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Exploration of IL-32 (rs45499297) Gene Variations in Epstein-Barr virus and Multiple Sclerosis Patients in Iraq

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Abstract

Interleukin-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 (MS). Studies about found relationship between them are rare and inconclusive, especially when MS patients with Epstein-Barr virus (EBV) positive. Recently, IL-32 identified increase and considers proinflammatory after EBV infection. A case-control study (79 MS patients and 108 controls) for the purpose of investigating T/C genotype of (rs45499297) in all participants then identify their role in MS and EBV patients by Restriction fragment length polymorphism (RFLP) technique. The results appeared that alleles and genotype (rs45499297) was no significant differences between MS patients and healthy controls (HC), also between groups of expanded disability status scale (EDSS) in MS patients but with EBV infection was results appeared that allele C of (rs45499297) more effector in MS patients than controls. The mean of EBV load among multiple sclerosis patients according to sex, EDSS, and therapy line the results revealed that elevated viral load in male, EDSS ≥ 3.0 and in second line therapy compared to opposite groups. In conclusion, the study indicated the role of sex and MS activity in susceptibility to EBV reactivation. However, analysis of the IL-32 gene variants (rs45499297) may influence susceptibility to EBV along with MS associated with the allele C occurrence.

Keywords: Multiple sclerosis, Herpes viruses, Interleukin- 32 gene polymorphism, Restriction fragment length polymorphism, Line of therapy.

اكتشاف التغاير الجيني ل انترلوكين -٣٢ (rs45499297)لمرضى ابشتاين بار فايروس والتصلب المتعدد

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الخلاصة

انترلوكين ٣٢ هو سايتوكين متعدد الوظائف مرتبط بمجموعة من الامراض والاضطرابات الالتهابية، ويتأثر الجهاز العصبي المركزي بمرض الالتهاب المزمن المعروف بأسم التصلب المتعدد. الدراسات حول وجود علاقة بينهما نادرة وغير حاسمة خاصة عندما يكون مرضى التصلب المتعدد ايجابياً مع ابشتاين بار فيروس. ابشتاين بار فيروس هو فيروس الهربس الذي يسبب عدوى كامنة، حالياً شخصت زيادة في انترلوكين ٣٢ الذي يعتبر محرض التهابي يلي الاصابة بالفايروس. دراسة الحالات والشواهد (٧٩ مريضاً بمرض التصلب العصبي المتعدد و١٠٨ من الاشخاص السليمين) بغرض فحص النمط الجيني T/C ل(rs45499297) في جميع المشاركين، ثم حدد دورهم في مرضى التصلب المتعدد وابشتاين بار فايروس. اظهرت النتائج ان الاليلات والنمط الجيني (rs45499297) لم يكن هناك فروق ذات دلالة احصائية بين مرضى التصلب المتعدد والاصحاء، وكذلك بين مجموعات مقياس حالة الاعاقة الموسعة في مرضى التصلب المتعدد، ولكن مع الإصابة ب ابشتاين بار فايروس ظهرت النتائج ان الاليلات C ل (rs45499297) اكثر فاعلية في مرضى التصلب المتعدد من السيطرة. متوسط حمل ابشتاين بار فيروس بين مرضى التصلب المتعدد حسب الجنس، EDSS وخط العلاج اظهرت النتائج ان الحمل الفيروسي مرتفع في الخط الثاني، عند الذكور، EDSS ≥ 3.0 مقارنة بالمجموعة المقابلة.

الكلمات المفتاحية: التصلب المتعدد، هربس فايروس، تعدد الاشكال الجيني ل انترلوكين ٣٢، تعدد شكل طول جزء الحصر، خط العلاج

Introduction

Multiple sclerosis (MS) is an autoimmune illness and most common neurological impairment that impact to central nervous system, frequently results in significant physical, cognitive incapacity and neurological issues in young people [(1)]. MS is thought to affect 2.8 million people globally; it is affects women more frequently than it does men about twofold [(2)]. MS marked by persistent inflammation, demyelination, gliosis, and loss of neurons. MS subtypes are thought to be significant for both prognosis and therapy choices are involve relapsing remitting MS (RRMS), primary progressive MS (PPMS), secondary progressive MS (SPMS), and progressive relapsing MS (PRMS) [(3)].

