A REPERTOIRE OF ANTIBODY AFTER COMPLETE VACCINATION OF COVISIELD CONCERNING SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS-2 - AN OBSERVATIONAL STUDY

ABSTRACT

Aim: To assess the repertoire of antibody after complete vaccination of covishield concerning SARS-CoV-2.

Study Design: Observational Study

Place And Duration of Study: The study was conducted in community area in Kerala for a duration of six months from January 2022 to June 2022.

Methodology: The study was conducted in community area using 30 samples. The participants who fulfill inclusion criteria were selected and samples were collected by convenience sampling from the community and analyzed by the DDRC(Doctor's diagnostic and research centre) by using Electrochemiluminescence immunoassay. The collected data was statistically interpreted using SPSS software version 26.0 and the development of antibody after complete dose of vaccination was analyzed.

Results: In this study blood samples were collected from 30 subjects fulfilling the inclusion criteria. Out of 30 subjects, 12 were females and 18 were males. The mean interval between the doses were 91.63. All the 30 samples produced antibodies greater than 250 U/ml. The study helps to provide qualitative evidence that the vaccine produces antibody response.

Conclusion: Covid-19 vaccines are being available into public for human use after limited testing and trials. Many efforts are being directed towards development of vaccines against SARS-CoV-2. The information made available from this study can be utilized to prove that covishield vaccine is effective for producing antibodies. Most of the people took vaccines since it was mandatory rather than concerning its benefits. By providing evidence of antibody development after complete vaccination can improve the public attitude towards the vaccination.

Keywords: *Covid-19, Vaccination, Covishield, Antibody, ECLIA,* SARS-CoV-2.

1. INTRODUCTION

Covid-19 is a pathogenic viral infection caused by severe acute respiratory syndrome coronavirus 2, which resulted in a global pandemic and significant loss of life. Corona virus identified in 1965 from human embryonic organ culture obtained from the respiratory tract of adult with a common cold. The infectious agent was first demonstrated by Tyrel and Bynoe by inoculating the medium of the cultures intranasally in the human volunteers, this resulted in colds in the subjects. SARS emerged in 2002-

2003 as a corona virus from Southern China, during this it spread to 29 countries in North and South America, Asia and Europe.[1]

In December 2019 and outbreak of pneumonia occurred at the workers in a human sea food wholesale market in Hubei, China which was characterized by fever, fatigue, dry cough and occasional gastro intestinal symptoms. Long term pulmonary cardiology complications are noted by this illness. Following this outbreak there was shut down in January 1 2020. By February 6, 2020, WHO had documented a total of 28276 confirmed cases with 565 deaths world-wide involving 25 countries.[2]

Coronaviruses belongs to the family coronaviridae due to its crown or halo like appearance of the glycoprotein studded envelope under electron microscopy.[3] The genome analysis of the genome of SARS-CoV-2 is 96% identical to the bat coronavirus showing its origin from the bats.[4]

Coronaviruses are single stranded RNA viruses enclosed by a 5'cap and 3'poly (A) tail. The electron microscopy of the virus showed virion diameters varying from 60 to 140 nano meter and distinct spikes of 9 to 12 nano meter. There are four structural proteins in the coronaviruses such as spike (S), membrane (M), envelope (E), nucleocapsid (N).[5]

1.1 Spike Proteins

The spike proteins aid the viral entry to the host cells. The spike proteins have two subunits S1 and S2, the S1 allows virus to recognize the host receptors and helps in viral attachment and fusion of virus with host cell membrane occurs with the help of S2 subunit of spike proteins.[6]

1.2 Membrane Proteins

M proteins give a proper shape to viral envelope and it is the most abundant protein present in the virion. It helps in the viral assembly.[7] The M protein consists of transmembrane domains, a short amino acid terminus and a long carboxy terminus.[8]

1.3 Envelope Proteins

Envelope protein is a small membrane polypeptide that act as an ion channel. The E protein consists of short hydrophilic amino terminal, a large hydrophobic transmembrane domain and a C terminal domain.[9]

1.4 Nucleocapsid Proteins

N protein plays an important role in regulation of viral RNA synthesis, transcription and regulation of infected cell metabolism proteins protects the genomic RNA by packaging them.[10]

