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STOOL VIRUSES AMONG PAEDIATRIC PATIENTS FROM A NAIROBI CLINIC, KENYA

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ABSTRACT

Objective: **To document the clinical presentation and prevalence of stool viruses among children presenting with symptoms of acute gastroenteritis (AGE) at Gertrude's Garden Children's Hospital, Nairobi, Kenya.**

Design: **Retrospective case-control study.**

Setting: ***A*** **private paediatric clinic in Nairobi.**

Results: **Viral antigen was detectable in the stool samples of 21 (rotavirus alone in ten cases, adenovirus alone in seven cases, and both viruses in four cases). Diarrhoea was almost universally present (20/21 cases) and was reported more frequently than in a control group of ten children with clinical acute gastroenteritis whose stools tested negative for viruses. Fever, an elevated total leukocyte count, and neutrophilia were commonly observed in patients with viral gastroenteritis. Eight children with viral AGE were treated with antibiotics and eight children were admitted to hospital.**

Conclusion: **A viral etiology can frequently be identified among children in Nairobi with AGE. Fever, an elevated leukocyte count, and neutrophilia were not helpful in differentiating viral from non-viral AGE in this series. Supportive management consisting of outpatient oral rehydration therapy without antibiotic treatment should be considered in the non-toxic child with AGE.**

INTRODUCTION

Acute gastroenteritis (AGE) is a significant cause of morbidity and mortality worldwide, annually affecting an estimated 700 million children under the age of five(1). Mortality in this age group is estimated at 3-5 million deaths per year, occurring mainly in developing countries(1). Viruses have been implicated as aetiologic agents of AGE since 1972, when Norwalk virus was discovered during a gastroenteritis epidemic. Shortly thereafter, rotavirus(2) and adenovirus(3) were identified in the clinical specimens of affected children. Currently, rotavirus accounts for 20-60% of cases of diarrhoea requiring hospitalisation. Adenovirus is the aetiologic agent in 2 to 31% of childhood diarrhoea in developing countries(1).

Rotaviruses are non-enveloped, double-stranded RNA viruses, with a double layer protein capsid and icosahedral structure. The VP6 antigen of the inner capsid layer was used in this study for immunologic detection of rotavirus in stool samples. Adenoviruses are non-enveloped DNA viruses with icosahedral symmetry and a protein capsid composed of hexones and pentones; the hexone antigen was used for adenovirus detection in this study. While multiple adenovirus serotypes cause human disease including upper respiratory tract infection and conjunctivitis, serotypes 40 and 41 are most frequently implicated in AGE. The pathogenesis of both agents involves infection of enterocytes, with villous atropy, compensatory hyperplasia of secretory crypt cells, and subsequent mal-absorption and fluid loss(1).

Treatment of viral gastroenteritis is symptomatic, aiming at preventing or correcting dehydration through oral, nasogastric or intravenous fluids. The role of antibiotics in AGE is limited to infections with *Shigella* spp., *Vibrio cholera,* severe unresponsive infections with *Salmonella, Yersinia, Aeromonas, Campylobacter, Plesiomonas* spp., and *Clostridium difficile,* and antibacterials are contra-indicated in enterohemorrhagic *Escherichia coli* infection(4). Oral rehydration is an effective and safe supportive measure, with a failure rate requiring IV hydration in only 3.6% of cases in one meta-analysis(5).

This study aimed to elucidate the aetiology of AGE among children presenting to a private paediatric practice in Nairobi, Kenya. This information may be of assistance to local physicians who care for children with AGE in guiding empiric therapy, potentially reducing the inappropriate use of antibiotics in a predominantly viral illness and the rate of hospitalisation for a self-limited disease which can be effectively

managed with oral rehydration. This study adds to previously published information on aetiologic agents of AGE in Kenya, gathered from specific urban, coastal, and rural populations(6-8).

MATERIALS AND METHODS

The setting of this study was a private paediatric clinic in Nairobi, Kenya between 25 August 2003 and 31 January 2004. Results of 49 stool samples from children with a clinical diagnosis of acute gastroenteritis submitted to the microbiology laboratory at Gertrude's Garden Children's Hospital were retrospectively reviewed. Samples were routinely analysed for the presence of parasitic ova and cysts, and were routinely cultured for the presence of bacterial pathogens *(Salmonella* spp., *Shigella* spp., *E.Coli).* Rapid antigen detection for rotavirus and adenovirus was performed in selected cases, based on clinical presentation. A commercially available test (Simple Rota-Adeno, Operano, Spain) utilised latex-conjugated rotavirus antibodies against antigen VP6 of group A rotavirus (red), and latex-conjugated antibodies against the adenovirus hexon antigen (blue) to detect the viruses from stool samples.

