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Correlations of *PD-1/PD-L1* Gene Polymorphisms with Susceptibility and Prognosis in Non-Hodgkin's Lymphoma among Iranian Populatio

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Abstract

Background: The activities of programmed cell death 1 (PD-1) and programmed death ligand-1 (PD-L1) have already been identified in various cancers. However, in non-Hodgkin's lymphoma (NHL), the prognostic value of *PD-1/PD-L1* gene polymorphisms and expression levels remains unclear.

Objectives: The present study aimed to investigate the relationship between the genetic polymorphisms of *PD-1/PD-L1* genes and NHL in the Iranian population.

Methods: Four single-nucleotide polymorphisms (SNPs) of *PD-1/PD-L1* genes were examined in 134 NHL patients and 134 healthy controls using polymerase chain reaction-restriction fragment length polymorphism. The expression levels of *PD-1/PD-L1* genes were analyzed using real-time polymerase chain reaction.

Results: The obtained results of the current study demonstrated that PD-L1 rs2890685 (A>C) SNP (P<0.0001) was significantly associated with the increased risk of NHL. The AA genotype of PD-L1 rs2890685 polymorphism was observed to be more prevalent in the NHL patients, compared to that reported for the healthy controls. There was no significant association between PD-L1 rs4143815, PD-1 rs11568821, and PD-1rs2227981 SNPs with the risk of NHL. Furthermore, the obtained findings showed that the messenger ribonucleic acid transcription levels of both *PD-1* and *PD-L1* were significantly higher in the NHL patients than those reported for the healthy controls (P<0.001).

Conclusion: According to the results of the current study, there was an association between functional PD-L1 rs2890685 polymorphism and risk of NHL, suggesting that the genetic variant of *PD-L1* might be a possible prognostic marker for the prediction of the risk and development of NHL.

Keywords: Non-Hodgkin's lymphoma (NHL), Programmed cell death 1 (PD-1), Programmed death ligand-1 (PD-L1), Single-nucleotide polymorphisms (SNPs)

1. Background

Cancer incidence and mortality rates are rapidly growing worldwide. Cancer is the leading cause of death in high-income countries and second leading cause of global mortalities (1). The term lymphoma refers to a diverse group of blood cancers arising in lymphatic tissues with a broad variety of clinical characteristics and genetic abnormalities. Lymphomas are generally classified as either Hodgkin's lymphoma or non-Hodgkin's lymphoma (NHL) and further categorized based on the type of cells from which cancer and other features are originated. Lymphomas are collectively the fourth most common cancer and sixth leading cause of cancer mortality in the USA. The NHL is considered the sixth most common type of cancer and ninth leading cause of cancer deaths among both males and females.

Previous experiments have shown that immune evasion plays an important role in the development of human cancers. Cancer cells can activate a variety of immune checkpoint pathways, such as programmed cell death protein 1 (PD-1) and cytotoxic T lymphocyte antigen-4, to induce immunosuppressive functions. The PD-1/programmed death ligand-1 (PD-L1) signaling pathway has a crucial role in immune tolerance and prevention of autoimmune disorders (2). The PD-1 is mainly expressed on the surface of activated immune cells, such as T cells, natural killer cells, B cells, macrophages, and dendritic cells (3). Human PD-1 belonging to the immunoglobulin CD28 family is encoded by the *PDCD1* gene and composed of 288 amino acid residues (4). The PD-1 has two known ligands, namely PD-L1 and PD-L2, which are expressed on the surface of both normal and immune cells.

The PD-L1 belongs to the B7 family, which is expressed much more frequently than PD-L2, and can be upregulated in some tissue and tumor cells in response to inflammatory factors (5). The PD-L1 is generally necessary for the maintenance of immune homeostasis under normal physiological conditions. This ligand bounds to PD-1 and down-regulates T cell activity to protect normal cells. In cancer, PD-L1 plays an important role in the immune escape of tumor cells. Tumor cells overexpress this ligand at a high level to induce apoptosis in tumor-specific T cells. In

the tumor microenvironment, the PD-1/PD-L1 pathway inhibits T cell proliferation, cytokine release, and T cell-dependent cytotoxicity, leading to the apoptosis of tumor-specific T cells and tumor escaping (6, 7).

