INTRODUCTION
Rotavirus is the leading cause of severe acute gastroenteritis in infants and young children and is found in all countries, and almost every child in the world will suffer at least once by its infection by the time they are three years old. An estimated 60,000 children worldwide die each year from rotavirus gastroenteritis, 80% of whom leave in developing countries. (Parashar et al., 2006; Niddowson et al., 2009).

Rotavirus is one of 9 genera amongst the family Reoviridae. It is non enveloped, 75 nm in diameter with a triple layered icosahedral protein capsid with 11 segments of double-stranded (ds) ribonucleic acid which code for six structural and six non-structural proteins (Fisher et al., 2004). The rotavirus genus is divided into serological groups (A to E) based on the reactivity of the middle capsid protein VP 6. Most rotavirus strains infecting humans belong to group A. Group B through G have been associated with human disease less commonly and are variably called pararotavirus, atypical rotavirus, rotavirus-like rotavirus and adult diarrhea rotavirus (Steele 1999; Pang et al., 2009).

Diagnosis has been performed using electron microscopy, which is still occasionally used in centers where it is available. However routine diagnosis is now performed by antigen detection from faeces using commercially available simple, rapid immunochromatographic dipstick style kits which have superseded the earlier latex agglutination and enzyme immunoassays (Sherlock et al., 1989). Reverse transcription polymerase chain reaction (RT-PCR) of faeces is available in some reference and research centers for diagnosis and is particularly useful for identification of outbreaks due to serogroups other than group A (Pang et al., 2004).

The currently licensed rotavirus vaccines have undergone some of the largest and most stringent testing in clinical trials ever seen for any vaccine. This has in part been because of the concerns regarding the previous vaccine called Rotashield®, licensed in the United States in 1998/99. Approximately one million children were vaccinated over a 9 month period of whom about 100 developed a type of bowel obstruction called intussusception resulting in withdrawal of Rotashield® from U.S.A. market (Dennehy, 2008).
In 2006, two new vaccines against rotavirus A infection were shown to be safe and effective in children. Rotavirus vaccines are licensed in more than 100 countries, but only 17 countries have introduced routine rotavirus vaccination (Widdowson et al., 2000). Indian vaccine makers are also in the process of developing live rotavirus vaccines. For instance, Shantha Biotechnics has been developing a multivalent rotavirus vaccine in collaboration with PATH, an international NGO.

The antigenic complexity and difficulty of cultivating rotaviruses isolated from humans has hampered serological characterization of human virus strains and seroepidemiologic surveys of rotavirus outbreaks. Molecular techniques such as analysis of the electrophoretic mobility of the 11 double-stranded RNA segments of rotaviruses by polyacrylamide gel electrophoresis (PAGE) are now most commonly used for epidemiological studies (Estes et al., 1984). It has subsequently been widely employed to determine the electropherotypes of human rotavirus strains circulating in different geographical areas often providing important epidemiological information (Lourenco et al., 1981, Rodger et al., 1981, Steele et al., 1987, Ushijima et al., 1984). Although electropherotype alone cannot be used to identify the serotype of a virus isolate, the detection of a new electropherotype may be used to predict the circulation of a different serotype that could play an important role in subsequent outbreaks. Such information is necessary for prevention of rotavirus infection and for the development of efficient vaccine. Therefore in this study an attempt has been made to investigate different rotavirus electropherotypes circulating in the population of Akola District. The study also aimed to carry out microscopic examinations of stools and detect the levels of protein and sugar in stools of diarrheic patients.

MATERIAL AND METHOD

Patients and study design – The present study was conducted in the Department of Biochemistry, Shri Shivaji College of Arts, Commerce and Science, Akola. A total of 106 patients (children) aged from 5 months to 30 months suffering from diarrhea admitted in various pediatric hospitals of Akola district were enrolled for study.

Kits & Chemicals: Rotavirus latex test kit was procured from Plasmatec Laboratory Products Ltd., (UK). Tris buffer, Acrylamide and Glycine were obtained from Himedia, India. All other chemicals and reagents used were of analytical grade.

Electropherotype Study: Stool specimens were obtained during the patients hospital stay, preferably within 24 hours of admission. Stool specimens were then tested for presence of rotavirus using Plasmatec Rotavirus Latex test kit. The Plasmatec test reagent is composed of latex particles sensitized against a pool of different Rotavirus isolates, both human and animal, allowing detection of antigen by slide agglutination. For study of rotavirus electropherotypes, stool specimens tested positive for rotavirus were used. The technique of PAGE based on the method of Herring et al (Herring et al., 1982) & Rodger and Holmes (Rodger et al., 1979) was employed.

