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Evaluation of the Frequency of (rs2227981 and rs2282055) Single Nucleotide Polymorphisms in Programmed Cell Death 1 and Its Ligand Genes of Iraqi Patients With Multiple Sclerosis

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Abstract

Multiple sclerosis (MS) is a central nervous system disease that causes demyelination and persistent inflammation and most of them among young adults. The current study aimed to assess genetic polymorphism in Programmed Cell Death 1(PDCD1) and Its ligand (PDLCD1) genes and the frequency of two single nucleotide polymorphisms (SNPs) (rs2227981 and rs2282055). The study design is case-control that has been achieved at Medical City and Al-Yarmouk teaching hospital from November 2021 to February 2022, Baghdad, Iraq. MS patients (n=100) were divided into two groups; newly diagnosed (n=42), and patients with ongoing treatments (58). These groups were compared to healthy subjects (n=55). The study for gene polymorphism of PDCD1 and PDLCD1 and the Frequency of Two SNPs (rs2227981 and rs2282055) were measured by Real-time Polymer Chain Reaction (RT-PCR) by using High-Resolution Melting (HRM). The results of this study found that the genotypic frequencies of MS patients were 31% (n=31) normal AA and 48 % (n=48) heterozygous AG. Mutant homozygous was found in GG 21 % (n=21). In controls, the results demonstrate 69% (n=38) wild-type AA, 27.27% (n=15) heterozygous AG, and mutant homozygous GG 3.6% (n=2). The odds ratio for the GG genotype was 12.8 (0.2-5.9) with p=0.001 indicating that the homomutant genotype. In PDCD1 gene polymorphism rs2227981 GG was at a higher risk of MS than the wild-type PDCD1 gene polymorphism rs2227981 AA. The PDCD1 gene polymorphism rs2227981 AG genotype has the second risk of MS after PDCD1gene polymorphism rs2227981GG with 3.92-fold high risk than the wild type AA (OR =3.92 (1.8-8.2) p=0.0003). In conclusion, the population-based case-control study, PDCD1, and PDLCD1 SNPs may be related to the pathogenesis of MS. PDCD1gene SNP loci rs2227981 and PDLCD1gene SNP loci rs2282055 may be a new candidate polymorphic locus for MS

Keywords: Multiple sclerosis, Polymorphism, PDCD1, PDLCD1, rs2227981, rs2282055.

تقييم تكرار (rs2227981 و rs2282055) تعدد أشكال النوكليوتيدات المفردة في جينات موت الخلية المبرمج ١ وجينات موت الخلايا الترابطية المبرمجة ١ في المرضى العراقيين المصابين بالتصلب المتعدد

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الخلاصة

التصلب المتعدد هو أحد أمراض الجهاز العصبي المركزي الذي يسبب إزالة المايلين والالتهاب المستمر ومعظمها بين الشباب البالغين. هدفت الدراسة الحالية إلى تقييم تعدد الأشكال الجيني في موت الخلايا المبرمج ١ (PDCD1) وجينات موت الخلايا الترابطية المبرمجة ١ (PDLCD1) وتكرار اثنين من تعدد أشكال النوكليوتيدات المفردة (SNPs) (rs2227981 و rs2282055). تصميم الدراسة هو حالة المقارنة مع الاشخاص الاصحاء تم انجازه في مدينة الطب ومستشفى اليرموك التعليمي من تشرين الثاني ٢٠٢١ إلى شباط ٢٠٢٢ ، بغداد ، العراق. مرضى

