**Rotavirus: What we know till date.**

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**Abstract**

Acute gastroenteritis in children below five years of age, causing severe diarrhea continues to be one of the important causes of morbidity and mortality in the world. Rotavirus infection is acquired by children via the fecal-oral route. Patients usually present themselves with loose stool to severe diarrhea and vomiting, which without proper management further leads to dehydration, electrolyte disturbances, shock and death. This review brings together all the information available till date about rotavirus structure, etymology, classification, mortality, morbidity, serotypes, genotypes, epidemiology, pathophysiology, factors, laboratory diagnosis, treatment and surveillance.

1. **Diarrhea**

Diarrhea is described as the condition in which an individual passes 3 or more loose or liquid stools per day, or more frequently than is normal. It is usually a symptom of gastrointestinal infection which can be caused by a variety of parasitic, viral and bacterial organisms. The spread of Infection is through contaminated food or drinking water or from person to person due to unhygienic living conditions [1]. E.coli, Salmonella, V.cholerae, Campylobacter and Shigella are the main causes of Bacterial diarrhea [2]. Rotavirus, human astrovirus, norovirus, enteric adenovirus, sapovirus are the main causes of Viral gastroenteritis and accounts for more than 75% of the cases [3]. 1,80,000–4,50,000 deaths of children
under the age of five, especially in developing countries were found to be due to rotavirus infection [4,5]. Infant deaths due to severe diarrhea have been reported since the 17th century [6,7].

2. Etymology of Rotavirus

A methodical study with histological examination of the duodenum in children affected by acute gastroenteritis in 1970 revealed severe inflammation of the upper intestine with villous atrophy in one third of patients [8,9]. Culture of intestinal aspirates was negative for known bacterial and viral pathogens [10]. Concurrent studies in 1973 examining ultrathin sections of duodenal mucosa from children with acute gastroenteritis with electron microscopy showed abundant viral particles in the epithelial cells lining the upper villous surface [11]. The viral particles could be identified by electron microscopy in diarrheal feces [12]. The viral structure of 70nm resembled a wheel and was thus named Rotavirus (Latin word for wheel) [13,14]. Soon, Rotavirus was confirmed to be the most common cause of severe acute dehydrating diarrhea in young children throughout the world [15].

3. Morbidity and Mortality of children under the age of five years due to acute gastroenteritis

Morbidity and mortality of children under the age of five years is a major public health problem in India of which a major cause is acute gastroenteritis. Contribution of rotavirus infection to this health condition and its importance in preventive health practice is reviewed here. The estimate of global child mortality rates show a decline over the years from 53% in 1990 to 43% in 2015 [16]. The decline being encouraging but still preterm birth complications, intrapartum-related complications, diarrhea, pneumonia and congenital abnormalities were found to be predominant causes of child mortality in 2015 [17]. The estimates in India, in 2015 showed 1,17,285 children in the age group of 0-4 years to have died due to diarrhea alone [17]. Premature and neonatal birth complications (39%) are the main causes of mortality followed by 14.9% of pneumonia and 9.8% of diarrhea in the under-five age group [16]. Hence it is considered that diarrheal disorder is the third most common cause of under 5 mortality and the 2nd most common morbidity after infancy.
4. Epidemiology of Rotavirus Gastroenteritis

4.1. Seasonality

Contrasting seasonal patterns have been described for rotavirus–gastroenteritis with the incidence peaking during winter months in the temperate regions. Seasonal variations have been found to be less in the tropics, which has been found throughout the year with peaks during summer and fall following monsoon [18]. Only a few studies have correlated rotavirus infection with a seasonal incidence [19,20]. Reports associating rotavirus infection with seasonality in India have also been found to differ geographically, though mainly it has been found to occur during the months of October to February in most parts of the country [21].

4.2. Transmission

Rotavirus infection is highly communicable and mode of acquiring rotavirus infection is through transmission of the virus via the fecal-oral route. Transmission has been found to be common among infants and young children. The incubation time period of the virus is approximately 2 days and the virus is shed in large quantities by the infected person in their stool 2 days prior to the onset of diarrhea and for up to 10 days after onset of the symptoms. The virus is shed most when they are sick and during the first 3 days after they recover from rotavirus disease [22].

