Reports of Biochemistry & Molecular Biology
Vol.11, No.1, Apr 2022

Original article

www.RBMB.net

Correlation of -475 IL-2 Promoter Gene Polymorphisms and the Levels of Serum IL-2 on the Risk of Multiple Sclerosis

Thekra Abdulaali Abed[1], Anwar Ali Abdulla*[1]

Abstract

Background: The aim of present study is to asset the IL-2 promoter gene (SNP -475) as a candidate gene for multiple sclerosis (MS) susceptibility.

Methods: This study included 70 patients with relapsing – remitting multiple sclerosis (RRMS) and 50 healthy controls. Following the extraction of genomic DNA from peripheral blood, frequency of genotypes and alleles of SNP -475 was calculated using Restriction fragment length polymorphism-polymer chain reaction (RFLP-PCR) and then the results were analyzed statistically.

Results: The results revealed the unusual ratio for the heterozygous (AT) was 1.6972 indicating that heterozygous patients were at higher risk of multiple sclerosis than wild homozygous (AA), and homomutant (TT). The results show protective role for - 475 IL-2 promoter among individuals with multiple sclerosis, (O.R: 0.4872; C.I. 95%: 0.1617- 1.4680) and (O.R: 0.9275; C.I. 95%: 0.2476 - 3.4745) for both AA and TT genotypes, respectively.

Conclusions: Our results showed that in this population of Iraqi patients, the AT genotype / A allele of -475 IL-2 promoter gene SNP may include attributed factors for MS predisposition.

Keywords: IL-2, Multiple sclerosis, PCR-RFLP SNP.

Introduction

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system affecting young adults. The result is the demyelination and dense astrogliosis of the white central nervous system (CNS) substance in multiples (1,2). It is assumed that the pathogenesis of MS may involve an aberrant immune response to myelin antigens (3). Multiple sclerosis (MS) has the potential to decrease CNS connectivity, which can cause a variety of signs and symptoms, including physical, emotional, and occasionally psychological issues (4). In the clinical epidemiology study, around 2,300,000 people were worldwide affected by MS in 2013, with approximately 20,000 deaths in MS in the same year (5). The Middle East and North Africa countries are situated in a low- to moderate risk region for MS according to MS Atlas (6). Multiple sclerosis is a complex disease arising from the combined action of several genes of susceptibility and one or more environmental factors. Interleukin-2 (IL-2) is a type of cytokine that regulates the immune system functions, it is a 15,000-kDa alpha-helical cytokine produced mainly by CD4+ and CD8+ T cells that are activated, the latter to a lesser extent. lnterleukln-2 (IL-2) is essential for T-lymphocytes to proliferate and differentiate (7). Three Single nucleotide polymorphisms (SNPs) have been identified at positions 384, 475, and 631 in the 800-bp 5' upstream region of the human IL-2 gene (8,9). In the current study we have investigated the effects of the - 475 A/T SNP on serum IL-2 levels on the risk of multiple sclerosis in Iraqi patients versus healthy people.

1: Department of Biology, College of Sciences, University of Babylon, Iraq.
***Corresponding author: Anwar Ali Abdulla; Tel: +96 47727336672; E-mail: alhussainybio@yahoo.com.**
Received: 22 May, 2021; Accepted: 24 Oct, 2021

[Downloaded from rbmb.net on 2025-10-27]

[DOI: 10.52547/rbmb.11.1.83]

Materials and Methods

Subject and Control Groups

This research was approved by Research Ethical Committee, MOH and MOHSER in Iraq.

The case-control study was involved during February 2019 to April 2021 on 70 patients (26 males and 44 female). The patients visited Bagdad's medicinal hospital consultation clinic and other provinces of Iraq and the neurologist based on the McDonald criteria Multiple Sclerosis Department (10). Additionally, 50 individuals (21 males and 29 females) were selected as healthy control group. A consent form and questionnaire were filled for each individual.

Blood Sampling

Five ml of blood were collected by vein puncture, one ml was put into EDTA tubes for molecular analysis and stored at -20 ºC until analysis, and the other 4 ml in gel tube for serum separation.

