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

hosnie hoseini (hosniehoseini@gmail.com)
Islamic Azad University
Parichehreh Yaghmaei
Islamic Azad University
Gholamreza Bahari
zahedan medical science
saeed aminzadeh
Islamic Azad University

Research

Keywords: programmed death-1 (PD-1), programmed death-1 ligand-1 (PD-L1); single nucleotide polymorphisms (SNPs); Non-Hodgkin lymphoma (NHL)

Posted Date: August 31st, 2020

DOI: https://doi.org/10.21203/rs.3.rs-67229/v1

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.

Introduction

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 And Methods

Study population

A total of 134 patients with clinically diagnosed non-Hodgkin lymphoma and 125 healthy controls with no history of cancer or inflammatory diseases were recruited for the study. Before attending this study, all patients had written informed consent for research use, and All experiments have been carried out in accordance with the ethical guidelines for medical research.

Primers designing

The sequences of PD-1 and PD-L1 genes were obtained from NCBI and target primers were designed using AlleleID v7.5 software (Premier Biosoft International, Palo Alto, CA). Subsequently, the designed primers were validated by OligoAnalyzer3 and checked in the NCBI database.

DNA extraction and genotyping

The genomic DNA of lymphoma patients and healthy controls were extracted from peripheral blood leukocytes using standard salting-out method describing elsewhere(8) and stored at -20 °C until use. The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was carried out to detect the genotypes of the PD-1 and PD-L1 polymorphisms. To this, two selected polymorphism containing regions of each target genes were amplified with specific primer listed in table-1. The PCR reaction was prepared using Ampliqon universal PCR master mix (12.5 µL) with adding 1 µL of genomic DNA, 0.5 µL of each primers and 10.5 µL of nuclease-free water.

Subsequently, the PCR products were digested with desired restriction enzymes (Fermentase) according to manufacturer's instruction and analyzed by agarose gel electrophoresis (Table-1).

Table 1
Primer sets and related restriction enzymes used for PD-1 and PD-L1 SNPs analysis by PCR-RLFP method.

polymorphism	Primer sequence (5 $\rightarrow$ 3)	Restriction Enzyme	Fragment (bp)
PD-1 rs2227981 C > T	F: TGAGCAGACGGAGTATGCC R: CTGAGGAAATGCGCTGACC	PvuII, AluI	CC: 207 TC: 207 + 133 + 74 TT: 133 + 74
PD-1 rs11568821 G > A	F: CTCACATTCTATTATAGCCAGGACC R: TAAGATAAGAAATGACCAAGCCCAC	PstI	GG: 290 AG:290 + 197 + 93 AA: 197 + 93
PD-L1 rs2890658 C > A	F: GCAAGAGGAAGTGAAATAATCAAG R: GATACCTGTGTTAAAATGGGAACAG	HaeIII	CC: 226 + 25 CA: 251 + 226 + 25 AA: 251
PD-L1 rs4143815 G > C	F: CCTGGAGGGAGACCTTGATACTTTC R: CAACACTGAGACTCTCAGTCATGCAGTA	Hpy8I	CC: 227 CG: 227 + 199 + 28 GG: 199 + 28

PD-1 and PD-L1 expression analysis

Expression of PD-1 and PD-L1 were analyzed via real time RT-PCR technique. Briefly, EDTA-treated blood sample were collected and the total RNA was extracted using TRIZOL reagent (Invitrogen) according to manufacturer instruction. The extracted RNA then reversely transcribed into cDNA using Takara cDNA synthesis kit (Takara, Japan). Real time PCR was performed using a StepOnePlus real time PCR system (Applied Biosystems) using RealQ Plus 2x Master Mix (Ampliqon) and designed primer listed in Table 2. The β-actin primer was used as the housekeeping control gene.

Table 2
Primer sets used for PD-1 and PD-L1 expression analysis

	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

The SPSS statistical software version .. was used for all statistical analyses. Differences in characteristics among the genotypes and mRNA expression were compared using t test for categorical data. Genotypes were analyzed separately and as combined allelic groups. The patient's baseline characteristics and the genotype of PD-1/PD-L1 were analyzed using one-way analysis of variance (ANOVA) for continuous variables. P values < 0.05 were considered significant.

