

BRIEF REPORTS

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## **Rotavirus Infection in Children with Acute Diarrhea as Detected by Latex Agglutination, ELISA and Polyacrylamide Gel Electrophoresis**

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*We examined prospectively, stool specimens from 135 children, 0 to 3 years old, referred for fever, abdominal pain, vomiting and/or acute diarrhea. Rotavirus antigens were detected from fecal samples by latex agglutination (LA), ELISA and polyacrylamide gel electrophoresis (PAGE). Rotavirus antigen positivity by Latex, ELISA and PAGE were 15, 55%, 12.59% and 11.85%, respectively. With PAGE test as reference, the sensitivity and specificity of LA and ELISA tests was 93.75%, 94.96% and 100%, 99.16%, respectively, The positivity ratio between 13-24 months group was meaningful with all tests ( $P = 0.042$  for LA;  $P = 0.05$  for ELISA;  $P = 0.031$  for PAGE). ELISA and LA use found to be as sensitive and specific as PAGE in the diagnosis of rotavirus diarrhea.*

**Key words:** *Diarrhea, Gastroenteritis, Rotavirus.*

Gastroenteritis is a leading disease causing death among infants in developing countries(1). In our country, the incidence in rural area is reported to be 28% and increases

to 33-40% in the region of East and Southeastern Anatolia(2). Incidence is related with age, living conditions, cultural attitudes and individual factors. Deaths from gastroenteritis are usually due to dehydration, malnutrition and dysentery(2). The aim of this study was the detection of rotavirus infection among children living in Middle Anatolia region in Turkey by using latex agglutination (LA), enzyme linked immunosorbent assay (ELISA) and polyacrylamide gel electrophoresis (PAGE) and comparison of these diagnostic tests.

### Patients and Methods

We investigated fecal samples of 135 children with acute diarrhea (46.66% female, 53.33% male), aged from 0 to 3 years, presenting to Selcuk University School of Medicine Pediatric Clinics between September 1999 and May 2000 from different parts of Middle Anatolia, Turkey.

Rotavirus antigens in stool specimens were checked by LA, ELISA and PAGE at Kocatepe University School of Medicine, Department of Microbiology laboratories. Stool samples were examined for macroscopic and microscopic evaluation. After microscopy and cultures, stool specimens were stored at  $-20^{\circ}\text{C}$  until examination.

*Latex Agglutination (LA) and ELISA:* Diarlex.ROT A (Orion Diagnostica, Espoo, Finland) and Rota-screen (Mercia Diagnostics, Surrey, UK) ELISA kits were used for detecting rotavirus antigens in stool samples. The kits contain latex particles coated with anti-rotavirus antibodies.

*Polyacrylamide Gel Electrophoresis(PAGE):* This was the gold standard for the study. Stool specimens were diluted with 1% sodium dodecyl sulphate (SDS) solution and 0.1 M sodium acetate. Specimens were mixed with 0.6 mL acid phenol (pH 5.0), 0.4 mL

chloroform(24:1) and izoamilalkol. Electrophoretic migration of rotavirus RNA was examined on acrylamide 10% acrylamide-gel.

*Statistical analysis:* Data were analyzed with SPSS10.0 (SPSS Inc., Chicago, IL). Relations among groups were analyzed with  $\chi^2$  test, and continuous variables (like age, gestational weeks) by Student's *t*-test and where appropriate. A P-value less than 0.05 were considered statistically significant.

### Results

One hundred and thirty five children with diarrhea were studied. Rotavirus in stool specimens was detected in 15.55% by LA, 12.59% by ELISA, 11.85 % by PAGE (Table I) There was no statistical difference in detection of rotavirus incidence among three different methods LA, ELISA and PAGE according to sex (P = 0.434). When antigen positivity in relation with age groups, the positivity ratio between 2 years old group was meaningful with all 3 tests (P 0.04 for LA, P = 0.05 for ELISA; P = 0.03 for PAGE). Table II shows the specificity, sensitivity ratios and negative-positive predictive values of LA and ELISA compared to PAGE.

### Discussion

Rotavirus is responsible for 20-30% patients with diarrhea younger than 5 years, and 30-50% hospitalized patients in Europe(5). In Turkey, rotavirus positivity in childhood diarrhea cases ranges from 16.3-36.1%(6-8). Most commonly used tests in diagnosing rotavirus infections are electron microscopy, LA, ELISA, PAGE, immunofluorescence and molecular tests(3). All of these, LA and PAGE are sensitive while electron microscopy, polymerase chain reaction(PCR) and PAGE have low sensitivity but high specificity(3).

Saravanan, *et al.*(9), detected positivity in

**TABLE I**—*Rotavirus Antigen Detection by Different Methods in Children with Diarrhea.*

| Age          | Total |       | LA |       | ELISA |       | PAGE |       |
|--------------|-------|-------|----|-------|-------|-------|------|-------|
|              | N     | %     | N  | %     | N     | %     | N    | %     |
| 0-12 months  | 28    | 20.74 | 3  | 10.71 | 2     | 7.14  | 2    | 7.14  |
| 13-24 months | 45    | 33.33 | 12 | 26.67 | 10    | 22.22 | 10   | 22.22 |
| 25-36 months | 62    | 45.93 | 6  | 9.68  | 5     | 8.06  | 4    | 6.45  |
| Total        | 135   | 100   | 21 | 15.55 | 17    | 12.59 | 16   | 11.85 |

LA: Latex agglutination; ELISA: Enzyme linked immunosorbent assay; PAGE: Polyacrylamide gel electrophoresis.

