DOI: 10.7860/JCDR/2024/66884.18997

Original Article

Microbiology Section

T-cell Response after COVID-19 Vaccination: A Cross-sectional Study

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ABSTRACT

Introduction: Even if antibody titers wane over time, T-cells demonstrate longevity and remain unaffected by viral variants. This study was done with the rationale of examining the virus-destroying cells post-vaccination, specifically focusing on their ability to produce the vital antiviral cytokine Interferon gamma (IFNγ) upon potential reinfection and investigates the impact of the Covishield vaccine on T-cell response, which is a largely unexplored domain due to the novelty of the disease.

Aim: To study T-cell response by doing IFN γ release assay by employing Enzyme Linked Immunosorbent Spot (ELISpot) method.

Materials and Methods: This was a cross-sectional study conducted from March 2021 to May 2023 at Sree Balaji Medical College and Hospital in Chennai, Tamil Nadu, India. The study included participants from all categories who were staff members of the College and Hospital. It is part of an extensive study done by the authors on immunological changes following Covishield vaccination. As part of this study, T-cell response was also separately studied by collecting an additional 2 mL of blood from 90 individuals one year after initiating the vaccination, specifically after administering the Covishield vaccine at 0 and 3 months. For T-cell response analysis, 2 mL of blood was collected and processed to separate Peripheral Blood Mononuclear Cells (PBMC). The Interferon Gamma Release assay (IGRA) was performed using ELISpot method, utilising a 96-well plate. The spots appearing in the sample wells {Spot Forming Cells (SFC)=T-cells} were quantified using an automated ELISpot reader. Sample wells demonstrating more than 12 spots were considered positive. The results were analysed using various statistical tests, including Chi-square test, One-way Analysis of Variance (ANOVA), Kruskal-Wallis Test, Karl Pearson correlation coefficient, t-test, and Mann-Whitney U-test.

Results: The number of spots in the wells containing blood samples from volunteers ranged from a minimum of 2 to a maximum of 631. Importantly, all participants had detectable spots in their sample wells. Out of 90 participants, 84 (93.4%) had more than 12 SFC, while 6 (6.6%) had less than 12 SFCs. High Immunoglobulin G (IgG) levels were positively correlated with good T-cell responses (SFC). Participants under 60 years of age and females exhibited superior responses. Individuals with co-morbidities had lower levels of T-cell response compared to the healthy/normal participants.

Conclusion: The volunteers in this study exhibited robust humoral and cellular immunity, with females showing a significantly better response. The T-cell response remained strong even nine months after the second dose of the vaccine.

Keywords: Enzyme linked immunosorbent spot, Interferon gamma release assay, Spot-forming cells

INTRODUCTION

Coronavirus Disease-2019 (COVID-19), an unprecedented global health crisis, has significantly disrupted our lives over the past three years. The rapid development and approval of vaccines have offered a ray of hope amid the turmoil. However, scientists seeked to delve deeper into the effects of these vaccines on the human immune response. The available literature on humoral immunity, when the authors started this study, indicated that the effect of antibodies wanes in a few weeks following infection or vaccination. The cellular response to viral infections is crucial in eliminating the virus and curing the disease. The mortality rate was very high during the first and second waves of the disease. Hence, it was attempted to study the T-cell response after Covishield vaccination.

The two major divisions of adaptive immunity, antibody and T-cell-mediated, are mainly directed at different targets. Antibodies usually function by binding to free viral particles and thereby block infection of the host cell. In contrast, T-cells act primarily by recognising and destroying virus-infected cells. Resolution of infection relies more on T-cell function than on antibodies. Although antibody waning after vaccination remains a concern, T stem cell memory subsets are induced after vaccination, raising hope that cellular immunity will remain more robust. According to Garcia VP et al., despite the durability and maintenance of serum antibodies, circulating memory B cells, and T-cell responses at 12 months after the original infection, COVID-19 convalescents have pronounced deficiencies in functional spike-specific T-cell responses and the ability to neutralise the current Variant of Concern (VOC) [1]. Schwarzkopf S et al., suggest that the majority of persons with undetectable systemic IgG may presumably be protected by specific T-cell immunity [2].

