WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 9, 1116-1125. Research Article ISSN 2277– 7105

RELATION BETWEEN GENETIC POLYMORPHISM IN *PD-1* AND THE SUSCEPTIBILITY TO TYPE 1 DIABETES MELLITUS IN IRAQI PATIENTS

Olla E. Khudhair*, Hameed M. Jasim and Ibrahim A. Ahmed

College of Biotechnology/Al-Nahrain University.

Article Received on
05 July 2020,

Revised on 25 July 2020,
Accepted on 15 Aug. 2020,

DOI: 10.20959/wjpr20209-18384

***Corresponding Author**
Olla E. Khudhair
College of Biotechnology/Al-Nahrain University.

ABSTRACT

Background and Objective: The programmed death *PD-1* pathway plays an important role in regulating T cell activation and maintaining peripheral tolerance, where the *PD-1* pathway was involved in the development of type 1 diabetes (T1DM), since the genetic background of type 1 diabetes differs greatly among different populations. The aim of this study is to assess the relationship between *PD-1* gene polymorphism and the incidence of T1DM in Iraqi patients. **Materials and Methods:** A total of 62 Iraqi patients with T1DM, and 62 apparently health controls were enrolled in this study to investigate the relationship between genetic polymorphism rs11568821 (G A) intron-4 and rs10204525 (G A) in 3′ untranslated region of *PD-1* gene and the susceptibility to T1DM. The levels of glycated hemoglobin (HbA1C), fasting blood glucose (FbG), C-peptide and lipid profile were monitored. Genomic DNA was extracted from 4 mL peripheral blood samples collected from each subject. Genotyping of the selected SNPs of PD-1 was achieved in T1DM patients and healthy controls. **Results:** The results demonstrated significantly higher levels of fasting plasma glucose, HbA1c; TC, TG, LDL, VLDL, and significantly decrease in serum levels of C-peptide in T1DM patients compared to those of healthy controls, while there is no significant difference in serum levels of high-density lipoprotein. Furthermore, results of the present study clearly showed for the first time, that there is no association between *PD-1*gene polymorphisms and T1DM in Iraqi diabetic patients. **Conclusion:** Our results indicate that rs11568821 and rs10204525 SNPs in *PD-1* are not significantly associated with T1DM and the prognosis of patients with T1DM.

KEYWORDS: Type 1 diabetes mellitus; Programmed cell death -1; PD-1.3; PD-1.6; Genetic polymorphism; Single nucleotide polymorphism; rs11568821; rs10204525.

INTRODUCTION

Type 1 diabetes mellitus or insulin dependent diabetes mellitus is a T cell-mediated autoimmune disease described by beta cell destruction and dysfunction. It has a complex etiology in which many genetic and environmental factors and the immune system are implicated.[1] This type of diabetes usually presents with sever hyperglycemic symptoms include polyuria, thirst, tiredness; weight loss and drowsiness usually indicate the upcoming ketoacidosis.[2] Still, T1DM has a lower incidence compared with type 2 diabetes mellitus (T2DM), even so, the genetically susceptible individuals at risk of T1DM believed to be at a variable age, which can range from the very young (6 months), to over 70 years. There are over 20 regions in the human genome that are associated with TIDM, but most of it make only small contribution to the susceptibility of type 1 diabetes.[3] Programmed cell death 1 (*PD-1*), is a transmembrane protein that consider as immunoinhibitory factor belonging to the CD28/B7 family. Accumulated studies showed that *PD-1/PD-L1* pathway was involved in the development of type 1 diabetes.[4] It plays a vital role in important role in down-regulating the immune system by enhancing T cell activation as a core costimulatory molecule. Many studies showed that blockage of the interaction between *PD-1* and *PD-L1* ligands can assisted with better prognosis in various malignant tumors [5-6]. Low *PD-1* might increase T cell proliferation and activation which cause the destruction of beta cells, producing a possible mechanism for T1DM. Since single nucleotide polymorphisms (SNPs) play critical roles in the transcription and translation of genes as well as they have associations with the appearance and development of diseases, studies had been committed to the associations between gene polymorphisms with T1DM susceptibility. Researchers suggested that *PD-1* and *PD-L1* SNPs were associated with T1DM susceptibility in different populations.[4,7] According to these findings, this study was aimed to investigate the relationship between rs11568821 and rs10204525 *PD-1* gene polymorphism and the susceptibility to T1DM in Iraqi patients.

