Advanced Studies in Biology, Vol. 9, 2017, no. 4, 193 - 204
HIKARI Ltd, www.m-hikari.com
https://doi.org/10.12988/asb.2017.7411

Expression Alternations of CCR5 in New Case and Treated Patients with Multiple Sclerosis

Shima Bayat*

Meymeh University, Isfahan, Iran
*Corresponding author

Mitra Ataei

National Institute for Genetic Engineering and Biotechnology
Tehran, Iran

Seyed Masood Nabavi

University of Tehran, Tehran, Iran

Mojgan Ghahari

Shahid Beheshti University, Iran

Mohammad Hossein Sanati

Murdoch University, Australia

Abstract

Multiple sclerosis (MS) is a multifactorial autoimmune disease which is characterized by CNC inflammation, demyelination and various degrees of axonal damages, disrupting the communication ability of nervous system. Numerous genes are interfering with MS. Studies indicate that CC chemokine and their receptors including CCR5 play a notable role in MS procedure.

Particular domain of medicines is used to cure MS which is considered as an attempt to reduce attacks and improve function after an attack. *Interferon* is one of

them which are believed to fulfill biological activities by some specific receptors on the cells surface. However, their mechanism still remains unknown and this would make trouble predicting the final results of the treatment.

We studied the expression of CCR5 in 30 cases of MS patients which were not on any kind of drug treatment with an age of onset at 20 to 61 years old. 25 patients were on drug treatment and 25 as healthy subjects with a similar sex, age, race, physiological statues, life style situation and education matched to patients along with applying Quantitative RT-PCR.

A remarkable CCR5 low-expression has observed in MS groups in comparison with healthy subjects ($p<0.05$). Moreover, a significant CCR5 over-expression has perceived in sedated patients comparing to the healthy group ($p<0.05$).

Results demonstrated that CCR5 chemokine receptor could be considered as a determining receptor interacting with interferon owing to the fact that CCR5 chemokine existing in the patients' peripheral blood who has received interferon was undeniable comparing to the others and this result could be a depiction of an effective interaction with chemokine CCR5 receptors.

Keywords: CCR5; multiple sclerosis (MS); nervous system; interferon

Introduction

Multiple Sclerosis is one of the most common diseases in the brain and spinal cord. MS is considered as both autoimmune and inflammation neurodegenerative diseases caused by demyelinating (Sarkijarvi et al., 2006). T cells' migration along with macrophages from blood to the central nervous system is an important process during its development (Cheng and Chen, 2014). Patients have plenteous T helper lymphocyte in cerebrospinal fluid. Hence, T cells expression development means more leukocytes migration to inflammation part as well as more damages (D'Angelo, 2011).

$CD4^+$ types of lymphocytes produce a protein called chemokine which activates B cells and macrophages. Chemokines are small peptide inductors directing pathogenic cells to inflamed areas. Each Th cells can express various chemokines receptor which is monopolistically effective on MS pathogens. Recent studies indicated that the surface of some chemokines and its receptors has increased in MS patients' blood and cerebrospinal fluid (Eikelenboom et al., 2002).

The expression pattern of each chemokine receptors inducts an exclusive property of migration which leaves the chemokine receptors in charge (Orjea-Guevara et al, 2012). CCR5 chemokine receptors play a pivotal role in utilization of pathogenic T cells in patients. Also CCR5 helps macrophages' survival during inflammation besides inflammation, in addition to the recruitment of Th1 cells. It is observed in two studies that CCR5+CCR2 receptors play a crucial role in MS development and are able to produce a vast amount of IL-17 and IFN- γ (Kes et al., 2012). MS medicine treatment is consist of some drug groups, meanwhile IFN-β is the most popular one among RRMS type patients (V. Foti Cuzzola et al., 2012).

Molecular mechanisms which are highly responsible for responding IFN- β are extensively complex and there is still a minor understanding of them. The remedy is executed by IFN- β in order to decrease the disease progression (O`Doherty et al., 2009). Frankly, IFN- β are those cytokines which are secreted by fibroblasts and utilized as a suppressive immune system's drug in MS treatment.

