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Human Coronaviruses In Severe Acute Respiratory Infection (SARI) Cases in Southwest India

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Acute viral respiratory infections (AVRI) are a leading cause of morbidity and mortality among all age groups globally. Except for Influenza virus and Respiratory Syncytial virus, mostly viral aetiology of AVRI remains undiagnosed. Lately, human coronaviruses (HCoVs) have emerged as an important aetiology of AVRI. A laboratory based retrospective cross sectional study was conducted in which respiratory samples (throat swabs) of patients (n = 864), with Influenza negative SARI, of all age groups between Jan 2011–Dec 2012 were tested for HCoVs including MERS-CoV using Conventional and real time PCR assays. The prevalence of HCoV among SARI cases was 1.04% (9/864) [95% CI: 0.36–1.72]. Of these four (44.44%) were identified as HCoV OC43, three (33.33%) as HCoV NL63 and two (22.22%) as HCoV 229E. No HCoV HKU1 was detected. The samples were also negative for SARS-CoV and MERS-CoV. The results of this study documents low prevalence of human coronaviruses in SARI cases in south western India and the absence of highly pathogenic human coronaviruses. As the study included only SARI cases the prevalence reported could be an under estimate when it is extrapolated to community. ***J. Med. Virol. 88:163–165, 2016***.

KEY WORDS: HCoV; SARI; SARS; MERS; prevalence; southwest India

INTRODUCTION

Acute respiratory illnesses (ARI) are a major health problem in people of all ages, according to the World Health Organization (WHO) [Dominguez et al., 2009]. Lower respiratory tract infections particularly contribute to the world's disease burden [Dominguez et al., 2009]. Except for Influenza virus and Respiratory Syncytial virus, mostly viral aetiology of AVRI remains undiagnosed. Human coronaviruses (HCoVs) have a history way back in the late 1960s, identified as a group of viruses with potential to infect humans and animals [Principi et al., 2010]. Six human coronaviruses have been identified till date, which are HCoV-229E, HCoV-HKU1, HCoV-NL63, HCoV-OC43, Severe Acute Respiratory Syndrome coronavirus (SARS-CoV) and the recent Middle East Respiratory Syndrome coronavirus (MERS-CoV). Four human coronaviruses HCoV-229E, HCoV-HKU1, HCoV-NL63, and HCoV-OC43 have been found to be associated with a wide range of respiratory symptoms, including fatal outcomes such as pneumonia and bronchiolitis. Specifically, HCoV-NL63 has been associated with croup [Abdul-Rasool and Fielding, 2010] and HCoV-HKU1 with febrile convulsion [Gaunt et al., 2010].

The worldwide epidemic of SARS in 2002–2003, due to a newly discovered coronavirus, the SARS-CoV (SARS-associated coronavirus), reinforced the interest into the *Coronaviridae* family [Geller et al., 2012]. Globally more than 8,000 cases were reported during the 2002–2003 SARS epidemic, which affected 30 countries across five continents with a case fatality rate of 9% [Bhatnagar et al., 2008].

Ten years after the detection of SARS-CoV, a novel coronavirus known as MERS-CoV was discovered in 2012 [Corman et al., 2012]. The nearest human coronavirus related to MERS-CoV is SARS-CoV [Khan, 2013].

Though HCoVs were identified as respiratory pathogens, hardly any data was available on their role in respiratory infections till 2003. There is no data available in literature on HCoV from India.

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METHODS

Severe Acute Respiratory Infection (SARI) cases, referred to Manipal Centre for Virus Research from January 2011–December 2012 from Kerala and Karnataka States of India, which are tested negative for Influenza A and B viruses, formed the study population. Respiratory samples were collected when the cases presented with symptoms of Influenza like Illness (ILI) (fever, cough, coryza, nasal catarrh) within a week of onset of illness. Respiratory samples (throat swab) collected in Viral Transport Medium were aliquoted and stored at −70°C. SARI cases were defined as a person having acute respiratory infection with history of fever or measured fever of ≥38°C, cough and breathlessness, requiring hospitalization.

Using a cross sectional study design, a total of 864 samples were selected by stratified random sampling method, by assuming a prevalence of 10% with a relative error of 20%, representing different age groups and different time period.

Clinical as well as epidemiological data of the SARI cases included in the study were obtained from the laboratory requisition forms. All samples and data records were annonymised to protect the privacy of the cases.

Institutional Ethical Committee, Manipal University, Manipal reviewed and approved the protocol on 14/05/2013.

Viral RNA Extraction and Amplification

RNA extraction. Viral RNA was extracted from the clinical samples using QIAmp® viral RNA mini-kit (QIAGEN).