4.3. Age and gender distribution

Children under the age of 5 years are normally affected, majority of the cases of rotavirus gastroenteritis occurring in children below 2 years of age [21]. Children of developing countries are most affected in their first year of life but large number are affected in their second year too [23,24]. 36.5% and 38.9% of hospitalized cases in India were rotavirus associated, in infants in the age group, 6–11 months and children aged 12–23 months respectively, as shown by the Indian Rotavirus Surveillance Network [25]. Earlier reports have shown males to be more frequently affected than females [26].
5. Clinical presentation of rotavirus associated gastroenteritis

Patients usually present themselves with loose stool to severe diarrhea and vomiting which further leads to electrolyte disturbances, dehydration and shock and death without proper management [1]. The estimated incubation time period is about 48 hours. Mild cases present with watery diarrhea and has a short duration. In severe cases, it is accompanied with fever, vomiting, dehydration, electrolyte imbalance, shock and death [27]. The onset of rotavirus infection usually shows up with fever (>39ºC) and vomiting, and watery diarrhea starts after 24 to 48 hours. The duration of vomiting lasts for less than 24 hours, and other gastrointestinal problems disappear within 3 to 7 days [28]. Dehydration is a usual complication, due to severity of diarrhea, associated with vomiting [29].

6. Factors responsible for rotavirus gastroenteritis

Infection, malnutrition, faeces contaminated water source and poor hygiene cause diarrhea [30]. Low birth weight, premature births, requirement for neonatal intensive care facilities, malnutrition or immunosuppression may increase the risk of getting infected by the virus [31]. The virulence factors involved in the induction of diarrhea are complex and involve both host and viral factors [32]. Recent reports indicate the association of rotavirus infection with Histo-blood group antigens.

6.1. Association of rotavirus infection with Histo-blood group antigens

Studies for a specific host receptor for the virus have revealed numerous host receptors including the heat shock proteins, integrins and gangliosides (Liu et al.,2012) [33]. Huang et al in 2012 found that the recombinant VP8* proteins of a number of human RVs interacted with the human histo-blood group antigens (HBGA’s) (Huang et al., 2012) [34]. This interaction showed to have a genetic predisposition and was found to be specific for each strain. The P types, P[4] and P[8], were found to bind the Lewis b (Leb)
and H-type 1 antigens, while the P[6] recognized H-type 1 only. Further investigations indicated that the human HBGAs play an important role in infection and therefore evolution of the virus [33]. Recent studies by the same group have indicated that P[19] RVs may bind to other oligosaccharides or ligands and have the potential to spread widely among humans [35]. Crystallographic studies of the cell attachment protein VP8* of a human rotavirus has showed that it specifically interacts with A-type histo blood group antigen [36]. It has also been reported that both Lewis and Secretor status mediate susceptibility to rotavirus infections in a genotype dependent manner [37]. These studies put forward the need for rotavirus epidemiologic studies in relationship with host HBGAs phenotypes [38]. A 2015 study indicates that the binding pattern of rotavirus to different HBGA's is strain dependent [39]. Efforts have been made to analyze this evidence clinically. Three recent clinical studies on the association of rotavirus positive gastroenteritis and ABO blood groups have given varied reports. Trang et al in their cross-sectional study of 260 children with paired stool and saliva samples in children hospitalized with diarrhea in Northern Vietnam demonstrated that HBGAs phenotype is a key susceptibility factor for rotavirus infection in children [40]. The association of rotavirus infection with blood group A was also detected in another study from Turkey [41]. However, no quantitative relationship has been found between rotavirus gastroenteritis and major blood groups in one more study in Turkey [42].

7. Structure of rotavirus

The Rotavirus belongs to the Reoviridae family and is a double-stranded (ds) RNA, non-enveloped virus. The 11 ds RNA segments of the rotavirus genome code for six structural proteins (VP1-8) and six non-structural proteins (NSP1-NSP6) [43]. The inner core or capsid of the virus is surrounded by a thick shell. The core is composed of the 11 dsRNA segments of the viral genome and is associated with one copy of the RNA-dependent RNA polymerase called VP1, and the RNA capping enzyme called VP3 [44,45]. Surrounding this core is the innermost layer of the TLP (thick triple layered particle), a T = 1 icosahedral shell which is made of 60 asymmetric dimers of the VP2 protein [46,47]. This layer is also known as the single layered particle (SLP) houses the ds RNA genome within a protein layer composed of 120 copies of VP2 arranged in an unusual T=1 icosahedral lattice with two molecules in the icosahedral asymmetric unit.
This is surrounded by the double layered particle (DLP) which has 260 trimers of the VP6 protein ordered in an icosahedral T = 13 symmetry [46,47,48]. The outermost layer of this DLP has 260 trimers of the VP7 glycoprotein, ordered in a T = 13 icosahedral lattice. Each of these trimers is connected to a protrusion in the VP6 layer by a flexible N-terminal arm [48,49]. Outermost layer has sixty spikes of trimers of the protein, VP4 which are attached to depressions in the VP6 layer [47]. It is this outermost layer of TLP with the VP4 protein spikes which the virus uses to identify, then binds and penetrates the intestinal epithelial cell [50,51]. Another important structural feature is the three different types of large channels which penetrate through the layers of VP7 and VP8*. 12 numbers of Type I channels are found at the five-fold vertices of the capsid, 60 numbers of type II channels are present at each of the pentavalent locations surrounding the type I channels, near to which the VP7 and VP6 are attached to VP4 and 60 type III channels surround the icosahedral three – fold axes on the capsid and which are located at the remaining hexavalent positions [43]. Aqueous and biochemical substances pass in and out of the capsid through these channels.

