Correlations of PD-1/PD-L1 Gene Polymorphisms With Susceptibility and Prognosis in Non-Hodgkin Lymphoma in Iranian Population

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Abstract

Background, Programed cell death 1 (PD-1) and its ligand (PD-L1) activity have already detected in various cancers. In non-Hodgkin lymphoma (NHL), however, the prognostic value of PD-1/PD-L1 genes polymorphisms and expression levels remains unclear. In the present study we aimed to investigate the relationship between genetic polymorphisms of PD-1/PD-L1 genes and non-Hodgkin lymphoma in Iranian papulation.

Methods, four single nucleotide polymorphisms of the PD-1/PD-L1 genes, including 134 NHL patients and 125 healthy controls were examined using PCR-RFLP method. The expression level of PD-1/PD-L1 genes were analyzed using real time PCR method.

Results, our data demonstrated that the PD-L1 rs2890685 (A>C) SNP ($p<0.0001$) significantly was associated with increased risk of NHL. The AA genotype of PD-L1 rs2890685 polymorphism was found to be more prevalent in NHL patents compared to healthy controls. No significant association were found between PD-L1 rs4143815, PD-1 rs11568821, PD-1rs2227981 SNPs and the risk of NHL incidence. Furthermore, our data showed that the mRNA transcription levels of both PD-1 and PD-L1 were significantly higher than normal healthy controls ($p<0.001$).

Conclusion, Collectively, our finding demonstrated that the functional PD-L1 rs2890685 polymorphism was associated with NHL risk, suggesting that genetic variant of PD-L1 might be a possible prognosis marker for prediction of NHL risk and its development.

Background

Cancer incidence and mortality are rapidly growing worldwide. Cancer is the leading cause of death in high income country and is the second leading cause of death globally(1). The term of lymphoma refers to a diverse group of blood cancers that arise in lymphatic tissues with a broad variety of clinical characteristics and genetic abnormalities. Lymphomas generally are classified as either Hodgkin lymphoma or non-Hodgkin lymphoma, and are further categorized based on the type of cell which cancer originated and other features. Lymphomas collectively are fourth most common cancer and the sixth leading cause of cancer death in USA. NHL is considered the sixth most common type of cancer and the 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 cancer. 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 (CTLA4) to induce immunosuppressive functions. The PD-1/PD-L1 signaling pathway plays an important role in immune tolerance and prevention of autoimmune disorders(2).

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, encoded by the PDCD1 gene and composed of 288 amino acid residue(4). PD-1 has two known ligands, PD-L1 and PD-L2, which expressed on the surface of both normal and immune cells.

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 answer to inflammatory factors(5). PD-L1 is generally necessary for the maintenance of immune homeostasis under normal physiological conditions. This ligand bound to PD-1and downregulate T cell activity to protects normal cells. In cancer, PD-L1 plays an important role in the immune escape of the 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 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 PD-1 and PD-L1 genes. These polymorphisms can have an effect on both tumor growth and cancer treatments. However, little study had investigated the relationship between of the PD-1 and PD-L1 gene polymorphisms and the risk of lymphoma incidence. In the meantime, whether these polymorphisms have an effect on the transcription levels of the PD-1 or PD-L1 protein and its impact on the occurrence, growth, prognosis and treatment of lymphoma should be investigated. In the present study, we investigated the association between four polymorphism of the PD-1 or PD-L1 genes and the risk of lymphoma cancer in Iranian papulation of Zahedan province.

Material

Subjects

This study included 134 newly diagnosed patients with clinically diagnosed non-Hodgkin lymphoma and 125 healthy controls with no history of cancer or inflammatory diseases were recruited for the study. patients were histologically confirmed as having non-Hodgkin lymphoma at the Ali Ebn Abitaleb Hospital of Zahedan University(Sistan and Baluchestan Province.Iran). 284 subjects were randomly selected from individuals who were participating in a routine cancer-screening program for the early detection of non-Hodgkin lymphoma cancer during the same period. All the control subjects were found to lack non-Hodgkin lymphoma lesions by cytology test. Two controls were matched to each case by age at enrollment (within ±5 years).