Results

A total of 284 patients were recruited in this study, 134 NHL patients and 125 healthy controls. The mean age of NHL patients and the healthy controls were 46.16 ± 17.1 and 44.9 ± 12 respectively. No significant difference was observed in age distribution between the NHL patients and control group (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 table-3. 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 3. 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 3: The frequencies of genotypes and alleles distribution of all SNPs (both PD-1 and PD-L1) in NHL patients and healthy controls.

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

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

Conclusion

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

Availability of data and materials

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

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.

Competing interests

The authors declare that they have no competing interests

Funding

This received financial support from the Islamic Azad University of Zahedan (Grant no: 14-11-5-784)

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.

Acknowledgements

The authors wish to thank the personnel of Ali ibn Abi Talib Hospital in Zahedan for their cooperation in this study.

References

1. Mahase E. Cancer overtakes CVD to become leading cause of death in high income countries. BMJ: British Medical Journal (Online). 2019;366.
2. Salmaninejad A, Valilou SF, Shabgah AG, et al. PD-1/PD-L1 pathway: Basic biology and role in cancer immunotherapy. J Cell Physiol. 2019;234(10):16824–37.
3. Li Y, Li F, Jiang F, et al. A mini-review for cancer immunotherapy: molecular understanding of PD-1/PD-L1 pathway & translational blockade of immune checkpoints. Int J Mol Sci. 2016;17(7):1151.
4. Boussiotis VA. Molecular and Biochemical Aspects of the PD-1 Checkpoint Pathway. N Engl J Med. 2016;375(18):1767–78.
5. Davis AA, Patel VG. The role of PD-L1 expression as a predictive biomarker: an analysis of all US Food and Drug Administration (FDA) approvals of immune checkpoint inhibitors. Journal for ImmunoTherapy of Cancer. 2019;7(1):278.
6. Zak KM, Grudnik P, Magiera K, Dömling A, Dubin G, Holak TA. Structural Biology of the Immune Checkpoint Receptor PD-1 and Its Ligands PD-L1/PD-L2. Structure. 2017;25(8):1163–74.
7. Jiang X, Wang J, Deng X, et al. Role of the tumor microenvironment in PD-L1/PD-1-mediated tumor immune escape. Molecular Cancer. 2019;18(1):10.
8. Chacon-Cortes D, Griffiths LR. Methods for extracting genomic DNA from whole blood samples: current perspectives. Journal of Biorepository Science for Applied Medicine. 2014;2014(2):1–9.
9. Kong EK, Prokunina-Olsson L, Wong WH, et al. A new haplotype of PDCD1 is associated with rheumatoid arthritis in Hong Kong Chinese. Arthritis rheumatism. 2005;52(4):1058–62.
10. Lee SH, Lee YA, Woo DH, et al. Association of the programmed cell death 1 (PDCD1) gene polymorphism with ankylosing spondylitis in the Korean population. Arthritis research therapy. 2006;8(6):R163.
11. Prokunina L, Castillejo-López C, Oberg F, et al. A regulatory polymorphism in PDCD1 is associated with susceptibility to systemic lupus erythematosus in humans. Nat Genet. 2002;32(4):666–9.
12. Zou W, Wolchok JD, Chen L. PD-L1 (B7-H1) and PD-1 pathway blockade for cancer therapy: Mechanisms, response biomarkers, and combinations. Science translational medicine. 2016;8(328):328rv4-rv4.
13. Zhou RM, Li Y, Liu JH, et al. Programmed death-1 ligand-1 gene rs2890658 polymorphism associated with the risk of esophageal squamous cell carcinoma in smokers. Cancer Biomark A. 2017;21(1):65–71.

