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International Journal of Infectious Diseases 90 (2020) 21–25

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Human Parvovirus 4: A harmless bystander or a pathogen of severe acute respiratory illness

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ARTICLE INFO

Article history:
Received 16 July 2019
Received in revised form 30 September 2019
Accepted 1 October 2019
Corresponding Editor: Eskild Petersen, Aarhus, Denmark

Keywords:
PARVO 4 virus
Genotype
Severe acute respiratory illness
India

ABSTRACT

Introduction: Severe Acute Respiratory Infection (SARI) is an important cause of morbidity and mortality worldwide, caused by a large number of viral and bacterial agents. PARV4 is a recently identified virus detected in human blood and variety of tissues, but its disease association with SARI could not be established.
Objective: In the present case control study, we aim to investigate the association of PARV4 with SARI.
Methods: The Nasal and Throat swab (NS/TS) samples of 241 cases and 146 healthy controls were tested for most common respiratory viruses and PARV4 by real-time PCR.
Results: PARV4 was detected in 64(26.55%) SARI cases and only one healthy control (0.68%). PARV4 was the most common viral agent detected in SARI cases. A strong association of PARV4 is seen with severe respiratory illness.
Conclusion: Detection of PARV4 in a significantly higher number of SARI cases, in comparison with controls, suggests association of PARV4 with SARI. PARV4 genotype 2 is the only circulating strain detected in our study.

Introduction

Severe Acute Respiratory Infection (SARI) is an important cause of morbidity and mortality worldwide, particularly in developing countries and among children under five years of age (GBD LRI Collaborators, 2017; GBD 2015 LRI Collaborators, 2017; World Health Organization, 2011). SARI is associated with viral and bacterial agents. Common viral agents are influenza A (Infl A) and B (Infl B) viruses, parainfluenza viruses (PIV), coronaviruses, respiratory syncytial viruses (RSV), adenoviruses (AV), and rhinoviruses. Influenza virus infection, in particular, is very common followed by respiratory syncytial virus (RSV) infection (CDC, 2016; Laguna-Torres et al., 2011; Chakhunashvili et al., 2018; Kenmoe et al., 2016; Tjon-Kon-Fat et al., 2016). Newer respiratory pathogens are also emerging. In view of this, we investigated the association of human PARV4 virus with SARI.

Parvovirus 4 (PARV4) is a newly discovered human Parvovirus, first reported in 2005 in serum of intravenous drug users infected with hepatitis B virus (Jones et al., 2005). PARV4 is a single stranded non-enveloped DNA virus belonging to the *Parvoviridae* family of genus *Tetraparvovirus* (Matthews et al., 2017). PARV4 infects a diverse range of hosts and has been classified into 3 genotypes; 1–3. Generally genotypes 1 and 2 are found in North America, Europe, and Asia, and genotype 3 is found in sub-Saharan Africa.

PARV4 has been detected in blood/plasma, autopsy samples, stool, nasopharyngeal swab, the bone marrow and CSF; however, until now, there are no established clinical manifestations of PARV4 infection (Simmonds et al., 2007a). It remains uncertain whether PARV4 actually causes the observed disease, or is a bystander that was coincidentally detected in a highly exposed population. Seroprevalence of PARV4 in the general population has been varying in different parts of the world ranging from 0% to 25% (Sharp et al., 2012; Lavoie et al., 2012). A study from Africa reports PARV4 in 0.8% of nasal swab specimens, whereas a study from Scotland did not observe PARV4in respiratory samples (Drexler et al., 2012; Manning et al., 2006).

To the best of our knowledge, no previous study has reported the association of PARV4 with severe acute respiratory illness (SARI). It is also not clear whether PARV4 is transmitted by the

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https://doi.org/10.1016/j.ijid.2019.10.001

respiratory route or causes respiratory infections. Here, we aim to investigate the association of human PARV4 with SARI.

