DOI: 10.7860/JCDR/2022/55401.16592

Original Article

Pathology Section

Clinico-pathological and Genomic Characteristics among Children with SARS-CoV-2 Infection

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ABSTRACT

Introduction: There is inadequate information on infections with the Severe Acute Respiratory Syndrome Virus 2 (SARS-CoV-2) in children. Their clinical, as well as pathological correlation, is poorly understood. In India, children and adolescents account for 12% of all Coronavirus Disease 2019 (COVID-19) cases reported. Children accounted for roughly 11% of those impacted globally last year. However, this year, we are seeing around 20-40% of youngsters in positive instances over the world. Even babies and infants are testing positive for COVID-19, although their illness is under control and seldom becomes fatal. Children aged 5 to 12 years, on the other hand, are at a higher risk.

Aim: To study the clinical, pathological and genomic characteristics among children with SARS-CoV-2 infection.

Materials and Methods: This cross-sectional study was conducted among 48 paediatric positive patients for SARS-CoV-2 at Government Institute of Medical Sciences, Noida, Uttar Pradesh, and CSIR-Institute of Genomics and Integrative Biology, New Delhi, India, from 1st April 2021 to 31st May 2021. The laboratory testing was done by the real-time Reverse Transcription-Polymerase Chain Reaction (RT-PCR) method. The patients were classified as mild, moderate, severe, or asymptomatic. Their clinical and pathological findings were recorded in the case sheet. Genomic analyses were done for identifying the genetic variant in the nine selected samples. Data entry and analysis were performed using Statistical Package for Social Sciences (SPSS) version 26.0. Chi-square test was used for categorical variables and the t-test was used for continuous variables.

Results: The study group has median age of 12 years. Male:Female ratio was 2:3. Most children had acquired infection from the community and 30% had the moderate illness and were admitted. Serum Glutamic-Oxalacetic Transaminase (SGOT) and Glutamic-Pyruvic Transaminase (SGPT) were raised in six patients. Alkaline Phosphatase (ALP) was raised in 21 patients and bilirubin was raised in two patients. The average duration of hospitalisation was six days (range 2-13 days). No mortality among the 48 paediatric patients studied was identified in the hospital. Delta variant (B.1.617.2) was identified in seven patients with D614, P681R, L452R mutations and B.1.617.2 was identified in two patients. Delta variant was present in the paediatric patients, but it did not prolong the hospital stay or cause mortality.

Conclusion: The findings of the study suggest that children may be a potential source of infection in the SARS-CoV-2 pandemic while having an asymptomatic to mild illness.

Keywords: Coronavirus disease 2019, Delta variant, Liver enzymes, Monoclonal antibodies, Severe acute respiratory syndrome virus 2, Spike protein gene

INTRODUCTION

The World Health Organisation (WHO) announced a pandemic on 11th March 2020, when a novel coronavirus dubbed Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) originated in China in late December 2019 and expanded worldwide [1]. As of May 2021, a total of 25,07,15,502 million instances of the new respiratory disease, Coronavirus Disease 2019 (COVID-19) had been reported worldwide, with 50,62,106 deaths [1].

For the first time, Chan JFW et al., described human-to-human transmission of SARS-CoV-2 when a family member without travel history to Wuhan, China, got infected few days after interaction with kinfolk who had returned from Wuhan [2]. There is inadequate information on infections with the Severe Acute Respiratory Syndrome Virus 2 (SARS-CoV-2) in children and their clinical, as well as pathological correlation is poorly understood. In India, children and adolescents account for 12% of all COVID-19 cases reported. Children aged ≤12 years, on the other hand, are at a higher risk as they are not vaccinated. Only few studies have been published on pediatric population and highlight either clinical or genomic data [3,4]. In light of this continuing COVID-19 epidemic, research of their clinical, pathological and genomic characteristics is critical for guiding screening, containment, and prevention efforts. The study focused on the clinico-pathological and genomic characteristics among children with SARS-CoV-2 infection in India.

