Egyptian Journal of Medical Microbiology Volume 34 / No.3 / July 2025 15-23 Online ISSN: 2537-0979

ORIGINAL ARTICLE

Association between Autophagy-Related Protein-5 and Epstein-Barr virus Infection in Multiple Sclerosis Patients

[1]Mona G. Nada*, [1]Nadia H. Ouda, [1]Nehad K. Hussein, [2]Enas Alsayyad, [1]Maha M. Kotb, [1]Rania Y. Shash

[1]Department of Medical Microbiology and Immunology, Faculty of Medicine, Cairo University, Egypt
[2]Department of Neurology, Faculty of Medicine, Cairo University, Egypt

Key words:
Autophagy, ATG5, EBNA1 IgG, EBV, MS

Corresponding Author:
Dr. Mona Gamal Nada
Department of Medical Microbiology & Immunology, Faculty of Medicine, Cairo University, Egypt
Tel.: +201223568853
monagnada@gmail.com
mona.nada@kasralainy.edu.eg

ABSTRACT

Background: *Epstein-Barr virus (EBV), a common human lymphotropic herpes virus infecting over 95% of the overall individuals. Strong evidence from recent research that EBV is a contributing factor in multiple sclerosis (MS). The exact mechanism by which EBV might exacerbate MS in risk groups is unknown. Objectives: To determine plasma levels of Autophagy-related proteins 5 (ATG5) and Epstein-Barr virus nuclear antigen1 (EBNA1) IgG. Additionally, assessing EBV DNA load in MS patients in relation to disease activity. Also, studying the relation between EBV DNA burden and the plasma levels of ATG5 and EBNA1 IgG in MS patients.* ***Methodology:*** *The study was conducted on 23 relapsing remitting MS (RRMS) patients in active attack, 23 RRMS patients' in-between attacks and 23 healthy controls. Enzyme-linked immunosorbent assay (ELISA) method was used to measure the amount of ATG5 and EBNA1 IgG in plasma. Real-time polymerase chain reaction (real-time PCR) was used to assess the EBV DNA load in peripheral blood mononuclear cells (PBMCs).* ***Results:*** *No statistically significant difference in ATG5 or EBNA1 IgG level between MS patients and healthy controls. However, all MS patients (100%) were EBNA1 IgG positive while 91.3% of the control group were EBNA1 IgG positive. EBV DNA load did not show statistically significant difference between MS patients and healthy controls. Significant positive correlation between EBV DNA load and ATG5 plasma level in MS patients in between attacks, P value =0.019.* ***Conclusion:*** *ATG5 levels and EBV DNA load could be utilized to predict disease-related activities in predisposed individuals or MS patients.*

INTRODUCTION

MS is a neurodegenerative, inflammatory, and demyelinating disease of the central nervous system (CNS). It is a heterogeneous, multi-factorial, immune-related illness caused by interplay of environmental and genetic interactions. MS is characterized by a number of demyelinating white and grey matter lesions in the brain and spinal cord. [1].

The exact cause of MS is uncertain; however, Multiple sclerosis is a disease with multiple causes. It seems to be a mix of a non-genetic trigger (environment, metabolism, or virus) and genetic predisposition that results in an autoimmune illness that causes recurring immune responses in the central nervous system. Significantly, infectious agents have been proposed to contribute in the development of MS disease. EBV has been strongly considered a triggering infectious factor for MS and it likely to be implicated throughout the clinical range of MS., including early pediatric-onset MS, relapsing remitting MS (RRMS), and primary progressive MS (PPMS), in addition those with both mild and severe illness courses[2,3]. However, a genetic predisposition and environmental factors like smoking or a lack of vitamin D must be present in addition to an EBV infection for MS to occur. [4].

According to Morandi et al.[5] EBV triggers the presentation of autoantigens by modulating the processing of myelin through autophagy. From yeast to mammals, autophagy is a largely conserved homeostatic mechanism. A Greek word meaning "self-eating". Autophagy is thought to be the controlled cellular breakdown of specific intracellular substances and organelles. First, the undesirable cytoplasmic contents are engulfed, and then lysosomal fusion and destruction take place. Mammals often have baseline (constitutive) autophagy under physiological conditions, which can be heightened by hunger or a number of illnesses, such as oxidative, toxic, immunological, and ischaemic insults[6,7].

The ATG proteins play a crucial role in the autophagy mechanism. ATG5 is essential for the creation of autophagic vesicles, and at least 41 more ATG genes have been found. Autophagy can be downregulated when ATG5 is knocked down, indicating that ATG5 is a key player in autophagy and is

frequently targeted in autophagy gene editing experiments[6].

