ISSN: 0067-2904
GIF: 0.851

Genetic Polymorphisms of *Interleukin -1 beta* Gene in Association with Multiple Sclerosis in Iraqi Patients

Ehab D. Salman*
Department of Biotechnology, College of Science, University of Baghdad, Baghdad, Iraq

Abstract

Interleukin-1 β (IL-1 β) is considered to be one of the most important mediators in the pathogenesis of inflammatory diseases, particularly in neurodegenerative diseases such as multiple sclerosis (MS). MS is a chronic inflammatory disease characterized with demyelination in central nervous system (CNS). There was believe that single nucleotide polymorphisms (SNPs) in *IL-1β* gene can alter the structure and function of the *IL-1β* and consequently may have play role in MS disease. In this this study the *IL-1β* gene polymorphism (rs16944, rs1143634) and their association with MS in Iraqi patients were investigated. Two SNPs including $IL\text{-}1\beta_{\text{-}511}$ (rs16944) in promoter and *IL-1B+3962* (rs1143634) in encoding region, were studied using Polymerase Chain Reaction- Sequence Specific Primer (PCR-SSP) technique. The results revealed that $IL\text{-}1\beta_{\text{-}511}$ SNP has three genotypes (CC,CT, TT) with non- significant difference in the SNP genotype and allele frequencies of MS patients compare with control group, it was also noticed that the risk allele was (C) with relative risk (RR=1.55) and higher frequency (60.77%) than T (39.23%) allele in RRMS patients. On other hand the three genotypes (CC,CT, TT) of *IL-1B+3962* SNP it seem to have the same picture, that mean there was no significant difference in their frequencies between RRMS patients and control group , and the highest genotype frequency was for (CC) genotype (68.92%) and the lowest frequency was for(TT) genotype (2.88%) in patients, it was also noticed that the risk allele was (C) with odd ratio (2.44) and that the (T) frequencies were (16.98%*vs.* 33.33%) while (C) frequencies were (83.02 *vs.* 66.67%) in patients and control group respectively. Concerning with these findings, one can suggest that *interleukin-1β* gene polymorphisms may not be relevant to susceptibility to MS in Iraqi patients, this is probably due to many reasons such as ethnic diversity, the relevant of haplotype for this gene with other haplotype of cytokine encoding genes and finally the limitation of sample size which play critical role in SNPs studies.

Keywords: Interleukin, Multiple Sclerosis, Single Nucleotide Polymorphism.

تعدد أشكال النوكليوتيدات المفردة لجين إنترلوكين-1بيتا وعلاقته بالتصلب العصبي اللويحي المتعدد لدى المرضى العراقيين

إيهاب داود سلمان*
قسم التقنيات الأحيائية، كلية العلوم، جامعة بغداد، بغداد، العراق

الخلاصة

يعتبر إنترلوكين – 1 بيتا (*IL-1β*) واحدا من أكثر المركبات الوسيطة ذات الأهمية والمسببة للأمراض الألتهابية خاصة الأمراض التي تصيب الجهاز العصبي مثل مرض التصلب العصبي اللويحي المتعدد Multiple sclerosis والذي يعتبر من الأمراض العصبية الألتهابية المزمنة ويتميز بأزالة مادة المايلين (demyelination) من الغشاء العازل للعصبونات الجهاز العصبي المركزي . لقد لوحظ أن تعدد

