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Accompaniment of multiple sclerosis with varicella zoster virus; a systematic review and individual participant data meta-analysis

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Abstract

Numerous studies and meta-analyses have been conducted on the role of infectious agents in susceptibility to multiple sclerosis (MS). In this study we aimed to investigate the role of varicella zoster virus (VZV) in susceptibility to MS as an individual participant data (IPD) meta-analysis. After screening and applying eligibility criteria 19 studies were imported for qualitative systematic review and 11 studies were imported for meta-analysis as different subgroups. No significant result was obtained for association of VZV IgG seropositivity with susceptibility to MS. Positive history of VZV infection was significantly associated with susceptibility to MS. Synthesis of IPD showed that presence of VZV DNA was associated with MS($P<0.001$) both in peripheral blood mononuclear cells (OR= 22.40 [5.85-85.71]) and in cerebrospinal fluid (OR= 14.42 [5.29-39.29]). In general VZV can be a risk factor for MS; but since VZV infection history is highly prevalent in populations without vaccination and on the other hand MS has low prevalence, this association should not be used as a prognostic or predictive value. The exact mechanism should be investigated in future.

Keywords: Meta-analysis, multiple sclerosis, neuroimmonology, neuroscience, varicella zoster

INTRODUCTION

Multiple sclerosis (MS) is a chronic inflammatory and autoimmune disorder that involves central nervous system (CNS). The characteristics of MS is demyelination, axonal injury and axonal loss.[1,2] Incidence of MS increases between 20 and 40 years of age. It has grown in last decades especially in women, and in such patients life expectancy is decreased by 7-10 years. The etiology of this disorder is unknown, though genetic and environment play important roles and the interplay between these factors is under evaluation. MS is not hereditary disease although genetic factors contribute to increase the risk of getting MS.[3] Genetic factors are associated with the human leukocyte antigen (HLA) region located on chromosome 6.[2] Latitude (UV-light exposure) and vitamin D level, smoking and microorganisms can be associated factors of MS. There is a correlation between smoking and the risk of conversion to secondary progressive MS.[4,5] Individuals who have a history of infectious mononucleosis seem to be more susceptible to getting MS, chiefly if the infection occurs after maturation.[6] Natural killer (NK) cells have two subtypes immune regulatory and cytotoxic. Most of them have improving effect on MS and some other autoimmune disorders because of both its regulatory effect and facilitating effect on induction of the antibody dependent cell-mediated cytotoxicity (ADCC) needed for treatment with monoclonal antibodies.[7,8]

Accumulation of evidence suggests an importance for environmental factors such as viruses. About the infectious etiology of MS it has been suspected a large variety of viruses such as Epstein-bar virus (EBV), human herpes virus (HHV)-6, varicella zoster virus (VZV), human endogenous retro viruses (HERVs) and measles virus. MS has a disease heterogeneity model and it may indicate that MS is not triggered with just one kind of a specific virus. A lot of data linking MS to viruses have been created with studying on different parts of virus such as RNA, DNA or proteins in tissues or body fluids. If some of the proteins of pathogens have homologous amino

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acid sequences with self-proteins it is occurred molecular mimicry that this process can create viral induced autoimmunity. Unfortunately multifold viral sequences have been ascertained to be homologous to proteins of myelin. The activated auto-reactive T cells pass blood-brain barrier and begin reaction with antigens that are in this locus. Microglial cells and astrocyte cells are stimulated by secretion of pro-inflammatory cells. The result of this inflammatory process and cross-reaction is myelin damage. At the same time repair of the damaged tissue and remyelination is possible. Axonal injury is depended on secondary myelin damage with the damage ongoing from myelin to the axon. In progressive disease course, diffuse ongoing destruction of gray and white matters and atrophy of brain has been demonstrated.[2,9,10]

Several studies and meta-analyses have been conducted on the role of infectious agents in susceptibility to MS; for instance EBV.[6,11,12] In this study we aimed to investigate the role of varicella zoster virus (VZV) in susceptibility to MS as an individual participant data (IPD) meta-analysis; because dorsal rote ganglions of spine and trigeminal ganglions of brain will not be cleared after being infected with VZV.

METHODS

This study is a systematic review with IPD meta-analysis conducted using preferred reporting items for systematic reviews and meta-analysis (PRISMA) guidelines.

Search and sources

Databases PubMed and Web of Science (WOS) core collection were used. We searched in titles and found 46 documents in WOS and 55 documents in PubMed in 2017. After exclusion of duplicates 62 documents remained. After exclusion of review papers, letters, commentaries, editorials and congress abstracts 27 articles were screened (Figure 1).

Eligibility criteria

The articles published before 2000 were not eligible, because most of them were expert opinions based on low and middle quality evidence with different methods and design. Hence 19 articles remained for qualitative systematic review; among them 11 articles categorized in different subgroups for meta-analysis (Figure 1).