Immunological effects of Interferons are consist of increasing the T cells activity's suppression, reducing the production of pre-inflammatory Cytokines, regulating the activity of existing Antigens as well as preventing lymphocytes' aggregation inside the central nervous system (Poser et al., 1983, Kurtzke, 1983).

Due to the Heterogeneous characteristic of MS, existing treatments are focused upon the clinical symptoms, preserving the MRI lesions inactivation and keeping the EDSS at a low level (Oertsches and Zettl, 2007). Results demonstrated that the peripheral blood mononuclear cells (PBMC) of the MS patients which are not sedated yet have some slight differences while are recognizable during their comparison with the control group due to the immune expression level and cells expression cycle (Oertsches and Zettl, 2007). Studies indicated that in MS patients who were under medication with IFN- β, the CCL3, CCL4 and CCL5 types of chemokines production, especially for CCR5, would probably face an expression's decline. The exact mechanism of IFN- β on genes expression remains anonymous; however the ongoing treatment would probably regulate the gene expression and the proteins on their signaling route (Latt Aung et al., 2010). Interferon's medicines are effective on reducing the disease recurrence and also declining the damages during MRI's analysis.

MS is a polygenetic disease which includes both gene-gene numerous interactions and post-transcription's regulation mechanisms, meanwhile the gene expression alteration are synched to immunological alterations on the proteins, cells level.

Therefore, results support the probability of the recovery process owing to the alteration profile of genes expression including CCR5 afterward the IFN- β medication. Considering the fact that the medication was followed through using IFN- β, analyzing the MRI and controlling clinical symptoms, the alteration of chemokines expression's pattern, especially for their CCR5 receptor, could be utilized in a vast amount as a potential biomarker to medications management or medications method in the future.

Materials and Methods

In this study, in order to select the qualified patients, the first step was clinical examination as well as the clinical inspections determined by an expert physician to diagnose the disease. Then, asking for patients' permissions if they are totally content, in order to provide the moral aspect of the research which is considered highly important.

Patients are divided into two groups by the total number of 50. The first group is those who had diagnosed as Cis or have not been using any immune systems suppressive drugs during the last three years including the first line of medications called IFN- β (β interferons). The second group is those who were in RRMS type and were under interferons' medication for at least two years by β interferons include Avonex, Rebif, Betaseron, along with constant clinical symptoms, and 25 normal subjects.

In each group 25 patients have studied which are mentioned completely by specifications as shown in Table 1. The under-study patients have examined clinically and MRI analyzing onset every 2 to 3 months by physicians. In case of necessity EDSS changes and the remedy trends review were made.

Table (1): details of studied groups

	The age average ±	**The EDSS average ±**	**Female/Male**	**The average of drug use**	**The duration of infection**
With no usage of MS medicine	±37.7	±2	72%	±3years	±9.4
Use MS interferon	±29	±1	28%	±2 years	±3.5

Afterwards, samplings are taken of 50 volunteered patients by 5ml venipuncture and have transferred to EDTA contained falcons.

RNA Extraction

RNA extraction is the very first step to study the amount of gene expressions, separate leukocytes of peripheral blood to determine the extraction of Total RNA and finally the RNA extraction in accordance with RNA extraction kit which is related to Roche Inc. Also, the NanoDrop spectrophotometer is used to analyze the amount of extracted RNA.

While after the RNA extraction stage, is to study the transformation of Total RNA into cDNA gene expression, meanwhile cDNA synthesis is provided by using *RevertAIDTM First sts End* kit which is crafted in Formantage Inc. containing Oligo (dt) Primer.

Designing Primer and Real Time PCR

In this study, β-actin gene is used as housekeeping genes. Besides the Primer design is determined for CCR5 as well as β-actin by using Oligo 7.

The Gene Expression's Evaluation

The Reverse-Transcriptase Real Time method based on SYBER Green is utilized in order to study the IFN- β medical effects upon CCR5 expression's alteration. Following the gene's alteration, there could be the depiction of medicine's remedy statues. So as to running Real Time by using cDNA, the Amplicon kit (Termociclerroter-Gene6000 Corbett) is exploited. Equal conditions were determined for both CCR5 and β-actin.