Villemonteix J et al., in their article, have stated that there is a significant correlation between the results obtained by ELISpot and Intracellular Cytokine Staining (ICS) assays using Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2) peptide pools. However, when measuring low-level responses, the ELISpot assay seems more sensitive. It allows the detection of T-cell responses in patients with negative results in ICS and can be used to analyse immunization in vaccine trials [3]. The ELISpot assay uses pairs of antibodies directed against distinct epitopes of a cytokine, which capture molecules secreted by individual activated T-cells. The presence of non T-cells in the ELISpot assay does not interfere with the detection and quantification of CD8+ T-cells secreting IFN-γ [4]. These insights underline the need for a comprehensive investigation into the interplay between vaccination, humoral immunity, and cellular immunity in the fight against COVID-19, forming the impetus for this study.

When the authors started the study in March 2021, there was scarce information from Indian sources about “T-cell response”

following COVID vaccination using the Interferon Gamma Release assay by the ELISpot method. The aim of the study was to assess the response of T-cells by conducting IGRA using the ELISpot method. The primary objective of the study was to determine the extent of T-cell response following antigenic stimulation. The secondary objective of the study was to investigate whether the participants can successfully fight reinfection with the help of T-cells.

MATERIALS AND METHODS

This was a cross-sectional study conducted at Sree Balaji Medical College and Hospital, Chennai, Tamil Nadu, India. Institutional Human Ethical Committee clearance was obtained (No. 002/SBMC/IHEC/2021/1528 dated 12.03.2021). It is part of an extensive study that the authors have conducted on the immunological responses following Covishield vaccination. Initially, 154 volunteers were recruited, comprising hospital and college staff from all categories. The entire study spanned from March 2021 to May 2023 and included a detailed analysis of immunological changes following COVID-19 vaccination. Among them, 90 blood samples were collected separately for IGRA one year after the start of the study, which is nine months after receiving two doses of the vaccine. After obtaining written informed consent from the participants, detailed demographic profile was collected.

Inclusion criteria: Ninety vaccinated volunteers of both sexes and above 18 years of age were included in the study.

Exclusion criteria: Known cases of immunodeficiency, individuals who were on immunosuppressive drugs, and those who had already had COVID-19 were excluded from the study.

Sample size calculation: The sample size was calculated based on the reference given in the article by Salgado DRE et al., which states that 77% of individuals developed a strong humoral and cellular immune response. The calculation used a 95% confidence limit and 12% relative precision, applying the following formula [5]:

Formula for Sample size=$(z)^2 \times (1-p)/(p) \times (e)^2$

Z=1.96 prevalence of immune response=77% precision e=12%

Sample size (N)=$(1.96)^2 \times (1-0.77)/0.77 \times (0.12)^2$

N=3.84(0.176)/0.00824=80 subjects.

Since the total number of wells in the plate used for the study was 96, we took a sample size of 90.

A batch of 10 participants was advised to come for sample collection each day. Under aseptic precautions, 2 mL of blood was collected in Heparin vacutainer nine months after the 2nd dose of the vaccine (1 year after the 1st dose). Blood samples were collected at 0, 3 months, and 12 months for other studies as well. After the blood sample collection, the Covishield vaccine was administered over the left deltoid muscle each time.

For this special study on T-cell response using IGRA during the 3rd sample collection at 12 months (9 months after the 2nd dose), 2 mL of blood was separately taken (blood was also taken separately for other studies). This 2 mL of blood was used to isolate PBMC following standard protocols. IGRA was performed using ELISpot method, and the results were analysed.

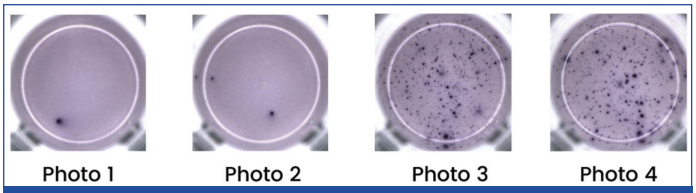
The isolation of PBMC [6] involved the following steps:

