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ABSTRACT

Nisreen Saadoun Jasim
https://orcid.org/0009-0003-6561-787X
Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq

Rana Saady Aboud
https://orcid.org/0009-0008-9853-0021
Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq

IMMUNOLOGICAL DETECTION OF HUMAN HERPES VIRUS-6 IN SERA OF IRAQI PATIENTS WITH MULTIPLE SCLEROSIS

Introduction: Multiple sclerosis (MS) is an autoimmune neuroinflammatory and neurodegenerative disease that infects and destroys the central nervous system (CNS). Many variables influence the start of multiple sclerosis disease. MS was thought to be mostly caused by viral infection, particularly infections with the human herpes virus 6 (HHV-6), Epstein-Barr virus (EBV), and other viruses.

Aim: the goal of the present study is to estimate the role of human herpesvirus-6 infection as a trigger factor for multiple sclerosis disorder and the role of some proinflammatory cytokine in early detection of this disorder.

Material and Methods: We measured the titer of IgM, IgG Ab for human herpesvirus-6 and proinflammatory tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), between October 2023 and February 2024, in the 90 blood samples that were drawn from individuals aged 13 to 75. The medical personnel of Dr. Saad Al-Witry Hospital for Neurosciences provided diagnoses for these patients. We divided the subjects into 3 groups: the first group included 27 patients who suffered from multiple sclerosis with HHV-6 infection, the second group included 33 patients who suffered from multiple sclerosis without HHV-6, and the third group included 30 people who appeared to be in good health. We used the Enzyme-Linked Immunosorbent Assay (ELISA) technology to perform measurements.

Results: The statistical analysis showed a significant increase ($P \leq 0.01$) in anti-HHV-6 IgM and IgG antibodies in the sera of patients with MS diseases and HHV-6 compared to the control group. There was also a significant increase ($P \leq 0.01$) in MS subjects without HHV-6 compared to the control group. Additionally, the statistical analysis of TNF-α level revealed a highly significant difference between MS patients with HHV-6 and those without HHV-6 compared with the control group.

Furthermore, the statistical analysis showed a significant elevation (P≤0.01) in interleukin-6 (IL-6) in sera of patients with MS and HHV-6 compared to the control group and in MS subjects without HHV-6 compared to the control group.

Conclusion: According to the current research, HHV-6 infection may be a major factor in MS.

Keywords: Human Herpesvirus-6; Interleukin-6; Multiple Sclerosis; Tumor Necrosis Factor-Alpha.

Corresponding author: *Nisreen Saadoun Jasim, Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq*
e-mail: nisreensjasam@gmail.com

ABBREVIATIONS

BBB: blood-brain barrier
CNF: central nerve fluid
CNS: center nerve system
ELISA: enzyme-linked immunosorbent assay
HD: healthy donor
HHV-6: human herpesvirus-6
IL-6: interleukin-6
MS: multiple sclerosis
OND: other neurological diseases
TNF α: tumor necrosis factor-alpha

INTRODUCTION

Multiple sclerosis is a persistent inflammatory disorder. Multiple sclerosis (MS) is typified by demyelination in the central nervous system (CNS) [1]. MS has an impact on the efficiency of individuals as well as the level of lifestyle for patients and their families. Tiredness, discomfort, weakness, changes in mood, a feeling of numbness, problems with the bladder, blurred vision, and lack of coordination are typical neurological manifestations. Approximately 2.8 million people worldwide are impacted by MS [2, 3]. The Arabian Gulf MS spreading percentage has increased at a rate of 2.3 per year since 1986 [4-6]. Approximately 40.4 per 100,000 people in Saudi Arabia and 61.95 per 100,000 Saudi nationals are affected by MS in the general population [7]. In Iraq, the prevalence of MS infection is considered lower; it is estimated at 11.7/100,000. The incidence of MS has grown from 0.05 in 2000 to 1.5 in 2017 [8]. There are basically four subgroups of MS that impact patients: relapsing-remitting multiple sclerosis (RRMS), progressive-relapsing multiple sclerosis (PRMS), primary progressive multiple sclerosis (PPMS), and secondary progressive multiple sclerosis (SPMS). 90% of sufferers are classified as RRMS; the condition is characterized by multiple bouts of appearance accompanied by temporary healing; however, the severity of neural insufficiency increases with every recurrence [9]. The blood-brain barrier (BBB) that separates and protects the central nervous system (CNS) on the vasculature bed is damaged when auto-reactive cells from the immune system in MS create destruction to the CNS, making the condition an autoimmune illness [10-12]. In genetically susceptible people, viral infection is the primary environmental element that triggers the autoimmune phenomenon [13, 14].

