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Review Article

Genomic Diversity in Rotavirus and the Current Scenario of Rotavirus Vaccines: A Brief Overview

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Abstract

Rotavirus associated infection still dominates the list of diarrheal illnesses in paediatric population despite the availability of effective Rotavirus vaccines. Frequent genetic reassortment in the genome of rotavirus leads to emergence of novel genotypes in different geographic areas worldwide. This review aims to give a brief overview of the genetic diversity prevailing in rotavirus worldwide with a focus on the vaccines available for rotavirus infection.

ABBREVIATIONS

RV: Rotavirus Infection; RNA: Ribonucleic Acid; EIA: Enzyme Immunoassay; ELISA: Enzyme Linked Immunosorbent Assay; IgA: Immunoglobulin A; IgG: Immunoglobulin G; PCR: Polymerase Chain Reaction; RT-PCR: Reverse Transcriptase PCR; VP: Viral Structural Protein; NSP: Non-Structural Protein; PAGE: Polyacrylamide Gel Electrophoresis; RRV: Rhesus Rotavirus, UIP: Universal Immunization Programme; WHO: World Health Organization.

INTRODUCTION

Rotavirus (RV), a member of the family Reoviridae, is non-enveloped viruses, about 76.5 nm in diameter and has 11-segmented double-stranded Ribonucleic acid (RNA) genome. The name Rota virus is derived because of their appearance as wheels, when observed under electron microscope [1]. Rotavirus infections are known to cause severe diarrhoeal illness in young paediatric population worldwide [2]. The virus is shed in very high concentrations (>10^{12} particles/gram) in the stools and vomitus of infected individuals for many days. Transmission occurs primarily by the faeco-oral route, directly from person to person or indirectly via contaminated fomites [3]. Infection may result in decreased intestinal absorption of glucose, sodium and water, with decreased levels of intestinal lactase, alkaline phosphatase, and sucrase activity leading to isotonic diarrhoea [4]. The graveness of rotavirus infection increases by the virtue of "genetic reassortment" in segmented genome in host cells, leading to frequent emergence of new strains with different genotypes. Enzyme immunoassay (EIA or ELISA) for detection of antigen (VP6, common to all group A rotaviruses) in stool; and Immunoglobulin G (IgG) and Immunoglobulin A (IgA) antibodies in serum may be used for the diagnosis of Rotavirus infection. Latex agglutination method, though less sensitive, may also be used. Electron microscopy and polyacrylamide gel electrophoresis are also useful. Molecular techniques like nested polymerase chain reaction (PCR), multiplex PCR and Reverse transcriptase PCR (RT-PCR) in stool samples can additionally help in the genotypic studies [5-7]. The genetic sequencing methods like microarray and real time PCR (RT-qPCR) are very sensitive methods for diagnosis [8].

Vaccine development and implementation has seen lots of ups and downs from RotaShield® to Rotarix® and RotaTeq®. Many are in the developmental stages [3]. The distribution of genotypes varies with time and change with the introduction of vaccines. Although various genotypic surveillance studies have been done to know the varying and predominant genotypic strains prevalent in different geographic areas, more studies are required so that it can be utilized for the formation of new and better vaccines for the effective control of rotavirus infection [9,10].

DISEASE BURDEN

According to WHO as of April 2016, 215 000 (197 000 - 233 000) child deaths occurred during 2013 due to rotavirus infection globally, compared to 528 000 (465 000-591 000) in 2000. Among children under five years of age, total death due to rotavirus infection ranged from 47-100 (India) to fewer than 5 deaths (79 countries). Among these, 22% of all rotavirus deaths occurred in India [11]. Studies have also shown the association of acute gastroenteritis and rotavirus infections among adolescent and adult population [12].

GENOMIC STRUCTURE OF ROTAVIRUS

Rotavirus comprises of 11 segmented double helix molecules of RNA containing 18,555 base pairs. The RNA is surrounded by a three-layered icosahedral protein capsid. Each helix is a gene, numbered 1 to 11 by decreasing size [13]. During the viral

replication cycle, "genetic reassortment" in the 11 gene segments may occur in coinfected host cells and is responsible for the wide variety of rotavirus strains found in nature; Reassortment between the animal and human strains have also been reported [3]. Each gene codes for one protein, except genes 9 and 11, both of which code for two proteins each. The virus particle is formed by six different structural proteins (VP) like VP1, VP2, VP3, VP4, VP6 and VP7. The nonstructural proteins (NSP) are NSP1, NSP2, NSP3, NSP4, NSP5 and NSP6, which are only produced in the infected cells [13].

VP1 is an RNA polymerase enzyme, located in the core of the virus particle. VP2 forms the core layer of the virion and binds the RNA genome. VP3 is an enzyme guanylyl transferase that catalyses the formation of the 5' cap in the post- transcriptional modification of mRNA. VP4 binds to molecules on the surface of cells called receptors and drives the entry of the virus into the cell. VP6 is a common antigen which is present in all rotavirus in their middle capsid and can be used to identify different rotavirus species. VP7 is a glycoprotein that is involved in immunity to infection [10].

