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Dışkı Örneklerinde Rotavirüs ve Adinovirüs Tanısında İki Farklı Serolojik Yöntemin Karşılaştırılması

Comparison of Two Different Serological Methods in The Diagnosis of Rotavirus and Adenovirus in Stool Samples

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ÖZ

Amaç: Tüm dünyada beş yaş altındaki çocuklardaki akut enfeksiyöz ishalin en sık nedeni rotavirüslerdir. Bu çalışma immünkromatografik ve Enzyme-Linked ImmunoSorbent Assay (ELİSA) yöntemlerinin karşılaştırılması, kullanılan yöntemlerin duyarlılık ve özgüllüklerinin belirlenmesi ve hastalığın doğru teşhis edilebilmesi için insanlığa ışık tutmayı amaçlamaktadır.

Yöntem: Toplam 1000 hastanın Rotavirüs hızlı antijen testi çalışılması amacıyla Diyarbakır Çocuk Hastalıkları Hastanesi'ne gönderilen dışkı örnekleri çalışıldı. Örneklerin incelenmesinde tek aşamalı Rotavirüs ve Adenovirüs Birlikte immünokromatografik ve ELISA yöntemleri kullanıldı. Hızlı test sonucu pozitif olan örnekler 1.5 ml'lik mikrosantrifüj tüplerine alındı, ELISA testi için toplanan pozitif antijen numuneleri -80°C'de çalışılacağı zamana kadar saklandı.

Bulgular: Analiz edilen 1000 dışkı örneğinin 345 kız çocuğunun 40' ında (%11.59) ve 655 erkek çocuğunun 60'ında (%9.16) toplam 100'ünde (%10) Rotavirüs antijeni ve 20'sinde (%2) Adenovirüs antijeni saptanmıştır (P>0,05). Viral antijen pozitif olgular en sık kış ayları (Aralık, Ocak, Şubat)'nda görüldü. Verilerin yaş ve cinsiyet yönünden istatistiksel olarak anlamlı bir farkı bulunmamıştır (P <0,05).

Sonuç: Bu çalışmada çocuk hastalarındaki ishal vakalarının onda birinde Rotavirus tespit edilmiş ve kış aylarında da mevsimsel artış gösterdiği ortaya konmuştur.

Anahtar Kelimeler: Rotavirüs, Gastroenterit, ELİSA, İmmünokromatografik, Pediatri.

ABSTRACT

Objective: Rotaviruses are the most common cause of acute infectious diarrhea in children under 5 years of age worldwide. This study aims to compare the immunochromatographic and ELISA methods, determine the sensitivity and specificity of the methods used, and shed light on humanity to diagnose the disease correctly.

Method: Stool samples of 1000 patients were sent to Diyarbakır Pediatric Hospital for rapid antigen testing of Rotavirus. Single-step Rotavirus and Adenovirus Co-immunochromatographic and ELISA methods were used in the examination of the samples. Samples with positive rapid test results were collected in 1.5 ml microcentrifuge tubes, and positive antigen samples collected for the ELISA test were stored at -80°C until the time of study.

Results: Of 1000 stool samples analyzed, 40 (11.59%) of 345 girls and 60 (9.16%) of 655 boys had Rotavirus antigen in a total of 100 (10%) and 20 (2%) had Adenovirus antigen (P> 0.05). Viral antigen-positive cases were most frequently seen in winter months (December, January, February). There was no statistically significant difference between the data in terms of age and gender (P <0.05).

Conclusion: In this study, Rotavirus was detected in one-tenth of diarrhea cases in pediatric patients and it was revealed that it increased seasonally in winter months.

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Keywords: Rotavirus, Gastroenteritis, ELISA, Immunochromatographic, Pediatrics.