Methods

Case definition (SARI)

A person of any age who presented with difficulty in breathing accompanied with fever ≥38 °C and cough with at least one of the following symptoms: poor general condition, thoracic pain, polypnoea or acute respiratory distress syndrome (ARDS) and required hospitalization (World Health Organization) (WHO, 2013). This study was ethically approved by the Institutional ethics committee (ethical clearance reference code: Registration No. ECR/262/Inst/UP/2013/RR/16). All cases and controls or their guardians provided written informed consent for participation in the study. Consenting subjects were consecutively enrolled. Demographic and clinical details were collected through a predesigned questionnaire.

Laboratory diagnosis

For conducting this case control study, NS/TS from cases referred to Virology Laboratory, Department of Microbiology, King George's Medical University, Lucknow, Uttar Pradesh, India, with clinical diagnosis of SARI and healthy volunteer controls (matched for age and sex), over a period of 15 months, i.e. January 2017 to March 2018, were collected and tested for viral etiology (Influenza A/Subtype H1N1/Subtype H3N2/ Influenza B, Para-influenza virus (PIV), Measles virus (MEV), Respiratory syncytial virus RSV, Adenovirus (AV), Bocavirus (BoV), as per previously reported method (Singh et al., 2014)) and human house-keeping control RnaseP (Shu et al., 2011). Each real time PCR consisted of positive, negative and extraction controls. The result was reported only if all the controls were valid. All the samples of both cases (SARI) and controls were also tested for presence of PARV4-DNA using primers and probes described in patent no. PCT/IN2015/000409 and WO2016071926A9 by Jain and Prakash (2016). Total viral nucleic acid was extracted from 200 μl NS/TS sample using PureLink DNA/RNA mini kit (Invitrogen, USA) as per manufacturer's instructions and processed for TaqMan real time PCR. The real time PCR reaction mix contained 12.5 μl 2× master mix (LightCycler 480 Probes Master, Roche Diagnostics, Nederland BV, Almere, the Netherlands), 1 μl of Parv4 forward and reverse primer (10 pm/μl) and 0.5 μl taqmanprobe (10 pm/μl)(IDT, USA) and 5 μl H_2O (DNAse and RNAse free). Finally, 5 μl extracted total nucleic acid was added to 20 μl of prepared mastermix, making it a final volume of 25 μl. Real time RT-PCR assays were performed on Quantstudio 7 (Life Technologies, USA).

DNA sequencing and analysis

PARV4 VP1 gene was amplified using an in-house designed nested PCR primer to yield a product size of 849–854 base pairs (bp). The amplified nested PCR product was purified using exonuclease I and Shrimp Alkaline phosphatase. The amplicon was processed for sequencing PCR using BigDye Terminator (BDT) cycle sequencing kitv3.1 (Applied Biosystems, Foster City, CA). After sequencing PCR the product was purified using an ethanol purification method and sequenced on Genetic Analyzer 3130 (Applied Biosystems) according to the manufacturer's instructions. Sequences were aligned and the phylogenetic tree was constructed using MEGA 7 software (Kumar et al., 2016; Tamura et al., 2004). Reference sequences were obtained from GenBank [genotype 1: EU546204.1, genotype 2: EU175855.1, genotype 3: JN183925.1]. Data were analyzed using GraphPad Prism software version 5. Intergroup comparisons were done using Chi square test. *P value*< 0.05 was regarded as statistically significant.

Results

A total of 241 cases of SARI and 146 normal healthy controls were included in the study. Groups were compared for gender and age distribution. The mean age ± SD of cases was 23.26 ± 23.34 (Median 14; range 0–63) and of the control group was 18.0 ± 15.51 (median 4.5; range 0k62). Peak positivity of PARV4 was noted among infants and young children (age, 0–10years) as 46.87% (n = 30/64) cases were younger than 5 years of age (Table 3). The seasonal distribution of PARV4 corresponds to the enrolled SARI cases. There was an upsurge in PARV4 positive cases in the month of January (Figure 1).

