

Cytomegalovirus Seropositivity in Iranian Multiple Sclerosis: A randomized case-control study

Fereshteh Ashtari¹, Farzad Fatehi², Mojtaba Akbari³, Shermineh Ghalamkari⁴

ABSTRACT

Objective: Multiple sclerosis (MS) is an inflammatory demyelinating disease of central nervous system. Environmental factors such as different viruses have proposed to be involved in the pathogenesis of multiple sclerosis (MS). This study was aimed to evaluate Cytomegalovirus (CMV) seropositivity in Iranian patients suffering from MS compared to controls.

Methodology: This case control study was conducted in Isfahan. Using ELISA method, IgM and IgG antibodies to Cytomegalovirus (CMV) in MS patients and age and sex matched controls were detected. CMV IgM titer higher than 25 and CMV IgG titer higher than 0.6 were regarded as seropositive according to laboratory Kit.

Results: Total 82 MS patients (64 females and 18 males) and 38 controls (29 females and 9 males) were included ($P > 0.05$). The median [IQR] titer of CMV IgM in MS group was 3.25[2.97-5.1] versus 3.5[2.5-5.7] in control group ($p = 0.66$); likewise, the median [IQR] titer of CMV IgG in MS patients was 15.15 [8.1-22.5] versus 7.75[5.3-22] in control group ($p = 0.011$). According clinical course median of IgG was 14.94[7.3-24] in RRMS and 20.0[9.8-22] in SPMS and median of IgM was 4.0[2.9-6.5] and 3.0[2.7-4.0] respectively.

Conclusion: Seropositivity of CMV was higher in MS patients than controls, therefore, it may have a possible role in MS pathogenesis, but further studies are needed to evaluate this result.

KEY WORDS: Multiple Sclerosis, CMV, ELISA.

Pak J Med Sci January - March 2012 (Part-II) Vol. 28 No. 2 263-266

How to cite this article:

Ashtari F, Fatehi F, Akbari M, Ghalamkari S. Cytomegalovirus Seropositivity in Iranian Multiple Sclerosis: A randomized case-control study. Pak J Med Sci 2012;28(2):263-266

1. Fereshteh Ashtari, MD, Associate Professor of Neurology,
 2. Farzad Fatehi, MD, Assistant Professor of Neurology, Tehran University of Medical Sciences, Tehran, Iran.
 3. Mojtaba Akbari, Epidemiologist,
 4. Shermineh Ghalamkari, MBA, ITM Research Centre, Isfahan University, Isfahan, Iran.
- 1, 3: Isfahan Neuroscience Research Centre, Isfahan University of Medical Sciences, Isfahan, Iran.

Correspondence:

Fereshteh Ashtari, MD, Associate Professor of Neurology, Isfahan Neuroscience Research Centre, Isfahan University of Medical Sciences, Neurology Department, Kashani MS Centre, Kashani Street, Isfahan, Iran. E-mail: f_ashtari@med.mui.ac.ir

- * Received for Publication: January 18, 2012
- * 1st Revision Received: February 15, 2012
- * 2nd Revision Received: February 24, 2012
- * Final Revision Accepted: February 27, 2012

INTRODUCTION

Multiple Sclerosis (MS) is an inflammatory demyelinating disease of the Central Nervous System (CNS), in which, the precise etiology is not known and genetic and environmental factors fail to completely explain its pathogenesis.¹⁻³

For a long time, an environmental agent has been proposed as an important factor in MS pathogenesis. Among putative environmental risk factors, numerous infectious agents, both viral and bacterial, such as herpes viruses have been suggested as being involved in the etiology of multiple sclerosis. Role of infection as a risk factor has supported by abnormal immunological finding that found in spinal fluid⁴ but so far no agent has been consistently associated with the disease. Many studies have linked infectious mononucleosis with MS⁵⁻⁸ furthermore, an inordinate number of other

viruses are scrutinized to detect the sinister virus including HHV6^{9,10} and HHV7 and HHV8.¹¹

CMV one of common viral infection of the herpes family was supposed to have association with many autoimmune diseases such as SLE.¹² There are some reports about seropositivity of CMV in AQP4 positive CNS autoimmunity.¹³ The prevalence of CMV is variable in different geographic areas, diverse racial populations and different socioeconomic levels such as of 50.4%, in the United States.¹⁴ It seems CMV can cause a primary infection at any age usually without recognized symptoms but establishes latent infection, so it could involve in chronic autoimmune disease such as MS.