Quantitative assessment of serum IL-2

The levels of IL-2 in the blood were assessed. The blood was kept at room temperature for 30 minutes to allow for clotting before being centrifuged for 15 minutes at 2000 g. The serum was held at -20 °C until it was analyzed. The concentrations of serum cytokines were calculated using an enzyme-linked immunosorbent assay (ELISA) as directed by the manufacturer (Human Interleukin 2 ELISA/ Bt-laboratory/ China). The serum concentrations were measured in (pg/ml).

Extraction of Genomic DNA

Genomic DNA was isolated from blood samples using gSYNC™ DNA Extraction Kit (Geneaid/ Taiwan). DNA concentration and purity were determined by spectrophotometry with NanoDrop.

Genotype of -475 IL-2 promoter Polymorphism

Two primers were selected (Forward: 5'-ATAGACATTAAGAGACTTAAAC-3') and (Reverse5'-GTAACTCAGAAAATTTTCTTTG-3') (Bioneer/Korea) (9) to amplify a fragment of 332 bp for alleles detection, aliquots of amplified DNA products were digested with *Mse*I (New England Biolabs/England). The PCR conditions were 95 ℃ for 2 min, amplifications were carried out in a GTC thermal cycler (Cleaver Scientific, UK) for 35 cycles, each with 30 s denaturation at 95 ℃, 30 s annealing at 50 ℃ and 40 s extension at 72 ℃. The final elongation step at 72 ℃ was for 5 min. The PCR product IL-2 promoter gene was digested with restriction endonucleases in a total volume of 25 µl containing 10 units of enzyme with buffers supplied by the manufacturer's instructions. The PCR products have been checked on 2% (w/v) of agarose gel for expected size. The molecular weight of the fragments was determined using a 50-bp DNA molecular weight marker (New England Biolabs/England) (11).

Biostatistical consideration

The SAS (Statistical Analysis System. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA. 2012) was used to investigate the differences between the study's variables. To compare percentage differences, a Chi-square test was used, at P-values of 0.05 and 0.01. The CI. 95% and odds ratio were determined.

Results

This research included 70 MS patients and 50 healthy controls (Table 1).

Mean age for the case group were (37.17±8.987) years, while the control group were (36.66±8.858) years. In the cases, there were 44 (62.8%) females and 26 (37.1%) males, while in the control groups, there were 29 (58%) females and 21 (42%). In regarding the gender distribution, there was no statistically significant difference between MS cases and healthy controls (0.0678 at $p \leq 0.05$).

[Downloaded from rbmb.net on 2025-10-27]

[DOI: 10.52547/rbmb.11.1.83]

Table 1. Demographic features of MS cases and controls.

Variables		Group		Total	p value
		Cases (70)	Control		
Gender	Female	44	29	**73**	**0.0791**
	Male	26	21	**47**	**0.4658**
Total		70	50	120	**0.0678**
P value		**0.0314 ***	**0.2579**		
Relapsing-remitting course Number (%)		70 (100%)	-		
Age at onset		33.28571 ± 9.146383			

*Significance differences ($p \leq 0.05$).

Estimation of IL-2

In MS patients, the mean serum level of IL-2 was significantly higher ($p \leq 0.01$) than in controls (1013.145±391.9535 pg/ml vs. 267.2318±207.7653 pg/ml) respectively (Table 2).

Table 2. Serum level of IL-2 in multiple sclerosis patients and controls.

	Control (N= 50) (Mean±SD)	Patients (N= 70) (Mean ± SD)	p value
IL-2(pg/ml)	267.2318±207.7653	1013.145±391.9535	0.0000 **

**High significance differences ($p \leq 0.01$).

Distributions of Genotypes and Allele Frequency of the SNP (-475) of IL-2 promoter gene Polymorphisms

The SNP (-475) of IL-2 promoter gene polymorphisms were amplified using PCR and unique primers. The results indicated that molecular size of -475 IL-2 promoter was approximately 332 bp in length.

Detection of the SNP (-475) of IL-2 promoter gene polymorphism, PCR-RFLP was achieved by a specific restriction enzyme *Mse*I for digestion of PCR product of -475 IL-2 promoter gene.