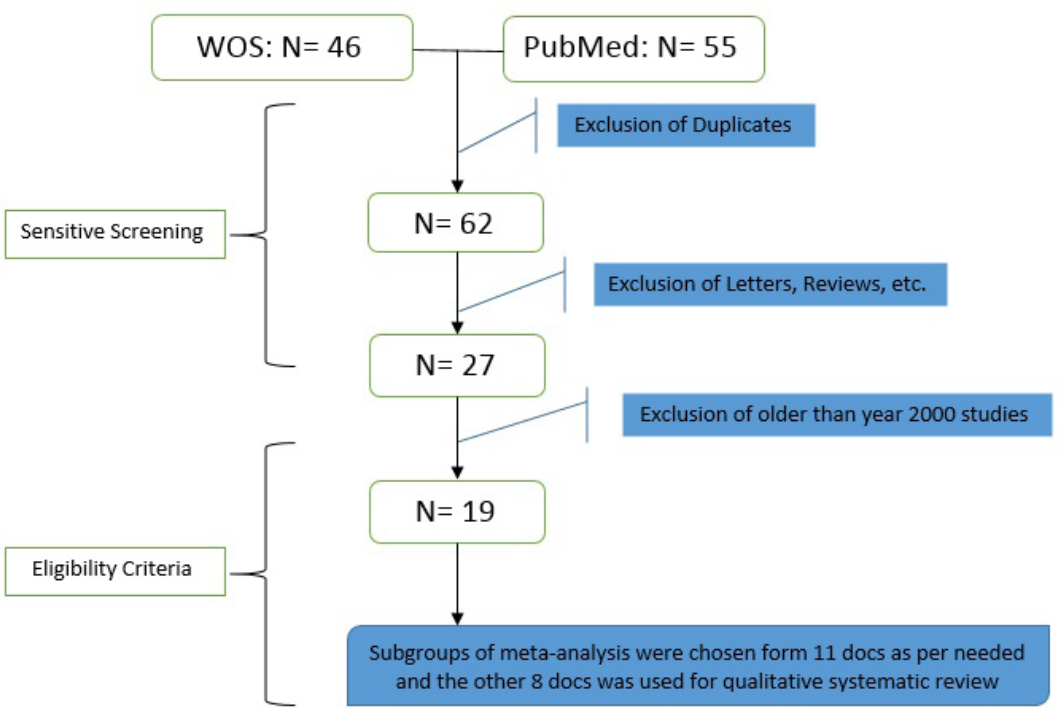

Figure 1. Searching and study selection algorithm.

Data collection process

The data were manually collected and integrated. The first subgroup of meta-analysis was based on case-control data of association of VZV IgG seropositivity with susceptibility to MS. The second subgroup of meta-analysis was based on case-control data of association of history (Hx) of VZV infection with susceptibility to MS. The third subgroup of meta-analysis was based on cohort data of following up VZV positive and negative patients. The forth subgroup was integration of the raw data of five studies with IPD approach. These data were qualitative and quantitative positivity of VZV DNA based on polymerase chain reaction (PCR) in cerebrospinal fluid (CSF) and peripheral blood mononuclear cells (PBMC) (supplement 1). Their PCRs were done on *ORF31* gene.

Statistical analysis

Pearson Chi-square, Chi-square with Yate's correction and Fisher's exact test were used according to their indications. In the cases of Yate's correction, odds ratios (OR) were corrected as well through applying ± 0.5 to2 by 2 table cells. The error bars of 95% confidence interval (CI) were used to design forest plot. Alpha 0.05 considered as significance level. The software Excel 2013 (Microsoft, US) and STATA 14 (StataCorp, US) were used.

RESULTS

Studies' characteristics

For qualitative systematic review 19 studies were investigated[13-31] (Table 1). Among them 11 studies were used for meta-analysis in four subgroups. Among them subgroups 1-3 had IPD approach based on study data and subgroup 4 had IPD approach based on raw data. For numerical data of subgroup 4 (number virus copies), no parametrical analysis was performed because of normality rejection.

Synthesis and analysis of data

In subgroup 1 no significant result was obtained for association of VZV IgG seropositivity with susceptibility to MS (Table 2). In subgroup 2 positive history of VZV infection was significantly associated with susceptibility to MS (P <0.0001; OR =4.15; pooled) (Table 3, Figure 2). In subgroup 3, there were two cohort studies. In first one, which was a-two-year follow up on clinically isolated syndrome(CIS) patients, there was a significant relation with VZV positivity and conversion of CIS to MS (P =0.03; OR =2.82; Yate's correction). In the second one which was a-one year follow up on more than one million Taiwanese individuals, VZV positivity increased the chance of MS(P <0.0001; OR =3.62) (Table 4, Figure 2). In subgroup 4 we found that presence of VZV DNA in CSF or PBMC was associated with susceptibility to MS (P <0.0001; Yate's correction) (Table 5, Figure 3).

DISCUSSION

Summary of evidence

For the first time Pierre-Marie discussed viral infection concept (1884), but it remained a possibility because no suitable argument has been found. Viral hypothesis is one of the several ideas that has been published for explanation of the origin of MS. One of the arguments which is adverse with viral infection concept is that persons who suffer from MS don't show the signs and symptoms being along with viral diseases like fever, general malaise, leukocytosis, catarrh. Endogenous reinfection and remission and relapse in MS does not suggest an infection cause although circumstantial evidence portends viral infection.[32,33] In one study on MS, researchers selected choroid plexus culture of embryonic brain tissue. The results of that study could not be evidence of presence of viral infection. Negative conclusion reported that virus might not isolate from degenerative lesions of brain or spinal cord of patients stricken to MS, before coming and development of signs. As a probable cause of MS, researchers conducted a study on brains of 13 patients with MS and 4 persons with other conditions using various tissue-culture methods in 4 years. They described isolation of a herpes like virus from brain of a patient that 27 years suffering from MS.[34] Finally in a paper entitled "measles virus a causative agent in multiple sclerosis" has been perused relation between measles virus and MS in 1968.[35] Among the meta-analysis, EBV was the most investigated virus. In one of them (2006) it has been reported that EBV was a risk factor for MS.[36] This meta-analysis has been updated two times (2010 and 2013) and same results were found.[6,37]