14. Hashemi M, Karami S, Sarabandi S, et al. Association between PD-1 and PD-L1 Polymorphisms and the Risk of Cancer: A Meta-Analysis of Case-Control Studies. Cancers (Basel). 2019;11(8):1150.
15. Salmaninejad A, Khoramshahi V, Azani A, et al. PD-1 and cancer: molecular mechanisms and polymorphisms. Immunogenetics. 2018;70(2):73–86.
16. Yin L, Guo H, Zhao L, Wang J. The programmed death-1 gene polymorphism (PD-1.5 C/T) is associated with non-small cell lung cancer risk in a Chinese Han population. Int J Clin Exp Med. 2014;7(12):5832–6.
17. Dong W, Gong M, Shi Z, Xiao J, Zhang J, Peng J. Programmed cell death-1 polymorphisms decrease the cancer risk: a meta-analysis involving twelve case-control studies. PloS one. 2016;11(3):e0152448.
18. Wang W, Li F, Mao Y, et al. A miR-570 binding site polymorphism in the B7-H1 gene is associated with the risk of gastric adenocarcinoma. Human genetics. 2013;132(6):641–8.
19. Shi X-L, Mancham S, Hansen BE, et al. Counter-regulation of rejection activity against human liver grafts by donor PD-L1 and recipient PD-1 interaction. Journal of hepatology. 2016;64(6):1274–82.
20. Pizarro C, García-Díaz DF, Codner E, Salas-Pérez F, Carrasco E, Pérez-Bravo F. PD-L1 gene polymorphisms and low serum level of PD-L1 protein are associated to type 1 diabetes in Chile. Diab/Metab Res Rev. 2014;30(8):761–6.
21. Vaddepally RK, Kharel P, Pandey R, Garje R, Chandra AB. Review of Indications of FDA-Approved Immune Checkpoint Inhibitors per NCCN Guidelines with the Level of Evidence. Cancers (Basel). 2020;12(3):738.
22. Nomizo T, Ozasa H, Tsuji T, et al. Clinical Impact of Single Nucleotide Polymorphism in PD-L1 on Response to Nivolumab for Advanced Non-Small-Cell Lung Cancer Patients. Sci Rep. 2017;7(1):45124.
23. Wu X, Gu Z, Chen Y, et al. Application of PD-1 Blockade in Cancer Immunotherapy. Computational structural biotechnology journal. 2019;17:661–74.
24. Nakanishi J, Wada Y, Matsumoto K, Azuma M, Kikuchi K, Ueda S. Overexpression of B7-H1 (PD-L1) significantly associates with tumor grade and postoperative prognosis in human urothelial cancers. Cancer immunology immunotherapy: CII. 2007;56(8):1173–82.
25. Gevensleben H, Dietrich D, Golletz C, et al. The Immune Checkpoint Regulator PD-L1 Is Highly Expressed in Aggressive Primary Prostate Cancer. Clinical cancer research: an official journal of the American Association for Cancer Research. 2016;22(8):1969–77.
26. Chen BJ, Dashnamoorthy R, Galera P, et al. The immune checkpoint molecules PD-1, PD-L1, TIM-3 and LAG-3 in diffuse large B-cell lymphoma. Oncotarget. 2019;10(21):2030–40.
27. Andorsky DJ, Yamada RE, Said J, Pinkus GS, Betting DJ, Timmerman JM. Programmed Death Ligand 1 Is Expressed by Non–Hodgkin Lymphomas and Inhibits the Activity of Tumor-Associated T Cells. Clin Cancer Res. 2011;17(13):4232–44.

Figures

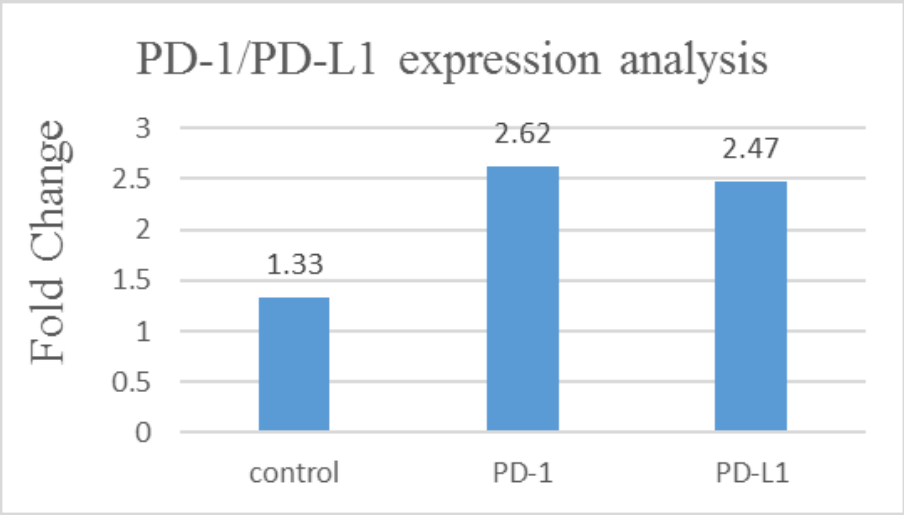

Figure 1

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 ($p<0.0001$)