Several single-nucleotide polymorphisms (SNPs) that may contribute to the susceptibility of the occurrence of cancer have recently been reported in both the *PD-1* and *PD-L1* genes. These polymorphisms can have an effect on both tumor growth and cancer treatment. However, a limited number of studies have investigated the relationship between the *PD-1* and *PD-L1* gene polymorphisms with the risk of lymphoma incidence. However, it is necessary to examine the effects of these polymorphisms on the transcription levels of the PD-1 or PD-L1 protein, occurrence, growth, prognosis, and treatment of lymphoma.

2. Objectives

The present study examined the association between four polymorphisms of the *PD-1* or *PD-L1* genes and risk of lymphoma cancer in the Iranian population of Zahedan province, Iran.

3. Methods

3.1. Study subjects

This study was carried out on 134 newly diagnosed patients with a clinical diagnosis of NHL and 134 healthy controls with no history of cancer or inflammatory diseases. The patients were histologically confirmed with NHL at Ali-ibn Abi Taleb Hospital of Zahedan University of Medical Sciences, Sistan and Baluchestan, Iran. A total of 284 subjects were randomly selected from the individuals participating in a routine cancer-screening program for the early detection of NHL cancer during the same period. All the control subjects were observed to lack NHL lesions by cytology test. Two controls were matched to each case by age at enrollment (with ±5 years).

3.2. Selection criteria

The current study evaluated studies performed on patients with NHL cancer and *PD1*/*PDL1* genetic polymorphisms as risk factors. The inclusion criteria applied to assess each study were 1) independent case-control studies evaluating the relationship between *PD1*/*PDL1* genetic polymorphisms and risk of NHL cancer, 2) all the patients diagnosed with NHL cancer confirmed by histopathological examinations demonstrating the occurrence of invasion, 3) studies with a number of evaluated cancer cases, 4) inclusion of at least 134 cases in the study, and 5) studies with genotype number and frequency information. The exclusion criteria were studies on familial and hereditary NHL cancer and studies on haplotypes alone. If there was a similar population in previous studies, only the most recent or largest sample size study was included in this study.

3.3. Ethical statements

Written informed consent was obtained from all the patients and controls before participation, and the protocol of this study was approved by the Institutional Ethics Committee of Ali-ibn Abi Taleb Hospital of Zahedan University of Medical Sciences.

3.4. Sample collection

In this study, 10 mL of venous blood were extracted from all the subjects after fasting for more than 12 h. The blood samples (4 mL) were anticoagulated with ethylenediaminetetraacetic acid and stored at -70°C. Then, the samples were incubated in an upright position for 1 h, followed by centrifuging at 3,000 rpm for 10 min at room temperature to isolate the peripheral blood mononuclear cells. Afterward, the genomic deoxyribonucleic acid (DNA) was isolated using a DNA extraction kit (Cat no.: DP318-03, Tiangen Biotech Beijing Co. Ltd., Beijing, China) according to the manufacturer's instructions. The remaining 6-mL blood samples were incubated in an upright position for 1 h and centrifuged at 3,000 rpm for 10 min at room temperature. Subsequently, the serum samples were extracted and stored at -70°C until use.

