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Positive Correlation of Human Herpes Virus 6 Immunoglobulin G and Immunoglobulin M Antibodies and Multiple Sclerosis Disease in Iraqi Patients

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ABSTRACT

Background: *Multiple sclerosis (MS) is the commonest cause of neurological impairment among young adults. MS is multifactorial, heterogeneous, autoimmune disease that is caused by complex environment–interactions.* ***purpose****: is to study the seroprevalence of human herpes virus 6 (HHV-6)in MS Iraqi patients.* ***Methods****: a case-control study includes 100 subjects: 50 patients previously diagnosed as MS or newly diagnosed without treatment and 50 healthy volunteers, we examined the presence of anti-HHV-6 IgM and anti-HHV-6 IgG antibodies by using ELISA technique* ***Results****: When comparing the participants with MS group to the control group, there was a significant rise in the level of HHV-6 IgM in patients 31.91 ng/L when compare to control group 12.62ng/L, respectively ($p < 0.001$). Also, the comparison of HHV-6 IgG level of MS group to control group revealed significantly rise in the level of HHV-6 IgG, 19.78 ng/L versus 9.5 ng/L, respectively ($p < 0.001$). there was no correlation between HHV-6 antibodies and age, sex, duration of disease and EDSS.* ***Implications****: MS patients have high level of seroprevalence of HHV-6 antibodies compare to control which mean positive correlation between MS disease and HHV-6 infection. Controversially, HHV-6 infection has no role in MS severity.* ***CONCLUSION:*** *MS patients have high level of seroprevalence of HHV-6 antibodies compare to control which mean positive correlation between MS disease and HHV-6 infection. Controversially, HHV-6 infection has no role in MS severity.*

Keywords: Multiple sclerosis, HHV-6, Seroprevalence.

Article Information

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INTRODUCTION

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS), characterized by a complex immune response. The etiology of MS is heterogeneous, leading to a multifaceted pathogenesis with varying types of disease manifestations and a diverse range of disease progression (1, 2). Infection with EBV and neurotropic HHV-6 has been inconsistently associated with elevated MS risk. The CD8+ T cell-mediated response to EBV and HHV-6 infected CNS cells may provoke proinflammatory reaction, resulting in damage to tissues and the subsequent release of sequestered substances, which activates self-activating lymphocytes and exacerbates auto-aggressive immune responses. Moreover, molecular mimicry occurs when EBV viral protein sequences resemble human myelin proteins and other CNS proteins and thereby induction autoimmunity against myelin and CNS antigens (3, 4). Infection with human herpes viruses has been suggested to contribute to multiple sclerosis (MS), while interaction between human herpes 6 (HHV6),

and MS remain unclear yet, HHV-6 has long been proposed as a potential etiological agent in multiple sclerosis (MS) (5). As a neurotropic virus, HHV-6 has been associated with a variety of neurological disorders, including neuromyelitis optica and MS (6). The potential link between HHV-6 and MS was first suggested in 1993 (7). The correlation between HHV-6 and MS has been proposed since 1993 (1). Multiple clinical research have established a correlation between multiple sclerosis (MS) and human herpesvirus 6 (HHV-6) infection, as highlighted in numerous investigations (8) Notably, when compared to healthy people or those with other neurological conditions, MS patients' serum has been found to have higher quantities of HHV-6 . DNA, a sign of an active infection (9, 10). HHV-6 DNA can either integrate into host cell chromosomes or persist in episomal form; in the general population, integration prevalence is roughly 85%. It is believed that neural cells act cells act as a reservoir for latent infections (2).

METHODS

The current study is a case- control study included fifty consecutive patients previously diagnosed as MS or newly diagnosed without treatment who observe in the Middle Euphrates Neuroscience Center in Al-Najaf / Iraq. In the period from January 2023 to September 2023and healthy volunteers to examines the correlation between multiple sclerosis disease and seroprevalence of HHV-6 in MS patients. The diagnosis of MS was depended on revised McDonald Criteria (3, 4). not all patients were on treatment (12 pre-treatment and 38 patients on treatment) and 50 sex-age matched healthy individual were randomly selected.

Under the supervision of neurologist specialists were included in this study and the information about each case was collected from the patient as well as the investigation that have been done in MS departments. such as (EMG, MRI, Laboratories assay including auto immune screen test, CSF analysis, routine blood test and in addition to parameters that found in questionnaire in appendix). The clinical assessment involved the determination of expanded disability status scale (EDSS) score (5). the EDSS of all patients were determined by a trained neurologist.

