BIOCHEMICAL STUDIES IN SERUM OF CHILDREN SUFFERING FROM ROTAVIRUS DIARRHOEA

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ABSTRACT

Rotaviruses are the commonest cause of diarrhoea in infants and children world wide, and account for about half the cases for children hospitalized for diarrhoea. The study was undertaken to determine the biochemical changes occurring in serum of diarrhoea affected children to find out changes in rotavirus and non rotavirus diarrhoea. The study consisted of hundred patients (children) with age group from 5 months to 30 months of age both males and females admitted to various pediatric hospitals of Akola district. Blood samples and stool samples were collected from pediatric hospitals. Serum was separated from blood samples which were used for the study of biochemical parameters and for SDS PAGE. Stool samples were used for detection of rotavirus diarrhoea patients. In the biochemical parameters, significant changes were observed in serum levels of total proteins, albumin, SGOT, Na and K of rotavirus as well as non rotavirus diarrhoea patients. However serum SDS PAGE did not reveal any change in band pattern of both rotavirus as well as non rotavirus diarrhoea patients as compared to normal healthy control group. Except SGOT, no significant changes were observed in serum of rotavirus compared to non rotavirus diarrhoea patients. Hence SGOT may be used as a marker to diffrentiate between rotavirus and non rotavirus diarrhoea.

Keywords- Potassium, rotavirus, serum, SDS-PAGE, SGOT, sodium, total protein.

INTRODUCTION

Rotavirus is the most common cause of severe diarrhoea in young children worldwide (Parashar *et. al* 2006). Rotavirus is estimated to cause about 40 percent of all hospital admissions due to diarrhoea among children under 5 years of age worldwide-leading to some 100 million episodes of acute diarrhoea each year that result in 350,000 to 600,000 child deaths (UNICEF/WHO, 2009).

Rotavirus is a genus of double–standard RNA virus in the family Reoviridae. There are five species of this virus referred to as A, B, C, D, and E (ICTV virus taxonomy: 2009). Rotavirus A, the most common causes more than 90% of infections in humans. By the age five, nearly every child in the world has been infected with rotavirus at least once (Velazquez *et al.,* 1996).

An Australian researcher Professor Ruth Bishop and colleagues originally described rotaviruses as the cause of infant gastroenteritis in 1973 (Bishop *et al.,* 1973).Rotavirus gastroenteritis is a mild to severe disease characterized by vomiting, watery diarrhea and low grade fever (Hochwald *et al.,* 1999). Rotavirus is transmitted by the faecal–oral route, via contact with contaminated hands, surfaces and objects (Butz AM *et al.,* 1993) and possibly by respiratory route (Dennehy PH, 2008).Sanitary measures adequate for eliminating bacteria and parasites seem to be ineffective in control of rotavirus, as the incidence of rotavirus infection in countries with high and low standards is similar (Dennnehy, 2000).

Specific diagnosis of infection of rotavirus A is made by finding the virus in the child's stool by enzyme immunoassay. There are several licensed test kits in the market which are sensitive, specific and detect all serotypes of rotavirus A (Smith TF *et al.,* 1993, Beards GM *et al.,* 1984). Other methods, electron microscopy and polyacrylamide gel electrophoresis are used in research laboratories (Beards GM, 1988). Reverse transcription-polymarase chain reaction (RT-PCR) can detect and identify all species and serotypes of human rotavirus (Fischer *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 1 million children were vaccinated over a 9 month period of which about 100 developed a type of bowel obstruction called intussusception resulting in withdrawal of Rotashield ® from the USA market (Ruiz-Palacios *et al.,* 2006).

The present study was conducted to investigate the changes occurring in biochemical parameters in serum of rotavirus and non rotavirus diarrhoea patients. Quantification and correlation of these factors in children with diarrhoea is

important for determining the mechanisms by which disease occurs and spreads throughout a population (Kristen *et al.,* 2005; Martin *et. al.,* 1999; Strand *et al.,* 2004). Such studies may lead to practical approaches for prevention, diagnosis and treatment of diarrhoea patients.

MATERIALS AND METHODS

Study design

The present study was conducted at Biochemistry Department of Shri Shivaji College of Akola (M.S.). The study included 100 patients (children) of both genders with age group from 5 month to 30 months suffering from diarrhoea admitted in various pediatric hospitals of Akola district. The study comprised of two groups. Group I consisted of patients suffering from rotavirus diarrhoea and Group II of non rotavirus diarrhoea. Each of the two groups was further divided into two sub groups according to age- the lower age group consisting of patients within 5 to 12 months of age and the higher age group consisting of patients within 13 to 30 months of age. In addition to study group there was control group consisting of 40 healthy age matched subjects.

