Original Article

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Paediatrics Section

Seroprevalence of SARS-CoV-2 among Children Visiting a Tertiary Hospital in Himachal Pradesh, India

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ABSTRACT

Introduction: Research on Coronavirus Disease-2019 (COVID-19) seroprevalence in children and adolescent population across the globe is quite limited. In India, there is a dearth of data on COVID-19 seropositivity, especially in unvaccinated paediatric population, particularly in the Himalayan region.

Aim: To estimate the seroprevalence of COVID-19 in children presenting in a tertiary care health institution.

Materials and Methods: A hospital-based cross-sectional serosurvey was conducted on 500 children, from October 2021 to March 2022 in paediatric age group, attending Indira Gandhi Medical College Shimla, Himachal Pradesh, India, for various health related concerns such as fever, cough, loose stools, vomiting and fast breathing using convenience sampling. Socio-demographic profile was recorded and blood sample was drawn for COVID-19 antibody titre estimation. Chi-squared and Fisher's-exact tests for proportions was used for testing statistical significance.

Results: A total of 500 children, age ranged from 12 hours to 17 years 7 months were enrolled with maximum children belonging to 01-05 years age group and there was slight male preponderance. Seropositivity in males (27.3%) was significantly higher than females (8.3%). Highest (42.3%) seropositivity was seen in age group of 06 months to 01 year. About 10.8% of cases were positive for Immunoglobulin (Ig)G antibody, 4.4% were positive for IgM antibody, while about 6.6% cases were positive for both antibodies.

Conclusion: The seroprevalence status of children and adolescents is quite low in this region, revealing the high susceptibility of children to SARS-CoV-2 in the study region. It further emphasises benefits of serological testing in children for SARS-CoV-2 as well as the need of safe and effective vaccination for the unimmunised, unprotected and vulnerable paediatric age group.

Keywords: Antibody, Children, Northern India, Severe acute respiratory syndrome-coronavirus-2

INTRODUCTION

Coronavirus Disease 2019 (COVID-19) caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has been a global public health problem and had brought down globe to an absolute stand-still. The World Health Organisation (WHO) declared COVID-19 as a "Public Health Emergency of International Concern" in 2020. SARS-CoV-2 infected nearly 200 million and killed more than 04 million people worldwide, causing massive disruptions to daily life and manifold economic losses [1-3]. In order to prevent SARS-CoV-2 transmission and to curb paediatric morbidity and mortality, the schools nationwide were closed for in-person teaching and classes were moved to online mode in March 2020 [4,5]. Despite all preventive measures, the infection transmission can never be eliminated completely. The paediatric infection rates and the true level of natural immunity in children are still unknown yet, and especially the region-specific data is lacking [6,7].

Moreover, the role of children in SARS-CoV-2 transmission has been a major area of concern [7,8]. As SARS-CoV-2 vaccines for children 12 years and below were not approved until late 2021, knowing infection rates in children seemed even more important, in order to implement protective measures to limit infection. Children typically experience mild COVID-19 illness and are more likely to have asymptomatic infections, allowing them to unknowingly transmit SARS-CoV-2 [8]. With the rate of asymptomatic infections estimated to be as high as 50%, estimating true infection rates remains a challenge [9,10]. Although nucleic acid testing can identify active SARS-CoV-2 cases, most infections clear within two weeks and leave asymptomatic cases undocumented [10,11]. Antibodies generated against previous infections can last for months to years and the presence of SARS-CoV-2 antibodies in blood indicate that a person was infected at some point in the past [11,12]. Serological studies can identify asymptomatic and symptomatic cases and more accurately estimate number of people infected with SARS-CoV-2 in the absence of known vaccination status [9-12].

The evidence on seropositivity in children and adolescent populations from all over the world as well as India is still evolving, especially in the unvaccinated population. Moreover, no research had been conducted on paediatric seroprevalence in Himachal Pradesh and there was dearth of region-specific data. This present hospital-based research was conducted to fulfil this major knowledge gap and estimate the seroprevalence of COVID-19 among paediatric age group; simultaneously providing an insight into the proportion of susceptible population amongst the young.

