Determination of the prevalence of viral aetiology of diarrhea in children less than 5 years of age in Baghdad province

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الخلاصة

اجريت الدراسة تحديد مدى انتشار المسببات المرضية الفيروسية والطفيلية والجرثومية المؤدية إلى التهاب المعدة والأمعاء في الأطفال بعمر أقل من 5 سنوات مع أعراض الإسهال في محافظة بغداد من مستشفيات مختلفة .

تم جمع ما مجموعه 250 عينة براز من الأطفال من مستشفيات مختلفة من محافظة بغداد تم التحري عن الممرضات المعوية enteropathogens البكتيرية والطفيلية باستخدام الفحص ألمجهري. وقد تم زرع عينات البراز على مختلف الأوساط الزرعية MacConkey, XLD, DCA, TCBS and campylobacter media, TCBS and selenite F broth وتمت تشخيص البكتيريا بواسطة المقايسة المعتمدة على (Api20E)والأمصال المضادة بالإضافة إلى خصائص المستعمرات البكتيرية. واستخدمت طريقة فحص مقايسة ألماز المناعي للأنزيم المرتبط ELISA لتحري فيروس الروتا Rotavirus ، Astrovirus وadenovirus المعوية.

تم فحص ما مجموعه 250 عينة براز من الأطفال دون سن 5 سنوات من العمر و من هؤلاء تم تشخيص 106 طفلا (42.4٪) كانت لديهم المسببات الفيروسية وان فيروس الروتا سجل معظم الحالات 91 (36.4٪)، ويأتي بعده adenovirus المعوية في 15 (6٪) من المرضى.

وكان 63 (25.2٪) مسببات بكتيرية منها 36 (14.4٪) كانت القولونية (ETEC) و 12 (4.8٪) كانت الشيغيلا الفلكسنرية، 6 (2.4٪) كانت Campylobacter jejunii و9 (3.6) كانت السالمونيلا الملهبة. وكانت المتحولة الحالة للنسج 22 (8.8٪)، والجياردية اللمبلية 22 (8.8٪) الطفيليات الأكثر شيوعا التي تم عزلها من عينات البراز

أيضا من اجمالي 250 عينة كانت 40 عينة (16٪) عينات البراز زحاري (الإسهال الدموي) ومسببات الأمراض الأكثر شيوعا المعزولة من عينات البراز الزحاري كانت المتحولة الحالة للنسج 22 (8.8٪) و12 (4.8٪) كانت الشيغيلا الفلكسنرية و 6 (2.4٪) كانت Campylobacter jejunii

كشفت الدراسة الحالية إلى ارتفاع معدل انتشار فيروس الروتا وكان السبب الأكثر شيوعا للإسهال في المستشفيات العراقية في الأطفال بعمر أقل من 5 سنوات .

Abstract

Determination   the prevalence of viral, parasitic and bacterial etiology of gastroenteritis in children less than 5 years of age with diarrhea in various hospitals of Baghdad province.

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A total of 250 stool samples were collected from various hospitals of Baghdad province .Samples were investigated for bacterial and parasitic enteropathogens using microscopic examination. Bacterial culture of stool samples was done in different culture media including MacConkey, XLD, DCA, TCBS and campylobacter media, TCBS and selenite F broth and bacterial growth was examined by analytical profile index (Api20E) system , commercial antisera and colonies characteristics. Enzyme immunoassays were used to identify Group A rotavirus ,Astrovirus and enteric adenovirus

Out of 250, 106 children (42.4%) were diagnosed as viral infection of which Rotavirus was found in the majority of cases 91 (36.4%), Adenovirus was detected in 15(6%) patients.

bacterial causes were 63 (25.2%) of which 36 (14.4%) were *Escherichia coli* (ETEC), 12 (4.8%) were *Shigella flexneri* , 6(2.4%) were *Campylobacter jejunii* and 9(3.6) were *Salmonella enteritidis* . *Entamoeba histolytica* 22 (8.8%) and Giardia lamblia 22 (8.8%) were the most frequent parasite which isolated from all diarrheal stool specimens.

Also from total of 250 samples, 40 samples (16%) were dysenteric stool specimens (bloody diarrhea) and the most common pathogens isolated from dysenteric stool specimens were *E.histolytica* 22(8.8%), *Shigella flexneri* 12(4.8%) and *Campylobacter jejunii* 6 (2.4%).

The current study revealed a high prevalence of rotavirus as most common cause of diarrhoea in children less than 5 years of age**.**

Introduction

Diarrheal disease is a major public health problem throughout the world, with over two million deaths occurring each year, mostly children under 5 years of age in developing countries (1). There is a wide range of recognized enteric pathogens such as viruses, bacteria, and parasites that cause diarrhea. Among bacteria, *Shigella spp*. and diarrheagenic Escherichia coli (DEC) are the

most common causes of diarrheal diseases in developing countries (2).