Of total 241 SARI cases, 64 (26.55%) were positive for at least one of the known tested respiratory pathogens. RSV [n = 19 (7.88%)] was the most common aetiology detected in cases, followed by Influenza A/H1N1 [n = 12 (4.97%)] and MEV [n = 12 (4.97%)] (Table 1). Four cases were positive for more than one virus [Influenza A/H1N1 + MEV (2), Influenza B + Adenovirus (1); PIV + MEV (1)]. PARV4-DNA was present in 64 cases. A total of 44(18.2%) cases had PARV4 DNA alone, whereas in 20 cases (8.2%) PARV4 was co-detected with other known pathogenic viruses (Table 2). Parvovirus 4 was significantly higher in cases as compared to controls (Relative risk 37.709595%; NNT (Harm) 3.868; 95% CI: 5.402 (Harm) to 3.013 (Harm); *p value*<*0.0003*).

Of 146 healthy controls, three subjects were positive for RSV, one for PIV, whereas in a single individual PARV4-DNA was found (Tables 1 and 2).

Out of total viral causes tested, a final aetiology could be established in 108(44.81%) cases which were positive for at least one of the tested viral agents. Of 64 PARV4 positive cases, 23

Table 1
Respiratory virus positivity among cases and control group excluding Parv4.

Virus	Positivity in SARI cases N = 241(%)	Positivity in conrols N = 146(%)
Influenza A/HINI	12(4.97)	0.00
Influenza B	11(4.56)	0.00
Para influenza virus (PIV)	6(2.48)	1(0.68)
Adenovirus	5(2.07)	0.00
Bocavirus	3(1.24)	0.00
Respiratory syncytial virus (RSV)	**19(7.88)**	3(1.20)
Measles virus (MEV)	12(4.97)	0.00
Human metapneumovirus (HMPV)	0.00	0.00
Total etiology positive	**68**[a]**(28.21)**	**4(2.73)**
Total cases positives	**64(26.55)**	**4(2.73)**

Abbreviation: Influenza A (InflA), Influenza B (Infl B), Parainfluenza virus (PIV), Measles virus (MEV), Respiratory syncytial virus (RSV), Adenovirus (AV), Bocavirus (BoV), Human Metapneumovirus (HMPV), Parvo 4 Virus (PARV4).

[a] 4 patients were positive for more than one pathogens (2 with Influenza A (H1N1) and Measles, 1 Influenza B with Adenovirus and 1 Measles with Parainfluenza virus).

Table 2
PARV4 virus positivity among cases and control group.

	SARI cases N = 241(%)	Healthy controls N = 146(%)	Statistical association (*p-value*)
Only PARV4 virus positive	**44(18.2)**	**1(0.68)**	***<0.05***
Infl A/HIN1 + PARV4	2(0.83)	0(0)	NA
Infl B + PARV4	6(2.49)	0(0)	NA
PIV + PARV4	1(0.41)	0(0)	NA
AV + PARV4	1(0.41)	0(0)	NA
RSV + PARV4	5(2.07)	0(0)	NA
MEV + PARV4	3(1.24)	0(0)	NA
Infl A/HIN1 + MEV + PARV4	1(0.41)	0(0)	NA
Infl B + AV + PARV4	1(0.41)	0(0)	NA
Total PARV4 positive	**64(26.55)**	**1(0.68)**	***<0.05***

Abbreviation: Influenza A (InflA), Influenza B (Infl B), Parainfluenza virus (PIV), Measles virus (MEV), Respiratory syncytial virus (RSV), Adenovirus (AV), Bocavirus (BoV), Human Metapneumovirus (HMPV), Parvo 4 Virus (PARV4).
NA – Not Applicable.

Table 3
Age and gender distribution of PARV4 positive cases.