Autophagic processes are deregulated in multiple sclerosis. In RRMS, the autophagy response is markedly elevated, and increased autophagy rate may contribute to the pathophysiology of MS [8]. The ATG5 level also correlates positively with clinical severity of EAE, suggesting a possible role of ATG5 in inflammatory demyelination[9]. Compared to individuals with quiescent RRMS, those with active RRMS have higher levels of ATG5 mRNA. Strong ATG5 immunoreactivity is detected in SPMS patients' postmortem brain tissue [9]. According to Castellazzi et al. [10] serum concentrations of ATG5 have been more elevated in active disease MS patients than in non-active MS patients. There is not much evidence linking ATG5 to multiple sclerosis. Increased ATG5 levels in MS animal models and postmortem brain tissue may not be enough to determine how ATG5 contributes to the pathophysiology of MS [6].

Host cells employ autophagy as a potent defence mechanism against viral infection. In particular, autophagy selectively breaks down immunological components linked to virus particles and triggers an innate immune response by collaborating with pattern recognition receptor signalling to produce interferon [11]. Additionally, autophagy facilitates adaptive immunity by transporting antigens produced from viruses to T cells. Nevertheless, viruses have developed the powerful capacity to employ autophagy to their advantage [11]. Many studies have been conducted on the interaction between EBV and autophagy in B cells. The EBV proteins, including EBNA1, EBNA3C, latent membrane protein (LMP) 1 and The regulation of autophagy start, progression, and completion for EBV lytic reactivation, viral particle production, and release has been linked to LMP2A [12]. Autophagy proteins, especially ATG5, are required for EBV reactivation. Accordingly, autophagy might play role as a link between EBV and MS [12].

We propose that EBV and autophagy through ATG5 might interplay together for MS development and disease activity. This could be a key for clarifying EBV role in MS development that might be a new hope for prevention and treatment of MS.

METHODOLOGY

This study was conducted as a case control performed between July 2022 to December 2023. A total of 23 RRMS patients in active attack, 23 RRMS patients in between attacks as well as 23 healthy individuals matched for age and gender who were considered as the control group. The 23 RRMS patients in active attack were admitted to the Neurology Department, Cairo University Hospitals, while the 23 RRMS patients in between attacks were attending the outpatient MS clinic. The purpose of the study was explained to each participant and written consent was obtained prior to the study. Approval for this research was obtained from the Research Ethics Committee of the Institutional Review Board, (Code: MD-1692022) Faculty of Medicine, Cairo University.

Inclusion criteria:

- Patients between the age of 18 and 60 years, who fulfill the 2017 revised McDonald criteria for the diagnosis of multiple sclerosis.
- MS patients in active attack before starting immunosuppressive or disease-modifying treatment for the attack.
- MS patients in active attack, either first attack or in-between attacks under maintenance treatment.
- MS patients in-between attack, either complaint to maintenance treatment or not.

Exclusion criteria:

- Age younger than 18 or older than 60 years
- Malignancy
- Pregnancy
- Other autoimmune diseases

All the included cases and healthy controls were subjected to:

Sampling: A volume of 5 ml of peripheral venous blood was withdrawn from all patients and controls and collected in vacuum tubes with ethylenediamine tetra acetic acid dipotassium (K2EDTA) as an anticoagulant by clean venipuncture under complete aseptic conditions. Each tube was labeled with the patient's or control's name and date of collection.

Separation of PBMCs and plasma: PBMCs were isolated from blood samples using Ficoll-Hypaque density gradient centrifugation .The upper layer (plasma) was first collected and stored at -20°C while the layer of mononuclear cells (buffy coat) was collected and stored at -80°C.

Quantitative detection of ATG5 protein plasma level by ELISA using Human ATG5 ELISA Kit **(SinoGeneClon Biotech Co.,Ltd)** according to the manufacturer's instructions.

Quantitative detection of EBNA1 IgG plasma level using SERION ELISA *classic* kit **(Institut Virion\Serion GmbH, Würzburg, Germany).**

Extraction of total DNA from PBMCs sample was performed, using QIAamp® DNA blood Mini kit and protocol according to the manufacturer's instructions **(Qiagen, Valencia, CA, USA).**

Amplification and quantification of EBV DNA by real-time PCR by using the Artus EBV Rotor-Gene (RG) PCR Kit, (ready-to-use system). The EBV RG Master contains reagents and enzymes for the specific amplification of a 97 bp region of the EBV genome, and for the direct detection of the specific amplicon in fluorescence channel Cycling Green of the Rotor-Gene Q MDx, or Rotor-Gene Q, or Rotor-Gene 6000, or

Cycling A.FAM™ of the Rotor Gene 3000 **(Qiagen, Valencia, CA, USA).**

Laboratory tests were performed at the Medical Microbiology and Immunology Department, Faculty of Medicine, Cairo University.

Statistical analysis:

Data were analyzed using the enclosed quantitation standards (EBV RG QS 1–4) were treated as previously purified samples and the same volume was used (20 µL). To generate a standard curve on Rotor-Gene Q Instruments, all 4 quantitation standards were used and defined in the "Edit Samples" dialog box as standards with the specified concentrations. The quantitation standards are defined as copies/µL. The following equation was applied to convert the values determined using the standard curve into copies/ml of sample material:

$$Result\ (copies/ml) = \frac{\text{Result (copies/µL) x Elution Volume (µL)}}{\text{Sample Volume (ml)}}$$

(result in µL is the result of the sample that measured according to standard curve, while result in ml is the estimated result of viral copies in one ml of the sample)

RESULTS

Detection of ATG5 protein plasma level:

Plasma level of ATG5 protein was positive in only 2 patients out of 23 of MS patients in active attack (8.7%) and positive in only one patient out of 23 of MS patients in between attacks (4.3%) and another one individual out of 23 healthy controls (4.3%) with no statistically significant difference (P value = 1) as shown in table 1 and figure 1.