Selection criteria

We evaluated studies on patients with non-Hodgkin lymphoma cancer and *PD1/PDL1* genetic polymorphisms as risk factors. The following inclusion criteria were applied to assess each publication for inclusion: (1) independent case-control study that evaluated the relationship between *PD1/PDL1* genetic polymorphisms and the risk of non-Hodgkin lymphoma cancer; (2) all patients diagnosed with non-Hodgkin lymphoma cancer were confirmed by histopathological examinations demonstrating the occurrence of invasion; (3) the number of evaluated cancer cases was provided; (4) at least 134 cases were included in the study; (5) the genotype number and frequency information were supplied. The exclusion criteria were the following: (1) studies on familial and hereditary non-Hodgkin lymphoma cancer; and (2) studies on haplotypes alone. If the same population was included in previous studies, only the most recent or largest sample size study was included.

Ethical statements

All patients and controls signed written informed consent before participating, and the protocol of this study was approved by the institutional Ethnics Committee of Ali Ebn Abitaleb Hospital of Zahedan University(Sistan and Baluchestan Province.Iran).

Sample collection

Ten mL of venous blood were extracted from all subjects after fasting for more than 12 h. The blood samples (4 mL) were anticoagulated with ethylenediaminetetraacetic acid (EDTA) and stored at −70°C. Then the samples were incubated in an upright position for 1 h, followed by centrifuging at 3000 rpm for 10 min at room temperature to isolate the peripheral blood mononuclear cells. Afterwards, the genomic 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 were centrifuged at 3000 rpm for 10 min at room temperature. Subsequently, serum were extracted and stored at −70°C until used.

SNP detection

Four SNP sites, 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 research. Locations and base pair positions of single nucleotide polymorphisms (SNPs) in PD-1 and PD-L1 genes are present in **Table1**. Restriction fragment length polymorphism polymerase chain reaction (PCR-RFLP) was used to analyze the genotype and allele frequency in 4 sites between the case and control groups. The PCR primers were designed using Primer Premier 5.0 software and synthesized by Shanghai Chemical Company (China). The amplification sites, primer sequences, fragment length, annealing temperature, and cycle number for the PCR-RFLP are presented in **Table 2**. The total volume of PCR reaction included 20 μl: 2 μl of 10×PCR reaction buffer, 2 μl of deoxy-ribonucleoside triphosphate (dNTP) (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, Shanghai, China), and 50 ng of genomic DNA. DNA was amplified during 30 cycles with 5 min predegeneration at 95°C, 30 s denaturation at 95°C, 30 s annealing at 58°C, and 40 s 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. Then the PCR products were digested with restricted enzyme RsaI and XmnI (New England Biolabs LTD., Beijing). 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. Then 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 detection of *PD-1 and PD-L1* polymorphisms using PCR-RFLP are presented in **Table3.**

Table 1. Locations and base pair positions of single nucleotide polymorphisms (SNPs) in PD-1 and PD-L1 genes.

Gene Name	db SNP rs # 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	

Table 2 The PCR primer sequences for *PD1/PDL1* single nucleotide polymorphisms.

Gene	SNP	Primer sequences	Annealing temperature (°C)	Circles
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

Table 3. The primers used for detection of *PD-1 and PD-L1* polymorphisms using PCR-RFLP

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

Viral RNA extraction and molecular detection

Viral RNA was extracted from 200 µl fluid samples by High Pure Viral Nucleic Acid kit (Roche, Germany) according to the manufacturer's manual. Then, Real-time RT-PCR was performed by RT-PCR kit (QIAGEN, Germany) using specific primers and probes in Stepone pulse instruments (Applied Biosystems, Foster City, CA) according to the protocol. The primer sequences used to identify are listed in **Table 4**.

Table 4 Real-time PCR primers and probes specifications

	Forward primer			Revers 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

Statistical analysis

Statistical analysis was conducted using SPSS 18.0 software (IBM Corporation, Somers, NY, USA). Continuous data are expressed as $\bar{\chi}$ ± standard deviation (SD), using *t* test or variance analysis for comparisons. Categorical data was presented with percentages and chi-square test was applied for comparisons between groups. Chi-square test was also used to verify whether the genotype distribution of the 4 SNPs met Hardy-Weinberg (HW) equilibrium. The genotype frequency and allele frequency between the case and control were calculated by OR (odds ratio) with 95%CI (confidence interval). All tests were 2-sided and differences were considered statistically significant at $P<0.05$.The association between PD-L1 rs2890658 C>A, PD-1 rs2227981 C>T , PD-1 rs11568821 G>A and PD-L1 rs4143815 G>C *PD1/PDL1* SNPs and non-Hodgkin lymphoma was assessed using OR with 95%CI.

