Population-Based Incidence of Intussusception and a Case-Control Study to Examine the Association of Intussusception with Natural Rotavirus Infection among Indian Children

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Background. A rotavirus vaccine previously licensed in the United States was withdrawn because it caused intussusception. Data on background intussusception rates in developing countries are required to plan pre- and postlicensure safety studies for new rotavirus vaccines. Also, it is unclear whether natural rotavirus infection is associated with intussusception.

Methods. Passive surveillance for intussusception in a large, well-defined, poor, urban population in Delhi, India, was conducted in 2 phases. Intussusception was confirmed by ultrasonography or surgery. Fecal samples obtained from patients with intussusception at study hospitals (irrespective of their residence in study areas) and healthy control subjects were tested for rotavirus with use of enzyme immunoassay. If available, resected intestinal tissue samples were tested for rotavirus with use of immunohistochemistical analysis and reverse-transcription polymerase chain reaction.

Results. The incidence of intussusception requiring hospitalization was 17.7 cases per 100,000 infant-years of follow-up (95% confidence interval, 5.9–41.4 cases per 100,000 infant-years). Detection rates of rotavirus in stool samples did not differ significantly between case patients and control subjects (4 of 42 case patients vs 6 of 92 control subjects), and no evidence of rotavirus was detected in any of the 22 patients with intussusception for whom intestinal tissue samples were available.

Conclusions. The incidence of intussusception among Indian infants appears to be lower than that reported in other middle- and high-income countries. Natural rotavirus infection does not appear to be a major cause of intussusception in Indian infants.

Rotavirus is an important cause of diarrhea associated with dehydration. It is estimated to be responsible for ~150,000 deaths annually among Indian children <5 years of age [1]. The tetravalent rhesus-human reassortant rotavirus vaccine was licensed in the United States in August 1998. In mid-1999, the use of this vaccine was reported to be associated with an increased risk of intussusception, and the vaccine was withdrawn from the market [2–4]. In 2006, phase 3 trials of 2 new rotavirus vaccines (Rotarix [GlaxoSmithKline], an attenuated human rotavirus vaccine, and RotaTeq [Merck], a pentavalent human-bovine reassortant rotavirus vaccine) that involved >60,000 children each in the Americas and Europe showed efficacy of >85% against severe rotavirus disease and no increased risk

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of intussusception [5, 6]. These vaccines have been introduced in national immunization programs in many countries, including the United States, Mexico, and Brazil. Trials are ongoing in several countries in Asia and Africa to demonstrate the efficacy of these rotavirus vaccines, and results are expected within the next 2–3 years.

Data on background rates of intussusception in developing countries are required to facilitate informed decision making about use of new rotavirus vaccines. These background rates are also needed for estimation of the sample size needed for studies to demonstrate safety both before and after licensure of new rotavirus vaccines. Such population-based data are not available in most developing countries, including India. Also, it is unclear whether natural rotavirus infection is associated with intussusception. If natural rotavirus infection increases the risk of intussusception, then some risk associated with rotavirus vaccines might be considered to be acceptable.

To address these issues, we conducted passive surveillance to estimate the incidence of intussusception in a well-defined, poor, urban population in Delhi over a period of 3 years. During the same period, a case-control study was conducted to examine the association of intussusception with natural rotavirus infection.

