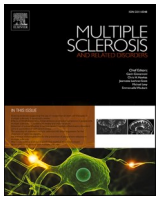
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Original article



Three doses of COVID-19 vaccines in multiple sclerosis patients treated with disease-modifying therapies

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ABSTRACT

Objectives and aims: Disease modifying therapies used in multiple sclerosis can decrease humoral response after COVID-19 vaccines. This problem must be adequately addressed because new variants evolve, and COVID-19 still poses a risk to patients with comorbidities and immunosuppression. We aimed to evaluate the antibody response after the third dose of the COVID-19 vaccine in people with multiple sclerosis on disease-modifying therapies.
Methods: People with multiple sclerosis who received the third dose of either mRNA or inactivated vaccine after two doses of inactivated vaccine were recruited for the study. Blood samples were collected at least two weeks after the third dose.
Results: Blood samples of 339 (female 72.5%) people with multiple sclerosis and 52 (female 71.2%) healthy controls were evaluated. Healthy controls (mean: 4.07 ± 0.66) have higher antibody titers than people with multiple sclerosis (mean: 2.79 ± 2.95). Seronegative cases were observed only in the fingolimod and ocrelizumab treatment groups. Patients on fingolimod who received mRNA as a third dose had significantly higher antibody titer than those who had inactivated vaccines. Longer disease duration, having inactivated vaccine as a third dose, and DMT use was associated with lower antibody response.
Conclusions: The study shows that even after inactivated vaccine schedule, mRNA still offers more protection in people with multiple sclerosis on disease-modifying therapies.

1. Introduction

Vaccines as a prophylactic source to fight the pandemic have shown their benefits by lowering the mortality and morbidity of the COVID-19 (Chen et al., 2022). Several vaccine types are available in different countries. The mounting evidence suggests that they are all effective, and the difference is with antibody titer that is elicited (Dong et al., 2020). Although there is no clear evidence which antibody titer against COVID-19 is adequate, some vaccines manage to boost a more significant humoral response than others (Ozakbas et al., 2022; Sormani et al., 2021). Studies show that immunocompromised patients can not mount good antibody response after a standard vaccine regime. Considering this fact and the knowledge that new variants are still causing a threat to the population and the humoral immunity against the COVID-19 vaccine is waning with time, a booster dose seems to be lifesaving for at least vulnerable members of the population (Krause et al., 2021; Kamar et al., 2021). At the start of the pandemic, it was shown that mortality and morbidity are related to age and some other comorbidities, immunosuppression being on the list (Wang et al., 2020; Wu et al., 2020). Though multiple sclerosis (MS) is an autoimmune disease whose pathology is tried to be explained by an altered immune system, it does not cause immunosuppression (Titus et al., 2020). But disease-modifying therapies (DMT) are potential candidates to diminish immune response after vaccines because they target immune cells directly or indirectly. Among the DMT, CD20 depleting agents were consistently associated with decreased antibody response against COVID-19. Other DMT show somewhat favourable results, but less than healthy people (Garjani et al., 2022; Tallantyre et al., 2021; Achiron et al., 2021).

Recent studies also showed that people with a history of COVID-19 infection have a greater immunologic response after vaccines (Sormani et al., 2021), which supports the idea that revaccination could boost humoral immunity. This study aimed to evaluate antibody

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Table 1
Demographic and clinical characteristics of the participants.

