consistent with the findings of our study.

A study has revealed that PD-L1 expression has a role in the poor consequence in CRC cases regarding a 5-year survival rate. This showed that PD-L1 may act as a notable prognostic tool. However, the findings of our study did not confirm mentioned study [9]. Additionally, a meta-analysis study by Cao et al. [16], which contained 15 research and 3078 subjects, revealed that excessive PD-L1

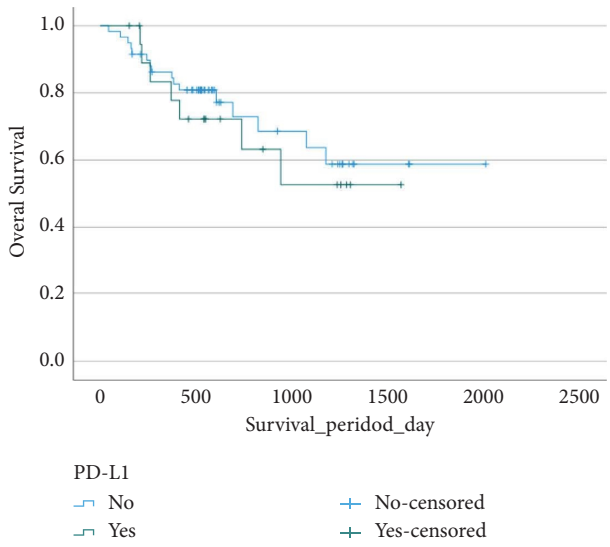

Figure 1: Survival analysis based on Kaplan–Meier method.

Table 2: The relationship between different variables and the mortality risk by using Cox regression modelling.

Variables	HR	95% CI	*p* value
Age at disease onset, years	1.007	0.978–1.038	0.626
Female	0.532	0.218–1.298	0.165
Metastasis	3.252	0.693-15.260	0.135
Stage			
2	—	—	—
3	1.720	0.298–9.931	0.545
4	0.302	0.041–2.223	0.240
CEA	0.812	0.345–1.914	0.635
PD-L1	0.867	0.332–2.262	0.771

CEA: carcinoembryonic antigen.

expression is associated with worse disease-free and overall survival. In contrast to our study, there is no significant difference between the differentiations of tumors in both groups [16].

A study showed that in nonmetastatic patients with CRC, high-density immune cells expressing PD-L1 significantly correlate with favorable disease-free survival [17]. Moreover, the same study confirmed that subjects with high-density PD-L1 expression through multivariate analysis have considerably longer disease-free and overall survival [17].

An investigation illustrated that a high PD-L1 level expression was associated with more prolonged disease-free survival, and in analysis with multivariate, it was an independent prognostic element [18]. These data contradict the Sivastava et al.'s [19] study, where overall survival was significantly higher among CRC cases with negative PD-L1.

The AJCC-UICC TNM system is the gold standard for staging CRC and illustrates the disease's local and distant extent, which helps guide prognosis and therapy. Tumor staging and nodal involvement are strong predictors of outcome in CRC, with higher T stages and involved lymph nodes related to worse overall and disease-free survival.

One of the most valuable prognoses and outcome predictors at diagnosis is distant metastasis. Adjuvant chemotherapy is recommended in node-positive disease to reduce the risk of recurrence and death. The most prevalent classification used for guiding adjuvant treatment and prognostication is TNM [20].

In our study, we did not have access to the cases mean age. Additionally, our investigation was cross-sectional, and we observed differences in our results compared to other studies. Hence, more research with a further sample population is required to confirm our data.

5. Conclusion

Based on our study findings, it can be concluded that patients' PD-L1 positive or negative does not significantly affect patient death or survival. In addition, other factors such as positive CEA, age at diagnosis, gender, metastasis, and disease stage did not significantly affect survival. However, given the higher prevalence of metastasis among PD-L1-positive patients, the positive status of patients in terms of this factor may increase the likelihood of metastasis. Nevertheless, to confirm the findings of this study, further studies are necessary in the future.

Data Availability

The nature of the data in this cross-sectional study includes information gathered from participants at a specific time. Access to the data can be obtained by making a reasonable request to the corresponding author. There may be restrictions on data access due to privacy or confidentiality concerns.

Ethical Approval

This study was performed according to the principles outlined by the World Medical Association's Declaration of Helsinki on experimentation involving human subjects, as revised in 2000, and has been approved by the ethics committee of the Tabriz University of Medical Sciences.

Consent

Written informed consent was obtained from the patient to publish this report and clinical images. Consent has been signed and collected in accordance with the journal's patient consent policy.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Alireza Zarbakhsh was responsible for resources and software. Amirreza Khalaji wrote the original draft, was responsible for writing, review, and editing. Amir Vahedi performed investigation, provided resources, and wrote, reviewed, and edited the manuscript. Roya Dolatkhah

conceptualized data and supervised and validated the study. Nasrin Gholami performed data analysis and wrote, reviewed, and edited the manuscript.

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