Gastroenteritis is defined as an inflammation of the stomach, large and small intestines (3) . It is one of the most common illnesses in humans worldwide (4). Although it can affect individuals of any age, it presents a significant health risk to those at extremes of age, the very young and the very old (5). It is the second most common cause of death among adults, and the leading cause of childhood death worldwide (6). In children under 5 years, there are more than700 million cases of gastroenteritis every year. The annual mortality associated with gastroenteritis has been estimated to be 3.5 to 5 million, with the majority of deaths occurring in developing countries (7).

Viral gastroenteritis is diseases worldwide and causing significant morbidity and mortality in children.(8) Four major viral pathogens are associated with gastroenteritis, three of them are RNA viruses (Rotavirus, Norovirus, and Astrovirus) and one DNA virus (enteric Adenovirus).(9)

The aim of this study was to determine the prevalence of bacterial, parasitic and viral etiology of gastroenteritis in children less than 5 years of age presenting with diarrhoea in various hospitals of Baghdad province

Patients and Methods

Study was carried out on 250 patients were suffering from diarrhea their ages below 5 years old (135 males and 115 females) attending to Baghdad Teaching Hospital, teaching laboratories ,Pediatric Al- Mansour teaching hospital in Baghdad Medicine city and Alsader hospital in Baghdad province. Stool samples were collected from each child in disposable clean containers and processed within 2 hours of collection.

Macroscopic examination of stool samples was done for the presence of worms (*Ascaris lumbricoides*, *Strongyloides stercoralis*, *Enterobius vermicularis*, *Trichuris trichiura* and *Taenia saginata*), blood and physical characteristics such as color, appearance and odor .Microscopic examination of stool samples

was done under 10x and 40x power after being stained with iodine for the presence of parasitic eggs and larvas. Bacterial culture of stool samples was done for the presence of a common enteric bacterial pathogens such as: *Salmonella*, *Shigella*, *Campylobacter*, Enteropathogenic *E.coli*, Enterotoxigenic *E.coli* and Vibrio cholera. These were performed by culturing the stool samples in different culture media including MacConkey, XLD, DCA, TCBS and selenite F MacConkey, XLD, DCA, campylobacter media, TCBS and selenite F broth broth culture media were incubated at 37ºC for 24 hours and samples incubated in selenite F broth were re-cultured in XLD media for another 24 hours at 37ºC. Campylobacter media were cultured at 42ºC for 48 hours Any bacterial growth was examined by standard microbiogical methods such as specific biochemical tests analytical profile index (Api20E) system and commercial antisera in addition to bacterial colonies characteristics.

Stools were screened for enterohemorrhagic E. coli by plating on sorbitol-MacConkey agar. All non-sorbitol-fermenting colonies were tested in an agglutinating assay with O157 and H7 antisera (2).
Samples were also tested for the presence of gastroenteritis viruses (Rotavirus, Adenovirus type 40/41 and Astrovirus) using antigen detection ELISA kits [ACON laboratories, incorporated (Inc), USA] as described in manufacturers instruction. In brief, washing buffer was diluted 1:10 with distilled water and samples were also diluted 1:11 with sample diluents. Hundred microliters of samples, positive and negative control were added to corresponding microtitre plate wells followed by the addition of 100Tl conjugate. Plates were then incubated at room temperature for 60 minutes before being washed 5 times with 300Tl diluted wash buffer using an ELISA washer (ETL testing laboratories Inc. USA). Hundred microliters of substrate were then added to all wells, before incubating the plates in dark for 15 minutes. Fifty microliters of stopping solution were then added before reading the results at an ELISA plate reader [E.ma, precision, microplate reader, USA] at 450 nm.

Results

Out of 250 children under 5 years of age were examined106 (42.4%) were diagnosed with viral etiology of which Rotavirus was found in the majority of cases 91 (36.4%), Adenovirus was detected in 15(6%) patients.

bacterial origin were 63 (25.2%) of which 36 (14.4%) were *Escherichia coli* (ETEC), and the other bacterial enteropathogens isolated from diarrheal stool specimens were *Shigella flexneri* 12 (4.8%) and *Campylobacter jejunii* 6(2.4%) and *Salmonella enteritidis* 9(3.6). The result of the serotyping of the bacteria in the Central Public Health Laboratories/ Ministry of Health in Baghdad city has ensured that these bacteria are *Escherichia coli* (ETEC), *Shigella flexneri*, *Salmonella enteritidis* and *Campylobacter jejunii*.

Also Data illustrated in table and figure (-1) also demonstrated that the most frequent parasite which isolated from diarrheal stool specimens were *Entamoeba histolytica* (22,8.8%) and *Giardia lamblia* (22,8.8%) also data reported in table and figure (-1) showed that (37,14.8%) children were with no causative agent isolated from their diarrheal stool samples.