*Email: ehab3232000@yahoo.com

أشكال النوكليوتيدات المفردة لجين إنترلوكين – 1 بيتا قد يؤدي الى إحداث تغيرات تركيبية ووظيفية للأنترلوكين–1بيتا مما ينتج عنه تأثيرا في أحداث مرض التصلب العصبي اللويحي المتعدد ، لذلك أجريت هذه الدراسة بهدف معرفة العلاقة بين تعدد أشكال النوكليوتيدات المفردة لجين إنترلوكين – 1 بيتا ومرض التصلب العصبي اللويحي المتعدد لدى المرضى العراقيين. أختير نوعين من تعدد أشكال النوكليوتيدات المفردة Single nucleotide polymorphisms (SNPs) للجين ، النوع الأول *IL-1β*$_{-511}$ (rs16944) واقع ضمن الحفاز بينما يقع النوع الثاني (rs1143634) 3962+ *IL-1β* ضمن المنطقة المشفرة للجين ، أستعملت تقنية التفاعل البلمرة المتسلسل ذات البادئ متخصص التتابع (PCR–SSP) لتحديد هذين النوعين من تعدد أشكال النوكليوتيدات المفردة للجين. أشارت النتائج للنوع الأول الى أن اليلات هذا النوع قد توزعت على ثلاث أنماط وراثية هي (CC,CT, TT) و أن التحليل الأحصائي لهذه الأنماط والنسبة المئوية لتكرار أليلاتها لم يشير الى فروقات معنوية بين مرضى التصلب العصبي اللويحي المتعدد ومجموعة السيطرة ، ولوحظ أن الأليل (C) يمثل العامل النسبي المؤثر حيث بلغت قمته (RR=1.55) وكان الأكثر تكرارا (60.77 %) مقارن بأليل (T) والذي بلغت قيمته (39.23%) لدى مرضى التصلب العصبي اللويحي المتعدد، من الناحية الأخرى لوحظ أن الأنماط الوراثية للنوع الثاني قد أظهرت نفس الصورة أعلاه حيث شملت هذه الأنماط على الأنواع الثلاث (CC,CT, TT) ولم يلاحظ فرق معنوي لتكرار هذه الأنماط لمرضى التصلب العصبي اللويحي المتعدد مقارنة بمجموعة السيطرة ، كذلك لوحظ أن أعلى تكرار كان للنمط (CC) وبلغت قيمته (68.92%) بينما كان النمط الوراثي (TT) أقل تكرارا (2.88 %) لدى مجموعة المرضى ، كذلك وجد أن العامل النسبي المؤثر يرجع للأليل (C) وبنسبة أرجحية (odd ratio =2.44) وبلغت النسبة المئوية الكلية لتكراره (83.02% ، 66.67%)، بينما بلغت هذه النسبة للأليل (T) (33.33% ، 16.98%) لكل من مجموعة المرضى والسيطرة على التوالي . على ضوء هذه النتائج يمكن القول أنه لا يوجد الى ما يشير لعلاقة تعدد أشكال النوكليوتيدات المفردة لجين إنترلوكين –1 بيتا بالتصلب العصبي اللويحي المتعدد لدى المرضى العراقيين ، و تفسير ذلك ربما يعود للعديد من الأسباب منها التنوع العرقي ، طبيعة نمط جين إنترلوكين –1 بيتا (haplotype) وعلاقته بالأنماط الجينية المشفرة للأنواع الأخرى من الأنترلوكينات وأخيرا صغر حجم العينة التي تم دراستها والذي يعتبر من العوامل المهمة والمؤثرة في دراسات تعدد أشكال النوكليوتيدات المفردة للجين .

Introduction

Multiple sclerosis (MS) is an autoimmune-inflammatory with a vast demyelination in central nervous system (CNS) [1, 2]. Cytokines play an important role in MS pathophysiology via demyelination in CNS as result of infiltration immune system cells (β and T) into CNS and producing antibodies and cytokines against myelin antigens [3].

It was noticed that Interluckin-1 (IL-1) present in and around MS lesions, and might be have a role in the disease progression through destruction of CNS myelin [4, 5]. IL- 1 consists of two distinct proteins, called interleukin-1 alpha (IL-1α) and interleukin-1 beta (IL-1β) [6]. IL-1α and *IL-1β* are pro-inflammatory cytokines with pleiotropic activities including differentiation and growth of T- and β -cells [7]. IL-1 β is released by monocytes, microglial cells, astrocytes and brain endothelial cells and seems to be involved in inflammatory reactions of the CNS either by a direct effect on the CNS cells or by a secondary effect via recruitment of leukocytes [8, 9].

It was found that *IL-1β* levels have been increase in lesions, cerebrospinal fluid (CSF) and serum of MS patients [10], moreover the level of IL-1 β production is influenced by different genotypes of IL-1 β gene that reflect gene polymorphism for this gene [11,12].