التصلب المتعدد (العدد = ١٠٠) تم تقسيمهم الى مجوعتين المشخصين حديثاً بالمرض (العدد = ٤٢) و المرضى الذين العلاجات المستمرة (العدد = ٥٨). هاتان المجموعتان تم مقارنتهم مع اشخاص اصحاء (العدد = ٥٥). تم عمل دراسة تعدد الأشكال الجيني لـ PDCD1 و PDLCD1 وتكرار اثنين من SNPs (rs2227981 و rs2282055) بواسطة تفاعل البوليمرات المتسلسل في الوقت الحقيقي (RT-PCR) باستخدام الذوبان عالي الدقة (HRM). وجدت نتائج هذه الدراسة أن التكرارات الوراثية لمريضى التصلب المتعدد كانت ٣١٪ (العدد= ٣١) AA و ٤٨٪ (العدد= ٤٨) AG, تم العثور على متحولة متماثلة الزايجوت في GG 21 ٪ (العدد = ٢١). من الاصحاء ، أظهرت النتائج ٦٩ ٪ (العدد = ٣٨) النوع البري AA، ٢٧,٢٧ ٪ (العدد = ١٥) AG متغاير الزايجوت ومتجانسة GG 3.6 ٪ (العدد = ٢). كانت نسبة الأرجحية للنمط الجيني GG ١٢,٨ (٠,٢ – ٥,٩) مع P=0.001 مما يشير الى ان النمط الجيني المتماثل في تعدد اشكال جين PDCD1 rs2227981 GG اكثر عرضة للاصابة من تعدد الاشكال الجيني PDCD1 من النوع البري rs2227981 AA. يحتوي النمط الجيني PDCD1 rs2227981 AG على خطر الاصابة الثاني بمرض التصلب المتعدد بعد تعدد اشكال جين PDCD1 rs2227981 GG مع مخاطر عالية بمقدار ٣,٩٢ اكثر من النوع البري AA (OR=3.92) (٨,٢-١,٨) p= 0.0003.

تلخص الدراسة الحالية إلى أن دراسة الحالات والشواهد المستندة على السكان ، PDCD1 PDLCD1 SNPs قد تكون مرتبطة بإمراضية مرض التصلب المتعدد. PDCD1gene SNP loci rs2227981 و PDLCD1gene SNP loci rs2282055 قد يكون مرشحا جديداً لتعدد الاشكال loci لمرض التصلب المتعدد.

الكلمات المفتاحية: التصلب المتعدد ، تعدد الأشكال ، موت الخلية المبرمج ١ ، جينات موت الخلايا الترابطية المبرمجة ١ ،rs2282055 ،rs2227981 .

Introduction

The central nervous system is affected by chronic inflammatory, autoimmune, and demyelinating diseases known as multiple sclerosis (MS) and most of multiple sclerosis patients are young adults [(1)]. Its four clinical presentations are progressive relapsing MS (PRMS), secondary progressive MS (SPMS), primary progressive MS (PPMS), and relapsing-remitting MS (RRMS). Relapsing-remitting MS (RRMS) which affects roughly 87 percent of patients, is characterized by acute attacks (relapses), which are followed by partial or total recovery (remission) [(2)].

Patients may exhibit a wide range of symptoms, such as vision alterations (unilateral visual loss, diplopia), weakness, poor coordination, sensory loss, or modifications to bowel and bladder movements. Cognitive shifts, exhaustion, and temper disruption are less diagnostic but nevertheless incapacitating symptoms. A severe disability could potentially be resulted from a disease's progression [(3)]. It is possible to treat MS symptoms using a variety of drugs and other methods. The treatment of patients with the relapsing forms of this disease has been transformed by the accessibility of disease-modifying medicines [(4)]. These drugs probably work by reducing immune-mediated inflammation to manage the underlying illness process. They neither treat the illness nor repair the harm caused by earlier occurrences. When these medications are administered to patients before more serious, widespread injury and incapacity have taken place, their effects typically seem to be more powerful [(5)]. The etiology of MS is complex, involving both genetic and environmental hazards [(6)]. There are several theories that address the causes of MS. The most generally recognized explanation is that of inherited causality. Genetic studies are essential in the case of MS.

According to the hypothesis put forth by Ortler et al., who also demonstrated for the first time that it was highly expressed in the inflammatory regions of white matter in MS patients and was likely expressed on activated microglia and macrophages, B7-H1 expression may reduce T cell activation and promote immune homeostasis in the CNS [(7)].