Table 1: ATG5 detection among MS patients and healthy controls

	ATG5 detection		P value
	Negative No. (%)	Positive No. (%)	
MS patients in active attack	21 (91.3%)	2 (8.7%)	1.000
MS patients in between attacks	22 (95.7%)	1 (4.3%)	
Healthy controls	22 (95.7%)	1 (4.3%)	

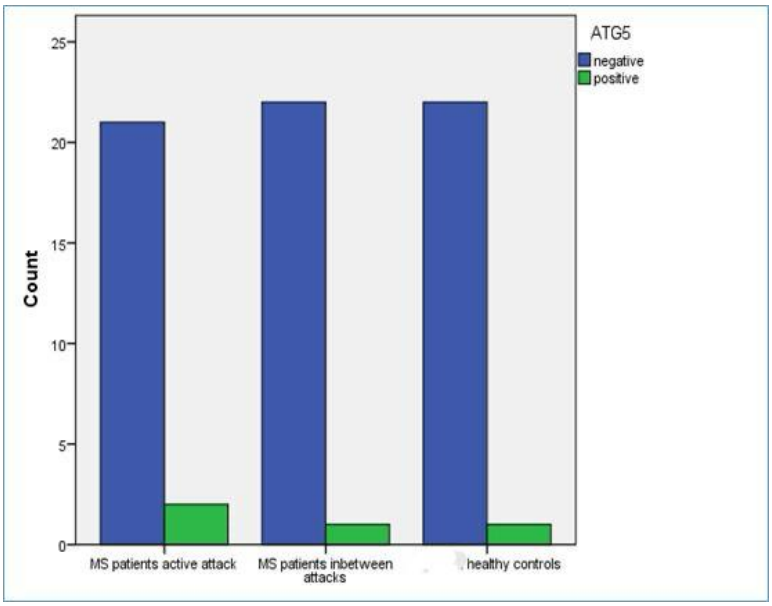

Fig. 1: ATG5 detection among MS patients and healthy controls

Detection of EBNA1 IgG plasma level:

The results showed that 100% of MS patients (in active attack and in-between attacks) were positive for EBNA1 IgG, indicating that all of them have been previously infected with EBV, and only two healthy controls were negative (8.7%) as shown in table 2 and figure 2.

Table 2: EBNA1 IgG detection in MS patients and healthy controls

	EBNA 1 IgG detection		P value
	Negative No. (%)	Positive No. (%)	
MS patients in active attack	0 (0.0%)	23 (100.0%)	0.324
MS patients in between attacks	0 (0.0%)	23 (100.0%)	
Healthy controls	2 (8.7%)	21 (91.3%)	

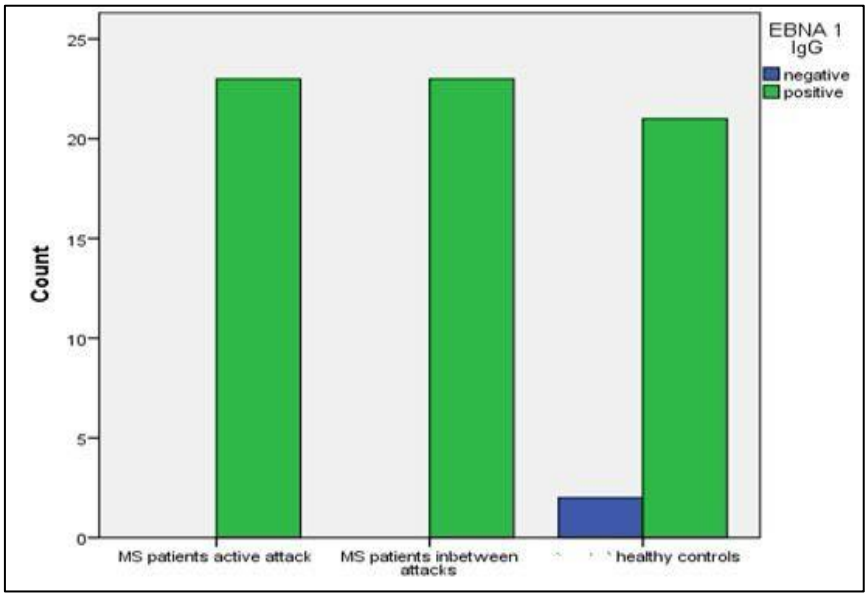

Fig. 2: EBNA 1 IgG detection in MS patients and healthy controls

EBNA1 IgG plasma level showed slight increase in MS patients (in active attack, mean = 11.966 and SD ± 3.005) (in between attacks mean = 11.751 and SD ± 2.532) than healthy control group (mean = 9.931 and SD±3.910). There was no statistically significant difference in EBNA1 IgG level between both MS patients and healthy controls (P value = 0.078) as shown in table 3.