MATERIALS AND METHODS

The present study was a hospital-based observational cross-sectional serosurvey to estimate the prevalence of seropositivity against COVID-19 in children (0-18 years), who attended the Department of Paediatrics, Indira Gandhi Medical College, Shimla, Himachal Pradesh, India, a tertiary care health institution for various health concerns, between October 2021 and March 2022 (06 months). Prior permission was taken from Institute Ethical Committee (Letter No. IGMC/HFW(MC-II) B (12) ETHICS/2022/3684, Dated 23.08.2022) to go ahead with the study. Institutional identifiers were omitted in order to maintain confidentiality and anonymity.

Inclusion criteria: Every alternate child/adolescent attending the outdoor department or admitted in the paediatric indoor facility during the study period, whose caretakers consented for the study were recruited in the study.

Exclusion criteria: All those children and guardians, denying consent were not enrolled in the study.

Every alternate child/adolescent fulfilling the inclusion criteria were recruited in the study,utilising convenience sampling technique, making a total of 500 study participants.

Study Procedure

A study tool was devised for collecting the relevant information for the research. It consisted of a semi-structured proforma comprising of both open and closed questions intended to collect information pertaining to socio-demographic parameters and awareness on personal protection measures.

The blood sample (05 mL) was collected using standard aseptic technique for the assessment of antibodies (IgG and IgM) against COVID-19. Serum was separated immediately and samples were stored in a deep freezer at -20°C. Serum samples were analysed for the estimation of IgG and IgM (anti-spike) antibody titres against COVID-19 virus in the immunohaematology laboratory of the institute. Quantitative estimation of IgG and IgM levels against COVID-19 virus was done using Immunoassay technique by the Department of Immunohaematology and Transfusion Medicine IGMC Shimla. Immunoassay kits (Dia Pro Diagnostic Bio probes Sesto San Giovanni Milano) were having 98% sensitivity and 98% specificity. Data was collected for serological status of enrolled cases for antibodies and their quantitative estimation. Titres of IgG and/or IgM antibodies were measured; IgG and IgM titres above 0.9 were taken as positive for COVID-19 serology. Positive serology was taken when IgG or IgM levels or both were above normal range [11].

STATISTICAL ANALYSIS

The data on demography, serological status, COVID-19 appropriate behaviour and vaccine status of caregivers of children enrolled was tabulated in Microsoft Excel sheet and processed and analysed with Epi Info (Epidemiological Information) version 7.2 with relevant statistical tests of significance. The qualitative data was presented as proportions as Chi-squared and Fisher's-exact tests for proportions was used for statistical significance testing; two tailed p<0.05 was considered as statistically significant.

RESULTS

Total 500 children/adolescents were enrolled in the study with age ranged from 12 hours to 17 years 7 months. About 54.2% of the enrolled participants were males. About 54 (10.8%) participants had tested positive for IgG with mean titre of 1.58±0.58, 72 (14.4%) had tested positive for IgM with mean titre of 2.07±0.71, 33 (6.6%) had tested for both IgG and IgM antibodies; therefore, a total of 93 (18.6%) of children had tested positive for COVID-19. Seropositivity in the males (27.3%) was significantly higher than females (8.3%).

[Table/Fig-1] shows the gender, locality distribution and COVID-19 appropriate behaviour of seropositive cases. Total seropositivity for any antibody was observed to be 18.6%. For IgG, IgM and both IgG and IgM; the seropositivity was 4.2%, 7.8% and 6.6%, respectively. Except for both IgG and IgM; the difference between males and females was found to be statistically significant. The overall seropositivity for urban and rural areas was 22.5% and 17.9%; however, there were no statistically significant differences in seropositivity based on residential locality. Barring social distancing measures, other COVID-19 appropriate preventive behaviours did not have statistically significant effect on extent of seropositivity.