MATERIALS AND METHODS

This cross sectional study was carried out on consented 62 already diagnosed type 1 diabetic patients (30 males and 32 females, 1 to 50 years) attending different health institutions (Central Children Teaching hospital, National Diabetes Center, and private laboratory) in

addition to 62 healthy individuals (31 males and 31 females) with normal blood glucose levels in the period from September/2019 to November/2019 in Baghdad governorate. Patients who were on lipid lowering medications were excluded. After an overnight fast, blood samples were collected for fasting blood sugar. C-peptide and lipid profile [which includes Triglycerides (TG), Total Cholesterol (TC), high density lipoprotein C (HDL-C), low density lipoprotein C (LDL-C), and very-low density lipoprotein-C (VLDL-C)]. Genomic DNA was extracted from peripheral white blood cells of T1DM patients and healthy controls, according to the protocol ReliaPrep™ Blood gDNA Miniprep System, Promega/USA.

Genotyping of the PD-1 rs11568821 polymorphism

PD-1.3 +7146 (G A) rs11568821 and PD-1.6 +3737 (G A) rs10204525 of *PD-1* gene were amplifided by using specific primers designed in this study shown in table 1. Polymerase chain reaction was performed in a total volume of 20µl mixture containing 3µl DNA, 1µl forward primer, 1µl reverse primer, 10µl master mix and 5 µl nuclease free water. The amplification conditions started with initial denaturation (95ºC) for 5 min., followed by 30 cycles of denaturation at 95ºC for 30 sec., annealing at 61 ºC for 30 sec., and extension at 72 ºC for 1min., then a final extension at 72° C for 7 min. PCR products were run on 1% agarose gels and visualized by UV transilluminator.

Table 1: Oligonucleotide primers used for amplification of *PD-1* gene

SNP	Sequence (5´→ 3´)	Tm (ºC)	Amplicon size (bp)
rs11568821	F: ATCTCTGTCCTCTAGCTCTG	61	998
	R: TCCTCTCTGGCTCAATGT		
rs10204525	F: TTCAGGGAAGGTCAGAAGAG	61	854
	R: CCGTGATGTTGGAGGAATTT		

Polymerase chain reaction was performed in a total volume of 20µl mixture containing 3µl DNA, 1µl forward primer, 1µl reverse primer, 10µl master mix and 5µl nuclease free water. The amplification conditions started with initial denaturation (95ºC) for 5 min., followed by 30 cycles of denaturation at 95ºC for 30 sec., annealing at 61 ºC for 30 sec., and extension at 72 ºC for 1min., then a final extension at 72° C for 7 min. PCR products were run on 1% agarose gels and visualized under by UV transilluminator.

Amplification products were sequenced and aligned with standard PD-1 gene in gene bank of the National Center for Biotechnology Information (NCBI) by using Geneious program.

Statistical analysis

The allele and genotype frequencies were evaluated by direct gene counting and compared with the controls using the chi-square test. The odds ratio (OR) and 95% confidence interval (CI) were examined for each allele and genotype, Hardy-Weinberg equilibrium (HWE), as well as linkage disequilibrium.[8]

RESULTS AND DISCUSSION

Sixty-two Iraqi patients with T1DM were recruited in this study, most of them were having symptoms including polyuria, thirst, tiredness, dehydration, and drowsiness, matched healthy unrelated controls that were randomly selected. Subjects were classified according to Fasting Blood Glucose (FBG) test as two groups: T1DM and the control group. Each group consisting of 62 females and males, total 64 subjects each matched in age. The clinical and biomarker for the two groups, T1DM and control sample are shown in Table 2.

Table 2: Clinical biomarkers for characterization of T1DM patients and healthy controls.

