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Research paper

Epidemiological features and genetic characterization of virus strains in rotavirus associated gastroenteritis in children of Odisha in Eastern India

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ABSTRACT

We have studied the clinical characteristics, severity and seasonality of rotavirus infection and prevalent genotypes in 652 non-rota vaccinated children in Odisha in eastern India. P genotypes were analysed for their association with host blood group antigens. P type of the virus is determined by the VP8* gene, and specific recognition of A - type of Histo - blood group antigen by P[14]VP8* has been reported. VP4, VP7 and VP6 genes of commonly identified G1P[8] strain were compared with genes of the same strain isolated from other parts of India, elsewhere and strains used for Rotarix and Rotateq vaccines.

In 54.75% of children with gastroenteritis, rota virus was found. 9.65% of children had moderate, 78.07% severe, and 12.28% very severe disease as assessed using the Vesikari scoring system. The incidence of infection was highest during winter months. There was no association between any blood group and specific P genotypes. G1P[8] was the commonest cause of gastroenteritis, followed by G1P[11], G3P[8], G9P[8], G2P[4], G2P[6], G9P[4], G9P[11] and G1P[6]. Predominant G genotypes identified were G1 (72.9%), G9 (10.81%), G2 (8.10%) and G3 (8.10%). Sequence analysis of the VP7 gene, placed the G1P[8] strain in lineage 1 and of VP6 gene placed nine G1P[8] strains in subgroup II and one in subgroup I. The VP7 gene segment of two Odisha G1P[8] strains were found to cluster relatively close to the VP7 sequences of Rotarix vaccine. Antigenic differences were found with vaccine strains. Ten G1P[8] strains sequenced for the VP4 gene had 91–93% nucleotide and 92–96% amino acid identity with Rotateq vaccine P[8]). Rotarix vaccine VP4 had 89–91% nucleotide and 90–92% amino acid identity. Our findings indicate genetic variability of rotavirus strains circulating in the region and are significant, given the introduction of rota vaccination in the State.

1. Introduction

Rotavirus infection in young children particularly in those below five years of age, resulting in severe diarrhea, is a cause of large number of infantile deaths all over the world, more so in developing countries such as India. It is well known that there are many different types of the virus and that some may be localised to certain areas while others have a global prevalence. Emergence, disappearance and re-emergence seems to be common with certain types (O'Ryan, 2009). Reassortment of genomes in virus strains belonging to the same group can lead to evolution of new viruses (Patton, 2012). Global rotavirus surveillance programmes, combined with advanced sequencing technologies have revealed the immense diversity of circulating rotavirus strains in different parts of the world (Patton, 2012).

The Indian Rotavirus Surveillance Network (IRSN) has helped, in collating data on the clinical, epidemiological and virological features of rotavirus gastroenteritis from various parts of the country (Kang et al., 2009). Studies in eastern India have been mostly in Kolkata centres of IRSN (Das et al., 2004, Mullick et al., 2014a, 2014b). A high disease prevalence has been reported from Bhubaneswar, another centre in eastern India (Kar et al., 2014, Sarangi et al., 2015).

Rotateq and Rotarix, live attenuated oral rotavirus vaccines have been licensed in India and more recently the Rotavac vaccine has also been introduced in Bhubaneswar. Given the significant diversity in circulating strains as observed in different regions of the country.

(Ramachandran et al., 1996, Jain et al., 2001) as well as in localities where the vaccines have been introduced (Kang et al., 2013, Kulkarni et al., 2014), identification of the circulating strains in Bhubaneswar assumes importance. There is no information on the strains circulating in Bhubaneswar except for a recent single hospital based study (Kar et al., 2014).

We have studied the clinical characteristics, severity and seasonality of rotavirus infection and prevalent genotypes in non-rotavirus vaccinated children admitted with diarrhea during the time period 2013–

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2015, in 3 hospitals in Bhubaneswar, Cuttack and Puri which serve a catchment area of 15 different districts of Odisha. The association between genotype and host blood group antigens has also been investigated. Rotarix and Rotateq vaccines have been designed based on the concept that VP4 and VP7 components of the virus are the primary determinants of immune responses against the virus. We, hence have characterized the VP4, VP7 and VP6 genes of the common strain isolated from patients in our study and compared them with the same strain identified from other parts of eastern India. The antigenic regions of the strain and the vaccine strains were also compared.

2. Materials and methods

2.1. Clinical data and sample collection

Children below 5 years of age admitted to hospitals with acute diarrhea were enrolled into the study. Stool samples were collected after obtaining consent from the parents for the children. Study was conducted between September 2013 and May 2015. Samples were collected from the Paediatric unit of Capital Hospital, Bhubaneswar, Sishu Bhavan, Cuttack and Puri Paediatric Hospital. These settings serve a catchment area of 15 different districts of Odisha (Fig. 1). Only those hospitalized children who had three or more watery stools within 24 h prior to admission (WHO definition), were included in the study. Demographic details and clinical presentation such as duration of diarrhea, maximum number of stools passed per day, duration and peak frequency of vomiting, degree of fever, presence and severity of dehydration, treatment and history of vaccination were recorded in a predesigned format. The severity of diarrhea was assessed using the Vesikari scoring system (Ruuska and Vesikari, 1990). A score of ≤5 was determined as mild, 6 to 10 as moderate, 11 to 15 as severe and 16 to 20 as very severe. Finger prick blood was also collected from the same children for blood grouping with prior consent from parents or guardians. The study was carried out with the approval of the Ethics Committee of the Institute.

2.2. Laboratory investigation for rotavirus antigen and of blood group

A 10% fecal suspension of each sample in phosphate-buffered saline was tested for rotavirus antigen positivity by enzyme-linked immunosorbent assay (Elisa) using the Ridascreen kit (R-Biopharm,Germany) Gautam et al., 2013 and performed according to manufacturers instructions. Blood group was ascertained by direct agglutination method using Monoclonal ERYSCREEN Tulip Diagnostics Ltd. (India) kit (Patil et al., 2013) against human A & B antigens. Chi square test was used to determine association between blood group and genotype of rotavirus.

2.3. Viral RNA extraction and reverse transcription polymerase chain reaction (RT-PCR)

Viral RNA was extracted and purified from 10% fecal suspensions in phosphate-buffered saline using a QIamp viral RNA minikit (Quiagen, Netherlands) according to manufacturer's instructions. The cDNA was generated from random hexamers(50 uM), (Thermo Fischer Scientific), using 200 u/ul (40,000 u) of reverse transcriptase (Invitrogen, Life Technologies, United Kingdom) PCR was carried out for genotyping the virus and for amplification of VP4,VP7 AND VP6 genes using the primers published earlier (Gentsch et al., 1992, Iturriza-Gomara et al., 2000, Gouvea et al., 1990). The PCR conditions involved PCR activation at 95 °C for 15 min, 40 cycles of amplification (1 min at 94 °C, 1 min at 50 °C and 2.5 min at 70 °C) with a final extension of 7 min at 70 °C. The VP7, VP4 and VP6 amplicons were sequenced as reported earlier (Arora et al., 2009).

2.4. Sequencing of the VP4, VP7 and VP6 genes of the G1P[8] strains circulating in Odisha and phylogenetic analysis

PCR amplicons were purified by exosap treatment and sequences in both directions were determined by the dideoxy nucleotide chain terminator method using a Bigdye Terminator cycle sequencing reaction

Fig. 1. Schematic map of Odisha showing number of children with rotavirus associated gastroenteritis/number of stool samples collected from different districts of Odisha.