Original Article

Enteric viral infections as potential risk factors for intussusception

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Abstract

Introduction: We aimed to identify potential risk factors for intussusception (ISS) among children presenting to two pediatric hospitals in Egypt.

Methodology: In this case-control study, enrolled children < 3 years old with ISS (confirmed radiologically and/or surgically) were matched by age and gender to controls admitted with acute non-abdominal surgical illnesses. Stool samples were collected and tested for various enteric bacteria, rotavirus, enteric adenoviruses (EA, 40 and 41) and astroviruses using commercially available ELISA diagnostic kits.

Results: From December 2004 to May 2009, 158 cases and 425 matched controls were enrolled. A history of diarrhoea and cough over the preceding four weeks of interview were more common in cases than controls, respectively. Children with mothers who had secondary education and above were 2.2 times more likely to have ISS than those whose mothers had a lower level of education. In spite of the low detection rate of EA infection (regardless of diarrhoea history) and asymptomatic rotavirus infection, they were detected in higher frequencies in cases than controls; however, infection with astrovirus and bacterial pathogens did not appear to be associated with increased risk of ISS.

Conclusions: History of diarrhoea and cough over the four weeks preceding the study and maternal education above secondary level were potential risk factors for ISS. EA and asymptomatic rotavirus infection were detected in higher frequencies among cases than controls; however, association with ISS cannot be confirmed. Additional research is needed to confirm these findings and evaluate the pathogenesis which may link such infections with ISS.

Key words: intussusception; adenovirus; rotavirus

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Introduction

Diarrhoea is one of the leading causes of childhood morbidity and mortality [1], and rotavirus infection is the main cause of severe acute diarrhoea among young children worldwide [2]. An estimated 527,000 children younger than five years of age die from rotavirus diarrhoea annually, with more than 85% of these deaths occurring in low-income countries in Africa and Asia [2]. The World Health Organization (WHO) reported that 20% to 30% of stool samples collected during 2009 from Egyptian children enrolled with acute gastroenteritis in the rotavirus surveillance, were positive for rotavirus (http://www.who.int/nuvi/rotavirus/en/). In Egypt, the incidence of rotavirus diarrhoea among children younger than three years was 0.19 episodes per person-year and it was the most common cause of severe diarrhoea causing hospitalization in children under five years [3-5]. The WHO recommended the introduction of rotavirus vaccine as a priority to decrease the morbidity and mortality from rotavirus gastroenteritis [6]. Two licensed rotavirus vaccines, RotaTeq (Merck Vaccines, Whitehouse Station, NJ, USA) and Rotarix (GlaxoSmithKline, Rixensart, Belgium), are now available against severe rotavirus diarrhoea [7]. As background knowledge for health policy makers in Egypt, one study estimated that vaccination for a birth cohort of 1.9 million Egyptian children would prevent about one million episodes of diarrhoea and save 2,873 lives [8]. The current

National Vaccination Program in Egypt does not include rotavirus vaccination.

The safety of the rotavirus vaccines became questionable after RotaShield (Wyeth Lederle Vaccines and Pediatrics, Marietta, PA, USA), the first rotavirus vaccine licensed in the United States, was withdrawn because of the association of the vaccine with the development of intussusception (ISS), a form of intestinal obstruction in which a segment of the bowel invaginates into a more distal segment [9]. The association of rotavirus vaccination with ISS raised some concerns not only about future rotavirus vaccines, but also about a potential link between natural rotavirus disease and other enteric infections and ISS. While a number of studies have looked at the association between rotavirus natural infection and ISS [10-16], additional data are needed to identify the potential risks that might be attributed to other common enteric infections in this age group. Our study aimed to contribute further to the understanding of the risk of enteric infection and other potential risk factors for ISS among infants presenting to two major pediatric hospitals in Egypt. Our study is unique in that, of other studies on ISS risk factors conducted in Egypt, no studies focused on enteric infections other than rotavirus.

Methodology

Study design

From December 2004 to May 2009, a prospective, observational, multicenter case-control study was conducted at Cairo University Children's Hospital and Alexandria University Children's Hospital in Egypt. These hospitals are large tertiary care referral centers with specialized care for pediatric patients, full-time pediatric surgery staff, and fully equipped radiological facilities.

Enrollment and specimen collection

Cases were patients under three years of age diagnosed with acute ISS at one of the study pediatric hospitals. Cases were diagnosed based on surgery, contrast enema or ultrasonography, thus meeting level I criteria for definite intussusception according to the Brighton collaboration criteria [17]. ISS cases were not eligible for enrollment if they had a congenital intestinal anomaly, or a history of abdominal surgery, Henoch-Schonlein purpura, or malignancy. The parents or guardians of eligible ISS cases provided informed consent for participation of their children.

