Prevalence of enteropathogenic diarrhea in Children up to 2 years in Kirkuk province

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Abstract

Diarrhea is one of the most common infectious gastroenteritis diseases in young children. To clarify the infectious etiology of diarrhea in children up to two years old, we conducted a cross-sectional study in 200 children (116 males and 84 females) admitted with diarrhea to Pediatric Hospital in Kirkuk province. In present study, we studied the distribution of enteropathogenic diarrhea (EPD) in children. Regarding to age group, the distribution of EPD was high 82(42.69%) in age group ranging from 7-12 Mon. On the other hand 106 (59.55%) male versus 72 (40.45%) females showed EPD. According to feeding nature, the incidence of EPD among breast feeding Children was 52(22.47%) patient while artificially feeding account for 56 (31.46%). The mixed feeding children with EPD also detected in 35 (39.32%) patient. We also studied enteropathogenic diarrhea (EPD) in children with persistent and acute diarrhea, out of 154 children with acute diarrhea, 136(76.4%) children showed EPD. While, from 46 children with persistent diarrhea, 42(23.6%) children were recorded to have EPD. Stool specimens were collected from subjects, and examined for several diagnostic laboratory routine tests to detect bacterial enteropathogens, parasites, and viruses. The results indicated that 178(89%) of diarrheal children passed an enteropathogenes (EP) in their stools, with 22(11%) negative cases. A total of 226 causative agents of diarrhea were isolated and identified including: bacterial causes comprised of 66 (33%) enteropathogenic E. coli (EPEC), 56 (28%) Salmonella spp., 10 (5%), human rotavirus 56 (28%), parasitic causes appeared in 54 (27%); this were E. histolitica 44 (22%), Giardia lambilia 10(5%), Candida albicans 50 (25%), with associated previous antibiotic therapy. To identify other causes of diarrhea we studied carbohydrate intolerance mainly lactase deficiency, among 200 stool specimen, 30 sample showed positive Benedict's test, which indicates disaccharide malabsorption.

Introduction

Diarrhea is an intestinal disorder characterized by abnormal fluidity and frequency of fecal evacuation, generally the result of increased motility in the colon; may be an important symptom of such underlying disorders as dysenteric diseases, lactose intolerance, gastrointestinal G1 tumors, and inflammatory bowel disease (1).

Diarrheal diseases are a major cause of childhood morbidity and mortality in the worldwide especially in developing world including Iraq. It contributes to the deaths of 4.6- 6 million children annually in Asia, Africa, and America; and 80% of these deaths occur in the first 2 years of life (2,3). In tropical climates it has been estimated that each child suffers up to 15 to 19 episodes of diarrhea per year, where in the United States, reports calculate 1.5 to 1.9 episodes per child per year (4,5,6).

There is a wide variety of enteropathogens that causes diarrhea, including bacteria, viruses, and parasites. The most common cause of acute diarrhea is a viral infection. *Rotavirus* infection is a frequent cause of viral gastroenteritis in children, and is known to be the most common cause of severe acute, watery diarrhea in children under 5 years of age in industrialized and developing parts of the world (7,8). Another group of viruses that can cause diarrhea in infants and children up to 2 years are *Adenoviruses, Norwalkvirsuses, Norwalk* like viruses, *Caliciviruses* and *Astoviruses* (3).Bacterial pathogens that

are capable of causing diarrhea include *Clostridrium difficile,* Enteropathogenic *Escherichia coli* (EPEC), *Salmonella Spp.*, *Shigella dysenteriae*, *Campylobacter* (mainly *Campylobacter jejuni*), *Vibrio cholerae*, *Yersinia enterocolitica*, and *Aeromonas hydrophila* (2,3,9). There is also a variety of parasites that cause diarrhea; among them are *Cryptosporidium parvum, Entamoeba histolytica, Hymenolepis nana, and Giardia lamblia* (3, 9).

Other causes include side effects of antibiotics (10), infections not related to the gastrointestinal (GI) system (11,12), and the non-infective causes of diarrhea may be attributed to carbohydrate intolerance mainly lactase deficiency, this phenomenon may be transient or congenital (3), or persistent diarrheas that occur especially in infants from communities with low socioeconomic status, and low sanitary conditions (6).

The present study, was conducted to clarify the most common infectious etiologies of diarrhea in children up to 2 years of age, beside carbohydrate malabsorption mainly lactase deficiency.

Materials and Methods

A cross-sectional study from 26th January 2003 to 3rd March 2003 was conducted in 200 children up to 2 years old who were admitted with diarrhea to the Pediatric Hospital in kerkuk province. Stool specimens were collected in sterile plastic vials, the parents of cases were questioned about clinical symptoms, and other relevant clinical information by using a questionnaire. The questionnaire included patient name, age, sex, type of feeding (breast, artificial, and mixed), type of diarrhea (acute or persistent), previous antibiotics therapy, and clinical features.