Pancoronavirus screening. Samples were screened for the presence of HCoVs using a pancoronavirus reverse transcriptase PCR (RT–PCR) assay targeting the 220 bp fragment of the polymerase 1 b gene [Adachi et al., 2004]. This assay was used to detect human coronaviruses including SARS-CoV. However, this assay does not detect MERS-CoV. In brief, 25 μl of reaction mixture contains 5 μl of eluted RNA, 12.5 μl of buffer mix (Ambion®, Life Technologies), 1.5 μl of each primer (from 10 μm working concentration), 1 μl of enzyme mix (Superscript™ III RT/Platinum® Taq Enzyme Mix, Life Technologies, Carlsbad, CA) and 3.5 μl of nuclease free water. Amplification was carried out using Veriti® thermal cycler (Life Technologies) with cycling conditions as described by D. Adachi et al (2004).

Typing for human coronaviruses. Further, the pancoronavirus cases were typed using a multiplex real time reverse transcriptase PCR assay (rRT–PCR) [Fast Tract Diagnostic, Luxembourg] in real time PCR system ABI7500 (Applied Biosystems, Inc., Foster City, Ca). This assay covered HCoV-229E, HCoV-HKU1, HCoV-NL63, and HCoV-OC43.

Samples were also screened for the presence of MERS-CoV by real time RT-PCR assay recommended by World Health Organization [Corman et al., 2012]. In brief, 25 μl of reaction mixture contains 5 μl of RNA, 1 μl each of forward and reverse primers (from 10 μM working concentration), 0.5 μl of probe (from 10 μM working concentration), 1 μl of enzyme mix and 4 μl of nuclease free water with following cycling conditions: 50°C for 15 min, 95°C for 10 min, 45 cycles of 94°C for 15 sec, 58°C for 30 sec.Coronavirus positive samples were screened for other common 13 respiratory viruses (Influenza virus A and B, Rhinovirus, Human Parainfluenza viruses (type 1,2,3,4), Human metapneumovirus A and B, Human bocavirus, Respiratory Syncitial virus, Parechovirus, Enterovirus, Adenovirus) using a multiplex real time reverse transcriptase PCR assay (rRT–PCR) [Fast Tract Diagnostic, Luxembourg].

Data was entered in Excel and then analysed using SPSS 16.0. The prevalence was reported with 95% CI.

RESULTS

A total of 1706 cases of influenza virus negative SARI were referred to MCVR during the study period. Of the 864 cases included, the mean age was 24.3 (SD = 21.9), median age was 20 (IQR = 3–40) and the male to female ratio was 1:1 (Table I). Geographically, 21.2% of SARI cases were from Karnataka and 78.8% from Kerala States of India.

Of the 864 samples tested, only nine samples were positive for HCoVs and the prevalence was 1.04% (95% CI: 0.36–1.72). The mean age of confirmed cases was 25.8 (SD = 17.5) and the median age was 24.0 (IQR = 16.5–32.0). Of the nine confirmed cases, four were positive for HCoV-OC43, three were positive for HCoV-NL63 and two were positive for HCoV-229E (Table I). Seven confirmed cases were seen in 2011 and only two cases were positive in 2012. No MERS-CoV was detected. Since, the pancoronavirus positive samples already revealed the HCoV types, the need to further screen for SARS CoV was negated.

DISCUSSION

This study reports low prevalence of HCoV among SARI cases from south west India during the period January 2011 to December 2012. This is in concordance with the findings of a similar study from Thailand [Dare et al., 2007]. However the prevalence reported here is less when compared to studies conducted in similar age groups in Brazil and United Kingdom [Dare et al., 2007; Gaunt et al., 2010; Cabecca et al., 2013]. But these studies included all respiratory infections and were not limited to SARI cases. While majority of HCoVs are associated with mild acute respiratory infection and our samples included only SARI cases, this could be the possible reason for the observed low prevalence of HCoVs in this study. Further, tropical climate in Southwest India also may have contributed to the low prevalence.