Table 3: EBNA 1 IgG plasma level in MS patients and healthy controls

	No.	EBNA 1 IgG plasma levels (U/ml)		P value
		Mean	SD	
MS patients in active attack	23	11.966	3.005	
MS patients in between attacks	23	11.751	2.532	
Healthy controls	21	9.931	3.910	0.078

Detection of EBV DNA load:

The results showed that 34.8% of each of MS patients in active attack and of healthy controls were positive for EBV DNA, while only 17.4% of MS patients in between attacks were positive for EBV DNA with no statistically significant difference (P value = 0.324) as shown in table 4 and figure 3. All EBV DNA positive samples were positive for EBNA1 IgG.

Table 4: EBV DNA detection in MS patients and healthy controls

	EBV DNA detection		P value
	Negative No. (%)	Positive No. (%)	
MS patients in active attack	15 (65.2%)	8 (34.8%)	
MS patients in between attacks	19 (82.6%)	4 (17.4%)	0.324
Healthy controls	15 (65.2%)	8 (34.8%)	

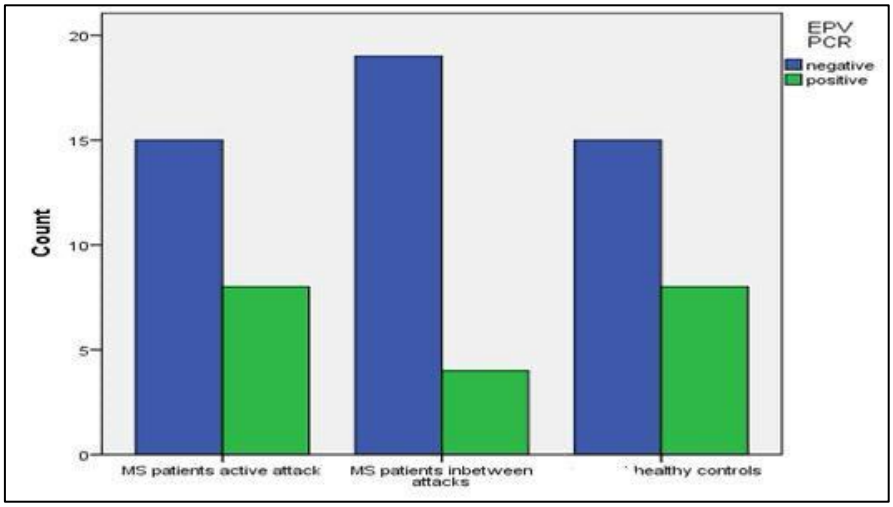

Fig. 3: EBV DNA detection in MS patients and healthy controls

The results showed no statistically significant difference in EBV DNA load in both MS patients and healthy controls (P value = 0.580) (Table 5). Positive samples among both MS patients and healthy controls were demonstrated in real time PCR curves as shown in figure 4 and 5.

Table 5: EBV DNA load in MS patients and healthy controls

	No.	EBV DNA load (copies/ ml)		P value
		Mean	SD	
MS patients in active attack	8	4472.500	6531.5558	0.580
MS patients in between attacks	4	1610.250	1808.4508	
Healthy controls	8	2863.750	2741.0795	

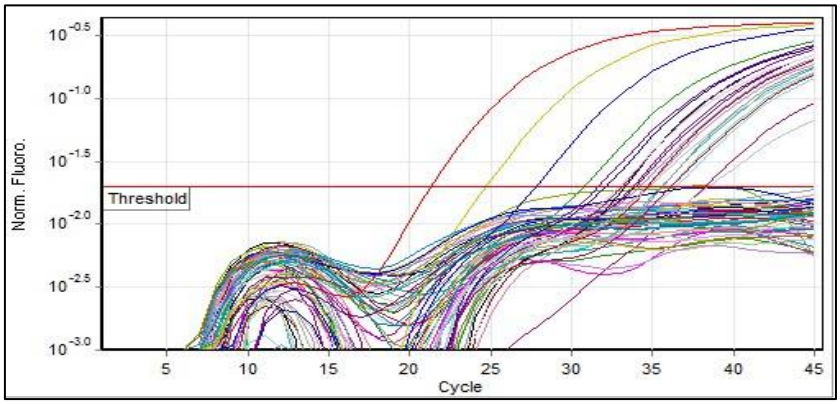

Fig. 4: EBV DNA load in MS patients and healthy controls

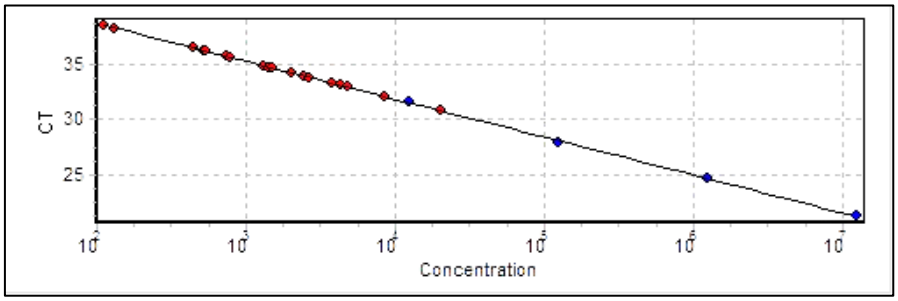

Fig. 5: Standard curve of EBV DNA PCR showing EBV DNA positive samples in both MS patients and healthy controls