As shown in [Table/Fig-2], maximum number of the children as well as antibodies positivity characterstic, corresponds to age group of 01-05 years. Highest (42.3%) seropositivity was seen in age group of 06 months to 01 year. [Table/Fig-3] shows the district-wise comparison of seropositivity. The highest number of cases was from Shimla district (54/291, 18.5%), while the highest seropositivity was seen in Bilaspur (11/27 patients, 40.7%); three districts had no patient admitted or referred to the institution. Only two of the enrolled children had family history of COVID-19 infection, but it was not associated with mortality. None of the caregivers/cases reported any history of febrile illness in the last three months. The immunisation coverage was relatively good in parents/caregivers of enrolled cases. Males (66.6%) had higher vaccination coverage of two doses of COVID-19 vaccine as compared to the females (48.8%).

Parameters	Sub-parameters	IgG+	IgM+	IgG+ and IgM+	Total seropositivity
Seropositivity	N=500 (%)	21 (4.2)	39 (7.8)	33 (6.6)	93 (18.6)
Gender	Male (271)	21 (7.7)	38 (14.1)	15 (5.5)	74 (27.3)
	Female (229)	0	01 (0.4)	18 (7.9)	19 (8.3)
	p-value*	**<0.001**	**<0.001**	0.388	**<0.001**
Locality	Urban (80)	03 (3.8)	09 (11.3)	06 (7.5)	18 (22.5)
	Rural (420)	18 (4.3)	30 (7.1)	27 (6.4)	75 (17.9)
	p-value*	0.826	0.209	0.724	0.328
Awareness of handwashing	Yes (383)	12 (3.1)	33 (8.6)	24 (6.3)	69 (18.1)
	No (117)	09 (7.6)	06 (5.1)	09 (7.6)	24 (20.5)
	p-value*	0.069	0.219	0.587	0.544
Awareness of social distancing	Yes (342)	06 (1.8)	14 (4.1)	06 (1.8)	26 (7.6)
	No (158)	15 (9.5)	25 (15.8)	27 (17.1)	67 (42.4)
	p-value*	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Awareness of mask usage	Yes (497)	21 (5.3)	39 (7.8)	33 (6.6)	93 (18.7)
	No (03)	0	0	0	0
	p-value**	>0.999	>0.999	>0.999	>0.999

[Table/Fig-1]: Seropositivity and various parameters.
*Chi-squared test for proportions
**Fischer-Exact test for proportions

Age group	Number (%)	IgG +	IgM +	IgG+ and IgM+
<1 days	08 (1.6)	0	0	0
1-7 days	39 (7.8)	0	0	0
8-<28 days	22 (4.4)	0	2 (5.1)	1 (3.1)
28 days ≤6 months	79 (15.8)	2 (9.5)	8 (20.5)	8 (24.2)
6 months ≤1 years	26 (5.2)	3 (14.3)	5 (12.8)	3 (9.1)
1 years ≤5 years	137 (27.4)	9 (42.9)	10 (25.6)	11 (33.3)
5 years ≤12 years	89 (17.8)	5 (23.8)	7 (17.9)	3 (9.1)
12 years ≤18 years	100 (20)	2 (9.5)	7 (17.9)	7 (21.2)
Total	500 (100)	21 (100)	39 (100)	33 (100)

[Table/Fig-2]: Age distribution and Seroprevalence in different age groups.
IgG: Immunoglobulin G; IgM: Immunoglobulin M; +: Present

District	Patients n (%)	IgG +	IgM +	IgG and IgM +	Total seropositivity
Shimla	291 (58.2)	12 (54.5)	22 (57.9)	20 (60.1)	54 (58.1)
Solan	50 (10)	02 (9.1)	01 (2.6)	02 (6.1)	05 (5.4)
Sirmaur	39 (7.8)	01 (4.5)	04 (10.5)	04 (12.1)	09 (9.7)
Bilaspur	27 (5.4)	02 (9.52)	06 (15.38)	03 (9.1)	11 (11.8)
Kinnaur	20 (4)	0	01 (2.6)	0	01 (1.1)
Mandi	46 (9.2)	03 (13.6)	03 (7.9)	02 (6.1)	08 (8.6)
Lahaul & Spiti	06 (1.2)	0	0	01 (3.1)	01 (1.1)
Kullu	17 (3.4)	01 (4.5)	02 (5.3)	01 (3.1)	04 (4.3)
Una	04 (0.8)	0	0	0	0
Total	500 (100)	21 (100)	39 (100)	33 (100)	93 (100)

[Table/Fig-3]: District-wise distribution of seropositivity.