Results

General Characteristics of the Subjects

In this case-control study, a total of 284 subjects were enrolled consisted of 134 NHL cases and125 healthy controls. The results from χ^2 test suggested that there are no significant differences between NHL cases and healthy controls with terms of age and gender distribution(p=0.2). The mean age of NHL patients and the healthy controls were 46.16 ± 17.1 and 44.9 ± 12 respectively. (p=0.2).

Association Between PD-1 polymorphisms and NHL

The frequencies of genotypes and alleles of all SNPs in both NHL patients and healthy controls are shown in **Table5**. The genotype distribution of PD-1 rs11568821 G/A (GG, GA, AA) in NHL and healthy controls were 93%, 7%, 0% and 94%, 6% ,0% respectively, as shown in table 3. No significant difference was observed between NHL and control group. In The PD-1 rs2227981 polymorphism, we found that the TT genotype showed an elevated level compared to the control group (TT: OR=2.07, 95% CI= (0.69-6.19), P=0.2). The genotype distribution of PD-1 rs2227981 C/T (CC, CT and TT) in NHL patients and healthy controls were 48%, 42%, 10% and 38% 58%, 4% respectively. Our analysis has also shown that this difference was not significant. As a net result, analysis of the PD-1 rs11568821 and PD-1 rs2227981 demonstrated no significant correlation between two selected PD-1 SNPs and NHL incidence overall.

Association Between PD-L1 polymorphisms and NHL

The genotype distribution of both PD-L1 rs4143815 and PD-L1 rs2890658 polymorphisms was shown in **Table 6**. Our results shows that the level of PD-L1 rs2890658 CA genotype was significantly higher in NHL patients compared to healthy controls (CA: OR=3.07; P<0.000). The genotype distribution of PD-1 rs2890658 C/A (CC, CA, AA) in NHL patients and controls were 36%, 60%, 4% and 64%, 34%, 2% respectively. Allele specific analysis revealed that the A allele of PD-L1 rs2890658 is more prevalent in NHL patients Compare to C allele (OR=2.17, 95% CI=(1.46-3.21), P<0.0001). The PD-L1 rs4143815 data analysis showed no significant difference between CC or GG genotype in NHL patients or control group (P=0.794). the genotype distribution of PD-L1 rs4143815 C/G (CC, CG, GG) were 36%, 54%, 10% in NHL patients and 38%, 53%, 9% in healthy controls respectively. This data suggested that the C<A genotype of PD-L1 rs2890658 is associated with higher prevalence of non-Hodgkin lymphoma.

Table 5
The frequencies of genotypes and alleles distribution of PD-1 in NHL patients and healthy controls.

Polymorphisms	Case n (%)	Control n (%)	OR (95%CI)	P
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

Table 6
The frequencies of genotypes and alleles distribution of PD-L1 in NHL patients and healthy controls.

Polymorphisms	Case n (%)	Control n (%)	OR (95%CI)	P
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

Table 7. Association of PD-1, PD-L1 polymorphisms with clinicopathological characteristics of lymphoma cancer patients.

Characteristic of patients	PD-L1 rs4143815			P value	PD-L1 rs2890658			P value	PD-1 rs11568821			P value	PD-1 rs2227981			P value
	CC	CG	GG		CC	CA	AA		GG	GA	AA		CC	CT	TT	
Age, years				0.004				0.035				0.200				0.845
≤50	44	71	04		47	83	0		023	07	0		56	70	4	
>50	13	66	3		31	48	3		86	7	0		34	46	2	
Tumor size, cm				0.060				0.241				1.000				0.447
≤2	01	27	0		07	22	0		36	3	0		15	12	1	
>2	43	104	07		63	87	3		150	06	0		65	84	5	
Histology				0.045				0.655				0.21				0.177
Lymph node metastasis				0.242				0.160				1.000				0.524
No	06	26	6		12	27	0		44	5	0		12	25	1	
Yes	31	80	7		53	63	2		016	04	0		52	62	4	
Grade				0.808				0.747				0.840				0.038
I	10	20	2		03	06	1		18	2	0		17	12	1	
II	22	56	6		30	54	1		85	00	0		35	43	6	
III+IV	23	58	8		38	50	1		79	10	0		35	54	0	
Stage				0.548				0.302				0.005				0.610
I	4	6	1		3	10	0		02	1	0		5	7	0	
II	17	63	7		34	52	1		005	3	0		32	51	4	
III	13	27	3		18	24	1		42	02	0		21	21	1	
IV	00	08	1		7	13	0		17	3	0		02	08	0	

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 mRNA expression levels were determined by real time PCR. Figure 1 represent the difference between various mRNA fold change respectively. The mRNA level analysis of both PD-1 and PD-L1 showed higher fold change in NHL patients compared to healthy controls. the independent t-test showed that the increased level of both PD-1 and PD-L1 mRNA were significant ($P<0.0001$).