Sample collection

Blood and stool samples of study group were collected from children's hospitals of Akola district. Blood samples were collected in plain vials at the time of admission of patient. The serum was separated by centrifugation at 5000 rpm.

Stools were collected preferably within 24 hours of the patients hospital stay, for detection of rotavirus diarrhoea patients.

Biochemical methods and kits used

Stool specimens were screened for presence of rotavirus using rotavirus latex test kit which was procured from Plasmatech Lab Products Ltd. (U. K.). Serum separated from blood samples was utilized for estimation of biochemical parameters. The parameters like serum albumin, serum total proteins, serum globulins serum GOT, serum GPT, serum alkaline phosphatase and serum CRP were processed on Robonic make Prietest Touch Biochemistry auto analyzer. For analysis of serum electrolytes (Na+, K+ and Ca2+), electrolyte analyzer of Carelyte make was used.

Serum samples obtained from rotavirus as well as nonrotavirus patients were then subjected to SDS PAGE for which standard Laemmli method containing 10% running gel and 5% stacking gel was used. Serum sample equivalent to 70 µgs of total protein was loaded in each well. Sample was prepared by mixing a quantity of serum sample containing 70 µgs of total protein with 10 µl of distilled water and 10 µl of SDS sample buffer and incubated in boiling water bath for 15 minutes. Electrophoresis was carried out at 50mA and then 250 mA for approximately 3 hours. Medium range molecular weight markers (Genei) were used as standards. After completion of electrophoresis gel were developed using commasie brilliant blue. The standard test used for investigation of biochemical parameters were: Serum albumin was estimated by bromocresol green end point assay method (Webster, 1977), serum total protein was estimated by modified biuret end point assay method (Vatzidis H, 1977), SGPT by IFCC method, kinetic (Bradley *et al.,* 1972), SGOT by IFCC method kinetic (Tietz N *et al*., 1986), Alkaline phosphatase by P Nitrophenyl Phosphate method (Wilkinson *et al.,* 1969), Quantitative estimation of serum CRP done by Latex Turbidometry method (lars – Olof Handson *et al.,* 1997). Serum electrolytes Na+, K+, Ca2+ were estimated on "Carelyte make" electrolyte analyzer (France).

STATISTICAL ANALYSIS

The results have been expressed as $\pm$ SD. Students't' test was employed for all statistical comparisons. Any value $p < 0.05$ was regarded as significant and $p < 0.01$ as highly significant.

RESULTS AND DISCUSSION

Serum biochemical constituents of normal as well as patients with rotavirus diarrhea and non rotavirus diarrhea are given in table 1 &2. A significant decrease in serum total protein was observed in both age groups of rotavirus as well as non rotavirus diarrhoea patients compared to normal healthy control group. However in lower age group the decrease was found to be slightly more in non rotavirus diarrhoea patients (6.503 $\pm$ 0.104 g/dL) compared to rotavirus diarrhoea (6.560 $\pm$ 0.110 g/dL). On the contrary the decrease was found to be more in case of rotavirus diarrhoea patients of higher age group (6.589 $\pm$ 0.082g/dL) than the non rotavirus diarrhoea patients (6.640 $\pm$ 0.114g/dL). Similarly serum albumin level was significantly lower in both rotavirus as well as non rotavirus diarrhoea patients compared to normal. But the decrease was slightly more in rotavirus diarrhoea patients of lower age group (3.638 $\pm$ 0.076g/dL) in comparison to non rotavirus diarrhoea (3.670 $\pm$ 0.052g/dL). But in higher age group the decrease was more in case of non rotavirus (3.528 $\pm$ 0.065g/dL) compared to rotavirus diarrhoea patients (3.611 $\pm$ 0.060g/dL).

Table 1: Serum Biochemical profiles of normal and diarrhea patients (Age group 5-12 months).