DISCUSSION

To date limited studies have been undertaken in paediatric population infected with SARS-CoV-2; though children usually are asymptomatic or have a milder form of infection with equivalent transmissibility [13-15]. The present study is one of the few studies conducted in Northern region of the country and was undertaken when immunisation for children had not yet started. In a China-based study, about 1% of children (<10 years) were found positive among 44,762 confirmed COVID-19 cases [16]. In another study, only 0.5% (0-4 years) and 1.3% (5-17 years) of children tested positive at public health laboratories in the United States [17]. These studies specified lower prevalence of disease as well as severity of common symptoms in children compared to adults. Presently, there is only in-vitro evidence of low SARS-CoV-2-specific Angiotensin-Converting Enzyme 2 receptors in children by the possibility of an active immune system and pre-existing antibodies against other viral infections in children [18,19]. Additionally, the SARS-CoV-2 sample collection method and type of specimens collected from children are other concerns as viral load may vary significantly. Furthermore, pathogenesis and transmissibility of COVID-19 may also differ in children and adults. Responsible factors for these differences include a low number of ACE2 receptors in children, a less mature enzyme against SARS-CoV-2 variants and presence of low inflammatory cytokines, which undergo substantial changes in adulthood. However, high levels of procalcitonin and interleukin-6 have been previously reported in COVID-19 positive children [18,19].

Overall, 93 (18.6%) participants of study population were found seropositive for COVID-19 antibody; meaning a significant 81.4% of the paediatric population was susceptible to COVID-19; the results were similar to a South Indian study where 19.6% prevalence was reported in the age group of 01 month to 17 years [13], and study by Misra P et al., where higher seropositivity of 62.3% was documented [20]. Seropositivity in the males was significantly higher as compared to females, which could be due to the fact that the males are more exposed as they are more mobile due to social and economic reasons. None of the newborns had tested positive for COVID-19 serology. Although not statistically significant, the present survey showed that the seropositivity among the rural population was slightly lower as compared to the urban population; this fact can be explained as social distancing is more natural in rural areas. Interestingly, the history of COVID-19 in the study participants and their caregivers was lacking. Only two of the cases had positive family history of COVID-19 infection in the past.

There was no history of any COVID-19 associated mortality in the families of the study population. None of the caregivers/cases reported had history of any febrile illness in last three months; suggesting subclinical or overlooked mild COVID-19 illness. The major challenge is thus to identify asymptomatic cases especially in children and older adults. Though, in various studies, few cases of symptomatic children with SARS-CoV-2 specific antibodies showed negative RT-PCR test [21]. The behaviour regarding prevention of transmission of COVID-19 such as the use of mask was seen almost universally in the study population, while the concept of hand washing and social distancing was lacking in quarter to one-third of population studied. The prevalence of seropositivity was higher in the group which did not observe the COVID-19 appropriate behaviour (particularly social distancing); further justifying universally advocated COVID-19 appropriate behaviour and preventive measures. All these observations cement the need of practicing personal protective measures for prevention of diseases with high communicability. Moreover, despite having infection, most of the children remained asymptomatic/mildly symptomatic; this further strengthened the need of early vaccination for the paediatric age group. In addition, in future, a possibility of COVID-19/Long COVID/Post-COVID should also be kept for children as the disease may present with vague symptoms and complications.

Limitation(s)

As it was a hospital-based study with a small sample size, possibility of compromised estimation of seropositivity cannot be denied.

CONCLUSION(S)

The study provides an insight into the proportion of children affected in the region of Shimla city which was once the hotbed of COVID-19. The results provide evidence that seroprevalence status of children and adolescents is quite low in the region, revealing high susceptibility of children to SARS-CoV-2. The findings also support the observation that children experience fewer and milder symptoms of infection which may often go unrecognised, warranting serological testing in children. Lastly, as the natural infection has shown a limited seroconversion as well as protection from infection, study justifies the need of strategic and effective vaccination for paediatric infection. In addition, it will be pertinent to continue monitoring of serological status of children especially in community, particularly in context of the introduction of vaccine in paediatric population as it will give us a good insight into postvaccination seroconversion.

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