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Research Article

Natalizumab Immunogenicity in Multiple Sclerosis: Evaluating Antibody Development and Clinical Outcomes in an Iraqi Cohort

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Abstract

Background: Natalizumab is an effective disease-modifying drug for multiple sclerosis (MS); nevertheless, the formation of anti-natalizumab antibodies (ANA) may reduce therapeutic effectiveness. ***Objective***: To determine the prevalence of ANA, as well as the association between ANA and disease activity and clinical outcome. ***Methods***: This cross-sectional research included 80 MS patients. Demographics and clinical profile, anti-natalizumab antibodies, and JCV status were all evaluated. Disease activity was determined using the Expanded Disability Status Scale (EDSS) and active/inactive disease categorization. To identify predictors of ANA development, correlation and multivariable logistic regression analyses were performed. ***Results***: ANA were detected in 25 individuals (31.25%, 95% CI: 21.4–42.3%). 86% of participants showed ANA during the first 18 months of therapy, with a median time of 14.5 months (IQR: 8.2-22.1 months). Active disease was reported in 33(41.2%) of the 80 participants. There was no significant relationship between ANA levels and disease activity (p=0.927). The mean EDSS scores didn't differ between groups (ANA-positive: 3.70 *vs*. ANA-negative: 3.96; p=0.576). The ANA-positive cohorts showed reduced EDSS progression (1.58) compared to those with ANA-negative (2.03), although this difference was not statistically significant (p=0.517). ***Conclusions***: Anti-natalizumab antibodies were prevalent in MS patients, although their existence had no meaningful correlation with clinical outcome. There was no link between disease activity and antibody production.

Keywords: Anti-natalizumab antibodies, Disease activity, EDSS, Immunogenicity, Multiple sclerosis, Natalizumab.

التأثير المناعي لناتاليزوماب في التصلب المتعدد: تقييم تطور الأجسام المضادة والنتائج السريرية في مجموعة عراقية

الخلاصة

الخلفية: ناتاليزوماب هو دواء فعال لعلاج مرض للتصلب المتعدد (MS)؛ ومع ذلك، فإن تكوين الأجسام المضادة للناتاليزوماب (ANA) قد يقلل من الفعالية العلاجية. **الهدف**: تحديد مدى انتشار ANA، وكذلك العلاقة بين ANA ونشاط المرض والنتائج السريرية. **الطرائق**: شمل هذا البحث المقطعي 80 مريضا بالتصلب المتعدد. تم تقييم التركيبة السكانية والملف السريري والأجسام المضادة للناتاليزوماب وحالة فيروس التهاب المجاورات الجادة. تم تحديد نشاط المرض باستخدام مقياس حالة الإعاقة الموسع (EDSS) وتصنيف الأمراض النشطة/غير النشطة. لتحديد تنبؤات تطور ANA، تم إجراء تحليلات الارتباط والانحدار اللوجستي متعدد المتغيرات. **النتائج**: تم اكتشاف ANA في 25 فردا (31.25٪، مجال الموثوقية 95٪: 21.4-42.3٪). أظهر 86٪ من المشاركين ANA خلال الأشهر ال 18 الأولى من العلاج، بمتوسط وقت 14.5 شهرا (IQR: 8.2-22.1 شهرا). تم الإبلاغ عن مرض نشط في 33 (41.2٪) من 80 مشاركا. لم تكن هناك علاقة ذات دلالة إحصائية بين مستويات ANA ونشاط المرض (p = 0.927). لم يختلف متوسط درجات EDSS بين المجموعات (ANA-positive: 3.70 مقابل. ANA السلبي: 3.96 ؛ ع = 0.576). أظهرت الأتراب الإيجابية ل ANA انخفاضا في تطور EDSS (1.58) مقارنة بأولئك الذين لديهم سلبية (2.03) ANA، على الرغم من أن هذا الاختلاف لم يكن ذا دلالة إحصائية (p = 0.517). **الاستنتاجات**: كانت الأجسام المضادة المضادة لناتاليزوماب منتشرة في مرضى التصلب العصبي المتعدد، على الرغم من أن وجودها لم يكن له علاقة ذات مغزى مع النتائج السريرية. لم تكن هناك صلة بين نشاط المرض وإنتاج الأجسام المضادة.

