Original Article

Impact of IL-21 Gene Polymorphisms (rs2055979) and the Levels of Serum IL-21 on the Risk of Multiple Sclerosis

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Abstract

Multiple sclerosis (MS) is a chronic autoimmune disorder of the central nervous system. Genetic and environmental factors have important roles in the induction, onset, and progression of MS. In this study, the IL-21 genotype (rs2055979) (G/T) in Iraqi MS patients was compared with a healthy control group to investigate the possible association of any particular genotype or allele with multiple sclerosis. This study included 70 patients with relapsing-remitting MS and 50 healthy individuals as control. Following the extraction of genomic DNA, polymerase chain reaction-restriction fragment length polymorphism, the frequencies of genotypes, and alleles were calculated and statistically analyzed. The results of the study revealed a significant reduction in the distribution of the wild homozygous genotype (GG) in MS patients, in comparison to a healthy control group (14.3% vs. 34 %; 0.0129 at $P \leq 0.05$; odds ratio [OR] 3.0909, 95% confidence interval [CI]: 1.2704-7.5203). However, MS cases and controls did not differ significantly in neither GT nor TT genotypes, 62.9% (OR 0.6402, 95% CI: 0.3064-1.3374) and 52% (OR 0.5494, 95% CI: 0.2074-1.4557), respectively. The data of allele frequencies in patients and controls showed that the G allele frequencies were 0.46 vs. 0.60 in patients and controls, respectively, while T allele frequencies were 0.54 vs. 0.40 in patients and controls, respectively. The current conclusions indicated that in the study group, the GG genotype of IL-21(rs2055979) could be related to MS.

Keywords: IL-21, rs2055979, Multiple sclerosis, PCR-RFLP

1. Introduction

Multiple sclerosis (MS) is a chronic autoimmune disorder of the central nervous system. Genetic and environmental factors have important roles in the induction, onset, and progression of MS (1, 2). Approximately 80% of patients are initially presented with relapsing-remitting (RR)-MS, which involves unpredictable attacks (remission). Interleukin (IL)-21 is part of the class I cytokines family, which consists of IL-2, IL-4, IL-7, IL-9, and IL-15 (3). It is a potent pleiotropic immunomodulatory cytokine with an impact on immune responses that are both innate and adaptive (4). During MS progression, IL-21 is a proinflammatory active substance (5). Several cell types can produce IL-21, which may cause the development of diseases, such as T helper (Th) 17, Th2, and natural killer (NK) cells (6). T helper 1 and Th17 cells that secrete proinflammatory cytokines, including interferon (IFN), tumor necrosis factor, and IL-17, tend to influence the pathogenesis of MS (7). The IL2/IL21 genetics have previously been established for various autoimmune diseases. Rheumatoid arthritis and psoriasis have been linked with this relationship (8, 9). B-cell proliferation is induced by IL-21, which improves their function by increasing antibody production and reducing cell death.

It is also been linked to controlling antibody development and preventing apoptosis and plasma cell differentiation (10). The cluster of differentiation 81 T cells, NK cells, and NK T cells are activated by IL-21, which enhances their cytotoxic activity and proliferation. This study aimed to examine any correlation between the single-nucleotide polymorphism (SNP) of the IL-21 gene (rs2055979) and MS and whether it could be used as a genetic marker of disease susceptibility; it also was conducted to check if there was a link between IL-21 serum levels and disease pathogenesis.

2. Materials and Methods

2.1. Study Design

This case-control study was conducted between February 2019 and April 2021. Blood samples were collected from 50 healthy individuals as a control (29 females and 21 males) and 70 RR-MS patients (44 females and 26 males, receiving treatment with drug IFNβ and other types) from Baghdad Teaching Medical Hospital and other provinces, Iraq. The study groups had mean age scores of 37.17 ± 8.987 and 36.66 ± 8.858 years for patients and control, respectively. Multiple sclerosis patients were diagnosed by neurologists based on clinical findings according to the revised 2010 McDonald criteria (11). All patients had the Expanded Disability Status Scale (EDSS) of 1.0-5.0.

