Indian J Med Res 156, October & November 2022, pp 659-668
DOI: 10.4103/ijmr.ijmr_3475_21

Quick Response Code:

Short Paper

A comparative study of antibody response, virus neutralization efficiency & metabolites in SARS-CoV-2-infected adults & children

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Received December 7, 2021

***Background & objectives*: COVID-19 has been a global pandemic since early 2020. It has diverse clinical manifestations, but consistent immunological and metabolic correlates of disease severity and protection are not clear. This study was undertaken to compare seropositivity rate, antibody levels against nucleocapsid and spike proteins, virus neutralization and metabolites between adult and child COVID-19 patients.**

***Methods*: Plasma samples from naïve control (n=14) and reverse transcription (RT)-PCR positive COVID-19 participants (n=132) were tested for reactivity with nucleocapsid and spike proteins by ELISA, neutralization of SARS-CoV-2 infectivity in Vero cells and metabolites by ^{1}H nuclear magnetic resonance (NMR) spectroscopy.**

***Results*: An ELISA platform was developed using nucleocapsid and spike proteins for COVID-19 serosurvey. The participants showed greater seropositivity for nucleocapsid (72%) than spike (55.3%), and males showed higher seropositivity than females for both the proteins. Antibody levels to both the proteins were higher in intensive care unit (ICU) than ward patients. Children showed lower seropositivity and antibody levels than adults. In contrast to ICU adults (81.3%), ICU children (33.3%) showed lower seropositivity for spike. Notably, the neutralization efficiency correlated with levels of anti-nucleocapsid antibodies. The levels of plasma metabolites were perturbed differentially in COVID-19 patients as compared with the naive controls.**

***Interpretation & conclusions*: Our results reflect the complexity of human immune response and metabolome to SARS-CoV-2 infection. While innate and cellular immune responses are likely to be a major determinant of disease severity and protection, antibodies to multiple viral proteins likely affect COVID-19 pathogenesis. In children, not adults, lower seropositivity rate for spike was associated with disease severity.**

Key words Antibody response - corona virus - COVID-19 - nucleocapsid - serosurvey - SARS-CoV-2 - spike

[#]Equal contribution

COVID-19 caused by the SARS-CoV-2 virus led to respiratory tract infection, which produced symptoms such as fever, sore throat, headache and shortness of breath, eventually resulting in multiple organ failure in some patients[1]. SARS-CoV-2 is an enveloped virus with positive-sense RNA genome of about 29.9 kb. It belongs to the Coronaviridae family. The genome encodes for 16 non-structural proteins and 4 structural proteins[2]. Spike, nucleocapsid, envelope and membrane proteins are the structural proteins, with spike and nucleocapsid as the major immunogenic proteins. Spike is a glycoprotein on the virion surface that interacts with angiotensin-converting enzyme 2 (ACE2) on epithelial cell surface, and mediates entry of the virus into the cell[3,4]. High sequence conservation among spike proteins of SARS-CoV-2 strains and negligible cross-reactivity with other coronavirus spike proteins make the spike protein an ideal candidate for vaccine and immunodiagnostics[5]. The nucleocapsid protein along with viral RNA enters the cell and aids in replication and packaging[6]. Antibodies to nucleocapsid can be detected within 5-10 days post-infection and have been found to be highly specific and sensitive, thus making nucleocapsid an important viral antigen for immunodiagnostics[7-9].

To gain insights into whether differences in antibody response to nucleocapsid and spike proteins and metabolite levels correlate with disease symptoms in COVID-19 patients, this study was aimed to assess patients plasma samples for antibodies to nucleocapsid and spike proteins, neutralization of SARS-CoV-2 infectivity in Vero cells and metabolites by ^{1}H NMR.

Material & Methods

Approval for collection and use of samples for this study was granted by the Institutional Ethics Committee of Centre for Cellular and Molecular Biology, Hyderabad and Gandhi Medical College, Secunderabad, Telangana, India (IEC-83/2020). Informed written consent was taken from all COVID-19 positive and naïve control participants before sample collection. Blood samples (2-3 ml) were collected in a heparin or EDTA vacutainer from the patients (n=132) only once 1-2 days after they were tested positive for SARS-CoV-2 by reverse transcription-(RT)-PCR test at Gandhi Medical College. Samples were collected during May-November 2020, and there was no information on prior exposure of SARS-CoV-2 at the time of sample collection. Samples were collected during the initial part of the pandemic in India, and patients were categorized into mild and severe groups based on ICMR criteria (*https://www.mohfw.gov.in/pdf/GuidelinesonClinicalManagementofCOVID1912020.pdf*). Patients with severe symptoms were admitted to intensive care unit (ICU), whereas those with mild symptoms or asymptomatic were admitted to ward for isolation at the Gandhi Medical Hospital. As naïve controls, 2-3 ml blood was collected in June 2020 from 14 healthy volunteers at the CCMB dispensary who were negative for SARS-CoV-2 RT-PCR test at the time of sample collection and free of any prior COVID-19 disease/exposure.