MS progression is thought to be influenced by PDCD1gene polymorphism. Patients with MS who have a PDCD1 gene polymorphism may have an impairment to their T cells' ability to suppress interferon (IFN) [(8)] It is possible that PDCD1 and PDLCD1 also have an immunosuppressive effect because MS patients have considerably more PDCD1 and PDLCD1 + interleukin (IL)-10+CD14+ and PDLCD1+IL-10+CD19+ cells during the remitting phase compared to the relapsing phase. Inhibiting PD-1 in lymphocytes during the acute phase of MS significantly increases the proliferation of CD4+ T cells and CD8+ T cells. However, this impact is absent when PDLCD1 expression in APC is inhibited, proving that PDCD1 is more effective than PDLCD1 in promoting lymphocyte mortality and reducing proliferation in MS [(9)]. Additionally, the PDLCD1 gene has a crucial causative role in the development of cancer, its low expression in cells serves as a reliable marker of how well a treatment is working [(10)]. The current study aimed to evaluate the frequency of two SNPs (rs2227981 and rs2282055) polymorphisms in PDCD1 and PDLCD1 genes of Iraqi individuals with multiple sclerosis in comparison with apparently healthy subjects.

Materials and Methods

This case control study has been achieved at Medical City and Al-Yarmouk teaching hospital from November 2021 to February 2022 in Baghdad, Iraq. A Total of (100) MS Iraqi patients (42 males and 58 females) were enrolled in the current study, divided into two groups 58 old MS patients (24 males and 34 females) and 42 newly diagnosed MS patients (18 males and 24 females), and compared with 55 healthy controls (24 males and 31 females). Permissions were obtained from the medical city hospitals in Baghdad, Iraq, and approved by the University of Technology's institutional ethical committee, Baghdad, Iraq (Ref. No. AS 1974-17-

10- 2021) in accordance with the Helsinki Declaration of 1975 revised in 2000[11]. All participants were informed about the study design and objectives and signed informed consent before the collection of any data or samples. The medical history for all patients has been taken, and detailed clinical checks with a particular questionnaire formula occupied for each patient contain; the duration of disease, age, height, body weight, type of drug, and symptoms include (vision problems, bladder dysfunction, muscle stiffness and spasms, tremor, slurred speech, fertility status, patient's family history of MS, and other diseases including diabetes mellitus or hypertension), The neurologists in the hospitals where the study was achieved used several strategies to determine criteria for an MS diagnosis and to rule out other possible causes of whatever symptoms the patients were experiencing. These strategies include a careful medical history, a neurologic exam, various tests including magnetic resonance imaging (MRI) and further testing with spinal fluid analysis, evoked potentials and additional imaging may be needed [12].

Blood sampling was three ml of whole blood from each participant had to be drawn straight from the vein into an EDTA-containing tube, and the operation had to be done in an aseptic manner. DNA extraction was done using the EasyPure® Blood Genomic DNA Kit, the DNA was extracted and purified from human whole blood samples (TransGen, biotech. EE121-01). Gel electrophoresis was performed to verify that the DNA had been extracted and was present on the Agarose gel following the extraction process [13]. The estimation of DNA concentration and purity was done Using a NanoDrop device, the concentration of DNA samples was estimated. In order to measure the concentration in ng/L at 260 nm wavelength and determine the purity of the DNA, one microliter of the extracted DNA was inserted in the lens of the device. For DNA, a ratio of (1.8 – 2.0) is generally regarded as "pure," and when the ratio falls below (1.8). It can be a sign of the presence of pollutants like protein, phenol, or other substances that absorb intensely at or close to 280 nm [14]. Genotyping of polymorphisms was done by using the Real Time Polymer Chain Reaction technique (RT-PCR) [15] to analyze the SNPs: PDCD1 for rs2227981 situated in exon 5 and PDLCD1 for rs2282055 located in intron 1. The primers were constructed in accordance with the full sequences of PDCD1 and PDCDL1, The DNA sequences for the PDCD1 and PDLCD1 genes were obtained from the GenBank database of the National Center for Biotechnology Information (NCBI). The PCR primers, were created using Premier 3 software (version 0.4.0), with annealing temperatures of 58°C , primer size PDCD1 for rs2227981 SNP were 88 bp and PDLCD1 for rs2282055 SNP were 52 bp .The company for Easy Pure ® "Genomic DNA" was "Trans Gen bio tech" ,China. Table 1 contains the primer sequence and prerequisites.

