

Cytomegalovirus Seropositivity and Its Impact on Clinical Progression of Multiple Sclerosis: A Case-Control Study from Northeastern Iran

ARTICLE INFO

Article Type Original Article

Authors

Amirali Ghahremani, MD¹

Mehdi Barati, PhD²

Hasan Namdar Ahmadabad, PhD^{2*}

¹ Department of Internal Medicine, North Khorasan University of Medical Sciences, Bojnurd, Iran

² Vector-borne Diseases Research Center, North Khorasan University of Medical Sciences, Bojnurd, Iran

* Correspondence

Vector-borne Diseases Research Center, North Khorasan University of Medical Sciences, Bojnurd, Iran. E-mail: h.namdar@nkums.ac.ir

How to cite this article

Ghahremani A, Barati M, Namdar Ahmadabad H. Cytomegalovirus Seropositivity and Its Impact on Clinical Progression of Multiple Sclerosis: A Case-Control Study from Northeastern Iran. *Infection Epidemiology and Microbiology*. 2025;11(3): 257-267.

Article History

Received: March 04, 2025

Accepted: May 20, 2025

Published: October 20, 2025

ABSTRACT

Backgrounds: Investigating the role of cytomegalovirus (CMV) in multiple sclerosis (MS) could clarify its potential impacts on the disease progression and pathogenesis. This study aimed to examine the relationship between CMV seropositivity and MS development as well as its impact on the disease clinical course and patients' disability status in North Khorasan province.

Materials & Methods: This case-control study assessed 98 MS patients and 100 age- and sex-matched healthy controls using the 2017 McDonald diagnostic criteria. Disability levels were evaluated via the Expanded Disability Status Scale (EDSS). The presence and levels of anti-CMV IgG and IgM antibodies in serum samples of both groups were quantified by ELISA.

Findings: All MS patients were seropositive for anti-CMV IgG and seronegative for anti-CMV IgM, akin to healthy controls. Interestingly, MS patients exhibited significantly lower serum anti-CMV IgG levels compared to healthy individuals (178.7 ± 61.92 vs. 342.9 ± 3.121 , $p < .0001$). Ethnicity and EDSS scores influenced anti-CMV IgG levels, with higher EDSS scores correlated with lower serum levels. In patients with relapsing-remitting multiple sclerosis (RRMS), serum levels of anti-CMV IgG exhibited significant variations based on age, EDSS scores, and the disease duration.

Conclusion: This study reveals a complex link between CMV infection and MS. While anti-CMV IgG antibodies were found in both MS patients and healthy individuals, lower levels in MS patients might suggest a protective effect against MS progression. Additionally, reduced anti-CMV IgG levels correlated with higher EDSS scores, indicating that diminished CMV-specific immunity could worsen disease severity, especially in RRMS patients.

Keywords: Cytomegalovirus, Multiple sclerosis, Relapsing-remitting, Seropositivity, Disability evaluation

CITATION LINKS

[1] Pardini M, Brown JW, Magliozzi R, Reynolds R, Chard DT. Surface-in... [2] Ghahremani A, Mosa Farkhani S, Baniyadi M, Hojjat SK, Namdar Ahmadabad H, Salarbashi D, et al. Personality... [3] Zhang G, Carrillo-Vico A, Zhang W, Gao S, Ayuso GI. Incidence... [4] Mirmosayyeb O, Shayannejad V, Bagherieh S, Hosseinabadi AM, Ghajarzadeh M. Prevalence... [5] Portaccio E, Magyari M, Havrdova EK, Ruet A, Brochet B, Scalfari A, et al. Multiple... [6] Yuan S, Xiong Y, Larsson SC. An atlas on risk... [7] Ghahremani A, Ahmadabad HN, Javadzadeh SM, Shafiei R. The potential... [8] Vanheusden M, Broux B, Welten SP, Peeters LM, Panagioti E, Van Wijmeersch B, et al. Cytomegalovirus... [9] Tarlinton RE, Martynova E, Rizvanov AA, Khaiboullina S, Verma S. Role of... [10] Bjornevik K, et al. Longitudinal... [11] Landry RL, Embers ME. The probable... [12] Zivadinov R, Nasuelli D, Tommasi MA, Serafin M, Bratina A, Ukmar M, et al. Positivity of... [13] Perri V, Zingaropoli MA, Pasculli P, Ciccone F, Tartaglia M, Baione V, et al. The impact of... [14] Lünemann JD, et al. Cytomegalovirus... [15] Pakpoor J, Pakpoor J, Disanto G, Giovannoni G, Ramagopalan SV. Cytomegalovirus... [16] Waubant E, Lucas R, Mowry E, Graves J, Olsson T, Alfredsson L, et al. Environmental and... [17] Thakolwiboon S, Zhao-Fleming H, Karukote A, Pachariyanon P, Williams HG, Avila M. Regional... [18] Dupont L, Reeves MB. Cytomegalovirus latency... [19] Shaheen SS, Hoque MA, Ferdous J. Seroprevalence of... [20] Glock B, Schistal E, Mayr WR. CMV DNA in... [21] Najafi S, Ghane M, Poortahmasebi V, Jazayeri SM, Yousefzadeh-Chabok S. Prevalence of... [22] Sanadgol N, Ramroodi N, Ahmadi GA, Komijani M, Moghtaderi A, Bouzari M, et al. Prevalence... [23] Karampoor S, Zahednasab H, Ramagopalan S, Mehrpour M, Etemadifar M, Alsahebhosoul F, et al. Cytomegalovirus... [24] Grut V, Biström M, Salzer J, Stridh P, Jons D, Gustafsson R, et al. Cytomegalovirus... [25] Maple PA, Tanasescu R, Gran B, Constantinescu CS. A different... [26] Langer-Gould A, Wu J, Lucas R, Smith J, Gonzales E, Amezcua L, et al. Epstein-Barr... [27] Alari-Pahissa E, Moreira A, Zabalza A, Alvarez-Lafuente R, Munteis E, Vera A, et al. Low cytomegalovirus... [28] Sundqvist E, Bergström T, Daialhosein H, Nyström M, Sundström P, Hillert J, et al. Cytomegalovirus...