TABLE I. Demographic Characteristics of SARI Cases and Confirmed HCoVs Cases From Jan 2011–Dec 2012 (n = 864)

Features	Description	All SARI cases % N (n = 864)	Human coronavirus OC 43 %N (n = 4)	Human coronavirus NL63 %N (n = 3)	Human coronavirus 229E %N (n = 2)
Age in years					
	Mean (SD)	24.3 (21.9)	26.25 (8.098)	33.67 (26.83)	13.25 (18.03)
	Median (IQR)	20 (3–40)	23.5 (21.–35.0)	24 (13–64)	13.25 (0.5–26)
Sex					
	Male	436 (50.5%)	1 (25.0%)	2 (66.7%)	1 (50.0%)
	Female	428 (49.5%)	3 (75.0%)	1 (33.3%)	1 (50.0%)
Place					
	Kerala state(INDIA)				
	Kozhikode	339 (39.2%)	1 (25.0%)	2 (66.7%)	1 (50.0%)
	Kasargod	33(3.8%)	1 (25.0%)	–	–
	Malappuram	35 (4.1%)	1 (25.0%)	–	–
	Trissur,	68 (7.9%)	1 (25.0%)	–	–
	Other districts	207 (23.8%)	–	–	–
	Karnataka state(INDIA)				
	Bangalore	18 (2.1%)	–	–	1 (50.0%)
	Dakshina Kannada,	31 (3.6%)	–	1 (33.3%)	–
	Other districts	133 (15.4%)	–	–	–

In this study we detected HCoV-OC43, HCoV-NL63, and HCoV-229E. However, HCoV-HKU1, SARS-CoV, and MERS-CoV were not detected during the period of study from Southwest India. HCoV infection was predominantly seen in adults. One of the HCoV-NL63 cases was co infected with rhinovirus.

A number of people from Southwest India travel to Middle East countries for employment, business or pilgrimage (Haj). Hence, there is a high possibility of introduction of MERS-CoV infection to Southwest India. However, the current study did not find any MERS-CoV case. As these expatriate Indians are in middle-eastern countries for specific work or pilgrimage they may not have the chance of getting exposed to MERS-CoVs and that may be the reason for its absence in the cases.

Though the retrospective design and laboratory based sampling are limitations of this study, it gave an insight into the role of HCoVs in SARI cases. The low prevalence of HCoVs found in the study could be possibly due to inclusion of SARI cases alone. This may be an underestimate of actual community prevalence of HCoVs in AVRI cases in community. A well designed cross sectional study in community is required to establish the distribution of HCoVs in the Indian subcontinent. Nevertheless, our findings form a strong base for future research on the epidemiology and disease burden of HCoVs in India.

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REFERENCES

Dominguez SR, Robinson CC, Holmes KV. 2009. Detection of four human coronaviruses in respiratory infections in children: A one-year study in Colorado. J Med Virol 81:1597–1604.

Principi N, Bosis S, Esposito S. 2010. Effects of coronavirus infections in children. Emerg Infect Dis 16:183.

Abdul-Rasool S, Fielding BC. 2010. Understanding human coronavirus HCoV-NL63. Open Virol J 4:76.

Gaunt ER, Hardie A, Claas ECJ, Simmonds P, Templeton KE. 2010. Epidemiology and clinical presentations of the four human coronaviruses 229E, HKU1, NL63, and OC43 detected over 3 years using a novel multiplex real-time PCR method. J Clin Microbiol 48:2940–2947.

Geller C, Varbanov M, Duval RE. 2012. Human coronaviruses: Insights into environmental eesistance and its influence on the development of new antiseptic strategies. Viruses 4:3044–3068.

Bhatnagar PK, Das D, Suresh MR. 2008. Molecular targets for diagnostics and therapeutics of severe acute respiratory syndrome (SARS-CoV). J Pharm Pharm Sci 11:1s.

Corman VM, Müller MA, Costabel U, Timm J, Binger T, Meyer B, Kreher P, Lattwein E, Eschbach-Bludau M, Nitsche A, Bleicker T, Landt O, Schweiger B, Drexler JF, Osterhaus AD, Haagmans BL, Dittmer U, Bonin F, Wolff T. 2012. Assays for laboratory confirmation of novel human coronavirus (hCoV-EMC) infections. Euro Surveill 17:49.

Khan G. 2013. A novel coronavirus capable of lethal human infections: An emerging picture. Virol J 10:66.

Adachi D, Johnson G, Draker R, Ayers M, Mazzulli T, Talbot PJ, Tellier R. 2004. Comprehensive detection and identification of human coronaviruses, including the SARS-associated coronavirus, with a single RT-PCR assay. J Virol Methods 122:29–36.

Dare RK, Fry AM, Chittaganpitch M, Sawanpanyalert P, Olsen SJ, Erdman DD. 2007. Human coronavirus infections in rural Thailand: A comprehensive study using real-time reverse-transcription polymerase chain reaction assays. J Infect Dis 196:1321–1328.

Cabecca TK, Granato C, Bellei N. 2013. Epidemiological and clinical features of human coronavirus infections among different subsets of patients. Influenza Other Respir Viruses [Internet] [cited 2013 Sep 8] http://onlinelibrary.wiley.com/doi/10.1111/irv.12101/full.