According to the evidence found in the literature review, there are the following approaches to associations of herpes viruses with MS; association of positive history of infection and susceptibility to MS, association

Table 1: Literature review matrix

Authors	Purpose	Methods	Results / conclusion
Pirtilla 2000[31]	Investigation of apoE level and *apoE* polymorphism in severity of disease in patients with MS or in patients with acute herpes zoster	ApoE level in 105 MS patients and 41 acu6te herpes zoster patients in Finland was investigated in serum and CSF with ELISA	Non-significant
Tarrats 2002[30]	To investigate the role of varicella infection, ephemeral breast feeding and eczema as risk factors of MS	A questionnaire-based case-control study in a Mexican population	Positive significant association with mutually additive effect No significant result was observed for characteristics and severity of MS
Ordonez 2004[29]	To investigate the role of VZV in the pathogenesis of MS	VZV DNA in PBMC and serum VZV IgG and IgM of 82 Mexican patients with RRMS were investigated using PCR and ELISA respectively	Viral activation can be associated with relapse of MS in a restricted period
Mancuso 2007[28]	To investigate the possible involvement of viruses (HSV-1, HSV-2, EBV, VZV, HCMV, HHV-6, JCV) in MS	CSF samples of 85 Italian patients were collected (38 MS patients, 28 patients with other neurological diseases and 19 controls) using PCR	VZV DNA had positive significant association particularly among the RRMS patients VZV was more important than other viruses
Sotelo 2007[27]	To investigate presence of DNA of different herpes viruses in PBMC from MS patients	VZV, HSV1 and 2, EBV and HHV6 DNA, DNA load and phenotypes in PBMC Mexican patients using PCR, real-time PCR and flowcytometry, respectively (40 MS patients during relapse, 131 MS patients during remission and 125 controls)	Positive significant association for VZV DNA was observed in relapse phase of MS VZV was more important than other viruses
Sotelo 2008[26]	To investigate of association between the exacerbations of MS and the reactivation of latent VZV	Viral particles were observed in CSF of Mexican patients using electron microscopy (15 MS patients during relapse, 19 MS patients during remission and 28 controls)	Positive significant association for VZV particles was observed in relapse phase of MS
Brettsch-neider 2009[25]	To investigate relevance of MRZR to predict conversion CIS to MS	MRZR was determined in a 2 year follow-up of 89 CIS patients in Germany using ELISA Brain and spinal cord MRI were performed	Positive significant association for MRZR was observed in conversion of CIS to MS

Authors	Purpose	Methods	Results / conclusion
Burgoon 2009[24]	To detect VZV DNA in CSF and PBMC of RRMS patients	Ultra-structural observation and VZV DNA assay in 15 patients using electron microscopy and PCR, respectively	Non-significant
Rodriguez-Violante 2009[23]	To investigate association of positive history of VZV infection with MS and its subtypes	A case-control study in Mexico including 126 MS patients and 157 controls.	Positive significant association for history of VZV infection was observed particularly for RRMS
Ordonez 2010[22]	To investigate association of VZV DNA and viral particles in CSF with MS	Ultra-structural observation and VZV DNA assay in 20 Mexican patients using electron microscopy and PCR, respectively	Positive descriptive findings
Kang 2011[21]	To investigate risk of MS following occurrence of herpes zoster infection	A nation-wide population-based cohort study in Taiwan	Positive significant association was observed Hazard of MS was 3.96 times greater for the study group than controls
Ricklin 2013[20]	To investigate the immune response against VZV in MS patients before and during treatment with fingolimod	VZV-specific immune response, proliferation assays and T-cell activation markers were studied using ELISA in Switzerland (38 patients before treatment, 34 patients after 3 months treatment, 33 untreated patients, 25 patients treated with IFN-β, and 22 controls) Viral replication was analyzed in 76 PBMC samples and 146 saliva samples using real-time PCR	Fingolimod treated patients showed a significant reduction in antiviral T-cell response This reduction response is accompanied by a subclinical reactivation of VZV or EBV in the saliva of 20% of patients treated with fingolimod
Hon 2014[19]	To investigate association of HHV-6 and VZV DNA existence with MS	HHV-6 and VZV DNA in PBMC of 31 Caucasian patients of south Africa with MS and 30 controls using PCR	Non-significant
Otto 2014[18]	To investigate association of intrathecal VZV IgG of the total intrathecal IgG in patients with MS and VZV reactivation in order to distinguish MS polyspecific immune response from VZV reactivation immune response	CSF samples of 25 MS patients in Berlin were taken for investigation of antibody index using ELISA and confirmation of VZV DNA using PCR (20 MS patients and 5 VZV reactivation patients)	VZV IgG was 35-fold higher in patients with VZV reactivation F_S anti-VZV value can be used to distinguish the source of immune response