**TABLE II**—*Sensitivity, Specificity, Accuracy and Predictive Values (%) for ELISA and LA tests (compared to PAGE).*

|       | Sensitivity | Specificity | Accuracy | Positive Predictive value | Negative Predictive value |
|-------|-------------|-------------|----------|---------------------------|---------------------------|
| ELISA | 100         | 99.16       | 99.26    | 94.12                     | 100                       |
| LA    | 93.75       | 94.96       | 94.81    | 71.43                     | 99.12                     |

LA: Latex agglutination; ELISA: Enzyme linked immunosorbent assay; PAGE: Polyacrylamide gel electrophoresis.

stool specimens as 42 of 211 by ELISA, 40 by modified PAGE, 33 by electron microscopy. They reported that electron microscopy was the least sensitive, ELISA most feasible for cohort screening, and PAGE most specific tests. Buesea, *et al.*(10) compared reverse transcription PCR, ELISA, PAGE and electron microscopy and found positivity rates of 30% for PCR, 29% for ELISA, 26.8% for PAGE and 25.4% for electron microscopy. Steele, *et al.*(11) compared two fast ELISA methods (Testpack, Abbott Laboratories; Chicago and Pinpoint, Cambridge Biomedical, UK) with normal ELISA and LA and confirmed results with EM and PAGE. These authors argued that fast ELISA tests were considerably sensitive (96% and 100%) and could be used for mass screening. Eing, *et al.*(12) compared two different ELISA tests (Ridascreen rotavirus and Pathfinder

rotavirus) in 393 specimens and found both test's sensitivity to be 100% and positive predictive values 93.7% and 57.7%, respectively. Chakravarty, *et al.*(13) investigated incidence of rotavirus in 145 children with gastroenteritis by LA (Rotastat, India), ELISA and PAGE. They detected 5.5% (8/145) positivity by all 3 tests. In this study for comparison of LA, ELISA and PAGE methods, frequencies of rotavirus positivity were found to be 15.55%, 12.59% and 11.85%, respectively. We accepted PAGE test as reference method for our investigation and found sensitivity and specificity of LA and ELISA tests to be 93.75%, 94.96% and 100%, 99.16%, respectively. Ibrahim, *et al.*(14) reported that LA was the most sensitive (96%) but least specific, electron microscopy and PAGE had 100% specificity and 73% and 84% sensitivity, respectively. In another

### Key Messages

- The sensitivity and specificity of ELISA and LA for diagnosis of rotavirus infection, was high when compared to PAGE.
- ELISA and LA may be used as a diagnostic test for detection of rotavirus.

study, three different ELISA (Rotavirus EIA; International Diagnostic Laboratories; Pathfinder Kallestad Laboratories and Rotaclone, Cambridge Bioscience) were compared with PAGE. Of 286 cases 30.8% were rotavirus positive; specificity and sensitivity rates were 91% and 100% for PAGE, 96% and 97% for EIA, 100% and 94% for Pathfinder and 96% and 97% for Rotaclone, respectively(15). Those results were in accordance with our results. Although most sensitive methods for determining rotavirus infections are electron microscopy. PAGE, ELISA and PCR, recently LA test has been reported sensitive and specific as ELISA (3,6,13). Buser, *et al.*(16) detected rotavirus in 27% children with diarrhea by LA (Meritec rotavirus, Meridian Diagnostics, USA) and 2 other rapid tests (Rotastick, Novamed, Combo Rota/Adeno Dipstick, All Diagn, France). They reported mild positivity by LA in three samples.

Dogan and Akgun(17) investigated 148 cases and found positivity 14.2% by LA and 17.5% by ELISA. They reported that infection was more common between 1-2 year age and in the months of January and February. Ozsan, *et al.*(18), screened rotavirus positivity by PAGE in stools of 86 children, aging from 3 to 30 months, between February and June, and reported the incidence as 22.09%. They reported that PAGE was considerably more sensitive than EM and ELISA. Albay, *et al.*(5) reported rotavirus incidence by ELISA to be 21.25% among 0-14 year old children. Turkoglu, *et al.*(19) investigated the virus

incidence by ELISA-PAGE in Istanbul and found 25.4% rotavirus positivity.

The incidence of rotavirus in our region was found to be lower than the other parts of our country. The sensitivity and specificity rates for ELISA and LA were found to be as high as PAGE. These methods may thus be used as a reliable test for diagnosis of rotavirus infection.

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*Contributors:* MA, SY and AS coordinated the study, drafted the paper and will act as guarantor. AD supervised the study. AC and HK did clinical data collection, analyzed the data and drafted the paper.

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