International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Clinical Validation of Effectiveness of Immunace Forte® & Ultra D3® Improving Immunogenicity of COVID-19 Vaccine in Healthy Individuals

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Abstract: ***Background: An effective immune response requires an adequate host nutritional status. The present clinical trial aims at exploring safety and effectiveness of Immuno adjuvant medication like Immunace forte® and Ultra D3® tablets administered with Covishield Vaccine in improving immunogenicity of the vaccine. Trial design: Trial was randomized controlled, parallel arm with 60 patients (n = 30/group). Methodology: The primary objective of the study was evaluating SARS-CoV-2 serum neutralizing antibody levels (Total Covid specific antibodies) in both groups and COVID 19 incidence. Results: At baseline the Covid Specific antibody levels in both group were comparable. At 1st assessment (after 12 weeks of first vaccine dose) the antibody levels in treatment group were increased by 7% whereas 31.37% reduction in control group. At assessment 2 (after 3 weeks of second vaccine dose) the antibody levels in treatment group were increased by 17.45% whereas reduced by 38.50 % in control group. Two subjects from control group demonstrated the COVID 19 incidence verses no subject from treatment group.6 subjects produced symptoms like cold, cough and mild fever from control group. Conclusion: The present study was instrumental in demonstrating the effectiveness of multi-nutrient supplementation in order to improve the response to vaccine in COVID 19.***

Keywords: Covishield, Vaccine, COVID-19, nutrition

1. Background and Study Rationale

In response to the COVID-19 pandemic, we have witnessed scientists all over the world have worked with determination and perseverance tirelessly to develop vaccines against the SARS-CoV-2 virus. Efficacy of vaccine can be measured by investigating ability to prevent disease (1). For COVID-19, which presents with a range of severities, measures of a vaccine efficacy can include reductions in asymptomatic infections, symptomatic infection, hospitalizations and deaths. Immunogenicity however, is a composite tool which measures the type of immune responses that the vaccine generates and their magnitude over time (2).

Vaccines work by priming of the immune system so if the pathogen gets encountered naturally, the immune system is able to react more quickly and effectively than if it were unprimed (3).

Neutralizing antibodies, developed after vaccination have been shown to be associated with protection against infection in preclinical disease models, though the level, or titer, is needed for protection is still not fully understood. As of the consensus out of the present data pre-clinically and clinically getting accumulated, more are the antibodies produced against the vaccine response stronger would be the probable immunogenicity and efficacy of the vaccine.

An effective immune response requires an adequate host nutritional status. Under nourished people may not gain the desired clinical protection from the vaccine. European Food Safety Authority (EFSA) has authorized nutrient function health claims for vitamins A (including β-carotene), B6, B9, B12, C and D, and the minerals Zn, Se, Fe and Cu (4).

It's well documented fact that the effectiveness of vaccine against influenza often ranges from only 30%–40% among the elderly. It has been considered either due to the nutritional deficiency, aging process, inflammatory state or comorbid condition. From a study conducted on elderly individuals with influenza vaccine it was observed that a reduced antibody response to influenza vaccine was associated with age-related decreases in protein, albumin, and micronutrient levels, since nutrients produce positive effects on the immune function. It should be noted that increasing age was not related to diminished immune response. However, poor health due to nutritional deficiencies was found to be associated with poor immune responses (5).

According to Food and Agriculture Organization, it is estimated that 190.7 million people are undernourished in India. Malnourishment could reduce the effectiveness of the COVID-19 vaccination campaigns. Proper use of nutrients in daily diets can support not only currently existing

therapies but also upcoming COVID-19 vaccines and drugs by enhancing their efficacy (6, 7).

Considering need of nutritional support to improve immunogenicity of vaccine as well as natural immune system of host, the present clinical trial aims at exploring safety and effectiveness of nutritional supplements Immunace forte and Ultra D3 tablets when administered with Covishield Vaccine in improving immunogenicity of the same vaccine in healthy adults eligible for vaccination.

Study objectives:
The primary objective of the study was to evaluate safety and efficacy of Immunace forte and Ultra D3 by assessing SARS-CoV-2 serum neutralizing antibody levels (Total Covid specific antibodies). The secondary objectives were to assess Percentage of participants reporting systemic events (After dose 1 and 2 of vaccine) as self-reported by subjects and percentage of subjects turning RT-PCR positive for Covid-19 after vaccination. The tolerability and safety was also evaluated.

Inclusion criteria:
Male and female of age between 18 to 70 of age and fit for Covid 19 vaccination were enrolled in the study. Subjects providing written informed consent and able to understand and agrees to comply with planned study procedures and be available for all study visits were enrolled. Subjects with Body Mass Index (BMI) 18.0-35.0 kg/m2 at screening were chosen for study. Any vulnerable population including pregnant, breastfeeding women were not included in the study. Comorbid patient but on stable prescription for last 3 months were included in the study.