Although the exact cause of MS is unknown, it may be said to be a complex illness caused by both genetic predisposition and environmental factors. The current study found that Epstien-Barr virus is crucial herpes viruses that associated to MS disease [4], one of the most frequent DNA viruses identified in humans affects around 95% of the adult population worldwide [5]. B lymphocytes and nasopharyngeal epithelial cells are where it first reproduces [6]. EBV has a number of essential characteristics that allow it to infect B memory cells latently and avoid the immune response [7]. Similarities between self-antigens and self-antigens EBV proteins offer a reasonable and fascinating pathway for disease induction [6].

Interleukin-32 (IL-32) is proinflammatory cytokine, the human IL-32 gene spans about 1.2 kilobase pairs and is found on chromosome 16p13.3, eight short exons in the IL-32 gene, and nine alternative splice variants of the IL-32 messenger RNA [8].IL-32 expressed via natural killer (NK) cells, T-cells, monocytes, and epithelial cell lines [9]. Macrophage inflammatory protein-2 (MIP-2), different chemokines and inflammatory cytokines, including interleukin (IL)-1, IL-6, IL- 8, and tumor necrosis factor-alpha (TNF-alpha), are said to be induced and produced in part by interleukin (IL)-32[10], these cytokines involved in the synthesis of interleukin-32 in epithelial cells and monocytes. It regulates a wide variety of cellular processes, such as differentiation, cytokine production and apoptosis (programmed cell death), in the pathophysiology of several disorders is pleiotropic [11]. Multiple autoimmune disorders, including inflammatory bowel disease, rheumatoid arthritis have been associated to abnormal IL-32 production, and a recent research highlighted IL-32's role in the etiology of type 1 diabetes [12]. It is unclear how IL-32 contributes to the development of autoimmune disease, although it may amplify the effects of other pro-inflammatory cytokines that have a role in the illness [13]. These results provide support for the idea that IL-32 may play a role in the development of multiple sclerosis.IL-32 has several SNPs, some are associated with autoimmune diseases, due to their function in changing the ratio of pro- and anti-inflammatory response. The variant of IL-32 gene (rs45499297) examined in order to determine their function in MS disease [14]. According to the researcher's best estimates, only one research looked and found no link to the disease particularly as accompanied to EBV infection, which diagnosis by RT-PCR technique.

Aim of the study

Due to the lack of knowledge around host variables that affect MS genetic risks, we looked at the possible role of IL- 32 gene variants in illness susceptibility and clinical response. So, this research seeks to shed light on how single-nucleotide polymorphisms (rs45499297) possible link to MS risk, when this variant modifies IL-32 expression and hence affect MS susceptibility.

Material and Methods

Case-control study conducted on 79 MS patients and 108 healthy controls during period start from October 2022 to January 2023, the patients were gathered from Baghdad Medical City, Neurology Department. Utilizing EDSS to evaluate physical impairment and patients suffer from MS were split into two groups in`cludes: <3.0 and ≥ 3.0, this scale runs from 0 indicate a typical ambulatory state to 10 patients with total disability then death [15]. First-line (IFN beta 1-alpha) or second-line (fingolimod or natalizumab) treatment was being administered to all patients. Group of HC consisted of healthy blood donors and medical staff members without neurological or immunological diseases. Patients with autoimmune diseases other than (MS), chronic disease, and relative patients were excluded. The local ethics committee of the College of Science at Baghdad University gave its approval to the current study protocol and addition to study was approved by the ethics committee of the Iraqi Ministry of Health.