1. INTRODUCTION

Viruses that cause diarrhea are in the virus family, such as Reovirus, Norwalk Agent, Rotavirus, Calicivirus, Adenovirus, Coronavirus, Echovirus, and Astrovirus (1). Infectious and non-infectious factors play a role in the etiology of acute diarrhea. The role of viruses in infectious diarrhea is 30%-70%; Rotavirus causes 50-80% of these cases (2). Rotavirus is responsible for 10-20% of patients admitted to hospital with diarrhea, 25-55% of severe diarrhea requiring hospitalization, and 20% of diarrhea-related deaths worldwide (3). Almost every child under the age of five in the world is infected with Rotavirus (4). In the United States (USA), there were between 55,000 and 70,000 hospitalizations and between 20 and 60 deaths per year before Rotavirus entered the vaccine schedule. In Europe, the number of hospitalizations due to Rotavirus gastroenteritis has been calculated to be 87,000 per year. Rotaviruses are responsible for 453,000 deaths in children under 5 years of age worldwide (5). Only group A, B, and C rotaviruses cause disease in humans. Group A Rotavirus (RVA) is endemically distributed worldwide, accounting for >90% of human Rotavirus gastroenteritis cases. Rotaviruses are highly contagious and the most common mode of transmission is the fecal-oral route (5). Rotavirus gastroenteritis presents with clinical findings in a spectrum ranging from subclinical infection with mild diarrhea to severe dehydration and fatal complications. It usually starts with fever and vomiting, and the clinical picture is followed by a sudden onset of watery diarrhea after 1-3 days of vomiting (6). Diarrhea usually lasts 5-7 days and more than 50% of patients have diarrhea and vomiting together. Prolonged diarrhea may cause secondary disaccharide deficiency. Fluid electrolyte loss, metabolic acidosis, malnutrition, malnutrition, and dermatitis are seen in acute gastroenteritis and are also common in rotavirus infection (7). Other viruses that can cause diarrhea after rotaviruses include Adenovirus, Norwalk virus, Norovirus, and caliciviruses. Rotavirus usually increases in winter and spring, while Adenovirus can be seen all year round (1). Laboratory methods are crucial in cases of gastroenteritis. Apart from immunochromatographic methods, ELISA is also available in electron microscopy, and nucleic acid hybridization methods, and is not preferred because it is expensive and requires advanced laboratory conditions (8). While 20-40% of deaths are caused by this disease, 80% of the patients are seen in infancy. Due to the deterioration of the villi of the intestine, they multiply in the cytoplasm of enterocytes and disrupt the transport mechanism (6). This study aims to compare the immunochromatographic and ELISA methods, determine the sensitivity and specificity of the methods used, and shed light on humanity to diagnose the disease correctly.

2. MATERIAL AND METHODS

This study was carried out with the approval of the Ethics Committee of Diyarbakır Pediatrics Hospital (Date: 13.09.2012 and Decision No: 04, No: 01).

2.1. Collection of specimens and patient population

In our study, stool samples of 1000 (345 female, 655 male) patients who applied to Diyarbakır Pediatrics Hospital with the complaint of acute gastroenteritis in 2015, were studied for Rotavirus rapid antigen testing. Single-stage Rotavirus and Adenovirus Co-

immunochromatographic and ELISA methods were used in the examination of samples. Samples with positive rapid test results were taken into 1.5 ml microcentrifuge tubes, and positive antigen samples collected from October, November, December, January, and February for the ELISA test were stored at -80°C until the time to be studied. The results were evaluated by reading in a spectrophotometer (Denley We Scan) at a wavelength of 450 nm. Samples with optical density greater than 0.50 according to the recommendation of ELISA kit protocol Positive; Samples <0.50 were considered negative.

2.2. Chromatography method

The one-step Rotavirus and Adenovirus co-cassette test is a quick and easy method for detecting the presence of Rotavirus and Adenovirus in human stool samples. The test cassettes contain antibodies that are specific to Rotavirus and Adenovirus. These antibodies react with the virus in the sample, resulting in the formation of blue or red lines in the respective regions. A positive result is indicated by the presence of a colored line, while a negative result is indicated by the absence of a colored line. The "C" line serves as a control and ensures the test is working correctly and the sample provided is adequate. To test for rotavirus antigen in stool samples, the following procedure was used. First, the samples were prepared using the chromatography method. To ensure accurate results, the stool samples were examined within hours of collection and stored at a temperature range of 2-8°C. For solid stool samples, a pea-sized amount of 50-100 mg was taken, and for liquid samples, 100 µl was taken and placed in 1 ml of extraction buffer. The mixture was vortexed and then allowed to stand for three minutes to form a homogeneous solution and to let any solid particles precipitate. Finally, four drops of the sample were taken from the supernatant and placed into the round window on the cassette. The introduction of solid particles into the liquid was prevented. The tapes were evaluated after a five-minute waiting period. In all tests, attention was paid to the red control line becoming evident. When the control line was not seen, the test was repeated. The test was considered negative when only the red control line was formed, and positive when the control line and red colors were also observed in the test band. Color changes occurring after ten minutes were evaluated.