Exclusion criteria:
Any subject having medical disease or condition that, in the opinion of the participating site principal investigator (PI) or appropriate sub-investigator, precludes study participation were excluded from the study. Subjects currently enrolled in or plans to participate in another clinical trial with an investigational agent were not considered for this study. Subjects with impaired immunity of any underlying psychiatric disorder were exclude. Subject with any significant hematological disorder of coagulation, chronic liver disease alcohol or drugs abuse were not selected. Subjects with documented infection of COVID-19 were not considered for the study.

2. Methodology

Male and non-pregnant female subjects of age >18 (both inclusive) attending outpatient department of study site (s) were screened for eligibility criteria. On screening visit, a written informed consent was obtained from subject for participation in the study. Subjects were able to understand and agrees to comply with the planned study procedures and be available for all study visits were enrolled. Subject's demographic details were recorded. Clinical examination (general and systemic examination including cardiac, respiratory, gastrointestinal, genito-urinary and nervous system) was performed for every subject. Subject's COVID specific antibodies levels test from blood sample was carried out. On baseline visit (day 0), subject were recruited in the study and be randomized to one of the twostudy groups as per the computer generated randomization list either in treatment or in control groups. Subject were assessed for any adverse event during screening period.

Treatment group activities-As per computer generated randomization list, subject were provided with the study medication and will have to consume it till 15 to 19 weeks one tablet each of Immunace and Ultra D3 once a day after a main meal. Subject were advised to continue concomitant medication other than antioxidant agents, vitamins, nutraceutical, Ayurvedic, or herbal medication. Drug compliance was assessed by the investigator on every follow up visit. If subject continuously missed dosing for >3 consecutive days or total missed dose > 6 during the 4 week period, subject will be treated as drop out. Subjects were advised to continue the diet and exercise regimen during the entire study period. Adverse events if any were assessed during study period. Subject's Covid 19 specific total antibody test was performed through blood sample on baseline within a week from first dose of vaccine (Covishield). Subject continued on medication till the second dose of vaccine (12-16 weeks of first dose) on that visit, total antibodies were assessed. Third assessment of antibodies was after 3 weeks of second Covid 19 vaccine. Till the last antibody assessment Subjects continued both medication on daily basis.

Control group activities-Subject were administered with first dose of COVID 19 vaccine along with COVID specific antibody testing on baseline. After 12-16 weeks of first dose of vaccination subject were called for follow up and the COVID specific antibodies were again tested along with the second dose of COVID vaccine. Third antibody assessment was performed 3 weeks after the second dose of vaccine. No treatment or any other nutraceutical product were consumed by the subjects in the control group.

Subjects from both groups were examined and evaluated for presence of any symptoms developed during the study period with any incidence of Covid 19. Subjects were insured and provided medical management wherever required.

Ethics committee and trial registry:
The clinical trial was approved by Institutional Ethics Committee Lokmanya Medical Research Centre and trial was carried out at Lokmanya Hospital Chinchwad, Pune. The trial was registered on CTRI (Clinical Trial Registry of India) with the registration number CTRI/2021/06/034256.

Intervention:
Immunace forte tablet, one tablet daily after main meal with key ingredients like elemental Magnesium, L-Lysine Hydrochloride, Vitamin C, L-Cysteine, L-Carnitine, Nicotinamide, Vitamin E, B6, B1, A, D, B12, elemental Zinc, Iron, calcium Pantothenate, Manganese, iodine, chromium, selenium, lycopene, Betacarotene and folic acid. Ultra D3 tablet, one tablet daily after main meal with Vitamin D3-1000 IU.

Sample size

As this study we proposed was exploratory study, we enrolled around 100 subjects in 1: 1 allocation of which 90 subjects completed the study. Based on this assumption from clinical experience of the investigator we chosen the sample size and carried out the study.

Randomization

We screened 118 participants based on the above-mentioned inclusion/exclusion criteria, of which 100 participants were found suitable for inclusion in the studyand were enrolled (Fig.1). They were randomized using a computer-generated randomization sheet to either in the treatment group orcontrol group (50 each group). There were 20 drop outs from each group as they lost to follow up. Figure 1 presents the flow of events for the trial. Finally we analyzed 60 subjects (30 in each group). Mechanism used to implement the random allocation sequence was sequentially numbered containers, as the trial is open label there is no blinding. We received randomization schedule from qualified statistician, investigator enrolled the participants to respective study groups.