Table 1. Designed Primers used in the present study.

	Sequence (5′→3′ direction)	**Band size (bp)**
	PDCD1 for rs2227981	
Forward	GTCCATCCTCAGGCCTCAG	88
Reverse	CCTAGCGGAATGGGCAC	
	PDLCD1 for rs2282055	
Forward	AAGTCAGATTCTCCTTGCTCT	52
Reverse	ACCTAGTAGAACCTGCCCTGT	

Real Time PCR (RT–PCR) for Genotyping Analysis was done by using the Qiagen Real-time PCR System was used to perform RT-PCR (Rotor gene with real time polymer chain reaction (RT–PCR) software 2.3.4). The TransStart®Tip Green RT–PCR Super Mix Kits (Eva green TransGen, biotech. EE101-01) components were used to quantify gene polymorphism by HRM as indicated in (Table 2). A non-template control (NTC), a non-amplification control (NAC), and a non-primer control were used as negative controls in each reaction that was carried out twice (NPC) [16].

Table 2. Reaction component and volume for RT PCR used in PDCD1 and PDLCD1 Genes SNP experiment.

Component	**Final Volume reaction (µl)**
Template	4
Forward Primer (10 µM)	1
Reverse Primer (10 µM)	1
2xEasyTaq® PCR SuperMix	12.5
Nuclease-free Water	6.5
Total volume	25

The heat profile shown in, was used to set the cycling protocol for the RT–PCR Reaction Run for HRM Analysis (Table 3).

Table 3. Thermal profile used in Genotyping SNPs.

Step	Temperature (ºC)	Duration	Cycles
Enzyme activation	94	60 sec	1
Denaturation	94	5 sec	40
Annealing	58	15 sec	
Extension	72	20 sec	
HRM	65-95	0.2sec for 1 degree	1

Statistical analysis

Statistical Analysis System (version 9.1) for Windows was used to conduct the statistical analysis. Both patients and healthy controls were used to test Hardy-Weinberg equilibrium (HWE). Per genotype and per allele analysis was performed using logistic regression, and the odds-ratio with a 95% confidence interval was estimated. The five percent significance level was used.

Results and Discussion

Genotypes and alleles frequency of rs2227981 SNP at PDCD1

This study examines rs2227981 SNP at PDCD1 among Iraqi patients with MS disease and in apparently healthy controls. The distribution of genotype and allele frequencies among groups compared with the control group for the PDCD1 gene polymorphism rs2227981 is shown in Table (4). The genotypic frequencies of MS patients were 31% (n=31) normal AA and 48 % (n=48) heterozygous AG. Mutant homozygous was found in GG 21 % (n=21). In controls, the results demonstrate 69% (n=38) wild-type AA, 27.27% (n=15) heterozygous AG, and mutant homozygous GG 3.6% (n=2). The results of genotype frequencies of MS patient analysis shown in Table (4) reveal that the wild-type genotype and wild-type allele were taken as reference. In PDCD1 gene polymorphism rs2227981, the odds ratio for the GG genotype was 12.8 (0.2-5.9) with p=0.001 indicating that homomutant genotype. In PDCD1 gene polymorphism rs2227981 GG was a higher risk of MS than the wild type PDCD1 gene polymorphism rs2227981 AA, The PDCD1 gene polymorphism rs2227981 AG genotype has the second risk of MS after PDCD1 gene polymorphism rs2227981GG with 3.92 fold high risk than the wild type AA (OR =3.92 (1.8-8.2) p=0.0003). The A allele frequency values were 83% and 55% for apparently healthy subjects and MS patients. Also, G allele frequency values were 17% and 45% for apparently healthy subjects and MS patients.