The average incubation period of SARS-CoV-2 is 5.1 days.[11] The symptoms start to appear in 11.5 days. An estimated 17.9 - 33.3% patients remain asymptomatic. The common clinical manifestations

shown by infected person includes fever, cough, shortness of breath and less commonly with a sore throat, dysgeusia, anorexia, nausea, shortness of breath, headache, malaise, myalgias and diarrhea.[12]

Vaccines are the weakened or killed microorganisms or toxins or other biological preparations containing antibodies, lymphocytes or mRNA to prevent disease. Vaccine provides active acquired and passive immunity against a specific harmful agent by stimulating the immune system to attack that agent. Vaccines are usually administered by injection but some are given oral or through nasal route.[13]

The first mass vaccination for covid-19 started in December 2020. Vaccines are assessed to make certain they meet applicable standards of safety and efficacy the usage of scientific trial records, manufacturing processes. The evaluation weighs the threat posed by the emergency as well as the advantage that could accrue from using the product towards any ability dangers.[14]

Three vaccines are available in India Covishield, Covaxin and Sputnik V. Serum institute of India authorised the emergency use of Covaxin and Covishield in 16 January 2021. Covishield is recombinant, non-replicating chimpanzee adenovirus vector encoding the SARS-CoV-2 S protein. It was produced in genetically modified human embryonic kidney (HEK) 293 cells. The vaccine only produce antibody, and are unable to replicate inside the body like other viral vector vaccines. It produces action by eliciting immune response and memory T-cell and B cell formation.[15]

The Covishield vaccine is composed of recombinant replication deficient chimpanzee adenovirus vector encoding the SARS-CoV-2 spike (S) glycoprotein, L-histidine, L-histidine hydrochloride monohydrate, magnesium chloride hexahydrate, polysorbate 80, ethanol, sucrose, sodium chloride, disodium edetate dihydrate (EDTA) and water for injection.[16]

Antibody response after covid vaccination is having an important role in developing immunity against the SARS-CoV-2 infection.[17] When the covid-19 virus is introduced into human system (either naturally or by vaccination) our bodies often create protective antibodies. On average immunoglobulin IgM antibodies develop in 7-9 days and IgG antibodies about 2 weeks after receiving the virus.[18] The antibodies produced can be detected from the blood samples by using the following methods:

- Haemagglutination test
- Enzyme linked immunosorbent assay
- Surrogate virus neutralisation test
- Ex-vivo interferon gamma ELISpot assays
- Chemiluminescence immunoassay.[19]

Chemiluminescence immunoassay - Chemiluminescent immunoassay (CLIA) is an immunoassay

technique where the label, i.e., the true “indicator” of the analytic reaction, is a luminescent molecule. When electron transitions from the excited to ground state, it emits visible or near visible radiations of wavelength 300- 800 nm. The potential energy is then released in the form of light.[20]

During its journey the coronavirus has mutated several times, the first mutations were observed in the spike proteins such as D614G and P323L respectively. During the second period ie., from August to December 2020 additional mutations in nucleocapsid and spike proteins were noted in multiple countries especially in Europe[21]

The lack of proper evidence related to the vaccine efficacy, fear of developing adverse effects were the common hesitancy issues. Studies to provide evidence are essential to reduce such hesitancy issues.

Most of the people took vaccination since it was made mandatory and unaware about the benefits of vaccination. The antibody response after the complete vaccination can assessed to be provide a better evidence and acceptance of vaccination among the population.

2. Methodology

This study was conducted in community area. 30 subjects were included in the study. The participants who do not have a history of Covid -19, subjects who have received complete vaccination with Covid-19, subjects within the age group of 18-55 years were included in the study. The participants who have severe comorbidities and not interested were excluded from the study. The samples were collected from the study subjects after receiving the informed consent and was assayed by using Electrochemiluminescence immunoassay at DDRC and detected for total IgG antibodies. The Electrochemiluminescence immunoassay was based on the principle of double-antigen sandwich immunoassay. The time required for the testing is 18 minutes. The main advantages of electro chemiluminescence immunoassay are high specificity reduced number of reagents and incubation time.

