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Original Research Article

Humoral immune response to COVID-19 vaccine in health care workers and the factors influencing it during vaccine drive - A cross-sectional study

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Abstract

Background: The COVID-19 vaccine drive by the public healthcare system had several safety and efficacy concerns in the minds of health care workers and common man for several vague reasons.
Objective: In this context; the study was planned to determine the humoral immune response in the vaccine recipients of the first indigenously prepared vaccines in India [Covaxin & Covishield]. And, to know the influence of various factors on it. Lastly, to note the adverse events reported by the recipients if any.
Materials and Methods: A cross-sectional point prevalence study with simple convenient sampling method was designed. Baseline data on demographics, presence of comorbid conditions, vaccination details, adverse events to vaccine and information on presence of COVID-19 disease in the past 3-6 months was obtained from participants using a validated questionnaire. Institutional ethical clearance was obtained. The participants' inclusion and exclusion criteria were established. SARS-CoV-2 IgG antibody was determined using ELFA method. Antibody index was estimated and used for interpretation of the test results. Statistical R software was used for data analysis.
Results: of the 86 participants, 84 were found eligible for enrollment. Male to female ratio was 0.9:1. The study participants were in the age group from 20-80 years. Around 29/84[34.52%] had comorbid conditions and the commonest comorbid conditions was hypertension. Overall, 72/84 [86%] of the HCWs were seropositive following vaccination. Greater number of fully vaccinated HCWs 60/62 [97%] were seropositive than partially vaccinated 12/22 [54%] with a p value of 0.001 and their corresponding antibody index was also higher. Among the various variables studied age, gender and presence of comorbid conditions the last one had a positive impact on seropositivity and antibody index values. Mild adverse events following vaccination were noted in majority. However, the antibody index was higher for them than in participants with no adverse events.
Conclusion: Our study highlights the efficacy and safety of vaccine by demonstrating seroconversion in 86% of the recipients and clears the vaccine hesitancy from the minds of HCWs and common man as well.

Keywords: Health care workers, Humoral immune response, SARS-CoV-2 IgG, Antibody index.

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1. Introduction

Vaccine acceptance against COVID-19 disease during the pandemic period had lot many myths and misconceptions both in the mind of health care workers [HCWs] and common man. This resulted in vaccine hesitancy and increased challenges for public healthcare system in its efforts to reduce the disease burden or its severity. Of the several reasons for vaccine hesitancy both in common man and HCWs, the fear of fatal outcomes or adverse events, questionable efficiency were in debate during the end of the first wave and beginning of the vaccination drive by the government of India in January 2021.[1] Published data on vaccine efficacy and adverse events following vaccination were meagre at the time when the vaccine drive was initiated.[1] Most of the vaccines for COVID-19 were released for emergency use during the pandemic period and had completed the initial phase 2 & 3 trials.[1-4] Covaxin from [Bharat biotech in partnership with ICMR & NIV, Pune, India] and Covishield from [Serum institute of India in partnership with Oxford- AstraZeneca]

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were launched and received emergency use authorization by DCGI for HCWs treating COVID-19 patients, and other frontline workers on priority basis in January 2021. Subsequently its use was expanded for immunization of elderly people and then general population.[1-4] Covishield [ChAdOx1-nCoV-19] is a recombinant replication deficient adenovirus vector-based vaccine carrying the SARS-CoV-2 spike protein administered in two doses 4-6 weeks apart for individuals above 18 years of age. Covaxin is a whole cell inactivated virion vaccine developed by Bharat biotech India in collaboration with ICMR & NIV administered in 2 doses and 28 days apart for individuals above 18 years of age. In this context the study was designed with the following objectives:

2. Aim

To determine the humoral immune response to COVID-19 vaccine in healthcare workers at a tertiary health care center in Telangana state.

1. To determine the influence of various variables on humoral immune response to COVID-19 vaccine in health care workers.
2. To determine the incidence of adverse event to vaccine administered.

3. Materials and Methods

Healthcare workers of Ayaan institute of medical sciences and research center at Kankamamidi village, Moinabad mandal, Ranga Reddy district Telangana state were enrolled in the study after the first wave of pandemic when vaccine drive against COVID-19 was initiated by the government of India for use in HCWs and front-line workers. IERB clearance was obtained prior to the study bearing no.2021/09.

