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Original Research Article

Efficacy of COVID-19 vaccine in elderly Indian population with Vitamin D and **Iron deficiency**

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ABSTRACT

Introduction: SARS-COV-2 is has led to a worldwide pandemic COVID-19 vaccine roll out proceeds worldwide, attention is increasingly being paid to vaccination of older people that can affect vaccine efficacy. Iron deficiency anaemia and Vitamin D deficiency is a leading global cause of years lived with disability.

Aims and Objective: To study the immune response in Vitamin D, Iron deficiency when compared to non deficient covid vaccinated recipients.

Methods: This is a hospital based observational cross-sectional study done between April 2021 to September 2021 in elderly Indian population. Total 360 vaccine recepients attending OPD post vaccination between 15-45 days were measured anti-RBD IgG antibody & anti-spike S1/S2 antibodies, vitamin D, Iron, TIBC, HB, levels in above 60 years.

Results: Out of 360 vaccine recipients, 46 had COVID-19, and we have excluded them from the study. Of the 314, They were grouped as Group I: Vitamin D deficient, Group II; Iron Deficiency, Group III :has both Deficiency of Vitamin D & Iron. GroupIV: has No Deficiency In Study I: We Compared age, sex hematological indices, vitamin D. In 314 Vaccine recipient 57.3% Male, 42.7% were female In Study II: 35% Vitamin D deficient ,30.6% Iron deficiency,23.6% had both deficiency 10.8% have no deficiency. In Study III Responses were evaluated post-vaccine RBD IgG concentration and Spike antibodies were each significantly higher among the No deficiency recipients, when compared to Iron & vitamin D Deficient recipients.

Conclusions: An integrated approach is required to better understand aging & how vaccines work in elderly which will help in improving the immune response after vaccination.

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1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) vaccine candidates are being evaluated, with the goal of conferring immunity on the highest percentage of people who receive the vaccine as possible. Importantly vaccine efficacy, depends not only on the vaccine, but also on characteristics of the vaccinated and many extrinisic and

intrinisic factors influence the immune response.¹ Vaccines are designed to give the adaptive immune system a lasting memory of viral or bacterial components, so that it can quickly and effectively respond when confronted with the actual pathogens. Recently researchers have highlighted nutrition as a possible factor influencing the effectiveness of the COVID-19 vaccine,² establishes a link between vitamin D levels, iron levels and an adequate immune response., The immune system, our first line of defense against infections, is dependent on the host nutritional status.³ Since

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the COVID-19 outbreak many scientific papers have been published regarding the role of selected nutrients in support of a well-functioning immune system.⁴ Factors influencing immunosenescence. Aging is influenced by multifaceted extrinsic and intrinsic factors leading to a progressive impairment of the function of various organs and systems. including the immune responsiveness, leading to increased susceptibility to infectious diseases and to reduced response to vaccination. Iron deficiency anaemia is a leading global cause of years lived with disability, especially affecting Low Income and Middle Income Countries populations.⁵Recent clinical, studies have converged to show that availability of iron is as key factor regulating the development of T and B cell responses to infection and immunisation.⁶ Exciting new evidence points to iron deficiency (ID) as a key modulator of innate and adaptive immune responses. A mutation in transferrin receptor-1 (which transports iron into lymphocytes) causes severe immunodeficiency in humans, with low levels of circulating IgG and decreased T- and Bcell proliferation.⁷ ID in mice attenuates T-cell-dependent and -independent antigen-specific antibody responses, and impairs B-cell proliferation.⁸ Iron deficiency anemia affects many older adults,⁹ who are vulnerable to COVID-19 infection.

Furthermore, it has long been recognized that a large percentage of the worldwide population has low levels of vitamin D, which could currently be exacerbated by restrictive measures and their impact on sun exposure. Vit-D deficiency occurs more frequently in older adults than in young ones because of their lower endogenous Vit-D synthesis, and because of their often reduced dietary intake.¹⁰ vitamin D receptor is expressed on immune cells such as B cells, T cells, and antigen-presenting cells, and because these cells can synthesize the active vitamin D metabolite, vitamin D also has the potential to modulate innate and adaptive immune responses.¹¹ There is substantial variation between individuals in the immune response to vaccination. Vitamin D enhances epithelial integrity and induces antimicrobial peptide (eg, cathelicidin) synthesis in epithelial cells and macrophages^{12,13} directly enhancing host defence., The effect of vitamin D on immune system has been recognized, ¹⁴ at a cellular and molecular level, They mainly targets helper T cell activity (Th1) by inhibiting the secretion of both IL-2 and IFN-gamma and by suppressing the secretion pro-Th1 cytokine IL-12 by antigen- presenting cells.¹⁵