Many Single Nucleotide Polymorphism (SNP) associated with MS have been described for *IL-1β* gene such as the SNP at the position of −511 C/T (rs16944) in the promoter region [13] and the SNP at position +3953 C/T (rs 1143634) [14] ,these SNPs were recorded in numerous studies which have shown the association of polymorphism in *IL-1β* and MS in the many populations at the regions surrounded Iraq country [1, 2 ,15] so this study was conducted to investigate the status of *IL-1β* gene polymorphism (rs16944 , rs1143634) and their potential association with MS in Iraqi patients .

Materials and Methods

Sixty eight unrelated relapsing-remitting multiple sclerosis (RRMS) patients (45females, 23 males) with clinically defined MS were recruited from MS Clinic of Baghdad during period from 2013 to 2014. Mean age of patients was 34.5±2.0 years. Mean age of the onset of the disease was 39.5 years and mean duration was 5.0 years. All patients had EDSS (Expanded Disability Status Scale) between 2.0 up 4.0. A random samples of twenty apparently healthy persons (15females, 5 males) were used as control group.

Blood samples were collected into 5ml vacutainer tube with EDTA. Two and half milliliter of blood were taken from patients and control group, all blood samples were kept in -20 until used.

DNA Extraction

Genomic DNA was extracted from whole frozen blood using Relia^{TM} Blood gDNA Miniprep System (Promega USA) and depending on manufacturer's instructions.

Genotyping of *IL-1β* Gene

The *IL-1β* gene polymorphism (rs16944, rs1143634) were detected *via* Polymerase Chain Reaction- Sequence Specific Primer (PCR-SSP) kit, which got from the University of Clinic Heidelberg –Germany. The genotyping was performed according to the working instruction supplied with the kit and the results were interpreted depending on manual No.A100 supplied with the kit.

Statistical Analysis

All data were analyzed using the Statistical Package for Social Science (SPSS), version 21 for windows [16]. A multivariate linear model was used to test the significant difference ($p<0.05$) for genotype and allele frequencies between patients and control group, Hardy-Weinberg equation was used for determining genotype /allele frequencies. Fischer exact test was performed by using winpepifile free ware package program for determining the relative risk, the etiological / protective factor (PF), and confidence of interval (Cl estimate at 95%).

Result and Discussion

IL-1B*$_{-511}$ gene SNP *(rs16944)

The results in Table-1 showed that *IL-1β*$_{-511}$ SNP have three genotypes (CC, CT, TT) with non-significant differences in the genotype and allele frequencies for both patients and control group. The CT genotype revealed the highest frequency in patients and control group (47.68% *vs*.50%) respectively while the lowest frequency was for TT genotype (15.39%) in patients ; on the other hand, the control group showed CT genotype has two-fold frequency (50%)than both CC and TT genotypes. Odd ratios for CC, CT, TT genotypes (1.54, 1.07, 0.54) respectively were compatible with the EF and PF values. This study also revealed that the risk allele was (C) with relative risk (RR) (1.55) and higher frequency (60.77%) than T (39.23%) allele in RRMS patients, this finding is in agreement with study [1] conducted on sample from Iranian population, which demonstrated that there was no significant difference in *IL-1β* -$_{511}$ allele frequencies between RRMS patients and control group and also between male and female ,moreover previous study [17] demonstrated that there was no association between MS susceptibility and polymorphisms of *IL-1*$_{-889}$, *IL-1β* $_{-511}$, *IL-1β* $_{+3953}$, and *IL-RA* VNTR, while other study [15] stated that the CT genotype associated significantly with early onset of MS in Turkish patients.

Table 1- The genotype and allele frequencies for *IL-1β* $_{-511}$ gene SNP.

Genotype – Allele Frequency	Patients		Control		OR[(2)]	EF or PF[(3)]	P-value	C.I [(4)] 95%
	No.	%[(1)]	No.	%[(1)]				
CC	27	36.93	6	25	1.54	0.146 (EF)	0.60	0.53-4.45
CT	25	47.68	7	50	1.07	0.026 (EF)	0.10	0.38-3.01
TT	13	15.39	6	25	0.54	0.145 (PF)	0.35	0.18-1.66
C	79	60.77	19	50	1.55	0.215(EF)	0.27	0.75-3.18
T	51	39.23	19	50	0.65	0.177(PF)	0.27	0.31-1.32

%[(1)]= Hardy –Weinberg allele frequency percentages for patients and control group, OR [(2)]= odd ratio, EF[(3)]=effective factor when odd ratio > 1, PF=preventive factor when odd ratio<1 , C.I[(4)] = confidence interval at 95% .