3.5. SNP detection

Four SNP sites, namely PD-L1 rs2890658 C>A, PD-1 rs2227981 C>T, PD-1 rs11568821 G>A, and PD-L1 rs4143815 G>C, were selected to conduct the current study. Table 1 tabulates the locations and base pair positions of SNPs in the *PD-1* and *PD-L1* genes. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to analyze the genotype and allele frequency in four sites between the case and control groups. The PCR primers were designed using Primer Premier software (version 5.0) and synthesized by Shanghai

Table 1. Locations and base pair positions of single-nucleotide polymorphisms in *PD-1*/*PD-L1* genes

Gene name	Single-nucleotide polymorphism Database reference # ID [a]	Chromosome position	Location	Base change	Amino acid change
PD-1	rs2227981	chr2:241851121	Upstream	C/T	-
	rs11568821	chr2:241851760	Intron	G/A	-
PD-L1	rs4143815	chr9:5468257	3′ UTR	G/C	-
	rs2890658	chr9:5465130	Intron	A/C	

PD-1: Programmed cell death 1; PD-L1: Programmed death ligand-1

Table 2. Polymerase chain reaction primer sequences for programmed cell death 1/programmed death ligand-1 single-nucleotide polymorphisms

Gene	Single-nucleotide polymorphism	Primer sequences	Annealing temperature (°C)	Circle
PD-1	rs2227981 C>T	F: TGAGCAGACGGAGTATGCC R: CTGAGGAAATGCGCTGACC	59	30
PD-1	rs11568821 G>A	F: CTCACATTCTATTATAGCCAGGACC R: TAAGATAAGAAATGACCAAGCCCAC	68	30
PD-L1	rs4143815 G>C	F: 5′-CCCCCATCATGTCTCCTCTCC-3′ R: 5′-CCAAGCAACTTGGTGTTTTGAGG-3′	67	30
PD-L1	rs2890658 C>A	F: GCAAGAGGAAGTGAAATAATCAAG R: GATACCTGTGTTAAAATGGGAACAG	61	30

PD-1: Programmed cell death 1; PD-L1: Programmed death ligand-1

Chemical Company (China).

Table 2 shows the amplification sites, primer sequences, fragment length, annealing temperature, and cycle number for PCR-RFLP. The total volume of PCR reaction included 2 µl of 10 × PCR reaction buffer (20 µl), 2 µl of deoxyribonucleoside triphosphate (2.5 mmol/L for each), 0.5 µl of each forward and reverse primer (10 pmol/µl), 2.5 U of Platinum Taq DNA polymerase (Invitrogen Trading Shanghai Co., Ltd., Shanghai, China), and 50 ng of genomic DNA. The DNA was amplified during 30 cycles with 5 min of predegeneration at 95°C, 30 sec of denaturation at 95°C, 30 sec of annealing at 58°C, and 40 sec of extension at 72°C. Then, the DNA was further extended for 5 min at 72°C and stored at 4°C. A total of 20 µl PCR production was extracted to construct the enzyme reaction system. Subsequently, the PCR products were digested with restricted enzyme RsaI and XmnI (New England Biolabs LTD., Beijing, China).

After incubation at 21°C overnight, the PCR products were placed in a water bath at 4°C for 15 min to terminate the reaction. Afterward, the enzyme-digested products were electrophoresis-separated by 2% agarose gel (containing ethidium bromide). The Gel Imaging System (Bio-Rad, USA) was used for genotyping interpretation. The primers used for the detection of *PD-1* and *PD-L1* polymorphisms using PCR-RFLP are presented in Table 3.

3.6. RNA extraction and molecular detection

Ribonucleic acid (RNA) was extracted from 200 µl fluid samples by the High Pure Viral Nucleic Acid kit (Roche, Germany) according to the manufacturer's manual. Then, real-time polymerase chain reaction (RT-PCR) was performed by the RT-PCR Kit (QIAGEN, Germany) using specific primers and probes in Stepone pulse instruments (Applied Biosystems Inc., Foster City, CA, USA). Table 4 tabulates the primer sequences used for the identification.