- 2 mL of blood was collected in heparin vacutainer tubes and diluted in a 1:1 ratio using Phosphate Buffered Saline (PBS).
- 3 mL of Ficoll-Paque (Ficoll) was transferred into a 15 mL Falcon tube, and 4 mL of the diluted blood was slowly added. Then, it was centrifuged for 30 minutes at 400g at room temperature.
- After centrifugation, the blood separated into four distinct parts/layers: plasma (top layer), greyish mononuclear cells (next layer), Ficoll (third layer), and RBC (fourth layer).
- The second layer containing greyish mononuclear cells was carefully removed using a pipette and transferred to a 15 mL Falcon tube. It was then filled with PBS.
- After centrifuging for 10 minutes at 400g, the supernatant was discarded, and the cells were resuspended with 10 mL of PBS. The mixture was centrifuged for 10 minutes at 400 g, and the supernatant was discarded to separate the cells.
- A freezing medium was prepared using 70% cell culture medium (Roswell Park Memorial Institute medium, i.e., RPMI 1640) supplemented with 1% Penicillin and Streptomycin, 20% Foetal Bovine Serum (FBS), and 10% Dimethyl Sulfoxide (DMSO). It was stored at 4°C.
- The pelleted cells were mixed with 1 mL of the freezing medium, and the contents were transferred into cryovials. The cells were stored in an Isopropanol box and kept at -80°C for 24 hours. Then the cryovials were transferred into a Liquid Nitrogen tank.

IGRA by ELISpot Method

The ELISpot assay was performed using the MABTECH AB kit from Sweden, specifically the Human IFN-γ ELISpot plus Kit (Product code: 3420-4AST-2), following the manufacturer's instructions. Prior to the ELISpot assay, the collected PBMCs were stored in liquid nitrogen and then thawed in RPMI medium supplemented with 5% FBS. They were incubated at 37°C with 5% CO_2. The precoated ELISpot plate (mAb1-D1K) was washed with PBS four times, and the plate was conditioned using 200 µL of RPMI medium with 5% FBS. They were incubated at 37°C for 30 minutes. After incubation, the media was removed, and 100 µL of peptide pool and 50 µL of cell suspension were added. The plate was then incubated in the incubator with 5% CO_2 for 18 hours. Cells were removed by emptying the plate and washing them five times with PBS. After incubation, the detection antibody biotin (0.5 µg/mL) was added and incubated at room temperature for two hours. Following this, the plate was washed, and Streptavidin-Alkaline phosphatase (Streptavidin-ALP) was added. The plate was incubated again for one hour at room temperature. After incubation, the plate was washed, and the 5-Bromo-4-chloro-3-indolyl phosphate/Nitroblue Tetrazolium (BCIP/NBT) plus substrate solution (100 µL) was added. Once the spots developed, the plate was washed in tap water to stop further spot development. They were then dried and counted using an automated ELISpot reader system. The ELISpot test operates on the sandwich Enzyme-Linked Immune absorbent Assay (ELISA) principle. In this assay, antigen-stimulated PBMC secrete the cytokine IFN-γ. The secreted IFN-γ is captured by a membrane-bound anti-IFN-γ antibody, leading to the formation of coloured 'spots' on the membrane around the secreting cell, which indicate the footprints of antigen-specific cytokine-secreting cells. Each T-cell produces one spot (each spot is one T-cell). Determining the cut-off value for SFC/10^6 PBMC posed challenges, as there were limited articles discussing this issue when the study began. Different scientists suggested different cut-off values. For example, Mak WA et al., reported robust responses in vaccinated volunteers stimulated with spike peptide pools, ranging from 12 to 480 SFC [7]. Lin H et al., stated in their article that if the negative control SFC were less than 5/10^5 PBMCs, a positive reaction was defined as SFC greater than 10/10^5. Otherwise, a positive reaction was defined as a result at least twice that of the negative control well. They mentioned that practically, if any peptide pool detection result is positive, it can be considered as positive [8]. Dubey S et al., mentioned in their article that more than 4-fold the negative control (SFC) can be considered positive for HIV vaccine-induced cell-mediated immunity [9]. Gallagher KME et al., stated in their article that for the analysis of ELISpot responses to variant pools, only responses with more than 6 SFU (SFC) were included in the statistical analysis [10]. Tebas P et al., mentioned in their article that the lower limit of detection was 12 spot-forming units, and anything above this cut-off was considered a signal of an antigen-specific cellular response [11]. In the present study, anything above 12 spots in the test wells of the volunteers' samples was considered 'positive'.

Some photos from ELISpot test (4 wells shown) [Table/Fig-1].



[Table/Fig-1]: Photos 1 and 2 are negative control wells. Photos 3 and 4 show Spot-Forming Cells (SFC), Spot-Forming Units (SFU)/T-cells.