In an ethylene-diamine tetra-acetic acid (EDTA) tube, 3 mL of venous blood were drawn from patients while they were in the clinic. Following the directions provided by the manufacturer (Geneaid Biotech Ltd., Taiwan), DNA was extracted from blood contain EDTA using a gSYNC DNA extraction kit. Isolated DNA was subjected to RT-PCR analysis to detect EBV qualitatively (positive or negative) and quantitatively (viral load). This analysis was carried out by use the Real-TM Quant kit in accordance with manufacturer's instructions (Sacace Biotechnologies Srl, Italy).

Polymorphism IL-32 GENE

IL-32 SNP (rs45499297) gene were examined, polymerase chain reaction (PCR) analysis was performed on isolated DNA to identify SNPs of interest. The PCR was carried out in total volume of 25µL that include 5µL PCR PreMix (Bioneer, Korea) with 1 µL Forward primer (5′-GATTGCTGAGACCAGTGA-3′),1µLreverseprimer(5′TCTCTGAGCCCAGGAATG-3′) (16), 5µL template DNA, and 13µL nuclease free water. The tube was then placed in an Eppendorf thermocycler (Germany) with set up for the optimum conditions shown below: initial denaturation cycle (95◦C for 3 min.), then followed by 35 cycles involve denaturation (94◦C for 3 sec.), annealing (62°C for 30 sec.), elongation (72°C for 45 sec.), and final elongation cycle (72°C for 5 min.). Product of the PCR (5 µl) digested with 0.5 µl restriction enzyme BamH1 according to restriction fragment length polymorphism technique, then incubated for 20 min at 37 ◦C. After

that, the PCR products were digested electrophoresed in agarose gel 1.5% agarose gel in

TBE buffer (1x) at 5 volts per square centimeter for 55 minutes to see the bands migrate. Two alleles (T and C) were linked to three genotypes (CC, TT, and TC) visible on gel electrophoresis.

Statistical Analysis

Continuous variable was provided mean and standard deviation or median and interquartile range were assessed for significant differences using a Welch corrected t-test or Mann-Whitney U test. Categorical variables (number and percentage) were utilized to characterize categorical data, and the two-tailed Fisher exact test or Pearson's Chi-square test was employed to determine statistical significance. Hardy-Weinberg equilibrium (HWE) was evaluated using Pearson's Chi- square goodness-of-fit test. The odds ratio (OR) and 95% CI were determined using logistic regression analysis. It was determined to be statistically significant when probability (p) ≤0.05. These statistical studies used GraphPad Prism version 8.0.0 (San Diego, California, USA) and IBM SPSS Statistics 25.0 (Armonk, NY: IBM Corp.).

Results

Main Characteristic of Multiple Sclerosis Patients and Healthy Controls

Multiple sclerosis (MS) patients and healthy controls (HC) were characterized for age, sex groups. The patients were additionally characterized in terms of Expanded Disability Status Scale (EDSS) and therapy status.

The patients who participated in the current study were split into two age groups: 59 (74.7%) in the

≤ 40 years old and 20 (25.3%) in the age of >40 years, Age is a significant risk factor, according to the statistical study, which revealed very significant ($p < 0.0001$) differences between the age group of multiple sclerosis patients.

In terms of sex for patients showed male 34(43%) and female 45(57%), and the difference was not significant between the two groups ($p = 0.535$).

Two distinct subsets of MS patients were identified using the EDSS; 44(55.7%) in the state <3.0 and 35(44.3%) in the state ≥ 3.0 that show no significant differences between two groups ($p = 0.311$).

Patients' therapy included first and second lines. Most patients received first-line therapy 62 (78.5%), while the remaining 17(21.5%) received second-line therapy and with significant differences between two lines ($p <0.01$), as shown in Table (1).