Table 4. Genotypes and alleles frequencies of the PDCD1 gene polymorphism rs2227981 for both patients and control group

PDCD1 **rs2227981**	**Frequencies (%)**		**P value**	**Odd ratio (95% CI)**
	Healthy (n=55)	**Patient (n=100)**		
AA	(n=38) 69%	(n=31) 31%	-----	1.00 (Reference)
AG	(n=15) 27.27%	(n=48) 48%	0.003	3.92 (1.8-8.2)
GG	(n=2) 3.6%	(n=21) 21%	0.001	12.8 (0.2-5.9)
A	(n=91) 83%	(n=110) 55%	----	1.00 (Reference)
G	(n=19) 17%	(n=90) 45%	0.0001	3.9 (2.2-6.9)

The distribution of genotype and allele frequencies among newly Patient group compared with the control group for the PDCD1 gene polymorphism rs2227981 is shown in table (5). The genotypic frequencies of newly multiple sclerosis patients were 40.47% (n=17) normal AA and 45.2 % (n=19) heterozygous AG. Mutant homozygous was found in GG 14.28 % (n=6). In controls, the results demonstrate 69% (n=38) wild type AA, 27.27% (n=15) heterozygous AG and mutant homozygous GG 3.6% (n=2). The results of genotype frequencies of MS patient analysis shown in table (5) reveal that the wild type genotype and wild-type allele were taken as reference.

In PDCD1 gene polymorphism rs2227981, the odds ratio for the GG genotype was 6.7 (0.1-3.6) with p=0.02 indicating that homomutant genotype In PDCD1 gene polymorphism rs2227981 GG was a

higher risk of MS than the wild type PDCD1 gene polymorphism rs2227981 AA, The PDCD1 gene polymorphism rs2227981 AG genotype has the second risk of MS after PDCD1 gene polymorphism rs2227981 GG with 2.8-fold high risk than the wild type AA (OR =2.8 (1.1-6.8)) with p=0.02.

The A allele frequency values were 83% and 63% for apparently healthy subjects and newly multiple sclerosis patients. Also, G allele frequency values were 17% and 37% for apparently healthy subjects and newly multiple sclerosis patients, respectively Table (5).

Table 5. Genotypes and alleles frequencies of the PDCD1 gene polymorphism rs2227981 for both the newly diagnosed patients and control group.

PDCD1 **rs2227981**	**Frequencies (%)**		**P value**	**Odd ratio (95% CI)**
	Healthy (n=55)	**Newly Patient (n=42)**		
AA	(n=38) 69%	(n=17) 40.47%	----	1.00 (Reference)
AG	(n=15) 27.27%	(n=19) 45.2%	0.02	2.8 (1.1-6.8)
GG	(n=2) 3.6%	(n=6) 14.28%	0.02	6.7 (0.1-3.6)
A	(n=91) 83%	(n=53) 63%	-----	1.00 (Reference)
G	(n=19) 17%	(n=31) 37%	0.002	2.8 (1.4-5.4)

The distribution of genotype and allele frequencies among patients with ongoing treatments group compared with Healthy for the PDCD1 gene polymorphism rs2227981 is shown in table (6). The genotypic frequencies of patients with ongoing on treatments of multiple sclerosis were 24.13% (n=14) normal AA and 50 % (n=29) heterozygous AG. Mutant homozygous was found in GG 25.86 % (n=15). In controls, the results demonstrate 69% (n=38) wild type AA, 27.27 (n=15) heterozygous AG and mutant homozygous GG 3.6% (n=2). The results of genotype frequencies of MS patient analysis shown in table (6) reveal that the wild type genotype and wild type allele were taken as reference.

In PDCD1gene polymorphism rs2227981, the odds ratio for the GG genotype was 20.3 (0.4-10.0) with p=0.0002 indicating that homomutant genotype In PDCD1 gene polymorphism rs2227981 GG was a higher risk of MS than the wild type PDCD1 gene polymorphism rs2227981 AA, The PDCD1 gene polymorphism rs2227981 AG genotype has the second risk of MS after PDCD1 gene polymorphism rs2227981 GG with 5.2 fold high risk than the wild type AA (OR =5.2 (2.1-12.5) with p=0.0002.The A allele frequency values were 83% and 49% for apparently healthy subjects and newly multiple sclerosis patients. Also, G allele frequency values were 17% and 50.9% for apparently healthy subjects and patients with ongoing multiple sclerosis treatments, respectively (Table 6.).

