

Does the status of micronutrients affect immune response to COVID-19 mRNA vaccination in rheumatoid arthritis patients?

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Abstract

Introduction: Rheumatoid arthritis (RA) is a chronic autoimmune disease that primarily affects the joints. It is recommended to start disease-modifying anti-rheumatic drugs (DMARDs) as soon as RA diagnosis is confirmed. The role of zinc, copper, magnesium, and ceruloplasmin on vaccine-induced immunoglobulin G (IgG) responses remains uncertain, particularly in the context of RA patients receiving ongoing immunosuppressive treatments. This study aimed to explore the association between baseline levels of these trace elements and IgG responses in RA patients following the second dose of the coronavirus disease 2019 (COVID-19) mRNA vaccine, within a 12–16-week interval. It also examined how DMARD treatments and trace element status relate to vaccine-induced immune responses, by comparing to a healthy control group.

Methodology: An observational cross-sectional study was conducted including 79 RA patients and 47 healthy controls. Serum levels of zinc, copper, magnesium, ceruloplasmin, and IgG were measured.

Results: Significant differences in zinc, copper, and magnesium levels were observed between vaccinated and unvaccinated individuals within both patient and control groups ($p < 0.05$). However, there were no significant associations between IgG positivity and trace element levels within any group ($p > 0.05$). Similarly, IgG positivity was not significantly associated with biological or non-biological treatment intake ($p = 0.439$).

Conclusions: COVID-19 mRNA vaccines produced a broadly consistent humoral immune response across participants, regardless of zinc, copper, magnesium, and ceruloplasmin levels. There were no significant associations between trace element status, RA treatment, and long-term antibody durability; suggesting that humoral immunity may be maintained across different nutritional and therapeutic profiles.

Key words: ceruloplasmin; DMARDs treatment; immunoglobulin G (IgG); mRNA COVID-19 vaccine; rheumatoid arthritis; trace elements.

J Infect Dev Ctries 2026; 20(9):1281-1289. doi:10.3855/jidc.22383

(Received 13 September 2025 – Accepted 09 March 2026)

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Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by persistent inflammation, primarily affecting small- to medium-sized joints and leading to pain, stiffness, functional impairment, and progressive joint damage [1]. Although RA can occur at any age, it predominantly affects individuals who are over 35 years and is more common in females than males [2]. In Iraq, the prevalence of RA in Diyala Province has been reported as 0.96%, exceeding the global average, with aging women, particularly unmarried individuals and those with diabetes mellitus, being at higher risk of symptomatic disease [3]. Early diagnosis and prompt initiation of therapy are critical to preventing irreversible joint damage and improving long-term outcomes. Accordingly, the European League Against Rheumatism (EULAR) recommends starting disease-modifying anti-rheumatic drugs (DMARDs) as soon as RA is diagnosed, since spontaneous remission is unlikely without treatment [4].

The coronavirus disease 2019 (COVID-19) pandemic, which emerged in late 2019, led to the rapid development of effective vaccines, with public health authorities recommending at least two doses to reduce the risk of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and severe disease [5]. This development has prompted increased scientific interest in factors influencing vaccine-induced immune responses [6,7]. Trace elements such as zinc (Zn), magnesium (Mg), selenium (Se), and copper (Cu) are essential for immune regulation, inflammatory control, and protection against oxidative stress, and have therefore attracted attention as potential modulators of vaccine responsiveness [8,9]. This topic is particularly relevant for patients with RA, who are at increased risk of infections and are frequently treated with immunomodulatory therapies that may alter immune competence [10].

Despite growing evidence linking micronutrients to immune function, the role of Zn, Cu, Mg, and ceruloplasmin in modulating vaccine-induced

immunoglobulin G (IgG) responses in RA patients, particularly those receiving DMARD therapy, remains insufficiently understood. Therefore, this study aimed to explore the association between baseline levels of trace elements (Zn, Cu, Mg) and ceruloplasmin with IgG responses in patients with RA following the second dose of an mRNA COVID-19 vaccine administered after a 12–16-week interval. The study also examined how DMARD treatment and trace element status relates to vaccine-induced immune responses and compared the responses to that of a healthy control group. This study was exploratory and hypothesis-generating.