Discussion

Previous studies have shown that PD-1 as well as PD-L1 polymorphisms are associated with different autoimmune diseases, such as rheumatoid arthritis (RA)(9), ankylosing spondylitis (AS)(10), and systemic lupus erythematosus (SLE)(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, we selected two potentially functional polymorphisms of each PD-1 (PD-1 rs2227981, PD-1 rs11568821) and PD-L1 (PD-L1 rs2890658, PD-L1 rs4143815) genes, and identified the association

between selected polymorphisms and the risk of lymphoma cancer in papulation of Zahedan Province, Iran. To the best of our knowledge, this is the first study to assess the relationship between four selected SNPs and risk of NHL occurrence. Our results demonstrated that among the four selected SNPs, the PD-L1 rs2890658 polymorphism significantly related to lymphoma cancer. We found that the A allele frequency of this SNP was more prevalent in NHL patients than C allele compared to the control group. Our result is the line with the Zhou study which reported that PD-L1 rs2890658 SNP is related with increased risk of esophageal squamous cell carcinoma in smokers(13). In the other side, Hashemi et al. reported that there is no significant association between this polymorphism and overall cancer risk(14).previous studies demonstrated that PD-1 rs2227981 polymorphism is associated with increased cancer risk in non-small cell lung, colon and ovarian cancer(15, 16). Moreover, some recent studies demonstrated that PD-1 rs2227981 polymorphism is related with increased risk of cervical cancer in Chinese and Swedish population(16), breast cancer in Chinese, gastric and digestive system cancer in Iranian and Chinese(17). These results conflict with our observation that there was no significant relationship between PD-1 rs2227981 polymorphism and the overall risk of lymphoma cancer in NHL patients. We have also observed the same pattern for PD-1 rs11568821 SNP. Previously, Dong et al. performed a meta-analysis discussed the association between this SNP and overall cancer risk(17). They result showed that Showed that the A allele of this SNP is associated with the decreased risk of cancer susceptibility(17). Our analysis, on the other hand, showed no significant difference between the frequency of A or G allele in NHL patients compared to the control group.Several studies suggested that PD-L1 re4143815 polymorphism, which is located in 3’ UTR, has medical significance. Wang and colleagues reported that the C/C genotype of PD-L1 re4143815 is 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. has shown that patients who received 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 liver graft of GG genotype donor showed the higher risk for late acute rejection (19). On the other side, Pizarro and his collaborators reported that the G/G genotype of PD-L1 re4143815 polymorphism was correlated with type I diabetic patients and lower serum PD-L1 level (20). In the present study, we found that there is no significant relationship between PD-L1 rs4143815 polymorphism and NHL malignancy. Our result indicated the similar frequency of CC, GC or GG genotypes between the NHL and control group. Currently, FDA has licensed a range of checkpoint inhibitors for cancer immunotherapy(21). Recently, Nomizo and colleagues 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 the advanced stage NSCLC patients who received nivolumab, the C allele of PD-L1 rs4143815 were significantly associated with better response rate(22). Our data indicated that the PD-1 rs2890658 SNP had a significant impact the occurrence of NHL. Moreover, the mRNA expression level of both PD-1/PD-L1 was notably higher than control group. Collectively, our finding suggested that this SNP may also have a positive impact on clinical outcome of NHL cancer treatment with checkpoint inhibitors, too.Finally, we analyzed the expression level of both PD-1 and PD-L1 in the present study. Previous study indicated that a large number of tumors exhibit higher expression of PD-1/PD-L1compare to healthy papulations(23-26). Andorsky et al. reported that PD-L1 expression was elevated in NHL patients and PD-L1 plays a pivotal role in DCBCL tumor microenvironment and results in an aggressive clinical phenotype and a worse outcome(27). In line with previous studies, the elevated PD-1 / PD-L1 expression was also observed in our study too. Our findings revealed that both PD-1 / PD-L1 mRNA levels increased significantly compare to healthy control.

