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Investigation of Diarrhea Agents Less Than 5 Years of Age in Summer in Gaziantep/Turkey

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Abstract: Pathogens causing summer diarrhea examined to detect among children less than 5 years of age in Gaziantep. We conducted among 100 children with diarrhea during summer at the pediatric hospital of Gaziantep. In stool samples from children, Rotavirus with Rotatect kit (Dade Behring, Germany), *Entamoeba* spp. with direct microscopy and bacterial pathogens with cultural techniques investigated. Cystic form of *Entamoeba* spp. was determined in 61 (61%) and Rotavirus antigen in 25 positive samples (25%). A predominant bacterium was determined in total 87 stool samples (87%). Despite of only cystic form of *Entamoeba* spp. was determined in seven, only bacteria in 22 and only Rotavirus in one; two of them were determined in 67 out of stool samples. According to comparison with stool samples belong to various months we have found that, Rotavirus and *E. coli* are the most pathogenic agents in August more than June and July.

Key words: Diarrhea, summer, *Entamoeba* spp., Rotavirus

INTRODUCTION

Acute diarrhea is the single most important health problem among all age groups in developed and developing countries. However, in especially developing countries, it is more important among infants and young children and it continues to be a significant cause of morbidity and mortality worldwide (Abu Elamreen *et al*., 2007). Acute diarrhoeal diseases rank second amongst all infectious diseases for the mortality of the in children below 5 years of age worldwide. Globally, 1.3 billion episodes occur annually, with an average of 2-3 episodes per child per year. In (Sur and Bhattacharya, 2006) Globally diarrheas diseases account for almost a fifth of all deaths of children under 5 years, with an estimated 2.2 million deaths annually. Of these deaths, a significant proportion related to episodes of persistent diarrhea. (Zulfiqar, 2006). This rate is higher in developing countries. It has reported that, bacteria were responsible for diarrhea in developing countries and virus is in developed countries. Rotavirus is one of the most common viral agents and the most important agent of childhood diarrhea (Blacklow and Grenberg, 1991; Koneman *et al*., 1993). Acute diarrhea is an infection of intestine. Agent features are identical to clinic symptoms. For this reason, to plan suitable treatment, patient with diarrhea is required to evaluate in details. Duration of symptoms, number of episodes of diarrhea, vomiting, fever and other symptoms should register (Erguven, 1994). Rotaviruses (RV) are the most common etiological agents in acute gastroenteritis (GE) in children in the first years of life. Data from the national scientific literature show that RV is responsible of 26% of all cases of hospitalisation for diarrhea in children, resulting the most frequently identified agent (Marocco *et al*., 2006). In present study, *Entamoeba* spp., bacteria and Rotavirus agents' cause of diarrhea examined and information concerning disease severity of patients evaluated.

MATERIALS AND METHODS

All children with diarrhea younger than 5 years of age who hospitalized at the pediatric hospital of Gaziantep from June to August 2005 were included to this study. Investigation group consisted of 100 children (62 boys and 38 girls). Anamneses of children with diarrhea were listened and pathogen microorganisms in collected stool samples have investigated. By socio-economic assessments have evaluated, whether pathogens are related to socio-economic conditions or not were investigated. Stool samples have placed on celenit F, bloody and EMB (Eosin Methylene Blue) medium. Reproduced colonies have identified by biochemical techniques (TSI, Urea, Citrate etc). For *Entamoeba* spp. investigation in stool samples were examined with direct microscopy and cystic and trophozoit forms of

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Entamoeba spp. were determined. However, their species were not determined. Rotavirus was determined with Rotatect kit, which is chromatographic test. Statistical analyses of collected samples performed by using SPSS (5.0) and chi-square test.