Human herpesvirus-6 (HHV-6) is a member of the Roseolovirus family, which also includes the separate viruses HHV-6A and HHV-6B [15, 16]. This virus caused the roseola clinical syndrome, which is characterized by a rash and a high body temperature. Babies make up the majority of HHV-6 afflicted members. HHV-6B remains the source of primary HHV-6 infections, which can progress to disorders of the central nervous system, such as febrile status [17]. Herpesviruses are capable of reactivation and latency. An immune system-required latency infection establishes a sustained infection in cells without devastation. In order to keep the immune system from discovering the infection, the production of viral proteins in latent infected cells should also be restricted [18]. Further investigations have demonstrated that HHV-6 is an additional plausible culprit, given its repeated associations with multiple sclerosis [19]. HHV-6 is thought to be a trigger factor for autoimmune responses in the pathophysiology of multiple sclerosis (MS). It has a function in the evolution of demyelination and has the

ability to enter the latent phase of infection in oligodendrocytes and cause harm when reactivating. It also attacks microglia cells, which causes the formation of a pro-inflammatory response [20, 21].

Proinflammatory and anti-inflammatory cytokines both release and regulate the immune system's response during infection. The balance of these cytokine groups determines how serious the immunological response is. Anti-inflammatory cytokines include IL-10, IL-1 receptor antagonist (IL1RA), and others [22]. Pro-inflammatory cytokines include IL-6, IL-8, IL-1B, TNF-α, and IFN-γ, which enable immune cells to penetrate the blood-brain barrier and cause axonal damage and demyelination [23, 24].

Objective: Multiple sclerosis (MS) spy usually alternates between periods of disease and healing. Neuronal axons become demyelinated as a result of inflammatory processes; according to new studies, those who have human herpesvirus-6 infection are more likely than those who do not contract multiple sclerosis.

MATERIALS AND METHODS

Studied subject sample

The study included ninety patients suffering from MS disease diagnosed by the consultant medical staff in Dr. Saad Al-Witry Hospital for Neurosciences. From October 2023 until February 2024. The patients' ages ranged from 15 to 75 years. A sterile syringe was used to collect blood samples (5ml), which were transferred to a vacuum gel plain tube and allowed to coagulate at room temperature for several hours. The tubes were then centrifuged at a speed of 3000 rpm for 5 minutes. All samples were tagged with the name, date, and numbers before being stored at (-20 °C) for immunological testing.

Estimation of ani HHV-6 IgM, IgG, and tumor necrosis factor-alpha (TNF-α)

All studied patient groups: MS with HHV-6, MS without HHV6, and controls which were healthy individuals, were evaluated with regard to the level of HHV-6 Ab IgM (Sun long/China), HHV-6 Ab IgG (Sun long/China), and tumor necrosis factor-α (TNF-α) (Cloud-Clone/USA) using enzyme-linked immunosorbent assay (ELISA) technique according to the protocol.

Statistical analysis

The Statistical Analysis System [25] program was used to detect the effect of different groups (patients and controls) on study parameters. To compare the differences between two means, we used the LSD test. Also, the Chi-square test was used to examine whether two categorical variables were independent in influencing the test statistics (0.05 and 0.01 probability). The correlation coefficient between variables was estimated in this study.

Ethical approval

Ethical approval for the study was obtained from the College of Science Research Ethics Committee /University of Baghdad (No. CSEC/0923/0061 on 15 September 2023).

RESULTS

The distribution of the sample study by gender in the various groups is displayed in Table 1. In male MS patients, there was a greater significant difference (P≤0.01) between the patient with HHV-6 infection group (14.81%) and the control group (43.33%) compared to the MS patients without HHV-6 (44.44%) (P value =0.0074). On the other hand, there was no significant difference (P≤0.05) in females.

Table 1 – Distribution of samples according to gender

Factor		Control (No = 30)	MS with HHV-6 (No = 27)	MS without HHV-6 (No = 33)	P-value
Sex: No (%)	Male	13 (43.33%)	4 (14.81%)	12 (44.44%)	0.0074 **
	Female	17 (56.67%)	23 (85.19%)	21 (55.56%)	0.108 NS
	P-value	0.287 NS	0.0001 **	0.0398 *	---
** (P≤0.05), ** (P≤0.01), NS: Non-Significant*					