Blood sample processing: Blood samples were processed in a biosafety hood in the BSL-3 facility at CCMB. The blood sample was centrifuged at 2000 g for 5 min at 4°C, and the supernatant was collected as plasma and stored at −80°C for further use. A 100 µl aliquot of the plasma was treated with Triton-X 100 (2%v/v final) at room temperature for 30 min to inactivate the virus, and used in ELISA.

Expression and purification of SARS-CoV-2 nucleocapsid protein: HEK293T-LX cells (a human embryonic kidney cell line) were cultured in DMEM (Dulbecco's Modified Eagle Medium) with 10 per cent fetal bovine serum (FBS) at 37°C in a CO_2 incubator. The cells were transfected at about 70 per cent confluency with PLVXEF1α-SARS-CoV-2-N-2x Strep-IRES-PuroNC plasmid (a kind gift from Nevan Krogan of UCSF, CA, USA)[10], and cultured in the presence of puromycin to generate stable cell lines. Multiple clones were screened for expression of nucleocapsid protein by western blot using horseradish peroxidase (HRP)-conjugated StrepMAB-Classic antibodies (IBA LifeSciences, Goettingen, Germany), and clones expressing the protein were used for production of recombinant nucleocapsid. For large-scale production, cells were harvested from six T175 cc flasks, resuspended in lysis buffer (100 mM Tris *p*H 8.0, 150 mM NaCl, 1 mM EDTA, 0.05 per cent NP40, protease inhibitors) at 4°C for 30 min and sonicated (two cycles of 5 min, each cycle for 15 sec on/15 sec off). The lysate was centrifuged at 11,000 g, the supernatant was incubated with pre-equilibrated Strep-Tactin resin (200 µl slurry/200 µl cell pellet; IBA LifeSciences, Goettingen, Germany) for 30 min at 4°C, the suspension was loaded onto a pre-equilibrated spin column, the resin was washed with wash buffer (100 mM Tris *p*H 8.0, 150 mM NaCl, 1 mM EDTA) and the protein was eluted with 150 mM biotin (in wash

buffer). The elution fractions were assessed for purity by SDS-PAGE and western blotting, eluates containing pure nucleocapsid were pooled, total protein amount was determined using bicinchoninic acid (BCA) and the protein was used for ELISA or stored at −80°C till further use.

Indirect ELISA with nucleocapsid and spike proteins: Recombinant nucleocapsid and spike (Cat No. 244-10001; RayBiotech, GA, USA) proteins were reconstituted in 0.2 M bicarbonate buffer (*p*H 9.2) at 100 ng/100 µl, and coated to the wells (100 µl/well) of a 96-well ELISA plate (MaxiSorp-Nunc immunoplate, Merck, New Jersey, USA). Unbound proteins were washed off with phosphate buffered saline (PBS), and the wells were blocked (blocking buffer: for nucleocapsid: 2% bovine serum albumin (BSA) in PBS with 0.05% Tween 20; for spike: 3% BSA in PBS with 0.05% Tween 20 and 0.25% gelatin). The plasma samples (at 1/250 dilution in blocking buffer) were added to the wells and incubated for one hour at room temperature. The wells were washed with PBS-T (PBS with 0.05% Tween 20), followed by the respective blocking buffer; 100 µl of HRP-conjugated anti-human IgG was added to each well (1/40,000 dilution in respective blocking buffer) and incubated for 45 min, followed by washing with PBS-T. Then, 100 µl of the ELISA substrate 1-Step™ Ultra TMB-ELISA (Thermo Fisher Scientific, MA, USA) was added to each well and incubated for 10-15 min, and the reaction was stopped using 1.0 N HCl. Absorbance was recorded at 450 nm using a BioTek PowerWave XS2 spectrophotometer (Agilent Technologies, CA, USA). Each plasma sample was tested in duplicates. Protein-coated wells probed with pooled naïve control plasma were used as a control. The background control wells contained substrate only. The absorbance value of background control was subtracted from those of naïve control and test plasma samples, and the data were analyzed using MS Excel 2016 (Microsoft, WA, USA) and GraphPad Prism 5.03 (CA, USA).