3.7. Statistical analysis

Statistical analysis was conducted using SPSS software (version 18.0; IBM Corporation, Somers, NY, USA). The continuous data were expressed as $\bar{\chi} \pm$ standard deviation, and the independent t-test or analysis of variance was used for comparisons. The categorical data were presented by percentages, and the Chi-square test was applied for comparisons between the groups. The Chi-square test was also utilized to verify whether the genotype distribution of the four SNPs met the Hardy-Weinberg equilibrium. The genotype and allele frequency between the case and control were calculated by odds ratio (OR) with a 95% confidence interval (CI). All the tests were two-sided, and p-values of less than 0.05 were considered statistically significant. The association between PD-L1 rs2890658 C>A, PD-1 rs2227981 C>T, PD-1 rs11568821 G>A, and PD-L1 rs4143815 G>C SNPs with NHL was assessed using OR with a 95% CI.

Table 3. Primers used for detection of programmed cell death 1 and programmed death ligand-1 polymorphisms using polymerase chain reaction-restriction fragment length polymorphism

Polymorphism	Restriction enzyme	Fragment (bp)
PD-L1 rs2890658 C>A	HaeIII	CC: 226+25 CA: 251+226+25 AA: 251
PD-1 rs2227981 C>T	PvuII	CC: 207 TC: 207+133+74 TT: 133+74
PD-1 rs11568821 G>A	PstI	GG: 290 AG: 290+197+93 AA: 197+93
PD-L1 rs4143815 G>C	Hpy8I	CC: 227 CG: 227+199+28 GG: 199+28

PD-1: Programmed cell death 1; PD-L1: Programmed death ligand-1

Table 4. Specifications of real-time polymerase chain reaction primers and probes

	Forward primer			Reverse primer			Product length
	Sequence (5'->3')	GC%	Length	Sequence (5'->3')	GC%	Length	
PD-1	CCGCACGAGGGACAATAG	61.1	18	GGTGGCATACTCCGTCTG	61.1	18	167
PD-L1	AGGGCATTCCAGAAAGATGAGG	50	22	GGGAACCGTGACAGTAAATGCG	54.55	22	88

PD-1: Programmed cell death 1; PD-L1: Programmed death ligand-1

4. Results

4.1. General characteristics of study subjects

This case-control study was carried out on a total of 268 subjects, including 134 NHL cases and 125 healthy controls. The results of the Chi-square test demonstrated that there were no significant differences between the NHL cases and healthy controls in terms of age distribution. The mean age of the NHL patients and healthy controls were 46.16±17.1 and 44.9±12 years, respectively (P=0.2).

4.2. Association between PD-1 polymorphisms and NHL

Table 5 tabulates the frequencies of the genotypes and alleles of all SNPs among both NHL patients and healthy controls. The genotype distribution rates of PD-1 rs11568821 G/A (i.e., GG, GA, and AA) were 93%, 7%, and 0% among the NHL patients and 94%, 6%, and 0% in the healthy controls, respectively (Table 5). No significant difference was observed between the NHL and control groups in this regard. In PD-1 rs2227981 polymorphism, it was observed that the TT genotype showed an elevated level in comparison to that of the control group (TT: OR=2.07; 95% CI: 0.69-6.19; P=0.2). The genotype distribution rates of PD-1 rs2227981 C/T (i.e., CC, CT, and TT) were 48%, 42%, and 10% in the NHL patients and 38%, 58%, and 4% among the healthy controls, respectively. The present analysis also indicated that this difference was not significant. As a net result, the analysis of PD-1 rs11568821 and PD-1 rs2227981 demonstrated no significant correlation between the two selected PD-1 SNPs and total incidence of NHL.

4.3. Association between PD-L1 polymorphisms and NHL

Table 6 shows the genotype distribution of both PD-L1 rs4143815 and PD-L1 rs2890658 polymorphisms. The obtained results of the present study showed that the level of PD-L1 rs2890658 CA genotype was significantly higher in the NHL patients, compared to that reported for the healthy controls. The genotype distribution rates of PD-1 rs2890658 C/A (i.e., CC, CA, and AA) were 36%, 60%, and 4% in the NHL patients and 64%, 34%, and 2% among the healthy controls, respectively.