Table 3 shows the distribution of the observed SNP (- 475) of IL-2 promoter gene genotypes, alleles frequencies, genotype frequencies of AA, AT, and TT of SNP (-475) of IL-2 promoter gene polymorphism in the control and cases individuals.

Table 3. Distribution of genotype and allele frequency of SNP (-475) of IL-2 promoter gene in cases and control.

Genotype of SNP (-475)	Control No. (%)	Cases No. (%)	Significance level	O.R.	C.I. (95%)
AA	5 (10%)	13 (18.57%)	0.2013	0.4872	0.1617- 1.4680
AT	41 (82%)	51 (72.85%)	0.2458	1.6972	0.6946- 4.1467
TT	4 (8%)	6 (8.57%)	0.9111	0.9275	0.2476- 3.4745
Total No.	50	70	-	-	-
A Allele Frequency	0.51	0.549	-	-	-
T Allele Frequency	0.49	0.449	-	-	-

*($P \leq 0.05$).

[Downloaded from rbmb.net on 2025-10-27]

[DOI: 10.52547/rbmb.11.1.83]

The results show that there were no significant differences of SNP (-475) IL-2 promoter genotypes cases and control groups. The odd ratio for the heterozygous (AT) was 1.6972 indicating that heterozygous patients are in higher risk of multiple sclerosis than wild homozygous (AA) and homo-mutant (TT), while the result show protective role for - 475 IL-2 promoter among individuals with multiple sclerosis. The allele frequency for case group (A), T were found 0.549 and 0.449, respectively, in patients, while for control group the allele frequency of A and T were 0.51 and 0.49, respectively.

Association between genotype of -475 IL-2 promoter gene and serum IL-2

Regarding the association between genotype of SNP (-475) of IL-2 promoter gene polymorphism and serum IL-2, there were no differences among case and control groups (Table 4). The findings of this analysis revealed that the -475 IL-2 promoter TA genotype reported the highest value serum level of IL-2 among MS patients (1069.031±393.0203 pg/ml), compared to the TA genotype (259.9776±187.4645 pg/ml) for control groups, but not significate.

Table 4. Relationship between genotype of -475 IL-2 promoter and serum IL-2 in cases and control.

Group	Genotype of SNP1 (IL-2(-475)	Mean±SD
		IL 2
Cases	TT	777.2907±325.8575
	TA	1069.031±393.0203
	AA	902.7519±375.7944
	P value	**0.1195**
	LSD	**NS**
Control	TT	192.6826±97.32115
	TA	259.9776±187.4645
	AA	386.3558±382.6499
	P value	**0.3383**
	LSD	**NS**

Discussion

IL-2 is a cytokine which is released by TH1 cells in response to mitogenic or antigenic activation (12). Several studies have shown that the level of serum IL-2 in MS patients is higher than healthy people (13-18).

In case of chronic progressive MS, *in vitro* studies revealed higher IL-2 level (20), whereas other researchers could not confirm this finding (21, 22). Trotter et al. (22) found a positive and significant linkage between serum IL-2 levels and disabilities in patients with progressive MS.

One study on the Iranian population found that the frequency of IL-2(-475) polymorphisms was slightly higher in MS patients compared to healthy controls, but these variations in frequency were not significant (23). So, our findings agree with these results (24,25). Sayad et al., noticed an increase in IL-2 plasma concentration in MS patients. Thus, some polymorphisms may play a key role in MS disease pathogenesis especially in the promoter region of the IL-2 gene (26).

According to the study by Fedetz et al. there is no correlation between IL-2 polymorphisms -475 and -631 and MS disease (9). Aĭtxozhina et al., found no evidence of IL-2 gene polymorphisms association with multiple sclerosis in an Iranian population (27).

[Downloaded from rbmb.net on 2025-10-27]

[DOI: 10.52547/rbmb.11.1.83]

Additionally, to study in Iranian achieved by Sayad et al., (23) they found no evidence of a connection between IL-2 polymorphisms -574 and -631 and MS disease. The SNP -475 IL-2 promoter is located in the IL-2 promoter's distal region Ward et al., showed that the distal region of the SNP -475 IL-2 promoter can act as a stable nucleation and/or initiator site (28).