Authors	Purpose	Methods	Results / conclusion
Sotelo 2014[17]	To investigate possible presence of VZV DNA during exacerbation of MS	PBMC and CSF samples of 53 Mexican patients with MS were taken for VZV DNA using real-time PCR (31 patients during relapse, 16 patients during remission and 6 patients with progressive MS)	Positive significant association was observed for presence of VZV DNA during relapse phase of MS
Kohlmann 2015[16]	To investigate VZV reactivation occurrence in MS patients treated with natalizumab	VZV IgG of 702 patients in Germany was investigated using ELISA (205 natalizumab treated MS patients, 402 healthy blood donors and 95 HIV infected patients)	Positive significant association for natalizumab treatment with subclinical VZV reactivation
Najafi 2016[15]	To investigate the prevalence of the VZV antibody and VZV DNA in patients with RRMS	PBMCs collected from 171 Iranian patients and controls were screened for VZV antibodies and VZV DNA using ELISA and PCR, respectively (82 MS patients and 89 controls)	Positive significant association was found for VZV DNA
Karampoor 2017[14]	To investigate seropositivity of CMV and VZV IgG in MS patients	A population-based study in Iran including 800 MS patients and 1000 healthy individuals using ELISA	Positive significant association was found for VZV and CMV IgG
Manouchehrinia 2017[13]	To investigate association of history VZV infection with MS	A questionnaire-based study in UK	Positive descriptive findings

MS: multiple sclerosis. ApoE: apolipoprotein E. PBMC: peripheral mononuclear cells. RRMS: Relapsing Remitting MS. HSV-1: Herpes Simplex virus 1. HSV-2: Herpes Simplex virus 2. EBV: Epstein-Barr virus. VZV: varicella zoster virus. HCMV: Human Cyto Megalo Virus. HHV-6: Human Herpes Virus 6. JCV: JC virus. PCR: Polymerase Chain Reaction. MRVR or MRZR: Measles, Rubella and Varicella zoster Reaction. ELISA: Enzyme-Linked Immunosorbent Assay. CNS: Central Nervous System.

of viral seropositivity and susceptibility to MS, association of viral antibody positivity in CSF and susceptibility to MS, association of viral DNA positivity in PBMC and CSF and susceptibility to MS, association of viral particles in ultra-structural studies and susceptibility to MS, association herpes viruses reactivation with relapse phase of MS. Evidence suggests that all of the above hypotheses may have positive significant association. Of course it is not clear whether MS relapse results in herpes reactivation or herpes reactivation results in relapse of MS.[13-31]

Table 2: Subgroup 1 of meta-analysis results (case-control studies on seroprevalence)

Study	Country	MS		Control		MS subtype	P value
		VZV IgG +	VZV IgG -	VZV IgG +	VZV IgG -		
Kohlman *et al*.[16]	Germany	203	2	397	5	NM	1 a
Najafi *et al*.[15]	Iran	78	4	82	7	RRMS	0.63 b
Pooled (IPD)		281	6	479	12	All	1 b

a) Fisher's exact test. b) Yate's corrected chi-square.
NM: not mentioned. RRMS: relapsing-remitting MS.

Table 3: Subgroup 2 of meta-analysis results (case-control studies of history of infection)

Study	Country	MS		Control		MS subtype	P value	OR (CI)
		VZV Hx +	VZV Hx –	VZV Hx +	VZV Hx–			
Tarrats *et al*.[30]	Mexico	79	15	81	129	NM	<0.0001 a	8.38 (4.52-15.56)
Rodrguez-violante *et al*.[23]	Mexico	83	43	66	91	All	<0.0001 a	2.66 (1.63-4.32)
Pooled (IPD)		162	58	147	220	All	<0.0001 a	4.15 (2.88-5.98)

a) Pearson's Chi-square.
NM: not mentioned. Hx: history

In general VZV can be a risk factor for MS, but since VZV positivity (whether historically or currently) is highly prevalent and on the other hand MS has low prevalence, this association should not be used as a prognostic or predictive value. The current presence of VZV DNA both in CSF and blood showed larger effect sizes among the other subgroups of our meta-analysis. As a suggestion for clinical trial, acyclovir can be used in MS patients with persistence of VZV DNA during relapse phase. The exact mechanism should be investigated in future.

Limitations

Because of different methods and designs of these 19 studies, we cannot perform a traditional meta-analysis with random and fixed effect pooled results. As well different subtypes of MS may have different associations. Since VZV positivity is highly prevalent in population without vaccination, finding its association with other diseases do not give us representative predictive values. Although all of such patients potentially have this virus, but PCR procedure is technician dependent. VZV vaccination is another bias affecting the investigated association.

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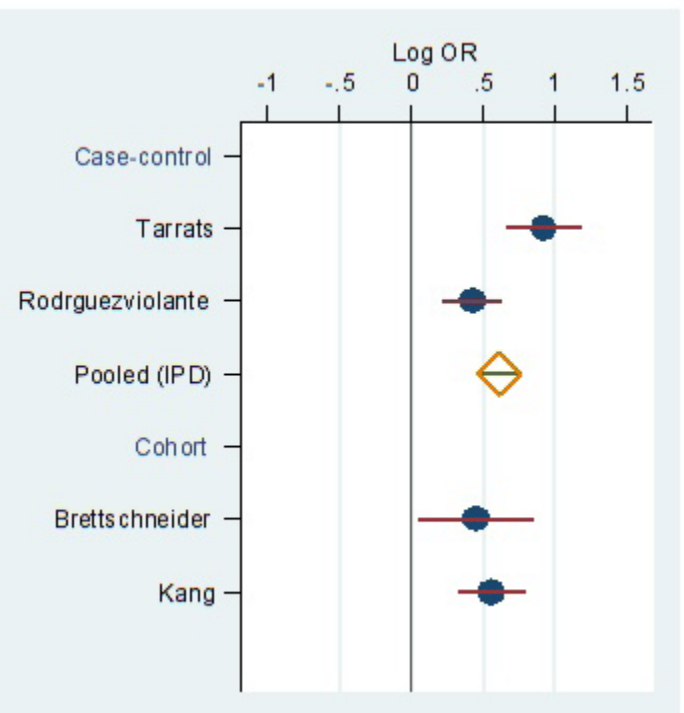

Figure 2: Forest plot of tables 3 and 4 (case control and cohort studies, respectively). OR=1 (Log OR=0) is the null hypothesis. The pooled bar for cohort studies is not shown, because they were different cohorts.