NSP1 is the product of gene 5 and is a nonstructural RNA-binding protein. NSP2, an RNA-binding protein, accumulates in cytoplasmic inclusion bodies called viroplasms, and plays a major role in genome replication. Rotavirus proteins and RNAs interact specifically in these viroplasms. NSP3, which is bound to viral mRNAs in infected cells, is responsible for the shutting down of cellular protein synthesis. NSP4 is a viral enterotoxin which induces diarrhoea. Genome segment 11 of rotavirus A encodes NSP5, which also accumulates in the viroplasm [10]. NSP6 is also encoded by gene 11 and is a nucleic acid binding protein. Due to the antigenic and genomic diversity, rotavirus has been further classified into 7 groups from Group A to G. With the predominance of Group A, Group B and C also cause infections in human beings. All the 7 groups cause disease in animals. Group A was further classified using the glycoprotein VP7 defining G types, and the protease-sensitive protein VP4 defining P types. The P-type is indicated by a number for the P-serotype and by a number in square brackets for the corresponding P- genotype. G-serotypes are similarly numbered but the G-genotype number is the same as the G-serotype. Approximately 14 G types and 20 P types have been reported, of which approximately 10 G types and 11 P types are identified in humans [13]. There are various G and P combinations that are identified and hence a binomial typing system is used to identify them.

METHODS FOR DETECTING DIFFERENT SEROTYPES AND GENOTYPES

Many molecular techniques can be used for the genotypic studies namely nested PCR, multiplex PCR and RT-PCR. They can be done in stool samples from the infected patients [5-7,9,12,14-21]. Selvarajan et al., used conventional hemi-nested VP7 and VP4 reverse transcriptional-polymerase chain reaction for genotypic studies after screening the samples by ELISA technique [14]. Mishra et al., performed the study using polyacrylamide gel electrophoresis (PAGE) and PCR (nested and multiplex) [7].

GENOMIC STUDIES IN ROTAVIRUS

Currently, 5 G-P combinations (G1P[8], G2P[4], G3P[8], G4P[8]) and G9P[8]) cause approximately 90% of all human rotavirus infections in large areas of the world; type G1P[8] is the most prevalent. Many different rotavirus types circulate simultaneously, particularly in developing countries. Moreover, the prevailing types may differ considerably from one season to the next, even within the same geographical area [3]. The prevalent genotypic strains may vary before and after the start of vaccination in an area, under the vaccine pressure [8,22-24].

New serotypes have been found in different geographic areas. A study from Kenya showed the diverse circulating genotypes with emergence of genotypes G3, G9, G12 and mixed genotypes G9/3 and recommended that vaccines should be formulated with a broad range of strains to include G9 and G12 [24]. During March-July 2014, rotavirus G8P[8] emerged as the predominant cause of rotavirus gastroenteritis among children in Hokkaido Prefecture, Japan [25]. G1P[8] was found to be the most dominant strain of rotavirus causing diarrhoea in children in Yogyakarta, Indonesia [26]. A study from Taiwan detected novel G9 rotavirus strains G9P [19] and G9P[13] co-circulating in children and pigs [27]. The study conducted in Turkey identified six different rotavirus G genotypes, 3 different P genotypes, 11 different G-P combinations and 5 different mixed genotypes combinations. G1, G9, G12 and P[8] were found to be the predominant genotypes. G12P[6] and G12P[8] genotypes were also reported [28]. Vizzi et al., reported the predominance of G2P [4] and reemergence of G1P [8] after the introduction of rotavirus vaccine (Rotarix® in the public health care sector, and RotaTeq® in private facilities) from a total of 912 fecal specimens, collected from children younger than 10 years of age with acute gastroenteritis in Caracas, Venezuela. The study also reported some uncommon genotypic combinations like G8P[14], G8P[4], G1P [4] and G4P[4] in <5% cases [23].

Several studies have also been done in different parts of India [7,12,15-21,29]. The genotypes identified by Mishra et al were G1 (38.0%), G2 (15.2%), G3 (16.5%), G9 (10.9%), G4 (5.1%) and mixed G types (10.1%) in 412 children with diarrhea from northern part of India. G1P[8], G3P[6], G1P[6] and G2P[8] were the most common G-P combinations [7]. In the study done by Tatte et al., sequencing and phylogenetic analysis of the VP4, VP6, VP7 and NSP4 genes revealed an infrequently reported NSP4-E6 genotype and circulation of heterogenous genotypes/lineages in the RV strains [12]. Chitambar et al reported the predominance of G1P[8] and G2P[4] strains, the continued circulation of G9 strains with the emergence of G9P[4] reassortant strains in Pune, western India [16]. Along with other common ones, G12P [6], has also been reported from Pune [18]. Anandan et al., reported G1P[8], as the most common genotype along with G1P[6], G1P[4], G9P[4], and mixed genotypic combination of G2 and G9P[4], from Southern India [19].