Age/gender distribution	Cases N = 241(%)		Total PARV4 positive cases 64 (%)		Cases positive for PARV4 only 44 (%)		Co-infected 20cases (PARV4 + any other aetiology positive (%)	
	Male	Female	Male	Female	Male	Female	Male	Female
0–5 yrs	67(27.80)	17(7.05)	22(34.38)	8(12.50)	12(27.27)	4(9.09)	10(50.00)	4(20.00)
>5–10 yrs	18(7.47)	3(1.24)	4(6.25)	0(0)	4(9.09)	0(0)	0(0)	0(0)
>10–15 yrs	14(5.81)	6(2.49)	7(10.94)	0(0)	6(13.63)	0(0)	1(5.00)	0(0)
>15–25 yrs	14(5.81)	12(4.98)	3(4.69)	3(4.69)	3(6.81)	1(2.27)	0(0)	2(10.00)
>25–40 yrs	11(4.56)	21(8.71)	2(3.13)	6(9.38)	2(4.54)	5(11.36)	0(0)	1(5.00)
>40–70 yrs	29(12.03)	21(8.71)	4(6.25)	4(6.25)	2(4.54)	4(9.09)	2(10.00)	0(0)
>70 yrs	6(2.49)	2(0.83)	1(1.56)	0(0)	1(2.27)	0(0)	0(0)	0(0)
Total	**159(65.97)**	**82(34.02)**	**43(67.18)**	**21(32.81)**	**30(68.18)**	**14(31.81)**	**13(65.00)**	**7(35.00)**

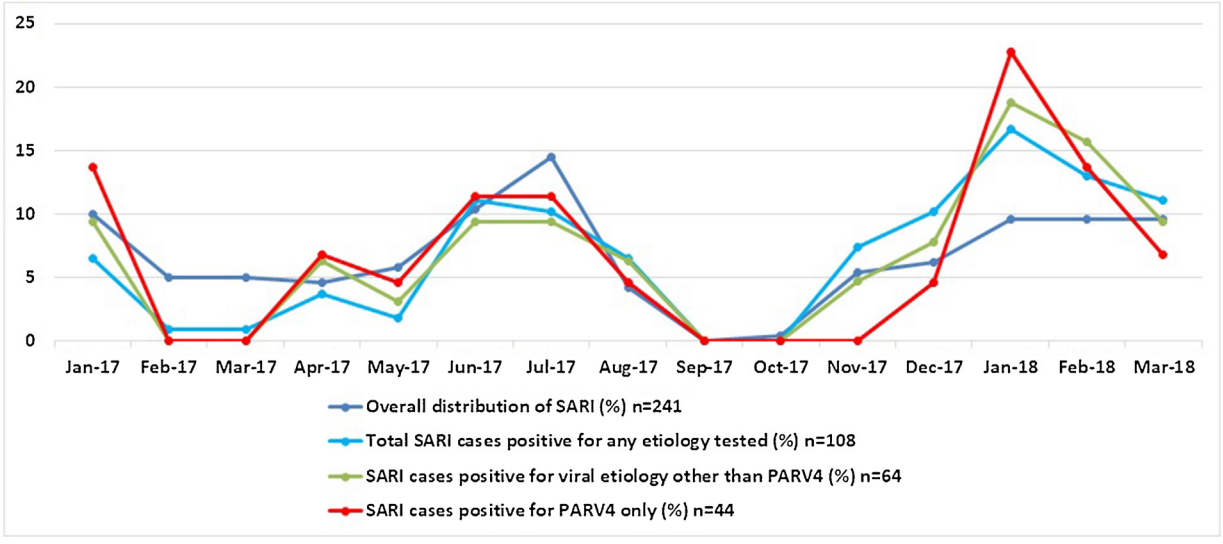

Figure 1. Month-wise distribution (%) of total cases tested and positive cases.

samples with high viremia (Ct value ≤30) could be amplified for the VP1 gene and sequenced. Phylogenetic analysis showed the presence of PARV4 genotype 2 in SARI cases (Figure 2). One strain from a healthy control was sequenced and was similar to other strains detected in cases. All the genetic sequences were submitted to GenBank (accession numbers MH939288 to MH939311).

