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The role of Epstein-Barr virus and some immunological parameters of multiple sclerosis in Iraqi patients

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ABSTRACT

Multiple sclerosis (MS) is autoimmune neuroinflammatory and neurodegenerative disease that infected and destroyed the central nerves system (CNS). Many variables influence start of multiple sclerosis disease. MS was thought to be caused by virus infection, particularly infection with Epstein-Barr virus (EBV) and other viruses. Sixty blood samples (the number of samples not standardized for patients and the control group because when the control number = 20 statistically it is acceptable and also to take into account the economic cost) were collected from patients with (MS) ranging in age from 18-75 years. The medical personnel of Dr. Saad Al-Witry Neurosciences hospital provided diagnoses for these patients. Thirty-five specimens of apparently healthy individuals were studied as a control group (criteria were used to select the control group, outwardly healthy people do not suffer from MS or any other autoimmune disease). All the studied groups were carried out to measure the level of EBV-CA IgG Ab and EBV-NA IgG Ab and some immunological parameters, IL-10, IL-32, and neurofilament light chain (NfL), by enzyme-linked immunosorbent assay (ELISA) technique. The results showed that EBV-NA was positive in 100% (60/60) of the cases, EBV-CA was positive in 100% (60/60), IL-32 was positive in 98.33% (59/60), while IL-10 was positive in 0% (0/60). Neurofilament light chain (NfL) was positive in 100% (60/60) of the cases. The statistical analysis shows a highly significant difference ($p<0.01$) in the level of anti-EBV IgG Ab (EBV-CA and EBV-NA) in the sera of patients with MS and EBV compared to control groups. Also, the statistical analysis shows highly significant differences ($p<0.01$) in the level of (IL-10 and IL-32) in sera of patients with MS and EBV compared to the control group. A highly significant difference ($p<0.01$) was shown in the level of neurofilament light chain (NfL) in the sera of patients with MS and EBV compared to the control group. It can be concluded by the present study that EBV plays an important role as a trigger factor for multiple sclerosis.

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Introduction

Epstein-Barr Virus (EBV) infection has been linked to several autoimmune related disorders including systemic lupus erythematosus, Sjögren's syndrome, rheumatoid arthritis, and multiple sclerosis (Ali et al. 2021). A study that examined transcription factor binding to the human genome revealed that the EBV EBNA2 protein binds to regulatory regions associated with SLE as well as other

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autoimmune disorders including multiple sclerosis, rheumatoid arthritis etc. (Harley et al. 2018). Multiple sclerosis (MS) is an autoimmune-mediated neurodegenerative disease of the central nervous system characterized by inflammatory demyelination with axonal transaction (Salman 2016). MS affects an estimated 900 000 people in the US. MS typically presents in young adults (mean age of onset, 20-30 years) and can lead to physical disability, cognitive impairment, and decreased quality of life (Kuhlmann et al. 2002).

Multiple sclerosis (MS) affects approximately 2.8 million people worldwide and is a neurological and inflammatory condition of the central nervous system. Advances in diagnosis, management, and data accuracy have contributed to an increasing number of cases. Epidemiological studies show that MS risk varies geographically, with low-risk zones near the equator (fewer than 30 cases per 100,000 people) and high-risk zones in North America and Northern Europe (over 100 cases per 100,000 people). Medium-risk zones fall between these regions (McGinley et al. 2021). MS significantly impacts productivity and quality of life for patients and their families, with symptoms such as fatigue, pain, weakness, mood swings, numbness, bladder dysfunction, visual impairment, and loss of coordination according to typical neurological manifestation (King 2020). Multiple epidemiological studies have associated EBV infection with MS and EBV-induced mononucleosis has been linked to an elevated risk of MS (Levin et al. 2010).

A study of over 10 million military personnel in the U.S. revealed an increased rate of EBV infection in MS patients compared to controls and EBV-infected individuals were 32 times more likely to develop MS. Furthermore, Neurofilament light chain (NfL) is a marker for neuroaxonal degeneration and NfL levels were significantly elevated in the sera of EBV-infected individuals with MS (Bjornevik et al. 2022). Another study reported the presence of cross-reactive cerebrospinal fluid (CSF) antibodies against EBV EBNA1 and human glial cell adhesion molecule (GlialCAM) in the central nervous system due to shared homology between the viral and human proteins (Lanz et al. 2022). Thus, molecular mimicry of viral and CNS myelin antigens is responsible for the pathological disease process and both anti-EBNA1 and anti-GlialCAM antibodies were found in MS patients. T cell responses against EBV proteins also cross-react with CNS proteins; for example, EBNA1-specific T cells in patients with MS cross-react with myelin antigens (Lunemmann et al. 2008).

Furthermore, in an autoimmune encephalomyelitis (EAE) mouse model of MS, injection of EBNA1 protein worsened disease (Lanz et al. 2022). Moreover, it has been reported that a significant number of brain-infiltrating B cells and plasma cells displayed EBV infection in 21 out of 22 post-mortem MS brain specimens (Serafini et al. 2007). Postmortem brains of MS patients also displayed CD8 cytotoxic T lymphocytes that recognize EBV latent and lytic proteins and interact with EBV-infected B cells (Serafini et al. 2019).

The aim of present study was to evaluate the relationship between EBV infection and multiple sclerosis (MS).

