Original Article

THE PROPORTION OF SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS 2 INFECTION AMONG THE PEDIATRIC POPULATION IN THE STATE OF TRIPURA, NORTH EAST INDIA.

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ABSTRACT

Introduction: COVID-19 is a global crisis caused by SARS-COV-2. The virus was first reported in Wuhan, Hubei province, China; in December 2019. The incubation period of the virus is 2 to 14 days, average around 6.5 days in children and 5.4 days in adults. Asymptomatic infections are common in children. **Method:** ICMR recognized Viral Research & Diagnostic Laboratory of Agartala Government Medical College for conducting RT-PCR test for screening and confirmation of SARS-COV-2. Subsequently, tests were undertaken from 5th March 2020. All the tests were performed as per the testing strategies of ICMR on Strategy for COVID-19 testing in India. Extraction of viral RNA was done as per the manufacturer protocol of QIAGEN for QIAamp Viral RNA Mini Kit. The screening was done by targeting the SARS-CoV-2-specific 'E gene'. Then the Confirmation was carried out by targeting the 'RdRp gene' and 'ORF-1b'. **Result:** During this study period out of 100995 samples tested 6571 (7%) were children. 98% (6447) were negative and 2% (124) were positive for SARS-COV-2 by RT-PCR. In positive children, 62% (n=77) was male and 38% (n=47) was female. The most affected age group was 6 to 10 years (33%). The median age was 8 (0-14) years. 81% (n=101) was asymptomatic and 19% (n=23) was symptomatic. Symptoms were similar as Influenza like Symptoms. **Conclusion:** This study shows that 2% of the tested children were positive for SARS-COV-2, majority of them had positive household contact. 81% were asymptomatic and 19% had moderate symptoms without any mortality. COVID-19 is going to be added as another viral agent of the respiratory panel for future diagnosis and research.

KEYWORDS: SARS-COV-2, COVID-19, Symptoms, Real time PCR, North-eastern State, Tripura.

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a global crisis in health care, caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-COV-2). The virus was first reported in Wuhan, Hubei province, China; in December 2019. Since then it has spread rapidly and affected more than 200 countries globally.[1, 2] The clinical aspect and disease progression in children as well as in young adults are milder compared to older individuals.[3] In the earlier epidemics of coronavirus, children accounted for 6.9% of SARS 2002-3 and 2% of Middle East Respiratory Syndrome (MERS) infection worldwide. The transmission capability of SARS-COV-2 is much higher than the closely related SARS 2002-3 and MERS. The mode of transmission of SARS-COV-2 is the inhalation of infected droplets (produced by the infected individual during coughing and sneezing) and touching a contaminated surface with the virus.[4] The

clinical symptoms are similar to other acute respiratory viral infections, which include fever, cough, sore throat, fatigue, breathlessness.[5] The incubation period for SARS-COV-2 is 2 to 14 days, average around 6.5 days in children and 5.4 days in adults. Children may get asymptomatic infections, which may advance the spread of the infection.[6, 7]