2.2. Blood Sampling

A total of 5 ml of blood was collected by vein puncture, of which, 1 ml was placed into ethylenediaminetetraacetic acid tubes for DNA extraction and stored at -20°C until analysis, and the other 4 ml was kept in a gel tube for serum after being centrifugated at 6,000 rpm for 15 min at 20°C. The serum was stored at -20°C.

2.3. Quantitative Assessment of Serum IL-21

The levels of IL-21 were determined in sera of MS cases and controls. Sandwich enzyme-linked immunosorbent assay (ELISA) was used to calculate concentrations (Human Interleukin 21 ELISA, Bioassay Technology Laboratory, China). The absorption, which is proportional to the cytokine concentration, was measured colorometrically using an ELISA plate reader (Biotech, UK). Serum IL-21 concentrations were expressed in pg/ml.

2.4. Extraction of Genomic DNA

Blood samples were the sources for genomic DNA extraction using (gSYNC™ DNA Extraction Kit). The optical density of the extracted DNA was measured at 260 and 280 nm wavelengths.

2.5. Genotype of IL-21 (rs2055979) (G/T) Polymorphism

To amplify a fragment of 213 bp for allele detection, two primers were chosen, namely (F-5'-GCTCTGAACCCAAACACTCTC -3) and (R- 5'-ACAGCCAGGAAACTCTGGAA -3') (Bioneer, Korea) (12). Aliquots of amplified DNA products were digested with Nla (restriction enzyme, New England Biolabs). For polymerase chain reaction (PCR), a GTC thermal cycler (Cleaver Scientific, UK) was used. The thermal cycle was as follows: 95°C for 5 min, followed by 35 cycles of 50-sec denaturation at 95°C, 50-sec annealing at 62°C, and 30-sec extension at 72°C. The final elongation step was performed at 72°C for 3 min. In a total volume of 25l, the PCR product IL-21 gene was digested with restriction endonucleases using 10 units of enzyme and buffers supplied by the manufacturer's instructions. On a 2% (w/v) agarose gel, the amplified PCR products were checked for expected size (13).

2.6. Biostatistical Consideration

Statistical calculations were carried out using SPSS (version 17.0, SPSS Inc, Chicago, USA) and Epi Info 2002 (Centers for Disease Control and Prevention, Atlanta, GA USA) statistical software packages. Genotype and allele frequency of IL-21 (rs2055979 G/T) SNP were compared in patients and normal groups using the Chi-square test or Fisher's exact test when proper. The association between continuous data (onset age, duration of disease, EDSS, and progression index) and different genotypes were determined by a one-way ANOVA test. The relationship between polymorphisms and categorized progression index was

assessed using the non-parametric Kruskal-Wallis test. Hardy-Weinberg's proportions were determined by applying the equation of p2 + 2pq + q2. A p-value of less than 0.05 was considered significant (14).

3. Results and Discussion

A total of 70 MS patients and 50 healthy controls participated in this study. Table 1 presents the general demographics for MS patients and reveals that the mean age scores were was 37.17±8.987 and 36.66±8.85 years for the cases and control group, respectively. The gender distribution was 44 (62.8%) females and 26 (37.1%) males among cases and 29 (58%) females and 21 (42%) males in the control group. Between MS patients and healthy controls, gender distribution did not differ significantly (0.0678 at $P \leq 0.05$).

Table 1. Distributions of gender and age in multiple sclerosis patients and controls

Group	Gender	Age (mean±SD)	Duration of the disease (mean±SD)	EDSS n	Age of onset (mean±SD)
Patients (n=70)	Female 44 (37%)	37.17±8.987	4.2±3.786322	EDSS ≤3 (n=59) EDSS >3 (n=11)	33.28571±9.146383
	Male 26 (63%)				
Controls (n=50)	Female 29 (58%)	36.66±8.858			
	Male 21 (42%)				

EDSS: Expanded Disability Status Scale

Multiple sclerosis severity was evaluated by the EDSS of Kurtzke (15), with a range of 0-10 from normal neurological patients to risk of death (Table 1).