MATERIALS AND METHODS

This cross-sectional study was conducted in Molecular Diagnostics and Research Laboratory at Government Institute of Medical Sciences, Noida, Uttar Pradesh, and CSIR-Institute of Genomics and Integrative Biology, New Delhi, India, from 1st April 2021 to 31st May 2021. Total 48 paediatric patients (≤18 years) presenting with symptoms of COVID-19 and diagnosed positive on real-time Reverse Transcription-Polymerase Chain Reaction (RT-PCR) for SARS-CoV-2 were enrolled in the present study. The study protocol was explained to the guardians or parents of the children participating in study. Their written consent was taken. The study protocol was approved by the Institutional Review Board and Ethical permission was obtained from both Institutes (GIMS/IEC/HR/2020/02).

A detailed history was taken from the parents and children were examined for signs of COVID-19. Laboratory investigations included-routine testing of paediatric patients for complete haemogram, renal function test, liver function test and measurement of erythrocyte sedimentation rate. Also Interleukin (IL6), D-dimer, C-Reactive Protein (CRP) were tested and chest radiograph was also done, as advised by the physician. In the absence of symptoms, asymptomatic SARS-CoV-2 cases were characterised as individuals with a positive SARS-CoV-2 PCR. The COVID-19 cases were identified by a positive SARS-CoV-2 PCR and the presence of compatible signs and symptoms. COVID-19 cases were classified, as mild, if they

would be handled in an outpatient environment, moderate if they would be admitted to the hospital and have a favourable outcome, and severe, if they would be admitted to the Intensive Care Unit (ICU) or have a fatal outcome. Fever was the only symptom of a febrile episode. The emergence of atleast one respiratory symptom was defined as an acute respiratory infection like cough, sore throat, dyspnoea. The onset of fever, weakness, headache, and/or myalgias in conjunction with cough, dyspnoea, and/or pharyngeal discomfort was characterised as Influenza-Like Illness (ILI). A case of respiratory infection in a patient having radiographic or computer tomography findings consistent with pneumonia was characterised as pneumonia. Persons under the age of ≤18 years shall be considered children. They were classified as mild, moderate, severe, or asymptomatic based on World Health Organisation (WHO) criteria. (Living guidance for clinical management of COVID-19. Available at: https://apps.who.int/iris/bitstream/handle/10665/349321/WHO-2019-nCoV-clinical-2021.2-eng.pdf. Accessed July 01,2022).

Procedure

Sampling for SARS-CoV-2 testing: Samples using swabs from nasopharyngeal, and oropharyngeal areas were taken as per standard protocol and put in a 3 mL sterile viral transport medium tube. Cold chain was maintained for all samples. Samples were processed immediately and stored at -80^{0}C after processing.

Ribonucleic Acid (RNA) Isolation: RNA extraction was carried out in a pre-amplification environment in a Biosafety level 2 facility as per established protocol. RNA isolation was done by standard protocol using the MagMAX™ with commercial Mag MAXCORE Nucleic Acid Purification kit (MMkit) [5]. Protocol design and validation MM kit protocol was done as per protocol established by Lázaro-Perona F et al., [5].

SARS-CoV-2 detection: The SARS-CoV-2 viral RNA detection was done using a multiplex RT-PCR kit (2019 nCoVAssay Kit v1). The kit targets the Orf 1ab gene, spike protein gene, nucleocapsid gene and human RNa-seP gene. All RT-PCR assays were performed in QuantStudio™ 6 Flex RT-PCR (Applied Biosystems). The reaction mixture (6.25 µL ofmaster mix+1.25 µL of the nCoV assay+1.25 µL of RNAse P assay +11.25 µL molecular grade water) was prepared volume 20 µL. To this 5 µL of nucleic acid was added. The final reaction volume was 25 µL. A positive control (1 µLnCoV Control+4 µL molecular grade water) and a negative control using (5 µL of molecular grade water) were used with each run. The reaction settings had uracil-N-glycosylase incubation at 25°C (2 min), reverse transcription at 50°C (15 min), activation at 95°C (2 min), denaturation at 95°C (3 seconds), annealing and extension for 40 cycles was done at 60°C (30 seconds per cycle). Signal in FAM{6-carboxyfluorescein (6-FAM)}, before 37 cycles was taken as positive for SARS-CoV-2, Signal in VIC (2′-chloro-7′phenyl-1,4-dichloro-6-carboxy-fluorescein) before 37 cycles was taken as positive for internal control of SARS-CoV-2. A signal detected in VIC, but not in FAM was considered invalid results.