RESULTS

In stool samples from 19 out of 71 (26%) children with stomachache symptom, Rotavirus antigen was determined and in 4 out of 29 (13%) children without stomach ache Rotavirus antigen was determined. 25 Children detected Rotavirus antigen, had vomiting symptom. Rotavirus antigen was not determined in 27 children with stomachache symptom. The total 20 out of 25 children (80%) detected for Rotavirus had fever complaint, 5 out of 22 children without fever complaint were tested positive for Rotavirus. In 10 of 18 children with dysenteric stool samples (50.5%), Rotavirus was determined in stool samples from 20 out of 25 children (80%) tested positive for Rotavirus not seen any leucocytes and erythrocyte was demonstrated in two samples of them. Rotavirus antigen was determined in 25.1% boys and 24.9% girls so there was not difference due to sexuality. In stool samples cultures from 18 out of 25 children tested positive for Rotavirus, *Klebsiella* spp. evaluated. Forty eight out of 61 children with *Entamoeba* spp. cyst had vomiting, 37 out of them had dysenteric stool, 39 out of them had yellow stool, 43 with stomach ache, in 38 leucocytes was determined and 38 with fever, was determined. Forty out of 50 children detected *E. coli* in stool samples had vomiting, dysenteric stool in 24, yellow stool in 3, stomach ache in 37, leucocytes in stool in 35 were tested. Fever was valued in 21, vomiting in 22, dysenteric stool in 20, yellow stool in 21, stomachache in 17, stool with leucocytes in 13 and stool with erythrocyte in 21 out of 30 children's positive stool samples for *Klebsiella* spp.

Sex and age of 100 children with acute diarrhea in this study, is represented in Table 1. At the result of hospital records, it was determined that 78 of them complained from fever, 77 vomiting and 71 stomachs ache (Table 2). The evaluation of reproductive results from cultures showed that, the signs of normal intestine flora were in the only three stool samples. Reproduces of bacteria was not seen in 10 samples, whereas was seen in 87. Overall, only bacteria was determined in 22, bacteria and *Entamoeba* spp. cyst together in 43, bacteria and Rotavirus antigen together in 13 and bacteria, cystic form of *Entamoeba* spp. and Rotavirus antigen together in 9 out of 87 stool samples (Table 3 and 4). The microorganisms that detected in the different month represented in the Table 5 and 6. Children in socio-economically developed families were tested 33.3%

Table 1: Sex and age of 100 children with acute diarrhea

Age (Months)	Female	(%)	Male	(%)	Total
0-3	9	45.0	11	55.0	20
4-6	6	40.0	9	60.0	15
7-9	6	35.3	11	64.7	17
10-12	17	58.8	10	41.2	17
13-24	6	35.3	11	64.7	17
25≤	4	28.6	10	71.4	14
Total	38	38.0	62	68.0	100

Table 2: Dispersion of symptoms and stools' characteristic in children

Characteristics	No.	(%)
Fever		
Exist	78	78.0
Non-exist	22	22.0
Vomiting		
Exist	77	77.0
Non-exist	23	23.0
Stomachache		
Exist	71	71.0
Non-exist	25	25.0
Unanswered	4	4.0
Appearance		
Yellowy particular	57	57.6
Green feces	16	16.2
Bloody	3	3.0
Fluid and mucus	23	23.2
Leucocytes		
Non-exist	64	64.6
2-3 Leuc. in each grid	12	12.1
>5 Leuc. in each grid	23	23.2
Erythrocyte		
Non-exist	77	77.8
2-3 Eryth. in each grid	12	12.1
>5 Eryth. in each grid	10	10.1

Table 3: Detection of different species in stool samples

Species	Unique bacterial colony	Bacteria + *Entamoeba* cist	Bacteria + Rotavirus	Bacteria + *Entamoeba* spp. cyst + Rotavirus	Total
E. coli	9	16	6	6	37
Klebsiella spp.	4	12	2	2	20
Enterococcus spp.	2	1	2	-	5
Proteus spp.	-	2	-	-	2
Pseudomonas spp.	-	1	-	-	1
Staphylococcus spp.	1	-	-	-	1
E. coli + *Klebsiella* spp.	1	5	1	1	6
E. coli + *Enterococcus* spp.	-	1	1	-	3
E. coli + *Proteus* spp.	-	2	-	-	2
E. coli + *Candida* spp.	-	1	-	-	1
Klebsiella spp. + *Enterococcus* spp.	1	-	-		1
Klebsiella spp. + *Candida* spp.	1	-	-		1
Enterococcus spp. + *Enterobacter* spp.	1	-	1	-	2
Enterococcus spp. + *Candida* spp.	1	2	-	-	3
Proteus spp. + *Candida* spp.	1	-	-	-	1
Total	22	43	13	9	87