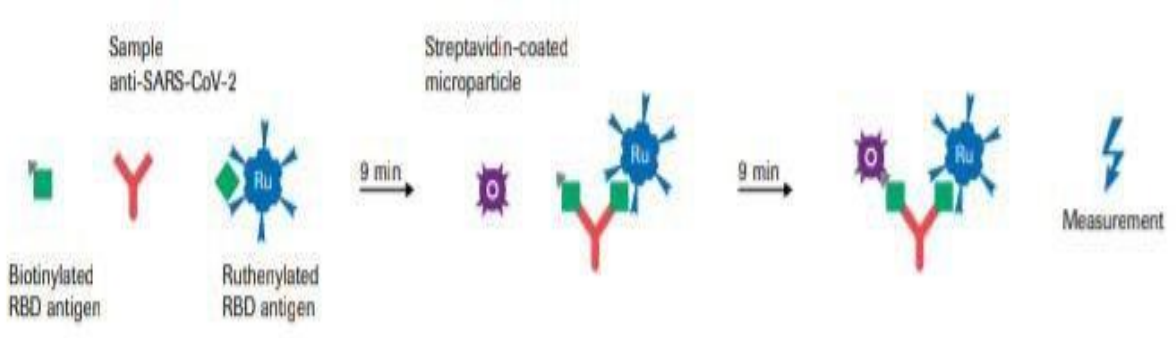


Fig 1 Electrochemiluminescence immunoassay

Electrochemiluminescence immunoassay: 20 microlitre of the sample from the subjects will be

incubated with a mix of biotinylated and ruthenylated RBD antigen. This results in the formation of the Double-antigen sandwich immune complexes in the presence of corresponding antibodies. Then a streptavidin coated microparticle is added to this which gets attached to the complex. The reagent mixture is then transferred into a measuring cell, and the microparticles will be captured by the electrode and unbound substances will be removed. The electrochemiluminescence is induced by applying the voltage and measured in a photomultiplier. The signal will be increased with an increase in the antibody titre values.

3. Results and Discussions

The samples were collected from 30 subjects. Out of this 12 were females and 18 were males. The mean age of the study group was 20.73 with minimum age of 18 and maximum age of 28 years. The maximum and minimum interval between the doses were 124 and 60 days respectively, with a mean interval between the doses of 91.63. It was clear from the results that among 12 females 83.3 % were below or equal to 20 years and 16.7% were above 20 years and among 18 males 50% were below or equal to 20 years and 50% were above 20 years. It was also found that mean interval between doses was 91.32 in subjects below or equal to 20 years of age with a standard deviation 14.788 and 92.18 in subjects above 20 years of age with a standard deviation of 17.145. The mean interval between doses were 86.17 days in females and 95.28 days in males.