The allele-specific analysis revealed that the A allele of PD-L1 rs2890658 was more prevalent among the NHL patients in comparison to the C allele (OR=2.17; 95% CI: 1.46-3.21; P<0.0001). The data analysis of PD-L1 rs4143815 showed no significant difference between CC or GG genotypes in the NHL patients or control group (P=0.794). The genotype distribution rates of PD-L1 rs4143815 C/G (i.e., CC, CG, and GG) were 36%, 54%, and 10% in the NHL patients and 38%, 53%, and 9% in the healthy controls, respectively. The analysis of the aforementioned data demonstrated that the C<A genotype of PD-L1 rs2890658 was associated with a higher prevalence of NHL (P<0.0001).

4.4. PD-1 and PD-L1 mRNA expression analysis

In order to establish the relationship between NHL prevalence and PD-1/PD-L1 pathway, the PD-1 and PD-L1 messenger ribonucleic acid (mRNA) expression levels were determined by RT-PCR. Figure 1 illustrates the difference between various mRNA fold changes respectively. The mRNA level analysis of both PD-1 and PD-L1 showed higher fold changes in the NHL patients in comparison to those reported for the healthy controls. The results of the independent t-test showed that the increased levels of both PD-1 and PD-L1 mRNA were significant (P<0.0001).

Table 5. Frequency of genotypes and alleles distribution of Programmed cell death 1 in non-Hodgkin's lymphoma patients and healthy controls

Polymorphism	Case n (%)	Control n (%)	Odds ratio (95% CI)	P-value
PD-1 rs11568821				
Codominant				
GG	124 (93)	126 (94)	1.00	-
GA	10 (7)	8 (6)	1.27 (0.48-3.32)	0.807
AA	0	0		
Allele				
G	258 (96)	260 (97)	1.00	-
A	10 (4)	8 (3)	1.25 (0.48-3.24)	0.811
PD-1 rs2227981				
Codominant				
CC	64 (48)	51 (38)	1.00	-
CT	57 (42)	78 (58)	0.58 (0.35-0.96)	0.042
TT	13 (10)	5 (4)	2.07 (0.69-6.19)	0.210
Dominant				
CC	64 (48)	51 (38)	1.00	-
CT+TT	70 (52)	83 (62)	0.67 (0.41-1.09)	0.138
Recessive				
CC+CT	121 (90)	129 (96)	1.00	-
TT	13 (10)	5 (4)	2.77 (0.95-8.00)	0.085
Allele				
C	185 (69)	180 (67)	1.00	-
T	83 (31)	88 (33)	0.91 (0.63-1.31)	0.710

PD-1: Programmed cell death 1; PD-L1: Programmed death ligand-1

Table 6. Frequency of genotypes and alleles distribution of Programmed death ligand-1 in non-Hodgkin's lymphoma patients and healthy controls

Polymorphism	Case n (%)	Control n (%)	Odds ratio (95% CI)	P-value
PD-L1 rs4143815				
Codominant				
CC	48 (36)	51 (38)	1.00	-
CG	72 (54)	71 (53)	1.07 (0.64-1.79)	0.794
GG	14 (10)	12 (9)	1.23 (0.52-2.94)	0.664
Dominant				
CC	48 (36)	51 (38)	1.00	
CG+GG	86 (64)	83 (62)	1.10 (0.67-1.80)	0.800
Recessive				
CG+CC	120 (90)	122 (91)	1.00	-
GG	14 (10)	12 (9)	1.18 (0.52-2.66)	0.836
Allele				
C	168 (63)	173 (65)	1.00	-
G	100 (37)	95 (35)	1.08 (0.76-1.54)	0.719
PD-L1 rs2890658				
Codominant				
CC	48 (36)	85 (64)	1.00	-
CA	80 (60)	46 (34)	3.07 (1.85-5.11)	<0.0001
AA	6 (4)	3 (2)	3.54 (0.84 -14.80)	0.083
Dominant				
CC	48 (36)	85 (64)	1.00	-
CA+AA	86 (64)	49 (36)	3.10 (1.88-5.11)	<0.0001
Recessive				
CC+CA	128 (96)	131 (98)	1.00	-
AA	6 (4)	3 (2)	2.04 (0.50-8.36)	0.500
Allele				
C	176 (66)	216 (80)	1.00	-
A	92 (34)	52 (20)	2.17 (1.46-3.21)	<0.0001