Materials and Methods

Population Samples

The practical study included sixty patients suffering from MS disease diagnosis by the consultant medical staff in Dr. Saad Al-Witry Hospital for Neurosciences (Samples were collected under the supervision of a consultant neurologist), the important diagnostic criteria for the disease that the specialist physician relies on are dissemination in space (DIS). This criterion requires evidence of lesions in different areas of the CNS: periventricular, juxtacortical/cortical, infratentorial, or spinal cord. MRI is the most common tool used to demonstrate DIS, dissemination in time (DIT).

DIT demonstrates that the disease has occurred at more than one time point. It can be shown via a new T2 lesion or gadolinium-enhancing lesion on follow-up MRI. Simultaneous presence of both enhancing and non-enhancing lesions, clinical presentation A clinical diagnosis of MS usually requires: At least two attacks (relapses) involving different CNS regions. Each attack is defined by a new neurological symptom lasting at least 24 hours, Exclusion of Other Diagnoses MS is a diagnosis of exclusion. Conditions such as infections, vitamin B12 deficiency, or other autoimmune diseases must be ruled out through lab tests and clinical history and cerebrospinal fluid (CSF) analysis.

CSF testing often reveals: Oligoclonal bands (OCBs) found in CSF but not in serum. An elevated IgG index, indicating intrathecal immunoglobulin production (Thompson et al. 2018, Teunissen et al. 2015). From October 2024 until February 2025. The patients' ages ranged from 18 to 75 years (that is logical in the study). 5 ml of whole blood were collected from all participant in the current work, a sterile syringe was used. The sample was transferred to a vacuum gel plain tube and allowed to coagulate at room temperature for several hours. (This clotting period allows for complete coagulation so the serum can be cleanly separated) (This procedure is logical). The tubes were then centrifuged at 3000 rpm for 5 minutes. (to separate the serum from the clot).

Thirty-five specimens of apparently healthy individuals were studied as a control group. The age average of the control group ranges between 15 to 75 years (there is an age group division between the groups).

The information which included the number that was given to the patient, name of patient and data of sample collection for all specimen patient and control were recorded in each tube.

Estimation of Anti-EBV IgG, IL-10, IL-32, and NfL

In all studied patient groups, MS with EBV and apparently healthy individuals, EBV Ab IgG, IL-10, IL-32, and NfL levels were estimated using ELISA (Indirect ELISA and Sandwich ELISA) according to the kit's protocol.

Statistical Analysis

The Statistical Packages of Social Sciences-SPSS (SPSS, 2019) program was used to detect the effect of difference groups in study parameters. T-test and least significant difference-LSD was used to significant compare between means. Chi-Square test was used to significant compare between percentages. Estimate of correlation coefficient between parameters in this study.

Results

This study concluded that there was a significant difference between male and female MS patients, Where the male patients 17(28.33%), while the female patients 43(71.67%) as shown in figure (1).

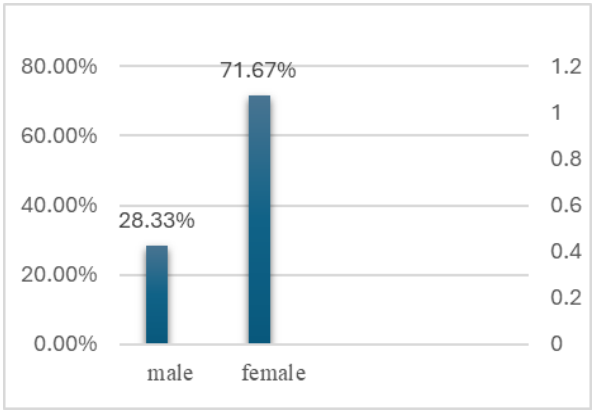

Fig 1. The difference in the percentages between male and female MS patients.

The study also conducted that, among different age groups, the patients with MS aged >50 years were 38 (63.33%), while the age group 40-50 years showed 18 (30.00%) of the patients with MS, with a highly significant difference ($p<0.01$) between the patients with MS and the control group. Also, there was a highly significant difference ($p < 0.01$) between the patients and the control groups in age < 40 yr., which represents (6.67%) and the control group (40.00%) (P value = 0.0001), as shown in table (1).

Table 1 Distribution of study samples according to age in control and patient groups.

Age groups	Control (No=35)	Patients (No= 60)	P-value
<40 yr.	14 (40.00%)	4(6.67%)	
40-50 yr.	21(60.00%)	18 (30.00%)	0.0001
>50 yr.	0 (0.00%)	38 (63.33%)	**

* ($p\leq0.05$): Significant, ** ($p\leq0.01$): Highly Significant, NS: Non-Significant.

Also, the statistical analysis reveals that there was a highly significant difference ($p\leq0.01$) in the level of EBV-CA in the patient group (1.491 ± 0.050 U/ml) as compared with the control group (0.492 ± 0.023 U/ml) and P-value = 0.0001 (Fig. 2).

