Prevalence of Rotavirus Infection in Children Up To Five Years Presenting With Diarrhea, In Tertiary Care Hospital, Jamnagar, Gujarat (India)

Dr. Pankti Champakbhai Pargi and Dr. Hitesh Kantilal Shingala

Abstract: Rotavirus is an intestinal virus belonging to the reoviridae family. Rotavirus is spread through the faecal-oral route by contaminated hands, water or food. The major clinical symptoms are severe diarrhoea and vomiting, including fever. This study, which was conducted in tertiary care hospital, Jamnagar. In my study children less than five years with watery diarrhoea from ward were included. About 15-20 ml of stool specimen was collected screened by Rotavirus Ag (stool) ELISA Kit. Total 170 stool samples from patient of acute diarrhoea were tested for rotavirus antigen and the prevalence of rotavirus diarrhoea was 33.5%. Among positive cases, prevalence was higher in male children (52.6%) than female children (47.4%). Maximum positive cases were found among 7-12 month (51%), followed by 25-60 month (19.3%), followed by 13-18 month (14%), followed by 0-6 month (10.5%), followed by 19-24 month (5.2%). Maximum positive cases (87.71%) of rotavirus were found in top feed children as compare to exclusive breastfeed (12.29%). Maximum positive cases (38.59%) of rotavirus infection is the most common cause of acute diarrhea in infants and young children and it may cause some to severe dehydration in acute diarrhoea in an unvaccinated child. And Study reveal exclusive breastfeed given protection against rotavirus infection.

Keywords: Rotavirus, Watery diarrhoea, Ag ELISA, breastfeed.

INTRODUCTION:

Rotavirus is an intestinal virus belonging to the reoviridae family (Dennehy, P.H. 2000). This type of virus was first described in the 1970s. Rotavirus can cause autumn diarrhoea. Seven types of rotavirus have been described, designated A to G. Among these types, group A causes 90% of the infections observed in human infants and children. Therefore, almost every child <5 years old has been infected by a rotavirus at least once (Velazquez, F.R. et al., 1996). However, a single virus infection strengthens the immunocompetence of an individual and will lower the chance of future infections (Linhares, A.C. et al., 1988).

Diarrheal disorders account for an estimated 1.5 million deaths globally every year making it the second leading cause of child mortality (Ahmad, B. Z. 2011). The burden of diarrhoeal disease is greatest in low- and middle income countries with the highest incidence rates found among children <12 months of age (Liu, L. et al., 2015). Apart from high morbidity and mortality associated with diarrhoeal diseases, diarrhoea also present an economic burden for the developing countries, with more than a third of the hospital beds for children being occupied by patients with diarrhoea, often involving the use of expensive intravenous fluids and drugs (WHO, UNICEF: 2005).

Rotavirus infection affects 95% of children under the age of 5 years regardless of the socioeconomic or environmental conditions and leads more frequently to dehydration than other aetiologies (World Health Organisation. 2000-2011; Banerjee, I. et al., 2006; & Chakravarti, A. et al., 2010). Rotavirus infection results in a significant disease burden and economic effect of direct medical costs, loss of work, quality of life and mortality. Vaccination is the only control measure likely to have a significant impact on the incidence of severely dehydrating rotavirus disease (Dennehy, P.H. 2008). Studies estimated that 90,000-
1,53,000 children die from rotavirus infection in India each year (Jain, V. et al., 2001; Parashar, U. D. et al., 2009).

The major clinical symptoms are severe diarrhoea and vomiting, including fever. However, infections can also be asymptomatic, especially in neonates, older children and adults (Lundgren, O., & Svensson, L. 2001). Cases of asymptomatic infections in older children and adults are probably due to active immunity. Usually all children have become infected several times during the first twenty-four months of life and by the time they reach 5 years of age most children have had repeated infections and developed a life-long lasting immunity to rotavirus disease (Lundgren, O., & Svensson, L. 2001).

