ENTERIC PATHOGENS AMONG CHILDREN WITH DIARRHEA IN BASRAH MATERNITY AND CHILDREN HOSPITAL WITH SPECIAL EMPHASIS ON *YERSINIA ENTEROCOLITICA*

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ABSTRACT

The study was carried out at Basrah Maternity and Children Hospital from April to September 1999 on 200 patients between 1 to 60 months of age who were admitted to pediatric units as cases of diarrhea from total of 1461 diarrheal cases admitted to the hospital in the same period. It was found that female to male ratio was 1:1.3 most of them were infants (83%), exclusive breast feeding was present in only 11.4% of them, and most patients were malnourished 68.5%. Bacterial enteropathogens were isolated in the present study in 16/200 (8%), they were distributed as: Y. enterocolitica 1%, Campylobacter jejuni 2.5%, Salmonella typhimurium 1%, Pseudomonas aeruginosa 1%, Shigella spp. 0.5% and Vibrio cholera 2%. Protozoa were isolated in 22/200 cases (11%) as Entamoeba histolytica 16/200 (8%), Giardia lamblia 6/200 (3%).

INTRODUCTION

Yersinia enterocolitica is rapidly emerging world wide as an enteric pathogen associated with wide spectrum of clinical and immunological manifestations[1,2]. Y. enterocolitica has suppressed Shigella, Salmonella and Campylobacter as a cause of acute bacterial gastroenteritis[2]. These bacteria are non-lactose fermenting gram-negative rods that are urease-positive and oxidase-negative, they grow best at 25C°[3]. The incidence of *Y. enterocolitica* infection in patients with acute endemic enterocolitis ranges from zero to 4% depending on geographic location, study method and population[3]. Animals, food, water are the major reservoirs for *Y. enterocolitica*[1], pig is the only animal regularly harbors pathogenic *Y. enterocolitica*[4]. The most common presenting signs and symptoms are abdominal pain and diarrhea with fecal leukocytes[1,2], pharyngitis sometimes in association with cervical adenopathy has been reported with or without gastrointestinal illness[2]. *Y. enterocolitica* bacteremia occurs in 20-30% of infants younger than 3 months, other predisposing factors include cirrhosis, hemachromatosis, acute iron poisoning, desferoxamine therapy, immuno-suppressive therapy, diabetes mellitus and malnutrition[2]. In establishing invasive diarrhea, culture is the most useful diagnostic test. Yersinia selective agar is inoculated with stool and incubated at reduced temperatures of 25-32C°[1,3]. Uncomplicated enterocolitis is a self-limited disease and a benefit of antimicrobial therapy has not been established. Culture proven septicemia should be treated with aminoglycosides in combination with third generation cephalosporins[1,2]. Aminoglycosides in combination with third generation cephalosporins were effective[1,5]. Other important bacterial enteropathogens are: *Escherichia coli, Vibrio cholerae, Salmonella spp., Campylobacter spp., Staphylococcus aureus, Clostridium difficile, Shigella sp., Aeromonas hydrophila, Pseudomonas aeruginosa, Bacillus cereus, Enterobacter spp., Klebsiella spp.* These bacteria cause diarrhoea by synthesis of enterotoxins like Vibrio cholerae or adhere to the brush border membrane of the small intestine causing severe mucosal damage like *E. coli* or possess enteroinvasive properties like Shigella species[1,4,5]. Other important causes of infective diarrhea in children are viral agents, which are the most common cause like Rotavirus, Astrovirus, Parvovirus[1,4,5]. Protozoa also cause infective diarrhea like *Giardia lamblia, Entamoeba histolytica, Cryptosporidium*[1,4,5]. The aim of our study is to identify the incidence of *Y.enterocolitica* gastroenteritis among hospitalized children in Basrah Maternity and Children Hospital.

PATIENTS AND METHODS

The study was carried out at Basrah Maternity and Children Hospital from April to September 1999 on 200 patients between 1 to 60 months of age who were admitted to pediatric units as cases of diarrhea from total of 1461 cases (admitted during the

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same period). After admission history was taken concentrating on type of diarrhea (acute, persistent or bloody), type of feeding, then patients were examined including hydration status and growth assessment. Degree of malnutrition was assessed by applying weight for length/height using CDC/WHO normalized reference weight for length, height and patients were described to have normal built, or -2SD, -3SD, -4SD below the mean. Regarding investigations stool specimens were examined microscopically using , saline and iodine preparation for direct examination and also to detect parasitic infections.

Isolation and identification

For isolation of *Y. enterocolitica* stool samples were inoculated onto MacConkey's agar at 22-28°C and examined carefully after 48 hours. *Y. enterocolitica* appear as non-lactose tiny colonies, diagnosis was further confirmed by gram stain, biochemical tests and motility at 22-28°C.

Lactose-
On PG + (at 22-28 °C)
Motility + (at 22-28 °C)
Motility - (at 37 °C)
Urease +
Citrate -
Oxidase -
Suspected isolates were further confirmed by API system.

For isolation of Salmonella and Shigella stool specimens were inoculated onto tetrathionate broth and incubated at 27°C for 24 hours, on the 2nd day a loopful of grown culture was subcultured on salmonella-shigella (SS) agar, and inoculated at 37°C for further 24 hours, colonies were further identified by H_2S production, IMVIC test and motility. Diagnosis was confirmed by serological studies. *Enteropathogenic E-coli* (EPEC) was isolated from typical colonies on MacConkey's agar and was further identified by IMVIC test and serological studies. For *Staph. aureus*, golden suspected colonies on mannitol agar were further identified by coagulase test. For isolation of Pseudomnas aeroginosa, smooth round colonies with a fluorescent greenish colour were further identified by oxidase test. Proteus retegeri were also isolated from MacConkey agar and further identified by biochemical tests.