Neutralization assay: The SARS-CoV-2 B.6 strain was grown in Vero cells[11]. For neutralization, Vero cells were seeded in a 96-well plate (30,000 cells/well; Thermo Fisher Scientific, MA, USA) and cultured in FBS-DMEM medium for 12 h. Serial 2-fold dilutions of the patient plasma and naïve control plasma (1:2 to 1:4096) were made in DMEM, each dilution with 300 infectious SARS-CoV-2 particles, and incubated at 37°C for 1 h. The cells were washed with PBS and incubated with the virus-plasma mixtures for 2 h at 37°C in a CO_2 incubator for infection, the medium was replaced with fresh FBS-DMEM and the plate was incubated for 6 days at 37°C in a CO_2 incubator. The medium was removed, the cells were fixed (4% formaldehyde in PBS) for 20 min at 37°C, the plate was washed, the cells were stained with 0.1 per cent trypan blue and each well was observed for cytopathic effects (CPE). CPE was quantitated according to the Reed and Muench method as has been described previously[11] [(mortality at dilution next above 50%) − (50%)/(mortality next above 50%) − (mortality next below 50%)], and expressed as neutralizing antibody titre. The assay was performed in quadruplicates.

NMR spectroscopy to measure metabolites: Plasma (200 µl) was mixed with 350 µl of PBS (prepared in H_2O:D_2O at 20:80% v/v) with 1 mM sodium formate and 0.25 mM sodium trimethyl siline propionate (TSP). The mixture was filtered using a 10 kDa cut-off centrifugal filter at 12,000 g and 4°C. The filtrate was transferred to a NMR tube for analysis. ^{1}H NMR spectroscopy was carried out using a 600 MHz Microimager (Bruker Biospin, Germany). ^{1}H NMR spectra were acquired using pulse acquire sequence with the following parameters: data points=65,536, spectral width=20.03 kHz, repetition time=7.04 sec and number of averages=256. An NMR spectrum of a control sample was acquired with 20 sec repetition time, and the intensity ratio of the spectra in 20 *vs*. 7.04 sec was used to correct any loss in the signal intensity of different metabolites due to shorter (7.04 sec) repetition time. The free induction decays were apodized using a Gaussian window function (lb= -0.15, GB= 0.15), zero filled to 262,144, Fourier transformed and phase and baseline corrected. The intensity of metabolites was determined relative to the formate (8.47 ppm) signal. The concentrations of the metabolites were calculated relative to formate (1 mM).

Statistical analysis: Data were compared using unpaired two-tailed MannWhitney U test to determine the significance of the difference between the two groups. Linear regression analysis was performed to determine the best curve fit or correlation between groups and Wilcoxon analysis was used to find the Spearman's coefficient. All the analyses were performed using GraphPad Prism 5.03 (GraphPad, CA, USA).

Results & Discussion

Nucleocapsid and spike proteins of SARS-CoV-2 are major targets of humoral response in COVID-19 patients[12,13]. Full length nucleocapsid and spike proteins were used to develop an ELISA platform for analyzing patient plasma samples. Recombinant nucleocapsid was purified from HEK293T-LX cells and confirmed by western blotting (Fig. 1). Attempts to express spike in mammalian cells were unsuccessful, hence commercially available spike protein was used. To determine optimum plasma dilution and assay conditions, 100 ng/well of these two proteins was used in ELISA for assaying naïve control and test plasma samples. Test plasma dilution of 1:250 and ≥2 fold reactivity as compared to that of naïve control was defined as a positive readout for the presence of specific IgG antibodies.

Serological study of SARS-CoV-2 patients: Samples were collected once 1-2 days after the SARS-CoV-2 RT-PCR-positive test from 132 individuals, including 59 adults and 73 children (<18 yr). Of these, 73 were male and 59 were female (Table I). Twenty eight individuals with severe symptoms were admitted to ICU and the remaining were admitted to ward. Blood samples were processed to obtain plasma to test for antibodies to nucleocapsid and spike proteins. Selected samples were also evaluated for low-molecular-weight metabolites and neutralization of the virus.

Of the 132 participants, 72 per cent had antibodies against nucleocapsid (mean reactivity: 12.8) and 55.3 per cent had antibodies against spike (mean reactivity: 4.8; Fig. 2A and Table I), indicating that antibody response to nucleocapsid developed earlier and was stronger than that to spike. This supports the use of nucleocapsid in antigen-antibody diagnostic assays, particularly for diagnosis of early infections. Males exhibited a higher seropositivity rate to both nucleocapsid and spike than females (Table I and Fig. 2B). Sex-specific differences in immune responses to SARS-CoV-2 have been reported in many studies[14,15], and our results are consistent with a report of higher antibody response in males[16].