Table 1 : Socio-Demographic Details of Study Participants

GENDER	NUMBER OF PARTICIPANTS	PERCENTAGE
Male	18	60%
Female	12	40%
Total	30	100%

Fig 2 **Gender wise distribution of study participants**

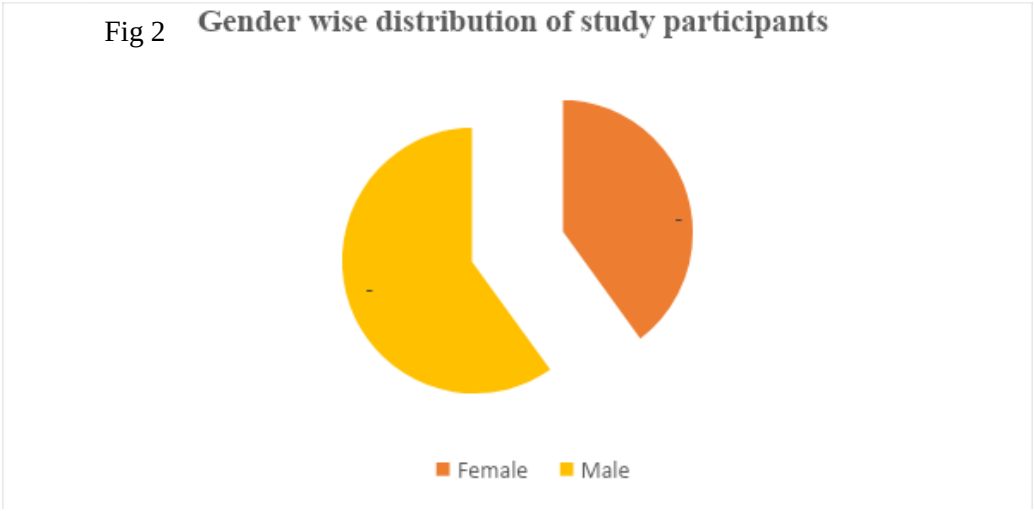
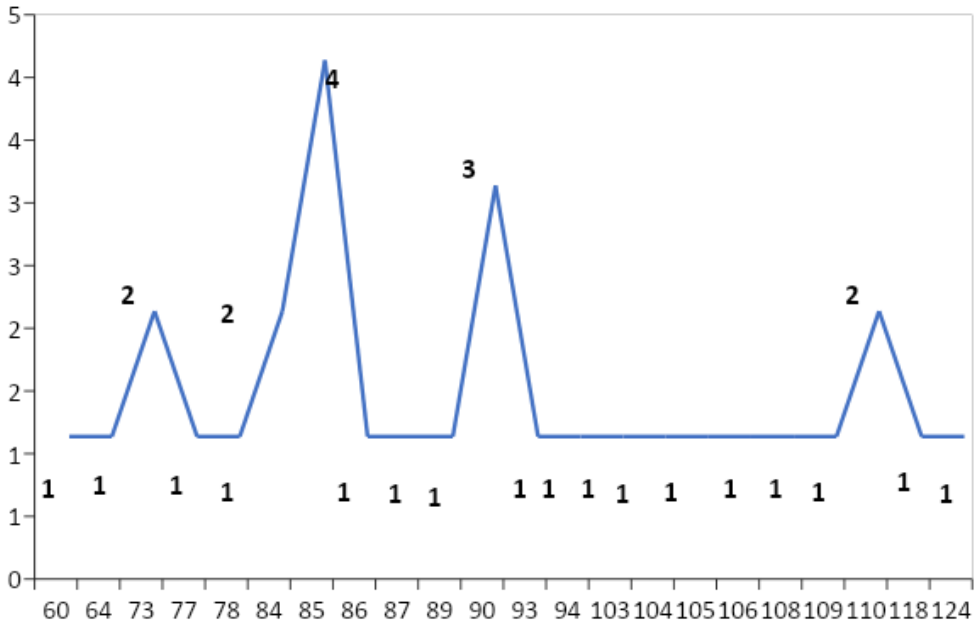


Figure 2 shows Socio-Demographic Details of Study Participant

Out of 30 subjects 12 (40%) were females and 18 (60%) were males.

Table 2 : Distribution of Interval Between Doses

MEAN INTERVAL	STD. DEVIATION	MINIMUM INTERVAL	MAXIMUM INTERVAL
91.63	15.40	60	124



Interval Between two Doses (in days)

Figure 3 Distribution of Interval Between Doses

The mean interval between two doses is 91.63 with a standard deviation 15.40. The minimum interval is 60 days and maximum interval is 124 days.

Table 3 : Detection of antibody

ANTIBODY DETECTED	>0.80 U/mL	<0.80 U/mL
NUMBER OF PARTICIPANTS	30	0
PERCENTAGE	100%	0

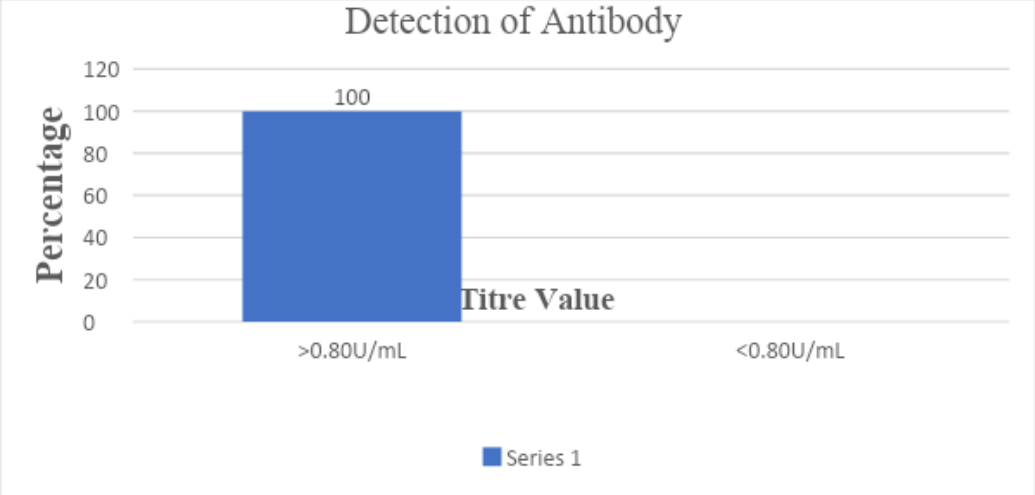


Figure 4 Detection of Antibody

All the samples collected were analysed by ECLIA and antibodies were produced in all the 30 samples. The end point value of the ECLIA assay used in the study was 250 U/ml indicating the presence of antibodies.