METHODS

Population-Based Surveillance for Intussusception

Passive surveillance for hospitalized infants with intussusception was conducted in 2 phases. The first phase lasted 2 calendar years (December 2000–November 2002) and linked data from active hospital-based surveillance of intussusception with census data for children <5 years of age who lived in the surrounding community. The community in South Delhi had a population of ~500,000 persons living in poor conditions. Trained field workers conducted a baseline house-to-house survey to record the age and sex of all the children aged <5 years. The age- and sex-specific population estimates were used as the denominator for calculating the incidence of intussusception. A total of 11,416 infants <1 year of age were identified. Infant-years of follow up during the 2 years of surveillance were calculated as twice this number, assuming that there was no change in the number during the second year of surveillance. Cases of intussusception were identified through surveillance at 6 hospitals that account for approximately two-thirds of all hospital admissions of children <5 years of age in the community. A study team led by a physician visited each of these hospitals daily, screened all children admitted to the pediatric medicine and pediatric surgery wards, and evaluated those with prespecified clinical signs of intussusception. These clinical signs included intermittent abdominal pain or excessive crying of unknown origin, bloody (currant jelly) stools, abdominal or rectal mass, recurrent vomiting not associated with acute diarrhea, and abdominal distension. A diagnosis of intussusception was made on the basis of confirmation by either ultrasonography or surgery. The team then recorded identification details of all patients to ascertain the number of patients who belonged in the study population because they resided in the defined study areas.

In the second phase, the participants were children enrolled in an ongoing randomized controlled trial of zinc supplementation. These children (age, 1–23 months) were enrolled from urban, poor areas in northern and northwestern Delhi and were followed up for up to 1 year. Infant-years of follow-up were calculated as the total number of months of follow-up that was contributed by study participants during the first year of life. Surveillance was conducted at 8 hospitals to identify study participants who were hospitalized and to ascertain the cause of hospital admissions. Children with the final hospital diagnosis of intussusception were considered to have the condition.

Case-Control Study of the Association between Rotavirus Infection and Intussusception

During the first phase of surveillance, infants admitted to any of the hospitals, irrespective of their place of residence, who had ⩾1 sign of possible intestinal obstruction and who were evaluated for possible intussusception were included in the case-control study if they were aged 2–12 months and if parents gave written informed consent. This study attempted to determine whether natural rotavirus infection was associated with intussusception.

Case patients. Infants, irrespective of their area of residence, were considered to be case patients if they were 2–12 months of age, had received a diagnosis of intussusception confirmed by either ultrasonography or surgery, and were admitted to any of the 6 hospitals.

Control subjects. Infants were considered to be control subjects if they were 2–12 months of age, were admitted to the same hospitals as the case patients, and received a diagnosis other than intussusception. Control subjects were enrolled concurrently with the case patients but were not matched with them by any criteria.

Assessment of case patients and control subjects. A questionnaire was completed for all case patients and control subjects, and stool samples were collected. Intestinal tissue specimens were also obtained whenever a case patient or control subject required an intestinal resection. Two stool samples each were collected from patients with intussusception and from control subjects; one was collected on the day of hospital admission, and the other was collected 48 h after admission. Samples were transferred to the laboratory at the All India Institute of Medical Sciences (New Delhi, India) on ice and were stored at −20°C until analysis. Rotavirus was detected in

Table 1. Characteristics of Patients with Intussusception and Control Subjects

Characteristic	Case patients (n = 47)	Control subjects (n = 110)
Age, months, mean	6.0	4.6
Weight, kg	6.4	4.8
Male sex	37 (78.7)	79 (71.8)
Duration of illness, days, median (IQR)	3 (2–6)	6 (3–21)
Clinical presentation		
Excessive crying and/or pain	29 (61.7)	43 (39.1)
Bloody and/or currant jelly stools	36 (76.6)	5 (4.6)
Abdominal and/or rectal mass	10 (21.3)	12 (10.9)
Recurrent vomiting	32 (68.1)	58 (52.7)
Abdominal distension	33 (70.2)	81 (73.6)
History of diarrhea in previous 2 weeks	10 (21.3)	22 (20.0)
Rotavirus detected in stool sample	4/42	6/92

NOTE. Data are no. (%) of participants, unless otherwise indicated. IQR, interquartile range.

stool samples with use of commercially available enzyme immunoassay kits (Rotaclone; Meridian Diagnostics).