Table 1. Main characteristic of multiple sclerosis patients against healthy controls

Characteristic		MS patient (n =79)	Healthy control (n=108)	*p*-value
Age; year		36 (28 – 41)	38 (28 – 49.8)	**< 0.0001**
Age group	≤ 40	59 (74.7%)	65 (60.2%)	**< 0.05**
	> 40	20 (25.3%)	43 (39.8%)	
	p-value	**< 0.0001**	**< 0.05**	
Sex	Male	34 (43%)	46 (42.6%)	$p = 0.535$
	Female	45 (57%)	62 (57.4%)	
EDSS group	< 3.0	44 (55.7%)	**NA**	$p = 0.311$
	≥ 3.0	35 (44.3%)		
Therapy	First-line	62 (78.5%)	**NA**	**< 0.01**
	Second-line	17 (21.5%)		

Median and interquartile range (for continuous data) or number and percentage (for categorial variable) are provided for ages; NA: Not applicable; *p*: probability of Mann-Whitney *U* test (to compare continues variables), two-tailed Fisher exact test or Pearson Chi-square test (to comparecategorical variables).

molecular analysis revealed that prevalence of Epstein-Barr virus (EBV) infection between male and female and age groups for all MS patients and HC significant difference ($p < 0.01$) as shown in Figure (1).

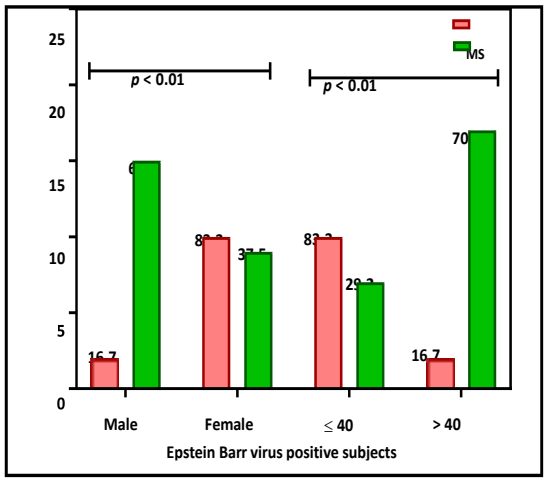

Figure 1. Epstein-Barr virus positive individuals with multiple sclerosis (MS) and healthy controls (HC) were dispersed by sex and age. Fisher's exact probability with two tails, abbreviated as p.

Although infections lowered in MS (12 infections) than 24 in HC, when compared to EBV-positive cases of HC, the mean EBV load in EBV-positive cases of MS was significantly higher (94.6 ± 84.2 vs. 35.87 ± 96.4 DNA copy/100 cells; p < 0.01). This indicates EBV is activated due to immunodeficiency and EBV infection in healthy control was latent as shown in Figure 2.

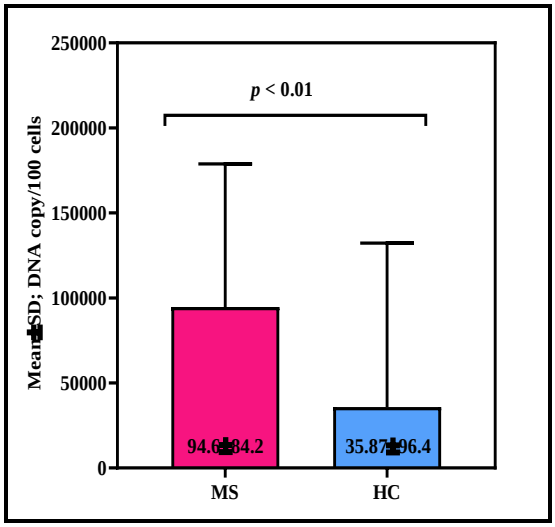

Figure 2. Welch's correction was used to the t-test probability to determine the mean viral load of the Epstein-Barr virus in patients with multiple sclerosis (MS) and healthy controls (HC).

There were no statistically significant variations in the median EBV load across the sexes, EDSS subgroups, or line treatment groups that contained EBV-positive MS patients.

In the case of MS sex, results obtained in the current study revealed that male higher EBV load compare to female, thus there was a statistically significant difference in the viral load (198.7 ± 114.5 male vs. 73.8 ± 65.7female DNA copy/100 cells; $p < 0.05$).

