

Article

Co-Existence of SARS-CoV-2 Antibodies with IgG Antibodies of Other Respiratory Viruses: A Possible Risk for Appropriate Adaptive Immune Response

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ABSTRACT: Respiratory viral infections are a major cause of public health concern worldwide. With the appearance of COVID-19 in 2019, the whole world has experienced severe and long-term complications associated with these infections. These respiratory viruses, their types and sub types are prevalent throughout the year and at varied seasons, and our body continues to fight against these pathogens through innate and adaptive immune responses. Facing multiple viral pathogens with a varied constitution of antigenic epitopes, the infected person generates a number of cytokines and also different paratopic induced antibodies, creating an immunological chaos. To understand this, the present study was undertaken. The antibodies against five major respiratory viruses, commonly reported in India, were targeted viz; SARS-CoV-2, rhinovirus, influenza virus, parainfluenza, and respiratory syncytial virus. An ELISA assay for the detection of all these viruses was made. It was observed that in most cases, there was a presence of antibodies against respiratory viruses (more than 3 respiratory viruses). When the overall status was checked, it was observed that the presence of IgG for Parainfluenza A virus was present in maximum study subjects (92.30 to 100%), along with IgG for SARS-CoV-2 (50–76.92%). We may thus assume that co-infection by multiple viruses may trigger the risk of multiple antibodies and an immunological chaos. Comorbidities that exist in the patients can also enhance the additional stress on the immune cells, with a possibility of B-cells losing their specificity to generate epitope specific antibodies.

Keywords: SARS-CoV-2; Respiratory virus; IgG; B cell; COVID-19

1. Introduction

All across the world, respiratory viral infections are very common and major contributors of morbidity and mortality. Being aerosol or airborne in nature, these viruses are highly transmissible and have the ability



to appear as epidemics and pandemics [1–4]. Studies have been published on co-infection with the recently emerged SARS-CoV-2 and other respiratory viruses, including Influenza, Rhinovirus, Metapneumovirus, Respiratory Syncytial Virus, and Parainfluenza Virus [5]. Netea and co-workers (2020) conducted research in which they found that majority of people infected with SARS-CoV-2 infection had mild infection, but coinfection can affect their immune systems by making the disease more serious [6]. If we look at the respiratory viruses, most of these are seasonal in appearance. Changes in temperature, humidity, rain, seasonal variations, host immune response, socio-economic settings, workplace, *etc.*, are some of the factors that influence the occurrence of respiratory viral infections at different times [7]. Human metapneumovirus, Rhinovirus, Adenovirus, *etc.*, are some of the viruses that appear throughout the year, while Coronaviruses, Respiratory Syncytial Virus, *etc.*, are considered to be winter viruses. We hypothesize that, since all of these viruses infect the respiratory system and utilize one or the other receptor on the respiratory epithelial cell, they may have a similar mechanism of activation of the adaptive immune response. Both the innate and adaptive immune systems may act targeted when infected by a single virus, as compared to the infection by multiple viruses. We hypothesized that infection through antigenically similar viruses may be hyper immune response in the patients through over cytokines secretions and at the same time, there could be fewer specific antibodies produced by B-cells, which could harm through high molecular weight proteins in the form of IgM and Ig G antibodies.

Present paper reports status of antibodies against multiple respiratory viruses to add to targeted clinical interventions for recovery of patients.

2. Materials and Methods

Blood Samples of patients reporting to Sharda Hospital, U.P, India (period 2021–2022) with respiratory like illness were taken with prior consent. Their symptoms were recorded. The vaccine status of COVID-19 was also recorded (considering the presence of antibodies generated against the vaccine). The blood samples were then brought to Centre of Excellence in Virology and Immunology (BSL-2 level lab) under proper biosafety conditions. The serum was separated, and as per individual protocol, each sample was subjected for ELISA tests. Five major circulating viruses, viz., SARS-CoV-2, Rhino virus, Parainfluenza virus, Influenza Virus A, and Respiratory Syncytial Virus, were selected for detecting IgG Antibody. The kits used were m/s NovaLisa, GmbH for Influenza virus, Parainfluenza Virus A and Respiratory Syncytial Virus, m/s MyBioSource, CA for Rhinovirus, and m/s J.Mitra, India for SARS-CoV-2 antibody detection.