3.1. Inclusion criteria

Health care workers of Ayaan institute of medical sciences who had been recruited on permanent basis, and were above 18 years of age, and who hadn't suffered from any confirmed COVID -19 illness during the first wave of the pandemic were enrolled for the study. Health care workers included were doctors, nursing staff, paramedics, housekeeping, administrators, and ministerial staff.

3.2. Exclusion criteria

Staff other than healthcare workers and those less than 18 years of age and who had suffered from confirmed COVID-19 disease during the first wave of the pandemic were excluded from the study. An informed consent was obtained from all the healthcare workers who volunteered to participate before administering a questionnaire to them on their demographic's, vaccine received, its type, time and number of doses, having suffered from confirmed COVID-19 disease during the first wave. Information on presence of any comorbid conditions like diabetes mellitus, hypertension, cardiac disease, kidney, liver, cerebro vascular disease, asthma or COPD etc. was noted. Later 5ml of blood was collected under aseptic precautions from them in a clot activator yellow top vacutainer for determining SARS-CoV-2 IgG antibody response using enzyme linked immunofluorescent assay [ELFA] technology from Biomerieux on Vidas®. This is a qualitative test to determine SARS-CoV-2 IgG antibody to recombinant spike protein antigen of the virus i.e. receptor binding domain [RBD] coated on the sample receptacle device [SRD] which correlated with neutralizing antibodies to S1 subunit of the SARS-CoV-2 as per the manufacturer claim. The antigen and antibody complex formed is in turn detected using monoclonal antibodies to human IgG tagged with fluorescent dye 4 methyl umbelliferone phosphate. On addition of alkaline phosphatase, the substrate is split into 4 methyl umbelliferon. The light emitted is proportionate to the amount of antibody in the test specimen and is measured in units as Antibody index [AI]. Test results were interpreted as AI of < 1= Negative, AI ≥1 is considered positive.

3.3. Study period

April 2021.

3.4. Study place

Ayaan institute of medical sciences and research center.

3.5. Study type

Cross sectional point prevalence study.

3.6. Sampling

Convenience sampling was opted based on health care workers' ease of accessibility, proximity and their willingness to participate.

3.7. Statistical tools

Test results are interpreted as frequencies, proportions in tables and graphs where applicable. Means± SD of the antibody index [AI] was also calculated for different variables studied. The level of significance was determined using student t test, fisher exact test using R software.

4. Results

Around 86 healthcare workers who gave an informed consent and filled the questionnaire on demographics, vaccine details like on time when taken and no. of doses received were documented. However, information on the type of the vaccine received was not documented by any of the participants. They also provided relevant information on COVID -19 disease, comorbid conditions and ADE. Of these 86 HCWs; two had suffered from confirmed COVID-19 disease and were found to be RT-rt PCR positive for SARS CoV-2 RNA during the first wave of the pandemic, therefore, were excluded from the study as the SARS-CoV-2 IgG immune response will be due to true infection and not vaccine induced.

Table 1: Health care workers humoral immune response to COVID-19 vaccine with respect to various variables studied

Health care workers vaccinated N= 86	**Total Sero positives participants N=72**	**Total Sero negatives participants N=12**	**p value**
Partially vaccinated N=22	12 [54.54%]	10 [45.45%]	Fisher value 22.814 p value -0.00115
Fully vaccinated N=62	60 [97%]	02 [3%]	
Males N= 40	32 [80%]	8[20%]	Chi square value =2.036 p value =0.153
Females N = 44	40 [90.90%]	4 [9.09%]	
Age group			Fisher's value = 4.961 p value – 0.378
21-30 years N=11	11 [100%]	0 [0%]	
31-40 years N=30	26 [86.66%]	4 [14.44%]	
41-50 years N=7	6 [85.71%]	1[14.28%]	
51-60 years N10	8 [80%]	2[20%]	
61-70 years N=21	18 [85.71%]	3 [14.28%]	
71-80 years N= 5	3[60%]	2[40%]	
Comorbid conditions present N= 29	25 [86.20%]	4 [13.79%]	Chi square =0.925 p value – 0.0087
Comorbid conditions absent N= 55	47[85.45%]	8[14.5%]	
Adverse events present N =56	49 [87.5%]	7[12.5%]	Chi square – 0.7519 p value – 0.385
Adverse events absent N=28	23[82.14%]	5[17.85%]	

Of the 84 participants there were 40 males and 44 females. The male to female ratio was 0.90:1. The age range of the study population was between 20-80 years. There were about 22/84 [26.19%] HCWs who had received only single dose of vaccine at the time of the study and around 62/84 [73.80%] of the healthcare workers completed two doses of the vaccine. Which coincided with the national COVID-19 immunization drive with the first dose of vaccine being administered to majority of the healthcare workers on 16th Jan. 2021 followed by 2nd dose in Feb. 2021 based on the feasibility, availability, of the vaccine and recipients' consent. Thus, the humoral immune response to vaccine was estimated in both the partially & fully vaccinated recipients nearly 30-45 days after words i.e. during the second week of April 2021.