Introduction

Multiple sclerosis (MS) is a progressive inflammatory autoimmune disorder of the central nervous system (CNS), characterized by demyelination and neuronal damage. Plaques, particularly in the white matter around the brain lateral ventricles and optic nerves, are indicative of the disease pathology. Cortical demyelination is correlated with clinical progression, severity, and subtypes of the disease, including relapsing-remitting and progressive forms ^[1, 2].

The epidemiology of MS varies significantly by region, with a lower prevalence observed in Asian populations than in Western populations. Recent studies have indicated increasing prevalence rates with notable discrepancies across northern and southern Asia ^[3]. The prevalence of MS in Iran (100/100,000) is higher than in southern Europe (e.g., Greece: 43.6/100,000) but significantly lower than in northern Europe (e.g., Scotland: 376/100,000) ^[4, 5]. This suggests that regional variations are influenced by genetic, environmental, and diagnostic factors.

MS presents with various clinical courses: relapsing-remitting (RRMS), primary progressive (PPMS), secondary progressive (SPMS), and progressive-relapsing (PRMS). RRMS is the most prevalent type, characterized by acute symptom exacerbations followed by remissions. The transition to SPMS involves a steady symptom worsening. PPMS presents with continuous neurological deterioration without remission, whereas PRMS includes occasional recoveries amidst the disease progression ^[5].

MS risk factors include genetic predispositions, particularly the HLA-DRB1*1501/1503 alleles, and demographic factors such as gender, with a higher prevalence in women. Environmental influences, notably latitude affecting sunlight exposure and vitamin D levels, along with lifestyle

choices (such as smoking) and microbial infections have also been implicated ^[6, 7]. However, the interplay and relative contributions of these factors to the risk and progression of MS remain poorly understood. It has been proposed that certain environmental factors, such as viruses, may serve as potential triggers for the disease ^[8].

The relationship between viral infections and MS is intricate, and certain DNA viruses, including members of the *Herpesviridae* family and human endogenous retroviruses (HERVs), have also been implicated as significant risk factors for the disease. These viruses could manipulate host gene expression, potentially causing immune dysregulation, myelin damage, and neuroinflammation ^[9]. They establish lifelong latent infections that may contribute to chronic neurological deficits. Recent research has elucidated a strong association between prior Epstein-Barr virus (EBV) infection and the development of MS. EBV has been identified as a key trigger in MS through immune-mediated mechanisms, suggesting that it may play a significant role in the disease onset and progression ^[10]. Other herpesviruses, including human herpesvirus 6 (HHV-6), varicella-zoster virus (VZV), and cytomegalovirus (CMV), have also been associated with multiple sclerosis (MS) pathogenesis; however, although these associations are noteworthy, the specific roles of these viruses in MS remain less clearly defined, and causal relationships have not yet been established ^[9, 11].

Numerous studies have explored the relationship between CMV and MS, yielding conflicting results regarding its effects, ranging from no impact to protective and deleterious influences. While some research has indicated that CMV may contribute to MS pathogenesis, other studies have suggested that it could limit the disease progression ^[12-15]. Factors such as genetic background, geographic location, age, and socioeconomic

status may affect these associations [11, 16, 17]. Therefore, further biological and epidemiological investigations are required.

Objectives: This study aimed to examine the relationship between CMV seropositivity and MS development as well as its impact on the disease clinical course and patients' disability status in a specific geographical region, North Khorasan province.