Rotavirus is spread through the faecal-oral route by contaminated hands, water or food (Viral Gastroenteritis. 2003). The amount of rotavirus shed in faeces has been shown to be 1010 virus particles/gram of stool (Greenberg, H.B., & Estes, M.K. 2009). There are few studies of infectivity but those indicate that only 10 or less particles are needed for an infection (Greenberg, H.B., & Estes, M.K. 2009; Graham, D.Y. et al., 1987). Probably, as the very infectious norovirus, causing “the winter vomiting disease”, rotavirus may also be spread by aerosol through vomits, since droplet spread of aerosolized rotavirus has been shown experimentally, using a mice model (Prince, D. S. et al., 1986).

Very few studies on the prevalence of rotavirus among children have been conducted in tertiary care hospital. This study aimed to identify the proportion of children with acute gastroenteritis infected with rotavirus through systematic sampling over a one year period in a tertiary care Government hospital.

MATERIAL AND METHODS:
Study Area
This study, which was conducted in tertiary care hospital for a period from November 2017 to September 2018.

Study Population
In my study 170 children less than five years with watery diarrhoea from ward were included.

Inclusion Criteria:
• Children under five years with acute gastroenteritis.

Exclusion Criteria:
• Children below five years with chronic diarrhoea (diarrhoea >14 days)
• Children under five year with immuno compromised state.

Clinical History:
Detailed history was taken from the parents/guardian of the patient and entered in the proforma as follows:

Name, age, gender, geographical area (urban/rural), Antenatal history- full term/preterm, birth weight, immunization, Onset and duration of diarrhoea, whether blood stained or not, vomiting, abdominal pain, and fever, Feeds- breast fed/bottle fed, Physical examination of the child to detect signs of dehydration-consciousness, whether the baby taking feeds, active, skin turgor. Macroscopically examination of stool specimen – watery, colour, whether blood stained or not.

Sample collection, transport and storage:
About 15-20 ml of stool specimen was collected in a sterile wide mouthed universal container during acute diarrhoea. Unpreserved samples should be kept at 4 c and tested within 24 hours of collection. Samples that cannot be tested within this time should be frozen at -20 c until used. Freezing the specimens does not adversely affect the test however, avoid respected freeze/thaw cycles. All dilutions must be made with the diluted wash buffer.

Preparation of Sample:
Prepare a 1:5 dilution of stool by adding 1 gram (approximately the size of a pea) to 4 ML of diluted wash buffer, mix well and allow the heavy particulates to settle. For diarrheal stools a lower dilution may be used (i.e., 1:2 dilution).

Procedure (Rotavirus Ag ELISA):
Rotavirus Ag (stool) ELISA Kit was used. ELISA plate reader capable of reading at 450/620-650 nm was used. Ensure all samples and reagents are at room temperature before use. Frozen samples must be thawed completely before use. The rotavirus Ag(stool) sandwich ELISA is an in vitro procedure for the qualitative determination of rotavirus antigen in feces.

Break the number of wells needed (number of samples plus 2 for controls) and place in strip holder. Add 100 µl of the negative and positive control to respective well. Add 100 µl of the stool supernatant to the appropriate test well. Incubate at room temperature for 30 minutes, then wash. Add 2 drops of Reagent 1 (blue solution) to each well. Incubate at room temperature for 5 minutes, then wash. Add 2 drops of Reagent 2 (red solution) to each well. Incubate at room temperature for 5 minutes, then wash. Add 2 drops of stop solution to each well. Mix wells by gently tapping the side of the strip.
holder with index finger. Read results visually or using an ELISA plate reader.

RESULTS AND ANALYSIS:
The present study, which was conducted in tertiary care hospital for a period from November 2017 to September 2018.

Table 1: Prevalence of rotavirus infection

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Total Samples</th>
<th>Positive Samples</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>170</td>
<td>57</td>
<td>33.5%</td>
</tr>
</tbody>
</table>

In this study, total of 170 stool samples were taken during study period from November-2017 to September-2018. Among this, 57(33.5%) were found to be rotavirus positive shown in Table 1.

Out of 57 positive rotavirus cases, majority of them were male children 30(52.6%) and 27(47.4%) were female children, showing in Table 2.