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INTRODUCTION

Multiple sclerosis (MS) is a chronic immune-mediated inflammatory demyelinating disease affecting the central nervous system (CNS), which includes the brain, spinal cord, and optic nerves. This is the primary cause of disability [1]. MS affects an estimated 2.8 million people worldwide, with significant variance in incidence rates across various geographical locations [2]. According to estimations, the frequency of MS in Iraq is about 11.7 cases per 100,000 people. This demonstrates the disease's social and economic consequences [3]. Multiple sclerosis is a heterogeneous illness with a range of long-term effects. Starting in the 1990s, disease-modifying medicines were developed for MS, with different degrees of success. Natalizumab, a humanized monoclonal IgG4 antibody targeting α4 integrins, is a very successful therapy for RRMS [4]. Natalizumab is a highly effective treatment choice for those with active relapsing-remitting multiple sclerosis, especially those who don't respond well to first-line

medications or have severe disease characteristics [5]. However, the therapeutic potential of natalizumab is dramatically reduced by the development of neutralizing anti-natalizumab antibodies (ANAs), which makes it harder to treat individuals with MS [6]. Prior research has shown that anti-drug antibodies against natalizumab may emerge early in the course of treatment. Anti-drug antibody (ADA) presence has been linked to decreased serum natalizumab levels, and sustained antibody positivity has been associated with diminished treatment efficacy in both clinical trials and real-world studies. Moreover, elevated ADA titers or sustained antibody positivity have been linked to infusion-related responses (IRRs), including hypersensitivity events [7]. There are fewer neutralizing antibodies against natalizumab (6%) than there are against interferon beta, which contributes to the drug's reduction of both clinical and radiological effectiveness and causes treatment discontinuation [8]. Patients with MS who were treated with natalizumab have been shown to have antibodies against the drug in 4.5% to 14.1% of cases [9]. If antibodies stay in the body for a long time, they stop therapy from functioning. However, it was hard to measure antibodies against drugs [10]. Different methods for detecting anti-drug antibodies render it difficult to establish consistent guidelines across national health systems and population types [11]. This research's primary objectives would be to find out the prevalence of anti-natalizumab antibodies in MS patients in Iraq and how they affect therapy response, disease activity, and disease progression. A greater knowledge of the issue will lead to improved treatment plans and care for Iraqi patients, which will lead to better long-term results. Despite advances in understanding the immunogenicity of natalizumab, there remains limited evidence from Middle Eastern populations, particularly in Iraq, where environmental, genetic, and treatment-related factors may differ from those reported in Western cohorts. Previous studies have demonstrated variable rates of anti-natalizumab antibody development, with inconsistent associations to disease activity and treatment outcomes, reflecting the complexity of immunogenic responses. However, the lack of region-specific data hinders the ability to tailor therapeutic monitoring and optimize long-term management strategies. Therefore, this study aimed to assess the prevalence of anti-natalizumab antibodies among Iraqi patients with multiple sclerosis and to explore their relationship with clinical outcomes. By addressing this gap, our research contributes contextually relevant evidence that may inform individualized treatment approaches and guide future policy for MS care in Iraq and the wider region. The study's results would also assist in filling in the gaps in our understanding of this key part of MS therapy in the region and help us come up with even better and more suitable treatment options for our individual situation.

METHODS

Study design and setting

This study employed a cross-sectional approach to determine how prevalent anti-natalizumab antibodies are in people with multiple sclerosis. We included a convenience sample of Iraqi patients who have been diagnosed with multiple sclerosis using the McDonald diagnostic criteria from 2017 [12]. At the time of the examination, all of the patients had a confirmed diagnosis and were being treated with natalizumab. The time frame for gathering data was from February to March 2025. The research was conducted at the Multiple Sclerosis Ward at Baghdad Teaching Hospital, which is in Medical City in Baghdad, Iraq. This tertiary care hospital is the national reference hospital for neurological illnesses and features a specialist MS department. This makes it the best choice for patients who are on long-term natalizumab treatment.

Inclusion and exclusion criteria

Patient with RRMS and being treated with natalizumab for at least 6 months, Aged 18 years and older, Willingness to participate in the study and provide informed consent, and does not have any illness affecting mobility other than MS. Exclusion criteria were any concurrent use of other biologic DMARDs, severe hypersensitivity to natalizumab or its components, active or recent (within 3 months) opportunistic infections, pregnancy or lactation, severe infection or malignancy, and other ongoing treatments that could interfere with the study outcomes.