Parameter	Control	Rotavirus positive	Rotavirus negative
Sex	1.5 ± 1.668	1.74 ± 0.094#	1.656 ± 0.085#
Age	10.7 ± 0.0448	10 ± 0.387#	10.06 ± 0.338#
Total proteins (g/dL)	7.121 ± 0.060	6.560 ± 0.110**	6.503 ± 0.104**
Albumin (g/dL)	4.028 ± 0.054	3.638 ± 0.076**	3.670 ± 0.052**
Globulin (g/dL)	3.093 ± 0.080	2.921 ± 0.102#	2.857 ± 0.106#
SGOT (IU/L)	34.71 ± 1.166	48.17 ± 2.754**	45.64 ± 2.283*
SGPT (IU/L)	20.11 ± 1.593	20.77 ± 1.797#	20.38 ± 2.268#
Alk. Phosphatase (IU/L)	197.6 ± 9.996	188.1 ± 11.17#	212.65 ± 18.88#
CRP (mg/L)	0.504 ± 0.063	4.748 ± 2.15#	5.204 ± 2.70#
Sodium (mmol/L)	140.4 ± 0.855	130.24 ± 0.867**	133.46 ± 0.650**
Potassium (mmol/L)	7.37 ± 0.075	6.29 ± 0.427#	6.091 ± 0.182**
Calcium (mmol/L)	11.06 ± 0.267	10.94 ± 0.275#	11.42 ± 0.300#

Values are expressed as mean ± SE, # Non significant,
*P<0.05 Significant at 5% level of significance,
**P<0.01 Significant at 1% level of significance.

Table2: Serum Biochemical profiles of normal and diarrhea patients (Age group 13-30 months).

Parameter	Control	Rotavirus positive	Rotavirus negative
Sex	1.5 ± 0.167	1.72 ± 0.084#	1.78 ± 0.101#
Age	20.6 ± 1.656	19.45 ± 0.073#	17.28 ± 1.060#
Total proteins (g/dL)	7.218 ± 0.115	6.589 ±0.082**	6.648 ± 0.114**
Albumin (g/dL)	4.099 ± 0.054	3.611 ± 0.060**	3.528 ± 0.065**
Globulin (g/dL)	3.121 ± 0.078	2.978 ± 0.081#	3.142 ± 0.086#
SGOT (IU/L)	30.8 ± 1.192	45.62 ± 1.770**	41.89 ± 4.203#
SGPT (IU/L)	19.69 ± 2.282	19.74 ± 1.338#	24.41 ± 4.517#
Alk. Phosphatase (IU/L)	235.05 ± 17.261	235.54 ± 20.656#	206.38 ± 19.54#
CRP (mg/L)	0.570 ± 0.069	7.277 ± 4.021#	5.72 ± 2.154#
Sodium (mmol/L)	140.39 ± 0.708	133.53 ± 0.750**	133.29± 0.618**
Potassium (mmol/L)	7.22 ± 0.163	5.534 ± 0.174 **	5.600 ± 0.278**
Calcium (mmol/L)	11.03 ± 0.113	11.38 ± 0.182#	10.96 ± 0.370#

Values are expressed as mean ± SE, # Non significant,
*P<0.05 Significant at 5% level of significance,
**P<0.01 Significant at 1% level of significance.

There was no significant change in serum globulin levels in rotavirus diarrhoea and non rotavirus diarrhoea patients compared to normal healthy control group. The serum GOT levels however were significantly elevated in rotavirus diarrhoea of both age groups (48.17 ± 2.745 & 45.62 ± 1.770 IU/L) as compared to normal (34.71 ± 1.166 & 30.8 ± 1.192 IU/L). However in non rotavirus diarrhea patients a significant rise in serum GOT level (45.64 ± 2.283 IU/L) but comparatively lower than rotavirus diarrhoea cases (48.17 ± 2.754 IU/L) was observed in lower age groups but there was no significant change in serum GOT level of non rotavirus group of higher age (41.89 ± 4.203 IU/L) compared to rotavirus group (45.62 ± 1.770 IU/L).

There was no significant change in serum GPT, Alkaline phosphatase and CRP level of both rotavirus as well as non rotavirus diarrhea patients compared to normal healthy patients.

Serum sodium and potassium concentrations decreased significantly in both rotavirus as well as non rotavirus diarrhoea children. However decrease of serum sodium levels was slightly more in rotavirus diarrhoea of lower age group (130.24 ± 0.867mmol/L) compared to non rotavirus diarrhoea of the same age group (133.46 ± 0.650 mmol/L). However the decrease in concentration of serum sodium levels was found to be almost the same in both rotavirus (133.53 ± 0.750 mmol/L) and non rotavirus (133.29 ± 0.618 mmol/L) of higher age group. The serum potassium levels were considerably decreased in non rotavirus diarrhoea (6.091 ± 0.182 mmol/L) compared to rotavirus diarrhoea (6.29 ± 0.427 mmol/L) of lower age group while a significant decrease in serum potassium concentration was observed in rotavirus diarrhoea patients (5.534 ± 0.174 mmol/L) than non rotavirus diarrhoea of higher age group (5.600 ± 0.278 mmol/L). However serum calcium levels did not change significantly in both diarrhoea cases compared to normal. Serum SDS PAGE revealed no change in band pattern of serum of rotavirus diarrhoea patients and non rotavirus diarrhoea patients with respect to the normal healthy control (Fig. 1).