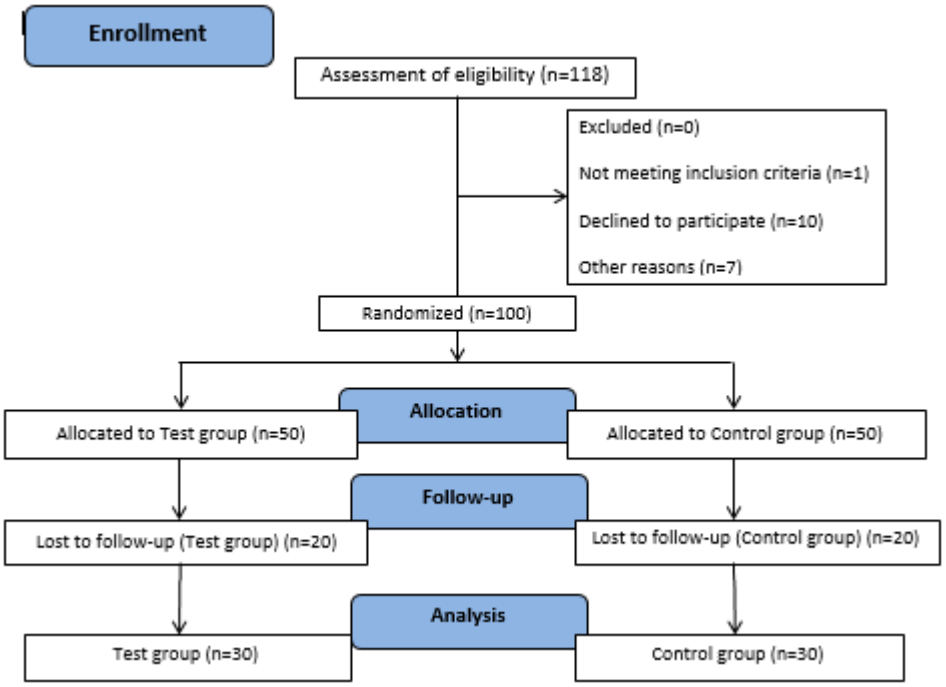


Figure 1: CONSORT diagram

Statistical Analysis:

Patients without any major protocol violation were included in the per-protocol population (pp), including those who had good treatment compliance, and those who did not take any prohibited medications during the study period with completed CRF. Both descriptive and inferential analyses were used for inferring the data. All p-values were reported based on a two-sided significance test and all the statistical tests were interpreted at a 5% level of significance. Continuous variables, such as age and Covid antibody levels, were summarized by using summary statistics, i. e. frequency, and mean, and standard deviation and analyzed by student t test. The adverse events, no. of subjects producing symptoms and incidences of COVID 19 were analyzed by Fisher Exact test. The study period was from June 2021 to October 2021.

3. Results

Demographic characteristics:

We analyzed 30 participants in each group. In the present study the mean age of subjects in test group was 30.94 ±5.90 years and control group 32.00 ±7.97 and were comparable and ranged from 19 to 53 years. All 60 subjects were male. The details are presented in table 1.

Table 1: Demographic details of study subjects

Group	Treatment (n=30)	Control (n=30)
Age (years)	30.94 ±5.90	32.00 ±7.97
Total Age (years)	19-53	

Data analyzed by student t test for between groups. Non-Significant at p>0.05

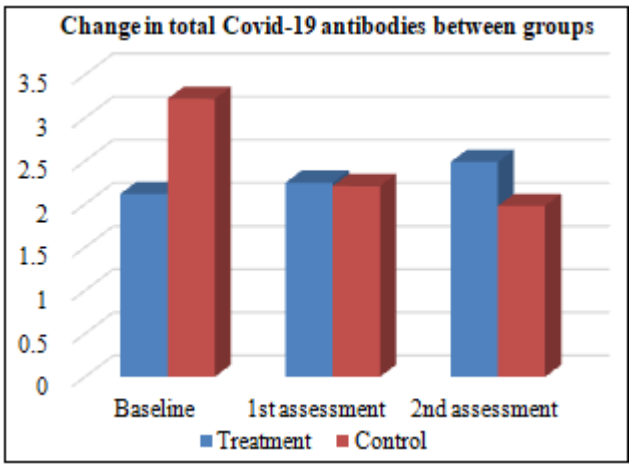
Changes in mean Covid Specific antibody levels between groups:

At baseline the Covid Specific antibody levels in both group were detected and comparable. At 1st assessment i. e. after 12 weeks of first dose of Covishield vaccine the antibody levels in treatment group were observed to be increased by around 7% whereas in control group there was decline in the antibody levels (31.37% reduction). At assessment 2; i. e. after 3 weeks of second dose of Covishield vaccine the antibody levels in treatment group were observed to be increased by around 17.45% whereas in control group there was decline in the antibody levels (38.50 % reduction) (Table 2, Graph 1 and 2).