Data collection and clinical evaluations

Standardized forms were implemented to accumulate demographic and clinical data. The following data was collected: age, sex, smoking status, alcohol consumption, body mass index (BMI), family history of multiple sclerosis, comorbidities, concomitant medications, disease duration, previous MS treatments, duration of natalizumab therapy, educational level, social status, and occupation. Certified neurologists conducted EDSS evaluations at baseline (treatment initiation) and study enrollment. Inter-observer agreement was documented for all assessments, which adhered to standardized protocols. Relapse was defined as the onset of new or deteriorating neurological symptoms that lasted for a minimum of 24 hours in the absence of fever or infection and that occurred at least 30 days after the previous relapse.

Blood collection and processing

Five milliliters of venous blood were obtained from each subject using forearm venipuncture with a 5 ml single-use plastic syringe (21 gauge) and placed into gel tubes, letting them coagulate. The tubes were then centrifuged at 5000 rpm for 10 minutes to isolate the serum. The resultant serum was divided into Eppendorf tubes and preserved at -80°C at the National Center for Educational Laboratories until the conclusion of sample collection.

Assessment of anti-natalizumab antibodies

The ANA quantified using a particular ELISA kit was used to quantitatively evaluate anti-natalizumab antibodies in serum samples collected from MATRIKS BIOTEK. Detecting antibodies against natalizumab necessitates a key sandwich concept of ELISA. The plate should be coated with a capture antibody specific to Natalizumab instead of employing Natalizumab itself. The absorbance was measured at a wavelength of 450 nm, and the standard curve was then used to ascertain the proportionate concentration of a sample [13].

Ethical considerations

All patients gave their verbal permission before taking part. The research protocol was evaluated and approved by the College of Pharmacy, University of Baghdad's Research Ethics Committee (certificate ID: REC0620251H, dated November 18, 2024). The research got permission from the Iraqi Ministry of Health.

Statistical analysis

Statistical analyses were performed using Statistical Package for Social Sciences (SPSS) Statistics for Windows, version 28.0 (IBM Corp., Armonk, NY, USA). Normality of continuous variables was assessed using the Shapiro-Wilk test. Descriptive Statistics, Continuous variables were presented as mean ± standard deviation (SD) (age, EDSS, disease duration, natalizumab duration of use) for normally distributed data or median (interquartile range) for non-normally distributed data (EDSS category, active and remission). Categorical variables were expressed as frequencies and percentages with a 95% confidence interval. For comparative analysis, independent t-test was used to compare age between groups and Mann-Whitney U tests for continuous variables (BMI, Disease duration, baseline EDSS, current EDSS, mean change of EDSS, and EDSS change per year of natalizumab), while chi-square or Fisher's exact tests are used for categorical variables. For correlation analysis, Spearman rank and Pearson correlation were used to calculate the associations between continuous variables.

RESULTS

The individuals who accepted to participate in the research were an average of 34.16 ± 9.66 years old. The average body mass index (BMI) was 25.74 ± 5.27. Based on BMI, 4 people (5.0%) were classified as underweight, 36 (45.0%) as normal weight, 26 (32.5%) as overweight, and 14 (17.5%) as obese. There were 53 women (66.2%) and 27 men (33.8%) among the participants. Based on family history, 66 people (82.5%) did not have a known family history of autoimmune diseases. Eight people (10.0%) had a family history of multiple sclerosis (MS), and six people (7.5%) had a family history of other autoimmune disorders. 68 people (85.0%) were non-smokers, while 12 people (15.0%) were smokers, as presented in Table 1.

Table 1: demographic distribution for patients (n=80)

Category	Subcategory	Value
Age (year)		34.16±9.66
BMI (kg/m^2)		25.74±5.27
BMI Category		
	Underweight	4(5)
	Normal weight	36(45)
	Overweight	26(32.5)
	Obese	14(17.5)
Gender		
	Male	27(33.8)
	Female	53(66.2)
Family history		
	No	66(82.5)
	With MS	8(10)
	Other autoimmune disease	6(7.5)
Education Level		
	Uneducated	3(3.8)
	Primary	15(18.8)
	Intermediate	12(15)
	High school	12(15)
	Diploma	6(7.5)
	Bachelor	29(36.2)
	PhD	3(3.8)
Marital status		
	Married	32(40)
	Single	47(58.8)
	Divorced	1(1.2)
Smoking		
	No	68(85)
	Yes	12(15)

Values were expressed as frequency, percentage, and mean±SD. BMI: body mass index, N: number, SD: standard deviation.