Discussion

The present study described a high prevalence of PARV4 DNA in cases presenting with SARI. An association of PARV4 positivity with SARI is also seen. Clinical impact of PARV4 infection is not well understood; still, a wide range of potential infection outcomes has been proposed. In the past, studies have described an association of PARV4 with influenza-like symptoms, encephalitis (Benjamin et al., 2011; Prakash et al., 2015), transient rash and hepatitis (Sharp et al., 2012), foetal hydrops (Chen et al., 2011) and acceleration of progression to AIDS in HIV-infected adults (Simmons et al., 2012). Transmission via respiratory secretions or faeces suggestive of respiratory tract infection or gastroenteritis is also suggested.[13] Groups with risk factors for parenteral infection and those infected with either HBV or HCV or HIV have associated PARV4 infections, suggesting parenteral transmission. Intravenous drug users and those with a history of multiple transfusions have a

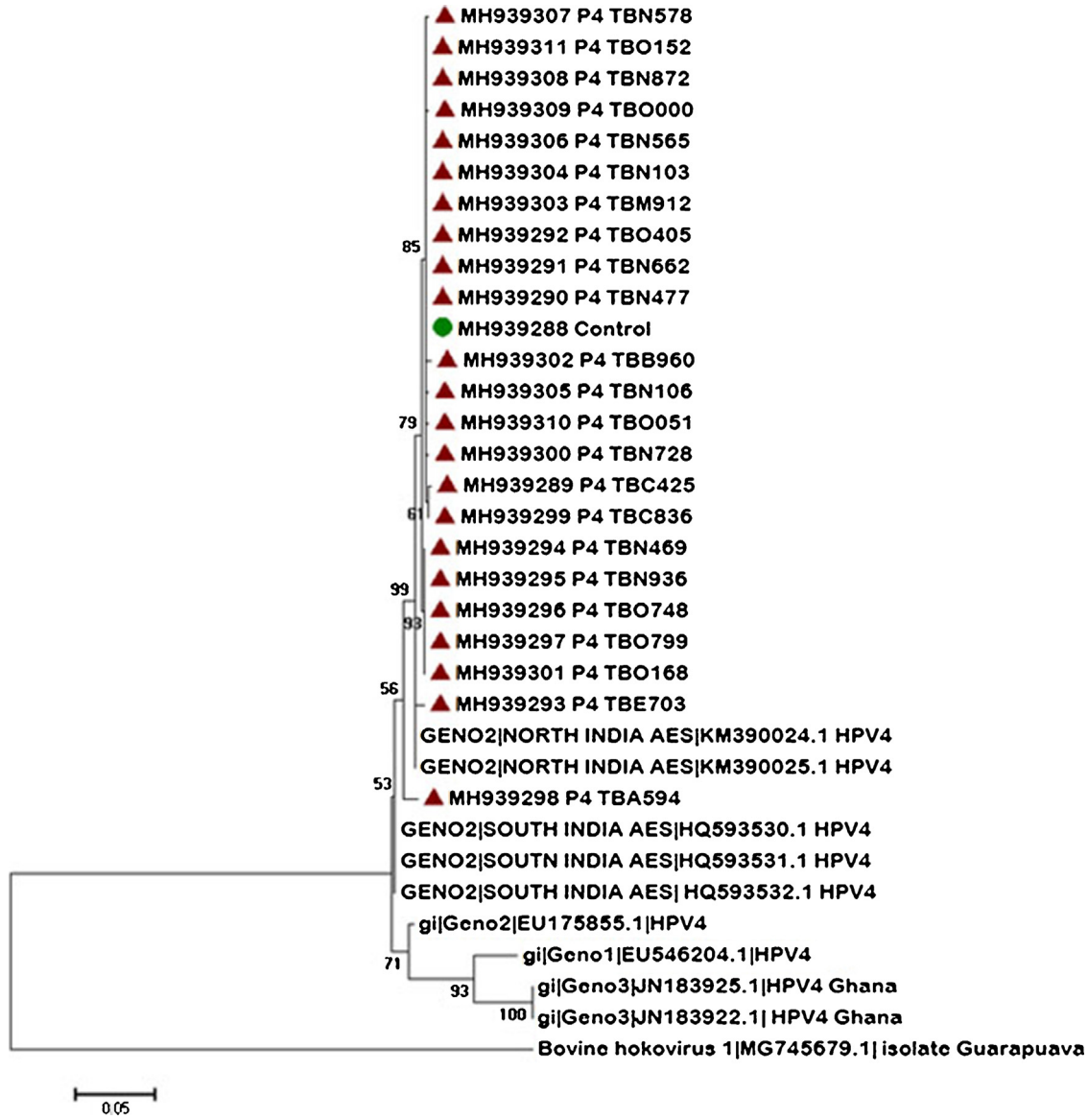

Figure 2. Phylogenetic analysis of PARV4 genome.
The evolutionary history was inferred using the Neighbour-Joining method. Each strain is labelled as country followed by GenBank accession number. The strains from the study cases are marked with red triangle and strain from one healthy control is marked as a green circle. The optimal tree with the sum of branch length = 0.44876634 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. There were a total of 723 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

high positivity of PARV4 (Jones et al., 2005; Simmonds et al., 2007b; Fryer et al., 2007a).

In the present study, we have tested respiratory samples from many healthy individuals and except for one, all were negative. Studies from Africa have reported the detection frequency of PARV4 IgG ranging from 30% to 50% in the general population, irrespective of their positivity for HBV/ HCV/ HIV (Lavoie et al., 2012; Drexler et al., 2012; Sharp et al., 2010). Acute infections with PARV4 DNA positivity were seen in 8.5% of young children in Ghana (Drexler et al., 2012).