Inclusion Criteria:

All patients diagnosed with MS that have any of four clinical phases of MS (RRMS, SPMS, CIS, RPMS), with remission or active phase and the age range from (15-60 years) include in this study.

Exclusion Criteria

Recent or Current infectious disorders or inflammatory in the past month, any type of malignancy, diabetes, pregnancy, or any other neurological condition were all considered excluding factors

Recent or Current infectious disorders or inflammatory in the past month, any other inflammatory diseases such as allergy, any type of malignancy, covid-19 infection other autoimmune diseases, diabetes, pregnancy, and heart and kidney diseases or any other neurological condition were all considered excluding factors

Approximately 3 ml of venous blood sample were drawn from each participant, it was taken through venipuncture by using disposable syringe. Three ml of blood was dispensed into a gel tube and allowed to clot then centrifugated 3500 rpm for 5min to separate the serum, the serum has been stored as aliquots by using small Eppendrof tubes and frozen at -20 to be used for immunological testes. we investigated the level of HHV-6 antibodies in the sera of MS patients and healthy individuals.

We examined the presence of anti-HHV-6 IgM and anti-HHV-6 IgG antibodies by using ELISA technique, the anti-HHV-6 IgM and anti-HHV-6 IgG measurements were performed in duplicate using an ELISA kit (HHV-6 IgG ELISA kit and HHV-6 IgM ELISA kit,

SunLong Biotech Co., China), which is available for purchase. This was done in accordance with the manufacturer's instructions. By measuring the optical density (OD) at 450 nm with a Microplate-Reader (ELISA) Bio-Rad/USA. This approach is utilised for the concentration of both HHV-6 Immunoglobulin G and Immunoglobulin M Antibodies. The data for both antibodies are represented as optical density readings plotted against their corresponding known concentrations of Human HHV6 antibodies Standard on the y-axis and x-axis, respectively. The sample concentration is determined by graphing the optical density of the sample on the Y-axis. We multiply the dilution factor to obtain the original concentration.

Statistical analysis

The collected data were analyzed by using the Statistics Package for Social Sciences (SPSS) version 23 to summarize, analyze, and present the data. By utilization of the the Kolmogorov-Smirnov test to estimate the normality distribution firstly. So the data divided into normally or not normally distributed, depend on the results of the Kolmogorov-Smirnov test we used the most suitable analysis test. We used the Chi-square test, ANOVA, Students T-test, Kruskal-Wallis test and Mann-Whitney U test. The Spearman correlation was utilized to assess the association between two numerical variables, Receiver Operator Characteristic (ROC) curve analysis was used, alongside the calculation of the AUC, specificity, sensitivity. A significance level of *P*-value ≤ 0.05 and a high significance at *P*-value < 0.001 was established.

RESULTS

Comparison of mean age and proportions of males and females between patients groups and control group is shown in **table 1.** Comparison of mean age between patients group (with treatment and without treatment) and control group revealed no significant difference ($p = 0.259$), 33.03 ±9.64 and 31.92 ±10.11 years versus 36.02 ±10.34 years, respectively. comparison between groups according to gender revealed no significant difference ($p = 0.360$).

Figure 1 revealed the frequency distribution of patients with MS according to age in which it appears that the most frequently involved age interval was 20-29 years (34 %) followed by the age interval 30-39 years (30 %) then by 40-49 years (26 %) then by 16-19 years (8 %) and lastly by 50-54 years (2 %).

Table 2, shows The duration of disease was ranging between a minimum of 0 years (recently diagnosed patients) to 16.5 years. Median duration was 3 years and the mean was 3.75 years. Recent diagnoses was reported in 11 cases (22 %), a duration of 1-5 years was seen in 26 (52 %), a duration of 6-10 years was seen in 10 (20 %) and a duration of > 10 years was seen in 10 (20 %).

Figure 2 illustrates the frequency distribution of individuals with multiple sclerosis classified by the clinical course of the disease. Clinically isolated syndrome (CIS) was reported in 6 %. Relapsing remitting multiple sclerosis was the most common and it was seen in 82 % of cases. Primary progressive MS (PPMS) was showed in 8 % and SPMS was reported in 4 %.

The comparison of HHV-6 IgM and IgG levels between MS patients and the control group is presented in **Table 3**. When comparing the participants with MS group to the control group, there was a significant rise in the level of HHV-6 IgM, 31.91 ng/L versus 12.62 ng/L, respectively ($p < 0.001$), **figure 3**.