2.3. Rotavirus Antigen ELISA

The ELISA kit was allowed to sit at room temperature for 30 minutes before use. To prepare the Wash Buffer, 25 mL of 20X Wash Buffer was mixed with 475 mL of distilled water (DS). For the preparation of samples, controls, and calibrator, about 1 gram of stool sample was put in numbered tubes. To dissolve the sample, 4 mL of 1X wash buffer was added to the samples and vortexed for about 5 minutes. Negative control (100 µL) was added to well A1 of the microplate, positive control (100 µL) to well B1, and diluted stool samples (100 µL) to the remaining wells of the microplate. The microplate was then incubated for 30 minutes at room temperature. Following incubation, the plate was washed three times with 1X Wash Buffer solution in an ELISA washing device (Medispec ESW300 ELISA PlateWasher) and finally aspirated twice and dried thoroughly. To ensure that no liquid remained, the plate was inverted on blotting paper and tapped several times. Two drops of Reagent 1 (anti-rotavirus monoclonal antibody) were added to each well. The plate was then incubated for five minutes at room temperature. Following this, it was washed three times with 1X Wash Buffer solution and

finally aspirated twice and dried thoroughly. Two drops of Reagent 2 (horseradish peroxidase-conjugated to anti-mouse antibody) were added to each well. The plate was incubated for five minutes at room temperature. Following this, the plate was washed three times with 1X Wash Buffer solution and finally aspirated twice and dried thoroughly. To ensure that no liquid remained, the plate was inverted on blotting paper and tapped several times. Two drops of chromogen substrate were added to each well and incubated for five minutes at room temperature. This resulted in the samples turning blue. After incubation, two drops of stop solution were added to each well. This caused a color change from blue to yellow. The results were evaluated by reading on a spectrophotometer (Denley We Scan) at a wavelength of 450 nm. According to the recommendations of the ELISA kit protocol, samples with an optical density greater than 0.50 were considered positive, while samples with values less than 0.50 were considered negative.

2.2. Statistical Analysis

All statistical analyses were performed using the Windows Statistical Package for Social sciences (SPSS) version 21.00 program. Two Kara tests were applied for group comparisons.

3. BULGULAR

In the samples collected in Diyarbakır children's hospital with diarrhea cases in October, November, December, January, and February, around 10% of the 1000 diarrheal cases, Rotavirus and Adenovirus antigens were detected in 2% of them. According to the results, an increase was observed in the autumn and winter months of the virus compared with other months (Figure 1). The distribution of patients by age and gender is shown in Table 1. The result of the study showed a deviation value of 2% between the ELISA (100%) and immunochromatographic (98%) methods. The majority of patients were male, and the most common symptoms were vomiting and diarrhea. When parents were queried, they reported that the stool was yellow or greenish in color and watery.

