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The genetic influence of PD-1/PD-L1 axis single nucleotide polymorphisms on the incidence of type 1 diabetes mellitus in pediatric Egyptian patients

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Abstract

Background: The increasing prevalence of diabetes mellitus (DM) is one of the most challenging public health issues. The destruction of insulin-producing cells in the islets of Langerhans is the hallmark of type 1 diabetes mellitus (T1DM) as an autoimmune disease. In the current case–control study, the role of single nucleotide polymorphisms (SNPs) was investigated within the programmed death-1 (PD-1)/programmed death ligand-1 (PD-L1) inhibitory axis and their association with T1DM susceptibility in a sample of Egyptian pediatric patients. The study included 80 T1DM pediatric patients and 76 healthy control subjects. The patients were recruited from Beni-Suef University Hospital's Pediatric Endocrinology Outpatient Clinic. Genotyping of PD-1 SNP (rs 34819629) and PD-L1 SNPs (rs 2297137 and rs 4143815) was performed by TaqMan allelic discrimination technique via real-time polymerase chain reaction (RT-PCR). The patients were subjected to a thorough clinical examination and history taking.

Result: Genotyping of PD-1 (rs 34819629) revealed that all of the enrolled patients and the control group inherited the same genotype (GG genotype). With regard to PDL-1 rs4143815 SNP and the risk of T1DM occurrence, our comparison did not reveal the presence of an association between the different genetic models (general, dominant, and recessive) of the SNP and the risk of T1DM ($p = 0.078$ and $p = 0.055$; for the general genetic model, $p = 0.061$ and $p = 0.169$ for the dominant and the recessive types, respectively). Regarding PDL-1 rs2297137 SNP, the results of this study demonstrated that the risk of T1DM was significantly associated with the recessive genetic model ($p = 0.007$) as the diabetic group's predominant G allele was higher compared to the control group.

Conclusion: The findings obtained supported the hypothesis that the predominant G allele of PD-L1 rs2297137 is associated with the development of T1DM. Chronic hyperglycemia and long-standing diabetes problems are linked to both PD-L1 SNPs (rs4143815 and rs2297137). Future studies with a more significant number of patients are required to support our results.

Keywords: Type 1 DM, PD-1, PD-L1, RT-PCR, SNPs

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Background

The increasing prevalence of diabetes mellitus (DM) is one of the most significant public health issues. Even though the incidence of type 2 DM is higher than type 1 diabetes mellitus, the prevalence of type 1 diabetes is higher in pediatric patients, and it has a more significant influence on their quality of life [1]. The destruction of

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insulin-producing cells in the Langerhans islets is a hallmark of type 1 diabetes mellitus as an autoimmune disease [2].

Type 1 DM is a multifactorial autoimmune disease with both environmental and genetic factors. The disease is characterized by the presence of variable combinations of genetic and environmental factors. The genetic risk factors are delineated by multiple combinations of alleles in the HLA region that affect the recognition of T cells and tolerance to autologous and foreign antigens [3]. Similarly, multiple other loci are involved in the regulation and modification of specific immune responses as well as the alteration of the β cells' sensitivity to inflammatory mediators [4].

Many studies have elucidated the function of immunological checkpoint inhibitors (ICI) in the immunopathogenesis of T1DM in both animals and humans. These studies have demonstrated that blocking PD-1 and PD-L1 causes nonobese diabetic mice to develop rapid-onset diabetes [5]. The PD-1/PD-L1 axis is crucial for maintaining the immunological balance in multiple organs, including tissues of the pancreas, as blocking the PD-1/PD-L1 axis in patients with cancer who were given monoclonal antibodies against PD-1 as a treatment has resulted in the occurrence of acute or chronic pancreatitis [6, 7]. In addition, evidence has indicated the importance of this inhibitory mechanism for maintaining tolerance in insulin-producing β cells and avoiding T1DM [8, 9].