4.1. Seropositivity

The overall seropositivity of HCWs was 72/84 [86%] and 12 /84 [14%] remained seronegative. Maximum sero negatives were observed in single dose recipients 10/22 [45.45%] while only 2/62 [3.2%] were noticed in fully vaccinated HCWs. Further, seropositivity varied with gender, age, and the presence of comorbid conditions which were not statistically significant except for comorbid conditions which had positive effect on seropositivity especially with diabetes and hypertension. Adverse events experienced by HCWs too following vaccination had a positive effect on seropositivity but statistically insignificant.(**Table 1**)

The overall mean antibody index [AI] of the vaccinated HCWs was 23.07±17.44. The AI of partially and fully vaccinated HCWs when compared varied significantly 12.15 vs. 26.94 with a fisher value of 9.7613 and p value of 0.0564. Great variation was observed in the mean, median, maximum, interquartile range as seen in the whisker plot1 below.(**Figure 1**)

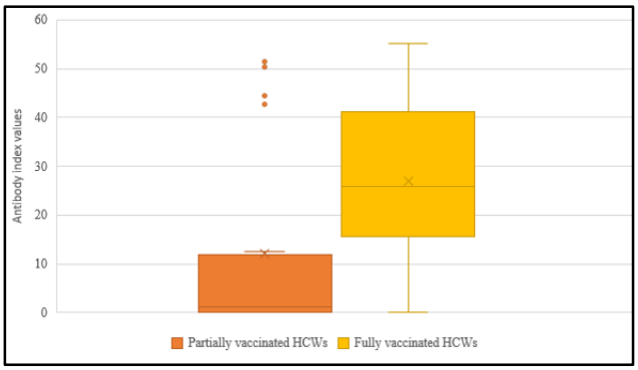

Figure 1: Whisker plot 1 showing SARS-CoV-2 IgG antibody index values of partially and fully vaccinated HCWs

4.2. Age

The overall median age of the participants was found to be 41 years. The median age of the males was 64 years and that for females was 36.5 years which is significant with a p value of 0.007 and t value of 1.9902 using t test as a statistical tool. The median age of the health care workers who were partially vaccinated was 38.5 years. In partially vaccinate recipients, the median age for males was 62 years and for females as 36.5 years which is again significant with a p value of 0.031 and t value of 2.1196. The median age of fully vaccinated HCWs was 43 years. In this group the median age of females was same as in partially vaccinated ones i.e. 36.5 years and that for males it was noted as 64.5 years which is once again significant with a p value of 0.001 and t value of 2.009. The overall median age of non-responders to vaccine was 59 years and for females it was noted as 36.5 years and males as 65 years with a p value of 0.036 and t value of 2.306. In partially vaccinated HCWs it was noted as 52.5 years. The overall median age of non-responder females in partially vaccinated group was 36.5 and for males as 52.5 years which was statistically insignificant with p value of 0.1008 and t value

of 2.35. In case of fully vaccinate HCWs and non-responders the median age was 70 years, and statistical tools cannot be applied for lack of representation from female HCWs in this group. The median age of participants who were partially vaccinated was lesser by 0.87 times compared to those who received two doses of vaccine. The median age of females was significantly lower compared to males in both the groups of vaccine recipients by 1.7 times.