	Sex (%)		Age-Mean (SD)	Disease duration Mean-year (SD)	MS type (%)				EDSS (SD)	Vaccine Type (%)		TBVS Mean-day (SD)
	Female	Male			CIS	RRMS	SPMS	PPMS		2 dose Inactivated Vaccine+1 dose mRNA BNT162b2	3 dose Inactivated Vaccine	
pwMS DMT	239 (72.6)	90 (27.4)	44.26 (12.1)	13.25 (8.3)	0 (0)	271 (80.4)	55 (16.3)	11 (3.3)	2.21 (2.1)	234 (71.1)	95 (28.9)	77.46 (56.8)
pwMS w/o T	7 (70.0)	3 (30.0)	40.20 (16.8)	7.97 (8.6)	2 (20.0)	5 (50.0)	2 (20.0)	1 (10.0)	2.25 (2.4)	3 (30.0)	7 (70.0)	76.40 (55.6)
Healthy Controls	37 (71.2)	15 (28.8)	41.04 (11.3)	0 (0)	–	–	–	–	–	46 (88.5)	6 (11.5)	75.05 (69.9)
Glatiramer Acetate	35 (92.1)	3 (7.9)	45.82 (14.3)	11.47 (10.4)	0 (0)	36 (94.7)	2 (5.3)	0 (0)	1.57 (2.0)	25 (65.8)	13 (34.2)	90.11 (60.6)
Interferons	26 (72.2)	10 (27.8)	41.78 (9.3)	12.78 (7.4)	0 (0)	36 (100)	0 (0)	0 (0)	1.13 (1.4)	24 (66.7)	12 (33.3)	77.94 (55.0)
Teriflunomide	14 (73.7)	5 (26.3)	49.47 (11.8)	13.9 (8.9)	0 (0)	17 (89.5)	2 (10.5)	0 (0)	1.42 (1.8)	15 (78.9)	4 (21.1)	92.79 (54.3)
Dimethyl Fumarate	9 (60.0)	6 (40.0)	39.27 (13.2)	3.67 (5.6)	0 (0)	15 (100)	0 (0)	0 (0)	1.47 (1.2)	13 (86.7)	2 (13.3)	70.67 (100.5)
Cladribine	5 (83.3)	3 (16.7)	40.33 (10.1)	10.0 (8.0)	0 (0)	6 (100)	0 (0)	0 (0)	0.50 (0.5)	6 (100)	0 (0)	42.0 (44.4)
Fingolimod	77 (72.6)	29 (27.4)	42.02 (10.5)	13.71 (7.6)	0 (0)	101 (95.3)	5 (4.7)	0 (0)	1.50 (1.6)	74 (69.8)	32 (30.2)	85.21 (53.7)
Natalizumab	28 (84.8)	5 (15.2)	37.12 (8.1)	9.94 (5.1)	0 (0)	32 (97.0)	1 (3.0)	0 (0)	1.62 (0.9)	27 (81.8)	6 (18.2)	65.30 (53.3)
Ocrelizumab	44 (60.3)	29 (39.7)	51.22 (11.0)	17.69 (7.4)	0 (0)	21 (28.8)	43 (58.9)	0 (0)	4.84 (1.8)	47 (64.4)	26 (35.6)	64.47 (49.9)
Azathioprine	1 (33.3)	2 (66.7)	54.0 (15.4)	15.67 (9.0)	0 (0)	2 (66.7)	0 (0)	1 (33.3)	2.50 (0.5)	3 (100)	0 (0)	64.33 (23.7)
Total	283 (72.4)	108 (27.6)	43.82 (12.0)	–	2 (0.5)	271 (69.3)	55 (14.1)	11 (2.8)	–	283 (72.4)	108 (27.6)	81.39 (65.0)

response after booster COVID-19 vaccines in people with multiple sclerosis (pwMS) treated with disease-modifying therapies.

2. Methods

This is a cross-sectional observational study of patients who already received two doses of inactivated COVID-19 vaccines and were enrolled in the comparative research after two doses of SARS-CoV-2 vaccines. Details reported previously (Ozakbas et al., 2022). The inclusion criteria for this study was having a third dose of either inactivated Sinovac or mRNA BNT162b2 vaccine. Patients from the previous survey were contacted by phone or e-mail, and eligible patients were invited to participate. The relatives and partners of patients and healthcare workers were recruited as healthy controls. University's Ethics Committee approved the study (2021/15-14). All participants gave the written informed concept. The blood samples were collected at least two weeks after the third vaccine dose.

2.1. Measurement

The primary outcome was quantifying antibody response after three doses of the COVID-19 vaccine. Antibody levels were transformed on a Log10 scale to normalize their distribution, and the 'AU/mL Log' name was used after that. For antibody titer less than the detectable limit of 21 AU/mL, to prevent missing data during Log10 transformation, a titer of 0.01 Au/mL was used. Vaccines were grouped into two categories: three-dose of inactivated vaccines and two doses of inactivated plus one dose of mRNA BNT162b2. Using the cut-off titer for seropositivity defined by the manufacturer, the antibody response was divided into two categories: seropositive (antibody titer $\geq$ 50 AU/mL) and seronegative (antibody titer$<$ 50 AU/mL). CIS and RRMS were classified as a relapsing group, SPMS with PPMS as a progressive group for multivariable analyses. Each DMT group and HC were compared in seropositivity, antibody titer, and vaccine type.

For all multiple comparisons, Bonferroni corrections were made. Two-sided hypothesis testing with a significance set at $p < 0.05$ was used. Statistical analysis was run on IBM SPSS STATISTICS software

Table 2
Comparison of having seropositivite and seronegative regarding vaccine groups.