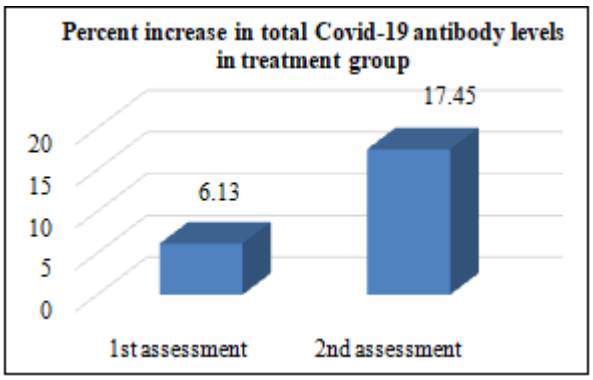
Table 2: Changes in mean Covid Specific antibody levels between groups

Mean Total Covid-19 Antibody level			
Duration	Treatment	Placebo	P Value Between group
Baseline	2.12±2.19	3.22±1.49	0.0258
1st assessment	2.25±2.36	2.21±1.57	
Mean Diff (Baseline-1st assessment)	-0.13±0.28	1.08±1.49	<0.001
2nd assessment	2.49±2.41	1.98±1.71	
Mean Diff (Baseline-2nd assessment)	-0.33±1.02	1.27±1.66	<0.001
P Value within group	0.8168	0.0087	

Data analyzed by ANOVA for within groups and student t test for between groups. Significant at $p<0.05$



Graph 1: Changes in Total Covid-19 antibody levels between groups.



Graph 2: Percent increase in total Covid-19 antibody levels in treatment group

Changes in subjects experiencing Covid related symptoms and Covid-19 incidence between groups:
In the present study, there were no subjects from treatment group experienced any symptoms whereas 20% subjects i. e.6 subjects from control group developed symptoms like cough, cold and mild fever. Out of six subjects two got positive RT-PCR report for Covid 19. Rest four didn't get tested for the RT-PCR for COVID 19 as they were relived of symptoms in average 3 days.

All the subjects experiencing the symptoms from control group were treated at home with rescue medication like antihistaminic, antipyretics and anti-inflammatory agents.

The RT-PCR positive incidence was recorded for two subject. The subject was home isolated and did not require hospitalization and any specific medication like antiviral. The subject got RT-PCR negative on 5th day and clinically recovered.

Changes in adverse events between groups:
There were no adverse events recorded in the study.

4. Discussion and Conclusion

Micronutrients have a noticeable effect on the host immune system regulation, as well as micronutrients insufficiencies where they can affect the host immune response against SARS-CoV-2 (8). The present study was instrumental in demonstrating the effectiveness of multi-nutrient supplementation in order to improve the response to vaccine in Covid 19.

We have chosen a practical study design starting a nutraceutical products like Immunace forte and Ultra D3 tablets (described above) from the first dose of Covishield up to 3 weeks later of second dose of the same vaccine, as it was very crucial to understand how host immunity interacts with the priming of vaccine to produce more neutralizing antibodies in order to protect host from Covid 19 incidence as well as severity.

It can be concluded from the present study that incorporation of the Immunace forte and Ultra D3 tablets restored and increased Total Covid antibody levels up to 17.5% at assessment 2; on the contrary the control group produced steady decline in Total Covid antibodies up to 38.5%. We have checked the Total Covid Specific Neutralizing antibodies and not the spike protein antibodies.

Deficiency of several micronutrients has been associated with prolonged hospital stay and various poor complications caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (9).

As the study period was not the period of Covid-19 wave in India, we naturally got less Covid 19 incidence. Two subjects from control group showed up the Covid 19 incidence and six subjects produced symptoms like cold, cough and mild fever from control group. All subjects recovered clinically with no requirement of hospitalization.

No subject from treatment group showed up Covid-19 incidence nor the symptoms related to Covid-19.

There were no adverse events observed in the study.

As the immune response to the vaccine efficacy is dependent largely on the nutritional status of the host cell, the present research can provide a proof of concept as we have observed the increased Covid specific antibody response greater in treatment group. Further research on larger sample size is warranted.

Supplementation with natural micronutrients may positively impact the course of COVID-19 and can be potentially

beneficial at the level of treatment and prophylaxis as well (10).

Acknowledgments

The authors would like to acknowledge the research team and the back-office team involved in the research work. We would like to acknowledge the support from all study site staff, investigators and back office team.

Declaration of conflict of interests: None

Funding: The study medication and testing charges were supported by Meyer Organics Pvt. Ltd.

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