Materials and Methods

Study population: This matched case-control study was conducted at Imam Hassan hospital in Bojnourd (Iran) on MS patients aged 18–55 years, referred by the MS Association. Participants were recruited from the North Khorasan MS Association, where their identity and contact details were registered. Patients were invited to voluntarily participate in the study while being informed of its benefits, including free consultation with a neurologist and potential access to necessary treatments and tests. Interested patients were referred to Imam Hassan hospital in Bojnourd. Diagnoses were confirmed by a neurologist using the 2017 McDonald criteria, all participants were in the remission phase of the disease, forming the case group for statistical analysis.

Exclusion criteria for the case group included: disease relapse within 30 days prior to enrollment, recent immunosuppressive or immunomodulatory treatment (within one month), severe concomitant diseases such as HIV/AIDS, primary immunodeficiency disorders, chronic kidney disease, liver cirrhosis or severe liver disease, active cancer or hematological malignancies, systemic autoimmune diseases, severe neurological disorders (other than multiple sclerosis), and severe chronic respiratory diseases. Additional exclusion criteria included pregnancy, antiviral drug use (including ganciclovir, foscarnet, acyclovir, valganciclovir, cidofovir, and letermovir),

disease-modifying treatments (DMT) within the past three months, family history of MS in close relatives, more than two relapses in the past two years, smoking, alcohol consumption, and drug abuse.

Healthy controls were recruited from the same geographic region as the patients and matched for age, gender, and race/ethnicity. They had no concomitant diseases and had not recently used antiviral, immunomodulatory, or immunosuppressive medications that could influence immune system function.

A standardized semi-structured questionnaire was administered to patients and healthy controls to gather demographic and clinical data, including personal background, education, employment, health history, and socioeconomic factors, which was then complemented with the results of clinical examinations and retrospective medical record reviews. The clinical course of MS was determined by evaluating the patient's history, neurological examinations, MRI findings, and symptom patterns. The Expanded Disability Status Scale (EDSS), developed by Dr. John F. Kurtzke, was used to quantify disability levels through neurological assessment from 0 (no disability) to 10 (death due to MS).

CMV serological assay: In this study, serum samples from all participants were collected after obtaining informed consent and stored at -70 °C prior to analysis. The presence of anti-CMV IgG and IgM antibodies was assessed using an ELISA kit (PishtazTeb Co., Tehran, Iran). The manufacturer reported that the anti-CMV IgG kit exhibited a sensitivity and specificity of 100%, while the anti-CMV IgM kit demonstrated a sensitivity of 100% and a specificity of 99%. Briefly, for the detection of anti-CMV IgM, 100 µL of patient serum samples along with positive and negative control sera were added to wells coated with cytomegalovirus (CMV) antigen and incubated at 37 °C for 30 min.

Following five washes, 100 μ L of HRP-conjugated anti-human IgM antibody was introduced to each well. After an additional 30-min incubation at 37 °C and five washes, 100 μ L of chromogen was added and incubated for 15 min at room temperature in the dark. Subsequently, 100 μ L of stopping

solution was added, and absorbance was measured at 450 nm. The cut-off value was defined as the mean optical density (OD) of negative controls plus 0.15; samples with a ratio exceeding 1.1 were deemed positive. For the detection of anti-CMV IgG, similar procedures were followed with serum

Table 1) Demographic profile of multiple sclerosis patients and controls

Variable	MS Patients: N=98	Healthy controls: N=100
Education levels, n (%)		
Illiterate	8 (8.16)	9 (9)
Elementary school	21 (21.43)	21 (21)
High School	11 (11.22)	12 (12)
College or university	58 (59.19)	58 (58)
Ethnicity, n (%)		
Persians	36 (36.73)	41 (41)
Khorasani Turks	20 (20.41)	10 (10)
Khorasani Kurds	37 (37.76)	42 (42)
Iranian Turkmens	2 (2.04)	5 (5)
Tats	5 (3.06)	2 (2)
Employment status, n (%)		
Homemaker	50 (51.02)	39 (39)
Unemployed	17 (17.35)	12 (12)
Full-time employee	18 (18.37)	35 (35)
Part-time employee	5 (5.10)	11 (11)
Retired	6 (6.12)	3 (3)
Not employed due to disability	2 (2.04)	0
Household income level, n (%)		
Low-Income	43 (43.88)	43 (43)
Middle-Income	14 (14.29)	17 (17)
High-Income	41 (41.83)	40 (40)
Place of residence, n (%)		
Village	17 (17.35)	17 (17)
City	81 (82.65)	83 (83)
Housing status, n (%)		
Private	57 (58.16)	40 (40)
Rented	26 (26.53)	33 (33)
With parents	15 (15.31)	27 (27)
Number of family members, n (%)		
3>	48 (48.98)	43 (43)
3-4	22 (22.45)	37 (37)
4<	28 (28.57)	20 (20)

samples, standards, and control sera, which were added to antigen-coated wells and incubated for 30 min at room temperature. After washing, HRP-conjugated anti-human IgG antibody was added, followed by chromogen and stopping solution, and absorbance was measured at 450 nm. The cut-off index was calculated by dividing the serum sample OD by the standard OD of 10 AU/mL; values above 1.1 were considered positive.