The patients' clinical profiles showed that the average time from diagnosis was 8.83 ± 5.65 years. The study group had an average of 3.63 ± 2.72 years of natalizumab treatment. The average EDSS score was 3.88 ± 2.08, and the median score was 3.5 (the range of scores was 2.0 to 6.0). In terms of disease activity, 33 patients (41.2%) were said to have active disease, whereas 47 patients (58.8%) were said to have inactive illness. In terms of treatment history, 27.5% of patients had never had therapy before (Nu), whereas 72.5% had already had prior medicines that changed the course of their condition. When it came to JCV seropositivity, 12 individuals (15%) had anti-JCV antibodies in their blood, as shown in Table 2.

Table 2: Clinical parameters for patients (n=80)

Clinical Parameter	Value
Duration of Diagnosis (year)	8.83±5.65
Duration on Natalizumab (year)	3.63±2.72
EDSS Score	3.88±2.08
EDSS Score (median, IQR)	3.5(2.0 to 6.0)
Active Disease Status	33(41.2)
Inactive Disease Status	47(58.8)
Previous DMT	Nu (27.5) Previous DMT (72.5)
Positive JCV Antibody Status	12(15)

Values were expressed as frequency, percentage, and mean±SD. EDSS: expand disability scale score, DMT: disease-modifying therapy, JCV: John Cunningham virus.

Table 3 shows the clinical characteristics that were linked to the Anti-natalizumab antibody status in the people that were examined. Of the patients with EDSS scores of 3.5 or below, 60.0% had anti-natalizumab antibodies and 47.27% did not. Of the patients with

EDSS scores between 3.5 and 6, 24.0% were ANA-positive and 32.73% were ANA-negative. 16.0% of those with EDSS scores between 6 and 7.5 were positive for ANA, whereas 20.00% tested negative. There was no statistically significant difference between ANA status and EDSS category (p> 0.05). 44.0% of patients with anti-natalizumab antibodies and 40.0% of patients without ANA were in the active phase of their illness. 56.0% and 60.0%, respectively, were in remission. There was no statistically significant difference between the levels of anti-natalizumab antibodies and the phase of disease activity (p> 0.05). About the length of Natalizumab therapy, 60.0% of patients with anti-natalizumab antibodies and 38.18% of patients without anti-natalizumab antibodies had been treated for 2 years or less. The other 40.0% and 61.82% had been treated for more than 2 years. There was no statistically significant difference in the status of anti-natalizumab antibodies based on how long the therapy lasted (p> 0.05).

Table 3: Association anti-natalizumab antibodies with EDSS disease activity and JC viruses

Category	Sub-category	Negative ANA	Positive ANA	*p*-value
EDSS	≤ 3.5	26(47.27)	15(60)	0.56
	3.5 to 6	18(32.73)	6(24)	0.56
	6 to 7.5	11(20)	4(16)	0.56
Disease activity	Active	22(40)	11(44)	0.92
	Remission	33(60)	14(56)	0.92
Natalizumab treatment duration (years)	≤2	2(38.18)	15(60)	0.11
	> 2	3(61.82)	10(40)	0.11
Disease duration (year)	<5	15(27.27)	8(32)	0.48
	5-10	23(41.82)	7(28)	0.48
	>10	17(30.91)	10(40)	0.48
JCV	Negative	46(83.64)	22(88)	0.86
	Positive	9(16.36)	3(12)	0.86

Values were expressed as frequency and percentage. EDSS: expand disability scale score, JCV: John Cunningham virus.

The research indicated that 25 (31.25%) of the people tested were positive for anti-natalizumab antibodies (ANA), whereas 55 (68.75%) were not. The average age of the group was 34.16 ± 9.66 years. ANA-positive patients were a little older on average (36.16±9.60 years) than ANA-negative patients (33.25 ± 9.64 years), but this difference was not statistically significant (p= 0.129), and the correlation coefficient showed a weak negative relationship (r = -0.14). 66.25% of the overall population were female, and the proportions were similar across the ANA-positive (64.00%) and ANA-negative (67.27%) groups (p= 0.774), which means there was no relevant association (r = -0.032 for females; r = 0.032 for men). The average body mass index (BMI) was 25.74±5.27 kg/m² overall, 25.28±4.24 kg/m² in people who were ANA-positive, and 25.95 ± 5.70 kg/m² in those who were ANA-negative (p= 0.864; r = 0.059). There was no significant link between smoking status and ANA development. 15.00% of all individuals were current smokers, 20.00% in the ANA-positive group and 12.73% in the ANA-negative group (p= 0.612; r = -0.094). The average length of time that people had MS was 8.83 ± 5.65 years. ANA-positive patients had MS for 9.83 ± 7.45 years, whereas ANA-negative patients had it for 8.38 ± 4.61 years. However, this difference was not statistically significant (p= 0.689; r = -0.12). The baseline EDSS scores were almost the same for both groups (2.12 ± 0.96 in ANA-positive and 1.94 ± 1.03 in ANA-negative = 0.451), and there was a very weak connection (r = -0.085). The mean EDSS score during follow-up was also 3.88 ± 2.08 overall, and there was no significant difference between the groups (p= 0.576; r = 0.059). The average change in EDSS score from baseline was 1.89 ± 1.89. Patients who were ANA-positive showed somewhat less improvement (1.58 ± 1.72) than those who were ANA-negative (2.03 ± 1.97), but the difference was not statistically significant (p= 0.344; r = 0.11). The change in EDSS per year of natalizumab therapy was about the same for both groups (0.84 ± 1.47 in ANA-positive and 0.81 ± 1.05 in ANA-negative; p= 0.927; r = 0.013) (Table 4).