PD-1: Programmed cell death 1; PD-L1: Programmed death ligand-1

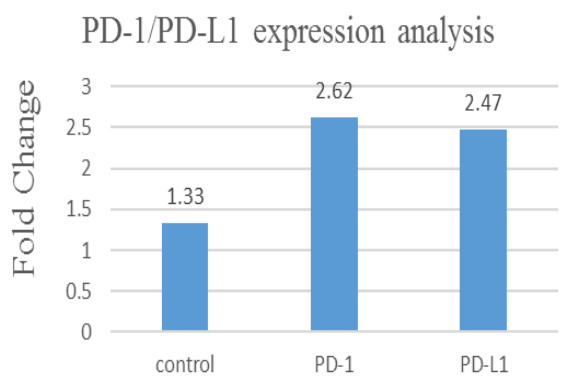

Figure 1. Messenger ribonucleic acid (mRNA) expression analysis of programmed death-ligand 1 (PD-L1) and programmed death-1 (PD-1) demonstrating significantly higher mRNA levels of PD-L1 and PD-1 in non-Hodgkin's lymphoma patients in comparison to healthy controls . patients in comparison to those reported for the healthy controls

5. Discussion

Previous studies have shown that PD-1 and PD-L1 polymorphisms are associated with different autoimmune diseases, such as rheumatoid arthritis (9), ankylosing spondylitis (10), and systemic lupus erythematosus (11). Based on their antitumor immune response suppression feature, PD-1 and PD-L1 may be regarded as effective biomarkers for new tumor development or cancer progression (12). In the present study, two potentially functional polymorphisms of *PD-1* (i.e., PD-1 rs2227981 and PD-1 rs11568821) and *PD-L1* (i.e., PD-L1 rs2890658 and PD-L1 rs4143815) genes were selected, and the association between the selected polymorphisms and risk of lymphoma cancer were identified in the population of Zahedan. To the best of our knowledge, this has been the first study carried out on the assessment of the relationship between four selected SNPs and risk of NHL occurrence.

The obtained results of the present study demonstrated that among the four selected SNPs, PD-L1 rs2890658 polymorphism was significantly related to lymphoma cancer. It was observed that the A allele frequency of this SNP was higher in the NHL patients than the C allele, compared to that reported for the control group. This finding is the line with the results of a study conducted by Zhou et al. indicating that PD-L1 rs2890658 SNP is associated with the increased risk of esophageal squamous cell carcinoma in smokers (13). On the other hand, Hashemi et al. reported that there is no significant association between this polymorphism and overall cancer risk (14).

The results of previous studies demonstrated that PD-1 rs2227981 polymorphism is associated with increased cancer risk in non-small cell lung, colon, and ovarian cancers (15, 16). Moreover, some recent studies have shown that PD-1 rs2227981 polymorphism is related to the increased risk of cervical cancer in Chinese and Swedish populations (16), breast cancer in the Chinese population, and gastric and digestive system cancer in Iranian and Chinese populations (17). These results are

inconsistent with the findings of the current study demonstrating that there was no significant relationship between PD-1 rs2227981 polymorphism and overall risk of lymphoma cancer in NHL patients. In the present study, there was also a similar pattern for PD-1 rs11568821 SNP.