Double stranded genome RNA of rotavirus present in stools was extracted with phenol–chloroform. Faecal material (0.25g) was suspended in 0.5 ml of 0.1 M sodium acetate buffer (pH 5.0) containing 1% (wt/vol) SDS, and an equal volume of 3:2 (vol/vol) phenol chloroform mixture was added. The mixture was shaken vigorously in a 1.5 ml microcentrifuge tube for 1 minute in a vortex mixer and centrifuged in a micro centrifuge at 6000 to 7000 rpm for 2 minutes. The clear upper aqueous layer containing double-stranded RNA was removed and a 40 µl aliquot was then mixed with a 15 µl of sample buffer (0.5 M Tris [pH 6.8], 25% glycerol, 0.2% bromophenol blue). Electrophoresis was done in slab polyacrylamide gel using Laemmli discontinuous system with SDS omitted from all buffers (David 2007). 10% polyacrylamide separating gel and 3% stacking gel was used 50 µl of each RNA preparation was carefully loaded into each well. Known Rotavirus-negative sample was loaded in one of the wells which served as a control. Electrophoresis was carried out at room temperature for 9 hours at a constant current of 100mA per slab gel. The separated double-stranded RNA in the slab gels were visualized by silver staining (Merril et al 1986).

Stool microscopy- Slides were prepared for microscopic examination of stools as per the standard procedures (WHO, 1980). Microscopic examination of all the stool samples was carried out. For examination of saline preparation x10 and x40 objective were used and for examination of iodine preparation x40 objective was used.

Detection of protein and sugar levels in the stools: Levels of protein and sugar in stools of rotavirus positive as well as rotavirus negative samples were detected using “Uristix Strips”. Uristix strips were dipped in the stool samples which were highly watery. Level of protein and sugar in the stool was indicated by change in colors of indicators present on the strip.

Statistical analysis: The results of protein and sugar level in stool were analyzed using unpaired student"
test and p values < 0.05 were considered as statistically significant.

RESULTS AND DISCUSSION
In the present study, stool samples from 106 children were tested for rotavirus of which 54 were found to be positive for Rotavirus, which were studied for rotavirus Electropherotypes. Present investigation showed occurrence of rotavirus strains with S (short pattern) electropherotype less frequently than strains with L (long pattern) electropherotypes [Fig. 1]. Electropherotype with faster migration of segment 10 & 11 is designated as L electropherotype as opposed to the short pattern in which segments 10 & 11 migrate comparatively slow. PAGE of rotavirus RNA from 54 patients studied, revealed 54 distinct electropherotypes, 7 with short, and 47 with long RNA profiles.

Rotavirus-negative samples showed absence of bands. None of the RNA migration pattern of rotaviruses showed presence of extra RNA bands (i.e. more than 11) showing absence of mixed infections with different rotaviruses. In this study atypical rotaviruses were not identified. These viruses also have 11 segments of ds RNA, as typical rotaviruses have but these RNA segments display different patterns when analyzed by means of PAGE (Sorrentino et al., 1986). All the rotavirus positive stool samples when examined microscopically were negative for parasites. Therefore rotavirus was the unique pathogen in positive faecal samples.

Results of detection of protein and sugar levels in the stools of rotavirus and non rotavirus diarrhea patients are shown in table 1 & 2. An overall significant rise in the levels of protein and sugar in stools of rotavirus group was observed over that of nonrotavirus group. The rise in protein level was found to be nonsignificant in case of males of rotavirus group compared to males of nonrotavirus group.

Table 1 : Levels of protein in stools of children suffering from Rotavirus diarrhoea and non Rotavirus diarrhoea.

<table>
<thead>
<tr>
<th>Group</th>
<th>Protein level (mg/100ml)</th>
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<tbody>
<tr>
<td></td>
<td>Rotavirus</td>
</tr>
<tr>
<td>Overall</td>
<td>7.6* ± 1.16</td>
</tr>
<tr>
<td>Males</td>
<td>7.419 ± 1.603</td>
</tr>
<tr>
<td>Females</td>
<td>7.89* ± 1.635</td>
</tr>
</tbody>
</table>

Table 2 : Levels of sugar in stools of children suffering from Rotavirus diarrhoea and non Rotavirus diarrhoea.