One of the inhibitory costimulatory molecules is PD-1 which is shown on the surface of active T cells, B cells, and monocytes. PD-1 has been associated with immunological tolerance. PD-1 is a member of the CD28 /B7 family of T-cell regulators [10], whereas PD-1 interacts with two B7 family ligands, PD-L1 and PD-L2. PD-L1 (CD274) is broadly dispersed on leucocytes and non -hematopoietic tissues in lymphoid and non-lymphoid, including the Langerhans cells. On the contrary, PD-L2 (CD273) is solely expressed on monocytes and dendritic cells [8]. When PD-1 binds to its ligands, it blocks the effector functions of T cells, reinvigorating exhausted T cells and thus improving the antiviral and anti-neoplastic effects [11, 12]. The findings have revolutionized cancer therapy, as PD-1 immune checkpoint inhibitors are being developed [13]. Nevertheless, these discoveries have been associated with the development of adverse effects such as the rapid development of autoimmunity, including T1DM [14].

T1DM has a different genetic background in different populations. Studies in Egypt on the genetic basis of T1DM in pediatric patients did not address the relationship between SNPs within the PD-1/PD-L1 axis and the danger of occurrence of T1DM, which may contribute to better management of T1DM. Consequently, the present study aimed to investigate the impact of SNPs within the PD-1/PD-L1 pathway and their association with T1DM susceptibility in a sample of Egyptian pediatric patients.

Patients and methods

Patients

The study included 80 T1DM pediatric patients (28 males and 52 females) diagnosed with type 1DM based on the American Diabetes Association's recommendations [15], with an age range of 1–13. The patients were recruited from the Beni-Suef University Hospital's Pediatric Endocrinology Outpatient Clinic. In addition, 76 unrelated healthy controls of the same ethnicity were enrolled. The study was carried out in the interval between December 2019 and May 2020. Patients with other related genetic abnormalities and those with T2DM were excluded from the study. All participants had a complete medical history taken, with an emphasis on the onset of diabetes mellitus and family history of DM and its complication (microvascular: nephropathy, retinopathy and neuropathy, and macrovascular: hypertension or coronary heart disease). All participants had a complete medical history taken, focusing on the onset of diabetes mellitus, family history of DM, and hypertension or coronary heart disease. A thorough clinical examination was performed, including arterial blood pressure and anthropometric measurements. The study was approved by the ethical committee of Beni-Suef University's Faculty of Medicine with an approval number FMBSUREC/01102019/Radwan. All participants gave their informed consent.

Methods

Sample collection

Seven milliliters of venous blood were drawn from each subject using a sterile venipuncture. The withdrawn blood was divided into two parts: 4 ml were used to assess HbA1c and fasting blood sugar. In an EDTA sterile vacutainer, 3 ml of the extracted blood were collected and used within 24 h of collection or stored at -20˚C for the detection of PD-1 SNP (rs 34819629) and PD-L1 SNPs (rs 2297137 and rs 4143815). A random morning urine sample was collected from each patient to detect the presence of microalbuminuria. The diagnosis of persistent microalbuminuria was confirmed if the urinary albumin/creatinine ratio was 30–299 mg/g creatinine in two of three consecutive urine samples taken three months apart [16].

Genotyping for the detection of PD-1 SNP (rs 34819629) and PD-L1 SNPs (rs 2297137 and rs 4143815), EDTA anticoagulated blood was used for DNA extraction using QIAamp DNA Mini Kit (Cat.no.51104, QIAGEN, USA) following manufacturer protocol. RT-PCR was used to

genotype PD-1 SNP (rs34819629) and PD-L1 SNPs (rs 2297137 and rs 4143815) through SNP genotyping assay using a TaqMan probe.

For SNP rs2297137, the TaqMan® MGB probes/extension primers were (VIC TGCAAAGGCATTCCACTG TTCAACA) and (6FAM CAATTATATTGAAGCTGA GTGGGAT) for the detection of the sequence of alleles 1 and 2, respectively, while for SNP rs4143815 were (VIC TTGCCTCCACTCAATGCCTCAATTT) and (6FAM TTTTCTGCATGACTGAGAGTCTCAG) for the detection of the sequence of allele 1&2 respectively, whereas for SNP rs34819629, they were (VIC TTCCAGAGCTAG AGGACAGAGATGC) and (6FAM GGTCACCATTCC CCAGGTGCAGGAC) for the detection of the sequence of allele 1&2, respectively.