Previously, Dong et al. performed a meta-analysis on the association between this SNP and overall cancer risk (17). The results of the aforementioned study showed that the A allele of this SNP is associated with a decreased risk of cancer susceptibility (17). On the other hand, the current analysis demonstrated that there was no significant difference between the frequency of A or G allele in the NHL patients, compared to that reported for the control group. Wang et al. reported that the C/C genotype of PD-L1 re4143815 correlated with an increased risk of gastric cancer as it interferes with miR-570 activity and possible suppression of the immunological tumor restriction by increasing PD-L1 expression (18).

Recently, Shi et al. have shown that patients receiving a liver transplant from a C/C PD-L1 rs4143815 allele donor have a reduced risk of developing a late acute immune response and organ rejection. In contrast, the patients who were transplanted with a liver graft of GG genotype donor showed a higher risk of late acute rejection (19). On the other hand, Pizarro et al. indicated that the G/G genotype of PD-L1 re4143815 polymorphism correlated with type I diabetic patients and lower serum PD-L1 level (20). In the present study, it was observed that there was no significant relationship between PD-L1 rs4143815 polymorphism and NHL malignancy. The results of the present study revealed a similar frequency of CC, GC, or GG genotypes between the NHL and control groups.

Currently, the United States Food and Drug Administration has licensed a range of checkpoint inhibitors for cancer immunotherapy (21). Recently, Nomizo et al. reported that PD-L1 polymorphisms could alter the immune checkpoint function and subsequently changed the clinical outcomes of response to immune checkpoint inhibitors in patients with lung cancer. They reported that in the advanced stage nonsmall-cell lung carcinoma patients receiving nivolumab, the C allele of PD-L1 rs4143815 was significantly associated with a better response rate (22). The collected data of the current study indicated that the PD-1 rs2890658 SNP had a significant impact on the occurrence of NHL. Moreover, the mRNA expression levels of both PD-1/PD-L1 were notably higher than those reported for the control group.

Overall, the findings of the present study suggested that this SNP may also have a positive impact on the clinical outcome of NHL cancer treatment with checkpoint inhibitors. Finally, the expression levels of both PD-1 and PD-L1 were analyzed in the present study. Previous studies indicated that a large number of tumors exhibit higher expression of PD-1/PD-L1 in comparison to healthy populations (23-26). Andorsky et al. reported that PD-L1 expression was elevated in NHL patients, and PD-L1 plays a pivotal role in the tumor microenvironment and results in an aggressive clinical phenotype and a worse outcome (27). In line with the results of previous studies, the elevated PD-1/PD-L1 expression was also observed in the current study. The obtained findings of the present study revealed that both PD-1/PD-L1 mRNA levels significantly increased, compared to those reported for the healthy controls.

6. Conclusion

In the present study, it was shown that PDL-1 rs2890658 SNP was significantly associated with NHL incidence and susceptibility. The results of the present study suggested that this SNP could be used as a risk factor for the prognosis and progression of NHL cancer. In addition, it was demonstrated that the expression levels of both PD-1 and PD-L1 mRNA significantly increased in the NHL patients in comparison to those of the healthy controls. Overall, the obtained findings indicated that PD-1 rs2890658 SNP could be utilized as a new biomarker for the prognosis and detection of NHL cancer. Moreover, the higher expression levels of PD-1 and PD-L1 make them suitable targets for checkpoint inhibitors.

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Footnotes

Authors' Contribution: All the authors contributed to data collection and wrote the manuscript. HH and PY conceived the structure of the manuscript and revised the manuscript. HH and GB drew the figures and tables. HH, PY, SA, and GB drafted the initial manuscript. All the authors read the manuscript. All the authors approved the current manuscript to be published, declared that they substantially contributed to the current study, and disclosed that there was no writing assistance. All the authors read and approved the final manuscript.

Conflict of Interests: The authors declare that there is no conflict of interest.

Ethical Approval: The current study was approved by the Ethics Committee of Ali-ibn Abi Talib Hospital in Zahedan University of Medical Sciences. Before the operation, informed consent was signed by all the patients after a detailed explanation of the therapeutic procedure to them. The current study was conducted according to the guidelines for case

series.

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