Conclusions

In the present study, we have shown that PD-1 rs2890658 SNP is significantly associated with NHL incidence and susceptibility. Our result suggested that this SNP could be used as a risk factor for the prognosis and progression of NHL cancer. In addition, we have shown that the expression of both PD-1 and PD-L1 mRNA has increased significantly in NHL patients compared to healthy controls. Taken together, our finding represents that PD-1 rs2890658 SNP could be used as a new biomarker for prognosis and detection of NHL cancer. Moreover, the higher expression levels of PD-1 and PD-L1 make them a suitable target for checkpoint inhibitors.

Abbreviations

PD-1*:* programmed death-1

PD-L1*:* programmed death-1 ligand-1

SNPs*:* single nucleotide polymorphisms

NHL*:* Non-Hodgkin lymphoma

cDNA: Complementary DNA

Declarations

Ethics approval and consent to participate

The current study was approved by the Ethics Committee of Ali ibn Abi Talib Hospital in the Affiliated Hospital of Zahedan Medical University. Before operation, informed consents were signed by all the patients after detailed explanation of the therapeutic procedure to the patients. The study is conducted according to the guideline for case series.

Consent for publication

Written informed consent for publication was obtained from all participants.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests

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Author Contributions

All authors contributed to data collection and wrote the manuscript.HH and PY conceived the structure of manuscript and revised the manuscript. HH and GB made the figures and tables. HH, PY, SA and GB drafted initial manuscript. All authors read manuscript. All authors approved the current manuscript to be published, attested that they contributed substantially to the current work, and disclosed that there was no writing assistance. All authors read and approved the final manuscript.

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Figures

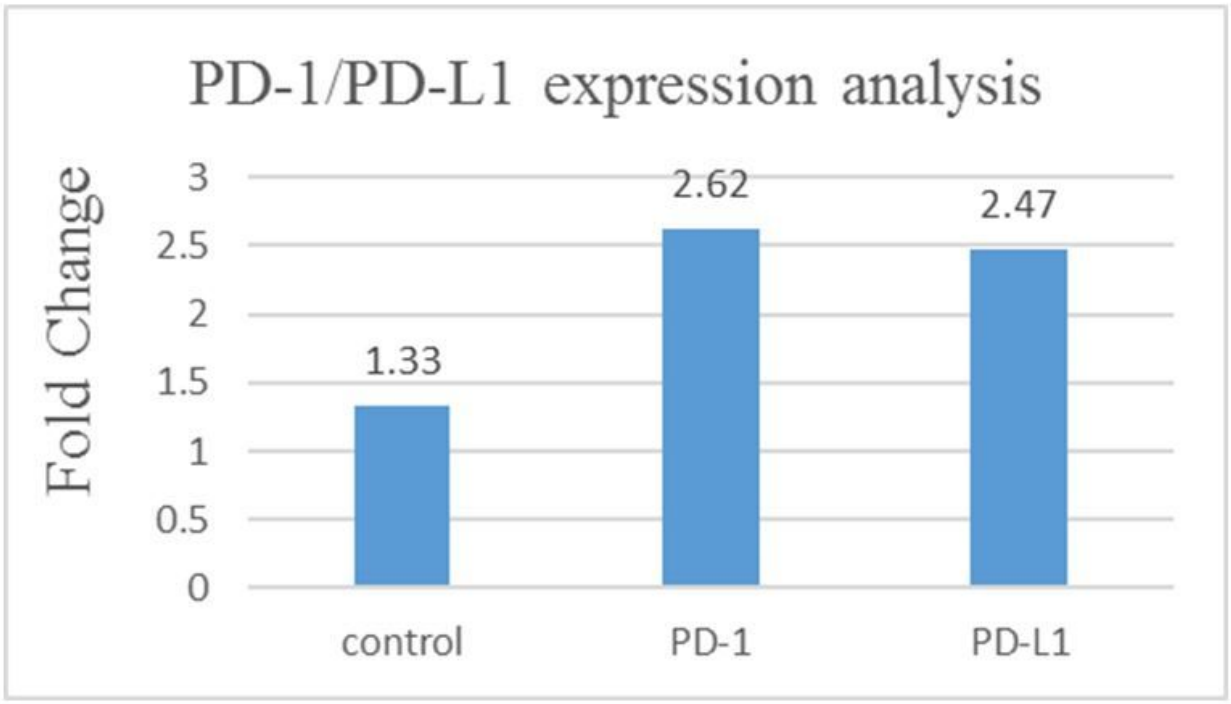

Figure 1

The PD-1 and PD-L1 mRNA expression analysis; the mRNA level of both PD-1 and PD-L1 were significantly higher in NHL patients compare to control group ($p<0.0001$)