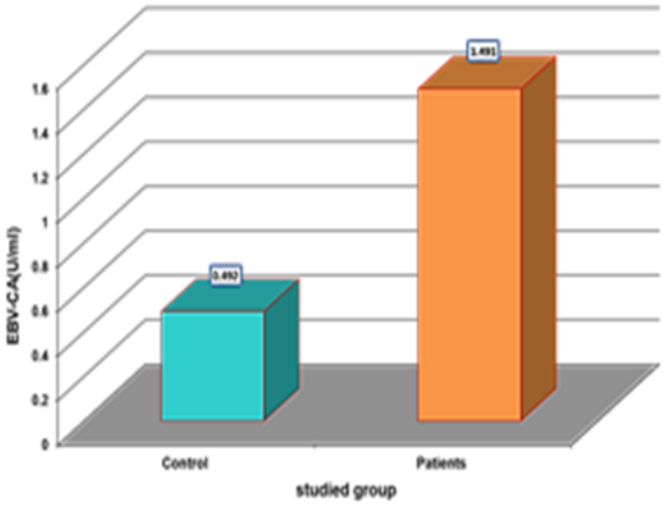

Fig 2. Mean level of EBV-CA IgG Ab (U/ml) in sera of patients with MS and the control group.

In this study the statistical analysis reveals that there was a highly significant difference ($p \leq 0.01$) in the level of EBV-NA in the patient group (1.622 ± 0.036 U/ml) as compared with the control (0.503 ± 0.019 U/ml) and P-value = 0.0001 (Fig. 3).

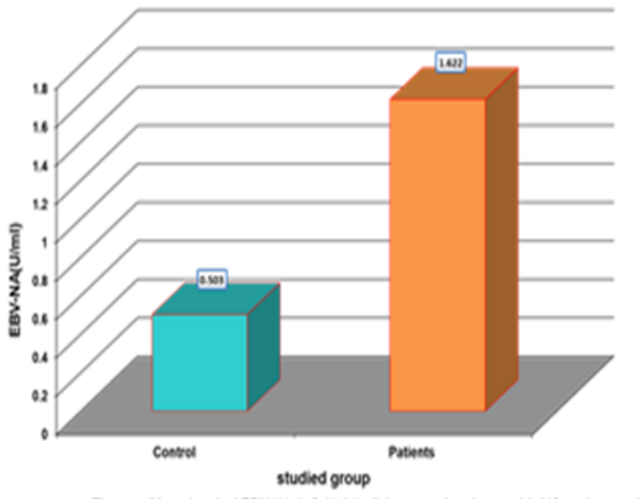

Fig 3. Mean level of EBV-NA IgG Ab (U/ml) in sera of patients with MS and the control group.

The present study showed that there was a highly significant decrease (p≤0.01) in the level of IL-10 in the patient group (42.91 ±1.22 pg/ml) as compared with the control (86.86 ±1.74 pg/ml) and P-value = 0.0001, as shown in (Table 2). In the current study the results showed that there was a highly significant difference (p≤0.01) in the level of IL-32 in patients' group (771.37 ±19.90 pg/ml) as compare with control (245.95 ±7.48 pg/ml) and P-value = 0.0001 (Table 3). While the results also showed that there was a highly significant difference (p≤0.01) in the level of NEFL in patients group (169.94 ±3.03pg/ml) as compare with control (51.38 ±1.84pg/ml) and P-value = 0.0001 (Table 4).

Table 2 Mean level of IL-10 (pg/ml) in sera of patients with MS and the control group.

Group	**Means ±SE**
	IL-10 (pg/ml)
Control	86.86 ±1.74
Patients	42.91 ±1.22
T-test	4.136 **
P-value	0.0001
**** (P≤0.01)**	

Table 3 Mean level of IL-32 (pg/ml) in sera of patients with MS and the control group.

Group	**Means ±SE**
	IL-32 (pg/ml)
Control	245.95 ±7.48
Patients	771.37 ±19.90
T-test	53.098 **
P-value	0.0001
**** (P≤0.01)**	

Table 4. Mean level of NfL (pg/ml) in sera of patients with MS and the control group.

Group	**Means ±SE**
	NEFL (pg/ml)
Control	51.38 ±1.84
Patients	169.94 ±3.03
T-test	8.375 **
P-value	0.0001
**** (P≤0.01)**	

Discussion

The present study was consistent with one study who discovered that women constituted 60% of Iraqi MS patients, compared to 40% of men. There was a significant difference (P≤0.01) which mean MS influence in female than male (Hammood & Mohammed 2022). Also, the present study was consistent with the study done by another article was showed that the percentage of males suffering from MS with HHV-6 was 14.81%, with a highly significant difference between the studied groups (Saadoun & Saady 2024). The mentioned results denoted a significant increase in multiple sclerosis among females rather than males.

These hormonal variations in female sex that include pregnancy, menopause, hormonal contraceptives, and a defect in the menstrual cycle could be explained by the raising of the MS disease ratio in females than males. Furthermore, genetic conditions or environmental factors with the psychological change of Iraqi people that result in high stress enhance autoimmune disease evolvement. In addition, these variations may be due to sample size and different circumstances and time of sample collection, besides different populations (Goodin et al. 2021). In a study done by (Ad'hiah et al. 2023), it was shown that EBV-positive MS cases were more common in females than in males (83.3 vs. 16.7%), while an opposite distribution was observed in HC (37.5 vs. 62.5%), and the difference was significant (p = 0.041). There is no well-documented data regarding this point, but it is well known that females are more likely to develop RRMS than males, and environmental factors (for instance vitamin D may have a role in this gender disparity (Sellner et al. 2011).