Also, the comparison of HHV-6 IgG level of MS group to control group revealed significantly rise in the level of HHV-6 IgG, 19.78 ng/L versus 9.5 ng/L, respectively ($p < 0.001$), **figure 4.**

Correlations of HHV-6 IgM and IgG levels to age, gender, duration of disease and EDSS are shown in **table 4.** No significant variation was reported ($p > 0.05$).

Comparison of HHV-6 IgM and IgG levels according to stage of disease are shown in **table 5** No significant variation was reported ($p > 0.05$), see **figure 3 and 4.**

Table 1: Comparison of mean age and proportions of males and females among study group.

Characteristic	Control group *n* = 50	MS with no treatment *n* = 12	Treated MS *n* = 38	*P*
Age (years)				
Mean ±SD	36.02 ±10.34	31.92 ±10.11	33.03 ±9.64	0.259 O NS
Range	20 -65	17 -46	16 -54	
Gender				
Male, *n* (%)	25 (50.0 %)	4 (33.3 %)	14 (36.8 %)	0.360 C NS
Female, *n* (%)	25 (50.0 %)	8 (66.7 %)	24 (63.2 %)	

SD: standard deviation; ***n***: number of cases; **O**: one way ANOVA; **C**: chi-square test; **NS**: not significant

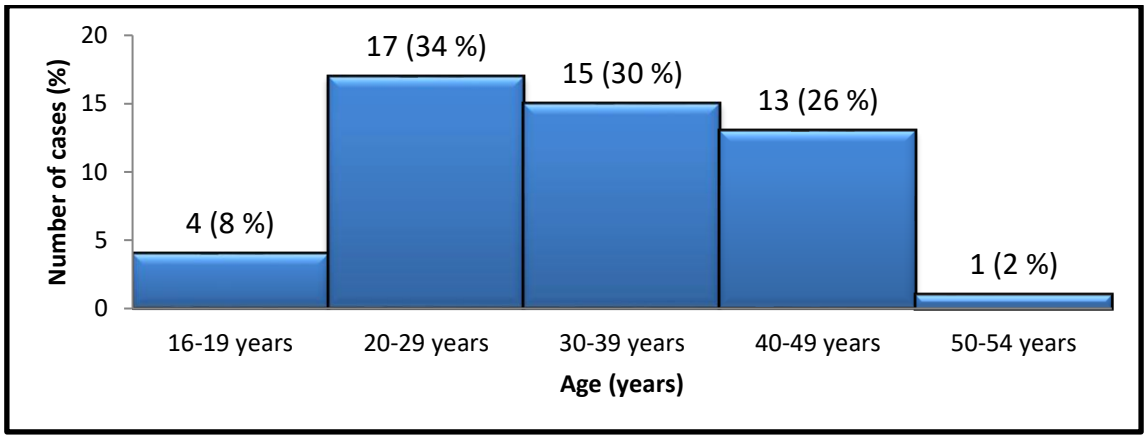

Figure 1: Histogram showing frequency distribution of individuals diagnosed with multiple sclerosis categorized by age

Table 2: The duration of disease.

Duration of disease	Results
Median	3
Mean	3.75
Standard deviation	3.92
Minimum	0.00
Maximum	16.5
Recent diagnosis, *n* (%)	11 (22 %)
1-5 years, *n* (%)	26 (52 %)
6-10 years, *n* (%)	10 (20 %)
>10 years, *n* (%)	3 (6 %)