Each SNP had a 20 µl reaction volume: 40 ng/ul gDNA, 10 µl 2 × master mix II (cat no. 4440043), 0.5 µl 20 × SNP assay mix, and a final volume of 20 µl adjusted with nuclease-free water. The reaction was carried out using a step-one real-time PCR (Applied Biosystems, Singapore). The cycling Life Technologies real-time instrument software (USA) plots the results of the allelic discrimination data as a scatter plot of allele 1 (VIC® dye) versus allele 2 (FAM™ dye). Each well of the 96 wells was represented as a separate point on the plot. The results of 10% of the amplification reactions were repeated twice for conformation and revealed to be identical.

Statistical analysis was done using IBM SPSS® statistics version 22 (IBM® Corp., Armonk, NY, USA). Numerical data were expressed in the form of mean and standard deviation (SD). Qualitative data were expressed as frequency and percentage. Pearson's Chi-square test or Fisher's exact test were used to examine the relation between qualitative variables, whereas to test the distribution of quantitative data, the Kolmogorov–Smirnov and Shapiro–Wilk tests were used. For normally distributed data, the student t test was used to compare two groups of quantitative data. Allele frequencies were determined using allele counting, and deviations from Hardy–Weinberg equilibrium were assessed using the Chi-square test. The risk was estimated using logistic regression and adjusted for age, gender, and BMI using an odds ratio (OR) with a 95 percent confidence interval (CI). Two-tailed tests were used in all of the tests. Genotype frequency data were used in haplotype construction. This analysis was performed by https://www.snpstats.net/analyzer.php. A p value of < 0.05 is considered statistically significant.

Results

Demographic, clinical, laboratory data, and anthropometric studies

This study included 80 children with type 1 diabetes, with males accounting for 35% of the group, while females made up 65%, and a mean age of 10.0 ± 3.2 years. The control group included 76 normal unrelated children of the same ethnic origin, 68.4% were made up of females and 31.6% of males with a mean age of 8.8 ± 2.8 years. The average age of onset of the disease was 2.36 ± 2.41 years. The patient group's mean BMI was 19.9 ± 5.0 SD, while the control group's mean BMI was 21.1 ± 1.2 SD. The percentage of patients with a high albumin/ creatinine ratio was 64%, and the percentage of patients with a normal albumin/creatinine ratio was 20%. The patients' mean fasting blood glucose level was 159.1 ± 38.6 mg/dl, while the control group's mean fasting blood glucose level was 78.1 ± 8.2 mg/dl. The features and laboratory investigations of the patients and controls are displayed in Table 1.

Analysis of the relation between T1DM and the selected SNPs

According to the distributions of different genotypes and alleles frequency with the Hardy–Weinberg principle, our findings revealed that the genotype frequency of PDL-1 rs2297137 in both the diabetic patients & control group was in accordance with the Hardy–Weinberg principle ($p = 0.727$ and $p = 0.867$, respectively). Regarding PDL-1 rs4143815 SNP, the genotype frequency distribution in both the patient and control groups was in agreement with the Hardy–Weinberg principle ($p = 0.281$, $p = 0.455$, respectively). First, we investigated the relationship between the incidence of T1DM and PD-1 and PD-L1 SNPs selected in the study (rs34819629, rs2297137, and rs4143815). Regarding PD-1 rs34819629 SNP, all patients and control subjects inherited the same genotype (GG), and hence no further comparison was performed between the SNP and its association with T1DM patients enrolled in the study (Table 2).

Table 1 Demographic features and laboratory investigations of the patients and control

Variables	Patients (T1DM)	Control	*p* value
Sex (%)			
Female	52 (65.0%)	24(31.6%)	**0.001**
Male	28 (35.0%)	52 (68.4%)	
Age (mean ± SD)	10.0 ± 3.2	8.8 ± 2.8	**0.011**
Age at diagnosis (years)	2.36 ± 2.41	–	–
BMI SDS (mean ± SD)	19.9 ± 5.0	21.1 ± 1.2	**0.043**
HbA1c (%) (mean ± SD)	9.8 ± 2.1	4.6 ± 1.2	**< 0.001**
Alb/creat. ratio (mg alb/g creat)			
Yes	64 (80.0%)	–	–
No	16 (20.0%)	–	–
Fasting blood glucose (mg/dl)	159.1 ± 38.6	78.1 ± 8.2	**< 0.001**