Methodology

Study population and design

This observational cross-sectional study was conducted to investigate the role of baseline levels of specific trace elements (Zn, Cu, Mg, and ceruloplasmin) in modulating the immune response in RA patients following the second dose of mRNA COVID-19 vaccine, administered after an extended interval of 12–16 weeks. The findings were compared with those from a healthy control group. The unvaccinated participants were analyzed separately to characterize baseline trace element profiles by disease and treatment status, providing context for interpreting results observed in vaccinated individuals.

A total of 126 subjects were included in the study: 79 RA patients and 47 healthy controls. Adult subjects (≥ 18 years old) who had received two doses of a COVID-19 vaccine were included. The exclusion criteria included use of vitamin or mineral supplements, pregnancy, history of allergic reactions to vaccines, recent COVID-19 infection (≤ 4 weeks), receipt of only one vaccine dose, cancer, recent myocardial infarction, liver disease, renal disease, and other autoimmune diseases.

RA patients were enrolled from the Rheumatology

and Medical Rehabilitation Department at Rizgary Teaching Hospital in Erbil, Iraq, between November 2021 and June 2022. Diagnosis was based on the 2010 American College of Rheumatology (ACR)/EULAR classification criteria or the 2012 ACR classification [11,12]. RA patients continued their treatments throughout the vaccination period. The effect of these treatments on vaccine immunogenicity was assessed by analyzing the IgG levels within each treatment class.

Sample collection and laboratory measurements

Blood samples were collected from all participants 12–16 weeks after receiving their second vaccine dose to measure serum levels of Zn, Mg, Cu, and ceruloplasmin. Vaccine-induced immune response was evaluated by measuring serum IgG antibody levels.

Trace element and ceruloplasmin concentrations were classified as below normal, within normal, or above normal range. These categories were then compared against the corresponding IgG levels to assess the impact of trace element status on vaccine-induced immunity. IgG responses were interpreted using a cut-off index (COI), calculated by dividing the optical density (OD) of each sample by the assay cut-off value:

$$\text{COI} = \frac{\text{OD of sample}}{\text{cutoff value}}$$

Based on this formula, values higher than 1 were considered positive.

Serum concentrations of Mg, Zn, Cu, and ceruloplasmin were determined using colorimetric methods (Randox, Crumlin, United Kingdom). Magnesium was measured using the colorimetric endpoint method (Roche/Hitachi Cobas c Analyzer, Indiana, USA), while ceruloplasmin levels were assessed via immunoturbidimetric assay (Roche/Hitachi Cobas c analyzer, Indiana, USA). Anti-spike IgG levels in serum were quantified using a

Table 1. Baseline characteristics of study participants.

Characteristics	Control		Rheumatoid arthritis patients		Total	p value
	Unvaccinated No. (%)	Vaccinated No. (%)	Unvaccinated No. (%)	Vaccinated No. (%)	No. (%)	
Age (years)						
< 40	22 (88.0)	21 (95.4)	10 (22.2)	5 (14.7)	58 (46.0)	
40–49	0 (0.0)	1 (4.6)	16 (35.6)	10 (29.4)	27 (21.5)	
50–59	2 (8.0)	0 (0.0)	17 (37.8)	9 (26.5)	28 (22.2)	
≥ 60	1 (4.0)	0 (0.0)	2 (4.4)	10 (29.4)	13 (10.3)	< 0.001**
Gender						
Male	8 (32.0)	6 (27.3)	4 (8.9)	7 (20.6)	25 (19.8)	
Female	17 (68.0)	16 (72.7)	41 (91.1)	27 (79.4)	101 (80.2)	0.047**
Residency						
Urban	21 (84.0)	21 (95.4)	37 (82.2)	30 (88.2)	109 (86.6)	
Rural	4 (16.0)	1 (4.6)	8 (17.8)	4 (11.8)	17 (13.4)	0.548**
Total	25 (100)	22 (100)	45 (100)	34 (100)	126 (100)	

*By Chi square test; **by Fisher's exact test.

Table 2. Distribution of COVID-19 vaccine types administered among participants.