Statistical analysis: Statistical analysis was conducted using GraphPad Prism software (Version 9.0). Normality was assessed using Kolmogorov-Smirnov test. Chi-square test was used to compare the prevalence of anti-CMV IgG and IgM antibodies between the control and case groups, while independent t-tests were used to evaluate anti-CMV IgG levels. Fisher's exact test was used to analyze the demographic and clinical characteristics of the seropositive and seronegative groups.

One-way ANOVA was employed to assess anti-CMV IgG levels among MS patients with multiple demographic and clinical characteristics. Data were reported as mean $\pm$ standard deviation, with significance set at $p < .05$.

Findings

A total of 198 individuals participated in this study, comprising 98 MS patients and 100 healthy controls. Among the patients with MS, 65 were female, and 33 were male, while the control group included 66 females and 34 males. The median age of MS patients was 39 years (95% CI: 36-42), while the median age of healthy controls was 40 years (95% CI: 36-42). Additionally, 76% of MS patients and 68% of controls were married (Table 1). In MS patients, the median age at the disease onset was 30 years (95% CI: 28-33), the median disease duration was 8 years (95% CI: 6-10 years), and the mean EDSS score was 2.37 ± 0.75 (Table 2). Serological analysis of MS group revealed that

Table 2) Clinical characteristics of multiple sclerosis patients

Characteristics	N=98
History of hospitalization, n (%)	
Yes	8 (8.16)
No	21 (21.43)
Age of the disease onset, mean $\pm$ SD	31.6 $\pm$ 9.2
Age of the disease diagnosis, mean $\pm$ SD	32.1 $\pm$ 9.7
Average duration of the disease, mean $\pm$ SD	8.3 $\pm$ 5.3
Clinical course, n (%)	
Primary progressive MS (PPMS)	12 (12.24)
Secondary progressive MS (SPMS)	13 (13.26)
Progressive relapsing MS (PRMS)	3 (3.06)
Relapsing remitting MS (RRMS)	61 (62.24)
First symptom of the disease, n (%)	
Darkness eye	24 (24.49)
Diplopia	22 (22.45)
Limb numbness	22 (22.45)
Paresthesia	20 (20.41)
Ataxia	3 (3.06)
Tremor	3 (3.06)
Motor deficit	4 (4.08)

Table 3) Comparison of anti-CMV IgG levels in MS patients according to various demographic characteristics

Variable		Anti-CMV IgG (AU/mL) mean $\pm$ SD	P Value
Gender	Male	191.0 $\pm$ 57.94	.17
	Female	173.5 $\pm$ 59.59	
Age	35 >	184.9 $\pm$ 45.73	.91
	35-45	181.1 $\pm$ 48.62	
	45 <	178.1 $\pm$ 73.12	
Ethnicity	Persians	187.1 $\pm$ 58.84	.042
	Khorasani Turks	205.5 $\pm$ 45.07	
	Khorasani Kurds	165.8 $\pm$ 60.99	
	Iranian Turkmens	149.0 $\pm$ 58.03	
	Tats	209.6 $\pm$ 68.53	
Employment status	Homemaker	175.2 $\pm$ 69.64	.95
	Unemployed	185.9 $\pm$ 51.87	
	Full-time employee	179.1 $\pm$ 77.02	
	Part-time employee	205.7 $\pm$ 67.02	
	Retired	178.3 $\pm$ 51.20	
	Not employed due to disability	189.9 $\pm$ 51.37	
Place of residence	Village	181.6 $\pm$ 57.15	.52
	City	192.4 $\pm$ 73.91	