Clinical information was gathered retrospectively by chart review on 21 specimens that tested positive for stool viruses and a control group of 10 samples which were negative for viruses. A third group of 18 samples in which testing for viruses was not performed (submitted only for routine microscopy and culture) was examined, but was not included in the statistical analysis since the virus status was unknown. Statistical comparison of proportions was by one-sided *z-test,* and comparison of groups by age and total leukocyte count (continuous variables) was by Student's *t-test* (one-sided).

RESULTS

Forty-nine stool samples from 48 patients were reviewed (on patient had two stool samples submitted during the study period). Patients were between the ages of one month and sixteen years and 80% were from Nairobi. Thirty-one samples were submitted for rapid detection of viral antigens. Patients whose stool samples were not submitted for viral analysis were generally older (mean age 5.8 years vs. 2.5 years; p=0.005), tended to have less diarrhoea (3/18=17% vs. 26/31=84%; p<0.001), and in four cases were being re-tested for parasitic infection. Ten samples were positive for rotavirus, seven for adenovirus, and in four samples, both adenovirus and rotavirus were detected. Other enteric pathogens detected were *Entamoeba histolitica* (3), *Blastocystis hominis* (2), *Endolimax nana* (1); furthermore, a diagnosis of shistosomiasis was made in two cases based on serologic testing. No enteric bacterial pathogens were identified in this series.

It was not possible to estimate the prevalence of stool viruses among children with clinical gastroenteritis from the 49 samples included in this series because they did not constitute a consecutive set. However, information was available on a subset of 56 consecutive stool samples submitted between 7 December 2003 and 21 January 2004. Thirteen stool samples (23%) were positive during this period. This represents a minimum estimate since 16 stool samples were not tested for viruses and clinical information was missing in a further 17 cases (these were assumed to be negative for stool viruses).

Among children with rotavirus-positive stools, 8/10 had fever, 9/10 had diarrhoea, 9/10 had vomiting and 2/10 had abdominal pain. Other symptoms and signs reported included dehydration(4), irritability(1), and anorexia(1). Among children with adenovirus-positive stools, none had recorded fever, all had diarrhoea, 2/7 had vomiting and none reported abdominal pain. Also reported were symptoms of upper respiratory tract infection(1), abdominal distention(1) and anorexia(1). When both viruses were detected, fever was present in 2/4 cases, diarrhoea in all cases, vomiting in 1/4 and abdominal pain in 1/4. A variety of symptoms were reported among patients with other pathogens including fever, abdominal pain, vomiting, headache, weakness, mucous in stool *(Entamoeba histolitica);* vomiting, abdominal pain, haematochezia, cough, haematemesis, lethargy (schistosomiasis); abdominal pain, myalgia *(Endolimax nana);* and lethargy *(Blastocystis hominis).*

A complete blood count (CBC) was obtained in *6/10* children with rotavirus, 2/7 with adenovirus, and in 2/4 with both viruses. Among children with stool viruses who had a complete blood count, an elevated total leukocyte count (TLC) was seen in 2/10 and neutrophilia was seen in 7/10.

Comparison was made between children who had virus-positive stools and a control group of ten children whose stools tested negative for viruses. This information is summarised in Table 2, as well as a third group of 18 cases for which viral testing was not conducted, although this group is not included in the statistical analysis since viral status was unknown. There was a trend toward lower age in the viruspositive group, although this difference was not statistically significant at the a=0.05 level (mean age 1.8 years vs. 3.9 years; p=0.08). Diarrhoea was present significantly more frequently among those with stool virus (20/21=95% vs. *6/10=60%;* p=0.02). No difference was observed in the prevalence of fever (p=0. 18) and vomiting (p=0. 19) between groups. Abdominal pain was noted less frequently in children who were stool virus positive than in the control group (3/21=14% vs. 6/10=60%; p=0.006). Total leukocyte count was similar in both groups (mean 10.4 vs. 8.8; p=0.47), as was proportion of children with neutrophilia (7/10=70% vs.3/4=75%;p=0.43).

Table 1

Comparison of clinical characteristics and management of patients testing positive (+) and negative (-) for stool viruses

	Stool virus positive (n=21)	Stool virus negative (n=10)	Stool not tested (n=18)	P-value (virus + vs virus-)
Demographics				
Mean age (years)	1.8	3.9	5.8	0.08
Proportion female (%)	33	40	45	0.36
Prevalence of				
Diarrhoea	95	60	17	0.02
Vomiting	62	40	6	0.19
Fever	43	30	17	0.18
Abdominal pain	10	60	33	0.006
Laboratory values				
Mean WBC count (x 10^6/L)	10.4	8.8	9.0	0.47
Neutrophilia (%)	33	30	6	0.43
Management				
Hospitalised (%)	38	0	6	<0.001
Antibiotics prescribed (%)	43	20	11	0.09