TIDM, type 1 diabetes mellitus; SD, standard deviation; BMI, body mass index; SDS, standard deviation score; Alb/creat. ratio, albumin/creatinine ratio

Regarding PDL-1 rs4143815 C/G SNP, a comparison of genotype and allele frequency distribution revealed that the GG genotype and the major G allele were more frequent in the diabetic group compared to the control (non-diabetic) group. Nonetheless, the comparison between the two groups revealed no statistically significant difference ($p=0.123$ and $p=0.071$, respectively) (Table 2). As regards PDL-1 rs2297137 A/G SNP, our comparison demonstrated that the frequency distribution of the GG genotype and the G allele was significantly more frequent among T1DM pediatric patients in relation to the control (healthy) group ($p=0.018$ and $p=0.005$) (Table 2).

In this analysis, we attempted to estimate the risk of T1DM associated with the PDL-1 selected SNPs in three genetic models (general or codominant, dominant, and recessive genetic models). As regards PDL-1 rs4143815 C/G SNP and the risk of T1DM occurrence, our comparison did not reveal the presence of an association between the general genetic model, dominant genetic model, and recessive genetic model of the SNP and the risk of T1DM; p value (0.078 and 0.055) for the general genetic model, p value (0.061 and 0.169) for the dominant and recessive genetic models, respectively (Table 3). Regarding PDL-1 rs2297137 SNP and the risk of T1DM occurrence, the current study's findings demonstrated that the risk of T1DM was strongly linked to the recessive genetic model ($p=0.007$) (Table 3).

Analysis of the relation between different inheritance genetic models, T1DM and HbA1c, and UACR

The patients were divided into two groups based on their HbA1c levels and the presence or absence of vascular complications. The first group included patients with HbA1c ≤ 7.0, indicating good glycemic control and the second category included patients with HbA1c > 7, denoting poor glycemic control [17]. The two levels of HbA1c were compared in the diabetic group and the different genetic models in the selected SNPs. As regards PDL-1 rs 2297137 SNP, the analysis revealed that concerning the general genetic model, in the patients' group with HbA1c ≤ 7.0, the number of patients having the GG genotype was higher than patients having the AG or AA genotypes (there was no p value since the subgroups had such a limited number of patients). Concerning the dominant genetic model, the number of patients with HbA1c level ≤ 7 was higher in patients with the GG or the GA genotypes (GA + GG) compared to patients with HbA1c level > 7 (no p value because the number of cases was small with the AA genotype). For the recessive genetic

Table 2 The relation between genotype and allele frequency distribution of PD-1 and PDL-1 in T1DM and control subjects

Variable	Patients (TIDM) *N* (%)	Control *N* (%)	Adjusted OR	95%CI	*p* value
PD-1 rs34819629					
GG	80 (100%)	76 (100%)	–		
GA	–	–			
CC	–	–	–		
PDL-1rs4143815					
Genotypes					
CC	2 (2.5%)	4 (5.3%)		0.123	
CG	30 (37.5%)	32 (42.1%)			
GG	48 (60.0%)	40 (52.6%)			
Allele					
C	34 (21.25%)	40 (26.3%)	1.675	0.957–2.934	0.071
G	126 (78.75%)	112 (73.7%)			
PDL-1 rs2297137					
Genotypes					
AA	2 (2.5%)	4 (5.3%)			0.018
GA	24 (30.0%)	28(36.8%)			
GG	54 (67.5%)	44 (57.9%)			
Allele					
A	28 (17.5%)	36 (23.7%)	2.523	1.325–4.802	0.005
G	132 (82.5%)	116 (76.3%)			

Rs, recognition site; CI, confidence interval; PD-1, programmed cell death protein 1; PDL-1, programmed death-ligand 1; OR, odds ratio

* Adjusted for gender, age, and BMI *Analysis of allele frequency distribution was done by the Hardy–Weinberg equation

Table 3 Risk of T1DM in the different genetic models of PDL-1 rs2297137 A/G and rs4143815 C/G SNPs