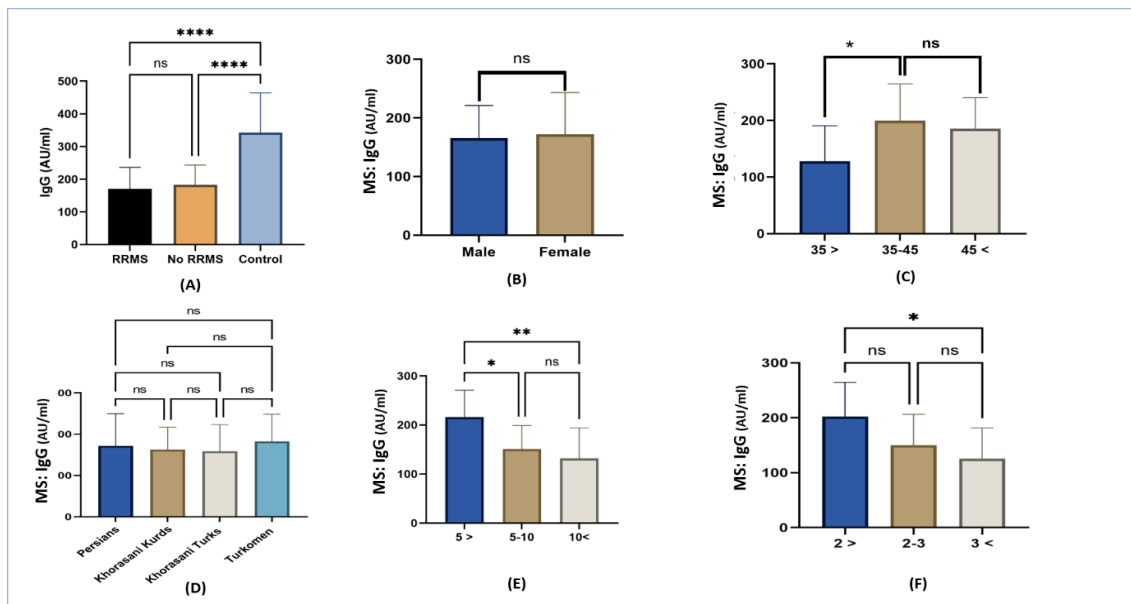


Figure 1) Serum levels of anti-CMV IgG in patients with relapsing-remitting multiple sclerosis (RRMS) based on various demographic and clinical characteristics. RRMS patients and individuals with other disease phenotypes (No RRMS) demonstrate significantly lower anti-CMV IgG levels than healthy controls (A). The levels of anti-CMV IgG vary by age (C), the disease duration (E), and Expanded Disability Status Scale (EDSS) scores (F), but do not differ significantly based on gender (B) or ethnicity (D). Data are presented as the mean $\pm$ standard deviation. NS: non-significant, *: $p < .05$, **: $p < .01$, ***: $p < .0001$.

all samples were positive for anti-CMV IgG, while none were positive for anti-CMV IgM. The healthy control group exhibited similar findings. Notably, the mean serum anti-CMV IgG concentration was significantly lower in MS patients than in controls (178.7 ± 61.92 vs. 342.9 ± 3.121 , $p < .0001$). Further statistical evaluation indicated no significant differences in anti-CMV IgG levels based on age, gender, or occupation in the MS group ($p < .05$); however, ethnicity significantly influenced anti-CMV IgG levels ($p = .042$), such that Khorasani Turks displayed higher anti-CMV IgG levels compared to Iranian Turkmens and Khorasani Kurds ($p < .05$) (Table 3).

In this study, no significant difference in serum anti-CMV IgG levels was observed between RRMS and non-RRMS patients ($p < .05$). However, RRMS patients exhibited significantly lower anti-CMV IgG levels than healthy controls (170.3 ± 65.9 vs. 342.9 ± 121.3 , $p < .0001$) (Figure 1A). Furthermore, no significant differences in anti-CMV IgG levels were found based on gender or ethnicity among patients with RRMS ($p < .05$) (Figure 1B and D). However, a significant difference in anti-CMV IgG levels was observed based on age ($p = .02$) (Figure 1C).

In patients with RRMS, no significant correlation was found between the age of the disease onset/ diagnosis and anti-CMV IgG levels ($p = .05$). However, a significant association was observed between the disease duration and anti-CMV IgG levels ($p = .002$) (Figure 1E). Additionally, a significant relationship was identified between EDSS scores and anti-CMV IgG levels in this patient population ($p = .014$) (Figure 1F), indicating potential implications for the disease progression and immune response.

Discussion

The relationship between CMV infection and MS pathogenesis remains controversial, with unclear implications as either a protective

factor or a potential risk enhancer. Limited research has explored the connection between CMV serostatus and disability levels in MS patients. Further seroepidemiological studies across diverse populations are essential to elucidate the role of CMV in MS development. Therefore, this study assessed the seroprevalence of CMV and its correlation with disability status and clinical disease progression among MS patients. The present study results indicated that all MS patients were seropositive for anti-CMV IgG antibodies while remaining seronegative for anti-CMV IgM antibodies, consistent with the findings observed in healthy control subjects. Notably, serum levels of anti-CMV IgG were significantly lower in MS patients compared to healthy controls. Additionally, variations in serum anti-CMV IgG levels among MS patients were correlated with factors such as ethnicity and EDSS scores. Specifically, in patients exhibiting the RRMS phenotype, serum anti-CMV IgG levels were dependent on patient age, EDSS scores, and the disease duration.