Correlation between EBV DNA load, ATG5 and EBNA1 IgG plasma levels in MS patients and healthy controls:

Our results showed that 8 MS patients in active attack positive for EBV DNA, all of them were positive for EBNA1 IgG while one of them was positive for ATG5. In other hands, only 4 MS patients in between attacks were positive for EBV DNA, all of them were positive for EBNA1 IgG and one of them was positive for ATG5. In healthy controls 8 of them were positive for EBV DNA, all of them were positive for EBNA1 IgG while one of them was positive for ATG5.

Through studying the correlation between EBV DNA load, ATG5 plasma level, and EBNA1 IgG plasma level in MS patients, both in active attack and in-between attacks, and healthy controls (Tables 6 and 7), there were no statistically significant correlations except for only one correlation; A statistically significant positive correlation was between EBV DNA load and ATG5 plasma level in MS patients in between attacks (P value = 0.019, r = 0.486) as shown in table 7 and figure 6.

Table 6: Correlation between EBV DNA load, ATG5 and EBNA1 IgG plasma levels in MS patients in active attack

		MS patients in active attack		
		ATG5 plasma level	EBNA 1 IgG plasma level	**EBV DNA load**
ATG5 plasma level	Correlation Coefficient	1.000	-0.192-	0.152
	P value		0.380	0.489
EBNA 1 IgG plasma level	Correlation Coefficient	-0.192-	1.000	-0.058-
	P value	0.380		0.792

Table 7: Correlation between EBV DNA load, ATG5 and EBNA1 IgG plasma levels in MS patients in between attacks

		MS patients in between attacks		
		ATG5 plasma level	EBNA 1 IgG plasma level	**EBV DNA load**
ATG5 plasma level	Correlation Coefficient	1.000	-0.321-	**0.486**
	P value		0.135	**0.019**
EBNA 1 IgG plasma level	Correlation Coefficient	-0.321-	1.000	0.151
	P value	0.135		0.492

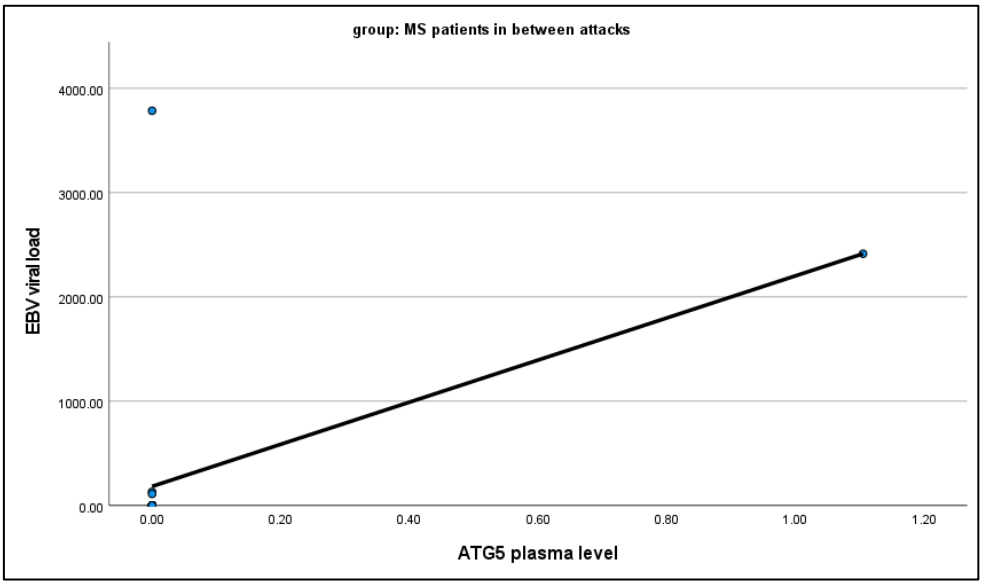

Fig. 6: Correlation between EBV DNA load and ATG5 plasma level in MS patients in between attacks

DISCUSSION

It's still difficult to pinpoint the exact processes by which EBV contributes to the development of MS. Targeting the pathways through which EBV is believed to contribute to the pathogenesis of MS offers a chance to develop novel medications for the treatment of MS [2].

Autophagy's significance in autoimmune illnesses, particularly MS, has been controversial recently. Autophagy has the potential to be both advantageous and detrimental to MS neuropathology, which makes it a double-edged sword in the disease's progression [13].