The results of the current study were agreed upon with research by (Prosperini et al. 2022), who observed an increased mean age at onset and a shortened mean interval to diagnosis over time (p<0.0001). Accordingly, there were more MS onsets at the older age classes of 40-49, 50-59, and ≥60 years (p<0.0001). In cases with age at onset ≥40 years, they also found an increased female-to-male ratio (p=0.007), more frequent spinal cord (p=0.0004), and less frequent supratentorial onset (p=0.008). The present study was incompatible with

other research by (Ajadi et al. 2024) who showed, the highest prevalence of 100% was recorded among the age groups 19-20 and 26-30, while the lowest prevalence of 88.8% was observed in the age group 31-35. Additionally, the age group 21-25 had a prevalence of 94.4%. A study by (Saadoun & Saady 2024) found a significant difference ($p < 0.01$) between MS patients aged 40-50 and control groups. Another study by (Hassan & Aboud 2024) revealed a significant correlation ($P \leq 0.01$) between H. pylori infection and multiple sclerosis.

The age distribution analysis demonstrated that the majority of MS patients, both with and without H. pylori, were above the age of 40. However, there was a significant difference in age groupings between the two patient groups. In experimental models, aged rats demonstrate a slower and less effective remyelination process compared to young rats. Similarly, elderly humans also showed reduced remyelinating capabilities; There are multiple hypotheses to explain this finding, including intrinsic characteristics that pre-determine maturation and aging of OPCs irrespective of extrinsic factors, and epigenetic changes known to occur during aging (Rist &Franklin 2008). Imaging studies suggest that during the fourth decade of life, myelination of the compact white matter slows and ceases in parallel with progressive white matter degeneration (Westlye et al. 2010). Because these age-related changes in myelination coincide with approximately the average age at which PwMS reach progression (mean age: 38–45 years), it has been proposed that the loss of remyelinating capacity with aging contributes to this transition (Tutuncu et al. 2013).

Hence, MS may be a disorder of a single stage, with an apparent shift in phenotype driven not by a fundamental change in pathology but by age-related changes (Scalfari et al. 2011). This study was compatible with (Ajadi et al. 2024) who revealed that 43 subjects (95.69%) tested positive for EBV, while 2 (4.4%) tested negative. Among different age groups, women aged 19-20, 26-30, and 36-40 showed a prevalence rate of 100%, with the lowest prevalence of 88.8% recorded among the 31-35 age group. Regarding the pregnancy stage, 100% was seen among first-trimester participants, while the lowest prevalence of 92.3% was noted during the third trimester. The majority of respondents were married, with those from polygamous families and single individuals showing a prevalence of 100%, This result could be attributed to a multifactorial interaction between genetic predisposition, environmental exposure to Epstein-Barr Virus (EBV), and sociocultural structures that influence both viral transmission and MS risk. Polygamous family settings, often characterized by larger household sizes and close intergenerational contact, may increase opportunities for early-life or delayed EBV transmission—an environmental factor strongly associated with the onset of multiple sclerosis (Bjornevik et al. 2022).

Social structures also play a critical role: marital status may reflect varying socio-economic conditions, healthcare access, and psychosocial support systems, all of which are known to influence immune health and disease progression (Mohr & Cox 2001, Kiecolt-Glaser et al. 2003). Single individuals, for example, may experience increased social isolation and stress—factors that have been linked to immune dysregulation and heightened susceptibility to autoimmune diseases. Conversely, being married may offer protective benefits through emotional support and stability. Thus, the elevated prevalence of MS in polygamous and single individuals may result from a complex interplay of biological, environmental, and social determinants. Arabs had the highest prevalence, whereas foreigners had the lowest. The result came with (Jassim et al. 2015) showed that the mean level of EBV VCA IgG Ab was 40.82 ± 2.36 NTU in RA (rheumatoid arthritis), while in the positive control groups the mean level of EBV VCA IgG Ab was 51.24 ± 2.19 NTU in AS (ankylosing spondylitis) patients and 38.24 ± 8.79 NTU in ReA (reactive arthritis) patients, respectively, compared to the control group, which was 2.55 ± 0.03 NTU.

The observed 95.6% seropositivity rate of EBV IgG antibodies aligns with findings indicating the presence of Immunoglobulin G (IgG) due to previous exposure to the virus. This rate also corresponds with (Schafer et al. 2015) assertion that over 90% of the global population possesses EBV IgG antibodies. The Epstein - Barr virus (EBV) Capsid Antigen (CA) comprises structural proteins that form the viral capsid, encapsulating the viral genome. These proteins are pivotal in the assembly and maturation of EBV virions (Wang et al. 2011). The appearance of immunoglobulin G against VCA at least 1 month after primary infection and persists for many years or throughout life (Lim 2009). (To clarify the role of EBV-CA in injury). The current study aligned with previous work by (Al-Obaidi et al. 2022), who found that anti-EBNA-1 IgG Ab was positive in 51.7% (62/120) of MS patients and 39.2% (47/120) of controls ($P=0.035$). The median of anti-EBNA-1 IgG levels in MS patients and controls were 81.08 U/ml and 67.73 U/ml, respectively ($P=0.043$). Additionally, EBNA-1 antibody was significantly higher in younger age groups. Patients with the first-line and second-line treatments showed no significant differences in anti-EBNA-1 IgG levels, while the median level in patients without treatment (newly diagnosed) was

higher. The study (Jassim et al. 2015) found that the mean of concentration EBVNA- IgG 1 Ab in ReA (Reactive arthritis) patients was 30.40 ± 9.40 while the mean in AS (Ankylosing spondylitis) group was 50.2± 17.83 U/ml and the mean of concentration of EBVNA-IgG 1 Ab was 29.01 ± 4.21 U/ml in RA (Rheumatoid arthritis) patients compared to control group 48.08 ± 5.83.