Library preparation, genome sequencing and data processing were done 20% randomly selected samples using protocols earlier established [6-9]. Phylogenetic analysis were also done using protocols earlier established by Rambaut A et al., [7]. Case detection for SARS-CoV-2 infection were reported to the Indian Council of Medical Research online portal and lab portals of state (https://labs.upcovid19tracks.in/). Data was collected from the Medical Record Department.

STATISTICAL ANALYSIS

Data entry and analysis were performed using Statistical Package for Social Sciences (SPSS) version 26.0. Chi-square test was used for categorical variables and the t-test was used for continuous variables.

RESULTS

A total of 48 paediatric patient, hospitalised due to COVID-19 illness were taken for the study. The study group has median age of 12 years. Male:Female ratio was 2:3. Most children had acquired infection from the community and moderate illness i.e, were admitted. Fever was the most common presenting symptom [Table/Fig-1,2]. Liver enzymes like Serum Glutamic Oxaloacetic Transaminase (SGOT) and Serum Glutamic Pyruvic Transaminase (SGPT) was raised in six patient. Serum Alkaline Phosphatase (ALP) was done in 35 out of 48 paediatric patients and was raised in 21 patients. C-Reactive Protein (CRP) was done in 15 paediatric patients and was raised in two only of them. D-dimer was raised in two out of seven paediatric patients (most of the parents did not show interest in sampling their children for the test as the test was done only once a week due to limited resources and kit availability). Interleukin 6 (IL-6) was raised in one paediatric patient. Erythrocyte Sedimentation Rate (ESR) was raised in nine out of 11 paediatric patients (11/48 were age less than 2 years and sufficient blood could not be collected for the ESR by manual method. Out of remaining 37 paediatric patients, only those who had both fever and cough were attempted for ESR from the amount of blood collected). Platelets were reduced in one out of 48 patients. Bilirubin was raised in two paediatric patients. The average duration of hospitalisation was six days (range 2-13 days). No mortality among the 48 paediatric patients studied was identified in the hospital.

Variables	Number of patients (n)
Underlying condition	
Chronic lung disease	2
Neurologic defecits	1
Hepatic disease	8
Prematurity	1
Spread of SARS-CoV-2 infection from	
Family members	10
Community	28
Travel history	0
School	0
Unknown	10
Category of SARS-CoV-2 infection	
Asymptomatic	10
COVID-19 illness	
Mild	2
Moderate	30
Severe	6

[Table/Fig-1]: Characteristics of 48 children with SARS-CoV-2 Infection.

Sign/Symptoms	Number of patients (n)
Fever	24
Cough	15
Weakness	6
Nausea/Vomiting	7
Sore throat	6
Diarrhoea	2
Loss of taste/or smell	2
Headache	1
Dyspnoea	1
Myaglia	0
Arthralgia	0
Abdominal Pain	0
Restlessness/Irritation	0

[Table/Fig-2]: Signs and symptoms of children with COVID-19.

Genomic analysis was done for randomly 20% of the patients which was taken as representative of subjects. Sequencing was not possible for all the positive patients and was neither indicated for any clinical reason so was not done. Genomic sequencing was done in nine paediatric patients randomly selected. Substitution in spike protein gene were found in seven patient. The substitutions were at T19R, G142D, 156del, 157del, R158G, L452R, T478K, D614G, P681R, D950N. These paediatric patients with the above mutations were identified as Delta variant (B.1.617.2). Delta variant is designated as a variant of concern, and was identified for the first time in India.

