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Deliberation of full-Genomic Sequence and phylogenetic analysis of circulating strains of group A rotavirus in Ahvaz city

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دکتر هیژیر جواهري زاده

معاون پژوهشی دانشکده پزشکی
Dedicated to my parents

My husband

My sister and brothers

Without whom none of my success

would possible

and

To whom I love the most
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Introduction: Group A rotavirus (RVA) mainly causes acute gastroenteritis exclusively in young children in developing countries. The prevalence and determination of the molecular epidemiology of rotavirus (RV) genotypes will determine the dominant rotavirus genotypes in the region. Many unusual combinations of G and P genotypes have been observed in rotaviruses circulating in developing countries. Mixed infection of a single individual with more than one strain is a mechanism by which genetic reassortants are formed with unusual G and P combinations. Full genome sequencing of rotavirus strains provide a strategy for the development of vaccine to prevent morbidity and mortality in children.
Materials and methods: A total of 100 faecal samples were collected from children below 5 years with acute gastroenteritis referred to Abooza Children’s Hospital of Ahvaz city during October 2015 to March 2016. All samples were screened by latex agglutination for the presence of rotavirus antigen. Rotavirus- positive samples were further analyzed by the semi-multiplex RT-PCR and the sequencing was done for the determination of G/P-genotyping. The full-genomic sequence was determined for strains G1P[8], G2P[4], G3P[8], G9P[8], and G12P[8] by RT-PCR and Biosystems 3730/3730xl DNA Analyzers Sequencing, Bioneer, South Korea.

Results: 32% of the specimens were RVA-positive by RT-PCR. Among the 32, VP7 genotyped strains, the predominant G genotype, was G9 (37.5%) followed by G2 (21.9%), G1 (12.5%), G12 (9.4%), G4 (9.4%), G2G9 (6.3%) and G3 (3.1%). Among the 31 VP4 genotyped strains, P [8] genotype was the dominant (62.5%) followed by P [4] (31.3%) and P [4] P [8] (3.1%). The genotypes for G and P were identified for 31 rotaviruses (96.87%) but only one strain, G9, remained nontypeable for the P genotype. The most prevalent G/P combination was G9P[8](28.5%), followed by G2P[4] (18.8%), G1P[8] (9.4%), G12P[8] (9.4%), G4P[8] (9.4%), G2G9P[4] (6.3%), G9P[4] P[8] (3.1%), G3P[8] (3.1%), G9P[4] (3.1%) G2P [8] (3.1%), and G9P [untypeable] (3.1%). A novel rotavirus strain, G12, for the first time was detected in patients from south-west Iran. Whole genome sequence and phylogenetic analyses revealed the existence of (i) 2-12 mutations in the VP7 genes, (ii) 6-16 mutations in the VP4 genes (iii) 2-6 mutations in the VP3 genes, (iv) 4-6 mutations in the NSP4 genes, (v) 2 mutations each in the VP1 and NSP3 genes, (vi) 1 mutation each in the VP2, NSP1 and NSP5 genes, and (v) no mutations in the VP6 and NSP2 genes.
**Conclusion:** The emergence of a new human rotavirus strain, G12, was identified in this region of Iran and sequenced for the first time. Furthermore, whole genome-based analyses are essential to understand the evolutionary dynamics of novel RVA strains such as G12P[8] strains. Comprehensive investigations are required to determine the prevalence of rotavirus genotypes in other regions of Iran to develop the region-specific vaccines.

**Key words:** Rotavirus, Genotype, Emergence, Novel, Sequence analysis, Iran
Chapter I
Introduction
A.1 Problem
Paediatric diarrhoea is frequently lethal since this illness causes severe dehydration (10). There are multiple causes of the disease including bacterial, parasitic and viral infections (2-4). Viruses, specifically of the rotavirus group A, are the predominant causes worldwide of viral gastroenteritis in children aged <5 years (5). Rotaviruses are repeated with high rates of morbidity and mortality in developed and developing countries, respectively (6).

Rotaviruses are transmitted via the faecal-oral route, which can happen directly from person to person, and contaminated drinking water (7). It is estimated about 125 million cases of diarrhoea and more than 453000 deaths occur worldwide annually due to gastroenteritis caused by rotaviruses (8, 9, 10).

Rotaviruses are non-enveloped, double stranded RNA and belonging to the Reoviridae family. The genome comprises 11 segments which encode six structural proteins (VP1-VP4, VP6, and VP7) and six nonstructural proteins (NSP1-NSP5/NSP6). According to the serologic cross-reactivity of the middle layer protein VP6, seven serogroups(A-G) have been firmly established, according to the VP6 genetic diversity there are likely to be at least eight(A-H) (11-13). The majority of human rotavirus infections belong to group A, although some strains of rotaviruses in groups B and C, too, can cause diarrhoea in humans. Rotaviruses are classified on the basis of their serological characteristics or genetic diversity of two outer capsid proteins, VP7 (glycosylated, G-type) and VP4 (protease sensitive, P-type) (14), since these protein targets for antibodies, are important for the broadening of the vaccine (15). So far, 27 different G and 37 different P-genotypes have been identified and approximately 73 G/P genotype of RVA have been reported to be responsible for acute diarrhoea in humans (16, 17). The major common rotavirus genotypes G1-G4, G9, P [8], P [4], and P [6] have been identified as the causative agents for gastroenteritis around the world (18, 19). Recently, the emergence of the novel G12 rotavirus has been reported in different
parts of the world (20-26). A high frequency of G12 associated with multiple VP4 genotypes has been reported in India, Bangladesh, and Nepal (27-30). Of all the possible combinations, six genotypes (G1P[8], G2P[4], G3P[8], G4P[8], G9P[8], and G12P[8]) have been observed in 80–90% of the isolated rotavirus infections (31-34).