Evidence of rotavirus in resected intestinal tissue samples from case patients and control subjects. For pathologic examination, all of the specimens were inspected grossly, and the presence or absence of intussusception and other details were noted. Multiple tissue sections were taken from different regions of the surgically resected specimen of intestine and were fixed in neutral-buffered formalin. Paraffin-embedded tissue blocks were prepared for histological, immunohistochemical, and reverse-transcription polymerase chain reaction (RT-PCR) studies. Methods described by Tatti et al [7] were used for immunohistochemical analysis and RT-PCR.

For histological studies, sections cut at 4 μ were stained with hemolysin and eosin. For immunohistochemical assays, rabbit polyclonal IgG antibodies were used as primary antibodies. A sepharose (Amersham) directed against the Wa strain was used on 4-μ–thick tissue sections. Before application, the primary antibody sections were digested in 0.1 mg/dL of proteinase for 30 min. Specificity of staining was confirmed with preimmune rabbit serum as primary antibody for negative control. Immunohistochemical staining was done with the streptavidine-biotin alkaline phosphates technique, as described elsewhere [7,8], with use of both positive and negative controls.

For RT-PCR, RNA was extracted from one 10-μm tissue section placed in a microcentrifuge tube. Paraffin was removed with xylene, and residual xylene was washed with alcohol. The RNA was further extracted using a commercial kit (R Naid Plus Kit; Bio 101). The 1-step RT-PCR kit and the conditions used for RT-PCR to detect RNA of the VP4 gene have been described in detail by Tatti et al [7]. The primer pair used to amplify a product of 212 base pairs spanning nucleotides 676–887 of the rotavirus VP4 gene (5′-TTG CCA CCA ATT CAG AAT AC-3′ [plus sense] and 5′–ATT TCG GAC CAT TTA TAA CC-3′ [minus sense]) was designed to be homologous to genotype P[4] and P[8] reference strains.

RESULTS

Incidence of Intussusception during the First Year of Life

During the first phase of surveillance, 2 confirmed cases of intussusception in infants aged <1 year who were from the study population were identified after 22,832 infant-years of follow-up. During the second phase, there were 3 confirmed cases of intussusception among the enrolled infants aged <1 year after 20,496 infant-years of follow-up. Therefore, the total incidence of intussusception requiring hospitalization was 17.7 cases per 100,000 infant-years of follow-up (95% confidence interval, 5.9–41.4 cases per 100,000 infant-years), after inflation of the crude incidence to adjust for ~65% coverage of surveillance.

Case-Control Study

Detection of rotavirus in stool samples from case patients and control subjects. A total of 47 patients with intussusception (2 of them in the study population and the remaining from outside the study area) and 110 control subjects were enrolled, irrespective of their area of residence (Table 1). Stool specimens were available for testing from 42 case patients and 92 control subjects, and detection rates of rotavirus did not differ significantly between case patients and control subjects (4 [10%] of 42 case patients vs 6 [7%] of 92 control subjects; age-adjusted odds ratio, 1.60; 95% confidence interval, 0.42–6.16).

Examination of rotavirus in resected intestinal tissue specimens from case patients and control subjects. Immunohistochemical staining failed to detect rotavirus antigen in sam-

ples from any of the 22 patients with idiopathic intussusception but yielded positive results for the positive control. None of the patients with intussusception who had resected intestinal tissue specimens examined for rotavirus had rotavirus detected in stool samples. None of the 20 RNA extracts from formalin-fixed, paraffin-embedded tissue samples from patients with intussusception were positive for rotavirus RNA by RT-PCR; in 2 cases, PCR could not be performed.