Genotype 2 was the only circulating genotype detected in the present study. Genotype-2 (formerly known as PARV5) has been identified in European and North American cohorts (Matthews et al., 2017; Simmonds et al., 2008; Fryer et al., 2007b). It was also the predominant strain reported from Asia (Matthews et al., 2017; Benjamin et al., 2011). In one of our earlier studies we also found genotype 2 in samples from cases of encephalitis (Prakash et al., 2015).There is a high similarity in strains of genotype 2 identified the world over.

Higher positivity in children less than 5 years of age and during winter is demonstrable with the current data. Most of the studies have commented on positivity in children; however, seasonality is not well described (Panning et al., 2010; Rosenfeldt et al., 2015; von Linstow et al., 2015). Co-infection with other respiratory viruses is also seen in the present study. It may be possible that PARV4 exacerbated other viral infections. However there is no supporting literature suggesting the same. Our study has a limitation that it does not include the co-existence or presence of non viral

etiologies. Our study also did not look at the prevalence in cases with milder illness for providing a comparison with prevalence in cases with SARI.

Conclusion

Detection of PARV4 in a significantly large number of SARI cases, in comparison with controls, suggests association of PARV4 with SARI. PARV4 genotype 2 is the only circulating strain detected in our study.

Conflict of interest

The authors have declared no conflict of interest.

Funding

The financial support was provided by the Department of Health Research, New Delhi.

Ethical reference number: ECR/262/Inst/UP/2013/RR/16.

Author's contributions

All authors reviewed and approved the final version. SP, SS & AJ consequently contributed to drafting and editing. HM, AKB & RV contributed to methodology and results of scientific work.

Acknowledgement

We acknowledge staff of the virology laboratory, Department of Microbiology, King George's Medical University, Lucknow, for their support.

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