	2 Dose Inactivated Vaccine+ 1 dose mRNA BNT162b2		3 Dose Inactivated Vaccine		P
	seropositive	seronegative	seropositive	seronegative	
pwMS w/o T	3 (100)	0 (0)	7 (100)	0 (0)	NA
Healthy Controls	46 (100)	0 (0)	6 (100)	0 (0)	NA
Glatiramer Acetate	25 (100)	0 (0)	13 (100)	0 (0)	NA
Interferons	24 (100)	0 (0)	12 (100)	0 (0)	NA
Teriflunomide	15 (100)	0 (0)	4 (100)	0 (0)	NA
Dimethyl Fumarate	13 (100)	0 (0)	2 (100)	0 (0)	NA
Cladribine	6 (100)	0 (0)	–	–	NA
Fingolimod	57 (77.0)	17 (23.0)	15 (46.9)	17 (53.1)	***0.002***
Natalizumab	27 (100)	0 (0)	6 (100)	0 (0)	NA
Ocrelizumab	20 (42.6)	27 (57.4)	9 (34.6)	17 (65.4)	0.507
Azathioprine	3 (100)	0 (0)	–	–	NA

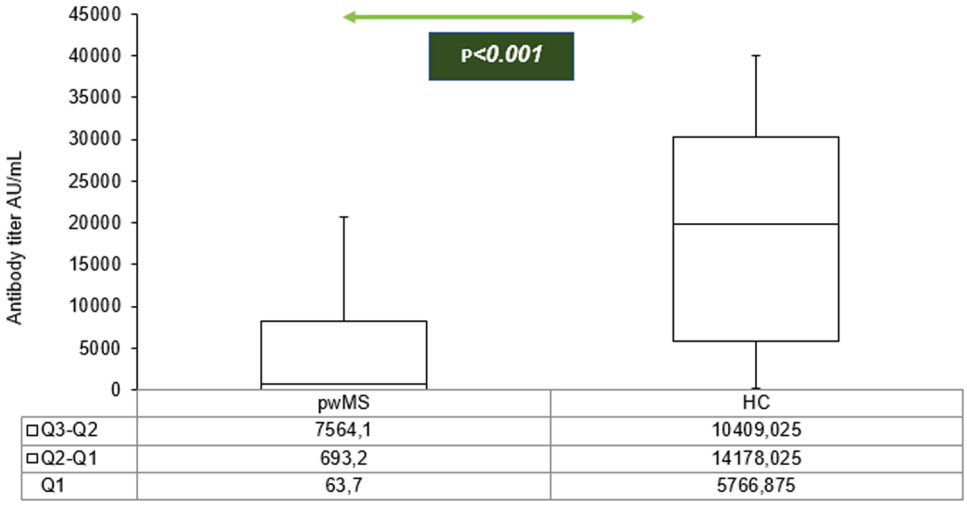


Fig. 1. The AU/mL Log level of the participants.

Table 3
Multivariable regression analysis assessing factors associated with antibody levels (AU/mL Log).

Variable	β (SE)	%95 CI	P
Sex (female vs male)	−0.046 (0.137)	−0.315–0.223	.737
Age	−0.004 (0.007)	−0.018–0.009	.501
Vaccine type			
2 Dose Inactivated Vaccine+1 Dose mRNA BNT162b2 Vaccine	reference		
3 Dose Inactivated Vaccine	−0.671 (0.133)	−0.933 – −0.409	**<0.001**
TBVS	0.000 (0.001)	−0.002–0.002	.761
EDSS	−0.031 (0.043)	−0.115–0.053	.472
Disease duration	−0.019 (0.010)	−0.038–0.000	**.044**
MS course (Relapsing vs progressive)	−0.223 (0.212)	−0.640–0.195	.295
DMT			**<0.001**
Mean difference			
No treatment	Reference		
Glatiramer acetate	−0.379 (0.347)	−1.496–0.739	>0.99
Interferons	−0.694 (0.352)	−1.829–0.440	>0.99
Teriflunomide	−0.743 (0.379)	−1.966–0.481	>0.99
Dimethyl fumarate	−0.413 (0.397)	−1.694–0.869	>0.99
Fingolimod	0.957 (0.328)	−0.102–2.016	0.138
Cladribine	−0.588 (0.493)	−2.179–1.003	>0.99
Natalizumab	−0.441 (0.354)	−1.583–0.701	>0.99
Ocrelizumab	1.957 (0.327)	0.901–3.013	**<0.001**

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3. Results

In total, 339 pwMS (329 pwMS using DMT, ten pwMSw/oT) and 52 healthy controls were enrolled in this study. Detailed information regarding clinical and demographic characteristics was presented in Table 1 according to DMT groups.