Variable	Patients (TIDM) *N* (%)	Control *N* (%)	Adjusted OR	95% CI	*p* value
PDL-1 rs4143815 C/G					
General model					
CC	2 (2.5%)	4 (5.3%)	1		
CG	30 (37.5%)	32 (42.1%)	34.9	0.672–1819.1	0.078
GG	48 (60.0%)	40 (52.6%)	47.2	0.924–2414.88	0.055
Dominant model					
CC	2 (2.5%)	4 (5.3%)	42.8	0.847–2169	0.061
CG + GG	78 (97.5%)	72 (94.7%)			
Recessive model					
GG	48 (60.0%)	40 (52.6%)	1.61	0.815–3.213	0.169
CC + GC	32 (40.0%)	36 (47.4%)			
PDL-1 rs2297137 A/G					
General model					
AA	2 (2.5%)	4 (5.3%)	1		
GA	24 (30.0%)	28 (36.8%)	3.52	0.334–37.24	0.295
GG	54 (67.5%)	44 (57.9%)	9.59	0.914–100.5	0.059
Dominant model					
AA	2 (2.5%)	4 (5.3%)	6.25	0.676–57.1	0.106
GA + GG	78 (97.5)	72 (94.7%)			
Recessive model					
GG	54 (67.5%)	44 (57.9%)	3.01	1.35–6.69	0.007
AA + GA	26 (32.5%)	32 (42.1%)			

CI, confidence interval; OR, odds ratio; PDL-1, programmed death-ligand 1; SNPs, single nucleotide polymorphisms; OR, odds ratio

model, patients with HbA1c levels greater than 7 were substantially more likely to have the GG genotype than those with the GA + AA genotype ($p = 0.001$).

In both the general and dominant genetic models of PDL-1 rs4143815, no significant difference was detected between patients with HbA1c ≤ 7 and those with HbA1c > 7. Regarding the recessive genetic model, the frequency of patients with the GG genotype was substantially higher for patients with HbA1c levels > 7% than for patients with the CC + CG genotypes ($p = 0.001$) (Table 4). We divided the patients into two groups to compare the association of the SNPs with the risk of diabetic nephropathy as a complication of T1DM. The first group included patients with urinary albumin creatinine ratio (UACR) < 30 mg/g creatinine, and the second group included patients with UACR ≥ 30 mg/g creatinine. As regards PDL-1 rs2297137 A/G, we found no significant relationship between the UACR and SNP in the three genetic models (general, dominant, and recessive) (no *p* value in the general genetic model and dominant genetic models due to a small number of cases having the AA genotype and $p = 0.474$in the recessive genetic model). Furthermore, in PDL-1 rs4143815 C/G SNP, we found no significant relationship between the SNP and UACR in the three genetic models (no *p* value in the general genetic model and dominant genetic models due to a small number of cases in the CC genotype group and $p = 0.819$ in the recessive model), as shown in Table 4.

Analysis of the relationship between several inheritance genetic models, T1DM, and demographic data

This analysis proved the relationship between demographic data, anthropometric measurements indicated by BMI, and different genetic models. The age of onset revealed no significant association between the inheritance of the different PDL-1 genetic models rs4143815 and rs2297137 (a *p* value could not be calculated for the general genetic model and dominant genetic model due to the small number of patients in the groups, while the recessive genetic model *p* value = 0.771 for rs4143815 and *p* value = 0.897 for rs2297137). Similarly, no significant difference between the different genetic models of inheritance could be detected in the relationship between gender variation and PDL-1 rs4143815 and rs2297137 (a *p* value for the general genetic model and dominant genetic model could not be calculated due to the small number of patients in the group, while the recessive genetic model *p* = value 0.180 for rs 4143815

Table 4 Different genetic models regarding hemoglobin A1c & urinary albumin/ creatinine ratio