The IgG specific for SARS-CoV-2 assay (BIOMERIEUX) was performed following the product literature using the Enzyme-Linked Fluorescent Assay (ELFA) technique. This assay helps determine if individuals may have been exposed and infected by the virus and if they have mounted a specific anti-SARS-CoV-2 IgG immune response. The assay principle combines a two-step sandwich enzyme immune assay method with final fluorescent detection.

For the convenience of presentation, the observed values of immunoglobulins were divided into three categories: 20.33 to 249, 250 to 499, and 500 and above. An IgG level greater than 20.33 BAU/mL is considered positive (BAU: Binding Antibody Units) [12]. The Cluster of Differentiation (CD) counts were performed by flow cytometry at HCG Anderson Laboratory in Chennai, with whom we have an Memorandum of Understanding (MoU).

STATISTICAL ANALYSIS

Demographic variables in categorical/dichotomous form were presented as frequencies with their corresponding percentages. Age, T-cell count, CD45, CD4, and CD8 counts were reported as mean and standard deviation. The T-cell count was assessed for positivity and negativity based on age and sex using the Chi-square test. The distribution of T-cell count across different age groups was evaluated using the non parametric Kruskal-Wallis test. The correlation between the mean T-cell score, age, CD45, CD4, and CD8 was determined using the Karl Pearson correlation coefficient method. The T-cell count distribution by sex and morbidity status was analysed using the non parametric Mann-Whitney U-test. One-way ANOVA and t-test was used. A p-value of ≤0.05 was considered statistically significant, and two-tailed tests were used to assess significance. The statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS, version 22.0) software.

RESULTS

Blood samples were collected from 90 volunteers for this specialised T-cell response study. The volunteers consisted of doctors, nurses, medical students, housekeeping staff, etc., from Sree Balaji Medical College and Hospital in Chennai, Tamil Nadu, India. Among the volunteers, 70 (78%) were females and 20 (22%) were males. Additionally, 81 (90%) of the volunteers were below 60-year-old, while 9 (10%) were above 60-year-old.

The number of spots {SFC=T-cells} in the wells containing samples from the volunteers ranged from a minimum of 2 to a maximum of 631. All the wells with samples from the volunteers exhibited SFC. Among the 90 volunteers, 84 (93.4%) had more than 12 SFC, while only 6 (6.6%) had less than 12 SFC [Table/Fig-2].

Demography		T-cell count (SFC)				n	χ^2	p-value
		Negative (≤12)		Positive (>12)				
		n	%	n	%			
Age group	18-45 years	2	4.17%	46	95.83%	48	11.47	0.01**(S)
	46-60 years	1	3.03%	32	96.97%	33		
	>60 years	3	33.34%	6	66.67%	9		
Sex	Male	4	20.00%	16	80.00%	20	7.35	0.01**(S)
	Female	2	2.86%	68	97.14%	70		

[Table/Fig-2]: T-cell count vs age and sex.

Positivity was more among those who were <60-year-old. Positivity was also more among females compared to males. It was calculated using Chi-square test. Out of 90 volunteers only 6 (6.6%) of them were negative of which three were above 60 years of age. No one had 0 counts [Table/Fig-2].

When the T-cell response (SFC) was compared with different age groups it was observed that the T-cell response was better in those who were below 60 years of age compared to those who were above 60 years [Table/Fig-3].

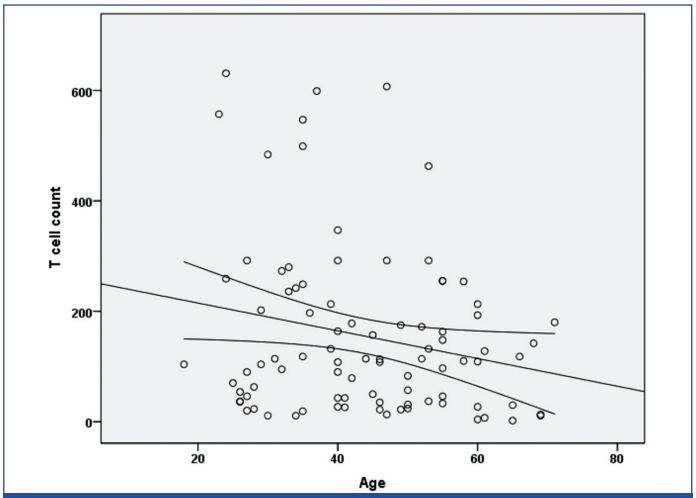
The following table clearly shows the correlation between the mean T-cell count and age [Table/Fig-4,5].