Stool were subjected to the following investigations: Microscopic examination was done for all stool specimens, looking for fecal leukocytes, mucus and worm or segments of tape worm, red blood cells, pus cells, presence of parasitic ova, trophozoite or cyst which considered as etiology of diarrhea, and *Candida spp.* also was demonstrated in the form of *Candida* with or without pseudohyphae (6).

Stool reaction and Benedict's test are the most simple, specific and sensitive laboratory tests which determine presence of carbohydrates in feces. Benedict's test was done according to method used by Baghdasarian, 1999(3).

Enterobacteriaceae (*E. coli, Salmonella spp., Shigella spp,*)were examined in fecal specimens as specified by Ewing (13). Selective and differential media were used for isolating *Salmonella, Shigella* species, *Escherichia coli*: SS agar (*Salmonella-Shigella*), MacConkey, and thiosulfate-citrate-bile salts agars then incubated at 37°C for 18 to 24 hours, and then examined for characteristic colonies, and performing the routine ordinary diagnostic biochemical tests (14). For further identification of Salmonella *spp.* and *Shigella spp.* antisera and agglutination test was performed by using *Salmonella* poly specific test sera (Sifin, Berlin), (6).

Identification of enteropathogenic *E. coli (*EPEC) was performed by slide agglutination with commercial polyvalent sera (Darford, England), (15). After using polyvalent *E.coli, biochemical* tests. Lactose-positive or –negative was evaluated as potential Enteroinvasive *E. coli* (EIEC) isolates (16).

For parasite detection, fresh direct microscopic examination with saline solution and iodine was carried out with feces, allowing the observation of living and moving trophozoite stages of protozoa.

Latex agglutination test was performed by using Slidex Rota kit (Biokit, Spain)(17).

Germ tube test: Germ tube test is a reasonably reliable confirmatory test for *Candida albicans*. In this test, a suspension of a yeast colony is made into 0.5 ml of fresh serum which is incubated at 37 ºC for (2-4) hours, then a drop of yeast suspension is examined microscopically for germ tube. *Candida albicans* isolates are positive for germ tube test only in an interval less than 24 hours (3).

Results

From a total of 200 diarrheic children, we found EPD at higher prevalence 76(42.69%) in age group ranged from 7-12 month, where less cases with EPD 50(28.08%) found in age group (1day-6mon), although EPD found in other age groups but in less cases, as shows in Table (1).

According to sex distribution, among 178 positive cases 106 (59.55%) were males, while 72 (40.45%) were females, as shown in figure (1). Male to female ratio was 1.47:1. The prevalence EPD among breast feeding, artificially feeding, and mixed feeding children was non significant ($P<0.05$). Among 66 diarrheic children with breast feeding, 52 (29.2%) patients were had EPD. Where from 60 children artificially feed, 56 (31.46%) were had EPD in their stool specimens. On the other hand from 74 diarrheic children with mixed feeding, 70 children were detected with EPD, as shown in figure (2).

The distribution of EPD according to type of diarrhea is illustrated in Table (2), from a total of 154 children with acute diarrhea, 136(76.4%) showed EPD. Whereas from 46 children with persistent diarrhea, 42(23.6%) were recorded to have EPD.

From a total of 200 children with diarrhea, 178(89%) children showed a potential enteropathogenic diarrhea (EPD) by 226 enteropathogenes including: bacteria 66(33%), Rotavirus 56(28%), parasite 54(27%), and fungi 50(25%).

Enteropathogenic *E. coli* (EPEC) was isolated from 56(28%) diarrheic children, where *Salmonella spp.* was found in 10(5%) children. Among all samples tested, a rotavirus was identified in 56(28%) children. The parasites observed in association with diarrheic feces were *Entamoeba histolitica* in 44(22%) children, and *Giardia lambilia* 10(5%), also fungi isolated from children as *Candida albicans* 50(25%), (Table 3), with associated previous antibiotic therapy.

Simple diagnostic laboratory test, were performed upon 200 diarrheic children to identify carbohydrate intolerance mainly lactase deficiency by measuring the pH of stool specimens using litmus filter paper, and then using Benedict's test. Among 200 samples, 30 samples were showed pH less than 5 and positive Benedict's test, which indicates disaccharide malabsorption; 22 case (73.3%) out of 30 positive cases were associated with EPD (Table 4), while 8 cases (26.6%) of 30 positive cases were showed no EP in their fecal specimens. From 22 positive patient with lactose deficiency were associated with EPD, Rota virus was the commonest predisposing factor for occurrence of lactose deficiency 10(45.45%), also other EPD associated with lactose deficiency which are, *E. histolitica* 8(36.36%), EPEC 2(9.09%), *G. lamblia* 2(9.09%).