Real Time PCR Statistical Data Analysis

As it previously mentioned, Corbett device is used through Real Time PCR approach based upon SYBER Green. By using melting curve analysis the productions variety is specified during the process. In order to analyze the CCR5 expression's alteration in the two defined groups and comparing them both, Quantitative PCR is used, and along with the resulted *ct*s and followed by calculating Δct, the quantitative analysis of gene expression is determined between the two defined groups.

The melting diagram of each pattern is performed by measuring the florescence alterations in various temperatures. To achieve a precise determination, the device is drawn standard curve referring to both intended gene and the threshold cycle. In order to do so we used Corbett and a sample's cDNA serial dilution. Also TE solution is used and the sample got diluted in a 1 to 2 proportion. Finally five different digits were obtained from cDNA (½, ¼, ⅛, 1.16, 1) afterwards it has ran on Real Time and then drew the standard curving device based on the resulted *ct*.

SPSS is used based on the *t* test for analyzing the resulted data. Therefore, for all samples, *ct* and *Δct* were applied to the software.

Results

The Quantitative results for both β-actin and CCR5 are as followed:

Figure1 has demonstrated the standard curve based on the resulted *ct*.

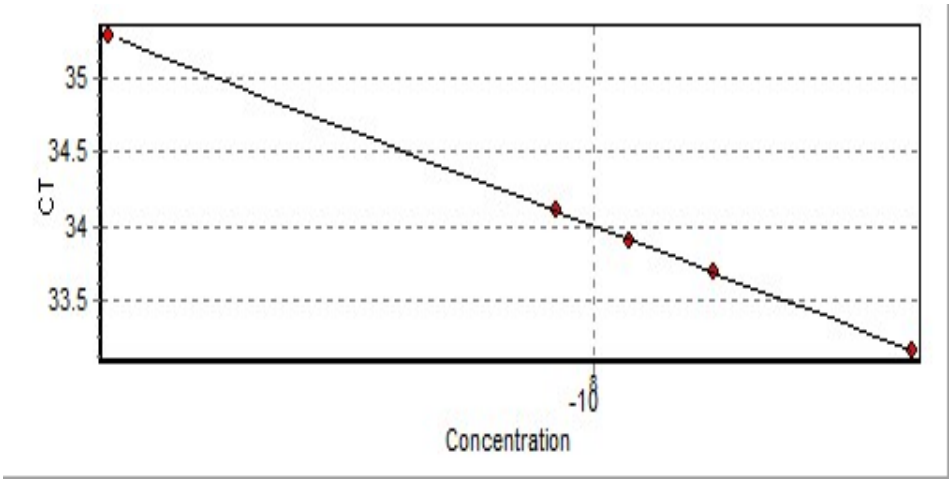

Figure (1): Standard curve (slope: -3, r=1, E: 1/15)

According to the resulted diagrams deprived from CCR5 expression's comparison between two existing groups, those who have used interferons had an increase in their CCR5 expressions in comparison with those who have not used interferons.