The levels of IgM and IgG in different groups of the study are shown in Table 2 and Figures 1 and 2. There was a highly significant difference (p≤0.01) in the level of HHV-6 IgM Ab in MS with HHV-6 infection groups (16.15±1.98 U/ml) as compared with healthy controls (7.19±0.18 U/ml), and there was a highly significant difference (p≤0.01) in the level of HHV-6 IgM Ab in MS patients without HHV-6 infection (6.67±0.15 U/ml) as compared with healthy controls (7.19±0.18 U/ml) (P-value = 0.0001). Also, there was a highly significant difference (p≤0.01) in the level of HHV-6 IgG Ab in MS patients with HHV-6 infection (43.85±4.92 U/ml) as

Table 2 – Comparison between difference groups in IgM and IgG

Group	Mean ± SE	
	HHV6 IgM (IU/ml)	HHV6 IgG (IU/ml)
Control	7.19 ±0.18 b	17.94 ±1.18 b
MS with HHV-6	16.15 ±1.98 a	43.85 ±4.92 a
MS without HHV-6	6.67 ±0.15 b	16.27 ±0.78 b
LSD	2.922 **	7.591 **
P-value	0.0001	0.0001

*Means with the different letters in the same column differed significantly, ** (P≤0.01)*

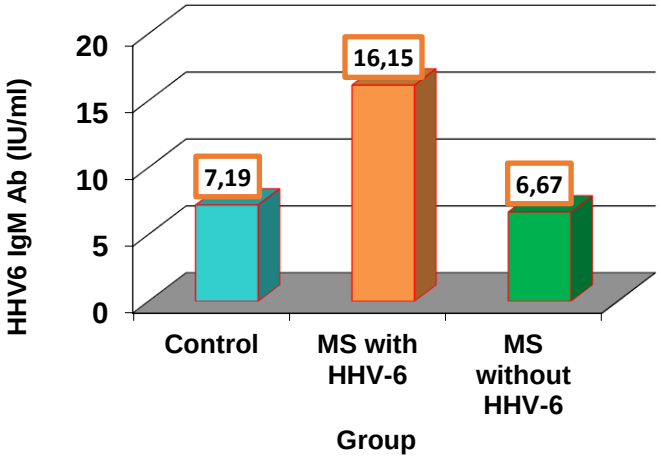

Figure 1 - Comparison between difference groups in HHV-6 IgM Ab

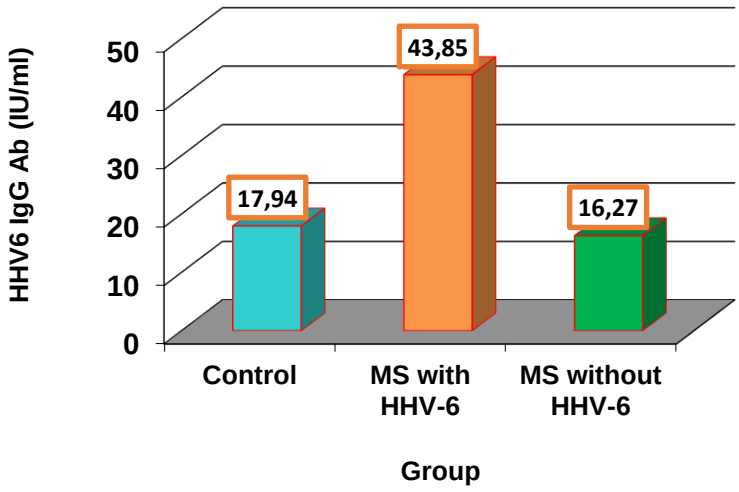

Figure 2 – Comparison between difference groups in HHV-6IgG Ab

compared with healthy controls (17.94±1.18 U/ml); and there was a highly significant difference (p≤0.01) in the level of HHV-6 IgG Ab in MS patients without HHV-6 infection (16.27±0.78 U/ml) as compared to healthy controls (17.94±1.18 U/ml) (P-value = 0.0001). The level of TNF-α and IL-6 in different groups of the study is shown in Table 3 and Figures 3 and 4. There was a highly significant difference (P≤0.01) in the level of TNF-α in MS patients with HHV-6 infection (206.93±7.88 Pg/ml) as compared to healthy controls (135.49±4.29 Pg/ml) and a highly significant difference (P≤0.01) in the level of TNF-α in MS patients without HHV-6

(203.90±6.24 Pg/ml) as compared to healthy controls (135.49±4.29 Pg/ml) (P-value for TNF-α = 0.0001). On the other hand, there was a highly significant difference (P≤0.01) in the level of IL-6 in MS patients with HHV-6 (55.92±2.99 Pg/ml) as compared with healthy controls (29.90±1.62 Pg/ml) and a highly significant difference (P≤0.01) in the level of IL-6 in MS patients without HHV-6 (51.74±2.14 Pg/ml) as compared with healthy controls (29.90±1.62 Pg/ml) (P-value = 0.0001).