Table 2: Gender wise distribution of rotavirus positive cases

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Gender</th>
<th>Total</th>
<th>Positive cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Male</td>
<td>97</td>
<td>30</td>
<td>52.6%</td>
</tr>
<tr>
<td>2</td>
<td>Female</td>
<td>73</td>
<td>27</td>
<td>47.4%</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>170</td>
<td>57</td>
<td>100%</td>
</tr>
</tbody>
</table>

Chart-1

Prevalence of Positive Cases

33.5%

66.5%

Chart-2

Gender wise distribution of positive cases

30

27

MALE

FEMALE

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Table 3: Age wise distribution of rotavirus positive cases

<table>
<thead>
<tr>
<th>Sr no.</th>
<th>Age (in month)</th>
<th>Positive cases</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0-6</td>
<td>06</td>
<td>10.5%</td>
</tr>
<tr>
<td>2</td>
<td>7-12</td>
<td>29</td>
<td>51%</td>
</tr>
<tr>
<td>3</td>
<td>13-18</td>
<td>08</td>
<td>14.0%</td>
</tr>
<tr>
<td>4</td>
<td>19-24</td>
<td>03</td>
<td>5.2%</td>
</tr>
<tr>
<td>5</td>
<td>25-60</td>
<td>11</td>
<td>19.3%</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>57</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table 3 shows that among the positive cases, 29(51%) cases were from the age group of 7-12 month, followed by 11(19.3%) were from 25-60 month, 8(14%) were from 13-18 month, 6(10.5%) were from 0-6 month and 3(5.2%) were from 19-24 month in my study.

Chart-3

Table 4: Seasonal distribution of rotavirus positive cases

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Month</th>
<th>Positive cases</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>November</td>
<td>07</td>
<td>12.3%</td>
</tr>
<tr>
<td>2</td>
<td>December</td>
<td>15</td>
<td>26.3%</td>
</tr>
<tr>
<td>3</td>
<td>January</td>
<td>03</td>
<td>5.3%</td>
</tr>
<tr>
<td>4</td>
<td>February</td>
<td>02</td>
<td>3.5%</td>
</tr>
<tr>
<td>5</td>
<td>March</td>
<td>02</td>
<td>3.5%</td>
</tr>
<tr>
<td>6</td>
<td>April</td>
<td>03</td>
<td>5.3%</td>
</tr>
<tr>
<td>7</td>
<td>May</td>
<td>02</td>
<td>3.5%</td>
</tr>
<tr>
<td>8</td>
<td>June</td>
<td>03</td>
<td>5.3%</td>
</tr>
<tr>
<td>9</td>
<td>July</td>
<td>04</td>
<td>7.0%</td>
</tr>
<tr>
<td>10</td>
<td>August</td>
<td>05</td>
<td>8.7%</td>
</tr>
<tr>
<td>11</td>
<td>September</td>
<td>11</td>
<td>19.3%</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>57</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table 4 shows that rotaviral diarrhoea was more prevalent in December month.

Chart-4
Table 5: Seasonal trends in rotavirus positive cases

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Season</th>
<th>Positive cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Winter</td>
<td>27</td>
<td>38.59%</td>
</tr>
<tr>
<td>2</td>
<td>Summer</td>
<td>10</td>
<td>12.2%</td>
</tr>
<tr>
<td>3</td>
<td>Monsoon</td>
<td>20</td>
<td>35.08%</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>57</td>
<td>100%</td>
</tr>
</tbody>
</table>

This table shows seasonal trends in rotavirus positive cases. During winter season (November to February) 27 (38.59%) cases were found positive which was maximum. During summer (March to June) 10 (12.2%) cases were found positive and during monsoon (July to September) 20 (35.08%) cases were found positive.

Table 6: Associated multiple clinical findings with watery diarrhoea in rotavirus positive cases

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Clinical feature</th>
<th>Positive case</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vomiting</td>
<td>33</td>
<td>57.8%</td>
</tr>
<tr>
<td>2</td>
<td>Abdominal pain</td>
<td>06</td>
<td>10.5%</td>
</tr>
<tr>
<td>3</td>
<td>Fever</td>
<td>23</td>
<td>40.35%</td>
</tr>
<tr>
<td>4</td>
<td>Dehydration</td>
<td>18</td>
<td>31.57%</td>
</tr>
<tr>
<td></td>
<td>Some</td>
<td>14</td>
<td>24.6%</td>
</tr>
<tr>
<td></td>
<td>Severe</td>
<td>04</td>
<td>7.0%</td>
</tr>
</tbody>
</table>

Table 6 shows, associated multiple clinical findings with watery diarrhoea in rotavirus positive cases.