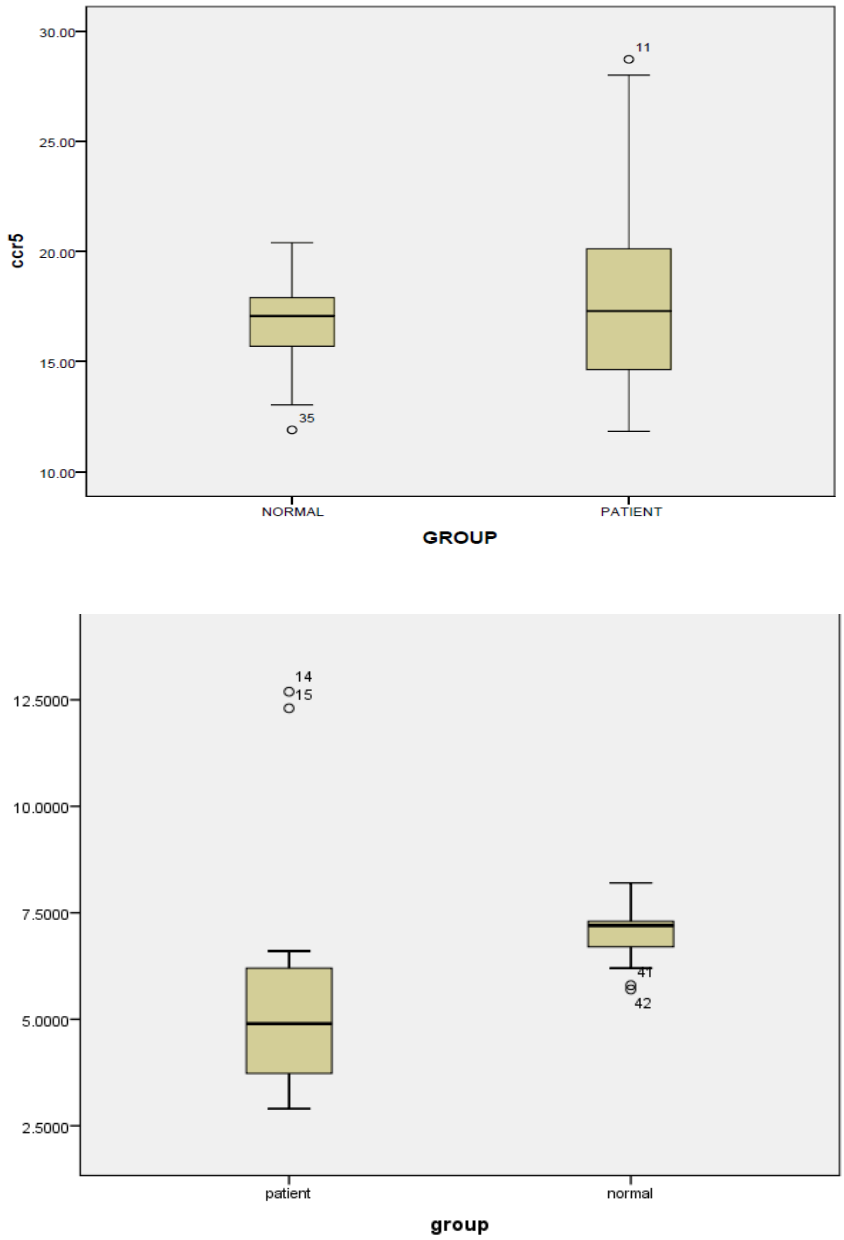

Figure (2): CCR5 expression's comparison between two existing groups of those who have used interferons and those who not.

Since the p value is 0.045 and less than 0.05, the difference between *Δct*s' averages is meaningful in both populations. Therefore, the observed expression is statistically meaningful. Consequently the subjects who were under interferons' sedation demonstrate an increased CCR5 expression which is also making sense considering statistics.

Discussion

Despite of implementing wide experiences to find the main reason of pathologic cells migration to CNC, yet no explicit depiction is specified of the process. However, it is obvious that a complex set of activities and molecular interactions would lead to immune cells migration and inflammation's development. During 2014 a study was provided which revealed the probability of inflammation process in result of the created chemo-toxic by chemokines (Cheng and Chen, 2014). The disease procedure is directly affected by chemokines interaction, hence the procedure is various due to each individual. A disorder might be disadvantageous in cytokines stability and causes more nervous damages and pathogenesis development. Therefore, coding genes of various cytokines are logically nominated to evaluate their relationship with MS (Orjea-Guevara et al, 2012). The alteration of CCR5 expression, which is a chemokine receptor in molecular interactions, is studied during this research as well as its expression alteration under the interferons drug use.

In 2004, results indicated that both understanding the role of CCR5 and analyzing the cytokine-cytokine as well as cytokine-neurotransmitter interactions which are considered crucial and presumably have a pivotal role in polygenetic disorders (Gade-Andavolu et al, 2004).