The expected reference values for Influenza Virus A detection were as follows: Optical Density (OD) for Negative Control (NC): 0.068, Cut off value: 0.732, and OD of Positive control (PC): 1.408. The expected reference values for Parainfluenza Virus detection were as follows: OD of blank: 0.047, OD of NC: 0.064, Cut off value: 0.926, and OD of PC: 1.831. The expected reference values for Respiratory Syncytial Virus detection were as follows: OD for blank: 0.055, OD of NC: 0.065, Cut off value: 1.097, and OD of PC: 2.312. The expected reference values for Rhino Virus detection were as follows: OD blank: 0.046, OD of NC: 0.114, Cut off value: 0.255, and OD of PC: 1.769. The expected reference values for Rhino Virus detection were as follows: NC $\bar{X}$ = 0.1585, Cut off value = 0.3585, PC = 2.066, final calculation for Covid antibodies unit = Sample O.D. ratio $\times$ 10.

3. Results

Tables 1–4 represent the occurrence of antibodies against various respiratory viruses, along with vaccination status, in each individual. It was observed that all the subjects were infected with more than one respiratory virus and that the maximum subjected had been administered the COVID-19 vaccine. Table 1 indicates that out of the total 92 subjects studied, 13 (14.13%) had antibodies for two respiratory viruses.

Of these, 84.6% had taken the COVID-19 vaccine. Of the 5 viruses studied, it was analyzed that antibodies for SARS-CoV-2 and Parainfluenza virus were found to coexist in maximum cases (Table 1).

Table 1. Presence of Antibodies against two Respiratory Viruses in study subjects.

S.No.	Sample Code	Age	Sex (M/F)	COVID Vaccination	SAR-CoV-2 Virus IgGAb	Influenza Virus A IgG Ab	Parainfluenza Virus IgGAb	Respiratory Syncytial Virus IgG Ab	Rhino Virus IgG Ab
1.	SUAb/2022/28	32	F	No	√	N	N	√	N
2.	SUAb/2022/203	18	F	√	√	N	√	N	N
3.	SUAb/2022/140	18	F	√	√	N	√	N	N
4.	SUAb/2022/70	24	F	√	√	Eq	√	N	N
5.	SUAb/2022/187	19	F	√	√	N	√	N	N
6.	SUAb/2022/105	19	F	√	√	Eq	√	N	N
7.	SUAb/2022/141	21	F	√	√	N	√	N	N
8.	SUAb/2022/236	25	F	√	√	N	√	N	N
9.	SUAb/2022/183	18	M	√	√	Eq	√	N	N
10.	SUAb/2022/121	25	M	√	√	N	√	N	N
11.	SUAb/2022/250	62	M	√	ND *	√	√	Eq	N
12.	SUAb/2022/269	71	M	√	ND *	√	√	N	N
13.	SUAb/2022/276	62	M	No	ND *	N	√	Eq	√
Total				11	10	2	12	1	1
% contribution				84.61	76.92	15.3	92.30	7.69	7.69

* ND = Not done; Eq = Equivalent; N = Negative; √ = Present.

Table 2 indicates that out of the total 92 subjects studied, 36 (39.13%) had antibodies for three respiratory viruses. Of these, 97.2% had taken the COVID-19 vaccine. The table showed that antibodies that co-existed in maximum cases were SARS-CoV-2, Influenza A, and Parainfluenza virus.

Table 2. Presence of Antibodies against three Respiratory Viruses in study subjects.

