Jundishapur Journal of Microbiology
Volume 16 · Issue 12 · e142026

OPEN PEER REVIEW

The Protective Effect of Serum Levels of Vitamins C, D, and E and IgG and IgM Antibodies in Individuals Vaccinated Against COVID-19 and Experienced Disease Relapse

Ashkan Alamdary, Alireaza Gholami, Maryam Shahali, Delaram Doroud, Rasul Moukhah, Mohammad Javad Hossein Tehrani, Rajab Mardani, Nayebali Ahmadi

SUBMITTED	REVISED	ACCEPTED	DOI
14 Oct 2023	14 Nov 2023	8 Dec 2023	10.5812/jjm-142026

About this report

This open peer review (OPR) report shows the public comments made on this article during peer review, by the reviewers, the associate editor and the editor-in-chief, for every revision round, together with the authors' replies. Comments appear only with the consent of the people who wrote them. Reviewers who chose to sign their report are named; the others are shown as "Referee". Comments are edited for language only, and confidential remarks to the editors are not included.

More about open peer review: brieflands.com/knowledgebase/opr

Contents

1 Original submission Referee 1 2
2 Revision 1 3
Authors' reply to reviewers 4
Reviewer and editor comments 13

1 Original submission

1 comment · 22 Oct 2023

Referee 1 Reviewer

MAJOR REVISION 22 Oct 2023

Regarding the manuscript entitled “The serum levels of vitamins C, D, and E, and levels of antibodies IgG and IgM has a protective effect in people previously vaccinated against COVID-19 with relapse of the disease”:

Below are some suggestions that the author may wish to consider to improve their manuscript:

Title:
It would be recommended to change the title to “The serum levels of vitamins C, D, and E, and levels of IgG and IgM antibodies have a protective effect in people previously vaccinated against COVID-19 with relapse of the disease”.

Introduction
The following sentence appears at the end of the introduction: “Two groups of hospitalized individuals have been investigated for C, D and E and compared with non-COVID-19 individuals”. What are the two groups of hospitalized individuals?

Method:
-Please mention the type of study in the method section (cross-sectional, retrospective cohort, etc.).
-Please change “100 patients from ICU (severe), 100 hospitalized patients (non-severe)” to “100 patients hospitalized in ICU (severe), 100 patients hospitalized in the wards (non-severe)”.
-Please mention the sensitivity and specificity of the ELISA kits used.
-The authors just mentioned swab sample collection; how about serum sample collection?
-Please mention the normal ranges of vitamins.
-What is the definition of disease relapse in this study?

Result
Table 4: Please rewrite “IgG Stat (+or -)” for clarity.

Discussion:
Please mention the full name of SHBG.

2

Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Thanks to the attention of the honorable Reviewers The requested amendments are submitted as an attachment

Reviewers 1

In the conclusion section the authors have claimed that:

Micronutrients act as immunomodulators and protect the host immune response, thus preventing immune evasion by pathogenic organisms. These findings could suggest that adequate levels of vitamins C, D, and E trio may contribute to preventing SARS-CoV-2 reinfection and the disease severity, especially if the vaccine efficacy is uncertain.

The conclusion section is not supported by the data. Claiming that micronutrients are immunomodelators cannot be inferred from this study. Many biological studies are needed to unravel the biological mechanisms and verify if these nutrients have immunomodelator effects. Also, you cannot infer that these vitamins can prevent Covid-19 infection, as having higher antibody levels does not necessarily predict the risk of covid-19 reinfection.

1-Vitamins C, D and E act as helpers in the immune system and help the host's immune response. These findings could suggest that adequate levels of vitamins C, D, and E may be helpful in preventing SARS-CoV-2 reinfection and disease severity, especially if vaccine efficacy is uncertain.

Keywords: the keyword, “Immunoglobulins” does not seem to be a good keyword for this study.

2-SARS-CoV-2, COVID-19, Vitamins, immune system

Table 1. Please write the exact number of the P values. Also please provide the mean (SD) or Median (IQR) for the measurements in each category.

	ICU VS Ward	ICU VS Control	Ward Vs Control
VitC level	NS P=0.06 1.58±0.069	+ (p<0.05) 1.61±0.084	NS P=0.08 1.79±0.17
VitD level	+++ (p<0.001) 24.71±2.28	+++ (p<0.001) 27.41±6.09	+++ (p<0.001) 29.02±3.8

VitE level	**+++ (p<0.001) 10.07±2.39**	**+++ (p<0.001) 10.88±3.54**	**+++ (p<0.001) 12.57±1.15**
IgM level	**+++ (p<0.001) 2.62±1.44**	**+++ (p<0.001) 2.42±1.72**	**++ (p<0.01) 1.4±0.28**
IgG level	**+++ (p<0.001) 7.88±4.86**	**+++ (p<0.001) 8.13±5.22**	**NS P=0.13 11.57±0.36**

Figure 1. Please indicate the unit of measurement in the Y axis.