<table>
<thead>
<tr>
<th>Group</th>
<th>Sugar level (mg/100ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rotavirus</td>
</tr>
<tr>
<td>Overall</td>
<td>364* ± 66.23</td>
</tr>
<tr>
<td>Males</td>
<td>385.4 ± 92.86</td>
</tr>
<tr>
<td>Females</td>
<td>328.95* ± 88.53</td>
</tr>
</tbody>
</table>

However the rise in protein level in stools of females of rotavirus group was found to be significant over females of non rotavirus group. Rise in sugar level was found to be nonsignificant in case of males of rotavirus group compared to males of nonrotavirus group. However females of rotavirus group showed a significant rise in sugar level compared to females of nonrotavirus group.

It is essential to understand the nature of rotaviruses circulating in different parts of India before currently available vaccines are tried in India. In this context we report the results of different rotavirus electropherotypes circulating in population of Akola district. Rotavirus causes outbreaks every year during the cooler months. Hence the study was undertaken from the month of October 2009 to February 2010 which is the peak period of infection. Approximately one half of the patients were found to be infected with rotavirus this
study has shown that most of the children hospitalized for diarrhea were under 2 years age. Present investigation showed occurrence of rotavirus strains with L electropherotypes more frequently than strain with S electropherotype. Generally all human rotaviruses having short or super short RNA electropherotype exhibit subgroup I specificity and those having long RNA electropherotype pattern have subgroup II specificity. Group A rotaviruses are classified according to 3 antigenic specificities, such as subgroup, G serotype, and P serotype. Subgroup I & II of human rotaviruses are identified by distinctive epitopes on VP6 using subgroup specific monoclonal antibodies(Kapikian et. al., 1990; Caprete, 2007).

In this study a higher incidence of subgroup II strain was found compared to sub group I strain. The subgroup II strain was 6 times more prevalent than subgroup I strain. In other parts of India too, subgroup II has been reported in higher frequency compared to subgroup I. In this study atypical rotaviruses and mixed infections with different rotaviruses were not identified. Mixed infections by different rotavirus strains are responsible for progressive alteration of genome within a community through genomic reassortment in vivo. A high human population density may facilitate transmission of rotaviruses providing ample opportunities of mixed infections resulting in antigenic diversity of rotavirus strains.

Antigenic diversity has important implications for diagnosis epidemiology and vaccine strategies. However such mixed infections were found to be absent in the present study. All the positive samples were negative for parasites indicating that rotavirus was a unique pathogen in positive faecal samples. Detection of levels of protein and sugar in stools of children suffering form diarrhea indicated the clinical severity of rotavirus diarrhea compared to non rotavirus diarrhea. This is evident from the fact that rotavirus infects enterocytes of villi of small intestine, leading to structural and functional changes of epithelium. Malabsorption that occurs secondary to destruction of enterocytes causes a large amount of proteins to be excreted in stools resulting in increase in levels of protein in stools of children infected with rotavirus diarrhea. The severity of disease was found to be more in females compared to males.

An increase in sugar levels in stools of rotavirus infected children may be due to secondary lactase deficiency which results from injury to small intestine. Lactose intolerance is caused by deficiency of enzyme lactase, which is produced by cells lining the small intestine (NIDDK, 2009). The rotavirus non structural protein NSP4 which is an enterotoxin, causes the enterocytes to become permeable and damaged healthy enterocytes secrete lactase into small intestine and milk intolerance caused by lactase deficiency is a particular symptom of rotavirus infection(Jourdan et al., 1998; Arya, 1984).

Consequently large amount of undigested lactose is excreted in stools. Glucose may also be present in stools as a result of undigested lactose. Thus showing an increase in sugar levels in stools of rotavirus infected children.

The findings of present study thus showed prevalence of subgroup II strain of rotavirus and absence of atypical rotavirus and mixed infections in Akola District. The study also showed rotavirus as a unique pathogen in all positive cases of infection. The clinical severity of rotavirus infection in comparison to non rotavirus infection was evident from the level of protein and sugar in the stools of children suffering from diarrhea. An increase in protein levels (7.6mg/100ml) in stools of rotavirus infected patients as against non rotavirus patients (4.78mg/100ml), as well as increase in sugar level (364mg/100ml) in stools of rotavirus infected patients as against non rotavirus patients (222.8 mg/100ml) indicated the clinical severity of rotavirus infection indicating the need of clinical treatment and control of rotavirus infections.

REFERENCES


Kapikian AZ, Chanock RM. 1990. Rotavirus. In:Fields BN,


Figure 1: Rotavirus Electropherotypes showing RNA migration patterns from 54 patients. Lane 1 to 6: Electropherotypes from Rotavirus diarrhoea patients. Lane 7: Known Rotavirus negative sample.