S.No.	Sample Code	Age	Sex	COVID Vaccination	SAR-CoV-2 Virus IgGAb	Influenza Virus A IgG Ab	Parainfluenza Virus IgGAb	Respiratory Syncytial Virus IgG Ab	Rhino Virus IgG Ab
1.	SUAb/2022/71	25	F	No	√	√	N	Eq	√
2.	SUAb/2022/224	25	F	√	√	Eq	√	√	N
3.	SUAb/2022/53	58	M	√	√	Eq	√	√	N
4.	SUAb/2022/42	40	F	√	√	N	√	√	N
5.	SUAb/2022/145	22	M	√	√	Eq	√	√	N
6.	SUAb/2022/55	30	F	√	√	N	√	Eq	√
7.	SUAb/2022/51	34	M	√	√	√	√	Eq	N
8.	SUAb/2022/91	22	F	√	√	√	√	Eq	N
9.	SUAb/2022/114	26	F	√	√	√	√	Eq	N
10.	SUAb/2022/189	18	F	√	√	√	√	N	N
11.	SUAb/2022/146	19	F	√	√	√	√	N	N
12.	SUAb/2022/123	18	M	√	√	√	√	N	N
13.	SUAb/2022/185	18	M	√	√	√	√	N	N
14.	SUAb/2022/199	21	M	√	√	√	√	N	N
15.	SUAb/2022/154	19	M	√	√	√	√	N	N

16.	SUAb/2022/01	66	M	√	√	Eq	√	√	N
17.	SUAb/2022/50	56	M	√	√	√	√	Eq	N
18.	SUAb/2022/06	31	F	√	√	N	√	√	N
19.	SUAb/2022/243	57	M	√	ND *	√	√	√	N
20.	SUAb/2022/244	68	M	√	ND *	√	√	√	N
21.	SUAb/2022/249	51	M	√	ND *	√	√	√	N
22.	SUAb/2022/252	60	M	√	ND *	√	√	√	N
23.	SUAb/2022/245	60	F	√	ND *	√	√	√	N
24.	SUAb/2022/264	36	M	√	ND *	√	√	N	√
25.	SUAb/2022/247	67	F	√	ND *	√	√	Eq	√
26.	SUAb/2022/255	75	M	√	ND *	Eq	√	√	√
27.	SUAb/2022/266	55	F	√	ND *	√	√	Eq	√
28.	SUAb/2022/270	73	F	√	ND *	√	√	Eq	√
29.	SUAb/2022/281	44	M	√	ND *	√	√	Eq	√
30.	SUAb/2022/284	30	M	√	ND *	Eq	√	√	√
31.	SUAb/2022/283	70	M	√	ND *	√	√	N	√
32.	SUAb/2022/280	67	M	√	ND *	√	√	Eq	√
33.	SUAb/2022/291	49	M	√	ND *	√	√	Eq	√
34.	SUAb/2022/289	27	F	√	ND *	√	√	Eq	√
35.	SUAb/2022/287	24	M	√	ND *	√	√	N	√
36.	SUAb/2022/286	25	F	√	ND *	√	√	Eq	√
Total				35	18	27	35	13	15
% contribution				97.2	50	75	97.2	36.1	41.6

* ND = Not done; Eq = Equivalent; N = Negative; √ = Present.

Table 3 indicates that out of the total 92 subjects studied, 33 (35.86%) had antibodies for four respiratory viruses, of which 93.9% had taken the COVID-19 vaccine. Table 4 indicates that out of the total 92 subjects studied, 10 (10.86%) had antibodies for five respiratory viruses, and all of these were administered the COVID-19 vaccine.

Co-infection status indicated that out of 92, there were 19 patients who suffered from breathlessness, one (SUAb/2022/253) had Tuberculosis, and one (SUAb/2022/282) had hypertension (since past 10–15 years) and lung infection.

Table 3. Presence of Antibodies against four Respiratory Viruses in study subjects.