Age (years)	N	SFC Mean±SD
18-45	48	197.58±136.28
46-60	33	136.58±130.92
>60	9	105.90±105.54
Total	90	155.05±152.14

[Table/Fig-3]: T-cell count vs age.
One way ANOVA F test=3.14 p=0.05 (S); Kruskal Wallis test χ^2=7.33 p=0.05 (S)

Correlation	Mean score Mean±SD	Karl pearson correlation coefficients
T-cell score vs age	155.05±152.14 vs 43.82±13.01	r=-0.22, p=0.05* (S) negative fair correlation

[Table/Fig-4]: Correlation between mean T-cell score (SFC) and age.
As the age increases, the T-cell count decreases



[Table/Fig-5]: Correlation between mean T-cell score (SFC) and age.

The scatter diagram, along with a 95% confidence interval regression line, clearly demonstrates a significant negative correlation between T-cell count (SFC) and age.

When T-cell response was analysed in different sex groups, it was observed that the SFC was higher in females than in males, indicating superior cellular immunity in females [Table/Fig-6].

Sex	N	Mean±SD
Male	20	95.30±105.96
Female	70	172.13±159.47

[Table/Fig-6]: T-cell count vs Sex.
t-test=2.03 p=0.05 (S); Mann-Whitney U-test z=2.49 p=0.05 (S)

Those volunteers who had no co-morbidity had more SFCs (T-cell response) than those who had co-morbidities like Diabetes, hypertension, etc., [Table/Fig-7].

Co-morbidity	N	Mean±SD
Yes	8	85.75±80.75
No	82	161.80±156.06

[Table/Fig-7]: T-cell count vs. co-morbidity.
t-test=1.35, p=0.18 (not significant); Mann-Whitney U-test z=1.51, p=0.13 (not significant)

When comparing Spike protein-specific IgG levels with T-cell count (SFC), it was observed that individuals with high levels of IgG also exhibited a high T-cell response (parallel) [Table/Fig-8]. There is a parallel increase in T-cell response as the IgG level increases.

IgG level BAU/mL	N	Mean (SFC)	Std. Deviation	One-way ANOVA F-test
20.33-249	11	120.36	120.54	F=0.33, p=0.72
250-499	17	154.94	129.01	
500 and above	62	161.23	163.58	
Total	90	155.05	151.780	

[Table/Fig-8]: T-cell count (SFC) vs. IgG level.
One-way ANOVA F=0.33, p=0.72; Kruskal-Wallis test χ^2=0.73, p=0.69; IgG level above 20.33 BAU/mL is considered positive; There is a parallel increase in T-cell response as the IgG level increases

When analysing the CD count, it was observed that the CD3+, CD4+, CD8+, and CD45+ counts remained within normal limits. There was no significant correlation between the CD counts and T-cell counts (SFC) [Table/Fig-9]. This indicates that CD counts are normal in the absence of antigenic stimulation. However, during antigenic stimulation such as infection or reinfection, T-cells release interleukins like Interferon gamma and eliminate the virus.

Correlation	Mean count Mean±SD	Karl pearson correlation coefficient	Interpretation
SFC count Vs CD45 count	155.05±152.14 2834.53±1124.36	r=0.11, p=0.32 (NS)	There is no significant correlation between SFC count (T-cell response) and CD45 count
SFC count Vs CD4 count	155.05±152.14 939.37±451.45	r=0.08, p=0.46 (NS)	There is no significant correlation between SFC count (T-cell response) and CD4 count
SFC count Vs CD8 count	155.05±152.14 622.20±351.89	r=0.13, p=0.22 (NS)	There is no significant correlation between SFC count (T-cell response) and CD8 count

[Table/Fig-9]: Correlation between Spot Forming Cell (SFC) count (T-cell response) and CD 45, CD4 and CD8 counts.

This shows that when there is no significant antigenic stimulus the CD counts remain within normal levels. However, when a significant antigenic stimulus occurs, T-cells respond appropriately, confirming that during infection, T-cells release interleukins like Interferon Gamma to eliminate the virus. This response is possible due to the presence of adequate memory cells following vaccination or infection.