***IL-1β* $_{+3962}$ gene SNP (*rs1143634*)**

Regarding with this SNP, it was also noticed Table-2 that *IL-1β* $_{+3962}$ SNP have three genotypes (CC,CT,TT) and non-significant differences were found in their frequencies of RRMS patients compared with control group, where the highest genotype frequency was for (CC) genotype (68.92%) and the lowest frequency was for (TT) genotype (2.88%) in patients. The results also showed that the risk allele was (C) with odd ratio (2.44) and that the (T) frequencies were (16.98%*vs*. 33.33%) while (C) frequencies were (83.02 *vs*. 66.67%) in patients and control group respectively, this finding disagree with the study [2] which found that there was a significant difference between RRMS patients and control group in (CC) and (CT) genotype of *IL-1β* $_{+3962}$ SNP. The conflict of data for this study with other results might be reflect the fact which state that SNPs have correlation with gene pools in each population, that mean the SNP affected with racial and ethnicity differences beside that there are other factors such as the relevant of haplotype for this gene with other haplotype of cytokine encoding genes as well as the limitation of sample size which play critical role in SNPs studies all these factors should be taken into consideration in order to evaluate genetic susceptibility of MS patients. In general one can concluded that there was no evidences refer to the relationship between *interleukin-1β* gene polymorphisms and MS in Iraqi patients.

Table 2- The genotype and allele frequencies for *IL-1β* $_{+3962}$ gene SNP (*rs1143634*)

Genotype – Allele Frequency	Patients		Control		OR[2]	EF or PF [3]	*P*- value	C.I [4] 95%
	No.	%[1]	No.	%[1]				
CC	36	68.92	7	44.44	2.42	0.399 (EF)	0.145	0.78-7.52
CT	16	28.2	6	44.44	0.65	0.141 (PF)	0.538	0.2-2.06
TT	1	2.88	2	11.11	0.13	0.117 (PF)	0.12	0.01-1.42
C	88	83.02	20	66.67	2.44	0.49(EF)	0.072	0.99-6.02
T	18	16.98	10	33.33	0.41	0.197(PF)	0.072	0.17-1.01

%[1]= Hardy –Weinberg allele frequency percentages for patients and control group, OR [2]= odd ratio, EF[3]=effective factor when odd ratio > 1, PF=preventive factor when odd ratio<1 , C.I[4] = confidence interval at 95%.

References

1. Heidary, M., Rakhshi, N., Kakhki, M. P., Behmanesh, M., Sanati, M. H., Sanadgol, N., Kamaladini, H. and Nikravesh, A. **2014**. The analysis of correlation between IL-1B gene expression and genotyping in multiple sclerosis patients. *Journal of the Neurological Sciences*. 343, pp: 41–45.
2. Sarial, S., Shokrgozar, M. A., Amirzargar, A., Shokri, F., Radfar, J., Zohrevand, P., Arjang, Z., Sahraian, M. A. and Lotfi, J. **2008**. *IL-1*, *IL-1R* and *TNFα* Gene Polymorphisms in Iranian Patients with Multiple Sclerosis. *Iran J. Allergy Asthma Immunol*. 7(1), pp: 37-40.
3. Lassmann, H. **2005**. Mechanisms of multiple sclerosis, J. Drug Delivery Today: *Disease mechanisms/Nervous system*. 2(4), pp: 448-452 (cited by Sarial *et al.*2008).
4. Schrijver, H. M., As, J. V., Crusius, J. B. A., Dijkstra, C. D. and Uitdehaag, B., M. J. **2003**. *Interleukin (IL)-1* gene polymorphisms: relevance of disease severity associated alleleswith IL-1b and IL-1ra production in multiple sclerosis. *Mediators of Inflammation*. 12(2), pp:89- 94.
5. Brosnan, C.F., Cannella, B., Battistini, L. and Raine, C.S.**1995**. Cytokine localization in multiple sclerosis lesions: correlation with adhesion molecule expression and reactive nitrogen species. *Neurology*. 45(6 suppl 6), pp: S16-S21.
6. Auron, P. E., Webb, A. C., Rosenwasser, L. J., Mucci, S. F., Rich, A.,Wolff, S. M. and Dinarello, C. A. **1984**. Nucleotide sequence of human monocyte interleukin 1 precursor cDNA. *Proc. Natl. Acad. Sci*. U.S.A. 81(24), pp: 7907–7911.
7. Kantarci, O.H, Atkinson, E.J, Hebrink, D.D., McMurray, C.T. and Weinshenker, B.G. **2000**. Association of two variants in IL-1beta and IL-1 receptor antagonist genes with multiple sclerosis. *J. Neuroimmunol*. 106, pp: 220–227.
8. de Jong, B. A., Huizinga, T. W., Bollen, E. L., Uitdehaag, B. M., Bosma, G. P., Van Buchem, M. A., Remarque, E. J., Burgmans, A. C.S., Kalkers, N. F., Polman, C. H. and Westendorp, R. G. J. **2002**. Production of IL-1beta and IL-1Ra as risk factors for susceptibility and progression of relapse-onset multiple sclerosis.*J.Neuroimmunol*. 126, pp: 172–179.