Coronavirus disease that spread world-wide which was caused by severe acute respiratory syndrome coronavirus - 2 and resulted in 4 million deaths. It was a serious issue during the year 2019 and hence termed covid 19. Mortality rate was increased during this period, there is a need for developing an effective and safe vaccine against particular infection. The outbreak resulted in the financial crisis and unemployement due to the lockdown which encouraged the pharmaceutical companies to

develop the vaccinations. As a result, save and effective vaccines were developed among them one developed by AstraZeneca with Oxford University (Covishield) and one by India firm Bharath Biotech (Covaxin) and was approved in Jan 2021 and third vaccine Sputnik-V by Russia was approved in April 2021 out of which Covishield showed more effectiveness among others. In this study Covishield vaccine was selected to investigate the response of antibodies after complete vaccination. Mainly there are 5 types of antibodies they are classified into IgG, IgM, IgA, IgD, and IgE. They are distributed and function differently in the body. IgG is the main antibody in the blood and it has a powerful ability to bind to bacteria and toxins, thus it provides biological defense system. IgM antibody is the other most abundant antibody constructed of five units of 'Y' shaped structures. In this method total antibodies against spike protein of receptor binding domain were estimated. IgA is mainly present as monomers (the shape of a single Y), but it forms dimers (a combination of 2 Ys) in secretions such as bowel fluid, nasal discharge, and saliva, to prevent bacterial invasion from a mucous membrane. It is also present in breast milk and protects the gastrointestinal tract of new-borns from bacterial and viral infection. Whereas IgD is present on the surface of B cells and it is reported to play a role in the induction of antibody production and the prevention of respiratory tract infections. It is believed that IgE was originally related to immunity reactions to parasites by binding to mast cells. Total antibodies estimated in the study includes IgG, IgM, IgA.

For this study 30 samples were collected from the community area among which 18 males and 12 females without a history of covid 19 and their age between 18 -28. The mean interval between the doses were 91.73 days. Blood samples were collected from those participants and analysed the presence of total antibody (spike) by ECLIA method. This method was based on the principle of double antigen sandwich assay. The patient sample is incubated with Biotinylated and Ruthenylated RBD antigen and it complexes in the presence of antibodies. By adding streptavidin coated microparticles, the complexes bind or interact with solid phase through the interaction of Biotin and streptavidin. After that the reagent is transferred measuring cell, the unbounded substances are subsequently removed. Electrochemiluminescence is then induced by applying a voltage and it is measured by using a photomultiplier and signal yield increases with the antibody titre.

The samples with total antibody above 0.80 U/ml was considered as positive and samples below 0.80 U/ml was considered negative. The maximum end point of antibody which can be measured here was 250 U/ml. The values above 250 U/ml requires further dilutions of the sample which is often expensive. The main limitation of this study is that only small number of individuals are included. However, we believe that these preliminary data are useful and that they raise important questions that should be investigated further in larger group of people and also whether the observed difference in antibody levels offer the protection against covid infection requires further investigation.

4. CONCLUSION

Vaccines were introduced into public for human use after clinical trials but there is a lack of detailed knowledge about the efficacy of vaccines this study aims to assess the development of antibody after complete vaccination. The information made available from this study can be utilized to prove that covishield vaccination is effective for producing antibodies. Most of the people took vaccines since it

was mandatory rather than concerning its benefits by providing evidence of antibody developed after complete vaccination can improve the public attitude towards the vaccination.
Here the study population includes participants who do not have a positive history of covid-19 but whether the participants had asymptomatic covid infection was not known. Therefore, it was difficult to validate whether the antibodies were produced because of vaccination or asymptomatic covid infection.

CONSENT

As per international standard, participants written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

The study was approved and certified by Institutional Research Committee (ECPS/RC-143/2022) of Ezhuthachan College of Pharmaceutical Sciences, Neyyattinkara, Trivandrum

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COMPETING INTEREST

Authors have declared that no competing interests existed.

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