Table 7: Rotavirus positive cases related with feeds

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Type of Feed</th>
<th>Positive cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Exclusive Breastfeed</td>
<td>07</td>
<td>12.29%</td>
</tr>
<tr>
<td>2</td>
<td>Top Feed</td>
<td>50</td>
<td>87.71%</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>57</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table 07 shows that maximum positive cases (87.71%) of rotavirus were found in top feed children as compared to exclusive breastfeed (12.29%).
DISCUSSION:
Rota virus is the most common and deadly cause of severe diarrhoea with dehydration in infants and young children. Even though diarrhoeal deaths have declined considerably through improved access to medical facility, the burden of the diarrhoeal disease continues to remain significant.

Table 8: Comparison of prevalence of rotavirus in different studies

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Study</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Present Study</td>
<td>33.5%</td>
</tr>
<tr>
<td>2.</td>
<td>Bhatnagar, S., &amp; Srivastava, G. (2017)</td>
<td>40.4%</td>
</tr>
<tr>
<td>3.</td>
<td>Ari. A et al., (2016)</td>
<td>29.8%</td>
</tr>
<tr>
<td>4.</td>
<td>Bonkoungou et al., (2010)</td>
<td>33.7%</td>
</tr>
<tr>
<td>5.</td>
<td>Indrani Banerjee et al., (2006)</td>
<td>27.4%</td>
</tr>
</tbody>
</table>

Table 08 shows comparison of prevalence of rota virus in different studies. In present study prevalence was 33.5%, which was more similar to Bonkoungou et al., (2010) and Ari. A et al., (2016) were respectively 33.7% and 29.8%.

Table 9: Gender wise Comparison of rotavirus in different studies

<table>
<thead>
<tr>
<th>Sr no</th>
<th>Study</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Present study</td>
<td>52.6%</td>
<td>47.4%</td>
</tr>
<tr>
<td>2.</td>
<td>Ari. A et al., (2016)</td>
<td>72.1%</td>
<td>27.9%</td>
</tr>
<tr>
<td>3.</td>
<td>Bonkoungou et al., (2010)</td>
<td>47%</td>
<td>53%</td>
</tr>
<tr>
<td>4.</td>
<td>Nakawesi et al., (2010)</td>
<td>58.8%</td>
<td>41.2%</td>
</tr>
<tr>
<td>5.</td>
<td>Rerksuppaphol, S., &amp; Rerksuppaphol, L. (2011)</td>
<td>61.4%</td>
<td>38.6%</td>
</tr>
</tbody>
</table>

Table 09 shows gender wise comparison of rota virus in different studies. In present study prevalence was more common in male children as compare to female children respectively 52.6% and 47.4%, which were more similar to Nakawesi et al., (2010) and Rerksuppaphol and Rerksuppaphol et al., (2011).

Table 10: Comparison of age group in rotavirus in different studies

<table>
<thead>
<tr>
<th>Sr.no</th>
<th>Age (in month)</th>
<th>0-6 month</th>
<th>7-12 month</th>
<th>13-18 month</th>
<th>19-24 month</th>
<th>25-60 month</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Present study</td>
<td>10.5%</td>
<td>51%</td>
<td>14.0%</td>
<td>5.2%</td>
<td>19.3%</td>
</tr>
<tr>
<td>2.</td>
<td>Ari. A et al., (2016)</td>
<td>18.3%</td>
<td>45.2%</td>
<td>20.2%</td>
<td>6.7%</td>
<td>9.6%</td>
</tr>
<tr>
<td>3.</td>
<td>Bonkoungou et al., (2010)</td>
<td>18%</td>
<td>47%</td>
<td>23.2%</td>
<td>6%</td>
<td>5.8%</td>
</tr>
<tr>
<td>4.</td>
<td>Indrani Banerjee et al., (2006)</td>
<td>12.9%</td>
<td>42.3%</td>
<td>26.7%</td>
<td>21.1%</td>
<td>-</td>
</tr>
<tr>
<td>5.</td>
<td>Wg C B M John et al., (2014)</td>
<td>1.7%</td>
<td>43.3%</td>
<td>35%</td>
<td>18.3%</td>
<td>1.7%</td>
</tr>
</tbody>
</table>