Numerous studies are provided to investigate the CCR5 role in MS. During 2002 chemokine expressions and their receptors were studied through separating mononuclear cells of peripheral blood in order to analyze the receptor effect's manner during the period of malady and the disease progress (Jalonen et al., 2002). Also other findings have considered the mutation highly effective in various alleles of CCR5 during MS procedure. Those patients who had at least a CCR5 mutation allele had some latency at beginning stages and had died sooner in comparison with others (Sellebjerg et al., 2000). Studies revealed that the PBMCs of patients who were not got under medicine remedy had some slight differences considering the immune expression's level and cells which involved in gene expression cycle (Oertsches and Zettl, 2007). In 2011 researchers demonstrated that genetic factors are highly effective on the expression and the proteins activity regulation as well as the medicines' pharmacokinetic and pharmacodynamics properties. Chemokines expressions, their receptors including CCR5 are altered and fluctuated during each phase of the disease, while the receptor's expression is changed under the influence of the disease intensity and

consuming suppressive medicines. The genetic variation which could be inspired by medicine effects might act as a biomarker utilized ante medication (O`Doherty et al., 2009). Both trusted and clinical biological biomarkers for MS are rare according to the fact that most of them would be fluctuated under different circumstances including the time of consuming medicine, the time of medicine suppression, the time of the disease relapsing, attacking times and the time when the disease is reported (Oertsches and Zettl, 2007).

Since there is no proper clinical definition for the reason behind accruing genetic alterations in MS patients, the patients conditions' equality is stressed both by clinical and laboratorial parameters in order to achieve a more trustful result. Therefore, by executing clinical examination along with a constant EDSS and MRI analyzing, redundancy would be prevented as far as possible.

Due to the heterogeneous behavior of MS disease, the existing remedies are focused upon clinical symptoms improvement and MRI lesions inactivation as well as the stabilizing EDSS at a low level. However, these relations demonstrated that these clinical symptoms are defective and should be completed by molecular parameters (Oertsches and Zettl, 2007).

In MRI analyzing, results indicated that Betaferons are led to decrease activated lesions in MS patients (Changsheng Du and Xie 2012).

Here, the study demonstrated that in those patients who have consumed interferons, the increasing amount of CCR5 expression could be considered as a crucial molecular parameter. By considering the obtaining results it can be stated that IFN- β has an effective interaction with CCR5 chemokine receptor during T cells migration to the CNS area in MS procedure. This interaction caused beneficial immunological alterations at molecular level in order to reduce diseases symptoms; because the resulting outcomes are entirely synchronized to both clinical symptoms and examinations. However, comprehending the clinical relations of genes expression, including CCR5 in MS patients requires the realization of complex molecular relations which occurs every single moment and highly affected by various elements.

References

[1] Changsheng Du, Xin Xie, G protein-coupled receptors as therapeutic targets for multiple sclerosis, Review, *Cell Research*, **22** (2012), 1108-1128. https://doi.org/10.1038/cr.2012.87

[2] W. Cheng and G. Chen, Chemokines and chemokine Receptors in multiple sclerosis, *Mediators of Inflammation*, **2014** (2014), Article ID 659206, 1-8. https://doi.org/10.1155/2014/659206

[3] M. Comabella, K. Vandenbroeck, Pharmacogenomics and multiple sclerosis: moving toward individualized medicine, *Current Neurology and Neuroscience Reports*, **11** (2011), 484-491. https://doi.org/10.1007/s11910-011-0211-1

[4] R. D'Angelo, CCR5Δ32 Polymorphism Associated with a Slower Rate Disease Progression in a Cohort of RR-MS Sicilian Patients, *Multiple Sclerosis International*, **2011** (2011), Article ID153282, 1-6. https://doi.org/10.1155/2011/153282

[5] M.J. Eikelenboom, J. Killestein, T. Izeboud, N.F. Kalkers, R.A.W. Van Lier, F. Barkhof, B.M.J. Uitdehaag, C.H Polman, Chemokine receptor expression on T cell is related to new lesion development in multiple sclerosis, *J. Neuroimmunol*., **133** (2002), 225-232. https://doi.org/10.1016/s0165-5728(02)00374-0

[6] R. Gade-Andavolu, D.E. Comings, J. MacMurray, M. Rostamkhani, Li S-C. Cheng, W.W. Tourtellotte, L.A. Cone, Association of CCR5 Δ32 deletion with early death in multiple sclerosis, *Genetics in Medicine*, **6** (2004), no. 3, 126-131. https://doi.org/10.1097/01.gim.0000127274.45301.54

[7] N. Kaminski, A. Achiron, Can Blood Gene Expression Predict Which Patients with Multiple Sclerosis Will Respond to Interferon?, *PLoS Medicine*, **2** (2005), e33. https://doi.org/10.1371/journal.pmed.0020033

[8] V.B. Kes, Iris Zavoreo, Vesna Serić, Vesna Vargek Solter, Marijan Cesarik, Sanja Hajnšek, Marija Bošnjak Pašić, Tereza Gabelić, Silvio Bašić, Silva Soldo Butković, Ivo Lušić, Lidija Dežmalj Grbelja, Ante Vladić, Ivan Bielen, Igor Antončić, Vida Demarin, Recommendatons for Diagnosis and Management of Multiple Sclerosis", *Acta Clin. Croat*., **51** (2012), 117-135.