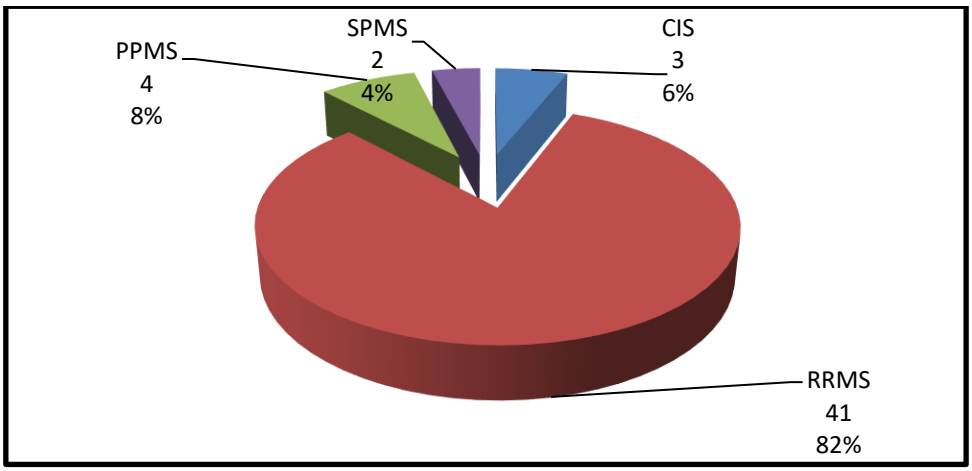

Figure 2: A pie chart representing the frequency distribution of patients diagnosed with multiple sclerosis, categorized by disease stage.

Table 3: Comparison of HHV-6 Antibodies (IgM and IgG) among study groups.

Characteristic	Control group *n* = 50	Non treated MS *n* = 12	Treated MS *n* = 38	*p*
HHV-6 IgM ng/L				
Median (IQR)	12.62 (7.1)	32.73 (33.27)	31.67 (25.02)	<0.001 K ***
Range	3.48 -178.57	19.71 -136.54	18.17 -193.58	
HHV-6 IgG ng/L				
Median (IQR)	9.5 (8.67)	18.47 (14.3)	19.78 (38.96)	<0.001 K ***
Range	2.52 -115.75	5.84 (81.42)	2.06 -537.44	

IQR: inter-quartile range; ***n***: number of cases; **K**: Kruskal Wallis test; ***: significant at $p \leq 0.001$.

Table 4: Correlations of NLRP3 gene expression level, human ASC, HHV-6 IgM and IgG levels to age, gender, duration of disease and EDSS.

Characteristic		HHV-6 IgM	HHV-6 IgG
Age (years)	*r*	0.010	-0.115
	p	0.921	0.256
Gender Male =1, Female =2	*r*	0.163	0.155
	p	0.105	0.124
Duration of disease (years)	*r*	0.091	-0.053
	p	0.531	0.717
Expanded Disability Status Scale (EDSS)	*r*	-0.137	-0.110
	p	0.341	0.447

EDSS: The Expanded Disability Status Scale; ***r*:** correlation coefficient, ***: highly significant at P ≤ 0.01; *: significant at P ≤ 0.05

Table 5: Comparison of HHV-6 IgM and IgG levels according to stage of disease.

Characteristic	CIS	RRMS	PPMS	SPMS	*p*
HHv-6 IgM					
Median (IQR)	38.98 ()	32.02 (26.12)	21.97 (6.41)	76.56 ()	1.000 K NS
Range	23.59 -68.46	18.17 -193.58	19.07 -26.53	20.34 -132.78	
HHV-6 IgG					
Median (IQR)	15.81 ()	20.06 (32.77)	19.66 (45.72)	22.57 ()	0.800 K NS
Range	5.75 -220.40	2.06 -537.44	5.84 -64.17	17.85 -27.28	

n: number of cases; **IQR**: inter-quartile range; **M**: Mann Whitney U test; **NS**: not significant