Variable		Model type	HbA1c		UACR	
			(Good glycemic control) ≤ 7.0%	(Poor glycemic control) > 7%	< 30 mg/g creatinine (Normal)	≥ 30 mg/g creatinine (Abnormal)
PDL-1 rs4143815	General genetic model	CC	2	0	2	0
		CG	24	6	24	6
		GG	20	28	38	10
		p value	–	–		
	Dominant genetic model	CC	2	0	2	0
		CG + GG	44	34	62	16
		p value	–	–		
	Recessive genetic model	GG	20	28	38	10
		CC + GC	60	6	26	6
		p value	**< 0.001**	< 0.819		
PDL-1 rs2297137	General genetic model	AA	2	0	2	0
		GA	20	4	20	4
		GG	24	30	42	12
		p value	–	–		
	Dominant genetic model	AA	2	0	2	0
		GA + GG	44	34	62	16
		p value	–	–		
	Recessive genetic model	GG	24	30	42	12
		AA + GA	22	4	22	4
		p value	**0.001**	0.474		

UACR, urinary albumin/creatinine ratio; rs, recognition site; PDL-1, programmed death-ligand 1

Bold values indicate the p-value < 0.01 is highly significant

and $p = 0.147$ for rs2297137). Finally, there was no significant difference between BMI and the two PDL-1 genetic models, rs4143815 and rs2297137 (a *p* value could not be calculated for the general genetic model and dominant genetic model due to the small number of patients in the group, while the recessive genetic model $p = 0.372$ for rs 4143815 and $p = 0.109$ for rs2297137), as depicted in Table 5.

Haplotype analysis of the selected SNPs and their associated risk with the selected SNPs

In the Egyptian population, haplotype analysis revealed the presence of linkage disequilibrium between rs2297137 and rs 4143815 SNPs ($D' = 0.7795$, $r^2 = 7102$, $p = 0.0001$). As shown in Table 6, the frequency distribution of the haplotypes GAC, GGC, and GAG was insignificant between the healthy control subjects and T1DM patients ($p = 0.12$, $p = 0.55$, and $p = 0.6$, respectively).

Discussion

The PD-1/PD-L1 axis is essential for maintaining immunological homeostasis in many organs, including pancreatic tissues. In 1.8% of cancer patients treated with anti-PD-1 antibodies, blocking this route resulted in acute or chronic pancreatitis. In addition, evidence revealed that this inhibitory pathway is particularly significant in maintaining immunological tolerance towards insulin-producing pancreatic β cells and, hence, plays a significant role in protection against T1DM [6, 18]. Studies on transgenic mice have highlighted the importance of an intact PD-1/PD-L1 axis in protecting against organ-specific autoimmune diseases. Compared to controls, transgenic mice have revealed a decrease in the severity of insulitis, delay in the onset of the disease, and reduction in the overall diabetes incidence. In addition, the transgenic mice demonstrated a different nature of lymphocytes with lower proliferative potentials than the controls [19].

In the present study, three SNPs within the PD-1/PD-L1pathway were investigated to detect a possible involvement in developing T1DM in pediatric patients. In terms of PD-1 rs34819629 SNP, the current investigation revealed that all patients and healthy control subjects inherited the same genotype (GG genotype). Due to the current results, we could not detect a correlation between PD-1 rs34819629 and the risk of occurrence of

Table 5 Relation between demographic data and PDL-1 rs4143815 and rs2297137 in the patient group

SNP		Genotypes	Age at diagnosis (years)	*p* value	BMI (Mean ± SD)	*p* value	Gender		*p* value
							Male	Female	
PDL-1 rs4143815 C/G	General	CC	4.15 ± 0.07	NA	44.6 ± 0.0	NA	0 (0%)	2 (3.8)	NA
		CG	2.71 ± 0.53		17.9 ± 2.4		14 (50%)	16 (30.8%)	
		GG	2.48 ± 0.61		18.5 ± 3.2		14 (50%)	34 (65.4%)	
	Dominant	CC	4.15 ± 0.07	NA	44.6 ± 0.0	NA	0 (0%)	2 (3.8)	NA
		CG + GG	2.97 ± 0.58		18.3 ± 2.9		28 (100%)	50 (96.2%)	
	Recessive	GG	2.84 ± 0.61	0.771	18.5 ± 3.2	0.372	14 (50%)	34 (65.4%)	0.180
		CG + CC	2.80 ± 0.63		19.5 ± 7.0		14 (50%)	18 (34.6%)	
PDL-1 rs2297137 A/G	General	AA	2.20 ± 0.14	NA	25.0 ± 0.0	NA	0 (0%)	2 (3.8%)	NA
		GA	2.89 ± 0.61		20.3 ± 7.7		12 (42.9%)	12 (23.1%)	
		GG	2.82 ± 0.62		18.1 ± 3.0		16 (57.1%)	38 (73.1%)	
	Dominant	AA	2.20 ± 0.14	NA	25.0 ± 0.0	NA	0 (0%)	2 (3.8%)	NA
		GA + GG	2.84 ± 0.61		18.8 ± 5.0		28(100%)	50 (96.2%)	
	Recessive	GG	2.82 ± 0.62	0.897	18.1 ± 3.0	0.109	16 (57.1%)	38 (73.1%)	0.147
		GA + AA	2.83 ± 0.61		20.6 ± 7.5		12 (42.9%)	14 (26.9%)	