In the case of EDSS, when compared to EBV-positive MS patients with EDSS < 3.0, EBV-positive MS patients with EDSS ≥ 3.0 had higher EBV loads. (68.5 ± 48.8 vs. 120.8 ± 95.9 DNA copy/100 cells; $p = 0.303$) and although the correlation between EBV load and EDSS but the difference was not significant as shown in Figure (3).

Also demonstrates results that correlation between EBV load and line therapy of MS was not significant difference (84.9 ± 65.9 first line vs. 143.1 ± 23.6second line DNA copy/100 cells; $p = 0.272$) in spite was found to be elevated EBV load in second line compared to first line therapy.

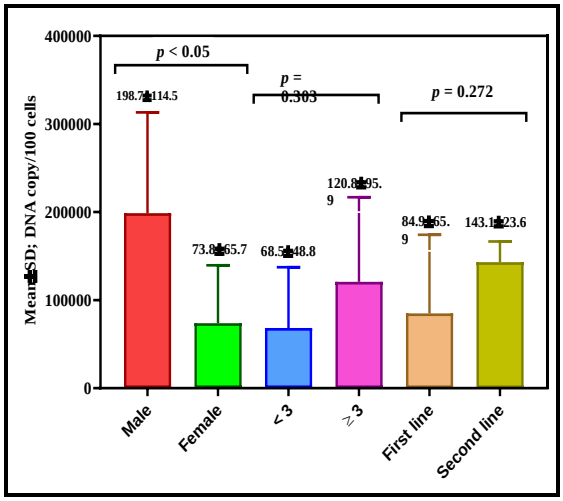

Figure 3. Epstein-Barr virus load in multiple sclerosis patients categorized by sex, age groups, therapy and expanded disability status scale (EDSS). p: Welch-corrected t-test probability.

The PCR product for the detection of SNP rs45499297 in IL-32 was amplified using conventional PCR technique, then the PCR product was digested with the restriction enzyme of BamH1, on gel electrophoresis three genotypes appeared (CC, TT, TC) that are associated by two alleles (T and C) as shown in Figure (4).