Biomarker	T1DM patients*	Healthy controls*	*P-value***
FBG (mg/dl)	300.30	108.11	0.0001
HbA1c (%)	9.27	5.23	0.0001
TC (mg/dl)	196.9	148.18	0.0001
TG (mg/dl)	176.58	101.61	0.0003
LDL (mg/dl)	113.13	82.90	0.0001
HDL (mg/dl)	51.55	57.2	0.151 (NS)
VLDL (mg/dl)	35.31	20.32	0.0003
C-peptide (ng/ml)	1.04	2.67	0.0001

*: Mean average; **: Significant (P<0.01); NS: Non-significant; FBG: fasting blood glucose; HbA1c: glycated hemoglobin; TC: Total cholesterol; TG; triglycerides; LDL: low-density lipoprotein; HDL: high-density lipoprotein; VLDL: very low-density lipoprotein.

In this study, we observed that mean value for FBS in diabetics was 300.30 mg/dl and mean value of HbA1C were found to be raised in diabetic patients to 9.27%. The results also demonstrated significantly higher levels (P<0.01) of lipid profile (TC, TG, LDL, VLDL), and significantly decrease in serum levels (P<0.01) of C-peptide in T1DM patients compared to those of healthy controls, while there is no significant difference in serum levels of high-density lipoprotein.

Genotyping of *PD-1* gene

This study was focused on the relation between *PD-1* gene polymorphism and the incidence of T1DM. However, there is no any study focused on the association between *PD-1* gene polymorphism and T1DM in Iraqi population. Therefore, this study was designed to investigate rs11568821 and rs10204525 SNPs in *PD-1* to ascertain whether this SNPs influence the susceptibility to T1DM. Genomic DNA was extracted from blood samples of patients and healthy controls with high concentrations (200-400 µg/ml), and purity (1.8-2.0), then *PD-1* was amplified, sequenced and genotyped. As shown in Table 3, frequencies of the GG, GA and AA genotypes for rs11568821were 83.33%, 10% and 6.67% in the cases, and 80%, 16.67% and 3.33% in the controls, respectively. Compared with GG, there was no difference in distribution of the GA and AA genotypic frequencies between cases and controls. In addition, no difference was observed in frequency of allele between the two groups. Frequencies of the genotype were in Hardy-Weinberg equilibrium in both groups. According to these results, the homozygous GG genotype considered as predominant genotype within the population of this study and the presence of GA and AA genotype in control samples reveal that it has no effect on the susceptibility to T1DM that may be occur due primarily to lifestyle factors and genetics as mentioned by Qian *et al.* [4], who found that there is no association between *PD-1.3* polymorphisms and T1DM susceptibility in Chines population.

Table 3: Distribution and allele frequency of *PD-1* gene polymorphism (rs11568821) in T1DM patients and health controls.

Genotype	Patients No. (%)	Control No. (%)	P-value	O.R. (95% C.I.)
GG	25 (83.33)	24 (80.0)	0.78 NS	0.54 (0.38-0.71)
GA	3 (10.00)	5 (16.67)	0.22 NS	0.67 (0.55-0.81)
AA	2 (6.67)	1 (3.33)	0.78 NS	0.54 (0.38-0.71)
Total	30	30	---	---
Allele Frequency				
G	0.88	0.88	1.0 NS	---
A	0.12	0.12	1.00 NS	---

However there were several studies indicated the correlation of this SNP with T1DM as the study which indicated that *PD-1.3* located in in intron 4 of the *PD-1* gene, alters the binding affinity of a transcriptional factor RUNX, and was highly associated with T1DM, other study suggested that the A allele of *PD-1.3* SNP is significantly associated with the susceptibility to type 1 diabetes [P=0.037, odds ratio (OR) =1.92] in Caucasians.[9] Its frequency was found to

be 12.2% of diabetic individuals against 6.8% of controls, hence indicating a significant association was confirmed in two separate populations of type 1 diabetes patients originating from two different regions in Denmark. Testing the pooled material further revealed that *PD-1.3* G/A and A/A genotypes are significantly associated with susceptibility to T1DM [10]. Some study conducted on the meta-analysis demonstrated a significant association of the *PD1.3* polymorphism with T1DM and RA susceptibility in Europeans and Asians populations.[11]