Table 2

Prevalence of rotavirus and adenovirus in stool samples of children with AGE

Country	Number of Patients	Rotavirus (%)	Adenovirus (%)	Reference
Africa				
Ghana	1717	39	n.r.	6
Kenya-Malindi	862	16.1	n.r.	7
Kenya-Nairobi	36	39	0	8
Kenya-Rift Valley	70	0	n.r.	9
Nigeria	108	33.3	6.7	10
South Africa	1142	55	n.r.	11
Asia				
Bahrain	805	13.9	0.6	12
Bangladesh	4409	23.6	2.3	13
Indonesia	3875	37.5	3.3	14
Korea	345	68	9	15
Thailand	103	18	10.7	16
Vietnam	123	39	1	17
South America				
Argentina-Buenos Aires	66	25	3	18
Argentina-Cordoba	133	35.3	1.5	19
Brazil-Goiana	2605	14.4	n.r.	20
Brazil-4 cities	1420	n.r.	1.55	21
Australia				
Australia	412	13.2	8.6	22
Europe				
England	305	27.9	7.9	23
France	438	17.3	0.7	24
Germany	217	47	8	25
Greece-Athens (inpatients)	132	14	4	26
Greece- Athens (outpatients)	294	11	2	27
Italy	417	18.2	7	28

n.r = not reported

Eight patients with stool viruses were hospitalised, seven of whom had rotavirus. This was significantly greater than hospitalisations among children without stool viruses (8/21=38% vs. 0/10=0%; p<O.OOI). Antibiotics were prescribed in eight patients with stool viruses, and in four patients in whom no enteric pathogen was identified. Anti-parasitic agents were used in patients with laboratory confirmed enteric parasites and an anti-malarial agent was used empirically in one febrile patient with rotavirus, prior to rapid antigen testing.

DISCUSSION

The purpose of this study was to characterise the aetiology of AGE in children presenting to a private urban clinic in Nairobi, Kenya. This information adds to our current understanding of the epidemiology of infectious diarrhoea in Kenya and emphasizes the prominent role of rotavirus and adenovirus as diarrhoeal pathogens. It is hoped that increased physician awareness of the importance of viruses in AGE will help minimize the inappropriate use of empiric antibiotics and perhaps reduce hospitalisations for self-limited viral infections that can often be effectively supported with oral rehydration therapy(5).

Table 2 shows prevalence rates of rotavirus and adenovirus among children with diarrhoea across the world. Rotavirus is globally the most important pathogen in AGE, present in 11% to 68% of affected children. Adenovirus accounts for 0.6% to 10.7% of cases, with the highest prevalence reported in the series from Thailand. Variability in the prevalence of stool pathogens is to be expected on the basis of variations in climate, genetic susceptibility of populations, and physician threshold for testing for stool viruses.

Data on stool virus prevalence from Africa are similar to other areas. A review of 43 published studies from 15 African countries (1975-1992) demonstrated that a median of 24% of children hospitalised for diarrhoea and 23% of outpatients with diarrhoea has rotavirus as the causative pathogen(29). In our series, viruses were the aetiologic agent in at least 23% of AGE cases, within the range of published figures from African and other countries.

Among three previous reports from Kenya, the prevalence of rotavirus varies widely, from 0% in a study of Maasai children(9) to 39% among children hospitalised for diarrhoea at Kenyatta National Hospital, Nairobi(8). These differences may be attributable to variation in living conditions or in methodology of viral testing. In our series, rotavirus was the most common aetiologic agent. Adenovirus is not reported as a diarrhoeal pathogen in the previous Kenyan studies. No cases of adenovirus were detected by complement-fixing antigen testing in the Nairobi series of 36 patients(8). Seven cases of adenovirus were observed in our series, in keeping with rates reported from Nigeria(10) and other geographical areas.

Bacterial pathogens were not seen in our series, perhaps because of a relatively small sample size or because of differences in access to clean drinking water in our relatively-affluent population attending a private paediatric outpatient clinic. This is in contrast to rates of bacterial gastroenteritis of 28%, 17% and 13% in previous Kenyan studies(7-9). Parasitic infections (Giardia *lamblia, Entamoeba histolitica, Blastocystis hominis, Cryptosporidium* spp.) are reported more commonly in series from Kenya(7,9) and Indonesia(14) than those from Australia(22), Greece(26) and Bahrain(12). Eight cases of parasitic infection were seen in our series.

Concurrent presence of adenovirus and rotavirus antigen was observed in four cases in this series. Mixed infection with two or more viral pathogens has been described previously in 5% of AGE in one Spanish study(30) and in 18% of cases in another series from Germany(25).