The presence of an antigen such as the myelin basic protein (MBP), a peptide derived from the myelin sheaths surrounding an axon having a homology to EBV viral proteins. (Kumar et al. 2013) have illustrated molecular mimicry of viral EBNA-1 to MBP that could prompt T-cell autoimmunity to myelin sheaths. For this reason, one of the most relevant non-self-antigens that is thought to induce MS is EBNA-1. Epstein - Barr virus (EBV) Nuclear Antigens (EBNAs) are pivotal in the virus's ability to establish latency and transform infected cells. The primary EBNAs include EBNA1, EBNA2, EBNA3A, EBNA3B, EBNA3C, and EBNA-LP, each serving distinct functions within the EBV life cycle and pathogenesis (Wilson et al. 2018).

Not all individuals produce EBNA-1 IgG antibodies, although most individuals do, and EBNA-1 IgG antibodies may secondarily be lost under circumstances such as immunosuppression and thus do not persist lifelong (Hess 2004). Antibody to EBNA (determined by the standard immunofluorescent test) is not seen in the acute phase of EBV infection. Instead, it slowly appears 2 to 4 months after the onset of symptoms and persists for the rest of a person's life (Wilson et al. 2018). In a study conducted by Pulvirenti et al. (2024) shown that the percentages of CD4+ cells expressing either (transcription factor EOMESODERMIN) EOMES or IL-10 alone were more variable (EOMES+14±10%, IL-10+3.3±3.8%, respectively) but did again not differ significantly between meningeal and parenchymal immune infiltrates. No IL-10 immunoreactivity was detected in intraparenchymal neural cells and microglia. Immunostaining for CD 20, and in situ hybridization for the non-coding EBV-derived RNA EBER, performed in consecutive brain sections, revealed the presence of numerous B-cells and EBV infected cells in the same immune infiltrates that contained EOMES+Tr1-like cells. Overall, this analysis indicates that EOMES+Tr1-like cells are present in the MS brain, some in the vicinity of CD8+T-cells and EBV-infected B-cells and are locally activated to produce IL-10.

Yasui et al (2008) shown that the cytosine-allele frequencies at hIL-10 T-819C were 32.9% in women and 30.9% in men. These are consistent with the published reports of Japanese and Chinese, but substantially lower than those of Caucasians (> 70%). It is a possibility that the T-819C polymorphism and the associated IL-10 levels may modulate the immune response and MS risk, given some observed correlation between the promoter polymorphism and the IL-10 levels in *in vitro/in vivo* (Schippers et al. 2005). It would be of interest to evaluate MS risk in relation to the hIL-10 T-819C genotype: 1. in populations where thymine-allele frequencies at hIL-10 T 819C are sufficiently high (e.g., Chinese and Japanese); and perhaps more importantly, 2. in any population stratified by gender. IL-10 production and proliferation of adaptive regulatory T-cells are controlled by T-cell receptor stimulation.

Therefore, the identification of the antigens that generate and activate these cells is a key issue to understand their functions, in particular in MS (Paroni et al. 2017). In the (experimental autoimmune encephalomyelitis) EAE model, T-cells, including adaptive regulatory T-cells, are deliberately primed by myelin-derived self- antigens. The failure to suppress myelin-targeted autoimmunity may explain the extensive CNS damage in MS. An unexpected key finding of this study is that regulatory T-cells in MS are activated by EBV, a persistent virus that has an essential, but also still enigmatic (Kuchroo & Weiner 2022), role in MS pathology (Bjornevik et al. 2022).

The results of (Abood & Fadhil 2024) show a non-significant difference in the IL-32 SNP (rs45499297) gene between MS patients and healthy controls, and this is considered the first study with MS, especially in the case of EBV positive. IL-32 may facilitate viral component neutralization (for instance, by inactivating viral nucleocapsid structures), and to measure the extent to which SARS-CoV-2 infection has damaged cells, IL-32 might be utilized as a biomarker (Abed-Alhussien & Fadhil 2023). Other studies found that IL-32 is elevated following EBV infection. EBV latent membrane protein 1 (LMP1) triggers the production of IL-32. IL-32 may be upregulated by LMP1 in a manner that allows EBV to retain latency (Lai et al. 2015). So, I noticed IL-32 was higher in healthy controls with EBV positive.

IL-32 is a unique intracellular cytokine which affects many cellular and physiological functions like cell death and survival, inflammation and response to pathogens. With numerous transcripts, more than one biologically active isoforms, IL32 drives its effect in diverse cellular functions. A cytokine restricted to higher mammals, it is known to fine tune multiple pathways involved in metabolic processes or infection. It modulates the immune response against diverse pathogens like *Leishmania, Mycobacterium* and HIV (Gautam & Pandit 2021). IL-32 is a multifunctional cytokine linked to a variety of illnesses and inflammatory disorders; the central nervous system is affected by the chronic

inflammatory disease known as multiple sclerosis (Abood & Fadhil 2024). However, the current study was compatible with (Kuhle et al. 2019) Who showed that NfL levels (pg/mL) were higher in MS patients than in healthy controls (30.5 and 27.0 vs 16.9, $p = 0.0001$) and correlated with T2 lesion load and number of gadolinium-enhancing T1 lesions ($p < 0.0001$, both). Baseline NfL levels, treatment, and number of new or enlarging T2 lesions during the studies predicted NfL levels at the end of study (all $p < 0.01$).