Delta variant was identified in India for the first time. It has following changes in spike protein of virus T19R, G142D, 156del, 157del, R158G, L452R, T478K, D614G, P681R, D950N. It has increased transmissibility asstronger affection to the Angiotensin Converting Enzyme 2 (ACE2) receptors. These mutations cause reduction in monoclonal antibodies treatment by its neutralisation. It is classified as a variant of concern.

The other two out of nine paediatric patients had kappa variant (B.1.617.1) with substitution in spike proteins gene at T951I, G142D, E154K, L452R, E484Q, D614G, P681R, Q1071H. [Table/Fig-3] showing phylogenetic graph of seven patient SARS-CoV-2 classified as delta variant.

DISCUSSION

The study reported the clinical, pathological and genomic characteristics in-hospital course and outcome of the 48 paediatric patients admitted to a COVID-19-dedicated hospital from North India.

In the present study mode of transmission was community-based in most of the children admitted this is in accordance with other studies conducted by Yonker LM et al., [8]. It is important to identify children with COVID-19 illness early, as they can be quarantined for infection control.

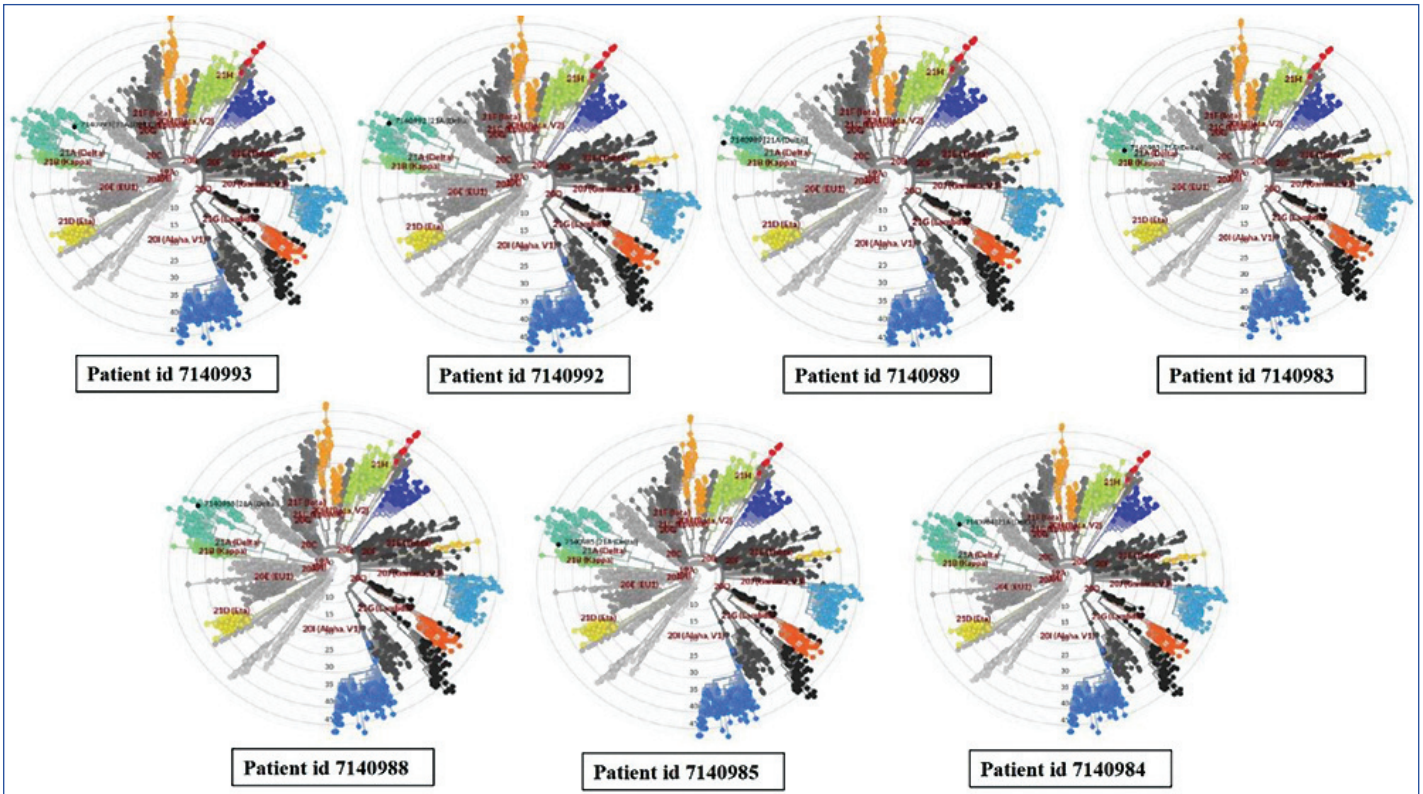
The present study had 12 patients with underlying conditions in form of hepatic, neurological, and respiratory involvement. The underlying co-morbidities worsen the clinical outcome in adults and the same is being observed in paediatric patients. Two patient had raised bilirubin levels due to neonatal jaundice (nine days and 13 days) but they did not have raised SGOT or SGPT. Raised bilirubin in the present study could be attributed to normal physiological phenomena in neonates born prematurely.