Discussion

Diarrhea is one of the gastrointestinal disturbances which is important cause of childhood morbidity and mortality in the developing world, although most of the episodes in infants, in Iraq remain a enormous public health problem (3). Approximately 48.9% of children admitted to three hospitals in Baghdad were suffering from diarrhea (1). Several studies of enteropathogens

associated with childhood diarrhea have been conducted in developing countries, but only a few analytical studies covering a broad range of newly discovered diarrheal agents have been undertaken in Europe (18,19,20,21). Many studies have focused on either bacterial or viral etiologies of diarrhea (22, 23,24)

In terms of the distribution of EPD in relation to age group, in this study children up to one years of age were more susceptible to EPD than older ones. EPEC strains are the major enteropathogenic agents in infants under one year of age, with the highest prevalence occurring in those under six months of age (25). Low incidence of EPD in children above one years of age might be due that childhood got immunity through intermittent or mild increasing exposure the infectious agents and being accustomed to have usual adult diet. The same results were reported in Mosul and Basrah (3, 26).

Regarding sex distribution, the infection between males and females did not differ statistically (P<0.05), male to female ratio was1.47:1 this was markedly in agreement with Mahdi (27).

In this study, the present of EP associated with breast feeding was 29.2% less than the percentage of bottle and mixed feeding which was recorded 31.46% and 39.32% respectively. The breast feed children passed EP in their stool specimen this may be due to poor hygienic condition of the child and the atmosphere that stay in, over crowding and over load in the world and contaminated air, but in bottle and mix feed childs the chance of passing EP in their stool specimen increase, this may be due to contamination during milk preparation. Contaminated complementary foods are primary sources of gastrointestinal pathogens (28).

Concerning to feeding nature, breast feeding have a protective effect in addition to simply shielding the infants

from exposure to possibly contaminated bottle feed. Breast milk has milk has specific protective elements including antibodies, macrophages and lymphocytes, also associated with improve growth, at least during the first months of life, their by reducing the risk of sever and persistent diarrhea (2). Other studies showed that both partial breastfeeding and no breastfeeding are significant risk factors for diarrhea. The authors conclude that promotion of breastfeeding is a major preventive measure against diarrhea in developing countries (29, 30).

With Respect to diarrhea nature, the rate of presence of EPD with acute diarrhea was found to be higher 136(76.4%) than persistent diarrhea 46(23.6%). The acute infectious diarrhea could be inflammatory or noninflamatory: Noninflamatory- caused by enterotoxin production by some bacteria, destruction of villus (surface) cells by viruses, and adherence or translocation by bacteria. Inflammatory - there is direct intestine invasion or citotoxins production by bacteria (31,32). EPEC strains are able to adhere to human intestinal tissue, inducing a typical lesion of attaching and effacing associated with fluid secretion and derangement of the digestive-absorptive enzymatic equipment, which is responsible for the persistence of diarrhea associated with malabsorption of the nutrients (33).

As indicated in Table (3) the bacterial enteropathogenes (EPEC, *Salmonella spp.*) were the most predominate etiology of diarrhea among children involved in present study, following by *Rota virus,* parasitic (*E. histolitica*, *G. lamblia*), and fungal diarrhea, these results agreed with other studies (2,28,34). In the study of diarrhea in man, especially in young infants, the principal microorganisms implicated are *salmonella*, *shigella* and enteropathogenic strains of *Escherichia coli* (EEC). The enteropathogenic strains

of *E. coli* are characterized by 2 factors: the ability to colonize the jejunum and the ability to cause movement of water and electrolytes across the intact intestinal epithelium into the bowel lumen (9), also the ability of *E. coli* to produce enterotoxin which is determined by a plasmid, designated as the "resistance transfer factor" or RTF, that can be transmitted from one strain of *E. coli* to another and to other species of bacteria by sexual conjugation (34).

Regarding to lactase intolerance, results in Table (4) shows that 30 patients were positive with lactase deficiency test, 22(73.3%) of them were associated with EPD, Rota virus was the commonest predisposing factor for occurrence of lactose deficiency, also other EP associated with lactose deficiency were *E. histolitica*, EPEC, *G. lamblia*, these result in agreement with that obtained in Mosul by Baghasarian (3). Transit form of the lactose deficiency is associated with EPD. Since the early seventies, have been advocating the use of lactose-free formula for young infants with severe acute diarrhea, because of the frequent occurrence of lactose intolerance reported, mainly in patients under six months of age or infected with EPEC strains (33,34). EPEC serogroups emerged as the most frequent enteropathogenic agent associated with food intolerance as a risk factor for death, mainly in infants under six months of age(10,34).