Table (-1) Distribution of etiological agents which caused diarrheal stools of children under five years age

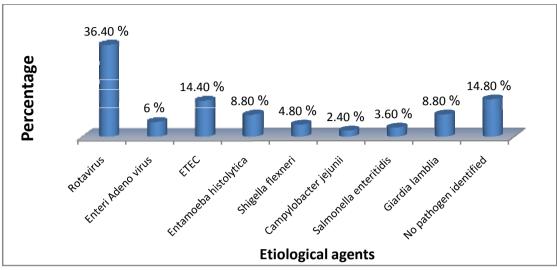| Etiological Agent	Number of cases	Percentage of cases
Rotavirus	91	36.4%
Enteri Adeno virus 40/41	15	6%
Enterotoxigenic Escherichia coli (ETEC)	36	14.4%
Shigella flexneri	12	4.8%
Campylobacter jejunii	6	2.4%
Salmonella enteritidis	9	3.6%
Entamoeba histolytica	22	8.8%
Giardia lamblia	22	8.8%
No pathogen identified	37	14.8%
Total	250	100%

Figure (-1) Distribution of etiological agents which caused diarrheal stools of children under five years age

Table (-2) showed the relation between the age of child and the incidence for each one of the causative agent of diarrhea , where all children who had Rotavirus as causative agent of diarrhea were below age 3 years , in contrast all children who had *Entamoeba histolytica* or *Giardia lamblia* as causative agent of diarrhea were above two years.

Also from the same table and figure all children who have unknown causative agent of diarrhea were under two years.

Table (-2) : Distribution of positive stool samples according to age differences.

Age group in years	Rotavirus	Adenovirus	ETEC	Entamoeba histolytica	Shigella flexneri	Compylobacter jejunii	Salmonella enteritidis	Giardia lamblia	No agent present
< year	20	4	5	---	---	---	---	---	28
1-2 year	31	6	14	---	3	---	---	---	9
2-3 years	40	5	17	4	3	1	---	---	---
3-4 years	---		---	6	6	5	4	5	---
4-5 years	---		---	12	---	---	5	17	---
Total	91	15	36	22	12	6	9	22	37

Of the total of 250 samples, 40 samples (16%) were dysenteric stool specimens (bloody diarrhea) and the most common pathogens isolated from dysenteric stool specimens were *E.histolytica* 22 (8.8%) *Shigella flexneri* 12(4.8%) and *Campylobacter jejunii* 6 (2.4%) as were observed in table (-3)

Table (-3) Distribution of etiological agent which caused dysenteric diarrheal stool from 150 diarrheal stool specimens.

Etiological agent	Number of cases	Percentage of cases
E.histolytica	22	8.8%
Shigella flexneri	12	4.8%
Campylobacter jejunii	6	2.4%
Total	40	16%

Table (-4) showed that non dysenteric stool specimens were observed in (84%) of all diarrheal stool specimens and the pathogens which isolated from non dysenteric stool were rotavirus (36.4%), adenovirus (6.0%) , Escherichia coli 14.4%, Salmonella enteritidis (4.7%) , Giardia lamblia 8.8% and unknown etiological agent (14.8%) .

Table (-4) : Etiological Agent which caused non dysenteric diarrheal stool

Etiological Agent	Number of cases	Percentage of cases
Rotavirus	91	36.4%
Adenovirus	15	6.0%
Enterotoxigenic Escherichia coli (ETEC)	36	14.4%
Salmonella enteritidis	9	3.6%
Giardia lamblia	22	8.8%
Unknown etiological agent	37	14.8%
Total	210	84%

Discussion

Diseases causing diarrhoea are the most important cause of morbidity and mortality in developing countries Five hundred million cases of acute diarrhoea occur annually in children aged less than five years throughout the world.(10,11)

The sex distribution among the children in our study showed a slight preponderance of diarrhoea in males compared with females (135 males and 115 females) . Similar findings have been reported in other studies in Iraq (12,13,14)

In the present study, enteropathogens were detected in 213/250 (85.2%) paediatric patients under five years of age. The present study clearly shows that Rotavirus is the most common cause of acute gastroenteritis among children 90 (36.4%). This agrees with Al-Khashab (12) and Al-Kaby (13) from Iraq, as well as (15) and Kamalaratnam et al. from India (16), and Gusmao et al. from Brazil (17) however, our rotavirus prevalence is slightly lower than that reported in Central Saudi Arabia, the prevalence was 44.3% in the under 5 year age group