High against low baseline NfL levels were associated with an increased number of new or enlarging T2 lesions relapses, brain volume loss, and risk of confirmed disability worsening. Fingolimod significantly reduced NfL levels already at 6 months, which was sustained until the end of the studies. Because of their specificity for neurons, increased NfL levels confirm that active inflammatory lesions are associated with neuroaxonal damage. The reasons for their low correlation with clinical disability must therefore primarily be sought in compensation and repair. Neuropathology studies showing a higher rate of axonal damage in early/relapsing than in progressive MS brains are also compatible with this view (Kuhlmann et al. 2002). In a study conducted by (Thebault et al. 2020) sixty-seven patients with MS had a median follow-up of 18.9 years (range 15.0–27.0).

The median serum NfL level in patient baseline samples was 10.1 pg/mL, 38.5% higher than median levels in 37 controls (7.26 pg/mL, $p = 0.004$). Baseline NfL level was most helpful as a sensitive predictive marker to rule out progression; patients with levels less than 7.62 pg/mL were 4.3 times less likely to develop an EDSS score of ≥ 4 ($p = 0.001$) and 7.1 times less likely to develop progressive MS ($p = 0.054$). Patients with the highest NfL levels (3rd tertile, > 13.2 pg/mL) progressed most rapidly with an EDSS annual rate of 0.16 ($p = 0.004$), remaining significant after adjustment for sex, age, and disease-modifying treatment ($p = 0.022$). The quantity of NfL in the serum is rapidly emerging as a convenient and important biomarker in MS, with evidence for its role in monitoring disease activity and treatment response (Siller et al. 2019). Although there is data to implicate its potential role early in MS in predicting short term outcomes (Costa et al. 2019), the data for long-term outcomes less well established. Neurofilament light chain is a biomarker that can be measured with immunoassays in cerebrospinal fluid and plasma and reflects axonal damage in a wide variety of neurological disorders (Thompson & Mead 2019). It is a useful arker for disease monitoring in amyotrophic lateral sclerosis, (Xu et al. 2016) multiple sclerosis, (Cai & Huang 2018) Alzheimer's disease, (Zetterberg & Schott 2019) and more recently Huntington's disease. It is also a promising marker for follow-up of patients with brain tumors. (Arslan et al. 2022).

Conclusion

This study suggests that EBV may play a significant role in triggering multiple sclerosis, highlighting the connection between viral infections and autoimmune responses. Treating MS remains a major challenge because of its complex and unpredictable nature. These findings emphasize the need for further research to better understand the role of EBV and to develop more effective strategies for managing this debilitating disease.

Declaration of interests

In this article, all authors declare that they have no known financial interests, competing interests, or personal relationships that could have influenced the research work described.

Declaration of ethics

Every participant agreed to give blood samples to the researchers. Per the Declaration of Helsinki, each subject gave their informed approval. The present work was approved by the Ethics Committee of the Baghdad health department, Iraq; the reference number was 485/11-8-2024.

References

Abed-Alhussien TAA, Fadhil HY. (2023). Exploring the Role of Caspase-3 and IL32 in SARS-CoV-2 Infection among Iraqi Patients. *Iraqi Journal of Science*, 64(8): 3837–3847.

Abood AR, Fadhil HY. (2024). Exploration of IL-32 (rs45499297) gene variations in EBV and MS patients. *Iraqi Journal of Pharmaceutical Sciences*, 33(2): 170–178.

Ad'hiah AH, Atiyah NS, Fadhil HY. (2023). Qualitative and Quantitative Molecular Analysis of Epstein-Barr Virus in Iraqi Patients with Relapsing-Remitting Multiple Sclerosis. *Iraqi Journal of Science*, 127–137.

Ajadi AE, Bale SI, Sorunke TA, Onifade SA, Oyawoye T, Babasola KM. (2024). Serological Evidence of Epstein-Barr Virus Capsid Antigen (EB-CA) Among Pregnant Women from Cottage Hospital, Ilorin. *UMYU Scientifica*, *3*(2): 94–101.

Ali R, Maulud S, Jalal P, Ahmed J. (2021). Molecular dual actions of hsa-miRNA and v-miRNA in oncogenic EBV. *Microbial Biosystems*, 6(1), 1-10. doi: 10.21608/mb.2021.87024.1034

Al-Obaidi AB, Ali ZA, Almashta RSA, Ghazi HF. (2022). The potential role of Epstein-Barr virus in

Multiple sclerosis molecular and serological study. *Wiadomosci Lekarskie*, 75(3): 691–696.

Amer S M, Alam N G, El-Shanshory M R, Ibrahim F A, El-shafey A S. (2024). Immunodiagnostic investigations for children with lymphadenopathy at Tanta University hospital. *Microbial Biosystems* Journal, 9(1), 33–37.

Arslan B, Arslan GA, Tuncer A, Karabudak R, Dinçel AS. (2022). Evaluation of cerebrospinal fluid neurofilament light chain levels in multiple sclerosis and non-demyelinating diseases of the central nervous system: clinical and biochemical perspective. *Bosnian Journal of Basic Medical Sciences*, 22(5): 699–706.

Bjornevik K, Cortese M, Healy BC, Kuhle J, Mina MJ, Leng Y, Ascherio A. (2022). Longitudinal analysis reveals high prevalence of Epstein-Barr virus associated with multiple sclerosis. *Science*, 375 (6578): 296–301.