In the present study, we hypothesized that there might be a correlation between EBV and ATG5 in MS pathogenesis. Our study included 23 MS patients in active attack, 23 MS patients in between attacks, and 23 healthy controls. All groups were investigated for ATG5 and EBNA1 IgG plasma levels using ELISA as well as for estimating EBV DNA load in PBMCs utilising real time-PCR.

Our study showed that two MS patients in active attack (8.7%) were positive for ATG5 versus only one positive patient among MS patients in between attacks (4.3%). In addition, one healthy control was positive among the control group (4.3%), however, these findings were not statistically significant.

To our knowledge, the present study is one of the earliest studies conducted to detect plasma level of ATG5 in MS patients. Previous studies were conducted to detect ATG5 level in serum, Serum levels of ATG5 were considerably greater in RRMS patients (who were not taking immunosuppressive medications) than in the healthy control group, according to Joodi Khanghah et al.[14]. Similarly, Castellazzi et al.[10] demonstrated the elevation of ATG5 levels in both serum and CSF in RRMS patients in active attack than MS patients in between attacks. Hassanpour et al.[8] also reported that levels of ATG5 were significantly elevated in both serum and CSF of MS patients than healthy control group.

Other studies evaluated ATG5 level by mRNA expression, Paunovic et al.[15] reported that ATG5 mRNA expression in PBMCs showed no significant difference between MS patients who were treatment-naïve and the healthy control group. However, Safa et al.[16] reported the absence of statistically significant difference in ATG5 mRNA expression blood levels between RRMS patients in remission and the healthy control group.

On the experimental level, Alirezaei et al.[9] observed a significant increase in the level of the ATG5-ATG12 complex in EAE mice, but cleaved ATG5 levels were lower than in control mice. Srimat Kandadai et al.[16] reported that the lack of ATG5 mRNA expression in microglia had no impact on the development of EAE in mice.

Results variations of different studies might be explained by different methods of ATG5 detection as well as different forms of ATG5 [9, 14], Moreover, it might be affected by the type of the sample taken whether serum, CSF, PBMCs or T cells [15, 10]. Interestingly, it was found that high ATG5 level is also associated with other neurological diseases. Han et al.[17] found that early onset Parkinson's disease patients had considerably greater plasma ATG5 levels than the control group.

In the current study, EBNA1 IgG was measured in plasma and found to be positive in 100% of MS patients (in active attack and in-between attacks) and in 91.3% of the healthy control group with no statistically significant difference. Similar results were demonstrated in a study conducted by Awwad et al. [18] who compared the seropositivity to EBNA1 IgG in MS patients and healthy controls and stated no significant differences. According to Kreft et al.[19] only 85% of healthy controls and 98.2% of MS patients revealed positive serum levels of EBNA1 IgG.

In the current study, the EBNA1 IgG plasma level showed a slight increase in MS patients However, no statistically significant difference reported. Additionally, Alemam and Maleek[20] discovered that the MS patients' group had a greater EBNA1-IgG level than the control group, While, Gieß et al.[21] and Agostini et al.[22] reported that EBNA1 IgG level was statistically significantly high in MS patients than in the healthy controls. The difference in findings could be attributed to the variability in EBNA1 IgG level in MS patients according to genetic variations in HLA [19].

In the present research, EBV DNA was found in PBMCs by real time -PCR in MS patients and healthy controls. According to our findings, EBV DNA was positive in 34.8% of MS patients in active attack with similar results observed in healthy controls, while only 17.4% of MS patients in-between attacks were EBV DNA positive with no statistically significant difference.

In accordance, Agostini et al.[23] study reported that EBV DNA from whole blood was more in MS patients (33%) compared to healthy controls (28%) with no statistically significant difference. In contrast, Agostini et al.[22] detected that EBV DNA in whole blood was statistically significantly elevated in MS patients than in healthy controls.

In the current study, EBV DNA load in PBMCs was measured in both MS patients as well as healthy controls. Our findings showed no statistically significant difference between MS patients and healthy controls (P value = 0.580). In accordance, Agostini et al.[23] study showed the similarity of whole blood EBV DNA load in both MS patients and healthy controls . In contrast, Soldan et al.[24] reported that EBV replication was higher in spontaneous lymphoblastoid cells derived from MS patients with an attack than in MS patients with stable disease or healthy controls.The reported differences might be due to the lack of standardization in EBV viral load assays. Many limitations in assays of EBV viral load includes: their variability, differentiating EBV

reactivation from periodic virus shedding is non specific, absence of standardization , the units of estimation for viral load, and the best samples used for DNA testing [25, 26].

However, it is less evident how EBV infection contributes to the onset of neurodegeneration and the development of MS [27]. There have been reports of a several-year delay between the start of clinical MS and the underlying EBV infection[28], due to the asymptomatic phases of MS[29], characterized by increased biomarker levels of neuro-axonal damage before the occurrence of the first neurological symptoms[30]. Accordingly, virus latency might be the major contributor to MS development. Also, spontaneous virus reactivation might act as a trigger for a new MS attack [31].