In a study conducted in Karachi, Pakistan, Rotavirus was detected in 112/818 (13.7%) of stool samples from infants with diarrhea (18). However, in another study carried out in India, Rotavirus was found in 22.6% of pediatric patients, who fell into the age group <1-3 years (19) . In the United Kingdom, Rotavirus infection prevalence was found to be 36% in children under 16 years of age (20) while the prevalence rate of Rotavirus in different cities of South Africa varied from 13% to as high as 55% in under 5 year of age (21) and in the USA the rate was 6.8% in children less than 6 years of age (22). In Lima, Peru, rotavirus was significantly associated with acute diarrhea(AD) as compared to children with persistent diarrhea (23). In northeastern Brazil, only 3.7% of children presenting to a clinic had rotavirus identified in their stool.(24)

Adenovirus was the second most common virus to be found with a prevalence rate of 6%. Other studies done in different parts of the world, showed prevalence rates ranging from as low as 0.8% in under 6 years old in the USA(22) to as high as 28% in children

under12 years of age in Ghana.(25) Our results were in accordance with the reported prevalence of Adenovirus but this is in contrast to one study which found Astrovirus to be more common than Adenovirus.(22) When compared to other studies done worldwide it was reported that the prevalence of Astrovirus ranged from0.4% in the under 5 year old age group in Tanzania(26) to 9.2% in <1 to 4 year old children in Saudi Arabia (27) .

Bacteria isolated in the current study were 25.2%, of which Escherichia coli (ETEC) made up 14.4%, 4.8% was due to *Shigella flexneri* ,3.6% was due to *Salmonella enteritidis* and 2.4% was due *Campylobacter jejunii*. The result of the serotyping of the bacteria in the Central Public Health Laboratories/ Ministry of Health in Baghdad city has ensured that these bacteria are *Escherichia coli* (ETEC), *Shigella flexneri*, *Salmonella enteritidis* and *Campylobacter jejunii*

In Brazilian children, ETEC was found to be associated with persistent diarrhea. (28) Whereas, in rural India, ETEC was found significantly more often in children with acute diarrhea than in controls.(29) ETEC was identified more frequently in children with PD compared to AD in rural Indian, but the association was not statistically significant.(30) In Vietnam, in a sample of children experiencing PD, ETEC was the most frequently identified etiologic agent

Salmonellosis has considered one of the most common causes of diarrheal diseases in in Iraq by (31) who found that children more than five years old are more susceptible to Salmonellosis and with study of (32) who record that the Children aged 2 are the most affected age group (incidence 391.6 per 100 000 population).

In two other studies done worldwide, Salmonella was found in 5.8% of patients in Australia in <1 up to 14 years of age(33) and Shigella was found in 6% in Palestine in children under 5 years of age.(34)

In addition our study's demonstrated that the most frequent parasite which isolated from diarrheal stool specimens were *Entamoeba histolytica* (22,8.8%) and *Giardia lamblia* (22,8.8%)

and all children who had *Entamoeba histolytica* or *Giardia lamblia* as causative agent of diarrhea were above 2 years age. We believe that children who aged younger than 2 years are under the supervision and care of their mothers, while older children are distance from their mothers and they play more that make them near from contaminated water sources that are the principle source of *Entamoeba histolytica* and *Giardia lamblia*. *Giardia lamblia* is a common intestinal protozoa present in 20-30% of people in the developing world.(35). In children living in northeastern Brazil, *Giardia* was frequently identified in children with persistent In other studies, an association was found between *Giardia* and PD in northeastern Brazil and in a New Delhi slum.(36) however, *Giardia* was identified infrequently in children living in Bangladesh and there was insignificant association

In this results have shown that 40 (16%) were dysenteric stool specimens (bloody diarrhea) and the most common pathogens isolated from dysenteric stool specimens were *E.histolytica* 22 (8.8%) *Shigella flexneri* 12(4.8%) and *Campylobacter jejunii* 6 (2.4%)

During the period of the current study, no clinical evidence of vibriosis due to *Vibrio cholerae* or food poisoning due to *Staphylococcus aureus* was found. other bacteria that are sometimes incriminated in diarrhoea among children, such as *Yersinia enterocolitica* (15)

This study have reported that 37(14.8%) children were with no pathogenic agent isolated from their diarrheal stool samples.

We have noticed that all children who have no pathogen identified of diarrhea were under two years, to interpret the high percentage of unknown causative agent of diarrhea ,this may be occurred following a change in the type of milk intake by child or the addition of a new type of food for the child, especially if it was not appropriate for the age also numerous other factors not related etiology have been identified as characteristics influencing of diarrhea. These include malnutrition (37-39) maternal education level(40,41) previous illness(42,43) time of weaning.

Conclusion

The current study revealed a high prevalence of rotavirus as most common cause of diarrhoea in children less than 5 years of age

Recommendation

Accordingly, we recommend routine general stool examination, stool culture/sensitivity and detection of rotavirus and enteri Adeno virus by ELISA methods for all patients with diarrhoea to identify the causative agent involved.

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