DISCUSSION

In this comprehensive study, the often underexplored realm of T-cell response following COVID-19 vaccination, yielding results that carry significant implications for public health strategies was studied.

In March 2021, the vaccination process was initiated and completed two doses at 0 and 3 months. At the 12-month mark, when blood was tested, 93.4% of the participants exhibited robust T-cell responses. Another study conducted by the same authors (currently awaiting publication) revealed that 95% of these volunteers had elevated levels of spike protein-specific IgG antibodies. This finding suggests that when antibody levels remain high, the T-cell response is also strong, indicating a robust humoral and cellular immunity in individuals vaccinated with Covishield.

Tebas P et al., reported that the percentage of responders at week 8 was 74% in the 1 mg dose group of the DNA vaccine (INO-4800) and 100% in the 2 mg dose group [11]. However, in the present study, 93.4% of the participants exhibited a good T-cell response (more than 12 SFCs) after nine months of receiving the second dose, with only 6.6% having less than 12 spots. The mean T-cell count (SFC) was 155.05±152.14. Arankalle V et al., stated in their article that they were able to demonstrate T-cell responses in the majority (77.8%) of Covishield recipients [13]. Sadoff J et al., mentioned that S-specific CD8+ T-cell responses, identified by the expression of interferon-gamma upon S-peptide stimulation, were absent at baseline in two cohorts: on day 15 in cohort 1a (18-55 years), and CD8+ T-cell response was detected in 51% of participants in the low dose group and 64% in the high dose group. However, in Cohort-3 (above 65 years), CD8+ T-cell responses were lower, with an incidence of 36% in the low dose group (vaccine dose 5×10^{10} viral particles) and 24% in the high dose group (vaccine dose 1×10^{11} viral particles) [14]. In the present study, the T-cell response remained strong even nine months after the second dose, with 93.4% of participants having more than 12 SFCs. Tormo N et al., found that IGRA-producing T-cells were statistically higher among those above 60 years of age [15]. Prendecki M et al., stated that there is no correlation between age and the degree of T-cell response [16]. However, in the present study, the T-cell response was more pronounced and better in individuals below 60 years of age. It supports the statement by Medeiros GX et al., regarding the diminished immune response in older men [17]. Naaber P et al., have mentioned that age has a less significant effect at later time points, such as six and 12 weeks, and six months after the second dose [18]. Takahashi T et al., stated in their article that female patients with COVID-19 had more abundant activated and terminally differentiated T-cell populations than male patients at baseline in unadjusted analysis [19]. Fischinger S et al., also reported that women exhibit stronger adaptive humoral and cellular immune responses compared to men [20]. The present findings of the authors confirm a gender-based difference in the T-cell response, with females outperforming their male counterparts. Chen Z et al., mentioned in their article that T-cell responses were maintained for at least six months after the booster dose [21]. However, in the present study, it was observed that the T-cell responses remained good even nine months after the second dose. Dan JM et al., stated that immune memory in at least three immunological compartments was measurable in 95% of subjects 5 to 8 months post-symptom onset, indicating the possibility of durable immunity against secondary COVID-19 disease in most individuals [22]. Ng OW et al., reported that CD8 T-cell responses continued to persist in a SARS-recovered subject for up to 11 years post-infection [23]. Lin H et al., mentioned that the functional response of SARS-CoV-2-specific T-cells can be maintained six months to one year after infection [8]. Schwarzkopf S et al., stated that cellular immunity was detectable for more than 17 years after infection with the coronavirus SARS-CoV-1. They also suggested that the majority of individuals with undetectable systemic IgG may still be protected by specific T-cell immunity [2]. Present study conducted a T-cell response study one year after the first dose and nine months after the second dose of Covishield, and the response was found to be very good. However, if the present study is repeated annually, it will provide insights into the duration of this cellular immunity. The authors observed that the immune response remained robust even nine months after the second dose. These present research findings align with the perspectives of another author, confirming that strong immunity remains intact for at least six months and potentially extends beyond this period after vaccination [24]. Repeating the test after some time will provide additional information regarding the duration of protection. Shiavoni I et al., have stated that they have found a positive correlation between humoral and cellular immune response before booster vaccination [25]. Hoffmann M et al., reported that the S protein of the Omicron variant evades antibody-mediated neutralisation with higher efficiency than any previously analysed S proteins of variants of interest and VOCs [26]. The authors' observations revealed a satisfactory and sustainable increase in IgG levels and T-cell responses after the second dose of the vaccine. Salgado DRE et al., state that spike-specific CD4+ and CD8+ T-cell responses peaked after the second dose of the mRNA-1273 vaccine [5]. The present study uncovers an intriguing connection between T-cell response and IgG levels. It was found that when