Table 4: Subgroup 3 of meta-analysis results (cohort studies; the pooled result is not shown because the cohorts were different)

Study	Country	VZV +		VZV -		MS subtype	Sample source	P value	OR (CI)
		MS +	MS -	MS +	MS -				
Brettschneider *et al*.[25] a	Germany	23	9	26	31	All	CIS	0.0301 b	2.82 (1.10-7.19) c
Kang *et al*.[21] d	Taiwan	29	315492	24	946602	All	Healthy	<0.0001 e	3.62 (2.11-6.22)

a) In this study VZV+ was defined as antibody index >1.5. The follow up period was 2 year. b) Yate's corrected chi-square. c) Adjusted with Yate's correction. d) In this study VZV+ was defined as previous clinical diagnosis. The follow up period was 1 year. e) Pearson chi-square.
CIS: clinically isolated symptom.

Table 5: Subgroup 4 of meta-analysis results (based on pooled analysis of individual participant data of supplement 1)

VZV	MS		Control a		P value	OR (CI)
	E +	E -	E +	E -		
History of infection	81	21	27	30	0.0001 b	4.28 (2.11-8.69)
DNA in CSF	30	13	7	50	<0.0001 c	14.42 (5.29-39.29)
DNA in PBMC	33	41	2	72	<0.0001 c	22.40 (5.85-85.71)

a) Control groups had other neurological disorders. b) Pearson chi-square. c) Yate's corrected chi-square.
E: exposure. CSF: cerebrospinal fluid. PBMC: peripheral blood mononuclear cells.

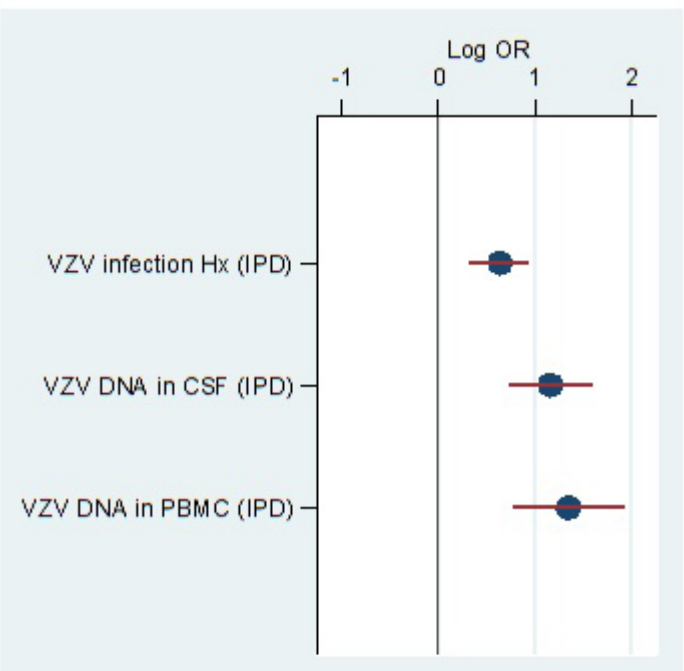

Figure 3: Forest plot of table 5. OR=1 (Log OR=0) is the null hypothesis. This plot is based on pooled analysis of individual participant data of the supplement file.