Study conducted by Ludvigsson JF, reported milder disease in children [9], however, this study had most of the cases as moderate illness. This could be due to the reason that the present study was conducted in hospital and the study by Ludvigsson JF, was conducted in community based settings.

Liver enzymes AST and ALT were elevated in 12.5% patients in the present study which is in accordance with the study conducted by Lazova S et al., wherein AST and/or ALT was raised in 12% cases only [10]. This involvement of liver in SARS-CoV-2 infection is due to its binding to ACE receptors in bile duct epithelial cells according to study by Feng G et al., in 2020 [11]. However, this has not been scientifically proven in children. The liver involvement in uncomplicated COVID-19 cases is temporary and usually resolves without treatment [12]. A study conducted by Zou YH et al., liver involvement in COVID-19 affects mainly 0-3 years as compared to the older children. Liver immaturity is perhaps the suspected reason for it [13].

The mean duration of hospital stay was six days only which suggests that children had a fast recovery from SARS-CoV-2 infection. It could be attributed to the fact that in children there is low expression of angiotensin-converting enzyme 2 receptors,Yonker LM et al., [8]. The SARS-CoV-2 enters host cell via ACE2 receptors expressed on human organs and the receptors have reduced expression in children [8,14]. Its expression increases with age.

Genomic sequencing of nine patients was a key feature of the present study. There is a scarcity of published data related to genomic sequencing in the paediatric patient. In the present study, nine patients have different mutations identified in SARS-CoV-2 genome. They had mutations in D614G, P681R, L452R that have clinical implications. Mutations D614G- moderate effect on transmissibility, increase infectivity and reduce spike protein shedding, L452R- Mutation on Spike protein Receptor-Binding Domain (RBD) increases binding affinity to human ACE2 receptor and decreases identification capability of the human immune system. It is also called as double mutant. The P681R (proline to arginine substitution at 681 position of spike



[Table/Fig-3]: Phylogenetic graph of seven patients SARS-CoV-2 classified as delta variant.

protein) is reported to boost the cell infectivity of B.1.617.2 variant by helping cleavage of the S precursor protein to the functional S1/S2 configuration [15].

Limitation(s)

Some of the investigation could not be performed at that particular point of time in pandemic during second wave on all the patients in the laboratory, due to limited availability of kits and resources.

CONCLUSION(S)

Based on the above clinical, pathological and genomic data we have learned more about the impact of SARS-CoV-2 on children. Variant of concern is present in paediatric patient, but it did not prolong the hospital stay or cause mortality. The study suggests that children with SARS-CoV-2 infection may be a potential source of spreading it in pandemic, even though they have a milder disease or are asymptomatic and must be treated timely.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]

- Plagiarism X-checker: Feb 02, 2022
- Manual Googling: Apr 06, 2022
- iThenticate Software: May 18, 2022 (24%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:

- Financial or Other Competing Interests: None
- 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: **Feb 02, 2022**
Date of Peer Review: **Mar 22, 2022**
Date of Acceptance: **Apr 07, 2022**
Date of Publishing: **Jul 01, 2022**