Cai L, Huang J. (2018). Neurofilament light chain as a biological marker for multiple sclerosis: a meta-analysis study. *Neuropsychiatric disease and treatment*, 2241–2254.

Costa DG, Martinelli V, Sangalli F, Moiola L, Colombo B, Radaelli M, Comi G. (2019). Prognostic value of serum neurofilaments in patients with clinically isolated syndromes. *Neurology*, *92*(7): e733-e741.

Gautam A, Pandit B. (2021). IL32: The multifaceted and unconventional cytokine. Human Immunology, 82(9): 659–667.

Goodin DS, Khankhanian P, Gourraud PA, Vince N. (2021). The nature of genetic and environmental susceptibility to multiple sclerosis. *PLoS One*, 16(3): e0246157.

Hammood FS, Mohammed BJ. (2022) Genetic identification of sample of multiple sclerosis in Iraqi patient. Iraqi *Journal of Biotechnology,* 21 (2): 255–235.

Harley JB, Chen X, Pujato M, Miller D, Maddox A, Forney C, Weirauch MT. (2018). Transcription factors operate across disease loci, with EBNA2 implicated in autoimmunity. *Nature genetics*, *50*(5): 699–707.

Hassan AJ, Aboud RS. (2024). The relationship between Helicobacter pylori infection and multiple sclerosis. *Iraqi Journal of Biotechnology,* 23(3): 174–184.

Hess RD. (2004). Routine Epstein-Barr virus diagnostics from the laboratory perspective: still challenging after 35 years. *Journal of clinical microbiology*, *42*(8): 3381–3387.

Jassim NS, Aboud RS, Joda AT, Fadil HY, Al_Humadani FG, Ahmed DM, Husaen MA. (2015). Detection of Epstein-Barr virus Capsid antigen (EBV CA) in Sera of Rheumatoid Arthritis, Reactive Arthritis and Ankylosing Spondylitis Patients. *Iraqi Journal of Science*, *56*(4B): 3130–3134.

Kiecolt-Glaser JK, Preacher KJ, MacCallum RC., Atkinson C, Malarkey WB, Glaser R. (2003). Chronic stress and age-related increases in the proinflammatory cytokine IL-6. *Proceedings of the national Academy of Sciences*, 100(15): 9090–9095.

King R. (2020). Atlas of MS 3rd edition. PART 1: mapping multiple sclerosis around the world key epidemiology findings. *Multiple Sclerosis International Federation: London, UK*..

Kuchroo VK, Weiner HL. (2022). How does Epstein-Barr virus trigger MS?. *Immunity*, 55(3): 390–392.

Kuhle J, Kropshofer H, Haering DA, Kundu U, Meinert R, Barro C, Kappos L. (2019). Blood neurofilament light chain as a biomarker of MS disease activity and treatment response. *Neurology*, *92*(10): e1007–e1015.

Kuhlmann T, Lingfeld G, Bitsch A, Schuchardt J, Brück W. (2002). Acute axonal damage in multiple sclerosis is most extensive in early disease stages and decreases over time. *Brain*, *125*(10): 2202–2212.

Kumar A, Cocco E, Atzori L, Marrosu MG, Pieroni E. (2013). Structural and dynamical insights on HLA-DR2 complexes that confer susceptibility to multiple sclerosis in Sardinia: a molecular dynamics simulation study. *PloS one*, 8(3): e59711.

Lai KY, Chou YC, Lin JH, Liu Y, Lin KM, Doong SL, Tsai CH. (2015). Maintenance of Epstein-Barr virus latent status by a novel mechanism, latent membrane protein 1-induced interleukin-32, via the protein kinase Cδ pathway. *Journal of virology*, *89*(11): 5968–5980.

Lanz TV, Brewer RC, Ho PP, Moon JS, Jude KM, Fernandez D, Robinson WH. (2022). Clonally expanded B cells in multiple sclerosis bind EBV EBNA1 and GlialCAM. *Nature*, 603(7900): 321–327.

Levin LI, Munger KL, O'reilly EJ, Falk KI, Ascherio A. (2010). Primary infection with the Epstein-Barr virus and risk of multiple sclerosis. *Annals of neurology*, *67*(6): 824–830.

Lim DNF. (2009). A case of Epstein–Barr virus (EBV) meningoencephalitis in a patient with rheumatoid arthritis. *Grand Round*, 9: 49–53.

Lünemann JD, Jelčić I, Roberts S, Lutterotti A, Tackenberg B, Martin R, Münz C. (2008). EBNA1-specific T cells from patients with multiple sclerosis cross react with myelin antigens and co-produce

IFN-γ and IL-2. *The Journal of experimental medicine*, 205(8): 1763–1773.

McGinley MP, Goldschmidt CH, Rae-Grant AD. (2021). Diagnosis and treatment of multiple sclerosis: a review. *Jama*, *325*(8): 765–779.

Mohr DC, Cox D. (2001). Multiple sclerosis: empirical literature for the clinical health psychologist. *Journal of clinical psychology*, *57*(4): 479–499.

Paroni M, Maltese V, De Simone M, Ranzani V, Larghi P, Fenoglio C, Geginat J. (2017). Recognition of viral and self-antigens by TH1 and TH1/TH17 central memory cells in patients with multiple sclerosis reveals distinct roles in immune surveillance and relapses. *Journal of Allergy and Clinical Immunology*, 140(3): 797–808.