Vaccine	Healthy control	RA patients	Total	<i>p</i> value
	No. (%)	No. (%)	No. (%)	
Pfizer	20 (90.9)	33 (97.1)	53 (94.6)	0.099*
AstraZeneca and Pfizer	2 (9.1)	1 (2.9)	3 (5.4)	
Total	22 (100)	34 (100)	56 (100)	

*By Fisher's exact test; RA: rheumatoid arthritis.

sandwich enzyme-linked immunosorbent assay (ELISA) approach with commercial kits (Pishtaz Teb Diagnostic, Tehran, Iran). The reference ranges for the analyzed biomarkers were as follows: Mg (1.6–2.6 mg/dL), Zn (60–120 µg/dL), Cu (70–155 µg/dL), and ceruloplasmin (15–38 mg/dL).

Statistical analysis

Data were analyzed using the Statistical Package for Social Sciences [13]. Normality of continuous variables was assessed using the Shapiro-Wilk test, and non-parametric tests were applied when indicated. The Chi-square test was used to compare the proportions of two or more groups. Fisher's exact test was applied when the expected frequency (value) was less than 5 for more than 20% of the table's cells. The Kruskal-Wallis test was used to compare mean ranks across study groups, and post-hoc tests were conducted using the Bonferroni correction. Spearman's rho correlation coefficient was calculated to assess the strength of associations between continuous variables.

Multiple regression analysis was performed with IgG level as the dependent variable to adjust for the effect of age and gender and to assess the independent contributions of other variables. A *p* value of ≤ 0.05 was

considered statistically significant.

Given the number of comparisons performed across study groups, including biomarkers, vaccination status, and treatment categories, the potential for inflated type I error due to multiple testing was considered. To mitigate this risk, Bonferroni correction was applied where appropriate for predefined primary comparisons. All remaining analyses, particularly subgroup and biomarker-level comparisons, were considered exploratory and were therefore interpreted cautiously. Primary analyses were specified a priori and focused on comparisons between the main treatment groups with adequate sample sizes, whereas secondary and exploratory analyses were conducted to identify patterns and generate hypotheses rather than to draw definitive conclusions.

Results

Four groups were included in the study: Group A, unvaccinated healthy control (*n* = 25); Group B, vaccinated healthy control (*n* = 22); Group C, unvaccinated RA patients (*n* = 45); and Group D, vaccinated RA patients (*n* = 34).

Table 1 shows a significant difference in age distribution between RA patients and controls (*p* <

Table 3. Serum trace element levels by vaccination status and study groups.

	Groups	Mean	SD	Mean rank	<i>p</i> value*	Groups ^a	<i>p</i> value **
Mg (mg/dL)	A. Unvaccinated healthy control	2.18	0.16	103.5	< 0.001	A x B	0.407
	B. Vaccinated healthy control	2.09	0.15	94.7		A x C	< 0.001
	C. Unvaccinated rheumatoid arthritis	1.37	0.32	31.3		A x D	< 0.001
	D. Vaccinated rheumatoid arthritis	1.69	0.30	56.5		B x C	< 0.001
Cu (ug/dL)					0.005	B x D	< 0.001
						C x D	0.002
	A. Unvaccinated healthy control	75.12	16.56	52.7		A x B	0.570
	B. Vaccinated healthy control	74.38	23.15	46.6		A x C	0.135
	C. Unvaccinated rheumatoid arthritis	86.44	28.12	66.3		A x D	0.007
	D. Vaccinated rheumatoid arthritis	93.48	26.25	78.7		B x C	0.038
						B x D	0.001
						C x D	0.135
Zn (ug/dL)	A. Unvaccinated healthy control	93.72	13.73	51.2	0.004	A x B	0.004
	B. Vaccinated healthy control	112.55	19.04	81.5		A x C	0.733
	C. Unvaccinated rheumatoid arthritis	100.11	35.72	54.3		A x D	0.023
	D. Vaccinated rheumatoid arthritis	116.26	45.31	73.0		B x C	0.004
Ceruloplasmin (mg/dL)					0.134	B x D	0.394
						C x D	0.024
	A. Unvaccinated healthy control	22.84	5.04	53.8			
	B. Vaccinated healthy control	26.59	10.65	64.0			
	C. Unvaccinated rheumatoid arthritis	24.27	7.06	60.0			
	D. Vaccinated rheumatoid arthritis	27.74	8.83	75.0			

*By Kruskal-Wallis test; **by post-hoc test (Bonferroni); A, B, C, D, represent the comparisons groups; for example, A x B refers to the comparison between group A (unvaccinated healthy controls) and group B (vaccinated healthy controls).