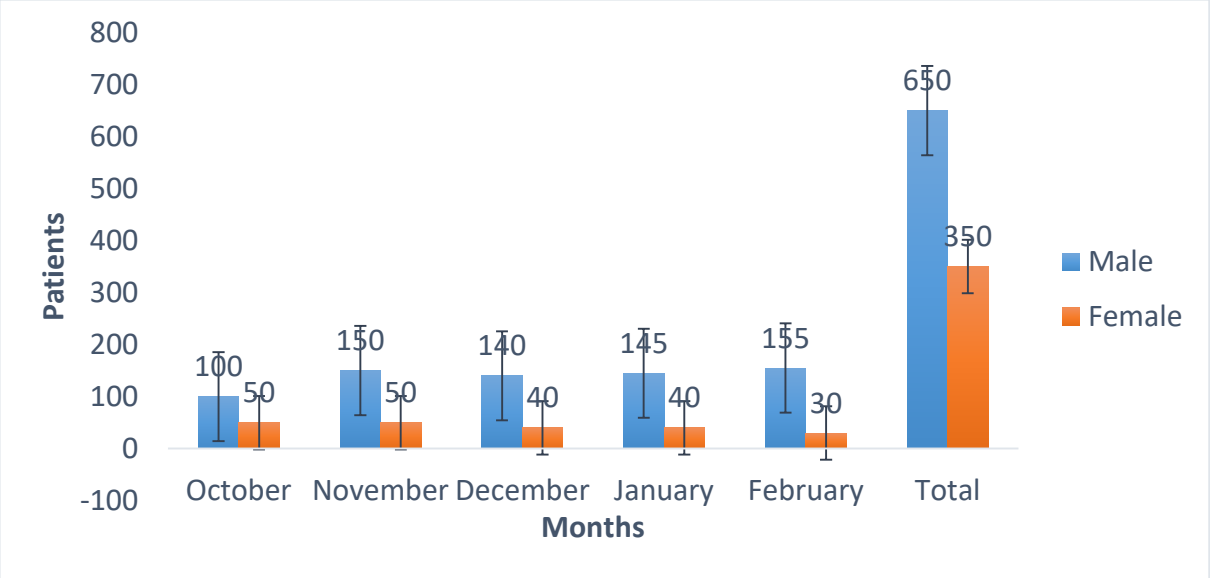

Figure 1.Distribution of patients by month and gender

The ELISA method is more robust and reliable than the chromatography method. Furthermore, the study with ELISA saves time, whereas the chromatography method is cheaper than the ELISA method, according to price standards. In this study, the percentage of rotavirus and adenovirus was determined to be 9.16% in men and 11.59% in women.

A study was conducted to evaluate the reliability of two diagnostic methods, chromatography, and ELISA, in the context of 100 positive cases of rotavirus infection with acute gastroenteritis. The results showed that the ELISA method had 100% accuracy in detecting antigens, while the immunochromatography method had a 98% accuracy rate with a deviation of 2%. From a microbiological perspective, rapid and reliable tests that identify the virus antigen are crucial for accurate diagnosis. However, the ELISA test is not particularly economical and may result in the loss of time. Nevertheless, the study indicates that ELISA tests are more sensitive than other methods.

Table 1. Positive and negative distribution by age and gender

Age (years)	M/R(+)	M/R(-)	%	F/R(+)	F/R(-)	%	M/A(+)	M/A(-)	%	F/A(+)	F/A(-)	%
0-1	12	110	9.83	9	70	11.4	4	118	3.27	3	68	4.22
>1-2	12	120	9.09	9	60	13	3	115	2.54	2	65	2.99
>2-3	13	120	9.77	6	70	7.9	2	132	1.49	1	66	1.49
>3-4	12	130	8.45	10	57	14.9	2	136	1.45	1	68	1.44
>4-5	11	115	8.73	6	48	11.1	1	142	0.70	1	70	1.40
Total	60	595	9.16	40	305	11.6	12	643	1.83	8	337	2.32

M/R: Male/Rota; F/R: Female/Rota; M/A: Male/ Adeno; M/F: Female/ Adeno
(Roa chi-square test 1.23; p>0.05, Adeno chi-square test 0.08; p>0.05).

4. DISCUSSION

Rotavirus was investigated by immunochromatographic rapid test and ELISA method in 1000 samples collected from patients aged 0-5 years who applied to our hospital with the complaint of gastroenteritis. Acute gastroenteritis is one of the most important causes of mortality and morbidity worldwide, especially in developing countries (9). Rotaviruses are the most common cause of acute infectious diarrhea in children under 5 years of age worldwide (10). Before the vaccination program in the USA, there were 55,000-70,000 hospitalizations, 205,000-272,000 emergency department admissions, and 410,000 outpatient visits per year due to rotavirus gastroenteritis (5). Rotavirus gastroenteritis is common all over the world and is transmitted by the fecal-oral route. Considering its seasonal distribution, it is more common, especially in cold seasons. The incidence of rotavirus increases in the United States and Europe in the December-March period, and the incidence of rotavirus is higher in Africa during the dry seasons (5). Rotavirus, which is mostly seen in winter months and children under 5 years of age in the temperate climate zone, occurs earlier in developing countries (6-9 months and 9-15 months, respectively) compared to developed countries and has a more severe clinical course (11). It usually occurs between the end of autumn and the middle of spring in regions located in the temperate climate zone such as our country. In a study by Kahraman et al. in Ankara, they found that the disease was most common in the first spring (12). Considering the season in our study, it was observed that the most frequent hospitalization was in the autumn season.