The relationship of IL-2 SNP to MS conditions has been reported already by Asouri et al. (29). They found that IL-2RA rs12722489 is significantly associated with the annual attack rate adjusted by the disease duration. Additionally, Moghadam et al., reported that decreased miR-18a-5p expression can be a prognostic marker for MS (30).

The present results showed the AT genotype / A allele of -475 IL-2 promoter gene SNP may include attributed factors for MS predisposition among Iraqi patients.

Acknowledgements

We thank the staff of hospitals and participants in the study.

There are no conflicts of interest declared by the authors.

References

1. Principles of Neurology, 6th Edition: Companion Handbook. Adams RD, Victor M, Maurice R, Ropper AH. Published by Mcgraw-Hill (Tx), 1998.
2. Charles J, Valenti L, Britt H. Multiple sclerosis. Aust Fam Physician. 2011;40(12):947-55.
3. Goverman J. Autoimmune T cell responses in the central nervous system. Nat Rev Immunol. 2009;9(6): 393-407.
4. Kampman MT, Eriksen EF, Holmoy T. Multiple sclerosis, a cause of secondary osteoporosis? What is the evidence and what are the clinical implications?. Acta Neurol Scand Suppl. 2011;(191):44-9.
5. Yousefipour G, Erfani N, Momtahan M, Moghaddasi H, Ghaderi A. CTLA4 exon 1 and promoter polymorphisms in patients with multiple sclerosis. Acta Neurol Scand. 2009;120(6):424-429.
6. Browne P, Chandraratna, DA, Good C, Tremlett H, Baker Ch, et al. Atlas of multiple sclerosis 2013: a growing global problem with widespread inequity. Neurology. 2014;83(11):1022-1024.
7. Cantrell DA, Smith KA. The mterleukm-2 T-cell system: a new cell growth model. Science. 1984;224(4655):1312-16.
8. John S, Turner D, Donn R, Sinnott P, Worthington J, Ollier WE et al. Two novel biallelic polymorphisms in the IL-2 gene. Eur J Immunogenet. 1998;25(6):419-20.
9. Fedetz M, Alcina A, Fernández O, Guerrero M, Delgado C, Matesanz F. Analysis of -631 and -475 interleukin-2 promoter single nucleotide polymorphisms in multiple sclerosis. Eur J Immunogenet. 2002;29(5):389-90.
10. Milo R, Miller A. Revised diagnostic criteria of multiple sclerosis. Autoimmune Rev.2014;13(4-5):518-24.
11. Sambrook, J. and Russell, D.W. (2001) Molecular Cloning A Laboratory Manual. 3rd Edition, Vol. 1, Cold Spring Harbor Laboratory Press, New York. Laboratory Press, Cold Spring Harbor, New York. 2001.
12. Farrar JJ, Benjamin WR, Hdfiker, ML, Howard M, Farra WJ, Fuller-Farrar J. The biochemistry, biology, and role of interleukin 2 in the induction of cytotoxic T cell and antibody-forming B cell responses. Immunol Rev. 1982;63:129-66.
13. Gallo P, Plccmo, M Pagm, S and Tavolato B. Interleukln-2 levels in serum and cerebrospmal fluid of multtple sclerosis patients. Ann Neurol. 1988;24(6):795-7.
14. Gallo P, Piccinno MG, Pagni S, Argentiero V, Giometto B, Bozza F, Tavolato B. Immune activation in multiple sclerosis: study of IL-2, sIL-2R, and gamma-IFN levels in serum and cerebrospinal fluid. J Neurol Sci. 1989;92(1):9-
15. Trotter J L, Van der Veen R, D B Clifford. Serial studies of serum mterleukm-2 m chromc progress, multiple sclerosis patient: occurrence of bursts and effect of cyclosporine. J Neuroimmunol. 1990;28(1):9-14.
16. Gallo P, Plcmno MG, Tavolato B, slain A. A longitudinal study on IL-2, SIL-2R, IL-4 and IFN-gamma in multiple sclerosis CSF and serum.