Studies have been healed on *PD1.3* SNP demonstrate the association of this SNP with different autoimmune diseases and cancers. Some studies have indicated that *PD-1.3* is associated with the development of SLE in European and Mexican populations.[12]

Furthermore, studies reported a significantly increased risk of SLE among Egyptian females carrying the dominant *PD1.3* GG genotype [P=0.0029, OR=1].[13] evaluation of the association of *PD-1.3* SNP including in Iranian patients with juvenile onset systemic lupus erythematosus (JSLE) and healthy controls was investigated, founding that there was a great influence of the *PD1.3* SNP on the development of JSLE in Iranian population.[14] Moreover, there was a statistical significance was shown between *PD-1.3* G/A polymorphism and risk of RA.[15] particularly, it has been proved that the *PD-1.3* (A/G) polymorphism is associated with colon cancer in an Iranian population with [P=0.015, OR=2.2].[16] However, no significant association has been reported for breast cancer in an Iranian population[17] and for hepatocellular carcinoma (HCC) in a Turkish.[18]

PD-1.3 G/A is located in intron 4, in general, polymorphic sequence variations are considered to be rather harmless, especially if located in noncoding parts of a gene. Even so, this SNP is a guanine (G) to adenine (A) polymorphism in the *PD-1* intron was suggested as an enhancer-like because of the presence of four tandem repeats that have multiple putative binding sequences of transcription factors, it has the ability to alter the binding of the runt-related transcription factor 1 (RUNX1) and modify the transcriptional regulation proficiency of the *PD-1* gene.[19]

Results indicated in Table 4, shown that the frequencies of the GG, GA and AA genotypes for *PD-1.6* (rs10204525) were 70.00%, 26.67% and 3.33% in the cases, and 63.33%, 30.00% and 6.67% in the controls, respectively. Compared with GG, there was no difference in distribution of the GA and AA genotypic frequencies between cases and controls. In addition,

no difference was observed in frequency of allele between the two groups. Frequencies of the genotype were in Hardy-Weinberg equilibrium in both groups. According to these results, the homozygous GG genotype considered as predominant genotype within the population of this study and the presence of GA and AA genotype in control samples reveal that it has no effect on the susceptibility to T1DM that may be occur due primarily to lifestyle factors and genetics . These result match with same studies conducted on T1DM patients in China and East Asian that proved there was no association between *PD-1.6* polymorphism and T1DM.[20][4]

Table 4: Distribution and allele frequency of *PD-1* gene polymorphism (rs10204525) in T1DM patients and health controls.

Group	Patients No. (%)	Control No. (%)	P-value	O.R. (C.I.)
GG	21 (70.00)	19 (63.33)	0.082 NS	0.794 (0.62-1.14)
GA	8 (26.67)	9 (30.00)	0.277 NS	0.562 (0.41-0.74)
AA	1 (3.33)	2 (6.67)	0.781 NS	0.538 (0.38-0.71)
Total	30	30	---	---
Allele Frequency				
G	0.83	0.78	0.748 NS	---
A	0.17	0.22	0.748 NS	---

However *PD-1.6* has been contribute in few studies to insure its correlation with other disease such as acute anterior uveitis[21], aplastic anemia[22], Takayasu's arteritis[23], Hodgkin lymphoma[24], gastric cardiac adenocarcinoma[25], and breast cancer[26], but all of them show that there was no correlation between the SNP and the incidence of these disease. On the other hand, Qiu *et al.*[27] proved that the *PD-1* rs10204525 GG homozygote genotype was associated with a borderline statistically decreased risk of esophageal cancer [OR = 0.68, 95%, *P* = 0.067].

PD-1.6 rs10204525, located in 3′ untranslated region, it may be involved in the modification of the inflammatory cytokines levels by modulating polyadenylation through the linkage disequilibrium with other nucleotide polymorphisms.[28]

REFERENCES

1. **Chiang, J.L.;** Kirkman, M.S.; Laffel, L.M. and Peters, A.L. (2014). Type 1 diabetes through the life span: a position statement of the American Diabetes Association. *Diabetes care*, 37(7): 2034-2054.