CMV typically establishes lifelong latency following an initial asymptomatic infection, with anti-CMV IgM antibodies indicating recent infection or reactivation [18]. In this study, all MS patients and healthy controls tested positive for anti-CMV IgG antibodies, reflecting past exposure, while all participants were seronegative for anti-CMV IgM, indicating no recent infection or reactivation. Several factors may account for this consistent seronegativity. Demographic characteristics and the size of our cohort might have contributed to this seropositivity, as previous studies have shown only 2-4% seropositivity for anti-CMV IgM in similar age groups [19, 20]. Furthermore, individuals might have transitioned from anti-CMV IgM to IgG due to prior exposure. The immune status of MS patients and the impact of DMTs may also play a role in preventing detectable

IgM responses.

In this study conducted in northeastern Iran, a seropositivity rate of 100% was reported for anti-CMV IgG antibodies among MS patients. In contrast, Najafi et al. (2016) reported a seropositivity rate of 79.3% in northern Iran^[21], Sanadgol et al. (2011) reported 87.2% in southeastern Iran^[22], and Karampoor et al. (2017) reported 98.8% in central Iran^[23]. However, studies conducted in Europe and the United States have indicated lower seroprevalence rates for anti-CMV IgG in MS patients, ranging from 36 to 65%^[13, 24-26]. Comparison of various studies indicates that the seroprevalence of CMV among MS patients in Iran has exceeded the rates observed in Europe and the United States, with a notable upward trend in recent years. In this study, no significant difference in CMV seroprevalence was found between MS patients and healthy controls. This finding aligns with the findings of other studies conducted by Najafi et al. (2016)^[21], Sanadgol et al. (2011)^[22], as well as a meta-analysis of 16 related studies^[15]. Conversely, Karampoor et al. (2023)^[23] reported higher seropositivity rates of anti-CMV IgG in MS patients compared to controls, suggesting a potential role of CMV in MS pathogenesis. Numerous studies conducted in European countries and the United States have demonstrated that the seroprevalence of anti-CMV IgG in MS patients is lower than in healthy individuals^[24-26, 27]. These studies have highlighted the potential protective effect of CMV against MS, which is often interpreted through the lens of the hygiene hypothesis. However, a recent study conducted in Italy in 2024 found no significant difference in the seroprevalence of anti-CMV IgG between MS patients and healthy controls^[13], suggesting the need for further investigation into this relationship. Thakolwiboon et al. (2020) reported that in Europe, CMV IgG seroprevalence was lower

in MS patients than in controls, while an opposite trend was observed in the Middle East, and no significant association was found between the two in North America^[17]. In this study, serum anti-CMV IgG levels were significantly lower in MS patients than in healthy controls. Additionally, there were no notable differences in anti-CMV IgG levels based on age, occupation, or gender among MS patients; however, ethnic disparities were found to influence anti-CMV IgG levels. This supports the findings of Langer-Gould et al. (2017), who similarly reported reduced anti-CMV IgG levels in MS patients compared to controls^[26]. Contrarily, studies by Zivadinov et al. (2006)^[12] and Sundqvist et al. (2014)^[28] reported no significant differences in anti-CMV IgG titers between MS patients and healthy individuals. Moreover, Sanadgol et al. (2011)^[22] found higher anti-CMV IgG levels in MS patients than in controls. While Zivadinov et al. (2006)^[12] confirmed no significant age or gender differences in anti-CMV IgG levels, Perri et al. (2024)^[13] indicated that age could affect CMV serostatus, but gender could not. Furthermore, research highlights that CMV seropositivity may vary according to race/ethnicity in MS patients^[26], aligning with the present study findings.

The present study findings align with those of previous research showing that CMV plays a complex role in MS. Lünemann et al. (2024)^[14] found that high CMV-specific immunity was associated with lower serum neurofilament light chain and disability scores, indicating a protective effect. In contrast, Alari-Pahissa et al. (2018)^[27] and Perri et al. (2024)^[13] noted that CMV infection might lead to altered immune responses such as increased differentiated T and NK cells, which could exacerbate disability in MS patients. Thus, low anti-CMV IgG levels may reflect a weak immune response, contributing to the disease progression.

Limited research has explored the relationship between CMV serostatus and disability in MS patients.

This study established a direct correlation, indicating that lower EDSS scores were associated with elevated serum anti-CMV IgG levels. This finding is in contrast to the findings of other studies conducted by Zivadinov et al. (2006) ^[12], who reported an inverse relationship, and Alari-Pahissa et al. (2018) ^[27], who found no significant association between CMV serostatus and EDSS scores in MS patients.