the IgG level is high, the T-cell responses also surge, suggesting a correlation between humoral and cellular immune responses. This co-elevation theory, if further validated, could significantly contribute to the understanding of post-vaccine immunity. Guo L et al., stated in their article that Omicron may be less likely to cause severe disease in those who have previously been vaccinated or infected due to T-cell cross-reactivity [27]. Canete PF and Vinuesa CG have mentioned that there is increasing evidence indicating that T-cells play a major role in resolving COVID-19 [28]. Cohen H et al., conducted a study on eight healthy individuals within 4-5 months of receiving the third BNT 162b2 vaccine and reported a predominant IFNγ response observed in all examined individuals, ranging from 50 to 400 secreting cells per 10^6 PBMC [29]. The present study by the authors also reveals a good T-cell response (interferon gamma release) in almost all vaccinated individuals, even nine months after the second dose. Tan AT et al., state, 'It is not known whether antibodies induced by vaccination will exhibit a rate of decline similar to that observed after natural infection beyond the six-month follow-up period, which emphasises the importance of analysing cellular immunity' [30].

The findings in the present study highlight the pivotal role that T-cells can play in combating COVID-19 and underscore the need for further research on the duration of immunity. Through this study, the authors have made significant efforts to contribute to the ongoing global research on the complex dynamics of the immune response following COVID-19 vaccination.

Limitation(s)

The ELISpot assay has become the gold standard for monitoring antigen-specific T-cell immunity in clinical trials of vaccines and other forms of immunotherapy. However, the kit and automated ELISpot reader used in the present study are very expensive. The procedure is time-consuming and requires training and sufficient experience. This kit allows for a maximum of 92 persons to be tested at a time. Therefore, the ELISpot assay is primarily suitable for studying the T-cell response in a large number of cases simultaneously. It is not practical or cost-effective for a single test or a few tests due to the associated expenditure.

CONCLUSION(S)

The study reassuringly indicates that 93.4% of the volunteers have successfully mounted a strong T-cell immune response, which holds promise in their defense against the virus. The enduring high levels of IgG also reflect robust humoral immunity, working in tandem with cellular defenses. Although the CD counts remained within normal limits, an impressive T-cell response to antigenic stimulus (93.4%) was observed, further supported by high IgG levels in vaccinated individuals. It is worth noting that an effective T-cell response may be key to protection against COVID-19 and its variants. Even if the IgG level decreases over time, the T-cells will still identify the antigen, mount a good response, and eliminate the virus. Therefore, the findings of the study provide valuable insights for the understanding and strategic planning against COVID-19.

Acknowledgement

Authors would like to acknowledge and express their gratitude to Prof. Dr. B.W.C. Sathiyasekaran M.D, M.S. Clinical Epidemiology (USA), Dean of Research at Sree Balaji Medical College and Hospital, for providing valuable suggestions and guidance during the preparation of the manuscript. Authors are also grateful for the involvement of Prof. Dr. Sumathy A, M.Sc, Ph.D, Professor in the Department of Biomedical Sciences at Sri Ramachandra Institute of Higher Education and Research, Chennai, for supervising the PBMC processing in her department. Authors extend their thanks to Dr. Vengatesan M.Sc, M.Phil, Ph.D for assisting with the statistical analysis. Additionally, authors would like to thank Mrs. Lakshmi Venkataramana M.A, Data Entry Operator, for her assistance with data entry. Special thanks are also due to the HCG Anderson Laboratory in Chennai for conducting the CD counts and providing regular reports without any inconvenience.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Aug 05, 2023
- Manual Googling: Nov 16, 2023
- iThenticate Software: Dec 26, 2023 (9%)

ETYMOLOGY: Author Origin

EMENDATIONS: 6

AUTHOR DECLARATION:
- Financial or Other Competing Interests: Funded by Bharat Institute of Higher Education and Research, Chennai.
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Aug 04, 2023**
Date of Peer Review: **Sep 05, 2023**
Date of Acceptance: **Dec 28, 2023**
Date of Publishing: **Feb 01, 2024**