Although some studies showed no association between MS and CMV^{15,16} but CMV was isolated from a chimpanzee with acute demyelinating disease¹⁷ and result of another study showing higher CMV antibody in vit D sufficient MS patients compare to controls¹⁸, therefore, the role of CMV in MS development is uncertain.

This survey was done to evaluate CMV seropositivity in Iranian patients suffering from MS compare to control in order to investigate association between this agent and MS.

METHODOLOGY

Study area: This case-control study was conducted in Isfahan, a large province that located in central of Iran between latitude 30 and 34 degrees north of the equator and longitude 49-55 degrees east with about 4 million population. People living in Isfahan are ethnically Persian belonging to Caucasian ethnicity. The total number of patients suffering from MS in Isfahan was 1391 with 35.5 per 100000 prevalence according to the one study in year 2006.¹⁹

Study description: Ninety clinically definite MS patients randomly allocated from Isfahan MS clinics. Randomization of patients was done according to a preexisting list produced by a computer program. Diagnosis of MS was confirmed base on MC Donald criteria. The patients have Relapsing Remitting (RRMS) or secondary progressive (SPMS) course and none of them was in relapsing phase. Eight patients were excluded because they refused entry or recently have acute relapse. Thirty eight sex and age matched healthy people were selected as controls.

After taking an informed written consent, conforming to the current revision of the Declaration of Helsinki, the baseline data were collected by questionnaire. In both groups, 5cc blood was taken from each person and serum samples were freezed in -20 after centrifugation.

Using enzyme linked immunosorbent assay (ELISA), by Liaison cmv IgG and IgM of Diasorin olfactory, USA, antibodies seropositivity and titers were determined in both groups. CMV IgM titer higher than 25 and CMV IgG titer higher than 0.6 were regarded as seropositive according to laboratory Kit. The Ethical Review Committee of Isfahan University of Medical Sciences approved the study protocol.

Statistical analysis: The demographic data were analyzed by t-test and CMV antibodies level (including IgM and IgG) were compared in two groups of MS patients and control by Mann-Whitney test.

RESULTS

A total of 82 MS patients (64 females and 18 males) and 38 controls (29 females and 9 males) were included ($P > 0.05$). Mean age was 33.3(9.96)[18-53] in patients group and 33.9(10.7)[18-57] in controls. Sixty six patients (80.5%) had Relapsing-Remitting (RRMS) and 16 patients (19.5%) Secondary progressive course (SPMS).

Common presenting symptoms were Optic Neuritis, sensory symptoms and motor signs consecutively. In MS group the mean (SD) interval between first and second attack was 1.74 (1.96) year, mean (SD) disease duration was 6.14 (4.66) year, mean (SD) number of attacks was 1.40 (0.83), and mean EDSS (SD) was 2.38 (1.80).

Table-I: Characteristics of groups.

Groups	MS patients (82)	Controls (36)	p-value
Female /Male	64/18	29/9	
Age(mean)	33.3(9.96)	33.9(10.7)	0.67
Disease course			
RR	66 (80.5%)		
S P	16 (19.5%)		
CMV IgG	15.15U/ml	7.75U/ml	0.011
Median[IQR]	[8.1-22.5]	[5.3-22]	
RR	14.94U/ml		0.57
	[7.3-24]		
SP	20.2U/ml		
	[9.8-22]		
CMV IgM	3.25U/ml	3.5U/ml	0.66
Median[IQR]	[2.97-5.1]	[2.5-5.7]	
RR	4.0U/ml		0.25
	[2.9-6.5]		
SP	3.0U/ml		
	[2.7-4]		

Data are presented as Median [IQR].

P-values calculated with Kruskal-Wallis test.