DISCLOSURE

Conflict of interest: None

REFERENCES

1. Soleimani M, Aghayan HR, Goodarzi P, *et al*. Stem cell therapy – Approach for multiple sclerosis treatment. *Arch Neurosci* 2016;3(1):e21564.
2. Shahsavar F, Mapar S, Ahmadi SAY. Multiple sclerosis is accompanied by lack of KIR2DS1 gene: a meta-analysis. *Genom Data* 2016;10:75-8.
3. Kamm CP, Uitdehaag BM, Polman CH. Multiple sclerosis: current knowledge and future outlook. *Eur Neurol* 2014;72(3-4):132-41.
4. Wingerchuk DM. Environmental factors in multiple sclerosis: Epstein- Barr virus, Vitamin D, and cigarette smoking. *Mt Sinai J Med* 2011;78(2):221-30.
5. Pantazou V, Schluep M, Du Pasquier R. Environmental factors in multiple sclerosis. *Presse Medicale* 2015;44(4 Pt 2):e113-20.
6. Almohmeed YH, Avenell A, Aucott L, Vickers MA. Systematic review and meta-analysis of the sero-epidemiological association between Epstein Barr virus and multiple sclerosis. *PloS one* 2013;8(4):e61110.
7. Boudreau JE, Hsu KC. Natural killer cell education in human health and disease. *Curr Opin Immunol* 2018;50:102-11.
8. Mousavi T, Poormoghim H, Moradi M, Tajik N, Shahsavar F, Soofi M. Phenotypic study of natural killer cell subsets in ankylosing spondylitis patients. *Iranian J Allergy Asthma Immunology* 2009;8(4):193-8.
9. Virtanen JO, Jacobson S. Viruses and multiple sclerosis. *CNS Neurol Disord Drug Targets* 2012;11(5):528-44.
10. Pawate S, Sriram S. The role of infections in the pathogenesis and course of multiple sclerosis. *Ann Indian Acad Neurol* 2010;13(2):80-6.
11. Pakpoor J, Disanto G, Gerber JE, *et al*. The risk of developing multiple sclerosis in individuals seronegative for Epstein-Barr virus: a meta-analysis. *Mult Scler* 2013;19(2):162-6.
12. Xiao D, Ye X, Zhang N, *et al*. A meta-analysis of interaction between Epstein-Barr virus and HLA-DRB1* 1501 on risk of multiple sclerosis. *Sci Rep* 2015;5.
13. Manouchehrinia A, Tanasescu R, Kareem H, *et al*. Prevalence of a history of prior varicella/herpes zoster infection in multiple sclerosis. *J Neurovirol* 2017;23(6):839-44.
14. Karampoor S, Zahednasab H, Ramagopalan S, *et al*. Cytomegalovirus and varicella zoster virus seropositivity of Iranian patients with multiple sclerosis: A population-based study. *J Neuroimmunol* 2017;309:4-6.
15. Najafi S, Ghane M, Yousefzadeh-Chabok S, Amiri M. The high prevalence of the varicella zoster virus in patients with relapsing-remitting multiple sclerosis: A case-control study in the North of Iran. *Jundishapur J Microbiology* 2016;9(3):e34158.
16. Kohlmann R, Salmen A, Chan A, *et al*. Serological evidence of increased susceptibility to varicella-zoster virus reactivation or reinfection in natalizumab-treated patients with multiple sclerosis. *Mult Scler* 2015;21(14):1823-32.
17. Sotelo J, Ordonez G, Pineda B, Flores J. The participation of varicella zoster virus in relapses of multiple sclerosis. *Clin Neurol Neurosur* 2014;119:44-8.
18. Otto C, Hofmann J, Finke C, Zimmermann M, Ruprecht K. The fraction of varicella zoster virus-specific antibodies among all intrathecally-produced antibodies discriminates between patients with varicella zoster virus reactivation and multiple sclerosis. *Fluids Barriers CNS* 2014;11(1):3.
19. Hon GM, Erasmus RT, Matsha T. Low prevalence of human herpesvirus-6 and varicella zoster virus in blood of multiple sclerosis patients, irrespective of inflammatory status or disease progression. *J Clin Neurosci* 2014;21(8):1437-40.
20. Ricklin ME, Lorscheider J, Waschbisch A, *et al*. T-cell response against varicella-zoster virus in fingolimod-treated MS patients. *Neurology* 2013;81(2):174-81.
21. Kang JH, Sheu JJ, Kao S, Lin HC. Increased risk of multiple sclerosis following herpes zoster: a nationwide, population-based study. *J Infect Dis* 2011;204(2):188-92.
22. Ordonez G, Martinez-Palomo A, Corona T, *et al*. Varicella zoster virus in progressive forms of multiple sclerosis. *Clin Neurol Neurosurg* 2010;112(8):653-7.
23. Rodriguez-Violante M, Ordonez G, Bermudez JR, Sotelo J, Corona T. Association of a history of varicella virus infection with multiple sclerosis. *Clin Neurol Neurosurg* 2009;111(1):54-6.
24. Burgoon MP, Cohrs RJ, Bennett JL, *et al*. Varicella zoster virus is not a disease-relevant antigen in multiple sclerosis. *Ann Neurol* 2009;65(4):474-9.
25. Brettschneider J, Tumani H, Kiechle U, *et al*. IgG antibodies against measles, rubella, and varicella zoster virus predict conversion to multiple sclerosis in clinically isolated syndrome. *PLoS One* 2009;4(11):e7638.
26. Sotelo J, Martinez-Palomo A, Ordonez G, Pineda B. Varicella-zoster virus in cerebrospinal fluid at relapses of multiple sclerosis. *Ann Neurol* 2008;63(3):303-11.
27. Sotelo J, Ordonez G, Pineda B. Varicella-zoster virus at relapses of multiple sclerosis. *J Neurol* 2007;254(4):493-500.
28. Mancuso R, Delbue S, Borghi E, *et al*. Increased prevalence of varicella zoster virus DNA in cerebrospinal fluid from patients with multiple sclerosis. *J Med Virol* 2007;79(2):192-9.
29. Ordonez G, Pineda B, Garcia-Navarrete R, Sotelo J. Brief presence of varicella-zoster vral DNA in mononuclear cells during relapses of multiple sclerosis. *Arch Neurol* 2004;61(4):529-32.
30. Tarrats R, Ordonez G, Rios C, Sotelo J. Varicella, ephemeral breastfeeding and eczema as risk factors for multiple sclerosis in Mexicans. *Acta Neurol Scand* 2002;105(2):88-94.
31. Pirttila T, Haanpaa M, Mehta PD, Lehtimaki T. Apolipoprotein E (APOE) phenotype and APOE concentrations in multiple sclerosis and acute herpes zoster. *Acta Neurol Scand* 2000;102(2):94-8.