The nucleotide sequence of all 11 rotavirus RNA segments for many rotavirus strains are known, and this forms the basis for the new classification system discussed earlier.(35) Each positive-sense RNA segment starts with a 5'-guanidine followed by a set of conserved sequences that are part of the 5' noncoding sequences. An open reading frame (ORF) coding for the protein product and ending with the stop codon follows, and then another set of noncoding sequences is found containing a subset of conserved terminal 3' sequences and ending with two 3' terminal cytidines. Almost all mRNAs end with the consensus sequence 5'-UGUGACC-3', and these sequences contain important signals for gene expression and genome replication. The last four nucleotides of the mRNAs function as translation enhancers.(36) The lengths of the 3' and 5' noncoding sequences vary for different genes, but the noncoding sequences of homologous strains are highly conserved (37).

The prototype simian SA11 strain was the first genome completely sequenced. The sequences from different rotavirus strains show general features of the structure of each genome segment. Matthijnssens and colleagues (2006) in Belgium showed that The Belgian rotavirus strain B4106, isolated from a child with gastroenteritis, was previously found to have VP7 (G3), VP4 (P[14]), and NSP4 (A genotype) genes closely related to those of lapine rotaviruses, suggesting a possible lapine origin or natural reassortment of strain B4106(16).
Tran and colleagues (2013) in India used full-genome sequencing to reanalyze a G3P[4] strain (107E1B) and a G2P[4] strain (116E3D) detected in India in 1993 and showed that 107E1B had virtually an identical nucleotide sequence with 116E3D, except the VP7 gene. Phylogenetic analysis revealed that the 107E1B VP7 gene was of typical human rotavirus origin, with a 99.3% nucleotide sequence identity with another Indian G3 VP7 gene. Thus, this study provided robust evidence for the formation of the G3P[4] strain through genetic reassortment in which a G2P[4] strain with a typical DS-1 genogroup background acquired the VP7 gene from a co-circulating G3 human rotavirus strain. This study established a basis on which to facilitate full genome sequence analysis of an increasing number of G3P[4] strains in China and elsewhere in the world (38).

Donato and colleagues (2014) in Australia reported a large outbreak of rotavirus gastroenteritis. The outbreak occurred 43 months after the introduction of the G1P[8] rotavirus vaccine RotarixH. Forty-three infants were hospitalized during the outbreak and analysis of fecal samples from each infant revealed a G1P[8] rotavirus strain. Whole genome sequencing demonstrated numerous amino acid differences compared to the RotarixH vaccine strain in the characterized neutralization epitopes of the VP7 and VP4 proteins. Phylogenetic analysis revealed a close genetic relationship to global strains, in particular RVA/Human-wt/BEL/BE0098/2009/G1P[8] and RVA/Human-wt/BEL/BE00038/2008/G1P[8] for numerous genes (39).

As mentioned studies, monitoring temporal changes in all 11 gene segments may help us to comprehend the nature and pattern of rotavirus evolution. Surveillance to monitor the strain diversity of circulating RV-A to detect possible strain replacement following the introduction of universal RV-A vaccine is a priority of the World Health Organization. Such studies are important to estimate potential impact of vaccination programs on circulating...
strains including whether escape mutants of known serotypes or novel strains that evade vaccine immunity will emerge (40).

**A.2 History of rotavirus**

Rotaviruses are the single most important cause of severe diarrheal illness in infants and young children in both developed and developing countries worldwide, accounting for 30% to 50% of these illnesses (41) Viruses were first discovered to be significant causes of diarrheal illness in the 1970s, with Norwalk virus being the first agent discovered in 1972 by Kapikian et al from an outbreak of gastroenteritis in a school in Norwalk, Ohio. Human rotaviruses were discovered in 1973, when particles were visualized by Bishop et al (42) in electron micrographs of thin sections of duodenal mucosa and later virus was identified in feces by electron microscopy. The 70-nm particles (44). from children’s feces were subsequently designated rotavirus and documented to be an important etiologic agent of severe diarrhea of infants and young children during the first 2 years of life in both developed and developing countries (45).

**A.3 Classification of rotavirus**

Rotaviruses are members of the genus Rotavirus within the family Reoviridae, and rotaviruses share common morphologic and biochemical properties. Early studies using negative-stain EM techniques underestimated the particle diameter, and the subsequent cryo-EM studies, in which no stains are used, established the particle diameter to be 100 nm including the spikes. Salient features are that (a) mature virus particles, including spikes, are about 100 nm (1,000 Å) in diameter and possess a triple-layered icosahedral protein capsid composed of an outer layer, an intermediate layer, and an inner core layer; (b) 60 protein spikes extend from the smooth surface of the outer shell; (c) outer capsid integrity requires calcium; (d) particles contain

1 - EM