METHOD

ICMR recognized Viral Research & Diagnostic Laboratory (VRDL) of Agartala Government Medical College (AGMC) for conducting Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) test for screening and confirmation of SARS-COV-2. Accordingly, as per ICMR guideline tests were undertaken from 5th March 2020. All the tests were performed by following the testing strategies of ICMR on Strategy for COVID-19 testing in India.[8] Detailed information including demographic data, travel and contact history, symptoms, and presence of comorbid conditions were taken in ICMR Specimen Referral Form (SRF) for COVID-19 (SARS-CoV2).[9] Nasopharyngeal and/or oropharyngeal swabs were collected and transported to the laboratory in Viral Transport Medium (VTM), maintaining a cold chain. Extraction of viral RNA was done as per the manufacturer protocol of QIAGEN for QIAamp Viral RNA Mini Kit.[10] In brief, the VTMs were thawed and thoroughly vortexed for 1 minute. Then 560 μl of prepared lysis buffer containing carrier RNA was taken in a 1.5 ml microcentrifuge tube. Then 140 μl of supernatant from the VTM was added to the solution. Then, mixed by pulse-vortexing for 15 seconds. Incubated at room temperature for 10 min. After brief centrifugation of the tubes, to remove drops from the inside of the lid, 560 μl ethanol (96–100%) was added and mixed by pulse-vortexing for 15 seconds. After mixing, the tubes were briefly centrifuged to remove drops from inside of the lid and 630 μl of the solution was added to the QIAamp Mini column (in a 2 ml collection tube) without wetting the rim. Then the columns were centrifuged at 6000 x g (8000 rpm) for 1 min. After centrifugation, the QIAamp Mini columns were transferred into a clean 2 ml collection tube, and the tubes containing the filtrate were discarded. The remaining solution is added to the column and centrifuged at 6000 x g (8000 rpm) for 1 min. After centrifugation, the QIAamp Mini columns were transferred into a clean 2 ml collection tube, and the tubes containing the filtrate were discarded. After that 500 μl, buffer AW1 was added to the columns and centrifuge at 6000 x g (8000 rpm) for 1 min. After centrifugation, columns were transferred into a clean 2 ml collection tube, and the tube containing the filtrate was discarded. Then 500 μl, buffer AW2 was added to the columns and centrifuge at full speed i.e. 20,000 x g (14,000 rpm) for 3 min. To eliminate possible buffer AW2 carryover, QIAamp Mini columns were transferred in a new 2 ml collection tube and the old collection tube with the filtrate was discarded. Then centrifuged at full speed for 1 min. After centrifugation, QIAamp Mini columns are transferred in a clean 1.5 ml microcentrifuge tube. The old collection tube containing the filtrate was discarded. 60 μl of buffer AVE, equilibrated to room temperature was added to the QIAamp Mini column. The caps were closed and incubated at room temperature for 1 min. Then centrifuged at 6000 x g (8000 rpm) for 1 min. After that, the extracted RNA was used for the downstream process or stored at -20oC for storage. The RT-PCR assays were performed in QIAGEN Rotor Gene Q as per the protocol of ICMR-NIV. The first-line assay (screening) was done by targeting the SARS-CoV-2-specific 'E gene'. Then the Confirmatory assays were carried out by targeting the 'RdRp gene' and 'ORF-1b'. The specimen was considered positive for SARS-COV-2 when reaction growth curves crossed the threshold line within 35 cycles for E gene, both RdRp and ORF-1b or either RdRp or ORF-1b.[11, 12, 13]

Statistical analyses: The data were analyzed using statistical package Graph pad Prism 7. Student t-test and Chi-square was used to determine the correlation between all the parameters. A P value <0.05 was considered significant.