2. **Ekman, I.;** Ihantola, E.L.; Viisanen, T.; Rao, D.A.; Näntö-Salonen, K.; *et al*., (2019). Circulating CXCR5− PD-1 hi peripheral T helper cells are associated with progression to type 1 diabetes. *Diabetologia*, 62(9): 1681-1688.
3. **Wong, F.S.** and Tree, T.I. (2019). Historical and new insights into pathogenesis of type 1 diabetes. *Clinical & Experimental Immunology*, 198(3): 292-293.
4. **Qian, C.;** Guo, H.; Chen, X.; Shi, A.; Li, S.; *et al*., (2018). Association of PD-1 and PD-L1 genetic polymorphyisms with type 1 diabetes susceptibility. *Journal of diabetes research*, 2018.
5. **Fujisawa, R.;** Haseda, F.; Tsutsumi, C.; Hiromine, Y.; Noso, S.; *et al*., (2015). Low programmed cell death-1 (PD-1) expression in peripheral CD4+ T cells in Japanese patients with autoimmune type 1 diabetes. *Clinical & Experimental Immunology*, 180(3): 452-457.
6. **Sobral-Leite, M.;** Van de Vijver, K.; Michaut, M.; van der Linden, R.; Hooijer, G.K.; *et al*., (2018). Assessment of PD-L1 expression across breast cancer molecular subtypes, in relation to mutation rate, BRCA1-like status, tumor-infiltrating immune cells and survival. *Oncoimmunology*, 7(12): 1509820.
7. **Pizarro, C.;** García-Díaz, D.F.; Codner, E.; Salas-Pérez, F.; Carrasco, E. and Pérez-Bravo, F. (2014). PD-L1 gene polymorphisms and low serum level of PD-L1 protein are associated to type 1 diabetes in Chile. *Diabetes/metabolism research and reviews*, 30(8): 761-766.
8. **Wasserstein, R.L**. and N.A. Lazar, 2016. The ASA's statement on p-values: Context, process and purpose. Am. Statistician, 70: 129-133.
9. **Ni, R.**; Ihara, K.; Miyako, K.; Kuromaru, R.; Inuo, M.; Kohno, H. and Hara, T. (2007). PD -1 gene haplotype is associated with the development of type 1 diabetes mellitus in Japanese children. *Human genetics*, 121(2): 223-232.
10. **Nielsen, C.;** Hansen, D.; Husby, S.; Jacobsen, B. B. and Lillevang, S. T. (2003). Association of a putative regulatory polymorphism in the PD-1 gene with susceptibility to type 1 diabetes. *Tissue antigens*, 62(6): 492-497.
11. **Lee, Y.H.**; Bae, S.C.; Kim, J.H. and Song, G.G. (2015). Meta-analysis of genetic polymorphisms in programmed cell death 1. *Zeitschrift für Rheumatologie*, 74(3): 230-239.