Using the hospital's master admission registry, the three successive patients after the case, of the same gender and within the same age range (0-6 months, 6-12 months, 12-24 months and 24-36 months) admitted for any medical cause other than acute gastroenteritis or acute respiratory infection or for non-abdominal surgical admissions, were identified as controls. Controls were not eligible if they had plasma exchange or blood transfusion within the previous three months or if there was any evidence of a congenital anomaly. If the parent/guardian of potential control declined to participate, the parent/guardian of the next patient meeting the criteria of a matched control was approached regarding study enrollment. Informed consent was obtained from the parents/guardians of all study participants.

For both cases and controls, demographic data and medical history were collected and physical examinations were performed. All data were recorded on pre-tested paper-based data collection forms. In addition, stools were collected from each child within 24 hours of admission for controls and before, during or directly after surgery for ISS cases. Stool samples were stored at 2°C to 8°C at the field site and then all laboratory specimens were sent to U.S. Naval Medical Research Unit No. 3's (NAMRU-3) diagnostic laboratory within three days of collection via a consistent cold chain. Samples were frozen and stored at -70°C until use.

Specimen processing and examination

Using conventional microbiologic techniques, stool specimens were cultured onto standard laboratory agar media for the recovery and identification of enteric bacterial pathogens including *Shigella* spp, *Salmonella* spp, *Campylobacter* spp, and *Vibrio* and Enterotoxigenic *Escherichia coli* (ETEC). The speciation for *Campylobacter* isolates was performed using hippurate hydrolysis, and *Shigella* isolates were serotyped by slide agglutination using commercial antisera (Difco Laboratories, Livonia, MI, USA). ETEC identification was performed as follows: five *E. coli*-like colonies were picked from each MacConkey plate and tested for the expression of heat-labile (LT) and/or heat-stable (ST) enterotoxins using a direct and an indirect GM-1 enzyme-linked immunosorbent assay, respectively [18]. Commercially available ELISA diagnostic kits were used according to the manufacturer's instructions to test for the presence of rotavirus (Premier Rotaclone R, Meridian Bioscience, Cincinnati, OH, USA), enteric adenovirus and astrovirus (IDEIA, DAKO Diagnostics Ltd., Cambridge, United Kingdom).

The study protocol DoD#NAMRU3.2005.0001 (IRB Protocol No.170) titled "Risk Factors For Intussusception in an Egyptian Hospital-based Pediatric Population" was approved by the Naval Medical Research Unit No. 3 Institutional Review Board in compliance with all applicable Federal regulations governing the protection of human subjects.

Statistical analysis

Data were entered and verified using MS access 2007 (Microsoft Inc, Redmond, WA, USA); statistical analyses were performed with SAS software (version 9.1, SAS Institute Inc, Cary, NC, USA). The McNemar test was used to determine the statistical difference among categorical variables between matched cases and controls and paired t-test or Wilcoxon matched-pairs signed-rank test were used for parametric and nonparametric testing, respectively. Multivariate conditional logistic regression models were used to evaluate the association between ISS and various risk factors, including rotavirus infection, infection with other enteric pathogens, and other predictor variables such as hospital location, vaccination status, and antibiotic use in the recent past. A backward step-wise approach was used where variables with p-value ≤ 0.2 identified in the univariate analysis were included in an initial model, which was refit after iterative removal of non-significant variables until all factors remaining in the model had a p-value of < 0.05.

Results

Case control study population and demographics

During the enrollment period between December 2004 to May 2009, a total of 156 children younger than three years of age were enrolled in the study and matched with 370 controls by age and gender. Eighty-seven cases were matched each to three controls, 41 were matched to two controls, and 28 were matched to one control. The varying number of controls was due to the inability to fill the inclusion criteria for enrollment in the study and/or matching criteria. In the end, 120 cases (77%) and 291 (79%) controls were enrolled from Cairo University pediatric surgery department and 36 cases (23%) and 79 (21%) controls were enrolled from Alexandria University's pediatric surgery department.

Overall, 95% of the cases were under one year of age (44% $\leq$ 6 months and 51% between 7 and 12 months), and the median age for cases was 7 months with an interquartile range (IQR) of 5 to 9 months (Table 1). Males represented 63% of cases and controls. There were significantly more ISS cases (41%) than controls (33%) who were referred from other hospitals ($p \leq 0.0001$). Education level and occupation of patient caregivers were used as surrogate measures of socioeconomic status (SES). Mothers with higher levels of education was more common among the cases ($p = 0.002$) than among the controls, while fathers' occupation was not different between cases and controls.