8. Molecular events and pathophysiology of rotavirus gastroenteritis

8.1. Infectivity

Cell releases the TLP which is digested in the lumen of the intestine. The VP4 protein is cleaved first by trypsin like proteases in the intestinal lumen to produce two fragments, the larger C-terminal VP5* and the N-terminal VP8*. The cleavage happens stepwise after three defined sites (Arg231, Arg241 and Arg247) the process ending in scission at Arg247 [52]. This cleaved TLP then becomes infectious. The three molecules of VP4 i.e VP4 A-C form the spikes [47] and are held together by non-covalent interactions [52]. There are suggestions that conformational changes in this complex spike structure release energy which is used by the virus to disrupt the cell membrane [52]. The distal lectin domains of the two VP8* molecules of chains A and B get bound to the receptor and this triggers reorganization of the spike components into an extended intermediate in which hydrophobic loops of the three VP5 β-barrels are inserted into the target cell membrane. Following this an unknown trigger brings back the extended
intermediate to a structure which is folded-back, in which the hydrophobic loops point toward the virus particle [51,53-56].

8.2. Transcription, replication and packaging

Viral replication takes place only in the terminally differentiated enterocytes of the intestine and begins only when the DLP is released into the cytoplasm [52] and becomes transcriptionally competent [57]. RNA segments are transcribed into viable mRNA molecules followed by template generation and viral protein production. The DLPs have been shown to remain structurally intact while the nascent transcripts pass out through the Type I channels [58]. The DLP has the enzymatic properties for synthesis of mRNA transcripts and facilitation of translation. The VP1, which is the RNA-dependent- RNA polymerase [59], the VP3, a guanyl transferase and methyl transferase carry out these functions [60]. The DLPs are transcriptionally competent both in vitro and in vivo, but the TLPs are transcriptionally incompetent [43]. On release of the transcripts, the virus starts replication. Viral proteins are synthesized by translation which is followed by replication, genome packaging and DLP assembly with the last step being budding of the newly formed DLPs into the endoplasmic reticulum and assembly of the outer layer to form mature TLPs [57]. The NSP3, which is the non-structural protein, is involved in the specific recognition of the rotaviral mRNAs [61-63]. The DLP assemblies occur in viroplasms which are perinuclear, non membrane-bound, electron dense inclusions. NSP2 and NSP5 have also been found to be involved in production of these viroplasms but also in the replication and packaging process [43]. The role played by NSP6 is still not clear. Once the DLPs bud into the endoplasmic reticulum they acquire an outer layer of composed of VP4 and VP7. NSP4 facilitates this process, which has a binding site for VP6 [43].

8.3. Pathophysiology of Rotavirus Gastroenteritis

Investigative study in an animal model by Ramig [64] has led to a clear understanding of infectivity and pathogenesis of rotavirus gastroenteritis. The endoplasmic reticulum releases Ca2+ as a result of intracellular events in which NSP4 may be involved. A number of cellular processes are triggered due to the increase in
intracellular Ca2+ concentration ([Ca2+]i) i.e. lowered expression of disaccharidases and other enzymes at the apical surface, disruption of the microvillar cytoskeletal network, necrosis and general inhibition of the Na+-solute cotransport systems. NSP4 is released specifically by a Ca2+-dependent, nonclassical secretion pathway prior to the lysis of the cell. This reduces the absorptive capacity of the epithelium, reduction in the Na+-solute cotransporters activity and reduction in the expression of digestive enzymes on the epithelial surface. The release of NSP4 from infected cells makes way for the occurrence of paracrine effects on uninfected cells by binding to these cells to specific, unidentified receptor(s) [32], which leads to the triggering of a phospholipase C-inositol 1,3,5-triphosphate (PLC-IP3) cascade that results in Ca2+ release from the endoplasmic reticulum, thus leading to the increase in [Ca2+]i. Action of NSP4 on enterocytes leads to paracellular permeability due to disruption of tight junctions. If NSP4 acts on crypt cells, which causes increase in [Ca2+]i further leads to activation of a Cl− transporter hence causing secretion in the crypt, which results in an increased secretory component of the diarrhea. NSP4, or other effector molecules released from infected cells, may also lead to the stimulation the enteric nervous system (ENS). It has been shown experimentally that rotavirus infection induces secretion via stimulation of the ENS. This shows the process by which a few infected cells which causes only little visible damage to the mucosa, but can develop a diarrheal response.