Seropositivity in different disease groups: Regular serosurveys together with RT-PCR test can be used to check if a person is carrying or exposed to infection. The antibody levels of symptomatic and asymptomatic patients were compared to check if there was any correlation between antibody levels and disease

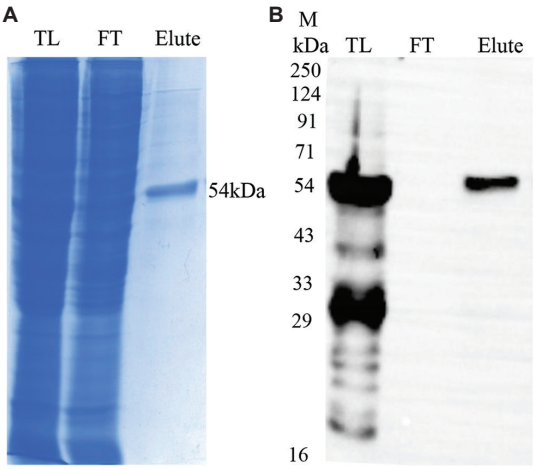

Fig. 1. Production of recombinant nucleocapsid. Nucleocapsid was expressed and purified as described in the Material and Methods section. (**A**) The Coomassie-stained SDS-PAGE gel shows total cell lysate (TL), flow-through (FT) and elution sample (elute) containing the purified protein of about ~54 kDa. (**B**) The western blot shows reactivity with the nucleocapsid protein. The lanes are as in (A) and protein marker sizes (M) are in kDa.

symptoms. Further, 71.4 per cent and 69.0 per cent of the asymptomatic patients (n=42) had antibodies to nucleocapsid and spike, respectively, whereas in the symptomatic group (n=90), 72.2 per cent had antibodies to nucleocapsid and 48.9 per cent had antibodies to spike (Table I), suggesting a role of anti-spike antibodies in protection from the disease. The ICU patients (n=28) showed a higher seropositivity rate for both nucleocapsid (82.1%) and spike (60.7%) than the ward patients (n=104), which showed 69.2 per cent seropositivity for nucleocapsid and 53.8 per cent for spike (Fig. 2C). Higher seropositivity rate and antibody levels in ICU patients than ward patients are intriguing, and have also been observed earlier[17]. In agreement with the published reports[18,19], our results indicated that antibody levels alone are not the protective indicator; rather, innate and cellular immune responses could be major factors for asymptomatic clinical manifestation of COVID-19 patients.

Antibody response in children compared to adults: The seropositivity rate in children (n=73) was less than adults (n=59), as 68.4 per cent of children were positive for nucleocapsid (mean reactivity: 8.5) and 52.1 per cent for spike (mean reactivity: 4.5) compared to 76.2 per cent of adults for nucleocapsid (mean reactivity: 17.5) and 59.3 per cent for spike (mean reactivity: 5.1; Table I and Fig. 2D). A previous study also reported lower seropositivity rate in children than adults[20]. This study also reported that children predominantly had

Table I. Antibody levels and seropositivity rate for spike and nucleocapsid proteins in different participant groups

Parameters	Study participant (n)	Nucleocapsid					Spike					*P* value
		Number of positive	Per cent positive	Reactivity			Number of positive	Per cent positive	Reactivity			
				Mean	SD	95% CI			Mean	SD	95% CI	
Total	132	95	72.0	12.8	10.9	11.0-15.0	73	55.3	4.8	1.8	4.4-5.2	<0.001
Males	73	55	75.3	14.5	11.6	11.4-17.7	47	64.4	5.1	1.9	4.5-5.6	<0.001
Females	59	40	67.8	10.4	9.5	7.3-13.3	26	44.1	4.2	1.4	3.6-4.7	<0.001
Symptomatic	90	65	72.2	14.9	12.0	12.0-17.9	44	48.9	5.0	1.9	4.5-5.6	<0.001
Asymptomatic	42	30	71.4	8.1	5.9	5.9-10.3	29	69.0	4.4	1.6	3.8-5.0	0.006
ICU	28	23	82.1	18.7	13.5	12.8-24.5	17	60.7	5.8	1.6	5.0-6.6	0.02
Ward	104	72	69.2	10.9	9.2	8.7-13.1	56	53.8	4.4	1.7	4.0-5.0	<0.001
Adults												
ICU	16	14	87.5	22.5	11.5	15.9-29.1	13	81.3	6.3	1.2	5.6-7.1	0.01
Ward	43	31	72.1	15.3	11.2	11.2-19.4	22	51.2	4.3	1.6	3.6-5.1	<0.001
Children												
ICU	12	9	75.0	12.7	14.9	1.2-24.2	4	33.3	4.2	1.5	1.8-6.6	0.15
Ward	61	41	67.2	7.6	5.6	5.8-9.3	34	55.7	4.5	1.8	3.8-5.1	0.016