12. **Prokunina, L.;** Castillejo-López, C.; Öberg, F.; Gunnarsson, I.; Berg, L.; *et al*., (2002). A regulatory polymorphism in PDCD1 is associated with susceptibility to systemic lupus erythematosus in humans. *Nature genetics*, 32(4): 666-669.
13. **Abo El-khair, S. M.;** Sameer, W.; Awadallah, N. and Shaalan, D. (2019). Programmed cell death 1 gene polymorphism as a possible risk for systemic lupus erythematosus in Egyptian females. *Lupus*, 28(12): 1427-1434.
14. **Mahmoudi, M.;** Rezaiemanesh, A.; Salmaninejad, A.; Harsini, S.; Poursani, S.; *et al*., (2015). PDCD1 single nucleotide genes polymorphisms confer susceptibility to juvenile - onset systemic lupus erythematosus. *Autoimmunity*, 48(7): 488-493.
15. **Zou, Y.;** Zhang, Z.; Liu, Y.; Liu, D. and Xu, W. (2017). Are programmed cell death 1 gene polymorphisms correlated with susceptibility to rheumatoid arthritis? a meta-analysis. *Medicine*, 96(35).
16. **Yousefi, A. R.;** Karimi, M. H.; Shamsdin, S. A.; Mehrabani, D.; Hosseini, S. V.; *et al*., (2013). PD-1 gene polymorphisms in Iranian patients with colorectal cancer. *Laboratory Medicine*, 44(3): 241-244.
17. **Haghshenas, M. R.;** Naeimi, S.; Talei, A.; Ghaderi, A. and Erfani, N. (2011). Program death 1 (PD1) haplotyping in patients with breast carcinoma. *Molecular biology reports*, 38(6): 4205-4210.
18. **Bayram, S.;** Akkız, H.; Ülger, Y.; Bekar, A.; Akgöllü, E. and Yıldırım, S. (2012). Lack of an association of programmed cell death-1 PD1. 3 polymorphism with risk of hepatocellular carcinoma susceptibility in Turkish population: a case–control study. *Gene*, 511(2): 308-313.
19. **Salmaninejad, A.;** Khoramshahi, V.; Azani, A.; Soltaninejad, E.; Aslani, S.; *et al*., (2018). PD-1 and cancer: molecular mechanisms and polymorphisms. *Immunogenetics*, 70(2): 73-86.
20. **Gu, Y.;** Xiao, L.; Gu, W.; Chen, S.; Feng, Y.; et al., (2018). Rs2227982 and rs2227981 in PDCD1 gene are functional SNPs associated with T1D risk in East Asian. Acta diabetologica, 55(8): 813-819.
21. **Li, Y.;** Hong, M.; Huang, X.; Zhong, L.; Gu, Y.; *et al*., (2019). PD-1 Polymorphisms Are Associated with Susceptibility of Acute Anterior Uveitis in Chinese Population. *DNA and cell biology*, 38(2): 121-128.
22. **Wu, Z.;** Miao, M.; Qiu, Y.; Qin, Z.; Wang, J.; *et al*., (2013). Association between polymorphisms in PDCD1 gene and aplastic anemia in Chinese Han population. *Leukemia & lymphoma*, 54(10): 2251-2254.

23. **Direskeneli, H.;** Tuna-Erdogan, E.; Gündüz, F.; Bandurska-Luque, A.; Alparslan, B.; *et al.*, (2012). PDCD1 polymorphisms are not associated with Takayasu's arteritis in Turkey. *Clin Exp Rheumatol*, 30(1 Suppl 70).
24. **Delamain, M.T.;** Gomez, G.V.B.; Lourenço, G.J.; de Souza, C.A. and Lima, C.S.P. (2019). Increased risk of Hodgkin lymphoma in males with inherited T lymphocyte receptor programed death-1 deficiency. *Leukemia & lymphoma*, 60(14): 3552-3556.
25. **Tang, W.;** Chen, Y.; Chen, S.; Sun, B.; Gu, H. and Kang, M. (2015). Programmed death-1 (PD-1) polymorphism is associated with gastric cardia adenocarcinoma. *International journal of clinical and experimental medicine*, 8(5): 8086.
26. **Ren, H.T.;** Li, Y.M.; Wang, X.J.; Kang, H.F.; Jin, T.B.; *et al*., (2016). PD-1 rs2227982 polymorphism is associated with the decreased risk of breast cancer in Northwest Chinese women: a hospital-based observational study. *Medicine*, 95(21).
27. **Qiu, H.;** Zheng, L.; Tang, W.; Yin, P.; Cheng, F. and Wang, L. (2014). Programmed death-1 (PD-1) polymorphisms in Chinese patients with esophageal cancer. *Clinical biochemistry*, 47(7-8): 612-617.
28. **Tejeda, C.;** Broadaway, A. K.; Ombrello, M. J.; Brown, M. R.; Ponder, L. A.; *et al.*, (2017). Case-control association study of autoimmunity associated variants in PDCD1 and juvenile idiopathic arthritis. *Current rheumatology reviews*, 13(3): 219-223.