RESULT

Total of 100995 samples were tested and 7% (n=6571) were children during this study period, among them 59% (n=3906) was male and 41% (n=2665) was female. A total of 6447 (98%) were negative and 124 (2%) were positive for SARS-COV-2 by RT-PCR. The median age was 8 (0-14) years. In positive children, 62% (n=77) was male and 38% (n=47) was female [Fig: 1]. The most affected age group was 6 to 10 years (33%) followed by 11 to 15 years (32%), 1 to 5 years (30%) and < 1 year (5%) [Fig: 2]. In respect to the clinical representation of the disease, 81% (n=101) was asymptomatic and 19% (n=23) was symptomatic [Fig: 3]. In the symptomatic children fever (37%) and cough (37%) was the most common symptom, followed by diarrhea (8%), body ache (6%), sore throat (6%), breathing difficulty (3%) and nasal discharge (3%) [Fig: 4].
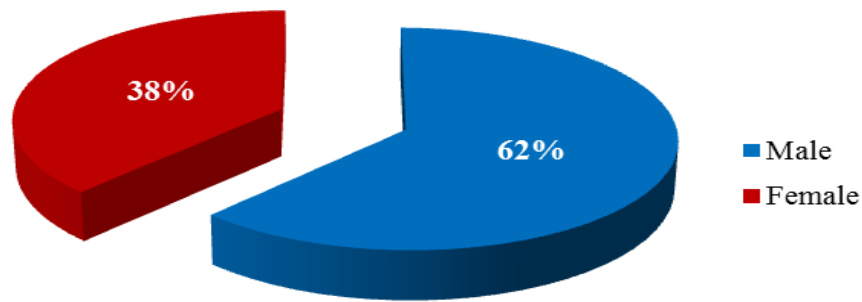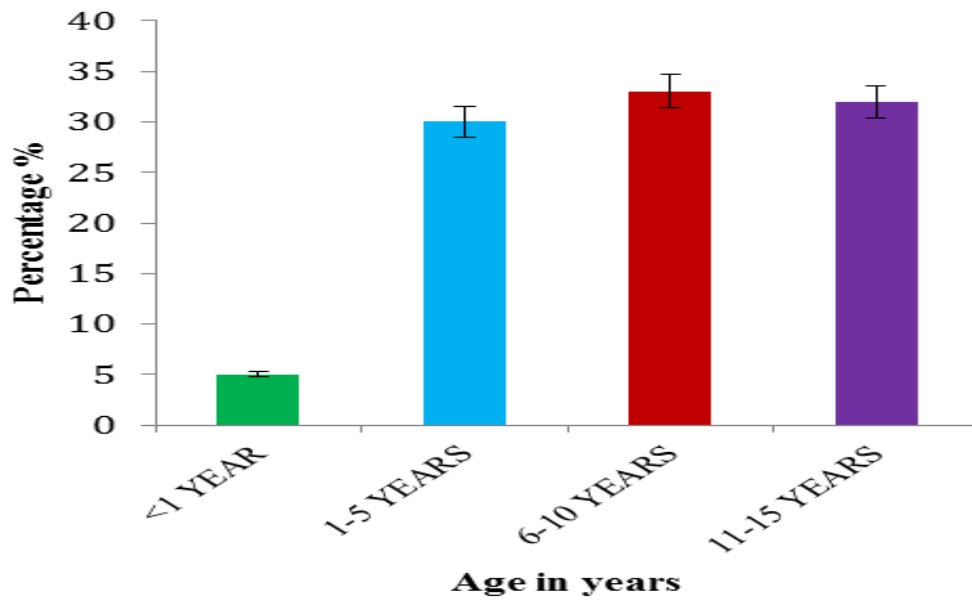
Fig. 1: Gender-wise positivity.

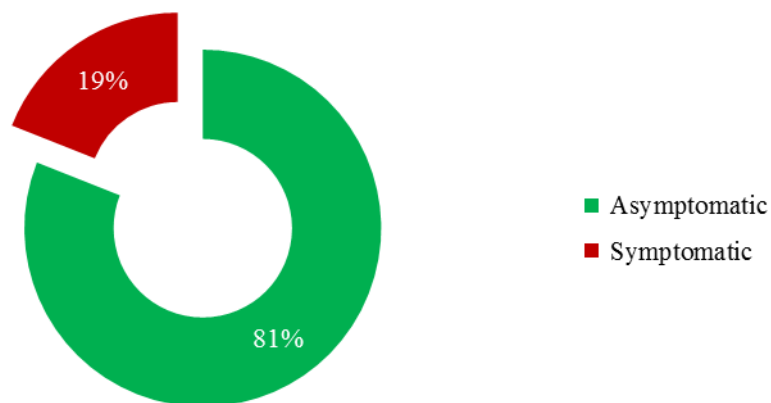**Fig. 2: Age-wise positive distribution.**

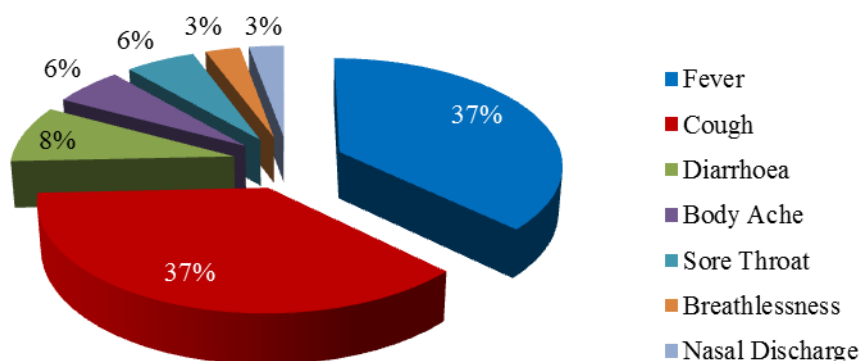
Fig. 3: Severity of the disease.