With the emergence of relatively vaccine-resistant SARS-CoV-2 variants, characterising the speed and duration of response to COVID-19 vaccines in iron deficiency, hypovitaminosis D is necessary to measure the expected benefits of the vaccination.¹⁶ Immune responses to COVID-19 vaccines appear to be slower to develop in older people. To date, only few studies tried to investigate the impact of hypovitaminosis D, Iron deficiency anemia on

-immune response in COVID-19 Vaccine efficacy.

Aim of study is to interrogate the immune responses to COVID-19 vaccines in elderly population and their effects of iron deficiency, Vitamin d deficiency on the immunogenicity of the vaccines.



Fig. 1: Aging mechanism 5

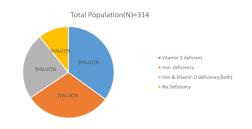


Fig. 2: Demographic representation of the deficiencies in elderly population

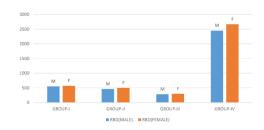


Fig. 3: The RBD antibodies patterns in all deficiency and no deficiency covid vaccine recipients

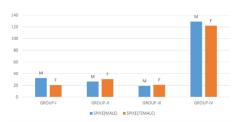


Fig. 4: The anti-spike antibodies pattern in vitamin D deficient coved vaccine recipients

Table 1: Demographic and Hemotalogical profile of study population (N = 314)

Parameters	Male	Female	
N (314)	180	134	
AGE	69±7.2	64 ± 9.4	
Hemoglobin(gm/dl)	14±5.6	11±4.2	
Iron $(\mu g/ml)$	70.5±14.3	60±15.6	
TIBC (μ g/ml)	220±62.9	115±72.6	
Vitamin D (ng/ml)	19.6 ± 11.0	16.7±10.6	

Table 2: Comparision of vitamin d deficiencient, iron deficiency, both deficiency and with no deficiency in vaccine recipient

Parameters	N(314)	Male(180)	Female(134)
Vitamin D deficient	110(35%)	65(36.1%)	45(33.6%)
Iron deficiency	96 (30.6%)	46(25.5%)	50(37.3%)
Iron & Vitamin D deficiency(Both)	74 (23.6%)	49 (27.2%)	25 (18.6%)
No Deficiency	34 (10.8)	20(11.1%)	14(10.4%)

Table 3: Comparison of characteristics vaccine responses in between vitamin d deficient iron deficient, both deficiency and no deficiency vaccine recepients

Parameters	SEX	RBD IgG Antibodies (U/ml)	Spike IgG Antibodies(AU/ml	P-Values		
				VIT- D/ND	ID/ND	BOTH/ND
Vitamin-D Deficient	М	550±241.1	32.5 ± 9.82	< 0.001	< 0.001	< 0.001
(VIT-D) F	F	570 ± 212.1	20.4 ± 8.27	< 0.001	< 0.001	< 0.001
Iron deficiency(ID)	М	460±220.6	26.4 ± 7.22	< 0.001	< 0.001	< 0.001
	F	500 ± 320.2	30.6 ± 6.88	< 0.001	< 0.001	< 0.001
Iron &VitaminD	М	284±222.1	18.9 ± 10.6	< 0.001	< 0.001	< 0.001
Deficiency(Both)	F	296±324.6	20.7±11.2	< 0.001	< 0.001	< 0.001
No deficiency(ND)	М	2450 ± 420	129±12.72	< 0.001	< 0.001	< 0.001
	F	2665±403.0	122 ± 66.7	< 0.001	< 0.001	< 0.001

M-Male: F-Female p<0.001 Highly significant

2. Materials and Methods

This is a hospital based Observational Retrospective cohort study of patients of 360 Vaccine recipients was done in Department of Biochemistry, Asian Institute of Gastroenterology, hospital Hyderabad in South India vaccinated either with ChAdOx1 nCoV-19 (Covishield) and BBV152 (Covaxin) vaccine.