9. Serotypes and Genotypes of Rotavirus

9.1. General classification

The three important antigenic specificities of rotaviruses are: group, subgroup, and serotype. Based on the group specificity which is determined by the VP6 protein, rotaviruses are classified into 7 groups (A to G) [65] and more recently Group H [29] and a new Group I rotavirus described in dogs [66]. The Groups A, B, C and H cause acute gastroenteritis in humans and in animals, Group B rotavirus has been detected in humans, pigs, rats, cattle, dogs and sheep, Group C rotavirus infects dogs, pigs, ferrets, cattle, and humans, Group H rotavirus affects humans and more recently pigs. Group D, E, F and G were only detected in animals and Group D, F and G affect only birds. Group E rotavirus was detected only in pigs [29]. Transmission between species and reassortment between human and animal rotaviruses are being increasingly reported [29].
9.2. Identified genotypes of Rotavirus

In the last few years following the advent of the whole-genome-based genotype classification system, the whole genomes of around 167 human group A rotavirus strains have been analyzed [67]. The 11 ds RNA segments in the genome codes for five non-structural (NSP1-NSP5) and six structural (VP1-VP4, VP6, VP7) proteins. VP7 and VP4 being the outer capsid proteins, serve as viral attachment proteins and neutralization antigens [68]. The proteolytic cleavage of VP4 activates it into two fragments—VP5* and VP8*. VP8* forms a globular domain for attachment at the tip of the VP5* stalk [47]. Under the binary classification system group A rotaviruses have 27 G and 37 P types [69,70] a classification which is based on neutralization specificities of VP7 (Glycoprotein) and VP4 (Protease sensitive protein).

9.3. Genotypes circulating globally

Globally, G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8] genotype combinations of rotavirus strains are the most common cause of human infections [71]. G1P[8] strains are the most predominant (37.7%) among these [71]. Repeated disappearance and re-emergence seems to be common with certain types of strains [72,73]. Genome reassortment in virus strains belonging to the same group can lead to evolution of new viruses [74]. Rotavirus surveillance programmes globally, combined with advanced sequencing technologies have revealed the immense diversity of circulating rotavirus strains in different parts of the world [74]. G1P[8] strains show diversity in the form of 11 G1 and 4 P[8] subgenotypic lineages [75,76]. G9P[8] strains over the past 10 years have often been detected and is currently the 5th most prevalent genotypes in humans [29]. Recently, G12P[8] has also been detected and seems to be found globally [66]. A Euro surveillance report has found that Sweden and France had the lowest genotype diversity and Bulgaria the highest [77].
9.4. Genotypes circulating in India

A significant diversity in circulating strains has been observed in the last decade in different regions of India [78,79]. Rotavirus surveillance programmes globally, combined with advanced sequencing technologies have revealed the immense diversity of circulating rotavirus strains in different parts of the world [74]. This information is important because of the introduction of vaccines for rotavirus infection in many countries. It has become all the more necessary to identify, presently circulating strains in different regions and find out if there are differences among these strains and those in the vaccines being administered. Hence, it has become very necessary to continuously monitor the circulating genotypes and determine the genetic variability's within the strains in order to determine better vaccine candidates.