Table 10 shows comparison of age group in rota virus in different studies. In present study prevalence was more common in 7-12 month of age group 51%, which were more similar to bonkoungou et al., (2010) and Ari. A et al., (2016) respectively 47% and 45.2%.
Table 11: Comparison of seasonal trends in rota virus in different studies

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Study</th>
<th>Winter</th>
<th>Summer</th>
<th>Monsoon</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Present study</td>
<td>38.59%</td>
<td>12.2%</td>
<td>35.08%</td>
</tr>
<tr>
<td>2.</td>
<td>Ari, A et al., (2016)</td>
<td>61.5%</td>
<td>9.7%</td>
<td>28.8%</td>
</tr>
<tr>
<td>3.</td>
<td>Bonkoungou et al., (2010)</td>
<td>53.3%</td>
<td>2.9%</td>
<td>43.8%</td>
</tr>
</tbody>
</table>

Table 11 shows the comparison of seasonal trends in rotavirus in different studies. In present study prevalence of winter season was 38.59%, which was higher among all seasons followed by 35.08% prevalence in monsoon and 12.2% prevalence in summer, which were similar to study of Bonkoungou et al., (2010) and Ari, A et al., (2016).

Table 12: Comparison of dehydration in rota virus in different studies

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Study</th>
<th>No</th>
<th>Some</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Present study</td>
<td>68.4%</td>
<td>24.6%</td>
<td>7.0%</td>
</tr>
<tr>
<td>2.</td>
<td>Rerksuppaphol and Rerksuppaphol et al., (2011)</td>
<td>63.9%</td>
<td>26.1%</td>
<td>10%</td>
</tr>
<tr>
<td>3.</td>
<td>Wg C B M John et al., (2014)</td>
<td>58.8%</td>
<td>30.8%</td>
<td>10.4%</td>
</tr>
</tbody>
</table>

Table 12 shows the comparison of dehydration in rotavirus in different studies. In present study prevalence of severe dehydration was 7% which was similar to study Rerksuppaphol and Rerksuppaphol et al., (2011), Wg C B M John et al., (2014), respectively 10%, 10.4%. Though approximately 30% patient had dehydration in various study, rotavirus infection had significant impact on morbidity and mortality.

Table-13: Comparison of rotavirus positive cases related with feeds.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Study</th>
<th>Top feed</th>
<th>Exclusive</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Present study</td>
<td>87.71%</td>
<td>12.29%</td>
</tr>
<tr>
<td>2.</td>
<td>B M John et al., (2014)</td>
<td>85.72%</td>
<td>14.28%</td>
</tr>
</tbody>
</table>

Table 13 shows that comparison of feeding in Rotavirus positive cases in different study. In present study, maximum cases (87.71%) were found in children who take top feed and minimum cases (12.29%) were found in children on exclusive breastfeeding which was similar to study B M John et al., (2014) in which maximum cases (85.72%) were found in children who take top feed and minimum cases 14.28% found in children on exclusive breastfeeding.

CONCLUSION:
Rotavirus infection is the most common cause of acute diarrhoea in infants and young children and it may cause some to severe dehydration in acute diarrhoea in an unvaccinated child. The peak incidence of rotavirus was noted in winter season. Study reveal exclusive breastfeed given protection against rotavirus infection due to maternal IgA rotavirus antibodies. Children with acute diarrhoea should be monitored for the development of severe dehydration. Introduction of supplementary feed increase the chance of infection unless proper hygienic measure such as a proper sterilization of feeding bottles/bowls, washing hands before preparing/feeding food strictly followed. There were few limitation as this study is hospital based, the prevalence of rotavirus might have been different from the actual prevalence in community.

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