Table 6. Genotypes and alleles frequencies of the PDCD1 gene polymorphism rs2227981 for both patients undergoing therapy and control group.

PDCD1 **rs2227981**	**Frequencies (%)**		**P value**	**Odd ratio (95% CI)**
	Healthy (n=55)	**Patients with ongoing treatments (n=58)**		
AA	(n=38) 69%	(n=14) 24.13%	----	1.00 (Reference)
AG	(n=15) 27.27%	(n=29) 50%	0.0002	5.2 (2.1-12.5)
GG	(n=2) 3.6%	(n=15) 25.86%	0.0002	20.3 (0.4-10.0)
A	(n=91) 83%	(n=57) 49.1%	----	1.00 (Reference)
G	(n=19) 17%	(n=59) 50.9%	0.0001	4.9 (2.6-9.1)

Genotypes and alleles frequency of rs2282055 SNP at PDLCD1

This study examines rs2282055 SNP at PDLCD1 among Iraqi patients with MS disease and in apparently healthy controls. The distribution of genotype and allele frequencies among groups compared with Healthy for the PDLCD1 gene polymorphism rs2282055 is shown in Table (7). The genotypic frequencies of MS patients were 42% (n=42) normal TT and 43 % (n=43) heterozygous TG. Mutant homozygous was found in GG 15 % (n=15). In controls, the results demonstrate 74.5% (n=41) wild type TT, 16.36% (n=9) heterozygous TG and mutant homozygous GG 9%(n=5). The results of genotype frequencies of MS patient analysis shown in table (7) reveal that the wild type genotype and wild type allele were taken as reference. In PDLCD1 gene polymorphism rs2282055, the odds ratio for the GG genotype was 2.9 (0.9-8.7) with p=0.05 indicating that homomutant genotype. In PDLCD1 gene polymorphism rs2282055 GG was at a higher risk of MS than the wild type PDLCD1 gene polymorphism rs2282055 TT, The PDLCD1 gene polymorphism rs2282055 TG genotype has the first risk of MS after PDLCD1gene polymorphism rs2282055 GG with 4.6-fold high risk than the wild type TT (OR =4.6 (2.0-10.7) p=0.0003). The T allele frequency values were 82.7% and 63.5% for apparently healthy subjects and MS patients. Also, G allele frequency values were 17.27% and 36.5% for apparently healthy subjects and MS patients, respectively (Table 7).

Table 7. Genotypes and alleles frequencies of the PDLCD1gene polymorphism rs2282055 for both patients and control group.

PDLCD1 **rs2282055**	**Frequencies (%)**		**P value**	**Odd ratio (95% CI)**
	Healthy (n=55)	**Patient (n=100)**		
TT	% (n=41) 74.5%	(n=42) 42%	----	1.00 (Reference)
TG	% (n=9) 16.36%	(n=43) 43%	0.003	4.6 (2.0-10.7)
GG	% (n=5) 9%	(n=15) 15%	0.05	2.9 (0.9-8.7)
T	(n=91) 82.7%	(n=127) 63.5%	----	1.00 (Reference)
G	(n=19) 17.27	(n=73) 36.5%	0.0005	2.7 (1.5-4.8)

The distribution of genotype and allele frequencies among newly Patient groups compared with Healthy for the PDLCD1 gene polymorphism rs2282055 is shown in table (8). The genotypic frequencies of MS patients were 42% (n=42) normal TT and 43 % (n=43) heterozygous TG. Mutant homozygous was found in GG 15 % (n=15). In controls, the results demonstrate 74.5% (n=41) wild-type TT, 16.36% (n=9) heterozygous TG, and mutant homozygous GG 9% (n=5). The results of genotype frequencies of MS patient analysis shown in Table (8) reveal that the wild-type genotype and wild-type allele were taken as reference. In PDLCD1 gene polymorphism rs2282055, the odds ratio for the GG genotype was 2.9 (0.9-8.7) with p=0.05 indicating that homomutant genotype. In PDLCD1 gene polymorphism rs2282055 GG was at a higher risk of MS than the wild type PDL1 gene polymorphism rs2282055 TT, The PDLCD1 gene polymorphism rs2282055 TG genotype has the first risk of MS after PDCD1 gene polymorphism rs2282055 GG with 4.6-fold high risk than the wild type TT (OR =4.6 (2.0-10.7) p=0.0003). The T allele frequency values were 82.7% and 63.5%for apparently healthy subjects and MS patients. Also, G allele frequency values were 17.27 and 36.5% for apparently healthy subjects and MS patients, respectively (Table 8).