Table 4. IgG positivity by trace element levels in each of the study groups.

	SARS IgG		Total No. (%)	p value
	Positive > 1 No. (%)	Negative No. (%)		
Unvaccinated healthy control				
Mg				
1.6–2.6	13 (52.0)	12 (48.0)	25 (100.0)	N/A
Cu				
< 70	5 (41.7)	7 (58.3)	12 (100.0)	
70–155	8 (61.5)	5 (38.5)	13 (100.0)	0.320*
Zn				
60–120	12 (50.0)	12 (50.0)	24 (100.0)	
> 120	1 (100.0)	0 (0.0)	1 (100.0)	1.000**
Ceruloplasmin				
< 15	1 (50.0)	1 (50.0)	2 (100.0)	
15–38	12 (52.2)	11 (47.8)	23 (100.0)	1.000**
Total	13 (52.0)	12 (48.0)	25 (100.0)	
Vaccinated healthy control				
Mg				
1.6–2.6	13 (59.1)	9 (40.9)	22 (100.0)	N/A
Cu				
< 70	7 (58.3)	5 (41.7)	12 (100.0)	
70–155	6 (60.0)	4 (40.0)	10 (100.0)	1.000**
Zn				
60–120	8 (53.3)	7 (46.7)	15 (100.0)	
> 120	5 (71.4)	2 (28.6)	7 (100.0)	0.648**
Ceruloplasmin				
< 15	1 (100.0)	0 (0.0)	1 (100.0)	
15–38	12 (63.2)	7 (36.8)	19 (100.0)	
> 38	0 (0.0)	2 (100.0)	2 (100.0)	0.156**
Total	13 (59.1)	9 (40.9)	22 (100.0)	
Unvaccinated rheumatoid arthritis				
Mg				
< 1.6	7 (20.6)	27 (79.4)	34 (100.0)	
1.6–2.6	2 (18.2)	9 (81.8)	11 (100.0)	1.000**
Cu				
< 70	2 (14.3)	12 (85.7)	14 (100.0)	
70–155	7 (23.3)	23 (76.7)	30 (100.0)	
> 155	0 (0.0)	1 (100.0)	1 (100.0)	0.756**
Zn				
< 60	0 (0.0)	1 (100.0)	1 (100.0)	
60–120	9 (23.7)	29 (76.3)	38 (100.0)	
> 120	0 (0.0)	6 (100.0)	6 (100.0)	0.455**
Ceruloplasmin				
< 15	0 (0.0)	3 (100.0)	3 (100.0)	
15–38	8 (20.0)	32 (80.0)	40 (100.0)	
> 38	1 (50.0)	1 (50.0)	2 (100.0)	0.431**
Total	9 (20.0)	36 (80.0)	45 (100.0)	
Vaccinated rheumatoid arthritis				
Mg				
< 1.6	6 (60.0)	4 (40.0)	10 (100.0)	
1.6–2.6	9 (37.5)	15 (62.5)	24 (100.0)	0.276**
Cu				
< 70	3 (60.0)	2 (40.0)	5 (100.0)	
70–155	12 (41.4)	17 (58.6)	29 (100.0)	0.634**
Zinc				
< 60	2 (66.7)	1 (33.3)	3 (100.0)	
60–120	6 (42.9)	8 (57.1)	14 (100.0)	
> 120	7 (41.2)	10 (58.8)	17 (100.0)	0.774**
Ceruloplasmin				
< 15	1 (100.0)	0 (0.0)	1 (100.0)	
15–38	13 (43.3)	17 (56.7)	30 (100.0)	
> 38	1 (33.3)	2 (66.7)	3 (100.0)	0.765**
Total	15 (44.1)	19 (55.9)	34 (100.0)	

*Calculated by the Chi square test; **calculated by Fisher's exact test.