Rotavirus affects primarily infants and young children: 38% of African patients hospitalised with rotavirus are less than six months of age and 81% are less than a year of age(29). In our series, the mean age of children tested for viruses was apparently less than that of untested children, reflecting a physician tendency to seek a viral aetiology principally among younger patients.

The clinical presentation of rotavirus AGE was relatively uniform in this series, as nearly all patients had fever, diarrhoea and vomiting. Abdominal pain was reported in only 20% of cases. Conversely, no patients with adenovirus as the sole pathogen had documented fever and only 2/7 reported vomiting. Diarrhoea was present in nearly all (20/21) cases of viral gastroenteritis, regardless of aetiology, and was present more frequently in this group than among ten control patients whose stools tested negative for viruses. This suggests that the presence of diarrhoea is a sensitive predictive factor for the presence of stool virus (sensitivity=95%, specificity=40%); i.e., patients without diarrhoea were unlikely to test positive for stool viruses. This observation may be useful for physicians concerned about the judicious use of laboratory testing, who may feel that stool virus testing is not justified in patients without diarrhoea due to low diagnostic yield. In contrast, fever and vomiting were not useful in discriminating viral from non-viral AGE. Abdominal pain was reported more frequently in patients with non-viral AGE, perhaps as a result of the trend toward older age in this group and therefore an increased likelihood that pain could be identified and localised in this group.

The presence of fever, and elevated total leukocyte count (TLC), and neutrophilia are frequently used as indications of bacterial infection. However, among children with viral AGE in this series, fever was present in 48%, an elevated TLC in 20%, and neutrophilia in 70%. Thus, these findings are not specific for bacterial infection, being common in viral AGE. Furthermore,

given that the mean TLC and the proportion of patients with neutrophilia was similar in both case and control groups, we suggest that in AGE, the TLC and differential has limited clinical value in distinguishing viral from non-viral aetiologies.

Eight patients with viral AGE were treated with antibiotics, and eight patients were hospitalised. While antibiotic use and hospital admission may not have been avoidable in all cases, the role of antibiotics in AGE (even of bacterial origin) is limited (4), and outpatient oral rehydration has proven efficacy (5). Costly and potentially harmful interventions might be minimised with the recognition of the common and self-limited nature of viral AGE.

Limitations of the current study include the method of virus testing, which may be subject to false positive and false negative results. The assay used in the microbiology laboratory at GGCH involves visual detection of agglutination of colored latex particles. False negative results may be seen at low levels of viral antigen as may occur beyond five days after onset of rotavirus diarrhoea(1). False positive results may occur with viral antigen tests at rates of approximately 11% for adenovirus(31) and 15.5% for rotavirus(16). Multiple adenovirus serotypes may be excreted in the stool and detected by hexon antigen testing up to several months after acute infection (e.g., upper respiratory tract infection), while only serotypes 40 and 41 are implicated as causes of AGE(I). Furthermore, rotavirus antigen was present in rectal swabs of 6.9% of asymptomatic children from Zimbabwe(32), implying that clinical symptoms do not necessarily correlate with rotavirus antigen carriage. The estimated prevalence of viral pathogens of 23% among children with AGE in this series is compromised by: (1) retrospective nature of the study, and lack of consistent clinical criteria for submission of stool specimens; (2) viral testing not performed on a subset of submitted stool samples; and (3) missing information on a further 17 cases within the consecutive set. Nonetheless, assuming the untested and unknown samples were negative for stool viruses provides a minimum estimate of 23% for a viral aetiology, which is consistent with figures reported in the literature. Finally, a host of possible viral pathogens were not tested for (e.g. Norwalk agent, astrovirus, and calcivirus) and may account for a portion of cases in which no pathogen was identified.

CONCLUSION

This report contributes to the existing information on patterns of diarrhoeal illness in Kenya. Data from a unique outpatient urban population build on earlier Kenyan reports, which studied coastal(7), urban inpatient(8) and rural(9) populations. As in other areas of Africa and around the world, rotavirus was the most important pathogen in childhood AGE. Adenovirus, which was not reported in earlier Kenyan studies(8), was detected as the sole pathogen in the stools of seven patients. Diarrhoea was nearly universally present in patients with viral AGE and was a sensitive predictor of virus-positive stool in this series. Fever, elevated TLC and neutrophilia were prevalent among children with viral AGE and were not distinguishable from the signs of non-viral aetiologies. Antibiotics were used in eight cases of viral AGE, and eight children with viral AGE were hospitalised. It is the hope of the authors that enhanced awareness among physicians on the importance of viruses as causative agents of acute childhood gastroenteritis may contribute to the judicious use of antibiotics and may reduce hospitalisations in favour of outpatient oral rehydration strategies, of proven efficacy in self-limited viral infections.

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