Table 8. Genotypes and alleles frequencies of the PDLCD1gene polymorphism rs2282055 for both the newly diagnosed patients and control group.

PDLCD1 **rs2282055**	**Frequencies (%)**		**P value**	**Odd ratio (95% CI)**
	Healthy (n=55)	**Patient newly (n=42)**		
TT	% (n=41) 74.5%	% (n=10) 23.8%	-----	1.00 (Reference)
TG	% (n=9) 16.36%	% (n=26) 61.9	0.0001	11.8 (0.4-3.3)

Continued Table 8.

GG	% (n=5) 9%	% (n=6) 21.4%	0.02	4.9 (1.2-19.4)
T	(n=91) 82.7%	(n=46) 54.8%	-----	1.00 (Reference)
G	(n=19) 17.27	(n=38) 45.2%	0.0001	3.9 (2.0-7.6)

The distribution of genotype and allele frequencies among patients with ongoing treatments groups compared with Healthy for the PDLCD1 gene polymorphism rs2282055 is shown in table (9). The genotypic frequencies of ongoing multiple sclerosis treatments were 55.17% (n=32) normal TT and 29.3 % (n=17) heterozygous TG. Mutant homozygous was found in GG 15.5 % (n=9). In controls, the results demonstrate 74.5% (n=41) wild type TT, 16.36% (n=9) heterozygous TG and mutant homozygous GG 9 %(n=5). The results of genotype frequencies of MS patient analysis shown in table (9) reveal that the wild-type genotype and wild-type allele were taken as reference. In PDLCD1 gene polymorphism rs2282055, the odds ratio for the GG genotype was 2.3 (0.7-7.5) with p=0.16 indicating that the homomutant genotype In PDLCD1 gene polymorphism rs2282055 GG was at a higher risk of MS than the wild type PDLCD1 gene polymorphism rs2282055 TT, The PDLCD1 gene polymorphism rs2282055 TG genotype has the first risk of MS after PDLCD1 gene polymorphism rs2282055 GG with 2.4-fold high risk than the wild type TT (OR =2.4 (0.9-6.1) p=0.06). The T allele frequency values were 82.7% and 69.8%for apparently healthy subjects and ongoing multiple sclerosis treatments. Also, G allele frequency values were 17.27% and 30.2%%for apparently healthy subjects and MS patients, respectively (Table 9).

Table 9. Genotypes and alleles frequencies of the PDLCD1gene polymorphism rs2282055 for both patients undergoing therapy and control group.

PDLCD1 rs2282055	**Frequencies (%)**		**P value**	**Odd ratio (95% CI)**
	Healthy (n=55)	**Patients with ongoing treatments (n=58)**		
TT	% (n=41) 74.5%	% (n=32) 55.17%	-----	1.00 (Reference)
TG	% (n=9) 16.3	% (n=17) 29.3%	0.06	2.4 (0.9-6.1)
GG	% (n=5) 9%	% (n=9) 15.5	0.16	2.3 (0.7-7.5)
T	(n=91) 82.72%	(n=81) 69.8%	-----	1.00 (Reference)
G	(n=19) 17.27%	(n=35) 30.2%	0.02	2.0 (1.0-3.9)