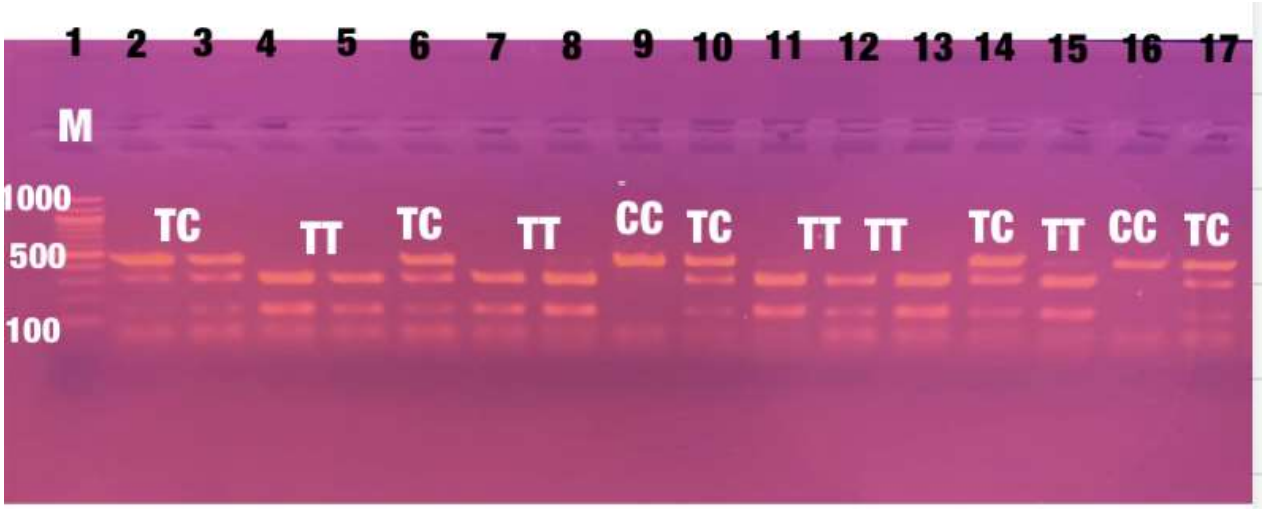

Figure 4. Gel electrophoresis of DNA-PCR amplified products (445 bp=CC/ 445,306,139 bp=TC/ 306,139bp=TT) for the SNP rs45499297 (T/C) on 1.5% agarose (5 volt/cm2 for 55 minutes) .Lanes2-17, lanes 1,2,5,9,13,16: TC, lanes 3,4,6,7,10,11,12,14: TT, lanes 8,15: CC. M: DNA Ladder (100 bp).

Analysis of Hardy-Weinberg equilibrium (HWE) revealed that genotype frequencies of SNP (rs45499297) in MS patients and control were in an agreement with the equilibrium as no significant differences, as shown in Table (2).

Table 2. Logistic regression and Hardy-Weinberg analyses of *IL-32* gene SNPs in multiplesclerosis patients and healthy controls

SNP	Allele/genotype	MS		HC		OR	95% CI	*p*-value
		N	%	N	%			
rs45499297 T/C	T C	126 32	79.75 20.25	175 41	81 19	eference1.08	0.65 -1.81	0.792
	TT	52	65.8	74	68.5	Reference		
	TC	22	27.8	27	25	0.86	0.45 -1.67	0.734
HWE-*p*-value	CC	5	6.3	7 0.06	6.5	0.98	0.31 -3.35	1.0

Multiple sclerosis (MS), Healthy Controls (HC), Single Nucleotide Polymorphism (SNP), Hardy-Weinberg Equilibrium (HWE), Odds Ratio (OR), Confidence Interval (CI), Two-Tailed Fisher's Exact Probability (p), and Probability (p)

In case of EDSS group, no significant difference of (rs45499297) IL-32 polymorphism frequency between EDSS patients' group was detected in this study.

Table 3. Multiple sclerosis patients' *IL-32* gene SNP allele and genotype frequencies were divided into categories based on the expanded disability status scale.

SNP	Allele/ genotype	EDSS				*p*-value
		< 3.0		≥ 3.0		
		N	%	N	%	
rs45499297	T	72	80.9	54	77.1	0.562
T/C	C	17	19.1	16	22.9	
	TT	29	65.9	23	65.7	0.208
	TC	14	31.8	8	22.9	
	CC	1	2.3	4	11.4	

SNP: Single nucleotide polymorphism; EDSS: Expanded disability status scale; *p*: Two-tailedFisher's Exact and Pearson Chi-square test probability

Further, allele and genotype frequencies showed significant statistical differences ($p < 0.001$) for EBV-positive MS cases compared to EBV-positive HC to the results of obtained from the current study, as shown in Table (4).

Table 4. Allele and genotype frequencies of *IL-32* gene SNP stratified by Epstein-Barrvirus in multiple sclerosis patients and healthy control.

SNP	Allele/ genotype	EBV positive				*p*-value
		MS patient		HC		
		N	%	N	%	
rs45499297	T	16	66.7	39	81.3	**0.036**
T/C	C	8	**33.3**	9	18.7	
	TT	6	50	15	62.5	**0.001**
	TC	4	33.3	9	37.5	
	CC	2	**16.7**	0	0	

EDSS is for the Expanded Disability Status Scale. SNP stands for single nucleotide polymorphism, and p stands for two-tailed Fisher's Exact and Pearson Chi-square test probability.

The study was for EBV infection in all (187) individual regardless if was patients or control. The results obtained from the current study showed no significant difference in frequency of allele T with positive and negative EBV infections [(76.4%) *vs.* (65.8%) and alleleC was (23.6%) *vs*. (34.2%); $p = 0.160$] but genotype frequency for all individual was significant higher TC genotype frequency between positive and negative EBV infection [(36.1%) *vs*. (28.8%); $p < 0.001$] as shown in Table (5).

