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Investigation of rotavirus infections by ELISA in children with gastroenteritis in Batman province

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ABSTRACT

Aim: Rotavirus (RV), which is one of the most leading causes of gastroenteritis in children. newborns and young children. In this study, it was aimed to investigate the prevalence of rotavirus with the ELISA test in patients aged 5 years and younger who applied to the hospital with the enteritis from Batman province and its districts.

Material and Method: Stool samples collected from children aged 5 years and younger with acute gastroenteritis who applied to Batman Gynecology and Children Hospital between January 1, 2015 and December 31, 2016 stored at -80°C until testing. The Rotavirus ELISA kit was used.

Results: Stool samples from 154 children were enrolled in the study. Enteritis due to RV was determined as 26.62% (41/154) in children aged 0-5 years. Among the positive samples, 8/41 (19.51%), 17/41 (41.47%) and 16/41 (39.02%) antigen positivity were determined for the age groups of 4-6 months, 7-12 months and 13-24 months, respectively

Conclusion: In our study, it was firstly reported that the prevalence of rotavirus in Batman was high. Hygienic measures and the potential benefit of RV vaccines should be considered in children, especially in rural areas.

Keywords: Children, ELISA, gastroenteritis, rotavirus, stool

INTRODUCTION

Rotavirus (RV), which is one of the most common and important causes of gastroenteritis in newborns and young children, is widespread all over the world. About 2 million children die from gastroenteritis every year, and 600,000 of these deaths are due to rotavirus gastroenteritis (1,2).

The clinical course and prognosis of rotavirus infection in children varies. The course of the infection ranges from asymptomatic disease to severe diarrhea with dehydration leading to death. The incidence of severe diarrhea is higher in RV infection when compared to infections caused by other factors progressing with gastroenteritis. Rotavirus is most common in children under the age of five, especially between the ages of 0.5 and 2 (2-5).

Rotaviruses are classified under 7 groups and although only group A, B and C rotavirus infections are seen in humans, but the most common rotavirus infections originate from group A. Group A rotavirus infections are reported to cause 21-65% of severe infantile gastroenteritis (1,6).

Although the outcome of the infection is different, rotavirus infections, which are the main cause of acute severe gastroenteritis in children under 5 years old, are seen with a similar incidence in both developed and developing countries. While mortality rates are low in developed countries, the rate of transmission is high, and the rates of hospitalization are quite high, such as 20-60%, and are similar to those in underdeveloped countries (3,4).

The causative agent is classified in the genus of Rotavirus within the Reoviridae family, viral RNA is double-stranded (dsRNA) and consists of 11 segments. Rotavirus is a non-enveloped virus with an icosahedral capsid and resembles a wheel when examined with an electron microscope. Because of this appearance, it was given the name "route", which means wheel in Latin. Considering the difference in the VP6 protein, seven groups named from A to G (A, B, C, D, E, F, G) have been reported (5,6). Rotavirus groups D, E, F and G infect only animals, while groups A, B and C include agents that cause infections in both humans and animals (7).

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RV is usually endemic and rarely causes epidemics. Rotaviruses, which cause 50-80% of viral gastroenteritis cases, are reported as the main cause of severe gastroenteritis in children under 5 years of age in both developed and developing countries (8). The causative agent is responsible for approximately 25% of diarrhea-related deaths worldwide. According to the data of the World Health Organization (WHO, World Health Organization) between 2000 and 2004, when the estimated number of deaths due to diarrhea in children under the age of 5 (1,566,000/year) is taken into account, it has been reported that there is an average of 611,000 (454,000-705,000) deaths due to rotavirus each year. this rate constitutes 20% of all deaths in children under the age of 5 (9). The vast majority of these deaths, 82% of which occur in developing countries, around 1,205 children die from rotavirus every day. In developed countries, although mortality is low, morbidity is high, and 220,000 children are hospitalized for rotavirus gastroenteritis each year, which corresponds to 1 in 50 children (10).

An ELISA kit is commercially available for the detection of rotavirus antigens in stool samples. The sensitivity of the Rotavirus ELISA test has been reported as 98-100% and the specificity as 100% (11). In this study, it was aimed to investigate the prevalence of rotavirus with the ELISA test, which was reported to have high sensitivity and specificity in previous studies, in patients aged 5 years and younger who applied to the hospital with the enteritis from Batman province and its districts.