4.3. Seropositivity with respect to age

Seropositivity of HCWs following vaccination exhibited a descending trend with respect to age. It was noted to be cent percent in HCWs belonging to 21-30 years of age as 11/11 [100%] followed by a descending trend with increasing age from 31- 80 years as 26/30 [87%] for 41-50 years of age as 6/7 [86%] for 51-60 years of age as 8/10 [80%], and 3/5 [60%] for 71-80 years of age. The only exception noted was for HCWs belonging age group 61-70 years were 18/21 [86%] were seropositive which exceeded the preceding age group 51-60 years as 8/10 [80%]. The mean antibody index of the vaccine recipients showed descending trend in antibody index values with few exceptions in the age group 41-50 years and 61-70 years where the antibody index values exceeded the preceding age counterparts as shown in the whisker plot 2 below.**Figure 2**

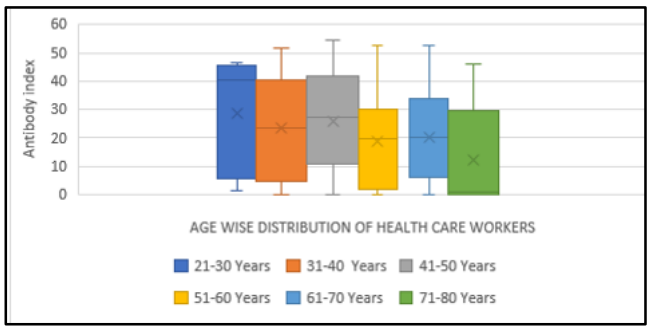

Figure 2: Whisker plot 2 showing age wise distribution of SARS-CoV-2 IgG antibody index values in HCW's.

4.4. Gender

Gender wise comparison of seropositivity following vaccination revealed a greater number of females to be seropositive as 40/44 [90%] than males as 32/40 [80%]. This pattern of higher rate of seropositivity continued to be observed even with either partially or fully vaccinated females than male HCWs. Greater number of males who had received either single dose 6/10 [60%] of vaccine or two doses of vaccine 2/30[7%] were seronegative compared to females as 4/12 [33%] and 0/32 [0%] as seen in **Table 2**. The overall mean AI for females also was higher than for males HCWs [23.48±17.06 vs 18.97±17.01] shown in whisker plot 3.(**Figure 3**)

Table 2: Gender wise mean antibody index of partially and fully vaccinated HCWs.

Vaccine dose received	Mean ±SD antibody index in Males	Mean ±SD antibody index in Females	P value
Partially vaccinated	10.65 ±16.21	13.52±18.48	Fisher value = 1.7247 & p value - 0.363
Fully vaccinated	24.04± 15.46	29.74±15.08	Fisher value 1.6706 and p value - 0.072

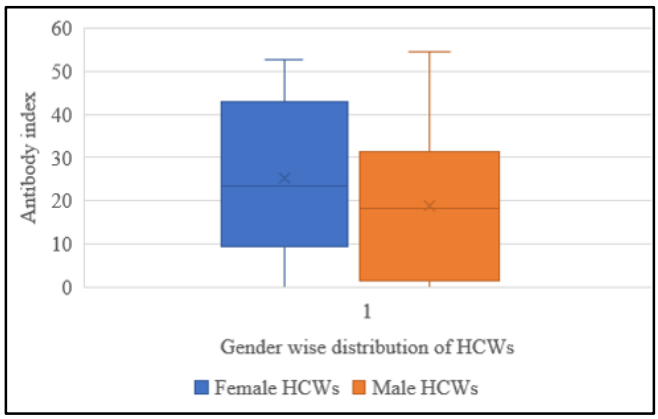

Figure 3: Whisker plot 3 showing antibody index values of SARS-CoV-2 IgG in HCWs with respect to gender

Table 3: Showing antibody index values of HCWs with single and multiple comorbid conditions

Comorbid conditions	Antibody index of SARS-CoV-2 IgG	P value
Diabetes mellitus	33.31 ± 19.32	Fisher value = 5.100242 p value – 0.004
Hypertension	30.51 ± 15.19	
Diabetes mellitus with hypertension/ hypertension with asthma	8.32 ± 6.48	
Diabetes mellitus with hypertension and coronary artery disease/ asthma	1.90 ± 0.99	
Coronary artery disease alone	14.99 ± 14.68	

4.5. Presence of comorbid conditions

Of the total 84 HCWs, 55/84 [65.47%] HCW's didn't had any comorbid conditions and only 29 /84 [34.525] suffered with comorbid conditions as seen in [**Table 1**]. Hypertension was the commonest comorbid condition noted as seen in 11/29 [38%] followed by diabetes mellitus with hypertension in 7/29 [24%], followed by diabetes mellitus alone in 5/29

[17.24%], followed by hypertension and coronary artery disease and coronary artery disease alone in 2/29 [7%] of the HCWs suffering with each type of the comorbidity disease, followed by hypertension and asthma, and diabetes mellitus with asthma and coronary artery disease in 1/29 [3%] of the HCWs suffering with each type of the comorbidity disease. The mean antibody titre of HCWs with no comorbid conditions was higher than that of the HCWs with comorbid conditions as 22.72± 17.06 vs.20.84 ± 17.76 as seen in whisker **Figure 4**. The mean antibody index of the HCWs with diabetes mellitus was higher compared to those with hypertension. Moreover, the mean antibody index of HCWs with more than one comorbid condition was lower than that of HCWs with only one comorbid condition as observed in the **Table 3**.