10. Laboratory diagnosis of rotavirus infection

Detection of the viral antigen in the stool is based on agglutination with latex particles (LA), enzyme immunoassay (EIA), immunochromatography (IC) and chemiluminescent immunoassay (CLIA) [91]. Enzyme immunoassay is very sensitive and specific for the detection of group A and C rotaviruses in fecal samples, when monoclonal antibodies are used [3]. The nucleic acid segments of the virus can be visualized on acrylamide gels after extraction and staining with ethidium bromide or silver nitrate. Human rotavirus groups - A, B, and C are distinguished by their specific patterns of gene-segment distribution designated as electropherotypes. The formation of a distinct electropherotype is considered diagnostic for the presence of individual rotaviruses of specific Groups A, B, and C [92]. Identification of rotaviruses in clinical specimens and determination of G and P types can be done by extraction of the viral RNA from fecal specimens and analysis by semi-nested RT-PCR with primers specific for regions of the genes encoding the VP7 (G-type) or VP4 (P-type) in order to obtain genotype-specific PCR products for analysis on an agarose gel or sequencing gel [10]. Rotavirus characterization is carried out by serotyping, genotyping and subgrouping with monoclonal antibodies. Determination of the G and P types can be done by analyzing the RT-PCR product. Primers described by Gentsch et al [93] and Gouvea et al [94] are used to genotype both VP4 and VP7.
proteins, respectively. Genotyping the specimen can show single or multiple infections (single cell being infected with different virus strains) [95]. Rotavirus is detected in clinical specimens by extraction of the viral RNA and analysis by electrophoresis on a polyacrylamide gel followed by silver staining. Electron microscopy allows very specific identification of the virus particle in stool specimens and its sensitivity can also be improved by Immune EM (IEM) by adding specific antibodies or immune sera to the sample. IEM differentiates between morphologically identical A, B and C rotaviruses serotypes [3]. But, routine use of this technique is not possible due to the high viral concentration need [96], electron microscopy experts and the costly equipment. Rotavirus isolates can be inoculated into MA-104 cells where they grow after serial passage [65]. The rotavirus causes cytotoxic effects causing cell lyses and loss of tissue integrity [97]. Specialized laboratories only perform this technique. The diagnosis of rotavirus infection in a peripheral health care setting is not feasible with the above tools for diagnosis as the laboratory facilities are lacking in most settings at sub district level. Therefore, an early diagnosis still remains a challenge and prevention could be the focus for public health management. Methods to Rotavirus have improved. The World health Organization in 2009 published a manual of methodology for identification of the virus [10]. One to two ml. or one to two gms of fecal samples are collected from the patient into clean containers and transported to the laboratory. Fecal suspensions are prepared, centrifuged, supernatants collected and stored at 4-8 ºC for short term and -70ºC for long term.

11. Treatment and Prevention of Rotavirus Infection

11.1. Treatment [98,99]

Prevention of death due to severe dehydration caused by diarrhea is very important. Reduced osmolarity ORS solution is administered for rehydration. The electrolytes and water lost during diarrhea are replaced by the ORS which is absorbed in the small intestine; there is severe loss of zinc during diarrhea. Zinc is administered for 14 days which greatly reduces the severity of the condition. Continuing nutrient rich foods during and after the diarrhea breaks the cycle of diarrhea and malnutrition. In case of acute dysentery, antibiotics could be administered. Cleanliness is very important.
11.2. Preventive strategies

11.2.1. Improving sanitation and hygiene.

Poor sanitation, contaminated water and unhygienic conditions play a major role in susceptibility to rotavirus infection [98].

12. Surveillance

The World Health Organization [100] has been coordinating the Global Rotavirus Surveillance Network since 2008 which is a network of sentinel surveillance hospitals and laboratories that report to the ministries of health (MoHs) and WHO on the clinical features and data on rotavirus testing on those children who are <5 years of age and hospitalized with acute gastroenteritis. The Global Rotavirus Laboratory Network (GRLN) is a fundamental component of the WHO RV surveillance system designed to conduct high quality diagnostic testing for RV diarrhea and characterize the most prevalent strain genotypes in different countries. Until January 2016, the network includes 115 laboratories including 68 sentinel hospital laboratories (SHL), 37 provincial and national laboratories, 9 regional reference laboratories (RRL) and one Global Reference Laboratory (GRL) [100]. After the identification of rotavirus gastroenteritis in India, there have been many reports on the epidemiology of the disease which were not very informative. The Indian Council for Medical Research (ICMR) in 2005 in collaboration with Centre’s for Disease Control and Prevention (CDC) in Atlanta, USA, a network for hospital based surveillance of rotavirus in different parts of the country was established [101]. The aim of the Indian Rotavirus Strain Surveillance Network were to generate timely and geographically representative information on the epidemiological, clinical and virological features of severe rotavirus disease using standardized protocols in Indian children for enrolment and diagnosis. It also had the aim to establish a model surveillance system for a vaccine preventable disease based on which further studies to evaluate the impact of vaccination could be conducted. It was the ICMR expert advisory group’s recommendation to include more clinical surveillance sites in hospitals and testing laboratories network in order to have more complete regional representation of the country [80].


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CONFLICT OF INTEREST

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