- : Not detected

Table 4: Detection of Bacteria, Rotavirus and *Entamoeba* spp. in stool samples

Species	No.	Species	No.	Species	No.
Bacteria (+)	87	Rotavirus (+)	25	*Entamoeba* spp. (+)	61
Only bacterial colony	22	Only Rotavirus	1	Only *Entamoeba* spp.	7
Bacteria + *Entamoeba* spp.	43	Rotavirus + *Entamoeba* spp.	2	*Entamoeba* spp. + Bacteria	43
Bacteria + Rotavirus	13	Rotavirus + Bacteria	13	*Entamoeba* spp. + Rotavirus	2
Bacteria + *Entamoeba* spp. + Rotavirus	9	Rotavirus + *Entamoeba* spp. + Bacteria	9	*Entamoeba* spp. + Bacteria + Rotavirus	9

Staphylococcus spp., *Candida* spp. and *Enterobacter* spp. were detected in stool samples

Table 5: The distribution and percentage of bacteria, *Entamoeba* spp. cysts and Rotavirus antigens detected in the stool samples

	No. of isolates			
Microorganisms	June	July	August	Total No. of isolates (%)
Entamoeba spp. cyst	20 (32.8)	18 (29.5)	23 (37.7)	61 (61)
Rotavirus	5 (16.1)	2 (6.7)	18 (46.1)	25 (25)
Bacteria	19 (61)	30 (100)	38 (97.4)	87 (87)

Table 6: Detected bacteria species such as *E. coli*, *Klebsiella* spp., *Enterococcus* spp., Candida spp., *Proteus* spp., *Enterobacter* spp., *Pseudomonas* spp. and *Staphylococcus* spp. in the stool samples

	No. of isolates			
Entero-pathogen	June	July	August	Total No. of isolates
E. coli	7	14	29	50
Klebsiella spp.	4	15	11	30
Enterococcus spp.	6	3	4	13
Candida spp..	3	2	1	6
Proteus spp.	2	2	1	5
Enterobacter spp.	1	1	0	2
Pseudomonas spp	-	1	-	1
Staphylococcus spp.	-	1	-	1

- : Not detected

Table 7: The distributional percentages of Rotavirus, bacteria and *Entamoeba* spp. cyst form rates according to Socio-Economic Conditions (SEC) of families

SEC	Rotavirus (%)	*Entamoeba* spp. Cyst (%)	Bacteria (%)
Low	23.4	63.9	66.7
Medial	6.6	29.5	30.0
Good	33.3	6.6	3.3

positive for Rotavirus, 6.6% positive for cystic form of *Entamoeba* spp. and 3.3% positive for bacteria (Table 7).

DISCUSSION

Blacklow *et al.* (1991) reported that while diarrhea was caused by bacteria which has invasion ability to intestine epithelium has showed fever symptom, viral gastroenteritis and diarrhea caused by parasites and enterotoxigenic *E. coli* symptoms has not show fever symptom however Rotavirus gastroenteritis has showed fever symptom. It was determined that fever symptom in 80% episodes with RV, in 78% episodes with *Entamoeba* spp. and in 76% episodes with bacteria had been considering that fever symptom was seen in all diarrhea caused by bacteria, virus and parasites. However, determination of fever symptom in 25% diarrhea caused