Through studying the correlations between ATG5 plasma level, EBNA1 IgG plasma level and EBV DNA load in MS patients and healthy controls, there were no statistically significant correlations between those parameters except for one significant positive correlation which was reported between EBV DNA load and ATG5 plasma level in MS patients in between attacks, P value = 0.025, r = 0.465.

The positive correlation between ATG5 plasma level and EBV DNA load in MS patients in between attacks could be attributed to that both ATG5 level and EBV DNA load might increase in MS patients just before the development of the attack [31].

CONCLUSION & RECOMMENDATIONS

The plasma level of ATG5 and EBNA1 IgG between MS patients and healthy controls showed no statistically significant difference. Additionally, EBV DNA load in PBMCs doesn't show statistically significant difference between MS patients and healthy controls. A significant positive correlation shown between EBV DNA load and ATG5 in MS patients in-between attacks. More research is required to determine how ATG5 contributes to the development of MS. Evaluation of the preferred specimen, method for detection, and form of ATG5 in MS patients and studying EBNA1 IgG level in relation to different MS risk factors especially genetic factors. Additional clinical investigations are required to assess the role of measuring the EBV DNA load and ATG5 plasma level in predicting MS and disease activity in high risk population. Also, raising the possibility that EBV-directed vaccinations could help the younger generation.

Conflict of Interest:

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media.

Funding: The study was funded by Cairo University, General Scientific Research Department.

REFERENCES

1. Filippi M, Bar-Or A, Piehl F, Preziosa P, Solari A, Vukusic S, Rocca MA. Multiple sclerosis. Nat Rev Dis Primers. 2018 Nov 8;4(1):43. doi: 10.1038/s41572-018-0041-4. Erratum in: Nat Rev Dis Primers. 2018; 22;4(1):49.
2. Du Toit A. EBV linked to multiple sclerosis. Nat Rev Microbiol. 2022; 20(4):189.
3. Bar-Or A, Pender MP, Khanna R, Steinman L, Hartung HP, Maniar T, Croze E, Aftab BT, Giovannoni G, Joshi MA. Epstein-Barr virus in Multiple Sclerosis: Theory and Emerging Immunotherapies. Trends Mol Med. 2020; 26(3):296-310. Erratum in: Trends Mol Med. 202;27(4):410-411.
4. Robinson WH, Steinman L. Epstein-Barr virus and multiple sclerosis. Science. 2022; 21;375(6578):264-265.
5. Morandi E, Jagessar SA, 't Hart BA, Gran B. EBV Infection Empowers Human B Cells for Autoimmunity: Role of Autophagy and Relevance to Multiple Sclerosis. J Immunol. 2017; 15; 199(2):435-448.
6. Ye X, Zhou XJ, Zhang H. Exploring the Role of Autophagy-Related Gene 5 (ATG5) Yields Important Insights Into Autophagy in Autoimmune/Autoinflammatory Diseases. Front Immunol. 2018; 17;9:2334.
7. Misrielal C, Mauthe M, Reggiori F, Eggen BJL. Autophagy in Multiple Sclerosis: Two Sides of the Same Coin. Front Cell Neurosci. 2020; 20;14:603710.
8. Hassanpour M, Cheraghi O, Laghusi D, Nouri M, Panahi Y. The relationship between ANT1 and NFL with autophagy and mitophagy markers in patients with multiple sclerosis. J Clin Neurosci. 2020;78:307-312.
9. Alirezaei M, Fox HS, Flynn CT, Moore CS, Hebb AL, Frausto RF, Bhan V, Kiosses WB, Whitton JL, Robertson GS, Crocker SJ. Elevated ATG5 expression in autoimmune demyelination and multiple sclerosis. Autophagy. 2009;5(2):152-8.
10. Castellazzi M, Patergnani S, Donadio M, Giorgi C, Bonora M, Fainardi E, Casetta I, Granieri E, Pugliatti M, Pinton P. Correlation between auto/mitophagic processes and magnetic resonance imaging activity in multiple sclerosis patients. J Neuroinflammation. 2019; 27;16(1):131.
11. Choi Y, Bowman JW, Jung JU. Autophagy during viral infection - a double-edged sword. Nat Rev Microbiol. 2018;16(6):341-354.
12. Yiu SPT, Hui KF, Münz C, Lo KW, Tsao SW, Kao RYT, Yang D, Chiang AKS. Autophagy-Dependent

Reactivation of Epstein-Barr virus Lytic Cycle and Combinatorial Effects of Autophagy-Dependent and Independent Lytic Inducers in Nasopharyngeal Carcinoma. Cancers (Basel). 2019; 26;11(12):1871.