Table 3 – Comparison between difference groups in TNF-α and IL-6

Group	Mean ± SE	
	TNF-α (pg/ml)	IL-6 (pg./ml)
Control	135.49 ±4.29 b	29.90 ±1.62 b
MS with HHV-6	206.93 ±7.88 a	55.92 ±2.99 a
MS without HHV-6	203.90 ±6.29 a	51.74 ±2.14 a
LSD	17.590 **	6.146 **
P-value	0.0001	0.0001
*Means with different letters in the same column differed significantly, ** (P≤0.01)*		

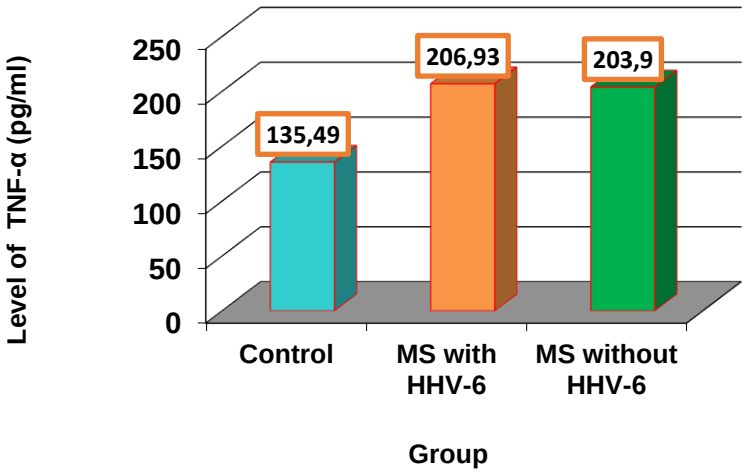

Figure 3 – Comparison between difference groups in TNF-α concentration (Pg/ml)

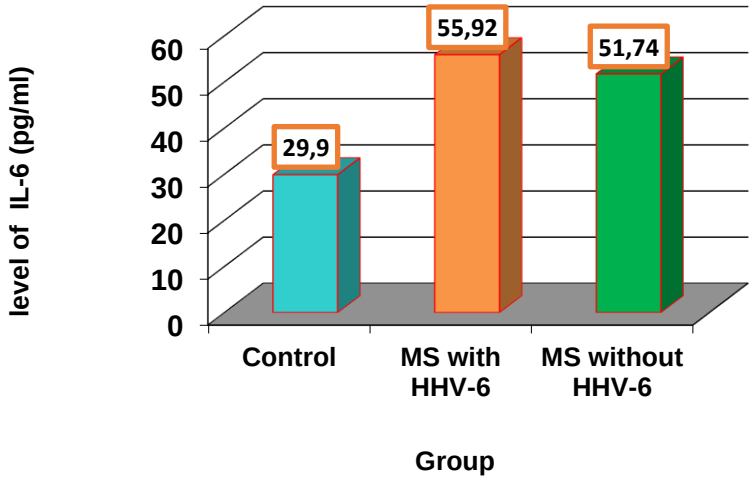

Figure 4 – Comparison between difference groups in IL-6 concentation (Pg/ml)

DISCUSSION

The hormonal variations in females that include pregnancy, menopause, hormonal contraceptives, and menstrual cycle disorders may have explained the increase in MS disease female vs. male ratio [26]. Our findings were consistent with the research by Hammood and Mohammed, who discovered that women constituted 60% of Iraqi MS patients, as compared to 40% of men. There was a significant difference (P≤0.01) in MS incidence in females vs. males [27]. These results corroborate Al-Faradahi's findings, which indicated that the female-to-male ratio was 2:1 (gender distribution – 66.67% : 33.33%) [28].