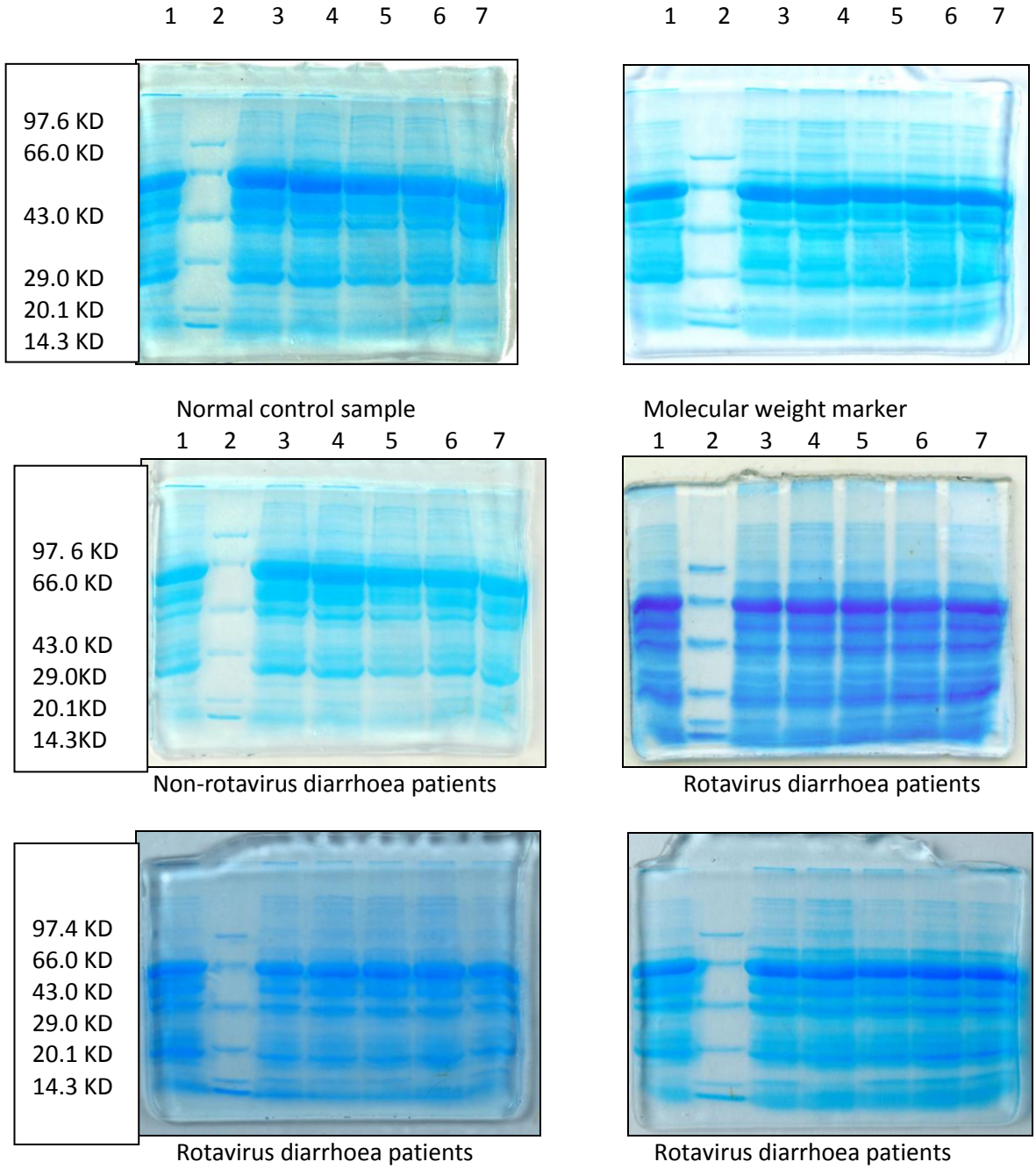

Figure 1: Comassie Brilliant Blue Stained SDS-PAGE pattern of serum samples of rotavirus and non rotavirus diarrhea patients.