Table 5. Allele and genotype frequencies of *IL-32* gene SNP stratified by positive andnegative Epstein-Barr virus in all studied groups.

SNP	Allele/ genotype	EBV				*p*-value
		positive		negative		
		N	%	N	%	
rs45499297	T	55	76.4	246	65.8	0.160
T/C	C	17	23.6	128	34.2	
	TT	21	58.3	105	69.6	**0.001**
	TC	13	**36.1**	36	23.8	
	CC	2	5.6	10	6.6	

The *p*: Pearson Chi-square and Two-tailed Fisher's Exact test probability; EDSS: Expanded disability status scale; SNP: Single nucleotide polymorphism.

Discussion

Though complex genetic-environment interactions probably definitely play a significant role, the underlying etiology of MS and the processes causing increase in worldwide are remain not fully understood [17]. According to observational research, viral infection is a major risk factor in triggering MS etiology [18]. The chance of getting MS is increased by infectious mononucleosis, which is brought on via delayed initial EBV infection [19]. Myelin-specific auto-reactive T-cells are activated against myelin proteins by antigens presented by memory B-cells, which may play a significant role in MS pathophysiology since they serve as repositories for EBV latency [20]. It is well acknowledged that the onset of MS entails a prolonged disruption of immunological homeostasis caused by complicated interactions between exposure to viral, genetic predisposition, and risk factors for inflammation such as low vitamin D level, smoked tobacco, obesity, and lack of sun exposure [21]. Approximately eighty-five percent of MS patients have a phenotype called RRMS, which is characterized by relapsing and remitting symptoms of neurological dysfunction, so this explain MS patient was higher with first line therapy group. Many researches of epigenetic modification support the idea that such changes may play a role in the development of multiple sclerosis [22].In (HLA-DRB1-15) or additional sites in significant linkage unbalance with it contain the majority of the genetic risk for developing multiple sclerosis (MS) [23]. Multiple sclerosis risk genes have been linked to over160 SNPs, according to genome-wide association studies (GWAS) [24]. Genome-wide association studies (GWAS) 1 have been very successful in discovering genetic variations linked to prevalent illnesses, but it has been challenging to establish which variants are causative and what function they play in disease. Numerous of these SNPs are located near to genes involved in immune function like IL7R, IL2RA, and BAFF and demonstrated implicated in development of multiple sclerosis [25]. The TNFRSF1A gene, which

codes for the tumor necrosis factor receptor 1 (TNFR1), has a single nucleotide polymorphism (SNP) that has been linked to multiple sclerosis (MS) by GWAS [26]. The current study examines IL-32 SNP (rs45499297) gene and role it in multiple sclerosis patients, consider first time study with (MS) special in case EBV positive. In other research suggested to assess the relationship between the (rs45499297) polymorphism and obesity [27].IL-32 may facilitate viral component neutralization (for instance, by inactivating viral nucleocapsid structures) and to measure the extent to which SARSCoV2 infection has damaged cells, IL32 might be utilized as abiomarker [28]. Elevate d IL-32 levels after HIV infection have been shown to inhibit viral replication [29]. Adults between the ages of 20 and 45 have the highest incidence of MS, as results in current study appeared (74.7) with ≤ 40 year and (25.3) with >40 year. Many studies showed that most people exposed to infection with herpesvirus at early age in their life and triggers in somehow immune system to attack body tissues called an autoimmune reaction lead to inflammation, which damage the myelin sheath and nerve fibers in bottom. Results of prevalence EBV infection among MS patients according to age and sex was higher appearance in female than male and age ≤ 40 year as in Figure 1. It is commonly recognized that women are more prone than men to acquire RRMS and that environmental variables (such as vitamin D) may play a part in this gender gap. Also sex hormones have a number of distinct effects on the immune system that are crucial to the pathophysiology of MS. Inflammatory DC are generated by estrogen, and estrogen may also interfere with B cell selection, allowing autoreactive B cells to get away [30]. Additionally, illness replace in female patients is linked to decreased estrogen levels [31]. Estrogen and progesterone are the main pregnancy-promoting hormones [32], that peak during pregnancy and modify systemic and maternal-fetal immune responses [33]. And at same time, many studies suggest that EBV seropositivity higher in female compare to male but EBV load in MS patients was higher than in healthy controls this confirm is activated in patients while was latent in (HC) $p < 0.01$ as shown in Figure 2. Reactivation of EBV is possible in certain people when specific immune condition occurs and it has been linked to the onset of a wide range of illnesses, including autoimmune disorders [34]. In this study EBV load in cases MS patients were classified by sex, EDSS, and line therapy groups. The findings showed that males had larger EBV loads than females did because women's humoral and cellular immune reactions were stronger than men's, thus there was statistically significant difference in the viral load $p < 0.05$. In the case of EDSS, when compared to EBV-positive MS patients with EDSS < 3.0, EBV-positive MS patients with EDSS ≥ 3.0 had higher EBV loads. In spite of, the correlation between EBV load and EDSS was difference not significant. There is no hard data connecting EBV load and EDSS but it has been hypothesized that EBV reactivation is linked to increased disease activity [35].