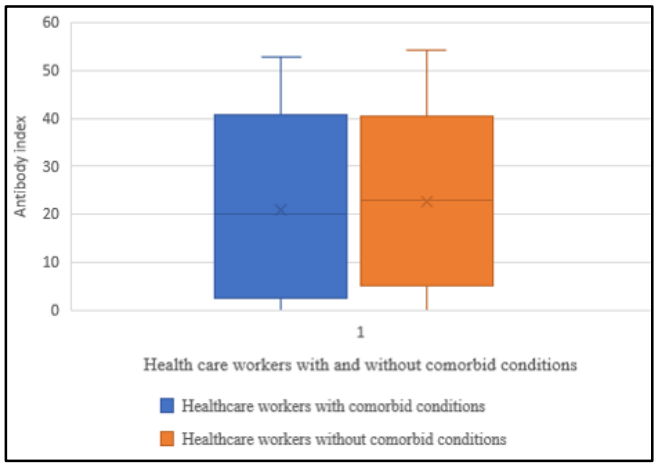

Figure 4: Whisker plot 4 showing SARS-Cov-2 IgG antibody index value in HCWs with and without comorbid conditions

Similar pattern was observed with a greater number of HCWs with no comorbid conditions being seronegative following vaccination than those with comorbid conditions as 8/55 [14.5%] and 4/29 [13.79%] seen in **Table 1**.

4.6. Adverse events following vaccination

Majority of the HCWs 56/84[66.66%] reported mild adverse events in the form of upper respiratory tract infection such as sore throat, myalgia, fever along with pain at the site of injection in the immediate 2-3 days post vaccination [table1]. The remaining 28/84 [33.33%] escaped any adverse events following vaccination [**Table 1**]. The mean antibody index, median, and IQR of the vaccinated HCWs with adverse events to vaccine against COVID 19 was more as 23.63 ± 17.57 when compared to HCWs without adverse events with AI as 19.55 ± 14.49. Moreover, less percentage of HCWs with adverse events were seronegative as 7/58 [12.5%] when compared to HCWs with no adverse events as 5/28 [17.85%] as seen in whisker plot 5 below. **Figure 5**

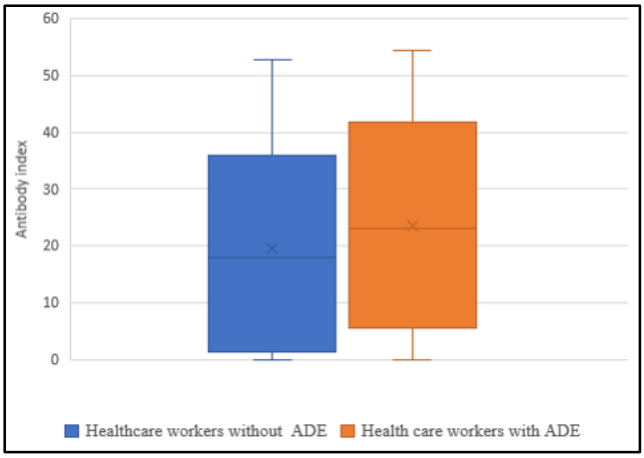

Figure 5: Whisker plot 5 showing SARS-CoV-2 IgG antibody index value in healthcare workers with & adverse events following vaccination