2.1. Inclusion criteria

All elderly people above 60 years attending outpatient were included in the study and divided. On inclusion, the eligibility criteria were assessed and medical history was recorded.

2.2. Exclusion criteria

Cases of previous covid, macrocytic blood picture, haemolytic anemia, CKD, CLD, patients with history of acute blood loss, blood transfusion, infections in the last 3 months, were not included in the study.

2.3. Specimen collection and processing

3ml blood was collected in a vial containing dipotassium EDTA for complete blood count. Fresh peripheral smear was made. 4 ml of venous blood was collected in an iron free plastic tube. Serum was separated for hematological and clinical chemistry were obtained. Serum was used for testing was for quantitative anti RBD IgG antibodies (Roche) by elecro chemiluminescence immunoassays (ECLIA), Total Antibodies against Nucleocapsid (Roche) ECLIA, anti-spike IgG S1/S2 antibodies(Diasorin) against SARS-COV-2 chemiluminescence immunoassays (CLIA), Vitamin D estimation with ECLIA (Roche) from all age group Above 60. Complete blood count was done using Beckman Coulter autoanalyzer. Peripheral smear: Wright stained peripheral smear was examined for RBC morphology Iron studies: Iron studies were done with serum iron (Iron-Ferrozine, Beckman Coulter) serum TIBC (Beckman Coulter AU5800). Definitions and cut offs of Vitamin D Vitamin D deficiency was defined as 25OHVitamin D level below 20 ng/mL, according to the last consensus report by Semposetal¹⁷

Ethical committee approval has been received and that the informed consent of all participating subjects was obtained. Ethical approval was given by AIG Hospitals Institutional ethics committee (Ref No: AIG/IEC-POST-EXP-28/9-9-2021)

2.4. Statistical analysis

SPSS program 19 version was used for the analysis of data. Data were presented as mean \pm SD. P-value < 0.05 was considered statistically significant.

3. Results

- 1. In study I: Comparison of 360 vaccine recipients, 46 were seropositive COVID-19 by analysing by IgG positive by Nucleocapsid and we have excluded them from the study. Of the 314 with no history of Covid-19,. The mean age in our study group was 69 ± 7.2 for male and 64 ± 9.4 for female. Both in male and female group, all the hematological and vitamin D were assayed. In our study we could see of male predominance than the female. Hb%,Iron,TIBC, Vitamin D were decreased in females than the males.
- In study II: In our study we observed 110 were Vitamin D deficient (65M;45 F) and 96 (46 M:50 F) were Iron Deficienct, both deficiencies were 74 (49M:25F) and vaccine recipients with no deficiency were 34(20M: 14 F). Predominantly Vitamin D deficiency is seen in elderly population.
- 3. In study III: Responses were evaluated after two doses after 15-45 days on an average post-vaccine. After two dose, anti-spike S1/S2 antibodies, anti RBD IgG concentration and were each significantly low among the Vit D deficient, Iron deficiency and both deficiency when compared to normal vaccine recipients who had no deficiency. In This RBD antibodies were significantly high in females when compared to male but anti-spike antibodies were significantly more in males when compared to females.

4. Discussion

However, it is important to note that there are no published nutrition studies in the context of SARS-Cov-2. In this study Though India is a subtropical country with adequate sunlight, vitamin D deficiency is prevalent. In this study we also compared both male and female vitamin deficiency to vitamin sufficient recipients All biochemical analyses were performed in batch. Nutritional deficiency and malnutrition are common in the elderly.

In Present study showed that the vaccine recipients with Vitamin D Deficient had lower anti-spike neutralizing antibodies of 32.5 ± 9.82 AU/ml in males and 20.4 ± 8.27 AU/ml in females, anti RBD antibodies in both 550 ± 241.1 AU/ml in male and 570 ± 212.1 in female and when compared to No deficiency vaccine recipients of 129 ± 12.72 AU/ml of anti-spike antibodies, 2450 ± 420 of anti RBD