This investigation revealed no significant differences in serum anti-CMV IgG levels between RRMS and other MS phenotypes, corroborating a previous study ^[28]. In patients with RRMS, anti-CMV IgG levels did not vary significantly by gender or ethnicity; however, significant variations were associated with age. Perri et al. (2024) also noted a significant correlation between CMV serostatus and older age ^[13]. In contrast, Sanadgol et al. (2011) indicated significant gender differences in anti-CMV IgG titers among RRMS patients ^[22]. While Najafi et al. (2016) found no age or gender differences in CMV serostatus ^[21], our data indicated significant associations between EDSS scores and disease duration with anti-CMV IgG levels, which is different from prior reports linking CMV serostatus with clinical variables ^[13].

This study had several limitations that might have impacted its findings. First, the absence of molecular methods, such as polymerase chain reaction (PCR), restricted our ability to detect active CMV infection in MS patients. Additionally, immune cell phenotypes or serum cytokine profiles were not assessed, which could elucidate the immunological effects of CMV on MS. Finally, since all MS patients were anti-CMV IgG seropositive, we were unable to differentiate the effects of active versus past CMV infections on clinical outcomes.

Conclusion

Taken together, this study underscores the complex relationship between CMV infection and MS. While the consistent presence of anti-CMV IgG antibodies in both MS patients and healthy controls confirms widespread prior exposure, the observed trend of lower serum anti-CMV IgG levels in MS patients, potentially indicative of a protective role, warrants further investigation. Crucially, the correlation between lower anti-CMV IgG levels and higher EDSS scores, particularly in RRMS patients, suggests a detrimental effect of diminished CMV-specific immunity on the disease severity. Future studies are essential to elucidate the precise mechanisms by which CMV influences MS pathogenesis and progression, thus paving the way for potential therapeutic interventions.

Acknowledgments

All author(s) would like to thank the members of the Division of Neurology, Department of Internal Medicine, North Khorasan University of Medical Sciences, Bojnurd, Iran, for their assistance in conducting this research project.

Ethical permissions: This study included human participants. Therefore, written informed consents were obtained from all participants. The study was approved by the Ethics Committee of North Khorasan University of Medical Sciences, Bojnourd, Iran (Ethic approval Code: IR.NKUMS.BLC.1401.003).

Author's contributions: H.N.A. conceived the study; A.G.H. designed the study; A.G.H. performed physical examination; M.B. completed the questionnaire; M.B. and H.N.A. performed the experiments; H.N.A. performed statistical analyses; A.G.H. and M.B. prepared the draft of the manuscript; and H.N.A. edited the manuscript. All authors read and approved the final manuscript.

Conflicts of interests: All authors declare

that they have no conflicts of interest. The authors have no affiliation with any organization with direct or indirect financial interest in the subject matter discussed in the manuscript.

Funding: This study was funded by North Khorasan University of Medical Sciences (Grant no. 4000223).

Consent to participate: All participants provided voluntary and informed consent to participate in this study.