BMI, body mass index; NA, non-applicable; SNP, single nucleotide polymorphism; SD, standard deviation; PDL-1, programmed death-ligand 1

* *p* value is non-applicable due to the small number of cases

Table 6 Haplotype frequency analysis of rs34819629, rs2297137, and rs4143815 and its association with risk of type 1 diabetes mellitus

rs34819629	rs2297137	rs4143815	Patients (TIDM)	Control	OR	95% CI	*p* value
G	G	G	0.7465	0.70989	1.0		
G	A	C	0.134	0.2089	1.66	0.88–311	0.12
G	G	C	0.0785	0.0542	0.74	0.28–1.98	0.55
G	A	G	0.041	0.0279	0.70	0.19–2.65	0.6

T1DM, type 1 diabetes mellitus; CI, confidence interval; rs, recognition site; OR, odds ratio

T1DM. Our findings are consistent with those of Qian et al. [20], who found no association between T1DM and the PD-1 rs34819629 [20]. In contrast, several other studies have revealed a correlation between the SNP and other autoimmune diseases, including allergic bronchial asthma, systemic lupus erythematosus, and ankylosing spondylitis [21, 22]. The absence of association could be attributed to the differences in the pathogenesis between T1DM and other autoimmune diseases [20].

In terms of PD-L1 rs 4143815 SNP, the investigation revealed that the GG genotype and the major G allele were more frequent within the patients' group than in the control subjects. However, there was no significant difference between the groups (*p* value of 0.123 and 0.071, respectively). Similarly, in the stratification of the diabetic group, according to three genetic models to estimate the risk of T1DM in the presence of PD-L1 rs4143815, the results demonstrated that the diabetic group had a greater level of the major G allele in the three genetic models (general, dominant, and recessive) compared to the control group. Nevertheless, the difference did not reach a significance level ($p = 0.078$ and $p = 0.055$ for the general genetic model, $p = 0.061$ for the dominant model, and $p = 0.169$ for the recessive model, respectively). The findings of this study are consistent with those of Pizzaro et al. [23], who indicated that the significant G allele was more significant in the diabetic group than in the control group, but the difference between the two groups was not statistically significant ($p = 0.058$) [23]. In contrast, the results of Qian et al. indicated that PD-L1 rs4143815 was significantly associated with T1DM as the inheritance of the G allele was significantly associated with autoantibodies against islets antigens are used for T1DM diagnosis [20].

With respect to the potential association of PD-L1 rs2297137 with T1DM, our findings indicate the presence of a potential association between the SNP and the T1DM as the results revealed that the GG genotype and the major G allele are the most frequently inherited among the diabetic group in comparison to the control

group ($p = 0.018$ and $p = 0.005$). Moreover, on stratification of the diabetic and control groups according to three genetic models (the general, dominant and recessive genetic models) to estimate the possible risk associated with the presence of rs2297137 and T1DM, the results demonstrated that in the recessive genetic model, the major G allele was found to be strongly related with the risk of T1DM ($p = 0.007$).

The present study's findings are consistent with those of Pizzaro et al. [23], who reported a significant difference in allelic distribution, with the G allele being the most prevalent among the diabetic group compared to the control group ($p = 0.035$) [23, 24]. In contrast, Qian et al. found no correlation between PD-L1 rs2297317 and T1DM [20]. HbA1c is a significant indicator of chronic hyperglycemia and correlates with the possibility of long-term problems of diabetes because it is a helpful measure of glycemic control [25]. The current study found that HbA1c levels were significantly related to both recessive genetic models in PD-L1 rs4143815 and rs22937137 (p values < 0.001 and 0.001, respectively). Consequently, this implies that both PD-L1rs4143815 and rs22937137 may be related to the risk of chronic hyperglycemia and long-term diabetes complications.