IMMUNITY AND VACCINATION

Neutralizing antibodies against VP7 and VP4 surface antigens play a role in protection after natural rotavirus infection, but their role in rotavirus vaccine-induced immunity is less clear. The concept behind the current live oral rotavirus vaccines is that immunity to the rotavirus surface antigens is essential for vaccine-induced protection. However, vaccines that elicit low levels of serum antibodies have been effective in field trials. The total serum anti-rotavirus IgA level, measured shortly after

infection, generally reflects intestinal IgA levels and appears to be the best marker of protection [10]. The focus on vaccine development revolves round the fact that it should be live, attenuated strains of rotavirus that would replicate in the gut [3].

Initially in 1998, a rhesus–human reassortant tetravalent rotavirus vaccine (RotaShield®, WyethLederle), was licensed in the United States after it was proved to be safe and efficacious and was recommended for routine use in infants. In this vaccine, Rhesus Rotavirus (RRV) backbone was reassorted with human rotavirus VP7 proteins representing the G-types G1, G2 and G4[30]. But in less than a year, the vaccine was withdrawn by the manufacturer following reports of an excess number of cases of intussusceptions which is the intestinal invagination resulting in obstruction, after about 2 weeks of vaccination [3].

CURRENTLY LICENSED VACCINES

At present, two vaccines namely Rotarix® and RotaTeq® are licensed for worldwide use. Rotarix® is a live oral vaccine which originated from G1P[8], strain isolated from a case of infantile gastroenteritis. After multiple passages in tissue culture, the resulting attenuated vaccine strain, RIX4414, has been propagated in Vero cells and subsequently found safe in extensive randomized, placebo-controlled safety and efficacy trials in Latin America and Europe. The lyophilized vaccine should be kept at 2-8 ˚C in its original package and should not be frozen. It is administered orally in a 2-dose schedule. The first dose should be given to infants at 6-12 weeks of age (but not later than 12 weeks of age) and the second following an interval of at least 4 weeks. The schedule should be completed by age 16 weeks, and not later than by 24 weeks of age [3]. This vaccine is unlikely to cause intussusceptions, because wild-type human rotavirus is not associated with the phenomenon [31].

RotaTeq® is a pentavalent human-bovine (WC-3) reassortant rotavirus vaccine. The WC-3 strain bovine rotavirus was reassorted with VP7 surface proteins of human rotaviruses G1, G2, G3 and G4, and one reassortant was made between the bovine rotavirus and human VP4 type P [8], formerly called P1A. The resulting 'pentavalent' vaccine is a mixture of these five reassortants. RotaTeq® is marketed in a squeezable plastic dosing tube which allows direct oral administration to infants. Each dose (2 ml) of the vaccine contains a minimum titre of approximately 1.2 x 10^{12} infectious units per dose. It can be stored and refrigerated at 2-8˚C for up to 24 months. RotaTeq® vaccine protects against severe RV gastroenteritis associated with any serotype that has been investigated [3,30].

Besides these, four additional vaccines have been licensed -Rotavac® and Rotasil® (which are licensed by local manufacturers in India, with phase I-III supporting trials), Lanzhou Lamb vaccine (in China) and Rotavin-M1® (in Vietnam) [32]. Rotavac® includes neonatal 116E rotavirus strain, which occurs naturally as a human-bovine reassortant strain of G9P[11] [33]. Rotasil® is a UK bovine reassortment vaccine composed of five reassorted strains, which is being developed in partnership with researchers from USA, India and Brazil. It is a heat stable vaccine [34]. Lanzhou Lamb vaccine was licensed in 2000 and is based on a rotavirus strain found in a local lamb, suffering from diarrhoea. It has an effectiveness of around 60- 78% [35]. Rotavin-M1® is developed from the strain G1P[8], which are similar to the strain in Rotarix® and were isolated from a child with diarrhoea in Vietnamese. Evaluation of different viral concentration and doses in a phase I adult – infant and phase 2 infant trials of Rotavin-M1®vaccine has also been done [36]. Some other candidate vaccines are also showing promising results and are in the process of development and research [30,37-39].

Rotavirus vaccine was introduced in 90 countries by the end of 2016 [40]. The clinical efficacy of rotavirus vaccines has been demonstrated mainly in the USA, Europe and Latin America. The efficacy of vaccines may vary in different regions and countries, so as per WHO, unless there is complete data from all regions of the world regarding the effectiveness of the existing vaccines, global inclusion of rotavirus vaccines into national immunization programmes cannot be recommended. But WHO strongly recommends the inclusion of rotavirus vaccination into the national immunization programmes of countries showing significant health impact [3].Studies from India suggests introducing rotavirus vaccine to Universal Immunization Programme (UIP) and increasing UIP coverage, which would be cost-effective and would greatly alleviate the disease and financial burden of vaccine-preventable diseases [41].

DISCUSSION AND CONCLUSION

There is a vast geographic variation in the existence of rotavirus genotypes worldwide. Regular surveillance studies for novel genotypes need to be done, which will definitely be of great help in the formation of newer vaccines providing wider coverage against rotavirus infection. Vaccine preventable diseases take heavy tolls of life annually [40], so there is a dire need for formation of dedicated organizations, which can help in carrying out more and more research works as regards to development of modern and effective vaccines.

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