5. Discussion

In the present study we have found an overall seropositivity to COVID-19 vaccine as 86% which is lesser compared to 98.1% & 98% demonstrated by Singh KA et al and Kataria S et al and by C. Jeewandara et al as 92.9%.[5-7] This could be most probably due to several of the reasons like variation in sample size, sampling method, study time & period, vaccine type. Host variables amongst which the most important one is the genetic constitution of the individual, followed by age, gender and presence of comorbid condition and finally the geographic variations.[1] Moreover, the test methodology used to detect humoral immune response also matters as the sensitivity and specificity of the assay directly correlates with the results obtained in the study as mentioned by Kumar R et al.[5,8,9] Seropositivity following single dose of vaccine showed modest increase in the antibody index but in participants who received full dose of vaccine the antibody titres, their mean, median and interquartile range were significantly high which was also noticed by Kumar R.et al.[5,8,9] On the contrary some authors have observed no significant improvement in antibody titre following second dose of the vaccine which was stated as no booster effect.[10-13] In majority of the previous studies the humoral immune response was checked at baseline and then follow up testing, done at interval of 2 weeks & 4 weeks. While some have checked 3-6 months later after the base line estimation and in some studies even up to 7-8 months the humoral immune response was monitored. During this time, they have noticed a slow decay of antibody levels in 72% of the vaccine recipients.[5,14] Some have even demonstrated decline in humoral immune response with a specific type of vaccine.[10-13] Some have even demonstrated sustainable immune response with one type of vaccine over 6 months when compared to other type of vaccine which has been reasoned as for the ability of one of the vaccines to induce better cell mediated immunity than antibody mediated immune response by demonstrating T lymphocyte subsets and TH1 response which is beyond the scope of our study and had some constraints.[5,7,8,15] Further it has been elucidated by

many of the authors that this decay in humoral immune response is not specific to SARS-CoV-2 vaccine alone; it has been noted with other vaccines too like MMR vaccine.[5,16,17] In some of the studies the scientist has gone a step ahead rather than just demonstrating antibody mediated immune response and have checked the memory B & T cells response, plasma blast and germinal B cells response to vaccine and has found that waning humoral response shouldn't raise any concerns as the vaccine induced a significant amount of T cell response both qualitatively and quantitatively.[7,8] The median age of the study participants in our study was 41 years of age which is like the findings by Singh AK but greater when compared with study by Rabish Kumar et al as he noted it as 28 years.[9] Previous studies by Singh AK et al showed no influence of variables like age, gender, comorbid conditions, BMI and blood group on antibody titres but in our study group we observed seropositivity varied significantly with age. In single dose recipients, it was noted to be maximum in participants in the age group 31-40 years followed by 21-30 years and in fully vaccinated group cent percent of the participants between 21-60 years of age were all seropositive this was not delineated in other studies except for.[5] In both the groups of vaccine recipients' individuals above 60 years of age were less seropositive as observed by Singh AK et al.[5] With respect to gender, it is noted in our study that maximum number of seropositive were females compared to males which is similar to the findings of Kumar R but different from the findings of C Jeewandara where he found males to be seropositive in greater numbers than females.[7-9] Their mean antibody index was also high compared to males. The mean antibody index of single dose recipients was lower compared to fully vaccinated participants which is also observed by other authors.[9-13] The commonest comorbid conditions noted were hypertension, followed by hypertension with DM then DM alone which is like other studies. [5] However, the mean AI of diabetics was found to be higher in our study which differed from the findings of Singh AK et al wherein he found hypertensive to be having high GMT for spike protein S1 subunit antibodies.[5] Majority of the participants reported adverse events but mild in nature which is like the findings of Kumar R.[9]

6. Conclusion

The findings of the present study clear the myths and misconceptions that were prevailing in the minds of both health care workers and common man about the vaccine safety and its efficacy. And sends a succinct message to its readers with few lacunae in the study methodology that vaccines are meant to reduce the disease occurrence or its severity as mentioned by the govt. of India that vaccine drive prevented 4.1 million diseases in a year.

7. Limitations

The present study had certain limitations like for example the study failed to demonstrate the baseline level of SARS-CoV-2 IgG in the healthcare workers before vaccination and moreover, follow up demonstration of same for extended period of time 3-6 months to check the duration of time the immune response was sustained in these individuals couldn't be carried out for purely financial constraints and to some extent HCWs willingness to continue their participation in the study. Further the present study focused only on humoral immune response in vaccine recipients and couldn't demonstrate cell mediated immunity by checking T helper 1 and helper 2 responses which is very crucial in viral infections. Hence the area remained unclear and demands in depth future study on it. However, some of the studies on the topic has demonstrated sustainable humoral immune response for a period 3- 6, 6-8 months following last dose of vaccine and sharp decay of antibody level by 72% with Covishield vaccine was noted after 6 months by Mishra et al.[10,14] Moreover, the present study involved convenient sampling based on ease of accessibility, willingness and proximity of the participants which had selection bias, lack of generalizability, low credibility, and missed diversity therefore, the results cannot be generalized due to small size.

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10. Conflict of interest

None.

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