Only the samples with reactivity ≥2-fold of the naive control were considered positive for reactivity with the proteins. *P* values represent the significance of the difference in reactivity between spike and nucleocapsid, SD represents variation within the group and the higher and lower limits of confidence interval are at 95% CI. SD, standard deviation; ICU, intensive care unit; CI, confidence interval

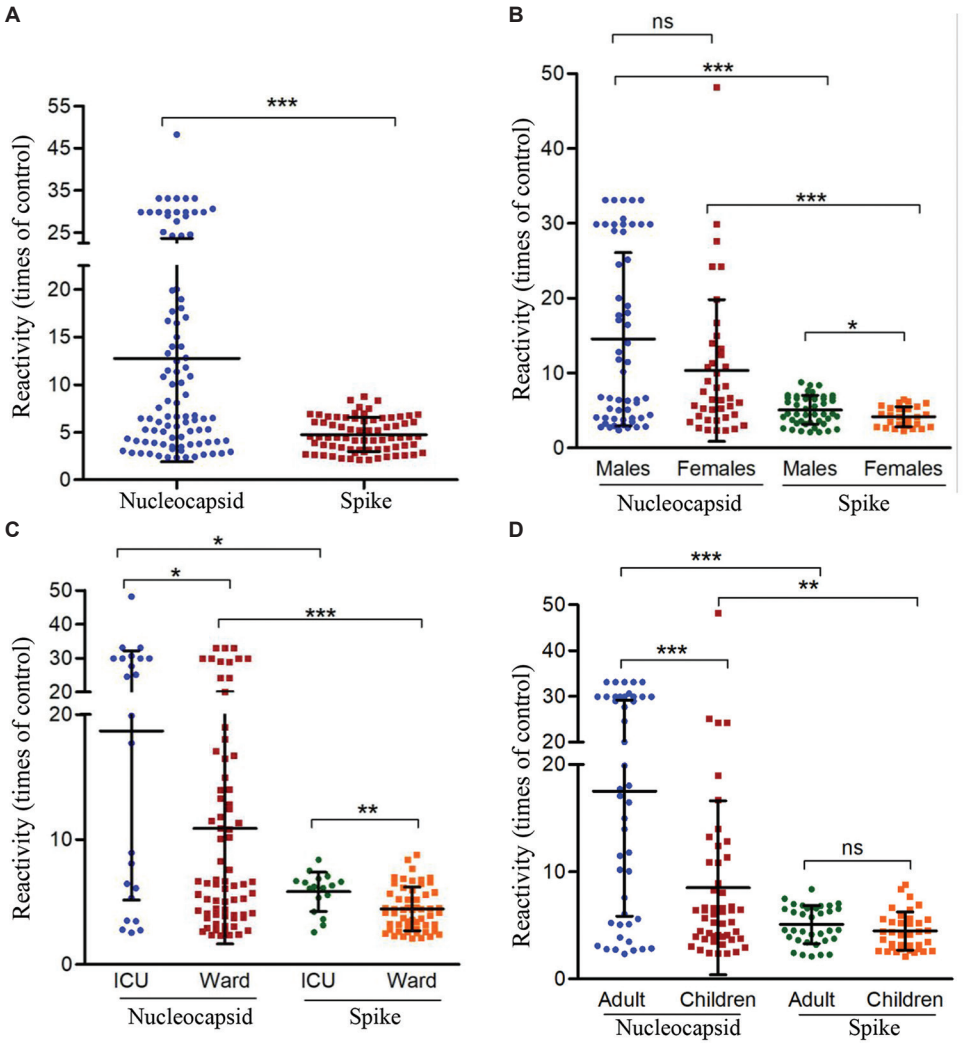

Fig. 2. Antibody reactivity to proteins. The figure shows a comparison of reactivity of plasma samples with nucleocapsid and spike proteins in different participant groups. The Y-axis represents the reactivity of the COVID-19 plasma as times of the reactivity of naive control plasma for the same protein. *P* values represent the significance of the difference between two groups (*P* *<0.05, ** <0.01, *** <0.001, ns is non-significant). Graph **A** is a comparison of reactivity of all COVID-19 patients for spike and nucleocapsid. Graph **B** is a comparison of reactivity between male and female patients. Graph **C** is a comparison of reactivity between ICU and ward patients. Graph **D** shows a comparison of reactivity of adult and children. ICU, intensive care unit.