Table 5. Serum trace element levels by type of biological treatment.

Trace elements	Biological treatment†	Mean	SD	Mean rank*	p value	Pairwise comparisons	p value**
Mg (mg/dL)	None	1.64	0.32	47.45	0.012	A vs B	0.021
	Infliximab	1.42	0.35	33.57		A vs C	0.600
	Rituximab	1.68	0.21	52.05		B vs C	0.019
Cu (ug/dL)	None	96.37	22.99	47.03	0.208	A vs B	N/A
	Infliximab	86.87	29.18	36.33		A vs C	N/A
	Rituximab	89.22	27.97	39.65		B vs C	N/A
Zn (ug/dL)	None	112.15	36.66	43.55	0.526	A vs B	N/A
	Infliximab	106.90	42.21	38.99		A vs C	N/A
	Rituximab	99.70	44.99	33.85		B vs C	N/A
Ceruloplasmin (mg/dL)	None	29.90	9.74	49.65	0.022	A vs B	0.009
	Infliximab	23.65	6.50	33.98		A vs C	0.652
	Rituximab	27.50	8.30	45.70		B vs C	0.136

*Calculated by the Kruskal-Wallis test; **calculated by post-hoc test (Bonferroni); †Adalimumab was not included for the sake of statistical analysis (n = 1 only). N/A, not applicable (because the difference was not significant); A: no biological treatment; B: Infliximab; C: Rituximab.

the majority of RA patients were aged ≥ 40 years. Among unvaccinated participants, females constituted a significantly higher proportion of RA patients compared with controls (91.8% vs 68%, $p = 0.047$). Most participants (87%) resided in urban areas and there was no significant difference in residence distribution between groups ($p = 0.548$).

Most vaccinated participants received the Pfizer vaccine, either alone (93%) or in combination with AstraZeneca (7%). There was no significant difference between the healthy control and RA groups in terms of the vaccine type received ($p = 0.099$) (Table 2).

Serum Mg levels differed significantly among the study groups ($p < 0.001$), with the highest mean ranks observed in unvaccinated healthy controls (Group A) and the lowest in unvaccinated RA patients (Group C); overall, Mg levels were higher in healthy controls than in RA patients. Cu levels also showed a significant intergroup difference ($p = 0.005$), with higher levels in RA patients than in controls; however, no differences were observed between vaccinated and unvaccinated subgroups, regardless of RA status. Zn levels varied significantly across groups ($p = 0.004$), with vaccinated individuals, both RA patients and controls, exhibiting higher levels than unvaccinated individuals, and all vaccinated–unvaccinated comparisons being statistically significant. In contrast, ceruloplasmin levels did not differ significantly among groups ($p = 0.134$), as shown in Table 3.

No significant associations were found between IgG positivity and the trace element levels within any of the study groups ($p > 0.05$), as shown in Table 4.

When stratified by treatment type, serum Mg levels differed significantly between patients receiving biological and non-biological therapies ($p = 0.012$), with the highest mean level observed in rituximab-treated patients (1.68 mg/dL). Slightly elevated Mg levels were also observed in patients not receiving biological treatment (1.64 mg/dL). Ceruloplasmin levels varied significantly across treatment groups ($p = 0.022$), with the lowest mean level observed in infliximab-treated patients (23.65 mg/dL) compared with patients not receiving biological therapy (29.9 mg/dL). In contrast, serum Cu and Zn levels did not differ significantly between treatment groups, as shown in Table 5.

No significant associations were observed between IgG positivity and DMARDs treatment type (biological vs. non-biological; $p = 0.635$), nor between IgG positivity and the type of biological treatment ($p = 0.310$), as, shown in Table 6.

Age emerged as an independent determinant of IgG levels in multivariable analysis, with each additional year of age associated with 0.026-unit increase in antibody responses. In contrast, RA status, gender, serum Mg, Cu, Zn, and ceruloplasmin did not show independent associations with IgG levels after adjustments, as shown in Table 7.

Table 6. IgG positivity across different types of treatments for rheumatoid arthritis.