[Downloaded from rbmb.net on 2025-10-27]

[DOI: 10.52547/rbmb.11.1.83]

Journal of the Neurological Sciences. 1991;101(2)227-232.
17. Bellamy AS, Calder VL, Feldmann M, Davlson, AN. The distribution of mterleukin-2 receptor bearing lymphocytes multiple sclerosis evidence for a key role of activated lymphocytes. Clin Exp Immunol. 1985;61(2):248-256.
18. Selmaj K, Plater-Zyberk C, Rockett K A, Maml R N, Alam R Perkm, G D Perkin, et al. Multiple sclerosis: increased expression of interleukin-2 receptors on lymphocytes. 1986;36(10):1392-5.
19. Merrill JE, Mohlstrom C, Uittenbogaart C, Kermaniarab V, Ellison GW, Myers LW. Response to and production of interleukin 2 by peripheral blood and cerebrospinal fluid lymphocytes of patients with multiple sclerosis. J Immunol. 1984;133(4):1931-7.
20. Selmaj K, Nowak Z, Tchórzewski H. Interleukin-1 and interleukin-2 production by peripheral blood mononuclear cells in multiple sclerosis patients. J Neurol Sci. 1988;85(1):67-76.
21. Brajczewska-Flsher W, Iwmska B; Kruszewska J, Kodak J, Czlonkowska A. Interleukin 1 and 2 production by peripheral blood mononuclear cells in subacute sclerosing panencephalitis and exacerbation of multiple sclerosis. Acta Neurol Scand. 1989;80(5):390-393.
22. Trotter JL, Clifford DB, McInnis JE, R C Griffeth, K A Bruns, M S Perlmutter, et al. Correlation of immunological studies and disease progression in chronic progressive multiple sclerosis. An Neurol. 1989;25(2):172-8.
23. Sayad A, Allameh A, Sayad A, Noruzinia M, Akbari MT, Sarzaeem A, et al. The association of -475 and -631 interleukin-2 gene polymorphism with multiple sclerosis. International Journal of Immunogenetics. Cell journal. 2013;41(4):330-334.
24. McDonald WI, Compston A, Edan G, Goodkin D, Hartung HP, Lublin FD, et al. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. Ann Neurol. 2001;50(1):121-7.
25. Van Heel DA, Franke L, Hunt KA, Gwilliam R, Zhernakova A, Inouye M, et al. A genome-wide association study for celiac disease identifies risk variants in the region harboring IL-2 and IL-21. Nat Genet. 2007;39(7):827-9.
26. Sayad A, Sayad A, Allameh A, Mohseni Y. Correlation of interleukin-2 and uric acid plasma concentration with multiple sclerosis in Iranian patients. Journal of Biology and Today's World. 2013;2(4):72-75.
27. Aĭtxozhina NA, Nigmatova VG, Khanseitova AK, Mendesh MA, Ashirbekov EE, Balmukhanov TS. Polymorphic markers of some genes associated with multiple sclerosis in the population of Kazakhstan. Genetika. 2011;47(6):847-52
28. Ward SB, Hernandez-Hoyos G, Chen F, Waterman M, Reeves R, Rothenberg EV. Chromatin remodeling of the interleukin-2 gene: distinct alterations in the proximal versus distal enhancer regions. Nucleic Acids Res. 1998;26(12):2923-2934.
29. Asouri M, Rokni H A, Sahraian M A, Fattahi S, Motamed N, Doosti R, et al. Association of HLA-DRAand IL-2RA Polymorphisms with the Severity and Relapses Rate of Multiple Sclerosis in an Iranian Population. Rep Biochem Mol Biol. 2020;9(2):129-137.
30. Moghadam ASF, Ataei M, Arabzadeh G, Falahati K, Roshani F, Sanati MH. Analysis of MicroRNA-18a Expression in Multiple Sclerosis Patients. Rep Biochem Mol Biol. 2020:8(4):429-437.

[Downloaded from rbmb.net on 2025-10-27]

[DOI: 10.52547/rbmb.11.1.83]