The findings of our research indicated that there was an increase in rotavirus prevalence during the autumn and winter months (November, December, January, and February). In contrast, this virus was in very limited numbers in the summer and spring months. The seasonal pattern plays an important role in patients with diarrhea. While bacterial diarrhea is common in the hot season, virus-induced diarrhea, especially rotavirus, is reported to be more common in cold weather (13). The immunochromatographic method, which has been used rapidly in recent years, is preferred in diagnosis due to its features such as speed and ease of use. In addition, the compatibility of antigen-positive results with ELISA results and high sensitivity and specificity are among the important features of this choice (14). In our study, the sensitivity of ELISA was 100%, while the immunochromatographic sensitivity was 98%. We can say that the immunochromatographic method, which is a cheaper, faster, and more easily applicable diyagnostik method, is preferred in hospitals due to its high level of compatibility. In studies conducted in our country, immunochromatographic methods were generally used, and Rotavirus antigen positivity was reported between 9.8% and 31.9%, and Adenovirus antigen positivity between 1% and 14.9% (15,16). In our study, Rotavirus antigen positivity was detected in 100 (10%) of 1000 cases by ELISA; Adenovirus antigen positivity was found in 20 (2%) of the patients. In addition, while it was mainly seen in the 0-1 age group (9.16%) in males, it was seen in the four age group (11.6%) in females. Experts have long agreed that a reliable vaccine is needed to reduce rotavirus-related morbidity and mortality. For this reason, the World Health Organization gives priority to Rotavirus vaccine development and administration (4). In our country, although Rotavirus vaccines are not yet included in the childhood national vaccination calendar, they are included in the childhood extended vaccination calendar, which also includes vaccines that can be covered by families or private health insurance, if any. Because history and clinical findings are not sufficient in the diagnosis of viral diarrhea, laboratory studies are needed. Because the clinical findings encountered during rotavirus diarrhea are nonspecific, various test techniques have been developed for diagnosis. These; electron microscopy, ELISA, immunochromatography, Real-time polymerase chain reaction (RT-PCR), Polyacrylamide Gel Electrophoresis for viral genomic RNA (PAGE), and viral culture (17,18). In conclusion, ELISA and immunochromatographic methods, which are easy to perform and have high sensitivity, have been the most common methods for the determination of viral antigens from stool or rectal swab samples. Genotyping of rotaviruses by molecular studies and determination of group and subgroup types are important in the creation of regional and national vaccines. A total of 1,000 stool samples were analyzed using chromatography and ELISA methods, and it was found that rotavirus antigen was present in 100 (10%) of the samples. The rotavirus antigen was detected in 40 out of 345 female samples (11.59%) and in 60 out of 655 male samples (9.16%). The analysis revealed no significant difference in the occurrence of rotavirus between the two genders ($P > 0.05$). Additionally, adenovirus antigen was identified in 2% of the 1,000 diarrheal cases. In the future, molecular studies should be carried out in our region and vaccines should be diversified by typing accordingly.

5. CONCLUSION

In our study, we have found that rotavirus infection is a severe illness that can lead to death if not diagnosed accurately. We have determined that the frequency of rotavirus-positive

cases is highest during the autumn and winter months when infection is most common. The sensitivity and specificity of chromatography and ELISA tests are crucial in making the right diagnosis and guiding the patient toward appropriate treatment. Our study shows that while the immunochromatographic method for detecting rotavirus antigen may have a 2% deviation, it is still more practical and cost-effective than the ELISA test and possesses advantages that should not be overlooked.

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Data Availability

The authors can confirm that all relevant data is included in the article

Conflict of Interest Statement

There is no conflict of interest.

Ethicial Considerations

This study was carried out with the approval of the Ethics Committee in Diyarbakır Pediatrics Hospital (Date: 13.09.2012 and Decision No: 04, No: 01).

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