In the present study the decrease in total proteins in children with diarrhoea may be related to its decreased synthesis by the liver or its increased rate of catabolism due to diarrhoea. Hypoproteinemia in children with diarrhoea may be due to enteric protein loss (Weizman et.al 2002). Fall in serum albumin level in children with diarrhoea may be due to dilution by an excess of protein free fluids (ORS) given during treatment and also due to protein loosing enteropathy (protein is lost from the GI tract during diarrhoea). Serum GPT & Serum GOT determine hepatocellular injury and may help in monitoring the status of liver during diarrheal infection. Serum GOT was significantly higher in rotavirus diarrhoea patients indicating damage of cells in liver, heart, kidney, pancrease and muscles. Hyponatremia may be due to sodium depletion during diarrhoea. The pathogenesis of Hyponatremia is due to a combination of serum and water loss and water retention to compensate the volume depletion. Hypokalemia noted in diarrhoea could due to increased potassium loss through vomiting and diarrhoea. However serum calcium levels remained unaffected. The present study indicated a significant loss of proteins in non rotavirus diarrhoea cases particularly of lower age group however as far as albumin levels were concerned, these were much affected in non rotavirus group of higher age.

CONCLUSION

As such no significant change has been observed in rotavirus diarrhoea as compared to non rotavirus diarrhoea in biochemical parameters and serum electrophoretic pattern except that of SGOT. Hence SGOT may be used as a marker to differentiate between rotavirus diarrhoea and non rotavirus diarrhoea.

LITERATURE CITED

Beards GM, Campbell AD, Cottrell NR, Peiris JS, Rees N, Sanders RC, Shirley JA, Wood HC, Hewett TH, 1984. Enzyme -linked immunosorbent assay based on polyclonal and mono clonal antibodies for rotavirus detection. *J. Clin. Microbiol.,* **19**(2):248-54.

Beards GM, 1988. Laboratory diagnosis of viral gastroenteritis. *Eur. J. Clin, Microbiol. Infect. Dis.,* **7**(1): 11-3.

Bishop RS, Devidson GP, Holmes IH, Ruck BJ, 1973. Virus particles in epithelial cells of duodenal mucosa from children with acute non bacterial gastroenteritis. *Lancet* **2**(7841): 1281.

Butz AM, Fosarelli P, Dick J, Cusack T, Yolken R, 1993. Prevalence of rotavirus on high risk fomites in day-care facilities. *Pediatrics* **92**(2):202-205.

Dennnehy PH, 2000. Transmission of rotavirus and other enteric pathogens in the home. *Pediatr. Infect. Dis. J.* **19**(10):103-105.

Dennnehy PH, 2008. Rotavirus vaccines: an overview. *Clin. Microbiol. Rev.* **21**(1): 198-208.

Fisher TK, Gentsch JR .2004. Rotavirus typing methods and algorithms. *Rev. Med. Virol.* **14** (2):71 – 82.

Green baum LA. 2004 . *Pathophysiology of body fluids and fluid therapy*. In: Behrman RE, Kliegman RM, Jenson HB (eds). *Nelson Text Book of Pediatrics Saunders 17th Edition.* 199-202.

Hochwald C, Kivela L, 1999. Rotavirus vaccine, live, oral, tetravalent(Rotashield). *Pediatr. Nurs*. **25**(2):203-207.

Kristen AN, Verge FV, O'Meare CF, Walker JL. 2005. High antidiuretic hormone levels and hyponatremia in children with gastsroenteritis. *Pediatrics* **116** (6):1402-1407.

Lars-Olof Hanson et. al. 1997. *Current Opinion in Infect Diseases.* **10**:196-201.

Parasher U, Gibson C, Bresee J, Glass R.2006.Rotavirus and severe childhood diarrhea. *Emerging Infectious Diseases*:**12:** 2

Ruiz Palacios GM, Perez-Schael I, Velazquez FR, 2006.Safety and efficacy of an attenuated vaccine against severe rotavirus gastroenteritis. *New England Journal of Medicine*. **354**(1):11-22.

Smith TF, Wold AD, Espy MJ, Marshall WF, 1993. New developments in the diagnosis of viral diseases. *Infect. Dis. Clin. North. Am*. **7**(2): 183-201.

UNICEF/WHO.2009. *Diarrhea: why children are still dying and what can be done.* Retrieved 23 May 2010.

Velazquez FR, Matson D O, Calva JJ, Guerrero L, Morrow AL, Carter – Campbell S, Glass RI, Estes MK, Pickering LK, Ruiz-Palacios GM, 1996. Rotavirus infections in infants as protection against subsequent infections. *N. Engl. J. Med*., **335** (14):1022-8.

Weitzman Z, Binsztok M, Fraser D, Deckelbaum RJ, Granot E, 2002. Intestinalprotein loss in acute diarrhea of early childhood.*J.Clin.Gastroenterol*. **34**(4):427-429.