Case diagnoses were confirmed either by radiography (91%; 11% by contrast enema and 80% by ultrasound), or during surgery (9%). The majority of patients (76%) had complete vital signs records; there was no difference between cases and controls with respect to fever, heart rate, or respiratory rate. Treatment of cases was either by pneumatic reduction (12%), surgical resection (38%) or simple surgical reduction (50%). Controls were admitted to the hospital with a variety of diagnoses, mainly inguinal hernia (34%), Hirschsprung's disease (11%), cleft lip/palate surgery (10%), and miscellaneous medical conditions (14%).

Univariate analysis of potential risk factors for ISS

Seventy-four cases (41%) received immunizations according to the National Immunization Program schedule (which does not include vaccination for rotavirus) compared to 158 (43%) of controls ($p = 0.2$). Median duration interval between last immunization and admission was one month in both cases and controls. As shown in Table 2, history of diarrhoea in the four weeks prior to admission date was twice as common in ISS cases ($n = 32$, 21%) compared to controls ($n = 33$, 9%) ($p = 0.001$); this association was also found when only the two weeks before admission were considered (cases 18% versus controls 11%; odds ratio (OR) 1.8; 95% confidence interval (CI) 1.1- 2.9). Among cases and controls, there were no differences in median duration of diarrhoea illness prior to hospitalization (three days versus four days, $p = 0.7$), median maximum number of loose stools (five versus five, $p = 0.9$), vomiting (71% versus 53%, $p = 0.1$), hospital admission (32% versus 19%, $p = 0.2$) or history of fever (65% versus 54%, $p = 0.4$). Neither antibiotic use during gastroenteritis illness nor health care seeking patterns were significantly different in cases versus controls.

A history of cough in the four weeks prior to admission date was reported in 25% of cases compared to 15% of controls, $p = 0.002$. Median duration of cough was five days among both cases and

Table 1. Comparison between intussusception cases and controls according to demographic characteristics and immunization history, Alexandria and Cairo Universities, December 2004 - May 2009

Characteristic	Cases (n = 156)	Controls (n = 370)	p-value
Age group in months, n (%)			
0 – 6	68 (44)	161 (44)	
7 – 12	80 (51)	188 (51)	
13 – 24	6 (4)	15 (4)	
25 - 36	2 (1)	6 (2)	
Median age, months (Interquartile range [IQR])	7 (5 - 9)	7 (4 - 10)	
Male gender, n (%)	100 (63)	236 (64)	
Maternal education, n (%)			
Not educated	47 (30)	143 (39)	1
Less than secondary	57 (37)	143 (39)	0.3
Secondary and above	52 (33)	81 (22)	0.002
Father's occupation, n (%)			
Farmer/Manual workers	36 (24)	94 (27)	1
Sales/Clerical	73 (49)	138 (38)	0.2
Professional	40 (27)	121 (36)	0.6
Any immunization received since birth, n (%)	74 (41)	158 (43)	0.2
Median duration since last immunization, month (IQR)	1 (0.1)	1 (0.1)	0.5

Numbers may not add up to n (%) due to missing answers.

Table 2. Comparison between cases and controls for potential risk factors of intussusception according to diarrhoea history, cough history and pathogen detected from stool, Alexandria and Cairo Universities December 2004 - May 2009

Characteristic, n (%)	Cases (n = 156)	Controls (n = 370)	OR (95% CI)*	p-value
Patient had diarrhoea in the preceding four weeks prior to interview	32 (21)	33 (9)	1.9 (1.2 - 3.1)	0.001
Patient had cough in the preceding four weeks prior to interview	38 (25)	54 (15)	2.4 (1.4-4.1)	0.002
Pathogen detected**				
Rotavirus	18 (12)	30 (8)	1.4 (0.8 - 2.6)	0.3
Adenovirus	11 (7)	9 (2)	3 (1.2 - 7.4)	0.01
Astrovirus	2 (1)	13 (4)	0.3 (0.1 - 1.5)	0.2

* Odds Ratio and 95 % confidence interval

** 155 out of 156 cases were tested for rotavirus, astrovirus and adenovirus; one stool sample was not available.

controls. Fever was present with cough in 59% of both cases and controls. Previous hospitalization due to the reported respiratory illness in the last month before the current admission was reported in 23% of controls compared to 6% of ISS cases, p = 0.04.