antibodies to spike but not nucleocapsid, whereas we observed a higher seropositivity rate for nucleocapsid (68.4%) than spike (52.1%). Seropositivity for spike in ICU children was 33.3 per cent (mean reactivity: 4.2) compared to 55.7 per cent in children in ward (mean reactivity: 4.5). This was in contrast to adults wherein seropositivity rates and reactivity for both nucleocapsid (87.5%) and spike (81.3) were higher in ICU patients than the ward patients (72.1% for nucleocapsid and 51.2% for spike; Table I). On the other hand, the seropositivity rate for nucleocapsid was 75 per cent (mean reactivity: 12.7) in ICU children compared to 67.2 per cent (mean reactivity: 7.6) in ward children. Although higher seropositivity rate for spike in ward children than the ICU children suggests a role of anti-spike antibodies in protection from the disease, the levels of anti-spike antibodies were similar in the two groups. The nature of antibody effector functions (cytotoxicity and neutralization) together with innate and cellular immune factors could play a major role in protection from the disease. The less seropositivity rate in children can be attributed to active innate immune response and less number of ACE2 receptors, which may result in reduced viral load for immune stimulation[21-23].

The paediatric ICU (n=12) and paediatric ward (n=11) plasma samples were also compared for neutralization of SARS-CoV-2 infectivity in *in vitro* cell culture model. The neutralizing antibody titres better correlated with the levels of anti-nucleocapsid antibodies than anti-spike antibodies irrespective of the disease symptoms (Table II and Fig. 3), suggesting that antibodies to membrane and envelope proteins could have also contributed to neutralization[24].

Dysregulation of plasma metabolite levels in COVID-19 patients: A typical ^{1}H NMR spectrum of the naive control plasma is presented in Fig. 4. For clarity, the expansion of different regions is shown in the upper panel. Well-resolved resonance of different metabolites, including amino acids, lipids and intermediates of metabolic pathways, are shown. The concentrations of metabolites were calculated relative to formate (Fig. 5). There was a differential change in the level of metabolites in adult COVID-19 samples. There was an increase in the level of lactate (P<0.001) and alanine (P=0.004), suggesting an uncoupling between glycolysis and oxidative phosphorylation in COVID-19 patients. Additionally, there was an increase in the levels of phenylalanine (P=0.001) and tyrosine (P=0.04) in COVID-19 patients. The levels of tricarboxylic acid (TCA) cycle intermediates (citrate and succinate) and floating lipids (choline and phosphocholine) were also increased in COVID-19 patients. In contrast, the level of acetate, the shortest chain ketone body, and glutamine

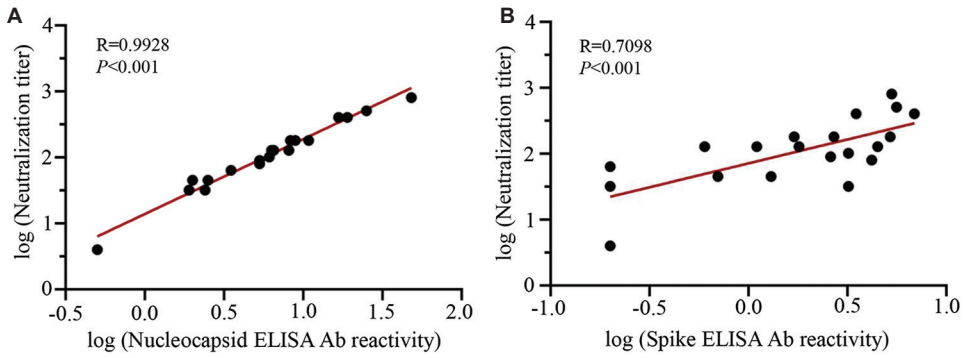

Fig. 3. Correlation between neutralizing antibody titres and antibody levels in paediatric patients. Antibody levels to (**A**) nucleocapsid and (**B**) spike in paediatric patients were analyzed for correlation with neutralizing antibody titres using the two-tailed Spearman rank-correlation test. The graph shows the best linear-fit relationship (red line) with R and *P* values.