	IgG positivity		Total	p value
	Positive	Negative		
Biological treatment intake				
Yes	17 (29.3)	41 (70.7)	58 (100.0)	0.635*
No	7 (35.0)	13 (65.0)	20 (100.0)	
Biological treatment intake**				
None	7 (35.0)	13 (65.0)	20 (100.0)	0.310*
Infliximab	16 (33.3)	32 (66.7)	48 (100.0)	
Rituximab	1 (10.0)	9 (90.0)	10 (100.0)	
Total	24 (30.8)	54 (69.2)	78 (100.0)	

*By Chi-square test; **Adalimumab was not included for the sake of statistical analysis (n = 1 only).

Table 7. Multiple linear regression analysis examining factors associated with SARS-CoV-2 IgG levels (dependent variable).

	Unstandardized coefficients		Standardized coefficients		95% Confidence interval for B		
	Unstandardized coefficients	Std. error	Beta	t	Sig.	Lower bound	Upper bound
(Constant)	1.023	1.200		0.852	0.396	- 1.355	3.400
Age (Year)	0.026	0.012	0.271	2.055	0.042	0.001	0.050
Gender (Female)	0.312	0.348	0.087	0.895	0.372	- 0.378	1.001
Rheumatoid arthritis	- 0.914	0.506	- 0.315	- 1.806	0.074	- 1.917	0.088
Vaccination	0.290	0.279	0.103	1.040	0.300	- 0.262	0.843
Mg (mg/dL)	- 0.066	0.495	- 0.020	- 0.133	0.894	- 1.047	0.915
Cu (ug/dL)	0.006	0.008	0.114	0.809	0.420	- 0.009	0.021
Zn (ug/dL)	- 0.005	0.004	- 0.132	- 1.290	0.200	- 0.014	0.003
Ceruloplasmin (mg/dL)	- 0.012	0.024	- 0.071	- 0.507	0.613	- 0.061	0.036

Discussion

This observational cross-sectional study investigated the role of baseline levels of specific trace elements (i.e., Zn, Cu, Mg, and ceruloplasmin) in modulating the immune response in RA patients following the second dose of an mRNA COVID-19 vaccine administered after an extended interval of 12–16 weeks. The study also evaluated the effect of DMARDs treatment on trace element status and its subsequent effect on vaccine-induced immune responses, by comparing with a healthy control group.

The primary finding of this study was that mRNA COVID-19 vaccine-induced IgG responses were not significantly associated with baseline levels of Mg, Zn, Cu, or ceruloplasmin in either RA patients or healthy controls. Despite significant differences in trace element concentrations between RA patients and controls, these differences did not translate into variations in IgG positivity following vaccination. This suggests that, under the conditions studied, baseline trace element status does not play a major role in modulating vaccine-induced humoral immune responses. These findings are consistent with previous studies reporting limited associations between baseline micronutrient levels and vaccine-induced antibody responses. Studies examining influenza and SARS-CoV-2 vaccines have shown that, although trace elements are essential for immune homeostasis, their influence on antibody production following vaccination may be modest in individuals without marked deficiencies [14,15].

The findings of this study showed that no significant differences in vaccine-induced humoral immune responses were observed among RA patients receiving different DMARD regimens, despite significant differences in serum Mg and ceruloplasmin levels across treatment groups. In particular, patients receiving non-biological DMARDs exhibited higher levels of Mg and ceruloplasmin than those receiving biological DMARDs. This finding aligns with prior studies evaluating COVID-19 vaccine immunogenicity

in RA patients, which have reported broadly preserved humoral immune responses across different DMARD treatment strategies [16,17]. Similarly, a study assessing the short-term immune response to the inactivated Sinopharm COVID-19 vaccine in healthy individuals found no significant association between baseline levels of Mg, Zn, or selenium and post-vaccination IgG responses [18]. Notably, that study differed from the present study in population (healthy individuals), vaccine platform (inactivated virus), and timing of immune assessment (2 weeks post-vaccination); whereas this study evaluated RA patients receiving an mRNA vaccine with immune responses assessed at 12–16 weeks. Together, these findings suggest that long-term antibody production following COVID-19 vaccination is largely maintained irrespective of baseline trace element status or RA treatment type.