3.1. Estimation of IL-21

There was a slight increase in IL-21 serum levels in MS patients, compared to the controls (104.8512±268.6606 pg/ml vs. 80.54524 ± 91.41666 pg/ml, respectively); however, it was not significantly different (Table 2).

Table 2. Concentration of IL-21 pg/ml for multiple sclerosis patients and controls

Groups	(Mean±SD)	P-value*
Patients (n=70)	104.8512±268.6606	0.5403
Control (n=50)	80.54524±91.41666	

*Significance differences at $P \leq 0.05$

Earlier analysis conducted by Vollmer, Liu (5) and Gharibi, Hosseini (16) showed that IL-21 functions as a proinflammatory cytokine during the development of MS and its serum level in MS patients was higher than in healthy controls. In other studies, decreased levels of IL-21 have been reported in MS patients undergoing treatment with alemtuzumab (17, 18). Therefore, IL-21 might be involved in MS immunopathology, particularly in the treatment patients of the current study. Ghalamfarsa, Mahmoudi (19) reported that increased serum levels of IL-21 were linked to Th17, Treg cell inhibition, Th2 cell growth, and IFN activity; all of these factors have been connected to MS etiology. As a result, IL-21 suppression could be a promising MS therapeutic target. Tzartos, Craner (20) confirmed that IL-21 was also expressed in gray matter neurons in MS patients.

3.2. Distributions of Genotypes and Allele Frequency of the IL-21 (rs2055979) Gene Polymorphisms

The IL-21 (rs2055979) gene polymorphisms were amplified using PCR and unique primers. As shown in

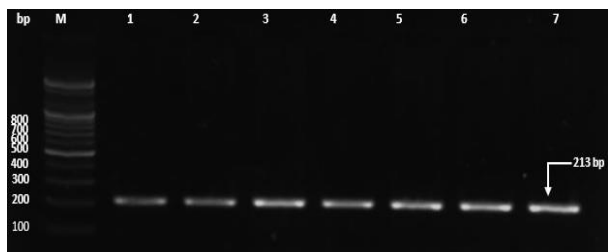figure 1, the molecular size of IL-21 (rs2055979) was approximately 213 bp.

Figure 1. PCR product of IL-21 (rs2055979) on 2% agarose at 70V for 90 min. Lane (1-4): case and Lane (5-7): control

Polymerase chain reaction-restriction fragment length polymorphism was used to detect the IL-21 (rs2055979) gene polymorphism, and a specific restriction enzyme *Nla*III was used to digest the PCR product of IL-21 (rs2055979), as shown in figure 2.
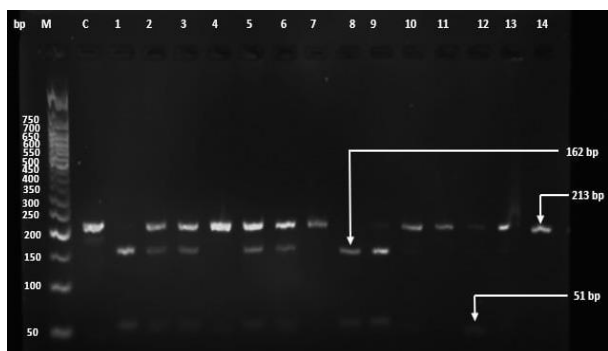

Figure 2. Polymerase chain reaction-restriction fragment length polymorphism product with digestion enzyme *Nla*III for IL-21 (rs2055979), on 2.5% agarose at 70 V for 2 h
Lane C: uncut 213bp. Lane (1-9): case. Lane (10-14): control, Lane (10, 11, 13, 14): one band 213 bp homozygous. Lane (1, 8, 9): two band 162 bp + 51 bp mutant homozygous. Lane (2, 3, 5, 6): three band 213 bp + 162 bp + 51 bp heterozygous.

The distribution of the observed IL-21 (rs2055979) genotypes and alleles frequencies in the control and case individuals are presented in table 3.