Table II. Correlation between neutralizing antibody titres and antibody levels for paediatric samples

Paediatric ICU				Paediatric ward			
Sample ID	ELISA reactivity		Neutralizing antibody titre	Sample ID	ELISA reactivity		Neutralizing antibody titre
	Nucleocapsid	Spike			Nucleocapsid	Spike	
S135	48.2	5.3	812.7	S16	19.0	6.9	406.4
S114	25.1	5.6	512.0	S106	16.7	3.5	406.4
S136	8.9	1.7	181.0	S13	10.8	5.2	181.0
S133	8.1	1.1	128.0	S19	8.3	2.7	181.0
S132	6.5	1.8	128.0	S36	6.4	4.5	128.0
S137	6.1	3.2	101.6	S103	6.3	0.6	128.0
S25	5.3	2.6	90.5	S28	5.3	4.2	80.6
S138	3.5	0.2	64.0	S29	2.4	3.2	32.0
S64	2.5	0.7	45.3	S10	1.9	0.2	32.0
S129	2.0	1.3	45.3	S115	0.5	0.2	4.0
S134	0.4	0.1	0.0	S33	0.4	2.6	0.0
S130	0.3	0.2	0.0				

The ELISA reactivity with nucleocapsid and spike proteins for the indicated sample was compared with neutralizing antibody titre of the same sample among ICU and ward paediatric samples. ICU, intensive care unit; ELISA, enzyme-linked immunosorbent assay

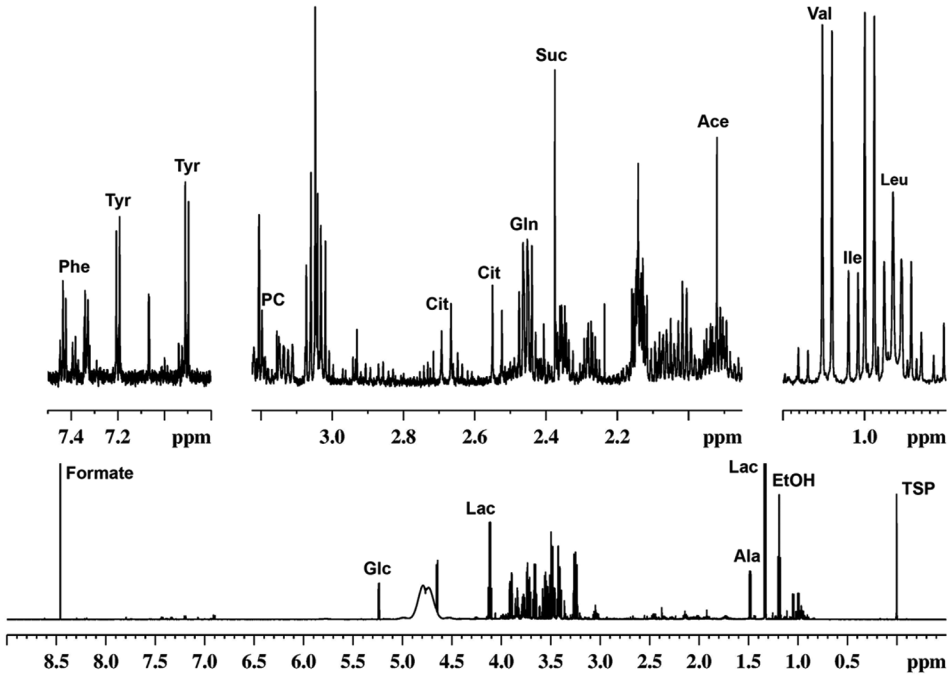

Fig. 4. A representative ^{1}H NMR spectrum of naive control plasma. The ^{1}H NMR spectrum was obtained using pulse acquired method. The upper panel depicts expanded spectrum. Ala, alanine; Cit, citrate; Gln, glutamine; Ile, isoleucine, Lac, lactate; Leu, leucine; PC, phosphocholine; Phe, phenylalanine; Suc, succinate; Tyr, tyrosine; Val, valine.