Specific identification of viral and bacterial pathogens isolated from ISS cases and controls is detailed in Table 2. Enteric adenoviruses (EA) were detected significantly more often in cases (7%) compared to controls (2%) (p = 0.01). Rotavirus was identified in 12% of cases compared to 8% in controls (p = 0.3). Astrovirus was detected in 1% of cases versus 4% of controls (p = 0.2). Routine bacterial culture identified an etiology in only two patients in the control group, both of which detected *Salmonella* spp. There was no significant difference in the presence of mixed infections in cases (1; astrovirus and rotavirus) and controls (4; 1 rotavirus and adenovirus and 3 rotavirus and astrovirus).

To clarify the role of active enteric infections in the last month prior to admission on the occurrence of ISS, cases and controls were stratified by presence or absence of positive history of diarrhoea in the preceding four weeks (Table 3). Among the subgroup of cases (n = 120) and controls (329) who had no history of diarrhoea in the preceding four weeks before the interview, rotavirus detection rates were higher in cases compared to controls (14% versus 8%, p = 0.06), as were the rates for EA (8% in cases, 2% in controls, p = 0.007). Astrovirus detection was not significantly different between cases (2%) and controls (4%) (p = 0.3). Of the 32 cases and 33 matched controls who reported diarrhoea in the preceding four weeks, EA was detected in comparable percentages among cases and controls (6%), while rotavirus was detected more frequently among controls (6%) versus cases (3%) although this was not statistically significant (p = 0.6).

Multivariate analysis

The finalized multivariate conditional logistic regression model revealed multiple independent risk factors for ISS (Table 4). ISS cases were found to be 2.7 (95% CI, 1.1- 7.1) times as likely to have an EA infection compared to controls. Cases were more twice as likely to have mothers who completed secondary education compared with controls (OR 2.2; 95% CI, 1.3 - 3.6). Furthermore, ISS cases were more likely to have had diarrhoea and cough in the preceding four weeks prior to hospital admission compared to controls (OR 2.4; 95% CI, 1.3 - 4.5 and OR 2.3; 95% CI, 1.3 – 4.3, respectively).

Discussion

ISS is a unique condition among children and infants, the etiology of which has been studied to determine whether enteric infections are a risk factor for ISS. This has been in part due to the fact that rotavirus is a common etiology of severe diarrhoea in pediatric patients [3,5,19], and the fact that a previous rotavirus vaccine (Rotashield) was removed from the market due to its association with ISS; in one study it was associated with a relative risk of ISS of 24.8 times within 3 to 7 days after the first dose, with an estimated risk of 1 case per 10,000 vaccinated infants [9]. A recent study also demonstrated that the monovalent attenuated human rotavirus vaccine was associated with short-term risk of ISS in approximately 1 of every 51,000 to 68,000 vaccinated infants [20]. Because of this observation, and the biological plausibility for a mechanism of infection-induced distal ileum wall thickness and adenopathy causing ISS [21], a number of studies have looked at the association between rotavirus natural infection and the occurrences of ISS. Our results regarding rotavirus showed that rotavirus infection was not associated with ISS when the whole set of data were analyzed. Studies on the association between rotavirus and ISS in both developed and developing countries reported mixed results. Some reported high rates of rotavirus

Table 3. Distribution of pathogen detected from stool of children with intussusception and their controls according to history of diarrhea during the preceding 4 weeks to enrollment, Alexandria and Cairo Universities, December 2004 - May 2009

	Diarrhoea in preceding 4 weeks (n = 65)			**No diarrhoea in preceding 4 weeks (n = 449)**		
Pathogen	Cases(32)	Controls (33)	p-value	Cases (120)	Controls (329)	p-value
Rotavirus n = 53	1 (3)	2 (6)	0.6	17 (14)	27 (8)	0.06
Adenovirus n = 21	2 (6)	2 (6)	0.97	9 (8)	7 (2)	0.007
Astrovirus n = 18	0 (0)	0 (0)		2 (2)	12 (4)	0.3

Table 4. Multivariate conditional logistic regression model of potential risk factors of intussusceptions, Alexandria and Cairo Universities, December 2004 - May 2009 (n = 574)

Variable	OR (95 % CI)*	p-value
Maternal Education, is Secondary or above	2.2 (1.3 - 3.6)	0.004
Diarrhea in preceding four weeks	2.4 (1.3 – 4.5)	0.005
Cough in preceding four weeks	2.3 (1.3 – 4.3)	0.05
Adenovirus	2.7 (1.1 – 7.1)	0.04

*Odds Ratio and 95 % confidence interval, conditional Logistic regression

infection among children treated for ISS [13,23], and other studies, including one from Egypt, showed no association between ISS and natural rotavirus infection [10,12,24-26]. Potential explanations for such conflicting results include the possibility of strain-specific association, [15] small sample sizes, [10,12] and possible host differences.