References

- Pardini M, Brown JW, Magliozzi R, Reynolds R, Chard DT. Surface-in pathology in multiple sclerosis: A new view on pathogenesis? *Brain*. 2021;144(6):1646-54.
- Ghahremani A, Mosa Farkhani S, Baniasadi M, Hojjat SK, Namdar Ahmadabad H, Salarbashi D, et al. Personality traits of patients with multiple sclerosis and their correlation with anxiety and depression levels: A cross-sectional case-control study. *Brain Behav*. 2022;12(5):e2596.
- Zhang G, Carrillo-Vico A, Zhang W, Gao S, Ayuso GI. Incidence and prevalence of multiple sclerosis in China and other Asian countries. *Neurologia*. 2023;38(3):159-72.
- Mirmosayyeb O, Shaygannejad V, Bagherieh S, Hosseinabadi AM, Ghajarzadeh M. Prevalence of multiple sclerosis (MS) in Iran: A systematic review and meta-analysis. *Neurol Sci*. 2022;43:233-41.
- Portaccio E, Magyari M, Havrdova EK, Ruet A, Brochet B, Scalfari A, et al. Multiple sclerosis: Emerging epidemiological trends and redefining the clinical course. *Lancet Reg Health Eur*. 2024;44:1-14.
- Yuan S, Xiong Y, Larsson SC. An atlas on risk factors for multiple sclerosis: A Mendelian randomization study. *J Neurol*. 2021;268:114-24.
- Ghahremani A, Ahmadabad HN, Javadzadeh SM, Shafiei R. The potential role of *Toxoplasma gondii* infection in multiple sclerosis development: A seroepidemiological study in North Khorasan province, Iran. *Acta Parasitol*. 2025;70(1):32.
- Vanheusden M, Broux B, Welten SP, Peeters LM, Panagioti E, Van Wijmeersch B, et al. Cytomegalovirus infection exacerbates autoimmune mediated neuroinflammation. *Sci Rep*. 2017;7(1):663.
- Tarlinton RE, Martynova E, Rizvanov AA, Khaiboullina S, Verma S. Role of viruses in the pathogenesis of multiple sclerosis. *Viruses*. 2020;12(6):643.
- Bjornevik K, Cortese M, Healy BC, Kuhle J, Mina MJ, Leng Y, et al. Longitudinal analysis reveals high prevalence of Epstein-Barr virus associated with multiple sclerosis. *Science*. 2022;375(6578):296-301.
- Landry RL, Embers ME. The probable infectious origin of multiple sclerosis. *NeuroSci*. 2023;4(3):211-34.
- Zivadnov 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):262-9.
- Perri V, Zingaropoli MA, Pasculli P, Ciccone F, Tartaglia M, Baione V, et al. The impact of cytomegalovirus infection on natural killer and CD8+ T cell phenotype in multiple sclerosis. *Biology*. 2024;13(3):154.
- Lünemann JD, Sao Avilés A, Tintoré M, Midaglia L, Fissolo N, Gutiérrez L, et al. Cytomegalovirus immune responses are associated with lower serum NfL and disability accumulation risk at multiple sclerosis onset. *Mult Scler J*. 2024;30(11-12):1445-54.
- Pakpoor J, Pakpoor J, Disanto G, Giovannoni G, Ramagopalan SV. Cytomegalovirus and multiple sclerosis risk. *J Neurol*. 2013;260:1658-60.
- Waubant E, Lucas R, Mowry E, Graves J, Olsson T, Alfredsson L, et al. Environmental and genetic risk factors for MS: An integrated review. *Ann Clin Transl Neurol*. 2019;6(9):1905-22.
- Thakolwiboon S, Zhao-Fleming H, Karukote A, Pachariyanon P, Williams HG, Avila M. Regional differences in the association of cytomegalovirus seropositivity and multiple sclerosis: A systematic review and meta-analysis. *Mult Scler Relat Disord*. 2020;45:102393.
- Dupont L, Reeves MB. Cytomegalovirus latency and reactivation: Recent insights into an age old problem. *Rev Med Virol*. 2016;26(2):75-89.
- Shaheen SS, Hoque MA, Ferdous J. Seroprevalence of cytomegalovirus among blood donor in transfusion medicine: Study from Bangladesh. *Int J Innov Res Med Sci*. 2020;5(01):1-4.
- Glock B, Schistal E, Mayr WR. CMV DNA in blood donors with IgM and IgG CMV antibodies. *Transfusion*. 2003;43(10):1493-4.
- Najafi S, Ghane M, Poortahmasebi V, Jazayeri SM, Yousefzadeh-Chabok S. Prevalence of cytomegalovirus in patients with multiple sclerosis: A case-control study in northern Iran. *Jundishapur J Microbiol*. 2016;9(7):e36582.
- Sanadgol N, Ramroodi N, Ahmadi GA, Komijani M, Moghtaderi A, Bouzari M, et al. Prevalence of cytomegalovirus infection and its role in total immunoglobulin pattern in Iranian patients with

- different subtypes of multiple sclerosis. *New Microbiol.* 2011;34(3):263-74.
23. Karampoor S, Zahednasab H, Ramagopalan S, Mehrpour M, Etemadifar M, Alsahebhosoul F, et al. Cytomegalovirus and varicella zoster virus seropositivity of Iranian patients with multiple sclerosis: A population-based study. *J Neuroimmunol.* 2017;309:4-6.
 24. Grut V, Biström M, Salzer J, Stridh P, Jons D, Gustafsson R, et al. Cytomegalovirus seropositivity is associated with reduced risk of multiple sclerosis: A presymptomatic case-control study. *Eur J Neurol.* 2021;28(9):3072-9.
 25. Maple PA, Tanasescu R, Gran B, Constantinescu CS. A different response to cytomegalovirus (CMV) and Epstein-Barr virus (EBV) infection in UK people with multiple sclerosis (PwMS) compared to controls. *J Infect.* 2020;80(3):320-5.
 26. Langer-Gould A, Wu J, Lucas R, Smith J, Gonzales E, Amezcua L, et al. Epstein-Barr virus, cytomegalovirus, and multiple sclerosis susceptibility: A multiethnic study. *Neurology.* 2017;89(13):1330-7.
 27. Alari-Pahissa E, Moreira A, Zabalza A, Alvarez-Lafuente R, Munteis E, Vera A, et al. Low cytomegalovirus seroprevalence in early multiple sclerosis: A case for the 'hygiene hypothesis'? *Eur J Neurol.* 2018;25(7):925-33.
 28. Sundqvist E, Bergström T, Daialhosein H, Nyström M, Sundström P, Hillert J, et al. Cytomegalovirus seropositivity is negatively associated with multiple sclerosis. *Mult Scler J.* 2014;20(2):165-73.