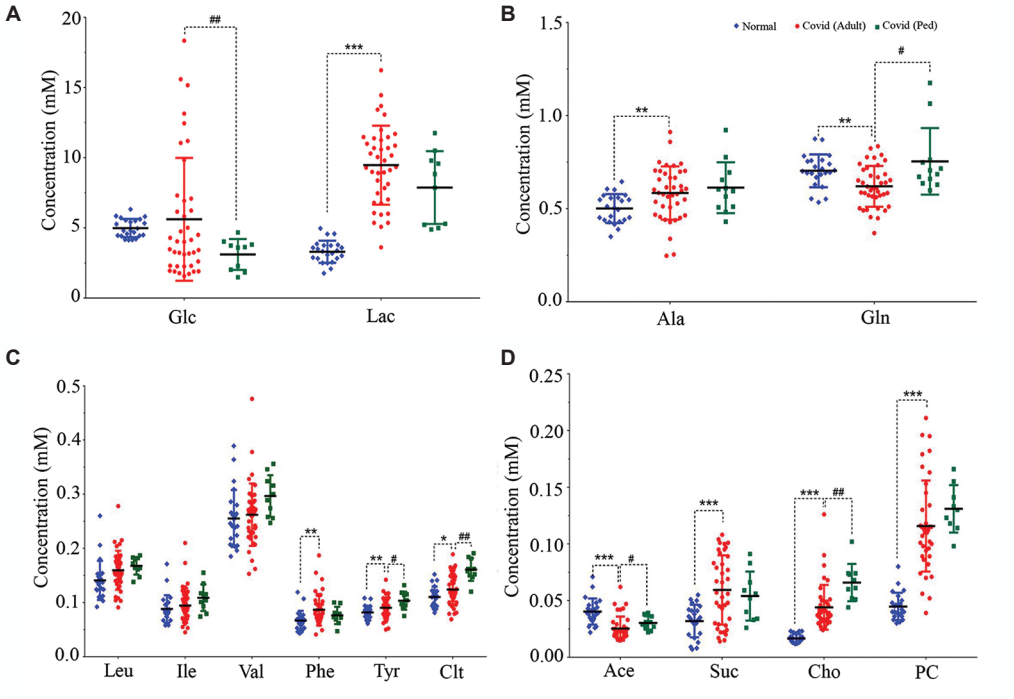

Fig. 5. Plasma metabolite profiles in COVID-19 patients. The levels (mM) of metabolites were measured in plasma samples of naïve control (Normal), adult and paediatric (Ped) COVID-19 patients by ^{1}H NMR spectroscopy relative to formate added during the sample preparation. The symbol represents the level in individual participants and the bar represents mean±SD. *P* *<0.05, **<0.01 and ***<0.001 are for the comparison of adult COVID-19 patients with naïve controls. *P* $^{\#}$<0.05 and $^{\#\#}$<0.01 for comparison of COVID-19 children with COVID-19 adults. The metabolites on X-axis include glucose (Glc), lactate (Lac), alanine (Ala), glutamine (Gln), leucine (Leu), isoleucine (Ile), valine (Val), phenylalanine (Phe), tyrosine (Tyr), citrate (Cit), acetate (Ace), succinate (Suc), choline (Cho) and phosphocholine (PC).

was found to be decreased in infected individuals. The metabolites with ≥2-fold change relative to the control included choline (2.67), lactate (2.87), phosphocholine (2.58) and succinate (1.86).

We also measured metabolites in paediatric samples (Fig. 5). There was no significant difference in the levels of metabolites in the COVID-19 paediatric and adult plasma samples. The changes in the levels of citrate and choline in paediatric patients were on the higher side as compared to the controls, suggesting that metabolomics changes upon COVID-19 could be similar in children and adults. The small-molecular-weight metabolite profiles of this study are in agreement with previous reports[25,26], indicating a general dysregulation of metabolism and increased metabolic stress in COVID-19 patients.

The results of this study highlighted the complexity of human immune response in COVID-19 patients. Although our data interpretation was limited due to single sample collection and lack of information on prior exposure and days post-sample collection, it was observed that individuals with higher antibody levels manifested severe disease, and those with mild symptoms or asymptomatic developed lower antibody levels. This suggests that, while innate and cellular immune responses are likely to be major determinants of disease severity and protection in COVID-19 patients, differences in antibody avidity could also affect the pathogenesis of SARS-CoV-2. In children, lower seropositivity to spike was associated with disease severity. Overall, higher seropositivity to spike was associated with asymptomatic SARS-CoV-2 infection. Hence, independent and timely trials of the COVID-19 vaccines and the study of pathogenesis of COVID-19 in children are necessary.

***Acknowledgment*:** The authors aknowledge Servshree Amit Kumar and Mohan Singh Moodu for their help in sample collection and processing.

***Financial support & sponsorship*:** This study was supported by funding from Council for Scientific and Industrial Research (CSIR), India through MLP0133 and MLP0136 projects.

***Conflicts of Interest*:** None.

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