by only RV and by only bacteria and in 80% diarrhea caused by *Entamoeba* spp. indicated that fever symptom widespread in all diarrhea episodes. Thus, fever symptom might be seen in diarrhea caused by RV, it's seen in most of diarrhea caused by *Entamoeba* spp. and it's seen in diarrhea caused by bacteria in a bacterial agent dependent manner, was determined. It has reported that determination of leucocytes in stool samples showed which agent could be bacteria has invasion capability (Hruska, 1991). Besides leucocytes was determined in viral gastroenteritis, diarrhea caused by parasites and enterotoxigenic bacterial diarrhea. Leucocytes observed as 62.3% of *Entamoeba* spp. cyst episodes and 20% of Rotavirus episodes (Guerant *et al*., 1992). Evaluation of episodes with leucocyte among all episodes-involved present study, showed that its presence rate was 37%. As 29% out of them seen in episodes caused by bacteria, 51% out of them seen in episodes caused by bacteria and *Entamoeba* spp. determined together. However, rate of RV detected episodes was 5%. It was determined that leucocytes detected in bacterial diarrhea, it detected in amoboidial diarrhea and it not detected in viral (RV) diarrhea. Liu *et al.* (2005) reported that bacterial agents are responsible for 11.2% episodes and viral agents are responsible for 39.6% episodes of diarrhea under 3 years of age. Rotavirus is responsible for 20-30% patients with diarrhea younger than 5 years and 30-50% hospitalized patients in Europe (Pickering, 1997). The presence of rotavirus in childhood diarrhea cases ranges from 16.3-36.1% in Turkey (Polat *et al*., 2000; Albay *et al*., 1996; Basustaoglu *et al*., 1995). Rodrigues *et al.* (2002) reported that bacteria and Rotavirus have detected in 41% out of stool samples collected children with diarrhea in a Brazilian study. In addition, stool samples with Rotavirus were consisted of 14.8% patients while samples with *E. coli* were consisted of 20.4% patients (Rodrigues *et al*., 2002). However, in present study Rotavirus antigen was determined in 25% of stool samples collected all children with diarrhea. Bacterial or diarrhea caused by parasites agents also determined in 60% out of them. Bacterial and diarrhea caused by parasites agents detected together in 36% out of them. The results of our study, RV positively rate in Turkey is resembled with the other studies reported from different part of the world. In 7 out of 61 samples with *Entamoeba* spp. cyst, only *Entamoeba* spp. cyst determined, *Entamoeba* spp. cyst + bacteria in 43, *Entamoeba* spp. cyst, bacteria and Rotavirus antigen determined in nine samples (Table 3). A study by Thomas *et al.* (2006) in Indonesia and Egypt suggest diarrhea related to Rotavirus was seen higher rate in late summer and at the beginning of winter and bacterial diarrhea was continuing during summer season. Out of 46 total diarrhea in this research, only 1% of diarrhea with Rotavirus determined in August. So evaluation of Rotavirus pathogen in summer will be useful for gastroenteritis episodes. As represented in Table 5, whereas cystic form of *Entamoeba* spp. quantity was equal in each of months, antigen of Rotavirus was 46.1% in August, 6.7% in June and 16.1% in July. Comparison of these months related to the presence of Rotavirus antigen showed that the number of Rotavirus antigen in August was higher than in that of others (Table 6). It reported that RV infections have a high rate in developed countries. The incidence of infectious diarrhea and the prevalence of a given causal agent are strictly associated with socioeconomic factors such as nutrition, sanitation and habitat of the population. In developing countries, major diarrhea agents are protozoans, bacteria and Rotaviruses. The latter is a universal entero-pathogen and accounts for most of the diarrhea cases in developed countries and in populations of high socioeconomic level of developing countries (Rodrigues *et al*., 2002). In present study, diarrhea caused by RV has a low rate in a comparison with bacterial and diarrhea caused by parasites. On the other hand, the RV has a higher value among the families that has higher standard of socio-economic conditions. The incidence of bacterial and diarrhea caused by parasites agents was determined much higher among families that are the undeveloped in the view of socio-economic conditions. It showed that hygiene and sanitation had a significant role for these pathogens infection. The incidence of Rotavirus found to be higher in the socio-economically developed families. It considered that why determination higher incidence of bacterial and parasitic agents in undeveloped countries. The correlation of the phenomena lays in the phrases of the higher incidence of viral agents in developed countries. According to these results, Rotavirus caused of approximately 1/4 childhood diarrhea in our Region. Majority of the doctors have started liquid replacement and antibiotics treatment in Turkey, if they do not have Laboratory for diarrhea associated with fever. On the other hand, with the recent technological improvement, the determination of Rotavirus take place with the advantages of being cheap, case-sensitive, fast and reliable. Moreover, it does not need any specific equipment or trained staff. Under this known circumstances, we believe that, using Rotavirus diagnostic techniques play remarkable role in treatment and prevent unnecessary using antibiotics in summer diarrhea among the children less then 5 years old. In addition, increasing of *Entamoeba* spp. episodes in summer has showed inadequate environmental sanitation and hygiene conditions in our region. Therefore, it is required to precaution for environmental sanitation and hygiene conditions. Besides that by investigation of the morbidity and the mortality of Rotavirus in Turkey in last decade, therefore, the vaccination studies should take into consideration as soon as possible.

In conclusion, it was determined that differential microorganisms such as bacteria, protozoas and virus as diarrhea agents. This study has noticed that in developing country like our country, such investigation should be to encourage municipalities to attach importance underground construction.

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