

Comparison of Enzyme Immunoassay, Immunochromatography, and RNA-Polyacrylamide-Gel Electrophoresis for Diagnosis of Rotavirus Infection in Children with Acute Gastroenteritis

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Abstract

Human rotavirus is a major etiologic agent for infantile diarrhea worldwide. It is responsible for up to 3.3 million deaths per year in children in developing countries. Various rapid and sensitive techniques have been developed to readily diagnose rotavirus gastroenteritis. In the present study, we compared the sensitivity and specificity of immunochromatography and RNA-polyacrylamide-gel electrophoresis (PAGE) methods with enzyme immunoassay (EIA) for diagnosis of group A rotavirus infection in 200 stool samples from children younger than 5 years old with acute gastroenteritis. Rotavirus was detected in 57 (28.5%) samples by EIA, 52 (26%) samples by ICG and 52 (26%) samples by RNA-PAGE. There was no significant difference between the three methods ($P=0.8$) nor between EIA and ICG ($P=0.57$) and EIA and RNA-PAGE ($P=0.57$). Furthermore, in comparing these methods with age variables, the present study found that the sensitivity and specificity of ICG and RNA-PAGE compared with EIA were 87.7%, 98.6% and 91.2%, and 100%, respectively ($P>0.05$). Results of the present study demonstrate that the sensitivity and specificity rates for ICG and RNA-PAGE were as high as EIA. It seems that all the three methods are reliable and suitable for detection of group A rotavirus infection in children affected by enteric diseases.

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Keywords • Rotavirus • enzyme immunoassay • immunochromatographic • acute gastroenteritis

Introduction

Human rotavirus (HRV) is major cause of enteric disease in infants and young children. Rotavirus is responsible for up to 60% of all cases of watery diarrhea in children. Early diagnosis is essential for effective treatment.^{1,2} The group A rotavirus can be detected by using different methods including enzyme immunoassay (EIA), electron microscopy (EM), RNA polyacrylamide-gel electrophoresis (RNA-PAGE), immunochromatography (ICG), and reverse transcriptase-polymerase chain reaction (RT-PCR).^{1,3} In the present study, we evaluated the sensitivity and specificity of ICG and RNA-PAGE methods and compared them with EIA as the golden standard technique for diagnosis of rotavirus gastroenteritis in children reside in Tehran, Iran.

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Patients and Methods

A total of 200 stool specimens were collected by simple random sampling from inpatient children younger than 5 years old admitted to Bahrami Pediatric Hospital (in Eastern Tehran), between March 2004 and April 2005. These patients had acute gastroenteritis and showed clinical features including fever, vomiting, dehydration, and watery diarrhea. The samples were transported to virology department of Pasteur Institute of Iran (Tehran), and kept in -20 °C until evaluation. Detection of group A rotavirus was performed by using three procedures. Complete clinical data were obtained from the patients' medical records. Data were analyzed using SPSS software (ver.11.5). The relation among the three methods and age variables with the methods were analyzed by χ^2 test. The sensitivity and specificity of these techniques were analyzed by standard procedures. The patients' fecal samples were examined for group A rotavirus antigen by EIA (IDEIA™, Dako-Denmark). The test utilizes a polyclonal antibody to detect group specific proteins, including the major inner capsid protein (VP6) present in group A rotaviruses. These were performed according to instructions supplied by the manufacturers with the kit. We also used the ICG "Rota-Strip" kit (Coris Bio Concept, Belgium) that consists of a strip sensitized with guinea pig anti-rotavirus polyclonal serum and goat anti-mouse IgG polyserum. The anti-rotavirus conjugate is produced with a mouse monoclonal antibody directed against human rotavirus group A VP6 antigens. We performed ICG test according to the manufacturer's instructions. RNA-PAGE was used for detection of rotavirus genome. The rotavirus double-stranded RNA was extracted from all rotavirus-positive specimens according to previously described methods.^{4,5} Briefly, a 10% emulsion was made in extraction buffer containing sodium dodecyl-sulfate (SDS). The suspension was then mixed with an equal volume of phenol/chloroform,

vortexed, centrifuged, and the top aqueous phase containing RNA was removed. The RNA was precipitated with 2 volumes of ethanol, collected by centrifugation, and resuspended in diethylene pyrocarbonate water. For analysis, 30 μ l of RNA was electrophoresed overnight at room temperature on a 10% polyacrylamide gel (0.5 mm thick) at 70 V. The gels were fixed in ethanol/acetic acid, stained with silver nitrate and photographed. The RNA extracted from strain simian rotavirus (SA-11) was used as positive control.