Several studies have shown that other bacterial and viral enteric infections may be associated with an increased risk of ISS; however, results have been inconclusive [25,27,28]. Our results demonstrated a risk of ISS association with EA detection in the stool at time of admission (presumably due to recent or current infection), which confirms findings from several previous studies [24,29,30]. However, the detection rate of EA in our study was low (8% in cases versus 2% in controls), and did not enable us to confirm the significant finding we observed. The EA detection rate from our study may help other researchers to correctly calculate sample size to confirm our finding. Unlike with the results from a previous study from Nigeria which demonstrated that astrovirus might be an independent risk factor for ISS [31], our study did not find an association with astrovirus. However, the Nigerian study was a small pilot study where astrovirus antigen was detected in only three of six stool specimens for ISS cases.

Interestingly, we noted higher rates of EA and rotavirus identification in ISS cases compared to controls among those who did not report any clinical history of diarrhoea in the preceding four weeks. In spite of the low detection rate of these viruses, this finding could suggest that there is a link with subclinical enteric virus infection and ISS through a more robust immune response and development and increased lymphoid tissue (Peyers' patch) hypertrophy, also known as the "leading edge" pathogenesis theory of ISS [21,32]. Another study reported that EA infection, specifically, is associated with inflammatory neuropathy that causes peristalsis disorder as a mechanism for ISS [33]. Alternatively, it could be possible that ISS itself (without antecedent diarrhoea) is a rare clinical presentation of acute infection through some other unknown mechanism. If these observations were to be confirmed, future investigation into the pathogenesis associated with asymptomatic enteric viral infection is warranted.

We found also that history of diarrhoea in the preceding four weeks was reported more frequently among ISS cases compared to controls. Despite an extensive microbiological work-up and the fact that the peak age for ISS is generally the same age period where gastroenteritis is more generally associated with bacteria rather than viruses, we did not find any association between bacterial pathogens recovered in cases compared to those found in controls, as has been described previously [34]. However, this lack of association may be due to the fact that the sampling for bacterial enteric pathogens was during asymptomatic periods after the illness had resolved and infection likely cleared. Meanwhile, the differential detection of viral pathogens may have been more likely due to the known persistence of shedding of these agents [35-37]. Serological assessment of enteric bacterial exposures may be considered to ascertain such associations in the future. Furthermore, we failed to find any association between antecedent immunization and ISS (including polio virus vaccine) in the preceding month prior to interview, a finding which supports the observations of other studies that concluded that prior vaccination with polio vaccine is not associated with ISS [28,38,39].

Reported cough in the four weeks preceding the interview was also a significant risk factor for ISS. This finding may indicate that a proportion of ISS cases were preceded by respiratory tract infection, a finding that is not supported by identification of respiratory pathogens or its immune response. Other studies had identified that respiratory adenovirus infection was a significant risk factor for ISS [25]. Identification of respiratory pathogens causing ISS may necessitate further studies.

Of the sociodemographic characteristics, maternal education was an independent risk factor for ISS. This

observation might be explained based on SES differences and access to care, which may have differentially affected health-care seeking or access between cases and controls. It could also be hypothesized that children of these higher SES mothers have better nutrition status and may thus develop a more robust immune response, or it could be a reporting bias of exposures among cases compared to controls.

Antibiotics were commonly used among cases and controls who had past history of diarrhoea prior to the interview; the difference was not statistically significant. In Egypt, patients have easy access to antibiotics in pharmacies with and without prescription; and use of antibiotics in treating diarrhoea is a common practice.

This study has several limitations. The relative timing of specimen collection in relation to the reported antecedent diarrhoeal illness, as well as methods of enteric pathogen detection, may have affected the ability to attribute temporality of infection and led to biased associations. Furthermore, control selection may have biased the associations. However, there did not appear to be any admission diagnoses which would have differentially affected the susceptibility to enteric or respiratory illnesses. Also, testing of stool samples for detection of other enteropathogens including parasites and norovirus was not performed and could be considered in future studies. The sample size we studied and the low detection rate of enteric viruses from cases and controls may have led to the misinterpretation of the role of enteric viruses as a risk factor for ISS. Studies with a larger sample size are required to confirm these findings.

Data from our study support a positive association of antecedent diarrhoeal illness or respiratory illness with increased risk for ISS. Future studies are needed to identify the pathogenesis of ISS and its relationship to enteric virus infections and immune response.

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