Prosperini L, Lucchini M, Ruggieri S, Tortorella C, Haggiag S, Mirabella M, Gasperini C. (2022). Shift of multiple sclerosis onset towards older age. *Journal of Neurology, Neurosurgery & Psychiatry*, *93*(10): 1137–1139.

Pulvirenti N, Righetti C, Clemente F, Serafini B, Pietroboni AG, Geginat J. (2024). Regulatory T-cells in multiple sclerosis are activated by Epstein-Barr Virus and produce IL-10 in the central nervous system. *bioRxiv*, 07.

Rist JM, Franklin RJ. (2008). Taking ageing into account in remyelination-based therapies for multiple sclerosis. *Journal of the neurological sciences*, 274 (1-2): 64–67.

Saadoun NJ, Saady RA. (2024). Immunological detection of human herpes virus-6 in sera of Iraqi patients with multiple sclerosis.Eastern. *Ukrainian Medical Journal,* 12(4): 846–855.

Salman ED. (2016). Genetic polymorphisms of interleukin-1 beta gene in association with multiple sclerosis in Iraqi patients. *Iraqi Journal of Science*, 594–598.

Scalfari A, Neuhaus A, Daumer M, Ebers GC, Muraro P. (2011). Age and disability accumulation in multiple sclerosis. *Neurology*, *77*(13): 1246–1252.

Schafer G, Blumenthal MJ, Katz AA. (2015). Interaction of human tumor viruses with host cell surface receptors and cell entry. *Viruses*, *7*(5): 2592–2617.

Schippers EF, van't Veer C, van Voorden S, Martina C A., Huizinga TW, le Cessie S, van Dissel JT. (2005). IL-10 and toll-like receptor-4 polymorphisms and the in vivo and ex vivo response to endotoxin. *Cytokine*, 29(5): 215–228.

Sellner J, Kraus J, Awad A, Milo R, Hemmer B, Stüve O. (2011). The increasing incidence and prevalence of female multiple sclerosis—a critical analysis of potential environmental factors. *Autoimmunity reviews*, 10(8): 495–502.

Serafini B, Rosicarelli B, Franciotta D, Magliozzi R, Reynolds R, Cinque P, Aloisi F. (2007). Dysregulated Epstein-Barr virus infection in the multiple sclerosis brain. *The Journal of experimental medicine*, 204(12): 2899–2912.

Serafini B, Rosicarelli B, Veroni C, Mazzola GA., Aloisi F. (2019). Epstein-Barr virus-specific CD8 T cells selectively infiltrate the brain in multiple sclerosis and interact locally with virus-infected cells: clue for a virus-driven immunopathological mechanism. *Journal of virology*, *93*(24): 10–1128.

Siller N, Kuhle J, Muthuraman M, Barro C, Uphaus T, Groppa S, Bittner S. (2019). Serum neurofilament light chain is a biomarker of acute and chronic neuronal damage in early multiple sclerosis. *Multiple Sclerosis Journal*, 25(5): 678–686.

Teunissen CE, Malekzadeh A, Leurs C, Bridel C, Killestein J. (2015). Body fluid biomarkers for multiple sclerosis—the long road to clinical application. *Nature Reviews Neurology*, 11(10), 585–596.

Thebault S, Abdoli M, Fereshtehnejad SM, Tessier D, Tabard-Cossa V, Freedman MS. (2020). Serum neurofilament light chain predicts long term clinical outcomes in multiple sclerosis. *Scientific reports*, 10(1): 10381.

Thompson AG, Mead SH. (2019). Fluid biomarkers in the human prion diseases. *Molecular and Cellular Neuroscience*, *97*: 81–92.

Thompson AJ, Banwell BL, Barkhof F, Carroll WM, Coetzee T, Comi G, Cohen JA. (2018). Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. *The Lancet Neurology*, 17(2): 162–173.

Tutuncu M, Tang J, Zeid NA, Kale N, Crusan DJ, Atkinson EJ, Kantarci OH. (2013). Onset of progressive phase is an age-dependent clinical milestone in multiple sclerosis. *Multiple Sclerosis Journal*, 19(2): 188–198.

Wang WH, Chang LK, Liu ST. (2011). Molecular interactions of Epstein-Barr virus capsid proteins. *Journal of virology*, *85*(4): 1615–1624.

Westlye LT, Walhovd KB, Dale AM, Bjørnerud A, Due-Tønnessen P, Engvig A, Fjell AM. (2010). Life-span changes of the human brain white matter: diffusion tensor imaging (DTI) and volumetry. *Cerebral cortex*, 20(9): 2055–2068.

Wilson JB, Manet E, Gruffat H, Busson P, Blondel M, Fahraeus R. (2018). EBNA1: oncogenic activity, immune evasion and biochemical functions provide targets for novel therapeutic strategies against

Epstein-Barrvirus-associated cancers. *Cancers*, 10(4): 109.

Xu Z, Henderson RD, David M, McCombe PA. (2016). Neurofilaments as biomarkers for amyotrophic lateral sclerosis: a systematic review and meta-analysis. *PloS one*, 11(10): e0164625.

Yasui Y, Hamajima N, Nakamura T, El-Din NS, Tajima K, Potter JD. (2008). Association of Epstein-Barr virus antibody titers with a human IL-10 promoter polymorphism in Japanese women. *Journal of Autoimmune Diseases*, 5: 1–5.

Zetterberg H, Schott JM. (2019). Biomarkers for Alzheimer's disease beyond amyloid and tau. *Nature medicine*, *25*(2): 201–203.