antibodies in male were detected, In Female 122 \pm 66.7 AU/ml of anti-spike antibodies and 2665±403.0 AU/ml of anti RBD antibodies were detected. Berryetal¹⁸ described an inverse linear relationship between vitamin D levels and respiratory tract infections in a cross-sectional study of 6789 British adults. Ginde AA et al. In agreement with this, data from the US Third National Health and Nutrition Examination Survey which included 18883 adults showed an independent inverse association between serum 25(OH)-vitamin D and recent upper respiratory tract infection.¹⁹ According to Urashima Metal Supplementation of Japanese schoolchildren with vitamin D for 4 months during winter decreased the risk of influenza by about 40%.²⁰ Martineau AR in his Meta-analyses have concluded that vitamin D supplementation can reduce the risk of respiratory tract infections.²¹ Insufficient vitamin D status, as defined by a low circulating level of 25-OH-D is associated with a reduced immune response to influenza vaccination, and to highlight, Ming-Dar et al. observed lower seroprotection rates of influenza A virus subtype H3N2 (A/H3N2) and B strain in vitamin D deficiency patients than patients with normal vitamin D levels.²² In Present study showed that the vaccine recipients with Iron Deficient had lower anti-spike neutralizing antibodies of 26.4±7.22 AU/ml in males and 30.6±6.88 AU/ml in females, anti RBD antibodies in both 460±220.6 IU/ml in male and in 500±320.2 IU/ml female and when compared to No deficiency vaccine recipients of 129±12.72 AU/ml of anti-spike antibodies, 2450 ± 420 of anti RBD antibodies in male were detected, In Female 122 ± 66.7 AU/ml of anti-spike antibodies and 2665±403.0 AU/ml of anti RBD antibodies were detected.²³ Frost JN et al., has showed that the key evidence that therapeutically targeting iron can support adaptive immunity is currently limited to the improved T cell and antibody responses seen in hypoferraemic mice and piglets, respectively, upon iron supplementation,²⁴ Direct evidence that serum iron deficiency regulates responses to vaccines in humans is limited because this parameter is rarely reported. Fülöp T et al. in his small study found that among elderly hospitalized patients receiving influenza vaccines, nonresponse was clearly associated with suppressed serum iron concentrations (mean, 8.34 µmol/L vs. 16.00 µmol/L in responders).²⁵ Stoffel NU et al. has shown that, iron supplementation at time of vaccination, which would be expected to raise serum iron, improved the antibody response to the measles vaccine in Kenyan infants. 17,26-28

Present Study we observed that the vaccine recipients with both Iron and Vitamin D deficiency had lower antispike neutralizing antibodies of 18.9 ± 10.6 AU/ml in males and 20.7 ± 11.2 AU/ml in females, anti RBD antibodies in both 284 ± 222.1 IU/ml in male and in 296 ± 324.6 IU/ml female and when compared to No deficiency vaccine recipients of 129 ± 12.72 AU/ml of anti-spike antibodies, 2450 ± 420 of anti RBD antibodies in male were detected, In Female 122 ± 66.7 AU/ml of anti-spike antibodies and 2665 ± 403.0 AU/ml of anti RBD antibodies were detected. Specific to the UK, the 2019 National Diet and Nutrition Survey showed 'a sustained worsening of the dietary intakes and chronic shortages of several of the nutrients involved in supporting the normal immune functions; these included vitamins A, B12, C and D and the trace minerals Zn, Se and Cu.²⁰ Such micronutrient deficiencies may limit the effectiveness of the COVID vaccines. However, human trials suggest that the intakes of some micronutrients (vitamins C, D and E and zinc and selenium) needed to optimally support the immune system are in excess of intakes that can easily be achieved through diet alone and in this case supplementation might be considered

5. Conclusion

At present, major gaps exist in our knowledge of the mechanisms behind the reduced ability of the aging immune system to respond appropriately to both infections and vaccinations. Vitamin D, Iron supplements may increase the levels of T regulatory cells in older individuals Thus, the foods or supplementation provide them can play a role in supporting the immune system. Iron deficiency, in elderly population who are at risk of poor vaccine responses, can be therapeutically optimizing serum iron levels to improve vaccination efficacy by iron interventions. Present study suggests improving lifestyle and consuming diets rich in nutrients, vitamins, iron supplementation, and by the optimal iron delivery regime for maximizing a durable vaccine response, and in which populations will improve immune responses, which is of utmost importance is providing information on COVID-19 vaccines. We propose that a nutritional supplement should be prescribed to all old age groups for a period of weeks before and after they receive the vaccine along with counselling on good nutrition for improved vaccine response and efficacy.

6. Ethical Approval

(AIG/IEC-POST-EXP-28/9-9-2021)

7. Source of Funding

None.

8. Conflict of Interest

None.

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