32. Innes JR, Kurland LT. Is multiple sclerosis caused by a virus? *Am J Med* 1952;12(5):574-85.
33. Thormar H, von M. Attempts to isolate virus from the cerebrospinal fluid of patients with multiple sclerosis. *Acta Neurol Scand* 1963;39:209-12.
34. Gudnadottir M, Helgadottir H, Bjarnason O, Jonsdottir K. Virus isolated from the brain of a patient with multiple sclerosis. *Exp Neurol* 1964;9:85-95.
35. Pette E. Measles virus: a causative agent in multiple sclerosis? *Neurology* 1968;18(1 Pt 2):168-9.
36. Thacker EL, Mirzaei F, Ascherio A. Infectious mononucleosis and risk for multiple sclerosis: A meta- analysis. *Ann Neurol* 2006;59(3):499-503.
37. Handel AE, Williamson AJ, Disanto G, Handunnetthi L, Giovannoni G, Ramagopalan SV. An updated meta-analysis of risk of multiple sclerosis following infectious mononucleosis. *PLoS One* 2010;5(9):e12496.

Appendix 1: Individual participant data of the patients.

Study	Year	Patient No.	Control subtype	VZV Hx	VZV Hx age	CSF VZV DNA	CSF VZV copy	PB VZV DNA	PB VZV copy	
Ordoez	2010	1	oth neurol dis	pos	10	pos	510	neg	0	Control group
Ordoez	2010	2	oth neurol dis	pos	5	neg	0	neg	0	
Ordoez	2010	3	oth neurol dis	pos	4	neg	0	neg	0	
Ordoez	2010	4	oth neurol dis	neg		neg	0	neg	0	
Ordoez	2010	5	oth neurol dis	neg		pos	1537	pos	98	
Ordoez	2010	6	oth neurol dis	pos	5	pos	220	neg	0	
Ordoez	2010	7	oth neurol dis	neg		pos	513	neg	0	
Ordoez	2010	8	oth neurol dis	pos	4	neg	0	neg	0	
Ordoez	2010	9	oth neurol dis	pos	3	neg	0	neg	0	
Ordoez	2010	10	oth neurol dis	pos	4	neg	0	neg	0	
Ordoez	2010	11	oth neurol dis	pos	7	neg	0	neg	0	
Ordoez	2010	12	oth neurol dis	neg		neg	0	neg	0	
Ordoez	2010	13	oth neurol dis	pos	4	neg	0	neg	0	
Ordoez	2010	14	oth neurol dis	neg		neg	0	neg	0	
Ordoez	2010	15	oth neurol dis	pos	9	neg	0	neg	0	
Ordoez	2010	16	oth neurol dis	pos	4	neg	0	neg	0	
Ordoez	2010	17	oth neurol dis	neg		neg	0	neg	0	
Ordoez	2010	18	oth neurol dis	pos	4	neg	0	neg	0	
Ordoez	2010	19	oth neurol dis	neg		neg	0	neg	0	
Ordoez	2010	20	oth neurol dis	neg		neg	0	neg	0	
Ordoez	2010	21	oth neurol dis	pos	5	neg	0	neg	0	
Ordoez	2010	22	oth neurol dis	neg		neg	0	neg	0	
Ordoez	2010	23	oth neurol dis	neg		neg	0	neg	0	
Ordoez	2010	24	oth neurol dis	neg		neg	0	neg	0	
Ordoez	2010	25	oth neurol dis	neg		neg	0	neg	0	
Ordoez	2010	26	oth neurol dis	pos	10	neg	0	neg	0	
Ordoez	2010	27	oth neurol dis	neg		neg	0	neg	0	
Ordoez	2010	28	oth neurol dis	neg		neg	0	neg	0	
Ordoez	2010	29	oth neurol dis	pos	11	neg	0	neg	0	
Ordoez	2010	30	oth neurol dis	neg		neg	0	neg	0	
Sotelo	2008	1	oth neurol dis	neg		neg	0	neg	0	
Sotelo	2008	2	oth neurol dis	neg		neg	0	neg	0	
Sotelo	2008	3	oth neurol dis	neg		neg	0	neg	0	
Sotelo	2008	4	oth neurol dis	neg		neg	0	neg	0	
Sotelo	2008	5	oth neurol dis	pos	6	neg	0	neg	0	
Sotelo	2008	6	oth neurol dis	neg		neg	0	neg	0	
Sotelo	2008	7	oth neurol dis	pos	9	neg	0	neg	0	
Sotelo	2008	8	oth neurol dis	neg		neg	0	neg	0	
Sotelo	2008	9	oth neurol dis	pos	4	neg	0	neg	0	
Sotelo	2008	10	oth neurol dis	pos	5	neg	0	neg	0	
Sotelo	2008	11	oth neurol dis	pos	14	neg	0	neg	0	
Sotelo	2008	12	oth neurol dis	pos	8	neg	0	neg	0	
Sotelo	2008	13	oth neurol dis	neg		pos	8	neg	0	
Sotelo	2008	14	oth neurol dis	pos	5	neg	0	neg	0	
Sotelo	2008	15	oth neurol dis	neg		neg	0	neg	0	
Sotelo	2008	16	oth neurol dis	pos	5	neg	0	neg	0	
Sotelo	2008	17	oth neurol dis	pos	8	pos	5	pos	11	
Sotelo	2008	18	oth neurol dis	neg		neg	0	neg	0	
Sotelo	2008	19	oth neurol dis	neg		neg	0	neg	0	
Sotelo	2008	20	oth neurol dis	pos	6	neg	0	neg	0	