The distribution of seronegative and seropositive participants regarding DMT groups is shown in Table 2. All participants have seropositivity in every DMT group except the fingolimod and ocrelizumab groups. While there was a significant difference between vaccinations in the fingolimod group (seropositive rate was higher in two doses of inactivated vaccine plus one mRNA BNT162b2), there were no significant differences in the ocrelizumab group.

The mean antibody titer Log level was higher in healthy controls (mean: 4.07 ± 0.66; minimum/maximum: 2.15/4.60) compared to pwMS (mean: 2.79 ± 2.95; minimum/maximum: −0.70/4.60). The AU/mL Log level of the participants is illustrated in Fig. 1.

When the analyses were repeated according to vaccination groups, healthy controls had higher titer in both three-dose inactivated vaccines ($p = 0.01$) and two doses of inactivated vaccine plus one dose of mRNA BNT162b2 ($p = 0.029$) than pwMS.

Multivariable regression analysis showed that the type of vaccine (the favor of mRNA BNT162b2), disease duration and DMT used by pwMS are significantly associated with the COVID-19 AU/mL Log level. Only ocrelizumab was found significantly differ among DMTs' (Table 3).

4. Discussion

In this study, we found that after the booster vaccine, there were no seronegative cases in pwMS except for those treated with fingolimod and ocrelizumab. mRNA vaccine is associated with higher antibody response even if given as a booster after the inactivated vaccine. These results are not surprising because studies have shown that fingolimod and ocrelizumab are responsible for the diminished immunologic response after COVID-19 vaccines (Garjani et al., 2022). The mechanism beyond this effect is that CD20 depleting agents such as ocrelizumab alter the new humoral response by decreasing the number of B-cells (Bar-Or et al., 2020). On the other hand, fingolimod keeps lymphocytes from entering the circulation by holding them hostage in lymph nodes (Mehling et al., 2011). Taking this notion further, we expect patients on azathioprine and cladribine to have lower antibody titer on comparative analyses, considering both as immunosuppressants (Corsini et al., 2000; Sipe et al., 1996). Though we did not observe these predictions, having fewer patients treated with these agents (three and six, respectively) prevented us from making statistically supported conclusions.

Study results show that mRNA as a booster after two doses of inactivated vaccine resulted in a statistically significantly higher number of seropositive cases in the fingolimod group but not in ocrelizumab. This positive effect could be attributed to the fact that although fingolimod prevents lymphocytes from egressing into the circulation, it does not deter B-cells from building the humoral response, although for this effect

to happen, a more potent trigger such as an mRNA vaccine might act on those lymphocytes.

We observed that pwMS treated with DMT had lower antibody titer than healthy controls. Of 339 patients, 73 were treated with ocrelizumab and 106 with fingolimod, making up 53% of the total study population on DMT. These two groups could shift the mean antibody titer of pwMS to the lower side.

By looking at the association of antibody titer after the third dose of the COVID-19 vaccine, we found that having inactivated vaccine as a booster, longer disease duration, and DMT use were correlated with lower antibody response. These findings are valuable because even if a patient has an inactivated vaccine as a standard dose, mRNA as a booster in these populations of MS patients could still mount an antibody response. We explained the relation of disease duration to lower antibody response by the negative effect of ocrelizumab, which is our choice of treatment in the progressive form of MS, and longer disease duration increases the odds of progression. This hypothesis is further supported by the finding that we did not find a significant relationship between age and antibody titer because longer disease duration could also mean advanced age.

Among the limitations of the study are the observational design of the research and the lower number of participants in DMT groups. Also, we did not record the information about previous COVID-19 infections, which could influence the antibody response after the vaccines. This is also true for asymptomatic encounters. This bias could have been prevented by looking at the antibody titer before the vaccination.

5. Conclusions

A vaccination strategy is vital for protection during the pandemic. MS is not a condition that causes immunosuppression per se; however therapeutic choices to treat the disease can alter the function of immune cells. Study results favor the mRNA vaccine as a booster for pwMS on DMT, even if the first two doses were the inactivated vaccine.

Declaration of Competing Interest

The authors report no declarations of interest.

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