The findings showed that lower ceruloplasmin and Mg levels in biologic-treated patients may plausibly reflect better disease control rather than a direct effect on vaccine responsiveness. Ceruloplasmin is a well-recognized positive acute-phase reactant, comparable to C-reactive protein, with circulating levels that typically increase in response to systemic inflammation [19]. Biological DMARDs, including tumor necrosis factor (TNF) inhibitors and interleukin-6 (IL-6) blockers, are known to exert more potent and rapid anti-inflammatory effects than conventional DMARDs [20], which may account for the reduced levels of ceruloplasmin observed in these patients. This interpretation is supported by previous studies demonstrating that biological DMARDs reduce oxidative stress and inflammatory markers more effectively than non-biological DMARDs [21]. However, in the absence of concurrent clinical or biochemical measures of disease activity (e.g. disease activity scores [DAS28], erythrocyte sedimentation rate [ESR], or CRP), this explanation remains speculative and should be considered hypothesis-generating.

The multivariable regression analysis showed that

age was the only independent predictor of IgG levels after adjustment for demographic, clinical, and biochemical factors. Trace elements, ceruloplasmin, RA status, gender, and treatment type were not independently associated with IgG responses. This finding is consistent with existing evidence indicating that vaccine immunogenicity is more strongly influenced by host-related factors such as age-related immune changes and inflammatory status, rather than by micronutrient status alone [22].

RA is more prevalent in females, and sex hormones play an important role in immune regulation. Estrogen is generally considered immunostimulatory and may contribute to heightened immune responsiveness [23]. However, despite the female predominance in the RA cohort, gender did not independently influence IgG responses in the adjusted analyses.

Limitations

This study has several limitations. First, the study's sample size limited the power of subgroup analyses, particularly within specific DMARD categories, increasing the risk of type II error; therefore, non-significant results should be interpreted cautiously. Second, age and gender distributions differed between RA patients and controls. Although statistical adjustment was performed, residual confounding cannot be entirely excluded. Third, only humoral immune responses were evaluated; cell-mediated immunity, and neutralizing antibody activity, which are important components of vaccine-induced protection, were not assessed. Fourth, unvaccinated participants were included to characterize baseline trace element profiles; however, this limits direct interpretability with respect to vaccine-induced immune responses. Finally, immune responses were assessed at a single post-vaccination time point, precluding evaluation of temporal dynamics or mechanistic relationships.

Strengths

Despite these limitations, the study has several strengths. The RA cohort was representative of the disease population, with a predominance of older female participants, consistent with the known epidemiological pattern [24]. Further, to our knowledge, this is the first study to investigate the association between baseline levels of Zn, Cu, Mg, and ceruloplasmin and mRNA COVID-19 vaccine-induced immune responses in RA patients.

Conclusions

Under the study conditions, COVID-19 mRNA

vaccines produced a broadly consistent humoral immune response across individuals, regardless of Mg, Zn, Cu, and ceruloplasmin levels. No significant association was detected between the examined trace elements and vaccine-induced antibody responses. These findings suggest that routine measurement or modification of trace element status is unlikely to be necessary for optimizing vaccine responses in RA patients or healthy controls.

Similarly, no significant association was observed between trace element status, RA treatment, and long-term antibody durability, indicating that humoral immunity may be broadly maintained across diverse nutritional and therapeutic profiles. However, these findings should be interpreted with caution, as the cross-sectional study design limits the ability to infer causality or temporal inferences.

Acknowledgements

The author would like to express her sincere gratitude to all the rheumatologists for their roles in the diagnosis of the study patients and for supporting throughout the course of this study: Dr. Avin Maroof, Dr. Neyaz Shekh Jawad Barzaji, and Dr. Shwan Qader; and to midline medical lab for their professional work. The author would also like to appreciate her family—Zainab Al-Khayat and Abbas Al-Khayat—for their continuous support.

Ethical statement

This study was conducted in line with the principles of the Helsinki Declaration. Approval was granted by the Hawler Medical University Ethics Committee (HMUCPh.120220-56). Informed consent was obtained from all individual participants included in the study prior to enrolment.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Author's contributions

The author solely conceived, designed, and conducted the study; and prepared the manuscript.

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Conflict of interest

No conflict of interest is declared.

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