S.No.	Sample Code	Age	Sex	COVID Vaccination	SAR-CoV-2 Virus IgG Ab	Influenza Virus A IgG Ab	Parainfluenza Virus IgG Ab	Respiratory Syncytial Virus IgG Ab	Rhino Virus IgG Ab
1.	SUAb/2022/96	39	F	No	√	√	√	√	N
2.	SUAb/2022/234	21	F	√	√	√	√	√	N
3.	SUAb/2022/226	23	F	√	√	√	√	√	N
4.	SUAb/2022/44	35	F	√	√	√	√	√	N
5.	SUAb/2022/101	21	M	√	√	N	√	√	√
6.	SUAb/2022/171	19	M	√	√	√	√	√	N
7.	SUAb/2022/36	45	F	√	√	N	√	√	√
8.	SUAb/2022/12	36	F	√	√	√	√	N	√
9.	SUAb/2022/7	26	F	√	√	√	√	N	√
10.	SUAb/2022/57	27	F	√	√	√	√	N	√
11.	SUAb/2022/109	35	F	√	√	√	√	Eq	√

12.	SUAb/2022/56	32	F	√	√	√	√	N	√
13.	SUAb/2022/27	41	M	√	√	√	√	N	√
14.	SUAb/2022/45	49	M	√	√	N	√	√	√
15.	SUAb/2022/66	28	M	√	√	Eq	√	√	√
16.	SUAb/2022/173	19	M	√	√	N	√	√	√
17.	SUAb/2022/174	18	M	√	√	N	√	√	√
18.	SUAb/2022/178	18	M	√	√	√	√	√	N
19.	SUAb/2022/176	19	F	√	√	N	√	√	√
20.	SUAb/2022/92	29	F	√	√	√	√	√	N
21.	SUAb/2022/108	27	M	√	√	√	√	√	N
22.	SUAb/2022/33	30	M	√	√	N	√	√	√
23.	SUAb/2022/34	40	M	√	√	√	√	√	N
24.	SUAb/2022/265	48	M	√	ND *	√	√	√	√
25.	SUAb/2022/253	65	F	√	ND *	√	√	√	√
26.	SUAb/2022/254	82	M	√	ND *	√	√	√	√
27.	SUAb/2022/262	70	M	√	ND *	√	√	√	√
28.	SUAb/2022/268	57	F	√	ND *	√	√	√	√
29.	SUAb/2022/282	71	M	√	ND *	√	√	√	√
30.	SUAb/2022/292	66	M	√	ND *	√	√	√	√
31.	SUAb/2022/275	78	M	√	ND *	√	√	√	√
32.	SUAb/2022/263	54	M	No	ND *	√	√	√	√
33.	SUAb/2022/11	67	F	√	√	√	√	√	N
Total				31	24	25	33	27	23
% contribution				93.9	72.7	75.7	100	81.8	71.8

* ND = Not done; Eq = Equivalent; N = Negative. √ = Present.

Table 4. Presence of Antibodies against five Respiratory Viruses & COVID-19 vaccination status in study subjects.

S.No.	Sample Code	Age	Sex	COVID Vaccination	SAR-CoV-2 Virus IgGAb	Influenza Virus A IgG Ab	Respiratory Parainfluenza Virus IgGAb	Syncytial Virus IgG Ab	Rhino Virus IgG Ab
1.	SUAb/2022/230	21	F	√	√	√	√	√	√
2.	SUAb/2022/100	22	F	√	√	√	√	√	√
3.	SUAb/2022/93	28	F	√	√	√	√	√	√
4.	SUAb/2022/38	27	F	√	√	√	√	√	√
5.	SUAb/2022/153	19	F	√	√	√	√	√	√
6.	SUAb/2022/43	31	M	√	√	√	√	√	√
7.	SUAb/2022/152	19	M	√	√	√	√	√	√
8.	SUAb/2022/94	29	F	√	√	√	√	√	√
9.	SUAb/2022/04	25	F	√	√	√	√	√	√
10.	SUAb/2022/10	26	F	√	√	√	√	√	√
Total				10	10	10	10	10	10
% contribution				100	100	100	100	100	100

√ = Present.