In serologic investigations for HHV-6, two types of antibodies were observed. Certain HHV-6 IgM antibodies first surfaced in the early going and then disappeared after a month, but specific HHV-6 IgG antibodies were detected when the elimination of IgM antibodies presented for an indefinite period of time, with some of those antibodies having the ability to neutralize viruses [29–31]. These results are consistent with those of other studies like Ablashi et al. (2000). Using an immunofluorescent antibody kit (IFA) for detecting HHV-6 IgM antibodies, the researchers discovered that seventy percent of the MS patients studied contained IgM antibodies for HHV-6, while only fifteen percent of the healthy donors and twenty percent of the patients with other neurological disorders had HHV-6 IgM antibodies [32]. The present study's findings are coherent with previous research showing that the mean percentage of anti-HHV-6A/B IgG was 98.0% (295/301) versus 93.4% (315/337) in controls who were healthy (p = 0.005). Between the test and control groups, a statistically significant difference was considered when p<0.05 [33]. The current study's findings agree with those of previous investigations, which demonstrate that 91.7% of individuals suffering from multiple sclerosis and 82.3% of healthy people were seropositive for HHV-6 IgG (p<0.001) [34]. In accordance with present results, previous studies demonstrated that the percentage of HHV-6 IgG antibody seropositive subjects was 100% (30 of 30) in multiple sclerosis patients, while in the patients suffering from neurological diseases, only 70% (14 of 20) were seropositive for HHV-6 IgG antibody, and in healthy individuals, this value was 75% (15 of 20). The statistical analysis shows that the mean index of HHV-6 Ab for the multiple sclerosis patients in the first sample collection was 2.68 versus 1.4 for both the control groups where (P value = 0.001) [35]. The differences in this study could be attributed to the small sample size, the age of the patients, the location from which the samples were collected, and the psychological conditions of the patients. Other factors that predispose to higher rates include low socioeconomic status and less education, in addition to genetic factors [36]. Cytokines are one immune factor that has been demonstrated to play a key role in the pathogenesis of MS [37]. Some of TNF-α functions related to the central nerve system (CNS) included control homeostatic functions: myelination, formation of nerve tissue, synaptic flexibility, and blood-brain barrier permeability [38]. Nevertheless, excessive or inappropriate activation of TNF-α signaling was associated with persistent inflammation and could lead to the development of clinical outcomes like autoimmune diseases [39]. Our findings are consistent with those from earlier research stating that sixty MS patients' had higher TNF-alpha levels in CSF, whereas none of the controls had any of the protein. It was found in the plasma of 7 (35.0%) controls and 38 (63.3%) MS patients, with considerably higher amounts in the MS group (p < 0.001). TNF-alpha values in CSF and plasma did not differ significantly in the MS group [40]. There was a significant increase in TNF-α level in the test group (26.55±2.68) compared to the control group (4.29±0.88) based on TNF-α gene expression [41].

Interleukin 6 (IL-6) pleiotropic mediators perform a different number of works and influence acute phase pathways, inflammatory processes, blood-brain barrier permeability, and other works. During injury or infection, IL-6 procure macrophages and lymphocytes at these sites [42]. The previously mentioned discovery provides additional evidence that the average serum concentration of IL-6 was considerably greater in MS patients compared to healthy controls (23.8±2.1 vs. 15.6±2.7 Pg/mL) (P value = 0.043) [43]. These findings are consistent with those of earlier investigations, which reported a rise in IL-6 in the serum of people with multiple sclerosis (12.1±1.8 Pg/ml) vs. healthy individuals acting as control group (6.6 ± 4.5 Pg/ml) [44]. Kallaur et al. (2013) measured some cytokine levels in South Brazil patients suffering from relapsing-remitting multiple sclerosis, and they found that serum IL-6 in this group was higher than in the controls [45]. These cytokines have a role in demyelination of the central nervous system (CNS) caused by immune system cells (β and T) penetrating the CNS and generating antibodies and cytokines against myelin antigens [46]. Finally, for the identification of human herpesvirus-6 and other infectious and immunological illnesses, we can suggest using molecular techniques like polymerase chain reaction (PCR), which was applied in different medical diseases [47–57].

CONCLUSIONS

- The percentage of females suffering from multiple sclerosis with human herpesvirus-6 infection was higher than males.
- A highly significant difference in the level of HHV-6 IgM, HHV-6 IgG Ab in MS with HHV-6 infection groups indicates that HHV-6 acts as a trigger factor to the development of multiple sclerosis by the formation of an autoimmune reaction in the host's body against components of nerve system.
- Significant increase in the level of proinflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin 6(IL-6) reveals the ability to use these parameters for early detection of multiple sclerosis disease.

AUTHOR CONTRIBUTIONS

All authors substantively contributed to the drafting of the initial and revised versions of this paper. They take full responsibility for the integrity of all aspects of the work.

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None.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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INFORMATION ABOUT THE AUTHORS

Nisreen, Saadoun Jasim - Msc. student
Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq
Email: nisreensjasam@gmail.com, nesreen.jassem2302@sc.uobaghdad.edu.iq.
Orcide: https://orcid.org/0009-0003-6561-787X

Dr. Rana Saady Aboud - PhD, Professor, Microbiology and Immunology,
Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq
Email: Rana.saadi@sc.uobaghdad.edu.iq
Orcid: https://orcid.org/0009-0008-9853-0021