We recommended further studies to identify other EPD particularly *Campylobacter jejuni*, *Yersinia enterocolitica*, *Aeromonas hydrophilia*, *Vibrio cholera*, and *Cryptosporidium parvum,* also Rota virus test should be performed on each child with diarrhea admitted to hospital.

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Table (1): The distribution of EPD in relation to age group.

Age group (months)	No. of Children	Positive EPD*	
		NO.	%
1 day-6 mon.	60	50	28.08
7-12 mon	82	76	42.69
13-18 mon.	26	24	13.48
19—24 mon.	32	28	15.74
Total	200	178	100

P> 0.05

*EPD = Enteropathogenic Diarrhea

Table (2): The distribution EPD according to type of diarrhea.

Type of diarrhea	No. of Children	Positive EPD*	
		NO.	%
Acute diarrhea	154	136	76.4
Persistent diarrhea	46	42	23.6
Total	200	178	100

P>0.05

*EPD = Enteropathogenic Diarrhea

Table (3): Prevalent of EPD among diarrheic children.

Organisms	No. of Children	%	EPD *	No. of Children	%
Bacteria	66	33	*EPEC* *Salmonella spp.*	56 10	28 5
Virus	56	28	Rota virus	56	28
Parasite	54	27	*E. histolitica* *G.lamblia*	44 10	22 5
Fungi	50	25	*C.albicans*	50	25
Total	226			226	

*EPD = Enteropathogenic Diarrhea

Table (4): The distribution of EPD in relation to lactase deficiency.

EPD*	No. of Children	%
Rota virus	10	45.45
E. histolitica	8	36.36
EPEC	2	9.09
G. lamblia	2	9.09
Total	22	100

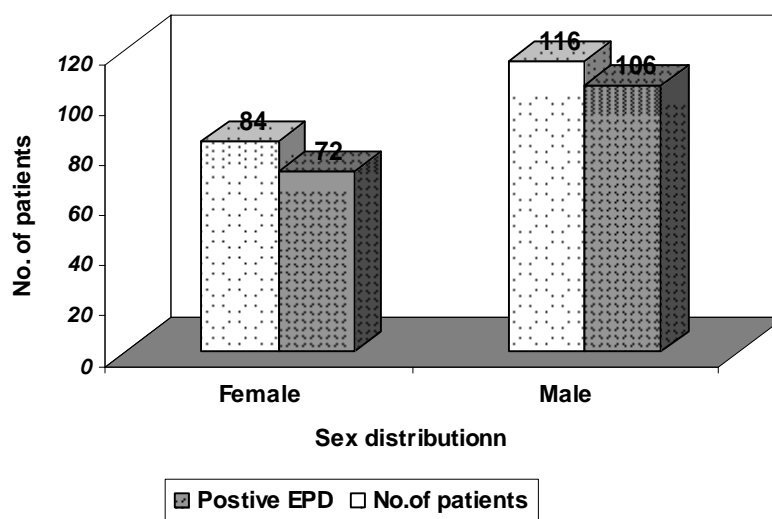*EPD = Enteropathogenic Diarrhea

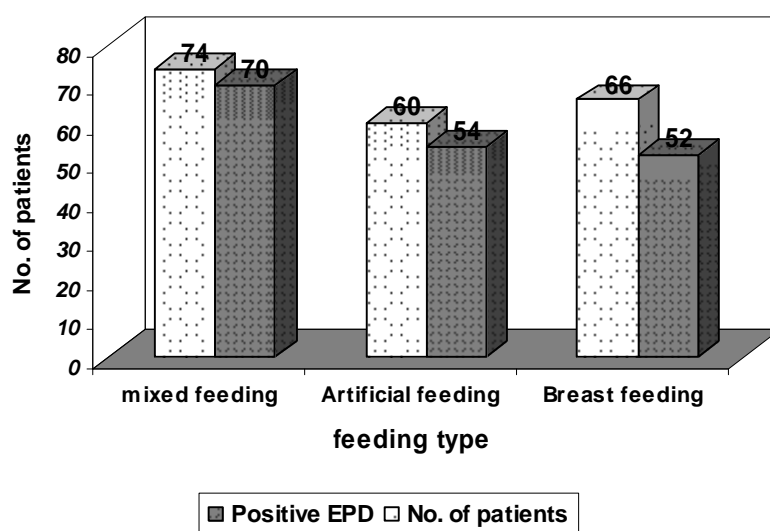**Figure (1):** The distribution of EPD according to sex distribution

Figure (2): The distribution of EPD according to feeding type.