When the overall status was checked it was observed that presence of IgG for Parainfluenza A virus was present in maximum study subjects (92.30 to 100%) along with IgG for SARS-CoV-2 (50–76.92%). Almost all these study subjects, 84.61–100%, were vaccinated during the COVID-19 period (Table 5).

Table 5. Summation of Presence of Antibodies against Respiratory Viruses in study subjects.

S.No.	IgG Categories and COVID-19 Vaccination Status	Presence of IgG Against Number (N) of Respiratory Viruses			
		N = 2	N = 3	N = 4	N = 5
1.	SAR-CoV-2 Virus IgGAb	76.92	50.00	72.70	100
2.	Influenza Virus A IgG Ab	15.30	75.00	75.70	100
3.	Parainfluenza Virus IgGAb	92.30	97.2	100	100
4.	Respiratory Syncytial Virus IgG Ab	7.69	36.1	81.8	100
5.	Rhino Virus IgG Ab	7.69	41.6	71.8	100
6.	COVID vaccination status	84.61	97.2	93.9	100

4. Discussion

IgM antibodies are produced by B cells at the rate of 2000/s. There are positive and negative feedback mechanisms that work during the production of antibodies. There is also addition of glucose residues and sialic acid residues in the crystal structure of the antibodies [8,9].

Production of antibodies by B cells against a specific pathogen is the result of a non-covalent binding between the amino acid ligand of the pathogen (epitope) and the B cell receptor or naïve membrane bound antibody molecules (paratope). The chemical reaction occurs between epitope and paratope as collective strengths of hydrogen bonds and Vanderwal's bond, *etc.* Reading an epitope amino acid constitution is conveyed to the nucleus of B-cells, where a gene rearrangement takes place, and programmed B cells produce specific antibodies. This entire process of antibody production is activated by the secretion of cytokines by T helper cells. In the event of co-infection by antigenically different, but sequentially similar (in amino acid sequence) respiratory viruses, there is a possibility of non-specific antibodies and an inappropriate level of cytokines. Therefore, possibility of cytokines storm and accumulation of non-targeted antibodies, which are high molecular weight protein and if not joined to epitopes, can lead to generation of antibody enhanced infection.

The limitation of the present study is that the SARS-CoV-2 antibody assay kits available in the market, including the one used in this study, are not able to distinguish vaccine-induced antibodies from those generated following natural infection. Another limitation of the present work is that there was not a sufficient number of study subjects for statistical analysis.

5. Conclusions

Results of the present study indicate the presence of more than three different types of IgG antibodies against respiratory viruses in COVID-19 patients. Interaction of multiple viral ligands with B cells and T cells may result in the production of less specific antibodies and hypo- or hypercytokine levels. The study reveals preliminary observations and necessitates further studies on antibodies and cytokines.

Author Contributions

Conceptualization, V.J.; Methodology, S.B., B.A., A.A., S.M.B., B.S. and N.B.; Validation, S.B., B.A., A.A., S.M.B., B.S., V.J. and N.B.; Formal Analysis, S.B., B.A., A.A., S.M.B., B.S., V.J. and N.B.; Investigation, S.B., B.A., A.A. and V.J.; Resources, S.B., S.M.B. and B.S.; Data Curation, S.B., S.M.B. and B.S.; Writing—Original Draft Preparation, S.B., B.A., A.A. and V.J.; Writing—Review & Editing, S.B., B.A., A.A., S.M.B., B.S., V.J. and N.B.; Supervision, V.J., B.A. and A.A.; Project Administration, V.J.; Funding Acquisition, B.A.

Ethics Statement

The study has been approved by the Institutional Ethical Committee, Sharda University, India (SU/SMS&R/76-A/2021/112, dated 03/09/2021).

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Data Availability Statement

All data pertaining to the study are included in this paper.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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