DISCUSSION

Our study presents the first population-based estimates of intussusception rates among infants in urban slums in Delhi, India, and is one of the very few estimates from a developing country. Intussusception rates among Indian infants were low (~18 cases per 100,000 infant years of follow-up). Studies from middle- and high-income countries in Europe, Australia, Asia, and the Americas have generally reported somewhat greater intussusception rates of 30–70 cases per 100,000 infant-years [9–18]; however, other studies from Asia have reported substantially greater rates of intussusception. A study among Vietnamese infants reported a rate of 302 cases per 100,000 infant-years [19], and a study from Japan reported a rate of 185 cases per 100,000 infant-years [20]. It is possible that differences in genetic predisposition or other factors possibly associated with intussusception (eg, prevalence of enteric infection, feeding practices, and prevalence of malnutrition) might explain the differences in rates of intussusception among various populations. Of note, a recent study from Israel found that the intussusception rates among Jewish children were more than twice those among Bedouin children [21]. Differences in access to medical care, methods of diagnosis (eg, ultrasound vs barium enema), and treatment practices (eg, surgical management vs outpatient enema reduction) might also explain some of the variation in rates of hospitalization for intussusception among various studies.

Infection with adenovirus has been well documented to be associated with increased risk of intussusception [22–27], but the literature on the association of intussusception with natural rotavirus infection is less clear. Several studies from the 1970s and 1980s reported high rates of rotavirus infection among children treated for intussusception [28–30]. However, these studies did not assess rotavirus infection among a healthy comparison group of infants; therefore, firm etiologic associations could not be derived, especially because many of these studies were conducted during the winter months, when rotavirus is common in hospitalized children who could be susceptible to nosocomial rotavirus infection. Around the time of the licensure of the rhesus rotavirus vaccine in the late 1990s, many ecological studies compared the seasonal patterns of intussusception and hospitalizations for rotavirus infection, and found that they differed substantially; this finding suggests that rotavirus is not a major cause of intussusception [10, 14, 16, 31–33]. An assessment of patients with intussusception and healthy control subjects with use of ultrasound reported that rotavirus infection was associated with increased distal ileum wall thickness and lymphadenopathy during the illness period, suggesting a plausible mechanism by which rotavirus infection could cause intussusception [34]. However, several epidemiologic studies using the case-control approach that have examined association of intussusception with either all diarrheal diseases or laboratory-confirmed rotavirus disease have failed to show an association [19, 35, 36]. Our findings support these observations and indicate that natural rotavirus infection is not associated with intussusception in Indian children or that the risk of rotavirus infection causing intussusception is extremely low.

Some potential limitations of the present study merit consideration. It is possible that our surveillance did not capture all patients in the source population, because they either did not go to the hospitals under surveillance or because they died at home without the parents seeking care in a hospital. We adjusted the observed rates for the efficiency of our passive surveillance (65% of all hospital admissions in the study area), which was estimated through a household survey in the study area. Even assuming that approximately one-half of the infants with intussusception died at home without being hospitalized, the actual incidence would still be <40 cases per 100,000 infant-years of follow-up. A limitation of the case-control study was the relatively small number of patients. However, we recruited all patients with confirmed cases of intussusception who were admitted to 6 of the largest hospitals in Delhi over a period of 2 calendar years. Poststudy calculations show that the current sample size had ~80% power to rule out an odds ratio of ⩾4.

In conclusion, the incidence of intussusception among Indian children appears to be lower than that reported in other middle- and high-income countries. The population-based rates of intussusception reported in this study would be useful for planning phase III and IV studies of rotavirus vaccines in India. The lack of a strong association of intussusception with natural rotavirus infection supports observations from other studies and indicates that live oral rotavirus vaccines free of this adverse effect can be developed. Although the exact pathogenesis of intussusception associated with the rhesus rotavirus vaccine is not known [33, 37], new rotavirus vaccines do not appear to be associated with intussusception on the basis of data from large prelicensure trials [5, 6] and available postmarketing data [38, 39]. However, because of the potential differences in the risk of intussusception caused by different rotavirus vaccines and variation in background rates of natural intussusception in various populations, continued assessment of this potential adverse event in association with rotavirus vaccines is important. Our data will provide valuable baseline

information to plan future trials of rotavirus vaccines in India and to monitor vaccine safety after implementation.

DELHI INTUSSUSCEPTION STUDY HOSPITAL GROUP

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