The comparison of immunoglobulin (positive>1.1) and vitamin levels(ng/ml) among patients and healthy cases. The mean of each bar is presented in the figures. A) All vitamin levels were decreased, inversely with the disease severity. B) The IgM level was highest in the ICU and lowest in the control group; oppositely, the IgG level was highest in the control and lowest in ICU patients.

Table 2: Please provide percent in each cell of the table.

		Studied cases			
		Ward	ICU	Control	Total
D,E, and C vitamins	Non-Deficient	99	94	100	293(97.66%)
	Deficient	1	6	0	7(2.33%)
D and C Vitamins	Non-Deficient	98	87	99	284(94.66%)
	Deficient	2	13	1	16(5.33%)
E and C Vitamins	Non-Deficient	99	96	100	295(98.33%)
	Deficient	1	4	0	5(1.66%)

Table 3. In the footnote of the table, please provide information on the type of statistical test, each P value has been resulted from.

The comparison of vitamins and immunoglobulins ratios between patients with the Student-t test, and the Mann–Whitney U test (severe & non-severe) and the control group. The p values<0.05 present significant differences.

Table 4. Please provide percent in each cell of the table.

		Studied cases Stat			
		Ward	ICU	Control	Total
IgG Stat (+or -)	Negative	3	20	1	24(8%)
	Positive	97	80	99	276(92%)
IgM Stat (+or -)	Negative	76	14	99	189(63%)
	Positive	24	86	1	111(37%)

Figure 2. The figure should be accompanied by a legend or table below the curve, that represent the cut of point and AUC for each biomarker.

Receiver operating characteristic (ROC) curves show the sensitivity and specificity of performed laboratory tests. The area under the curve (AUC) and p values were studied to find the best biomarker for the disease. In this study, IgM (AUC=0.783, p=0.000) and VitD-to-VitE ratio (AUC=0.573, p=0.000) have shown the best biomarker for disease diagnosis between patients and healthy cases. IgM (AUC=0.953, p=0.000) and IgG (AUC=0.684, p=0.000) also were proper biomarkers for comparison of ICU and healthy subjects. IgM (AUC=0.613, p=0.006) was a satisfactory biomarker among ward and healthy cases. In the comparison of ward and ICU patients, IgG-to-IgM ratio (AUC=0.947, p=0.000) and IgG (AUC=0.935, p=0.000) were found to be the best biomarkers, followed by VitE (AUC=0.853, p=0.000), VitE-to-VitC ratio (AUC=0.777, p=0.000), and VitD (AUC=0.664, p=0.000)

Reviewers 2

Regarding the manuscript entitled “The serum levels of vitamins C, D, and E, and levels of antibodies IgG and IgM has a protective effect in people previously vaccinated against COVID-19 with relapse of the disease:

Below are some suggestions that the author may wish to consider to improve their manuscript:

Title:
It would be recommended to change the title to “The serum levels of vitamins C, D, and E, and levels of IgG and IgM antibodies have a protective effect in people previously vaccinated against COVID-19 with relapse of the disease”

The serum levels of vitamins C, D, and E, and levels of IgG and IgM antibodies have a protective effect in people previously vaccinated against COVID-19 with relapse of the disease

Introduction
In the following sentence: Two groups of hospitalized individuals have been investigated for C, D and E and compared with non-COVID-19 individuals, what are the two groups of hospitalized individuals at the end of the introduction?

Considering that vitamins C, D and E are potentially involved in the management of covid-19, in the present study, serum levels of these vitamins were studied in previously vaccinated against covid-19 with disease relapse. Two groups of patients hospitalized in the ward and ICU in the hospital have been examined to measure the levels of vitamins C, D and E and compared with non-Covid-19 patients.

Method:
-Please mention the type of the study in the method section (Cross-sectional, retrospective cohort, etc.)
-Please change “100 patients from ICU (severe), 100 hospitalized patients (non-severe)” to “100

patients hospitalized in ICU (severe), 100 patients hospitalized in the wards (non-severe)"
-Please mention the sensitivity and specificity of the ELISA kits used.
-The authors just mentioned swab sample collection, how about serum sample collection?
-Please mention the normal ranges of vitamins.
-What is the definition of disease relapse in this study? **disease relapse (Body pain, shortness of breath, cough, fever, ...)**

In this study, which will be carried out as a case-control (case-control) , total of 300 subjects (148 males and 142 females), received two doses of vaccine with a mean age of 47.21 years were admitted to the Ayatollah Kashani Hospital (Tehran, Iran). The cases included 100 patients hospitalized in ICU (severe), 100 patients hospitalized in the wards (non-severe). The pharyngeal swab specimens were collected from all participants for SARS-CoV-2 PCR test (SANSURE Novel Coronavirus Nucleic Acid Diagnostic Kit) , And those who tested positive for Covid-19, 10 cc of blood samples were taken in tubes without anticoagulants for testing.

COVID-19 IgM and IgG detection

The IgM and IgG antibodies were quantified using antibody detection ELISA kits against SARS-CoV-2 from Padtan Gostar Issar Company (Tehran, Iran). The assays were performed according to the instruction manuals of the kits with a sensitivity of 81.82 and a specificity of 94.83 and normal range of positive>1.1.