13. Al-Kuraishy HM, Jabir MS, Al-Gareeb AI, Saad HM, Batiha GE, Klionsky DJ. The beneficial role of autophagy in multiple sclerosis: Yes or No? Autophagy. 2024;20(2):259-274.
14. Joodi Khanghah O, Nourazarian A, Khaki-Khatibi F, Nikanfar M, Laghousi D, Vatankhah AM, Moharami S. Evaluation of the Diagnostic and Predictive Value of Serum Levels of ANT1, ATG5, and Parkin in Multiple Sclerosis. Clin Neurol Neurosurg. 2020;197:106197.
15. Paunovic V, Petrovic IV, Milenkovic M, Janjetovic K, Pravica V, Dujmovic I, Milosevic E, Martinovic V, Mesaros S, Drulovic J, Trajkovic V. Autophagy-independent increase of ATG5 expression in T cells of multiple sclerosis patients. J Neuroimmunol. 2018; 15;319:100-105.
16. Srimat Kandadai K, Kotur MB, Dokalis N, Amrein I, Keller CW, Münz C, Wolfer D, Prinz M, Lünemann JD. ATG5 in microglia does not contribute vitally to autoimmune neuroinflammation in mice. Autophagy. 2021; 17(11):3566-3576.
17. Han J, Feng G, Wu J, Zhang Y, Long Z, Yao X. Association of ATG5 gene polymorphism with Parkinson's disease in a Han Chinese population. Acta Neurol Belg. 2022;122(4):1049-1056.
18. Awwad AM, Hanafi NF, Achmawi GA, Naguib AM. Epstein-Barr Virus Infection in Multiple Sclerosis Patients. Egypt J Immunol. 2017;24(1):49-55.
19. Kreft KL, Van Nierop GP, Scherbeijn SMJ, Janssen M, Verjans GMGM, Hintzen RQ. Elevated EBNA-1 IgG in MS is associated with genetic MS risk variants. Neurol Neuroimmunol Neuroinflamm. 2017; 12;4(6):e406.
20. Alemam A, and Maleek M. Is Epstein-Barr virus a risk factor for multiple sclerosis?. J. Neurol. Res. 2018; *8*(3), 19–25.
21. Gieß RM, Pfuhl C, Behrens JR, Rasche L, Freitag E, Khalighy N, Otto C, Wuerfel J, Brandt AU, Hofmann J, Eberspächer B, Bellmann-Strobl J, Paul F, and Ruprecht K. Epstein-Barr virus antibodies in serum and DNA load in saliva are not associated with radiological or clinical disease activity in patients with early multiple sclerosis. PloS one, 2017; 12(4), e0175279.
22. Agostini S, Mancuso R, Guerini FR, D'Alfonso S, Agliardi C, Hernis A, Zanzottera M, Barizzone N, Leone MA, Caputo D, Rovaris M, Clerici M. HLA alleles modulate EBV viral load in multiple sclerosis. J Transl Med. 2018; 27;16(1):80.
23. Agostini S, Mancuso R, Caputo D, Rovaris M, Clerici M. EBV and multiple sclerosis: expression of LMP2A in MS patients. Front Neurosci. 2024; 24;18:1385233.
24. Soldan SS, Su C, Monaco MC, Yoon L, Kannan T, Zankharia U, Patel RJ, Dheekollu J, Vladimirova O, Dowling JW, Thebault S, Brown N, Clauze A, Andrada F, Feder A, Planet PJ, Kossenkov A, Schäffer DE, Ohayon J, Auslander N, Jacobson S, Lieberman PM. Multiple sclerosis patient-derived spontaneous B cells have distinct EBV and host gene expression profiles in active disease. Nat Microbiol. 2024;9(6):1540-1554.
25. AbuSalah MAH, Gan SH, Al-Hatamleh MAI, Irekeola AA, Shueb RH, Yean Yean C. Recent Advances in Diagnostic Approaches for Epstein-Barr Virus. Pathogens. 2020; 18;9(3):226.
26. Maple PAC, Gran B, Tanasescu R, Pritchard DI, Constantinescu CS. An Absence of Epstein-Barr Virus Reactivation and Associations with Disease Activity in People with Multiple Sclerosis Undergoing Therapeutic Hookworm Vaccination. Vaccines (Basel). 2020; 28;8(3):487.
27. Ortega-Hernandez OD, Martínez-Cáceres EM, Presas-Rodríguez S, Ramo-Tello C. Epstein-Barr Virus and Multiple Sclerosis: A Convoluted Interaction and the Opportunity to Unravel Predictive Biomarkers. Int J Mol Sci. 2023; 17;24(8):7407.
28. Bjornevik K, Cortese M, Healy BC, Kuhle J, Mina MJ, Leng Y, Elledge SJ, Niebuhr DW, Scher AI, Munger KL, Ascherio A. Longitudinal analysis reveals high prevalence of Epstein-Barr virus associated with multiple sclerosis. Science. 2022; 21;375(6578):296-301.
29. Makhani N, Tremlett H. The multiple sclerosis prodrome. Nat Rev Neurol. 2021;17(8):515-521.
30. Bjornevik K, Münz C, Cohen JI, Ascherio A. Epstein-Barr virus as a leading cause of multiple sclerosis: mechanisms and implications. Nat Rev Neurol. 2023;19(3):160-171.
31. Soldan SS, Lieberman PM. Epstein-Barr virus and multiple sclerosis. Nat Rev Microbiol. 2023; 21(1):51-64.