COMPARISON OF LATEX AGGLUTINATION AND POLYACRYLAMIDE GEL ELECTROPHORESIS WITH ENZYME LINKED IMMUNOSORBENT ASSAY FOR DETECTING HUMAN ROTAVIRUS IN STOOL SPECIMENS

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ABSTRACT

One hundred and forty five stool samples from children below 2 years of age, hospitalized with diarrhea were tested for rotavirus antigen by enzyme linked immunosorbent assay (ELISA), latex agglutination test using commercially available kit Rotastat (Ranbaxy Diagnostic, India) and by polyacrylamide gel electrophoresis. Twenty eight samples were positive for the virus antigen by all the three assay systems. The sensitivity of latex agglutination (LA) and polyacrylamide gel electrophoresis (PAGE) was 91.4% (32/35) and 80% (28/35), respectively; the corresponding specificity was 98.18% (108/110) and 100% (110/110), respectiveiy. Latex agglutination was the least complex, required the least amount of apparatus and provided a result within a short time. It showed a high specificity and a reasonable amount of sensitivity and the results correlated well with ELISA and PAGE.

Key words: *Rotavirus, Enzyme linked immunosorbent assay, Polyacrylamide gel electrophoresis, Latex agglutination.*

Human rotavirus (HRV) are an important cause of diarrhea in infants and young children in developed as well as developing countries. Reports from India show the incidence of rotavirus infection to be upto 70%(1-3).

Rotaviruses are readily detectable in stool by various techniques(3). These range from assays like electron microscopy and polyacrylamide gel electrophoresis of viral nucleic acid and antibody based assays such as enzyme linked immunosorbent assay, immunofluorescence, radioimmunoassay and solid phase aggregation of coated erythrocytes (SPACE).

Latex agglutination test (LA) has been reported to be a simple, sensitive and rapid test(4). A rapid diagnosis of rotavirus gastroenteritis is essential since it obviates the unnecessary use of antibiotic therapy. Moreover, clinical and epidemiological information can be gathered. In view of this and because of the availability of the Rotastat kits (Ranbaxy, India), this technique has been evaluated with other standard techniques namely enzyme linked immunosorbent assay (ELISA) and polyacrylamide gel electrophoresis (PAGE) for the detection of rotavirus antigen from stool specimens.

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Material and Methods

The study was conducted from August, 1987 to March, 1988. One hundred and forty five children suffering from acute gastroenteritis below 2 years age admitted to the Pediatric Ward of Lok Nayak Jai Prakash Hospital were included in the study. Stool samples were collected from each of the above children on the day of admission.

A 10% suspension of each sample was prepared in phosphate buffered saline (PBS) and was homogenized thoroughly with glass beads on a vortex mixer. The homogenized suspension was clarified by centrifugation at 3000 rpm for 10 minutes. Supernatent was separated and kept at –20°C. This was used for detection of rotavirus by ELISA, PAGE and LA.

ELISA: Direct double antibody sandwich enzyme linked immunosorbent assay was carried out in accordance with the method described by Grauballe *et al.*(5). Reagents used for ELISA were procured from Dakopatts Denmark.

PAGE: All the specimens were electropherotyped on polyacrylamide gel by the method of Konno *et al.*(6). Discontinuous electrophoresis was carried out as described by Laemmeli(7). Gels were stained by silver staining according to Merril *et al.*(8).

LA: It was performed using the commercial latex agglutination kit (*i.e.,* Rotastat of Ranbaxy Diagnostic, India) with some modification. One drop of the supernatent was mixed with test latex coated with antirotavirus antibody and control latex was added to a second drop of stool supernatent whicl. served as a negative control. The test was done on a black coloured glass slide instead of using the black cards provided with the kit. This was left at room temperature and the reading was taken within five minutes. The test was considered positive for rotavirus if distinct agglutination was observed with test latex but not with control latex.

Results

Rotavirus antigen was detected in 35, 32 and 28 cases of gastroenteritis by ELISA, LA and PAGE, respectively. One hundred and thirty six (93.8%) were either positive or negative with all the three tests used while nine (6.2%) specimens gave contradictory results. Five specimens were positive by ELISA, while two specimens were positive by LA test. A further two specimens were positive by ELISA and LA but not by PAGE (*Table I*).

The comparisons were made on the assumption that the specimens tested with ELISA were genuinely positive or negative.

The sensitivity of LA and PAGE was 91.4% (32/35) and 80% (28/35), respectively. The specificity was 98.18% (108 of 110) for LA test and 100% (110 of 110) for PAGE. The corresponding accuracy was 96.5% (136 of 145), respectively.

Discussion

The purpose of this study was to evaluate the routine use of latex agglutination test and to compare the performance of this with ELISA and PAGE.

ELISA was performed as per the method of Grauballe *et al.* using Dakopatts ELISA kit which has been reported(9) to be as sensitive and specific as the world Health Organization ELISA kit for rotavirus detection. In the present study ELISA was the most sensitive assay system and this finding is in accordance with the obser-

TABLE I— *Comparison of the Results Obtained by Enzyme Linked Immunosorbent-assay (ELISA), Polyacrylamide Gel Electrophoresis (PAGE) and Latex Agglutination Test (LA).*

No. of samples tested	Technique		
	ELISA	PAGE	LA
28	+	+	+
2	+	−	+
2	−	−	+
5	+	−	−
108	−	−	−
Sensitivity		of PAGE $\frac{28}{35}$ (80.0%)	of LA $\frac{32}{35}$ (91.4%)
Specificity		of PAGE $\frac{110}{110}$ (100.0%)	of LA $\frac{108}{110}$ (98.2%)
Accuracy		of PAGE $\frac{136}{145}$ (93.8%)	of LA $\frac{140}{145}$ (96.5%).

vations of others(10) that solid phase immunoassays are more sensitive than LA test and PAGE for the detection of rotavirus antigen.

LA was performed as per the manufacturers instructions. The only variation in the technique was that it was performed on a glass slide instead of cards provided in the Kit since it was easier to take the reading and the result could be best assessed on a flowing drop. Although the sensitivity of the test was inferior to that of ELISA it is sufficiently specific and sensitive to be used reliably specially in acute diarrhea, where a rapid diagnosis is required. It was certainly more sensitive than PAGE. Similar observations have been made by others(10, 11).

PAGE was the least sensitive assay system although it had a high specificity. A large amount (about 1 g) of fecal material was required for extraction of viral RNA by the technique used in this study. The lower sensitivity of PAGE was probably due to inadequate amount of sample in some cases. Moreover, it was a labor intensive technique, required trained personnel and took 2-3 days to perform. The main advantage with PAGE is that the non group A strains can be detected by visualizing the RNA pattern on the gel.

Of the three assay systems, latex agglutination was the least complex, easy to interpret and provided a rapid diagnosis in a short time. It showed a reasonable amount of sensitivity and a high degree of specificity. It is a suitable screening test for rapid diagnosis of rotavirus gastroenteritis. The only limitation is the high cost (Rs. 800 for 20 tests). So it should be used selectively for the cases with severe diarrhea that requires rapid and special treatment.

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