Also demonstrates findings that correlation between EBV load and line therapy of MS was not significant differences in spite was found to be elevated EBV load in second line compare to first line therapy. Because relapsing-remitting multiple sclerosis (RRMS) is treated with fingolimod, an oral immunomodulatory medication[36].Also natalizumab use as treatment for multiple sclerosis after first line failed and two drugs consider immunomodulatory, specific for MS and without effect on mechanism of cell cycle and virus replication. In other hand, first line (INF beta 1 alpha) are a class of cytokines that are activated in response to viral infection and its main function as antiviral but also have role against multiple sclerosis. Therefore, noticed elevated EBV load Ms patients with second line[37].

Frequencies of rs45499297 among groups of MS and HC were no significant differences according to HWE analysis in Table 2, also in case EDSS group results revealed that no significant differences between MS patients in Table 3. In other hand results in Table 4 revealed that frequencies allele T in EBV positive HC was higher than in EBV positive MS cases but allele C higher appear in EBV positive MS compare to EBV positive HC this mean appear C allele33% encourage MS disease also in other research reported that allele C was more prevalent in MS patients than in controls and may enhance the risk of MS by as much as 1.6 times[38] and in case positive EBV because EBV is one of important causes that associated with develop of MS. Allele T and genotype TT appeared significant statistical differences between MS and HC $p < 0.001$ with higher frequencies in HC this indicate that wild type TT is protective from Ms. and in other studies found that IL-32 is elevated following EBV infection, and all LCLs examined show evidence of this upregulation. 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 [39]. So noticed IL-32 higher in healthy control with EBV positive. Finally, in Table 5 this study looked for EBV infection in all (187) people, whether they were patients or controls.The results showed no significant difference in frequency of allele T with positive and negative EBV infections $p = 0.160$, but individual with genotype TC was higher appear for EBV infection with significant differences $p < 0.001$, this meaning individual with TC genotype was more appear for infection. The limitation of this study, effect of IL-32 SNP rs45499297 on serum level has not been

investigated, and how IL-32 genes are expressed.

Conclusion

The study indicated the role of sex and MS activity in susceptibility to EBV reactivation. However, analysis of the IL-32 gene variants (rs45499297) may influence susceptibility to EBV along with MS associated with the allele C occurrence.

Conflict of Interest

All the authors certify that they have no conflict of interest to disclose the subject matter or materials discussed in the present study.

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Ethics Statement

The study was approved by the ethics committee of the Biology department with study protocol (Reference: CSEC 1122/0142), College of Science /University of Baghdad.

Author Contribution

Aya R. Abood: Planning the study, gathering samples with clinical information, examining samples, and drafting the report. Hula Y. Fadhil was responsible for the experiment's planning, oversight, statistical analysis, writing review, and paper modification.

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