9. Simi, A., Tsakiri, N., Wang, P. and Rothwell, N. J. **2007**. Interleukin-1 and inflammatory neurodegeneration. *J. Biochemical Society Transactions*.1, 35(5), pp:1122–1126.

10. Dujmovic, I., Mangano, K., Pekmezovic, T., Quattrocchi, C., Mesaros, S., Stojsavljevic, N., Nicoletti, F. and Drulovic, J. **2009**. The analysis of IL-1 beta and its naturally occurring inhibitors in multiple sclerosis: The elevation of IL-1 receptor antagonist and IL-1 receptor type II after steroid therapy. *J. Neuroimmunol.* 207, pp: 101–106.

11. Shu, K.H., Lee, S. H., Cheng, C.H, Wu, M. J. and Lian, J. D. **2000**. Impact of interleukin-1 receptor antagonist and *Tumor Necrosis Factor-α* gene polymorphism on IgA nephropathy. *J. Kidney International*. 58, pp: 783–789.

12. De- Jong, B. A., Huizinga, T. W., Bollen, E. L., Uitdehaag, B.M., Bosma, G. P., Van- Buchem, M.A,Remarque, E. J., Burgmans, A. C., Kalkers, N. F., Polman, C. H., and Westendorp, R. G. **2002**. Production of IL-1beta and IL-1Ra as risk factors for susceptibility and progression of relapse-onset multiple sclerosis. *J. Neuroimmuno*l. 126(1-2), pp: 172-179.

13. Di- Giovine, F., Takhsh, E., Blakemore, A. and Duff, G. **1992**. Single base polymorphism at −511 in the human *interleukin-1β* gene (*IL-1β*). *J. Hum. Mol. Genet* .1, p: 450.

14. Abtahi, S., Farazmand, A., Mahmoudi, M., Ashraf-Ganjouei, A., Javinani, A., Nazari, B., Kavosi, H., Amirzargar, A. A., Jamshidi, A. R. and Gharibdoost, F. **2015**. *IL-1A* rs1800587, *IL-1B* rs1143634 and *IL-1R1* rs2234650 polymorphisms in Iranian patients with systemic sclerosis. *Int. J. Immunogenet*. 42(6), pp: 423-427.

15. Isik, N., Arman, A., Canturk, I. A., Gurkan, A. C., Candan, F., Aktan, S., Erzaim, N., Duz, O. A., Aydin, T., Turkes, M. and List, E. O. **2013**. Multiple sclerosis: association with the *interleukin-1* gene family polymorphisms in the Turkish population. *Int. J. Neurosci*. 123(10), pp: 711-718.

16. SPSS. **2014**. Statistical Package for Social Sciences. Version 21 for windows. Chicago, U. S. A.

17. Hooper-van Veen, T., Schrijver, H. M., Zwiers, A., Crusius, J. B. A., Knol, D. L., Kalkers, N. F., Laine, M. L., Barkhof, F., Pen˜a, A. S., Polman, C. H., and Uitdehaag, B. M. J. **2003**. The *interleukin-1* gene family in multiple sclerosis susceptibility and disease course.*J. Multiple Sclerosis*. 9, pp: 535- 539.