Fig. 4: Distribution of symptoms.

Table 1: Epidemiological and Clinical Characteristics of Children with SARS-CoV-2 Infection.

Parameters	Number	Percentage (%)
Total tested	6571	
Positive	124	2
Gender-wise distribution		
Male	77	62
Female	47	38
The severity of the disease		
Asymptomatic	101	81
Symptomatic	23	19
Age-wise positive distribution		
<1 YEAR	6	5
1-5 YEARS	37	30
6-10 YEARS	41	33
11-15 YEARS	40	32
Symptoms		
Fever	13	37
Cough	13	37
Diarrhoea	3	8
Body Ache	2	6
Sore Throat	2	6
Breathing difficulty	1	3
Nasal Discharge	1	3

DISCUSSION

In this study, all the children were between 1 month to 15 years who were tested positive for SARS-COV-2 by RT-PCR. Most of them were detected in contact with the lab-confirmed case or family member and designated containment zones. Though the infection has been observed in all age groups, children of less than 5 years of age were found to be less affected, which may be due to less exposure to virions, being isolated at houses or maturation, distribution and functioning of the viral receptors such as ACE2 (Angiotensin Converting Enzyme 2); which plays a vital role in susceptibility to serve COVID 19.[15, 16, 17, 21] Other studies on pediatric age groups have also shown similar observation on symptoms, that fever with a mild cough, commonly followed by sore throat, rhinorrhea, sneezing, myalgia, diarrhoea and vomiting.[16, 18, 19] Recent reviews also show that most of the children were asymptomatic or have mild symptoms, which correlates with the study.[14, 20] On the severity of COVID 19 in children, several hypotheses have been proposed[21], though a confirmed outcome is still anticipated. However, all the positive children were recovered without any mortality, by isolation in COVID Care Centers (CCC) and supportive medication.

CONCLUSION

In conclusion, this study shows that 2% of the tested children were positive for SARS-COV-2, majority of them had positive household contact. 81% were asymptomatic and 19% had moderate symptoms without any mortality. COVID-19 is going to be added as another viral agent of the respiratory panel for future diagnosis and research.