The Median[IQR] titer of CMV IgM in MS group was 3.25U/ml[2.97-5.1] versus 3.5U/ml[2.5-5.7] in control group ($p=0.66$); likewise, the Median[IQR] titer of CMV IgG in MS patients was 15.15U/ml [8.1-22.5] versus 7.75U/ml[5.3-22] in control group ($p=0.011$).

Considering the clinical course, CMV IgG was 14.94U/ml[7.3-24] in RRMS and 20.2U/ml[9.8-22] in SPMS and CMV IgM was 4.0U/ml[2.9-6.5] and 3.0U/ml[2.7-4] respectively. There was not any significant difference between two groups according to CMV IgG ($p=0.57$) and CMV IgM ($p=0.25$). There were not any differences of CMV antibodies between MS patients according to course of disease compare to controls (Table-I).

DISCUSSION

Based on the results of the present study the median of CMV IgG in MS patients was significantly more than controls, but there was not any significant difference in MS patients according to clinical course. MS is considered an autoimmune disease of CNS. Immunity response to different environmental factors such as viral infection may have a role in development of this disease.

Many survey were done for detecting the role of viruses in developing of MS and some of them showed human herpesvirus infection is more frequent in the CSF and serum of MS patients compare to other neurological diseases.²⁰

Through the herpes family, EBV is one of the most important candidate as a risk factor of MS but based on different researches it cannot stand alone as a causal factor. One study showed that it is not directly related to MS as etiology²¹ and another one suggested it can play an indirect role as an activator of the underlying disease process.²² Therefore it seems reasonable to evaluate other members of this family as a possible risk factor for developing MS.

CMV isolated from a chimpanzee with acute demyelinating disease after inoculation of multiple sclerosis brain cells, so it may have a possible role in chronic inflammation and degeneration of myelin in CNS.¹⁷

CMV is common viral infection which belongs to herpes family, with diverse prevalence in different parts of the world: roughly under 85% in African-Americans by the age of 21, 96.8% after the age of seven in Turkey²³, to the average of 57% in Australia²⁴, 48.07% in China²⁵ and 97.69% in Iranian pregnant women in Fars province of Iran.²⁶ Indeed, it is a widespread agent and the prevalence of active CMV (IgM seropositivity) or previous infection

(IgG seropositivity) varies depending on the geographical location, age and socioeconomic status.

Recently, laboratory-based signs of active and latent CMV infection have been observed in association with the onset and course of different autoimmune diseases such as systemic lupus erythematosus²⁷, and systemic sclerosis (SSC).²⁸ Noticeably, in one survey, elevated levels of IgG anti-CMV were associated with the production of lupus-related autoantibodies to RNA or DNA-protein complex.²⁹

The mechanism by which CMV induce autoimmune phenomena in different disease such as MS is unknown but non-human studies showed that lifelong asymptomatic infection with CMV may create potentially autoreactive memory T cells, which can be reactivated after being exposed to the antigens released from central nervous injury. Reactivated memory T cells may cause autoimmune neurologic disease many years after initiating infection.³⁰ A few researches were done to evaluate association between CMV and CNS demyelinating disease.

One study showed CMV antibody levels in MS/CIS subjects with sufficient vit D level were higher than controls¹⁸ and another study compared Serum samples of CMV IgG antibodies in patients with AQP4 antibody positive CNS autoimmunity and relapsing-remitting MS. Compared to MS, AQP4 positive cases had a significantly higher CMV seropositivity rate.¹³ There is one case report of neuro-myelitis optica following CMV infection.³¹ Overall these results suggested a possible association between CNS demyelinating disease and CMV. Indeed our study showed higher CMV IgG in MS patients compare to controls (15.15U/ml versus 7.75U/ml) ($p=0.011$), so chronic infection with CMV may associate with MS as an autoimmune disease.

Despite of these findings, one recent study was suggested lower risk of MS in pediatric with a remote infection of CMV³² and another study has showed a protective role of CMV in MS patients by triggering some immunomodulating mechanisms which may decrease immune reactivity.³³ However these findings may relate to the age of acquisition of infection, therefore, more researches are needed to evaluate role of CMV in MS disease.