[9] M.D. Latt Aung, P. Fitzgerald-Bocarsly, S. Dhib-Jalbut, K. Balashov, Plasmacytoid Dendritic cells in Multiple Sclerosis: Chemokine and Chemokine Receptor Modulation by Interferon beta, *J. Neuroimmunol.*, **226** (2010), no. 1-2, 158-164. https://doi.org/10.1016/j.jneuroim.2010.06.008

[10] C. O`Doherty, A. Favorov, S. Heggarty, C. Graham, O. Favorova, M. Ochs, S. Hawkins, M. Hutchinson, K. O'Rourke, K. Vandenbroeck, Genetic polymorphisms, their allele combination and IFN-β treatment response in Irish Multiple Sclerosis patients, *Pharmacogenomics*, **10** (2009), no. 7, 1177-1186. https://doi.org/10.2217/pgs.09.41

[11] R.G. Oertsches, U.K. Zettl, MS Therapy Research Applying Genome-Wide RNA Profiling of Peripheral Blood, *International MS Journal*, **14** (2007), 98-107.

[12] C. Orjea-Guevara, J. Ramos-Cejudo, L.S. Aroeira, B. Chamorro and E. Diez-Tejedor, TH1/TH2 Cytokine profile in relapsing-remitting multiple sclerosis patients treated with Glatiramer acetate or natalizum, *BMC Neurology*, **12** (2012), article 95. https://doi.org/10.1186/1471-2377-12-95

[13] C.M. Poser, D.W. Paty, L. Scheinberg, W. Ian McDonald, F.A. Davis, G.C. Ebers, K.P. Johnson, W.A. Sibley, D.H. Silberberg, W.W. Tourtellotte, New diagnostic criteria for multiple sclerosis: Guidelines for research protocols, *Annals of Neurology*, **13** (1983), no. 3, 227-231. https://doi.org/10.1002/ana.410130302

[14] J.F. Kurtzke, Rating neurologic impairment in multiple sclerosis: An expanded disability status scale (EDSS), *Neurology*, **33** (1983), no. 11, 1444-1452. https://doi.org/10.1212/wnl.33.11.1444

[15] S. Sarkijarvi, H. Kuusisto, R. Paalavuo, M. Levula, N. Airla, Terho Lehtimäki, M. Koskenvuo and I. Elovaara, Gene expression profiles in Finnish twins with multiple sclerosis, *BMC Medical Genetics*, **7** (2006), no. 1. https://doi.org/10.1186/1471-2350-7-11

[16] F. Sellebjerg, H.O. Madsen, C.V. Jensen, J. Jensen, P. Garred, CCR5 delta 32, matrix metalloproteinase-9 and disease activity in multiple sclerosis, *J. of Neuroimmunology*, **102** (2000), 98-106. https://doi.org/10.1016/s0165-5728(99)00166-6

[17] Tuula O. Jalonen, Kati Pulkkinen, Maritta Ukkonen, Marika Saarela, Irina Elovaara, Differential intracellular expression of CCR5 and chemokines in multiple sclerosis subtypes, *Journal of Neurology*, **249** (2002), 576-583. https://doi.org/10.1007/s004150200067

[18] V. Foti Cuzzola, E. Palella, D. Celi, M. Barresi, S. Giacoppo, P. Bramanti and S. Marino, Pharmacogenomic update on multiple sclerosis: a focus on actual and new therapeutic strategies, *The Pharmacogenomics Journal*, **12** (2012), 453-461. https://doi.org/10.1038/tpj.2012.41

Received: May 2, 2017; Published: July 17, 2017