The wild homozygous genotype (GG) distribution was found to be significantly lower in multiple sclerosis patients than in controls in the current study (14.3% vs. 34%, 0.0129 at $P \leq 0.05$; odds ratio [OR] 3.0909; 95% confidence interval [CI]: 1.2704-7.5203) (Table 3). However, there were no significant variations among MS people between case and control groups, 62.9% (OR: 0.6402; 95% CI: 0.3064-1.3374) and 52% (OR: 0.5494; 95% CI: 0.2074-1.4557) for both GT and TT genotypes, respectively. The data of allele frequencies in patients and controls are summarized in table 3, revealing that the G allele frequencies were 0.46 vs. 0.60 in patients and controls, respectively, while T allele frequencies were 0.54 vs. 0.40 in patients and controls, respectively. It was found in the present study that the GG genotype of IL-21 (rs2055979) in the Iraqi population might be related to predisposing factors for MS. Akesson, Oturai (21) reported that several chromosomal regions have been involved in complete genome scans for MS sensitivity. In an Iranian study conducted by Gharibi, Kazemi (12), it was demonstrated that the distribution of genotypes and allele frequencies of IL21 rs2055979 SNP did not differ between MS patients and controls. Nevertheless, they reported the possible interactions of the IL-21 gene with MS clinical profiles and an association between the IL-21 gene polymorphism and the MS disease progression, suggesting that the G allele promoted or the T allele protected against disease progression. Additionally, Gharibi, Hosseini (16) reported that IL-21 played a proinflammatory role and might enhance the MS pathogenesis and progression.

Table 3. Distribution of genotype and allele frequency of IL-21 (rs2055979) gene in cases and controls

Genotype of IL-21 (rs2055979)	Control n (%)	Cases n (%)	Significance level	OR	95% CI
GG	17 (34%)	10 (14.3%)	*0.0129	3.0909	1.2704-7.5203
GT	26 (52%)	44 (62.9%)	0.2354	0.6402	0.3064-1.3374
TT	7 (14%)	16 (22.8%)	0.2283	0.5494	0.2074-1.4557
Total number	50	70	---	---	---
Allele	Frequency	Frequency			
G	0.60	0.46	---	---	---
T	0.40	0.54	---	---	---

*Significant level at $P \leq 0.05$

3.3. Association between Genotype of IL-21 (rs2055979) and Serum Level IL-21

There were no significant differences among case and control groups regarding the association between genotypes of IL-21 (rs2055979) gene polymorphism and serum IL-21 (Table 4).

Table 4. Relationship between genotype of IL-21 (rs2055979) and serum IL-21 in cases and controls

Group	Genotype of SNP4 IL-21 (rs2055979)	Mean±SD
		IL-21
Patients	GG	58.304±6.89221
	GT	136.7591±345.012
	TT	56.2004±4.864942
	P-value	0.5099
	LSD	NS
Control	GG	67.73541±9.292444
	GT	92.497±126.4766
	TT	67.26257±8.803391
	P-value	0.6387
	LSD	NS

In this study, the IL-21 (rs2055979) GT genotype was found to be correlated with the highest serum level of IL-21 among MS patients (136.7591±345.012 pg/ml), compared to the GT genotype (92.497±126.4766 pg/ml) for control groups; however, the difference was not significant (Table 4). Both innate and adaptive immune responses are known to be modulated by IL-21 (22). According to McAlpine and Compston (23), the condition is induced by a disruption of the blood-brain barrier, which causes the release of a range of cytokines and an inflammatory state. The findings of the current study indicated that the GG genotype/G allele of IL-21 (rs2055979) might be associated with MS predisposition in this group of Iraqi patients.

Authors' Contribution

Study concept and design: A. A. A.

Acquisition of data: T. A. A.

Analysis and interpretation of data: A. A. A.

Drafting of the manuscript: T. A. A.

Critical revision of the manuscript for important intellectual content: A. A. A.

Statistical analysis: W. R. A. A.

Administrative, technical, and material support: A. A. A.

Ethics

The subjects' confidentiality was observed at our institution. The experiments on animals or humans were reviewed by the ethical review committee, which uses the guidelines of the Ministry of Health and the Ministry of Higher Education and Scientific Research in Iraq. This project was reviewed and approved under number 336 on 12 February 2019.

Conflict of Interest

The authors declare that they have no conflict of interest.

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