RESULTS

This study was a prospective study included 200 cases, male: female ratio was 1.3:1. Regarding age distribution it was found that most cases were infants 83% (Table-1), only 11.4% of them were exclusively breast fed (Table-2). Most of the cases (68.5%) were malnourished (Table-3). Table-4 demonstrated the isolated enteropathogenes and it was found that *Y. enterocolitica* was present in 1% of cases.

Table 1. ***Distribution of cases according to sex and age.***

Age in months	*Male*		*Female*	
	No.	*%*	*No.*	*%*
1-24	101	50.5	66	33
25-48	9	4.5	10	5
49-60	6	3	8	4
Total	116	58	84	42

Table 2. ***Type of feeding in different age groups.***

Age in months	*Breast*		*Bottle*		*Mixed*		*Ordinary*	
	No.	%	No.	%	No.	%	No.	%
1-24	19	11.4	70	91.9	75	44.9	3	1.7
25-48	3	15.7	2	10.5	4	21	10	52.6
49-60	-	-	1	9	-	-	13	91
Total	22	-	73	-	79	-	26	-

Table 3. ***Nutritional status of cases.***

Status	*No.*	*%*
Normal	63	31.5
-2SD	53	26.5
-3SD	46	23
-4SD	38	19

Table 4. *Enteropathogens isolated from diarrhea cases.*

Enteropathogen	*No.*	*%*
Y. enterocolitica	2	1
Enteropathogenic E. coli	5	2.5
Shuggella spp.	1	0.5
Salmonella spp.	2	1
Pseudomonas aeruginosa	2	1
Vibrio cholera	4	2
Entamoeba histolytica	16	8
Giardia lamblia	6	3
Total	38	19

DISCUSSION

Y. enterocolitica has been described with increasing frequency in different countries, mostly of temperate climate[1,6,7]. Many studies were done to identify its incidence in different countries like Jordan, Italy, Denmark, USA, and UK and also Basrah [1,2,3,4]. In our study 200 diarrheal cases were investigated to identify the incidence of *Y. enterocolitica*, most of these cases were infants 83% (Table-1), this finding is consistent with the fact that diarrhea is most frequent in younger ages because infancy is regarded as one of the factors that increases susceptibility to infection with enteropathogens[1]. Regarding type of feeding exclusive breast feeding was present in only 11.4% of infants, so breast feeding is less in diarrheal cases, this result is explained by the role of breast milk in decreasing the infections because breast milk is a living tissue that consists of lymphocytes and macrophages with immune activity[1,5]. In relation to nutritional status, it was observed that most diarrheal cases were malnourished (68.5%) because diarrhea is common in malnutrition which is present with secondary immunodefficiency that increases susceptibility to infection with enteropathogens[5,9]. *Y. enterocolitica* was found in 1% of our cases (Table-4), while it was found in 2.7% of diarrhoeal cases in a study done in Tahreer Hospital (in Basrah) at 1997[3]. In a study done in Denmark in 1996, it was found in 13% of gastroenteritis cases[6], in other study at Northern Jordan in 1994, *Y. enterocolitica* was found in 4.5% of cases[10]. In Nigeria in 1993 *Y. enterocolitica* was found in 1.5% of cases[4]. The percentages of isolation of *Y. enterocolitica* in 3 studies done in 1994, 1997, 1999 in Nigeria, Island of Crete, and Tuscany were found to be 0.9%, 0.3% and 0.1%respectively [14]. While it was observed in a limited number of patients with diarrhea in a study done in Italy in 1994[15], it was not detected from stool samples of patients with diarrhea in two studies at United Kingdom and Singapore in 1994, 1992[11,16], so the incidence of *Y. enterocolitica* was variable, this can be explained by different geographic location of patients and study method and population, difference in reservoirs, or may be simply a consequence of more invasive surveillance and culturing techniques in these areas. Relatively few cases have been suggested that tropical climate may not favor propagation of the organism[4]. The infrequent occurrence of *Y. enterocolitica* in some areas of the world may be related in part to avoidance of certain environmental risk factors such as eating pork in Muslim countries[8,17]. Regarding other types of bacterial enteropathogens *Enteropathogenic coli*, *Vibrio cholera*, *Salmonella*, *Pseudomonas aeruginosa* and *Shigella spp.* were found at 2.5%, 2%, 1%, 1%, 0.5% respectively while their percentages were 27.8%, 0%, 1.5%, 2.3%, in a study done in 1997 in Basrah (Al-Tahreer Hospital). *Entamoeba histolytica*, and *Giardia lamblia* were present in 8%, 3% of our cases respectively (Table-4) while both were present in 44.9% of cases in Basrah City in a study done in 1991 by Al-Kerwi[18]. We did not demonstrate viral enteropathogens because no kits were available at the time of the study. From this study we conclude that *Y. enterocolitica* is an important enteropathogen and it is important to be put in mind when dealing with diarrheal cases whether on first assessment or when investigations are done to these cases because some cases are fulminate but treatable. Knowledge of incidence is important to encourage sanitary precautions while dealing with farm and domestic animals to help in prevention and control program to end in decreasing its occurrence. We recommend prolongation of period of study for two purposes. First, the chance to isolate *Y. enterocolitica* is more in cold whether and, second, to isolate more cases helping in study of their characteristics and presentations.

ACKNOWLEDGMENT

I would like to express my great thanks to Dr. Jehan M. Al-Mukh M.B.Ch.B., M.Sc. Microbiology Department in Basrah Maternity and Children hospital for her support and help.

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