T and B cell activation is primarily regulated by the inhibitory immunoreceptor, Programmed Cell Death-1 (PDCD1), a member of the B7/CD28 superfamily, which is expressed on T, B, and myeloid cells. Increasing evidence indicates that activation of PDCD1 by its ligands PDLCD1 (B7-H1) or PDLCD2 (B7-DC) suppresses T cell receptor-mediated proliferation and cytokine generation in activated T cells [(17)]. PDCD1 exclusively controls T cell function in suboptimal T cell receptor activation and CD28 co-stimulation circumstances, which is consistent with its function in preserving self-tolerance [(18)]. Cytokines (e.g., IL-6, IL-10), the degree of co-stimulation, and TLR signaling all have an impact on the result of PDCD1 activation. [(19)] Animal models of MS provide evidence (EAE) PDCD1/PDLCD1/2 pathway involvement in the etiology of MS is strongly confirmed [(20)] Taken together, these data point to the PD-1 molecule as a potential key player in the pathogenesis of MS. Pawlak-Adamska and his colleagues believed that the PDCD1 polymorphic variation may be MS risk as well as prediction factors in a direct or indirect manner because genetic variation may interfere with the proper expression and function of encoded molecules. From an autoimmune perspective, PDCD1-mediated suppression of T cell proliferation and IFN production (in a dose-dependent manner) indirectly affects the levels of PDCD1 following optimum stimulation by lowering basal and induced PDCD1 expression at early-to-intermediate stages of CD4+T cell activation. Therefore, it has been proposed that PDCD1 expression and function are subject to aberrant control [(21)]. Two more SNPs were included in the analysis: a synonymous mutation at PDCD1.5

rs2227981 (NP 005009.2: p. Ala268=) and a missense change at codon 215 (NP 005009.2: p. Ala215Val), both of which are found in exon 5 of the PDCD1. Only two studies have examined the relationship between polymorphism variation in the PDCD1 gene and multiple sclerosis to date, and none have examined the functional implications of those SNPs [22]. Investigating the possible role of this co-stimulatory pathway in MS patients suffering from various disease subtypes and to determine whether immunotherapy has any effects on the expression of these molecules. PDLCD1-expressing CD19 and CD14 cells in myelin basic protein MBP-stimulated cultures of all the participants in the study; results suggested that PDCD1/ PDLCD1interaction stimulates both IL-10 production and apoptosis of Ag-specific [23].

Conclusion

This study potentially demonstrates an association of PDCD1 and PDLCD1 gene polymorphisms, rs2227981 and rs2282055 in Iraqi patients with Multiple Sclerosis. The study found that the genotypic frequencies of MS patients were 31% normal AA and 48 % heterozygous AG. Mutant homozygous was found in GG 21 %. In controls, the results demonstrate 69% wild-type AA, 27.27% heterozygous AG, and mutant homozygous GG 3.6%. In PDCD1 gene polymorphism rs2227981 GG was at a higher risk of MS than the wild-type PDCD1 gene polymorphism rs2227981 AA. The PDCD1 gene polymorphism rs2227981 AG genotype has the second risk of MS after PDCD1 gene polymorphism rs2227981GG with 3.92-fold high risk than the wild type AA. There were substantially more PDLCD1 gene SNP loci in MS patients compared to controls. PDLCD gene SNP locus rs2282055 and PDCD1gene SNP locus rs2227981 might be potential MS candidate polymorphism loci and played a critical role in immune responses and subsequently the MS.

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Conflicts of Interest

There are no conflicts of interest.

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Ethics Statements

Permissions were obtained from the medical city hospitals in Baghdad, Iraq, and approved by the University of Technology's institutional ethical committee, Baghdad, Iraq (Ref. No. AS 1974-17-10- 2021) in accordance with the Helsinki Declaration of 1975 revised in 2000.

Author Contribution

The authors confirm contribution to the paper as follows: study conception and design: Sarah Abdulhameed Sahib, Ghassan Mohammad Suliman, Huda Jaber Waheed; data collection: Sarah Abdulhameed Sahib; analysis and interpretation of results: Sarah Abdulhameed Sahib, Ghassan Mohammad Suliman, Huda Jaber Waheed draft manuscript preparation: Sarah Abdulhameed Sahib, Huda Jaber Waheed. All authors reviewed the results and approved the final version of the manuscript.

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