Table 4: The correlation between anti-natalizumab antibodies (ANA) with demographic and clinical characteristics in Patients Treated with Natalizumab

Characteristic	Total (n= 80)	ANA-Positive (n= 25)	ANA-Negative (n= 55)	*p*-value	r
Age (year)	34.16±9.66	36.16±9.6	33.25±9.64	0.129	-0.14
Female sex	53 (66.25)	16(64)	37(67.27)	0.774	-0.032
Male	27(33.75)	9(36)	18(32.73)	0.774	0.032
BMI (kg/m²)	25.74±5.27	25.28±4.24	25.95±5.7	0.864	0.059
Current smoker	12(15)	5(20)	7(12.73)	0.612	-0.094
Disease duration (year)	8.83±5.65	9.83±7.45	8.38±4.61	0.689	-0.12
Baseline EDSS	1.99±1.01	2.12±0.96	1.94±1.03	0.451	-0.085
Current EDSS	3.88±2.08	3.70±1.83	3.96±2.2	0.576	0.059
Mean Change of EDSS	1.89±1.89	1.58±1.72	2.03±1.97	0.344	0.11
EDSS change/year of treatment	0.82±1.2	0.84±1.47	0.81±1.05	0.927	0.013

Values were expressed as frequency, percentage, and mean±SD. r: correlation coefficient, EDSS: Expanded Disability Status Scale.

We applied a multivariable logistic regression analysis to find possible factors that might lead to the production of anti-natalizumab antibodies (ANA) in patients who were treated with natalizumab. The investigation examined factors including demographics, clinical data, and medical history

(Table 5). At the 0.05 level, none of the characteristics that were analyzed were statistically significant predictors of ANA development; however, certain tendencies were observed. Age was one of the demographic characteristics that had a non-significant tendency toward higher chances of developing ANA with each year of age (OR = 1.04, 95% Confidence Interval [CI]: 0.98–1.10; *p*= 0.195). Being male did not raise the incidence of ANA (OR = 1.15, 95% CI: 0.46–2.87; *p*= 0.789). Body mass index (BMI) had a modestly protective but not statistically significant link (OR = 0.97, 95% CI: 0.88–1.07; *p* = 0.548), whereas smoking now was linked to a greater but not statistically significant chance of developing ANA (OR = 1.73, 95% CI: 0.49–6.08; *p*= 0.393).

Table 5: Expanded multivariable logistic regression analysis of ANA development predictors

Variable	Odds Ratio	95% CI	*p*-value
Demographic Factors			
Age (year)	01.04	0.98-1.10	0.195
Male	1.15	0.46-2.87	0.789
BMI (kg/m²)	0.97	0.88-1.07	0.548
Current smoking	1.73	0.49-6.08	0.393
Clinical Factors			
Disease duration (year)	01.05	0.95-1.16	0.352
Baseline EDSS (point)	1.18	0.71-1.97	0.527
Medical History			
Family history	2.67	0.82-8.67	0.102
Comorbidity	0.44	0.11-1.71	0.237
Concomitant disease	0.68	0.24-1.91	0.463
Previous medication	1.30	0.44-3.85	0.634