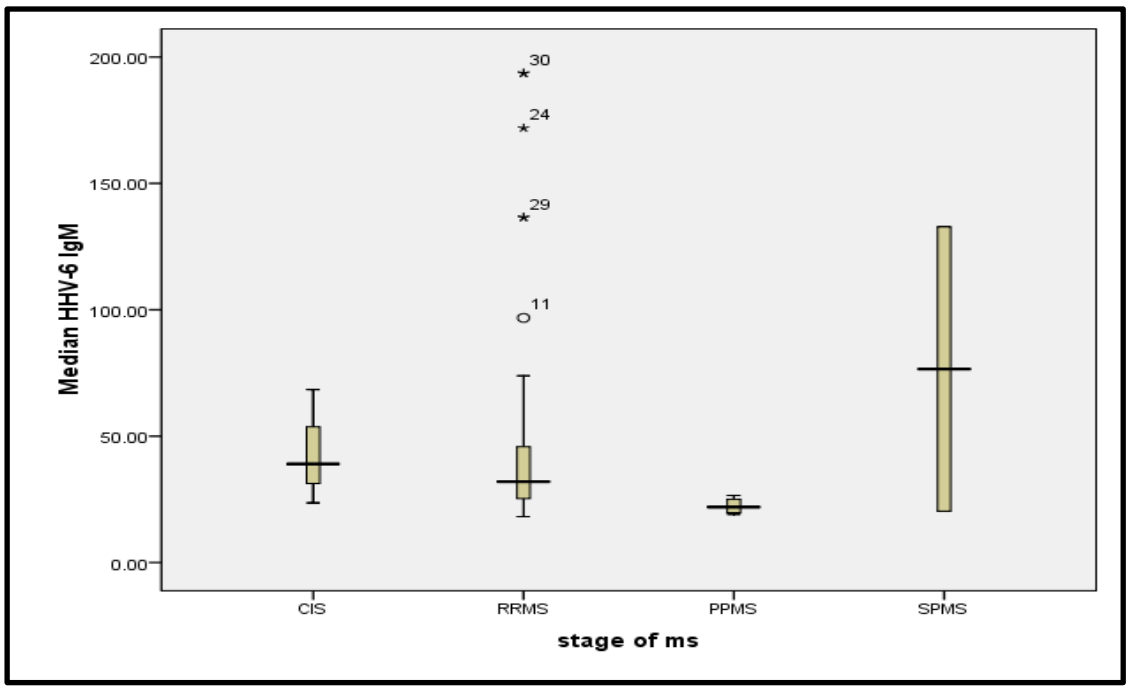

Figure 3: Bar chart showing comparison of HHV-6 IgM levels according to stage of disease.

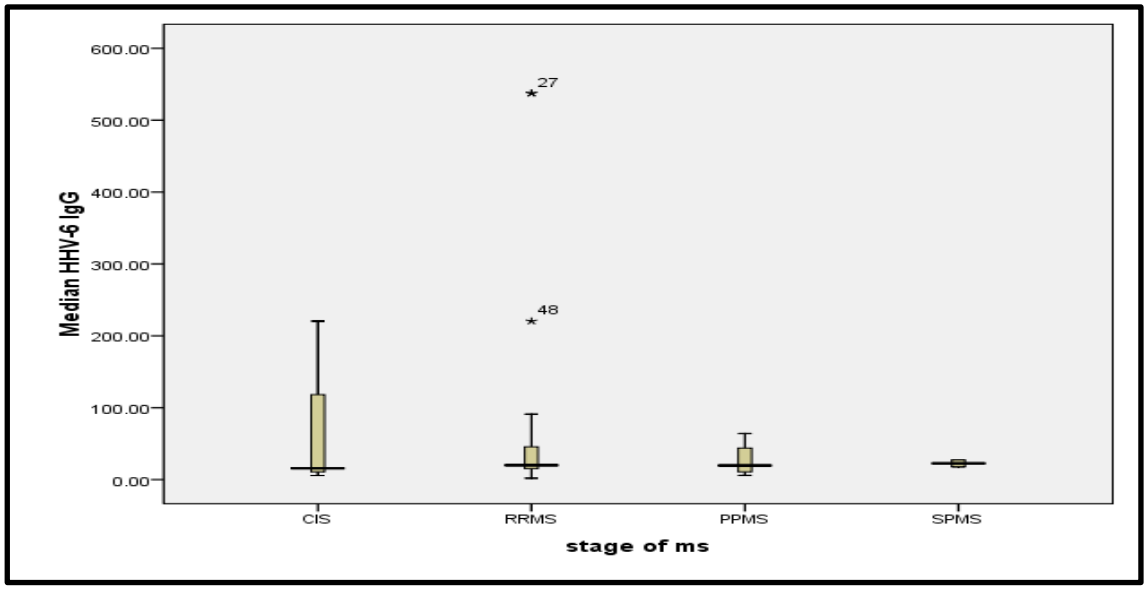

Figure 4: Bar chart showing comparison of HHV-6 IgG levels according to stage of disease

DISCUSSION

MS is a complex disease characterized by immune-mediated damage to myelin, oligodendrocytes, and nerve fibers in the CNS, resulting in a wide range of symptoms and disease presentations (6). Cortés B *et al* (2016) conducted a study. The average age was 41 years (SD 11.22). The maximum age was 66 years, the minimum age at diagnosis was 12 years, and the mean age at diagnosis was 32 years (SD 9.72), previous study in Iraq revealed that the age ranged from 14 to 52 years (mean ± SD = 33.3 ± 9.5 years). There was also no significant difference in the proportions of males and females between patients group and control group (p = 0.157) The frequency distribution of patients with MS according to gender is shown in table 1 . The disease was most common in females in comparison with males, 64 % versus 36 %, respectively and the male to female ratio was 1: 1.8. suggesting that hormones may play a significant role in determining susceptibility to MS (7, 8).
Women were consistently shown to have a higher incidence of MS compared to men. Extensive research has been conducted on the differences in the immune system and neurological system between women and men. These differences may be attributed to the influence of gonadal hormones, genetic variations, and distinct environmental exposures and modern lifestyles in both genders.