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REFERENCE

1. Singhal T. A review of coronavirus disease 2019 (COVID-19). Indian Journal of Pediatrics, 2020; 87: 281-6.
2. Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med, 2020; 382: 727-33.
3. Yang Lu, Qingbin Liu, Mingjin Wang, Yixing, Zhang, Anran, Jalali, Neda et al (2020). Epidemiological and clinical features of the 2019 novel coronavirus outbreak in China. DOI: https://doi.org/10.1101/2020.02.10.20021675.
4. Pedersen SF, Ho YC. SARS-CoV-2: a storm is raging. *J Clin Invest*, 2020; 130(5): 2202-2205. DOI:10.1172/JCI137647.
5. Guan, Wei-Jie & Ni, Zheng-yi & Hu, Yu & Liang, Wenhua & Ou, Chun-Quan et al (2020). Clinical Characteristics of Coronavirus Disease 2019 in China. New England Journal of Medicine. 382. https://doi.org/10.1056/NEJMoa2002032.
6. Cai, J., Xu, J., Lin, D., Yang, Z., Xu, L., Qu, Z. et al (2020). A Case Series of children with 2019 novel coronavirus infection: clinical and epidemiological features. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America*, ciaa 198. Advance online publication. https://doi.org/10.1093/cid/ciaa198.
7. Yang Lifen, Dai Zhenyuan, Duan Mengqi, Zhang Yang, Dong Wanqiu, Pan Li et al. Suggestions for medical staff from department of pediatrics during the treatment of 2019-nCoV infection/pneumonia[J]. JOURNAL OF NEW MEDICINE, 2020; 51(2): 77-84.
8. ICMR, Strategy for COVID-19 testing in India. Available at: https://www.icmr.gov.in/cteststrat.html. Accessed on 07th August 2020.
9. ICMR, ICMR Specimen Referral Form for COVID-19 (SARS-CoV2). Available at: https://www.icmr.gov.in/pdf/covid/labs/SRF_v11.pdf. Accessed on 07th August 2020.
10. QIAamp Viral RNA Mini Handbook, Available at: https://www.qiagen.com/us/resources/resourcedetail?id=c80685c0-4103-49ea-aa72-8989420e3018&lang=en. Accessed on 07th August 2020.
11. Gupta, N., Potdar, V., Praharaj, I., Giri, S., Sapkal, G., Yadav, P. et al (2020). Laboratory preparedness for SARS-CoV-2 testing in India: Harnessing a network of Virus Research & Diagnostic Laboratories. *The Indian journal of medical research*, *151*(2 & 3): 216–225. https://doi.org/10.4103/ijmr.IJMR_594_20.
12. ICMR, NIV Pune SoP for detection of COVID-19 in suspected human cases by rRT-PCR : First Line Screening assay. Available at: https://www.icmr.gov.in/pdf/covid/labs/1_SOP_for_First_Line_Screening_Assay_for_2019_nCoV.pdf. Accessed on 07th August 2020.
13. ICMR, NIV Pune SoP for detection of COVID-19 in suspected human cases by rRT-PCR : confirmation assay. Available at: https://www.icmr.gov.in/pdf/covid/labs/2_SOP_for_Confirmatory_Assay_for_2019_nCoV.pdf. Accessed on 07th August 2020.
14. Sarangi, B., Reddy, V. S., Oswal, J. S., Malshe, N., Patil, A., et al (2020). Epidemiological and Clinical Characteristics of COVID-19 in Indian Children in the Initial Phase of the Pandemic. *Indian pediatrics*, S097475591600218. Advance online publication.
15. Murray MA, Chotirmall SH. The impact of immunosenescence on pulmonary disease. Hindawi [Internet]. 2015 Jun 24. Available from: http://downloads.hindawi.com/journals/mi/2015/692546.pdf. Accessed on 07th August 2020.
16. Yuanyuan Dong, Xi Mo, Yabin Hu, Xin Qi, Fang Jiang, Zhongyi Jiang, Shilu Tong. Epidemiological Characteristics of 2143 Pediatric Patients with 2019 Coronavirus Disease in China. Pediatrics Mar 2020, e20200702; DOI: 10.1542/peds.2020-0702.
17. Lee, P. I., Hu, Y. L., Chen, P. Y., Huang, Y. C., & Hsueh, P. R. (2020). *Are children less susceptible to COVID-19?* Journal of microbiology, immunology, and infection = Wei mian yu gan ran za zhi, 53(3): 371–372. https://doi.org/10.1016/j.jmii.2020.02.011.
18. Cai, J., Xu, J., Lin, D., Yang, Z., Xu, L., Qu, Z., et al (2020). A Case Series of children with 2019 novel coronavirus infection: clinical and epidemiological features. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America*, ciaa198. Advance online publication. https://doi.org/10.1093/cid/ciaa198.
19. Lu X, Zhang L, Du H, Zhang J, Li YY, Qu J, et al. SARSCoV-2 Infection in children. NEJM. 2020 Mar 18. Available from: https://www.nejm.org/doi/full/10.1056/nejmc2005073. Accessed on 07th August 2020.
20. Meena, J., Yadav, J., Saini, L., Yadav, A., & Kumar, J. (2020). Clinical Features and Outcome of SARS-CoV-2 Infection in Children: A Systematic Review

and Meta-analysis. *Indian pediatrics*, S097475591600203. Advance online publication.

21. Hoffmann, M., Kleine-Weber, H., Schroeder, S., Krüger, N., Herrler, T., Erichsen, S., et al. (2020). SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell*, *181*(2): 271–280.e8. https://doi.org/10.1016/j.cell.2020.02.052.