25-Hydroxy vitamin-D3 quantification

The vitamin D was quantified with a 25-Hydroxyvitamin D3 competitive ELISA kit from Pishtaz Teb Company (Tehran, Iran). All the assay steps were performed by conforming to the manufacturer's instructions with a sensitivity of 96.56 and a specificity of 99.8 and normal range of 30-70 ng/ml .

Vitamin C and vitamin E quantification

The Abbexa Vitamin ELISA kit (Cambridge, UK) is based on competitive enzyme-linked immunoassay technology. In the kit vitamin C (with a sensitivity of 97.56 and a specificity of 98.4 and normal range of 1.2-2.0 ng/ml) (ascorbic acid) and vitamin E (with a sensitivity of 97.56 and a specificity of 98.4 and normal range of 5.5-17 ng/ml) (alpha tocopherol)) were previously coated with antibodies against each of them in a 96-well plate. Standards, test samples and biotin conjugation reagents were added to the wells and incubated according to the manufacturer's instructions. A competitive inhibition reaction between biotin-labeled and unlabeled vitamin molecules was performed for the coated antibody. The test was performed according to the manufacturer's instructions.

Result
Table 4: Please rewrite “IgG Stat (+or -)” in terms of clarity.

Table 4. The frequency of IgM and IgG positive(>1.1) and negative (<1.1) tests among studied cases.

Discussion:
Please mention full name: SHBG

sex hormone binding globulin

Reviewers 3

This study evaluate serum levels of vitamins C, D, and E, and levels of antibodies IgG and IgM as a protective effect in people previously vaccinated against COVID-19 with relapse of the disease.
This paper deserves to be taken into consideration for publication in JJM; however some revisions need to be addressed:

1) In introduction, it needs to add more details of the relationship between immune responses with vitamins C, D, E and also effect of vitamins on the development of immune response, especially humoral responses (as a background information).
There is interest in the antioxidant role of vitamin C, as research has found the vitamin to neutralize free radical molecules, which in excess can damage cells. Vitamin C is also involved in the body's immune system by stimulating the activity of white blood cells and Vitamins C and D are well-established as immune-supportive micronutrients
Vitamin D can modulate the innate and adaptive immune responses. Deficiency in vitamin D is associated with increased autoimmunity and an increased susceptibility to infection
and vit E helps keep the immune system strong against viruses and bacteria. also helps form red blood cells and widen blood vessels to keep blood from clotting inside them. Cells also use vitamin E to interact with each other
vitamins C, D and E stimulate Th2 response which in turn promotes humoral immunity.

2) In results, rephrase table 1 content to reflect the title (legend) of table. Such as include antibody /vitamin level ...

	ICU VS Ward	ICU VS Control	Ward Vs Control
VitC level	NS P=0.06 1.58±0.069	+ (p<0.05) 1.61±0.084	NS P=0.08 1.79±0.17
VitD level	+++	+++	+++

	(p<0.001) 24.71±2.28	(p<0.001) 27.41±6.09	(p<0.001) 29.02±3.8
VitE level	+++ (p<0.001) 10.07±2.39	+++ (p<0.001) 10.88±3.54	+++ (p<0.001) 12.57±1.15
IgM level	+++ (p<0.001) 2.62±1.44	+++ (p<0.001) 2.42±1.72	++ (p<0.01) 1.4±0.28
IgG level	+++ (p<0.001) 7.88±4.86	+++ (p<0.001) 8.13±5.22	NS P=0.13 11.57±0.36

3) In results, authors should determine what the bar on the graphs represents. (SEM or SD or .....)
The comparison of immunoglobulin (positive>1.1) and vitamin levels(ng/ml) among patients and healthy cases. The mean of each bar is presented in the figures. A) All vitamin levels (mean ± SD)were decreased, inversely with the disease severity. B) The IgM level (mean ± SD)was highest in the ICU and lowest in the control group; oppositely, the IgG level was highest in the control and lowest in ICU patients.

4) In results, rephrase table 1 content, because it is not statistically informative.
OK,DONE

5) In discussion, given that the two types of vaccines have two different platforms (Adeno-vector based and inactivated vaccines), can the effects of these vitamins in creating responses be different? State in the discussion.
However, no significant differences were observed between AstraZeneca or Sinopharm vaccines in terms of mean anti-SARS-CoV-2 immunoglobulin levels, as well as the effects of vitamins A, C, and D among the different study groups, and it can be stated that vitamins The beneficial effects show themselves regardless of the type of vaccine.

6) In discussion, is it possible that the pattern of results obtained in this study based on vaccines is the same as the pattern of natural infection? State in the discussion.

Practical evidence in this work showed that both induced immunity obtained by anti-Covid-19 vaccines and natural immunity obtained after SARS-CoV-2 infection have a protective function against SARS-CoV-2 infection, but with Due to the lack of more samples, it is not possible to point out this issue with certainty

2 Revision 1

0 comments

No public comments in this round.