Sotelo	2008	21	oth neurol dis	neg		neg	0	neg	0
Sotelo	2008	22	oth neurol dis	neg		neg	0		
Sotelo	2008	23	oth neurol dis	neg		neg	0		
Sotelo	2008	24	oth neurol dis	neg		neg	0		
Sotelo	2008	25	oth neurol dis	pos	10	neg	0		
Sotelo	2008	26	oth neurol dis	neg		pos	81		
Sotelo	2008	27	oth neurol dis	pos	5	neg	0		
Sotelo	2008	28	oth neurol dis	pos	6	neg	0		

Appendix 2: Individual participant data of the controls. Data provided from Ordonez *et al*. 2010[22], Burgoon *et al*. 2009[22], Sotelo *et al*. 2008[26] and Ordonez *et al*. 2004[29]

Study	Year	Patient No.	Control subtype	VZV Hx	VZV Hx age	CSF VZV DNA	CSF VZV copy	PB VZV DNA	PB VZV copy
Ordonez	2010	1	oth neurol dis	pos	10	pos	510	neg	0
Ordonez	2010	2	oth neurol dis	pos	5	neg	0	neg	0
Ordonez	2010	3	oth neurol dis	pos	4	neg	0	neg	0
Ordonez	2010	4	oth neurol dis	neg		neg	0	neg	0
Ordonez	2010	5	oth neurol dis	neg		pos	1537	pos	98
Ordonez	2010	6	oth neurol dis	pos	5	pos	220	neg	0
Ordonez	2010	7	oth neurol dis	neg		pos	513	neg	0
Ordonez	2010	8	oth neurol dis	pos	4	neg	0	neg	0
Ordonez	2010	9	oth neurol dis	pos	3	neg	0	neg	0
Ordonez	2010	10	oth neurol dis	pos	4	neg	0	neg	0
Ordonez	2010	11	oth neurol dis	pos	7	neg	0	neg	0
Ordonez	2010	12	oth neurol dis	neg		neg	0	neg	0
Ordonez	2010	13	oth neurol dis	pos	4	neg	0	neg	0
Ordonez	2010	14	oth neurol dis	neg		neg	0	neg	0
Ordonez	2010	15	oth neurol dis	pos	9	neg	0	neg	0
Ordonez	2010	16	oth neurol dis	pos	4	neg	0	neg	0
Ordonez	2010	17	oth neurol dis	neg		neg	0	neg	0
Ordonez	2010	18	oth neurol dis	pos	4	neg	0	neg	0
Ordonez	2010	19	oth neurol dis	neg		neg	0	neg	0
Ordonez	2010	20	oth neurol dis	neg		neg	0	neg	0
Ordonez	2010	21	oth neurol dis	pos	5	neg	0	neg	0
Ordonez	2010	22	oth neurol dis	neg		neg	0	neg	0
Ordonez	2010	23	oth neurol dis	neg		neg	0	neg	0
Ordonez	2010	24	oth neurol dis	neg		neg	0	neg	0
Ordonez	2010	25	oth neurol dis	neg		neg	0	neg	0
Ordonez	2010	26	oth neurol dis	pos	10	neg	0	neg	0
Ordonez	2010	27	oth neurol dis	neg		neg	0	neg	0
Ordonez	2010	28	oth neurol dis	neg		neg	0	neg	0
Ordonez	2010	29	oth neurol dis	pos	11	neg	0	neg	0
Ordonez	2010	30	oth neurol dis	neg		neg	0	neg	0
Sotelo	2008	1	oth neurol dis	neg		neg	0	neg	0
Sotelo	2008	2	oth neurol dis	neg		neg	0	neg	0
Sotelo	2008	3	oth neurol dis	neg		neg	0	neg	0
Sotelo	2008	4	oth neurol dis	neg		neg	0	neg	0
Sotelo	2008	5	oth neurol dis	pos	6	neg	0	neg	0
Sotelo	2008	6	oth neurol dis	neg		neg	0	neg	0
Sotelo	2008	7	oth neurol dis	pos	9	neg	0	neg	0
Sotelo	2008	8	oth neurol dis	neg		neg	0	neg	0
Sotelo	2008	9	oth neurol dis	pos	4	neg	0	neg	0
Sotelo	2008	10	oth neurol dis	pos	5	neg	0	neg	0
Sotelo	2008	11	oth neurol dis	pos	14	neg	0	neg	0
Sotelo	2008	12	oth neurol dis	pos	8	neg	0	neg	0
Sotelo	2008	13	oth neurol dis	neg		pos	8	neg	0
Sotelo	2008	14	oth neurol dis	pos	5	neg	0	neg	0
Sotelo	2008	15	oth neurol dis	neg		neg	0	neg	0
Sotelo	2008	16	oth neurol dis	pos	5	neg	0	neg	0
Sotelo	2008	17	oth neurol dis	pos	8	pos	5	pos	11
Sotelo	2008	18	oth neurol dis	neg		neg	0	neg	0
Sotelo	2008	19	oth neurol dis	neg		neg	0	neg	0
Sotelo	2008	20	oth neurol dis	pos	6	neg	0	neg	0

Control group

Sotelo	2008	21	oth neurol dis	neg		neg	0	neg	0
Sotelo	2008	22	oth neurol dis	neg		neg	0		
Sotelo	2008	23	oth neurol dis	neg		neg	0		
Sotelo	2008	24	oth neurol dis	neg		neg	0		
Sotelo	2008	25	oth neurol dis	pos	10	neg	0		
Sotelo	2008	26	oth neurol dis	neg		pos	81		
Sotelo	2008	27	oth neurol dis	pos	5	neg	0		
Sotelo	2008	28	oth neurol dis	pos	6	neg	0		