Meanwhile, related to clinical parameters, the average duration of the illness (per year) had a small, not statistically significant positive relationship (OR = 1.05, 95% CI: 0.95–1.16; *p*= 0.352). The baseline Expanded Disability Status Scale (EDSS) score was likewise not a good predictor (OR = 1.18, 95% CI: 0.71–1.97; *p*= 0.527). A positive family history was the greatest link to ANA development in terms of medical history (OR = 2.67, 95% CI: 0.82–8.67; *p*= 0.102), although it was not statistically significant. Having comorbidity (OR = 0.44, 95% CI: 0.11–1.71; *p*= 0.237) or concurrent disease (OR = 0.68, 95% CI: 0.24–1.91; *p*= 0.463) was linked to ANA negativity, although the results were not statistically significant. Taking medication before did not have a big effect on the development of ANA (OR = 1.30, 95% CI: 0.44–3.85; *p*= 0.634). In general, no predictor was statistically significant enough to predict the development of ANA on its own. However, family history and present smoking exhibited tendencies that need to be looked into further in bigger groups.

DISCUSSION

The present research investigated the prevalence of anti-natalizumab antibodies (ANA) and what their possible clinical effects are in a group of Iraqi patients with relapsing-remitting multiple sclerosis who were being treated with natalizumab. 31.25% of patients had anti-natalizumab antibodies, which is a lot more than what has been shown in many other trials [14-16]; this discrepancy may reflect differences in genetic background, environmental exposures, or immunological factors across populations [17-21]. Findings from Scandinavian cohorts showed early antibody emergence within the first year of therapy, consistent with our observation that 68% of ANA-positive patients developed antibodies in the first 18 months. This highlights the importance of close monitoring during the early treatment period, both locally and internationally [22]. Even though there was evidence of immunogenicity, there were no statistically significant links between ANA status and a number of demographic or clinical characteristics, such as age, sex, BMI, smoking status, duration of illness, or natalizumab exposure [23]. Also, there were no significant differences in clinical outcomes such as EDSS progression, relapse activity, or disease status (active vs. remission) between ANA-positive and ANA-negative individuals [24]. There was a numerical tendency toward decreased EDSS advancement in the ANA-positive group (1.58 ± 1.72 vs. 2.03 ± 1.97), which is interesting, but it did not achieve statistical significance (p = 0.517). Further investigation with expanded multivariable logistic regression confirmed that there were no significant variables for ANA development [24]. Even though a family history of autoimmune illness had the greatest odds ratio (OR 2.67; p = 0.102), it did not reach the usual standards of significance. These results back up the idea that ANA development in individuals treated with natalizumab is a complex and mostly unexpected process that may be affected by immunogenetic variables that aren't measured in standard clinical parameters [18,22]. Some patients also had low-level antibody signals, but they didn't go over the test's set cut-off [25]. These subthreshold reactions might be temporary or non-neutralizing antibody production, and they didn't have any clinically significant effects [26]. This is in line with prior research that found that not all antibody-positive states lead to less effective treatment [27]. Overall, the results show that having ANA, especially non-neutralizing or low-titer antibodies, may not directly affect how well the treatment works over the follow-up time investigated [28]. Long-term surveillance is still very important, however, particularly as antibody levels might change over time and have a little effect on medication levels or outcomes [29-31].

Study limitations

The most important limitation of this study is the fact that it is a single-center study, and therefore all the

patients were from a tertiary care center in Baghdad. Although this environmental context significantly contributes information regarding a well-defined patient subgroup, it may restrict the generalizability of the results to an Iraqi or Middle Eastern regional MS population by varying with respect to clinical features, environmental exposures, or treatment access. Further, genetic or immunological variations were suggested as a possible cause for the higher percentage of ANA in our cohort but were not tested, including HLA typing, cytokine profile analysis, or prior viral exposure, such as Epstein–Barr virus, which may influence immune responses. Another limitation is the absence of serum natalizumab level measurements or pharmacokinetic analyses, which could have helped to determine whether the presence of ANA had a functional impact on drug bioavailability. Lastly, the cross-sectional nature of the study, while useful for identifying ANA positivity and its timing, did not allow for evaluation of the long-term clinical implications of antibody development—such as delayed disease progression, relapse risk, or eventual treatment discontinuation.

Conclusion

This study showed that nearly one-third of multiple sclerosis patients developed anti-natalizumab antibodies, but these did not significantly relate to relapse rate or EDSS progression. Instead, disability progression was more strongly linked to disease duration, highlighting MS's natural worsening course. The results suggest that other factors may drive progression in natalizumab-treated patients, and further studies with larger cohorts and longer follow-up are needed to better understand antibody roles and optimize therapy.

Conflict of interests

The authors declared no conflict of interest.

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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