CONCLUSION

CMV seropositivity in Iranian Ms patients is higher than controls so it may suggest an possible association between CMV and development of MS disease and further investigations with large number of patients are needed to confirm this finding.

REFERENCES

1. Kallaur AP, Kaimen-Maciel DR, Morimoto HK, Ehara Watanabe MA, Georgeto SM, Vissoci Reiche EM. Genetic polymorphisms associated with the development and clinical course of multiple sclerosis (Review). *Int J Mol Med* 2011;28(4):467-479.
2. Ramagopalan SV, Dobson R, Meier UC, Giovannoni G. Multiple sclerosis: risk factors, prodromes, and potential causal pathways. *Lancet Neurol* 2010;9(7):727-739.
3. Wingerchuk DM. Environmental factors in multiple sclerosis: Epstein-Barr virus, vitamin D, and cigarette smoking. *Mt Sinai J Med* 2011;78(2):221-230.
4. Pawate S, Sriram S. The role of infections in the pathogenesis and course of multiple sclerosis. *Ann Indian Acad Neurol* 2010;13(2):80-86.
5. Giovannoni G. Epstein-Barr Virus and MS. *Int MS J* 2011;17(2):44-49.
6. Haahr S, Plesner AM, Vestergaard BF, Hollsberg P. A role of late Epstein-Barr virus infection in multiple sclerosis. *Acta Neurol Scand* 2004;109(4):270-275.
7. Kakalacheva K, Lunemann JD. Environmental triggers of multiple sclerosis. *FEBS Lett* 2011;585(23):3724-3729.
8. Munger K, Levin L, O'Reilly E, Falk K, Ascherio A. Anti-Epstein-Barr virus antibodies as serological markers of multiple sclerosis: a prospective study among United States military personnel. *Mult Scler* 2011;17(10):1185-1193.
9. Behzad-Behbahani A, Mikaeili MH, Entezam M, Mojiri A, Pour GY, Arasteh MM, et al. Human herpesvirus-6 viral load and antibody titer in serum samples of patients with multiple sclerosis. *J Microbiol Immunol Infect* 2011;44(4):247-251.
10. Opsahl ML, Kennedy PG. Early and late HHV-6 gene transcripts in multiple sclerosis lesions and normal appearing white matter. *Brain* 2005;128(Pt 3):516-527.
11. Opsahl ML, Kennedy PG. Investigating the presence of human herpesvirus 7 and 8 in multiple sclerosis and normal control brain tissue. *J Neurol Sci* 2006;240(1-2):37-44.
12. Barzilai O, Sherer Y, Ram M, Izhaky D, Anaya JM, Shoenfeld Y. Epstein-Barr virus and cytomegalovirus in autoimmune diseases: are they truly notorious? A preliminary report. *Ann N Y Acad Sci* 2007;1108:567-577.
13. Sellner J, Cepok S, Kalluri SR, Nestler A, Kleiter I, Kumpfel T, et al. Aquaporin 4 antibody positive central nervous system autoimmunity and multiple sclerosis are characterized by a distinct profile of antibodies to herpes viruses. *Neurochem Int* 2010;57(6):662-667.
14. Bate SL, Dollard SC, Cannon MJ. Cytomegalovirus seroprevalence in the United States: the national health and nutrition examination surveys, 1988-2004. *Clin Infect Dis* 2010;50(11):1439-1447.
15. Jaquiere E, Jilek S, Schluep M, Meylan P, Lysandropoulos A, Pantaleo G, et al. Intrathecal immune responses to EBV in early MS. *Eur J Immunol* 2010;40(3):878-887.
16. Sanders VJ, Felisan S, Waddell A, Tourtellotte WW. Detection of herpesviridae in postmortem multiple sclerosis brain tissue and controls by polymerase chain reaction. *J Neurovirol* 1996;2(4):249-258.