Type one diabetes can affect anyone at any age, but it is most common in children and young people. Since the incidence of T1DM is substantially lower in young adults than in children, most investigations have focused on children under the age of 15 [26]. Since 1938, diabetes mellitus has increased by around 2%, with no gender differences in children aged 0–14, although males aged 15–39 years have a two-fold higher prevalence than females [27]. This finding is consistent with the current study results, as comparing the relation of rs4143815 and rs2297137 with the age of onset of the disease and gender variation did not reveal a significant difference in the three genetic models. Furthermore, a comparison of the BMI of the diabetic group and rs4143815 and rs2297137 in the three genetic models revealed no significant relation.

The current study results on the association of PD-L1 rs2297317 and T1DM demonstrate the importance of PD-L1 as part of the PD-1/PD-L1 pathway in protection against T1DM. Evidence indicates the importance of an intact PD-1/PD-L1 axis in protecting against autoimmune diseases, especially organ-specific ones. This inhibitory pathway contributes to protecting against T1DM in humans and mice. T1DM develops in nonobese diabetic mice with PD-1 or PD-L1 deficiency [28, 29].

Preclinical studies have shown that in prediabetic nonobese diabetic mice, PD-1 or PD-L1 blockade was responsible for developing T1DM [5]. Other studies have shown that PD-L1 plays a protective role against autoimmune diabetes [30]. It has been hypothesized that PD-L1 expression on parenchymal cells may inhibit autoreactive $CD4^{+}T$ cell tissue destruction and effector cytokine production and hence plays a protective role against autoimmune DM [31, 32]. Moreover, PD-L1 blockade has led to the inhibition of T cell migration, extended T cell and dendritic cell engagement, increased the production of cytokines by T cells, augmented T-cell receptor (TCR) signaling, and abrogated peripheral tolerance [33]. Therefore, these findings may explain the results of the current study's findings and the impact of PD-L1 rs 2297137 on the risk of T1DM.

The discrepancies between our study's findings and other studies that investigated the association between the PD-1/PD-L1 pathway and T1DM could be explained by several factors, including variations in the analytical methods utilized in the research, discrepancies in the ethnic origins of the examined population, and difference in the sample size and characteristics of the studied populations.

Limitations

The present study has some limitations; first, pancreatic antibodies were not measured as anti-GAD65 antibodies, insulin autoantibodies, or islet cell antibodies to determine their correlation with susceptibility to the studied genotypes. Second, the current study could not interpret the correlation between genotype and allele frequency distribution with the prevalence of diabetic ketoacidosis at presentation. In order to determine the effect of the SNPs on gene expression, the expression pattern of the genes should be determined, and detection of the serum level of PD-1 and PDL-1 should be considered to correlate the results. In addition, the sample size was limited. Future studies with a larger number of patients are required to generalize the results.

Conclusion

The present study demonstrated that the predominant G allele of PD-L1 rs2297137 is associated with the development of T1DM. Both PD-L1 SNPs (rs4143815 and rs2297137) are associated with the risk of chronic hyperglycemia and long-term diabetes complications within the study groups.

Abbreviations

CD: Cluster of differentiation; HbA1c: Hemoglobin A1c; PD-1: Programmed death cell protein-1; PD-L1: Programmed death-ligand; rs: Recognition site; RT-PCR: Real-time polymerase chain reaction; SD: Standard deviation; SNPs: Single nucleotide polymorphisms; T1DM: Type 1 diabetes mellitus; TCR: T-cell receptor; UACR: Urinary albumin creatinine ratio.

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RA contributed significantly to the preparation of this study by analyzing and interpreting the patient data related to genotyping. MH was primarily responsible for the writing of the manuscript. All authors read and approved the final manuscript.

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Declarations

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Consent for publication
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Competing interests
The authors declare that they have no competing interests.

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