MATERIAL AND METHOD

This study was carried out with the permission of the Harran University Faculty of Medicine Ethics Committee (Date: 16.05.2016, Decision No: 05/06). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Stool samples used in this study were collected from children aged 5 years and younger with acute gastroenteritis who applied to Batman Gynecology and Children Hospital, affiliated to Batman Provincial Health Directorate, between January 1, 2015 and December 31, 2016. Personal information of the patients was not collected, only their age and gender were recorded, and the sampling details are shown in **Table 1**. Stool samples collected from children with acute gastroenteritis were stored at -80°C until testing. In this study, the Rotavirus Antigen ELISA (RIDASCREEN® Rotavirus; cat. No: C0901, Lot No: 15255E, R-Biopharm AG, Germany) kit for VP6 antigen detection was used. VP6 is the common group protein of all rotaviruses that infect humans, produced by the sixth viral gene. The test was performed according to the procedure recommended by the commercial firm. The image of ELISA positive samples is shown in the **Figure.**

Table 1. Number of stools sampled and distribution by age groups

Age groups (months)	number	%
0-3	15	9.74
4-6	33	21.43
7-12	54	35.07
13-24	44	28.57
25-60	8	5.20
Total	**154**	**100**

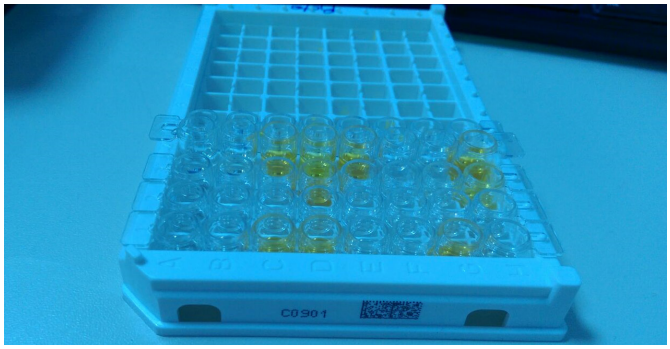

Figure. Image of ELISA positive samples

RESULTS

Enteritis due to RV was determined as 26.62% (41/154) in children aged 0-5 years who applied to the hospital hospitalized with diarrhea. It is thought that 73.36% (113/154) of the same cases may be caused by other infectious agents (as viruses, bacteria, parasites or food poisoning) having role in the etiology of diarrhea which were not covered in our study. ELISA test results and their distribution by age groups are presented in **Table 2**.

The cases included in this study were evaluated in five groups, as in many previous studies. The first group consisted of infants aged between 0-3 months and was 9.74% of all cases. This group was found to be negative for RV antigen. The ratio of the cases aged between three and six months in the whole sample was 21.43%. and 24.24% (8/33) of the cases in this age range were positive for rotavirus. The group aged between six months and one year comprised 35.07% of the patients in the study, and 31.48% (17/54) of the children in this age range were found to be virus positive. Cases aged one to two years constituted 28.57% of the children in the study, and the prevalence of RV was found to be 36.36% (16/44). RV was found negative in 5.20% of the cases at the age of two and over (**Table 2**).

Tablo 2. Samples found positive by ELISA according to age groups

Age groups (months)	Sample number	Number of rotavirus positive samples (%)	Number of rotavirus negative samples (%)
0-3	15	0 (0.00)	15 (100)
4-6	33	8 (24.24)	25 (75.76)
7-12	54	17 (31.49)	37 (68.52)
13-24	44	16 (36.36)	28 (63.64)
25-60	8	0 (0.00)	8 (0.00)
Total	**154**	**41 (26.62)**	**113 (73.38)**

Distribution of positivity in age groups was shown in **Table 3**. **Table 3** shows that the patients were aged between 4 and 12 months and a total of 41 samples were positive. Among the positive children, 8/41 (19.51%), 17/41 (41.47%) and 16/41 (39.02%) antigen positivity were determined for the 4-6, 7-12 and 13-24 age groups, respectively (**Table 3**).

Table 3. Distribution of positivity in age groups

Age groups (months)	Rotavirus positive sample number/ Total Positive Sample number	Number of rotavirus positive samples (%)
0-3	0/41	0.00
4-6	8/41	19,51
7-12	17/41	41,47
13-24	16/41	39,02
25-60	0/41	0.00
Total	**41/41**	**100**

DISCUSSION

The existence and prevalence of rotaviruses, which are common all over the world, have been revealed by many researchers. Soriano-Gabarro et al. (12) reported in a study they conducted in European countries that RV is responsible for hospital admissions due to acute diarrhea with rates varying between 21% and 59.8%. Huilan et al. (13) in the study of Asian countries including China, India, Mexico, Myanmar and Pakistan, children were evaluated for two years and RV was positive in 16% of the patients. Carlin et al. (14) found that 50% of the patients admitted to the hospital with the complaint of enteritis and admitted to the hospital in Australia were RV positive. In the United States, the most common cause of acute diarrhea appears to be RV. Infections that cause severe dehydration are more common among children aged 3-35 months, and it is reported that 4/5 of the children in the first 5 years of age have RV infection. RV is shown as 30-50% of hospitalizations due to diarrhea. It is reported that 20% to 25% of outpatient clinic examinations due to diarrhea are caused by rotavirus (MMWR 2006). Cunliffe et al. (15) conducted a study covering 15 countries in the African continent. In this study, they examined the studies conducted between 1975-1992 to determine the cause of diarrhea in children younger than 5 years old. As a result of this examination, it was determined that 24% of children hospitalized for diarrhea and 23% of outpatients were positive for rotavirus (15). Cunliffe et al. (15) in the same study reported positivity rates of rotavirus same as 34% in Egypt, 22-49% in Ethiopia, 29-41% in Kenya, 20-32% in Nigeria, 31% in Tanzania, 24% in Zambia and 13-55% in South Africa. The rate of 26.62% obtained in this study is close to or similar to the rates reported in various countries.