Results

Of the 200 samples from children with gastroenteritis, group A rotavirus was detected in 57 (28.5%) by EIA, 52 (26%) by ICG, and 52 (26%) by RNA-PAGE (fig. 1 and table 1). There was no significant difference between the three methods (P=0.8) nor was statistical difference between EIA and ICG (P=0.57) or between EIA and RNA-PAGE (P=0.57). Furthermore, comparing these methods with age variables yielded P=0.72, P=0.87, and P=0.75 respectively. Table 2 shows the sensitivity, specificity ratios, and negative and positive predictive values of ICG and RNA-PAGE compared with EIA.

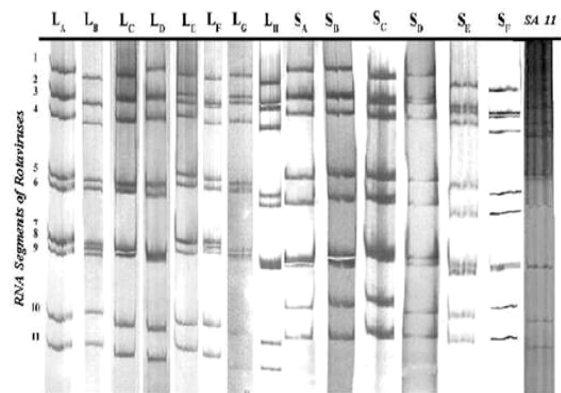


Figure 1: Genomic RNA electrophoresis of representative strains from rotavirus Electrophoretotypes identified in Tehran, between March 2004 and April 2005. Simian Rotavirus Strain (Sa-11) as Control.

Table 1: Rotavirus antigen and RNA genomic detection by different methods in children with acute gastroenteritis.

Age	Total	EIA	ICG	PAGE
	No. of cases	No. (+/-)	No. (+/-)	No. (+/-)
0-11 months	65	19/46	15/50	17/48
12-24 months	78	28/50	25/53	26/52
25-60 months	57	10/47	12/45	9/48
Total	200	57/173 (28.5%)	52/148 (26%)	52/148 (26%)

- EIA: Enzyme immunoassay, PAGE: Polyacrylamide gel electrophoresis, ICG: Immunochromatography

Table 2: Sensitivity, specificity, accuracy, confidence interval, and predictive values (%) for ICG and RNA-PAGE techniques (compared with EIA).

	Sensitivity	Specificity	Accuracy	Confidence interval	Positive predictive value	Negative predictive value
ICG	87.7	98.6	95.5	Sensitivity: 75.7-94.5 Specificity: 94.52-99.75	96.15	95.27
PAGE	91.2	100	97.5	Sensitivity: 79.95-96.72 Specificity: 96.73-100	100	96.6

EIA: Enzyme immunoassay, PAGE: Polyacrylamide gel electrophoresis, ICG: Immunochromatography

Discussion

Human group A rotavirus was diagnosed in 28.4% of the diarrhea episodes in hospitalized children younger than 5 years old with gastroenteritis. The infection peaked in cool seasons in Tehran.⁶ Rapid diagnosis of rotavirus infection in patients admitted to hospital with symptoms of gastroenteritis would yield to more effective treatment including isolation or discharge. This is important because in many cases effective rehydration can be achieved at home and most rotavirus infections are self-limiting. To ensure consistent performance in detection of rotavirus in stool samples, the methods of choice should exert high degree of sensitivity and specificity, high predictive values, and reproducibility.²

In the present study, we compared EIA, ICG, and RNA-PAGE methods for detection of rotavirus infection in fecal samples from hospitalized children with acute gastroenteritis. Rotavirus was detected in the stool specimens by EIA (28.5%), ICG (26%), and RNA-PAGE (26%). Other studies have reported rotavirus detection by EIA in 30-97% of cases,⁶⁻¹⁰ by ICG in 30.3- 68% of cases.^{11,12} and by RNA-PAGE in 84.4-97.8% of cases.^{6,7}

EIA is clearly the most sensitive method for detection of rotaviruses and is ideal for screening large numbers of fecal specimens in a single test.^{3,13} Recently, immunochromatographic test for rotavirus detection has become available. This test facilitates qualitative information of rotavirus infection based on the presence of a rotavirus specific band obtained by immunochromatography. In addition, the ICG test required less handling of the sample, and the results would be available in less time.^{14,15} The RNA-PAGE of the 11 segments of ds-RNA genome of group A rotavirus allows detection and classification of the viruses into two major patterns. Those are the long (L) and the short (S) electrophoretotypes based on the migration profiles of gene segments 10 and 11 on polyacrylamide gel.¹⁶ We applied EIA test as golden standard and found the sensitivity and specificity of ICG and RNA-PAGE tests to be 87.7%, 98.6% and 91.2%, 100%, respectively. Overall, sensitivity and specificity of these methods are more than 85%. The results of the above-mentioned studies do not show significant difference to those obtained in our study.

Conclusion

The present study showed that the sensitivity and specificity rates and positive and negative predictive values of RNA-PAGE are more than ICG, and that of EIA was more than both.

These techniques may be suitable for diagnosis of other enteric viral infections.

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Conflict of Interest: None declared

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