According to study done by Cantó, Barro (10) he found that the majority of patients is RRMS, the MS cohort consisted of 607 participants: 93 with CIS (15.3%), 435withRRMS (71.7%), 25with PPMS (4.1%), and 54with SPMS (8.9%) and study done in Nineveh province/ Iraq by Abdul-Fattah, Sulaiman (11) he found that In Nineveh Governorate, about 136 individuals are known to be affected by MS, with a relapsing-remitting pattern detected in 78.7% of patients, making it the most prevalent type of MS in the region which accordance with results of this study. An association between HHV-6 and MS has been hypothesized since 1993, and several studies have conducted. HHV-6 is a neurotrophic virus linked to various nervous disease orders, including neuromyelitis opticus and MS. There is a link between MS and HHV-6 infection which estimated by several clinical studies (12-14). The involvement of viral agents in the development and progression of MS has attracted significant scientific attention (15). Migration studies and familial aggregation demonstrate that exposure to environmental factors, both non-infectious and infectious, during adulthood and childhood significantly influences the risk of MS(16). Consequently, viruses have been the epicenter of study into MS pathogenesis for over 40 years (17). This result was consistent with the previous studies that proved a relationship between HHV-6 infection and MS. On geographic area near to Iraq there are two investigations identified elevated levels of HHV-6 IgG and IgM in MS cohorts relative to control groups, one conducted in an a Tunisian population (18) and the other in Iranian population. This comprehensive investigation of Iranian MS patients and controls done by Ramroodi, Sanadgol (19) who analyzes IgM and IgG antibodies to HHV-6, as well as the viral sequences in mononuclear cells from peripheral blood, saliva and serum, and expands upon previous findings of a systematically reactivated HHV-6 infection in MS patients relative to controls. Gender factors, which need to be taken into consideration while evaluating MS patients, are the biggest obstacle to the current study.

Our findings did not demonstrate a significant correlation between gender and the HHV6-MS connection. The meta-analysis study in stand with our finding found that no significant correlation between gender and HHV6 infection in MS patients (21). A previous study in stand with current results done by Villoslada, Juste (22) they found There was no correlation seen between sex and the

proportion of anti-HHV-6 IgM-positive patients (p 0.287) Current study demonstrated no associated between EDSS score and HHV-6 antibodies aprevious study in line with our finding done by Engdahl, Gustafsson (23) found that HHV-6B and HHV-6A serology was not correlated with two scores of multiple sclerosis Severity, the Age Related Multiple Sclerosis Severity Score (ARMSS) and the Multiple Sclerosis Severity Score (MSSS). Another study done by Amini, Zahednasab (24) they fiund that the mean of Expanded disability scale in MS patients was 2.348 ± 1.45 and in seropositive MS patients was (2.508 ± 0.36) which highly significant when compared to seronegative MS patients (2.188 ± 0.26) ($p < 0.01$). Ortega-Madueno, Garcia-Montojo (25) study the Anti-Human Herpesvirus 6A/B IgG association with Progression and Relapses in MS found that Anti-HHV-6A/B IgG titers increased in 62.9% (44/70) of MS patients who showed an increase in the EDSS after two years of treatment (Odd Ratio=4.3; p =0.0000003). Additionally, following two years of treatment, a higher percentage of patients remain free of progression the lower their anti-HHV-6A/B IgG titers were at the 24-month visit, which inconsistence with current finding, We can speculate that a larger group of MS patients, divide the patients into seropositive and seronegative by measure the cutoff which could not utilized in this, may produce more significant outcomes. In conclusion, these findings do not confirm a definitive correlation between HHV-6 and EDSS score in MS, however they are sufficiently convincing for requiring additional research.

CONCLUSION

MS patients have high level of seroprevalence of HHV-6 antibodies compare to control which mean positive correlation between MS disease and HHV-6 infection. Controversially, HHV-6 infection has no role in MS severity.

Statement of permission and conflict of interests

The others declare that there is no conflict of interests

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