There are many studies on rotaviruses as a cause of diarrhea in children in Turkey and varying rates have been reported by researchers in these studies (16-20). It has been observed that these studies are mostly conducted at the 0-5 age range, as in our study.

Keser M. (18) reported 82.3% (79/96) of children hospitalized for diarrhea to be RV positive. In another study, stool samples of 148 patients with acute diarrhea in the 0-6 age group in Eskişehir were studied by ELISA method, and RV was positive in 18.2% of the patients (17). Zarakolu et al. (20) found the rate of rotavirus to be 8.5% in 59 children with acute diarrhea in the 0-5 age group. Akdogan et al. (16) included 240 children with acute diarrhea in their study conducted in Kayseri in the 0-5 age group, and reported the prevalence of RV as 34.2% in their study with the stool ELISA test. 218 children aged 0-5 years with acute diarrhea were included in the study conducted in Şanlıurfa. The causative pathogen was detected in 43.1% of the patients, and RV was positive in 7.8% of them (19). In another study conducted in Ankara, the records of 1099 patients for whom stool RV antigen testing was requested were retrospectively reviewed; RV positivity was found at a total rate of 36.8% (21). Şay Coşkun et al. (22) found RV positivity at a rate of 8.1% in the study in which they included children aged 0-18 in Tokat. In a study carried out in Manisa, the frequency of rotavirus in patients who applied to health centers with acute diarrhea was investigated using the ELISA method, and RV was positive at a rate of 17.4% (23). Kurugöl et al. (24) found RV positive in 39.8% of the patients in a study in which the stools of children younger than five years old who were admitted to the hospital due to diarrhea in İzmir were examined by ELISA test (24). Bozdayi et al. (25) In a study conducted in Ankara in children younger than five years with acute diarrhea, RV positivity was found at a rate of 39.7%.

When these studies, which were conducted in different provinces of Turkey, at different times, with different methods, and whose study group was predominantly between 0-5 years of age, were considered, quite variable RV positivity rates were reported for RV, with a prevalence ranging from 7.8% to 82.3%. The 82.3% value they determined by Keser (18) is quite high not only compared to our study, but also compared to many other studies. The rate of 26.62% in our study is similar to other studies.

The fact that the frequency of infection is low after 24 months of age is due to the fact that natural infections reduce the incidence and severity of the next attack. In this study, a result consistent with this view was obtained and rotavirus antigen could not be detected in samples aged 2 years and above.

Another feature of RV diarrhea is the high rate of hospitalization due to diarrhea in infants and older children in both developed and developing countries (9). This view is supported by the literature information given above and the data of our research in addition to this view.

CONCLUSION

Our study was carried out to determine the prevalence of rotavirus in children aged 0-5 years with acute gastroenteritis in Batman region. It was revealed that the prevalence of rotavirus in Batman was high, with 26.62% positivity in the age groups of 0-5 years of age of 154 cases included in the study. Since there is no previous study in Batman, it was not possible to compare The low incidence of RV infection in children younger than 6 months is attributed to both maternally transmitted antibodies and breast milk (26). It is thought that feeding newborn babies with enough colostrum and continuing breastfeeding for an adequate period of time will be beneficial in reducing the incidence of the disease. RV diarrhea is responsible for significant mortality in developing countries. It also causes clinical and economic burden in developed countries. Along with feeding clean water and food, it is necessary to consider the potential benefit of RV vaccines (27,28). In Turkey, the etiological researches of RV diarrhea should be done more frequently to contribute to the formation of the country policy, and therefore, country-specific solution policies should be designed (29). Complications and deaths in RV diarrhea are mostly due to dehydration, electrolyte imbalance and acidosis. Although it causes mild, moderate and severe clinical presentation, RV diarrhea is characterized by persistent vomiting attacks and frequent defecation. As a result, it causes severe dehydration more frequently than other viral enteropathogens (30). It is very important to apply the right treatment options to hospitalized patients.

ETHICAL DECLARATIONS

Ethics Committee Approval: This study was carried out with the permission of the Harran University Faculty of Medicine Ethics Committee (Date: 16.05.2016, Decision No: 05/06).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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