17. Wroblewska Z, Gildea D, Devlin M, Huang ES, Rorke LB, Hamada T, et al. Cytomegalovirus isolation from a chimpanzee with acute demyelinating disease after inoculation of multiple sclerosis brain cells. *Infect Immun* 1979;25(3):1008-1015.
18. Mowry EM, James JA, Krupp LB, Waubant E. Vitamin D status and antibody levels to common viruses in pediatric-onset multiple sclerosis. *Mult Scler* 2011;17(6):666-671.
19. Etemadifar M, Janghorbani M, Shaygannejad V, Ashtari F. Prevalence of multiple sclerosis in Isfahan, Iran. *Neuroepidemiology* 2006;27(1):39-44.
20. Alvarez-Lafuente R, Garcia-Montojo M, De LHV, Dominguez-Mozo MI, Bartolome M, Benito-Martin MS, et al. Herpesviruses and human endogenous retroviral sequences in the cerebrospinal fluid of multiple sclerosis patients. *Mult Scler* 2008;14(5):595-601.
21. Fatima N, Toscano MP, Hunter SB, Cohen C. Controversial role of Epstein-Barr virus in multiple sclerosis. *Appl Immunohistochem Mol Morphol* 2011;19(3):246-252.
22. Christensen T. The role of EBV in MS pathogenesis. *Int MS J* 2006;13(2):52-57.
23. Ataman S, Colak D, Gunseren F, Senol Y, Colak T, Aktekin MR, et al. Investigation of cytomegalovirus seroprevalence in Antalya with a population-based cross-sectional study and review of related data in Turkey. *Mikrobiyol Bul* 2007;41(4):545-555.
24. Seale H, MacIntyre CR, Gidding HF, Backhouse JL, Dwyer DE, Gilbert L. National serosurvey of cytomegalovirus in Australia. *Clin Vaccine Immunol* 2006;13(11):1181-1184.
25. Zhao P, Ma D, Xue F, Ji C, Wang S, Zhang X, et al. Seroprevalence and risk factors of human cytomegalovirus infection in the eastern Chinese population. *Arch Virol* 2009;154(4):561-564.
26. Tabatabaee M, Tayyebi D. Seroepidemiologic study of human cytomegalovirus in pregnant women in Valiasr Hospital of Kazeroun, Fars, Iran. *J Matern Fetal Neonatal Med* 2009;22(6):517-521.
27. Ramos-Casals M, Cuadrado MJ, Alba P, Sanna G, Brito-Zeron P, Bertolaccini L, et al. Acute viral infections in patients with systemic lupus erythematosus: description of 23 cases and review of the literature. *Medicine (Baltimore)* 2008;87(6):311-318.
28. Contreras MA, Salas C, Andreu JL, Mulero J. Cytomegalovirus esophagitis as a treatable complication of systemic sclerosis. *J Clin Rheumatol* 2001;7(6):384-387.
29. Palafox Sanchez CA, Satoh M, Chan EK, Carcamo WC, Munoz Valle JF, Orozco BG, et al. Reduced IgG anti-small nuclear ribonucleoprotein autoantibody production in systemic lupus erythematosus patients with positive IgM anti-cytomegalovirus antibodies. *Arthritis Res Ther* 2009;11(1):R27.
30. 't Hart BA, Hintzen RQ, Laman JD. Multiple sclerosis - a response-to-damage model. *Trends Mol Med* 2009;15(6):235-244.
31. Tran C, Du Pasquier RA, Cavassini M, Guex-Crosier Y, Meuli R, Ciuffreda D, et al. Neuromyelitis optica following CMV primo-infection. *J Intern Med* 2007;261(5):500-503.
32. Waubant E, Mowry EM, Krupp L, Chitnis T, Yeh EA, Kuntz N, et al. Common viruses associated with lower pediatric multiple sclerosis risk. *Neurology* 2011;76(23):1989-1995.
33. Zivadinov R, Nasuelli D, Tommasi MA, Serafin M, Bratina A, Ukmar M, et al. Positivity of cytomegalovirus antibodies predicts a better clinical and radiological outcome in multiple sclerosis patients. *Neurol Res* 2006;28(3):262-269.