

Rotavirus-associated immune thrombocytopenic purpura in children: A retrospective study

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Abstract. Certain studies have previously indicated that an association may exist between rotavirus infection and primary immune thrombocytopenic purpura (ITP). The present retrospective study aimed to investigate whether rotavirus may cause ITP in children. Firstly, the incidence of ITP in children with or without rotavirus diarrhea was compared. A 14.58% incident rate was observed in children with rotavirus diarrhea compared with a 7.22% incident rate in children without rotavirus diarrhea. Subsequently, the clinical features of ITP children with or without rotavirus infection were compared. The results indicated that ITP children with rotavirus infection were significantly younger, showed significantly decreased mean platelet volume (MPV) levels and presented a significantly higher frequency of bleeding score of 3 against ITP children without rotavirus infection. In conclusion, these findings suggest that rotavirus serves a causative role in ITP.

Introduction

Immune thrombocytopenic purpura (ITP) is an acquired immune-mediated disorder characterized by isolated thrombocytopenia (1). ITP in infants is uncommon and appears to be predominantly benign. Although the exact cause of ITP remains unknown, infection is generally considered to serve an important role in the pathogenesis of ITP. Recently, increasing evidence has suggested an association between viral infection and ITP (2,3).

Rotavirus is a double-stranded RNA virus belonging to the Reoviridae family that was first described in 1973 (4). Human rotavirus is the major etiological agent of diarrhea in infants and children throughout the world (5). However, previous studies have indicated that rotavirus infection is not limited

to the gastrointestinal tract (6). Viremia and other systemic infections are commonly reported in patients and animals with rotavirus infections (7). Furthermore, rotavirus infection may be an etiological agent of autoimmune disease (8).

To date, the existence of an association between rotavirus infection and ITP remains unclear. The present retrospective study described a series of cases in children who suffered from both rotavirus diarrhea and ITP simultaneously in order to investigate whether rotavirus infection serves a causative role in ITP.

Patients and methods

Participants and study procedure. Initially, the incidence of ITP in children with or without simultaneous rotavirus infection was compared. A total of 601 children hospitalized for diarrhea in the Department of Hematology at Tianjin Children's Hospital (Tianjin, China) between December 1, 2002 and December 1, 2010 were included in the study. The rotavirus antigens were investigated in all children. Patients that were rotavirus-positive were classified as the rotavirus group, while rotavirus-negative patients served as the controls. The diagnosis of ITP was established according to the medical history of the patients, including the results of physical examination, complete blood count and peripheral blood smear that eliminated other causes of the thrombocytopenia.

Subsequently, the clinical features in ITP children with or without rotavirus infection were compared. The clinical features that we evaluated were as follows: i) Demographic characteristics; ii) seasonal variation; iii) untreated platelet count; iv) mean platelet volume (MPV); v) bleeding severity; and vi) response to therapy.

Examinations. Fecal specimens collected from the patients were examined on admission for the presence of rotavirus antigen using Group A Rotavirus Antigen Rapid Test Kit (colloidal gold method; Gentaur, Kampenhout, Belgium).

Statistical analysis. The STATA statistical software (version 12.2; StataCorp, College Station, TX, USA) was used to analyze statistical correlations in the collected data. Student's t-test or Fisher's exact test were used to calculate statistically significant differences, which were indicated by $P < 0.05$.

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Results

Incidence of ITP in children with or without rotavirus infection. Among the children with rotavirus infection, 21 (14.58%) demonstrated simultaneous ITP. Among the 457 children with non-rotavirus-associated diarrhea, 33 (7.22%) presented ITP.

As shown in Fig. 1, the inner circle represents the 144 children with rotavirus diarrhea, which includes 21 children (14.58%) with simultaneous ITP (blue color) and 123 children (85.42%) who did not present ITP (red color). Similarly, the outer circle represents the 457 children with non-rotavirus diarrhea, including 33 children (7.22%) with ITP (blue color) and 424 children (92.78%) without ITP (red color).

Demographic characteristics and clinical features of ITP children with or without rotavirus infection. Table I lists the demographic and clinical features of the included patients. The median age of children with simultaneous ITP and rotavirus infection was 18.42 months, with a range of 4-49 months. The median month age of the 601 children presenting ITP without rotavirus infection was 35.85 months, with a range of 5-98 months. Children presenting ITP with rotavirus infection were significantly younger in age ($P<0.05$) compared with children presenting ITP without rotavirus infection. In addition, the male:female ratio in children presenting ITP with rotavirus infection was 2.2:1, while the ratio was 1.5:1 in children presenting ITP without rotavirus infection. Thus, the possibility of developing ITP is higher in males with rotavirus infection.

Seasonal variation. The seasonal variation in children with ITP and rotavirus infection was as follows: 1 case in spring, 6 cases in summer, 1 case in autumn, and 13 cases in winter. Therefore, the majority of ITP cases with rotavirus infection occurred during the winter months (13/21). The seasonal variation in ITP cases without rotavirus infection was as follows: 7 cases in spring, 10 in summer, 8 in autumn and 8 in winter. Thus, the number of ITP cases in children without rotavirus infection occurred evenly throughout the seasons, without a significant seasonal variation observed.

Untreated platelet count. The mean platelet count in children presenting ITP with and without rotavirus infection was 23.51×10^9 and $22.69 \times 10^9/l$, respectively. No statistically significant difference was observed between the two groups (Table I; $P>0.05$).

MPV measurement. The MPV in children presenting ITP with and without rotavirus infection was 7.88 and 9.21 fl (9.6-13.0 fl), respectively. A statistically significant decrease in MPV was observed in ITP children with rotavirus infection when compared with those without rotavirus infection (Table I; $P<0.05$).

Bleeding severity. The scoring system described by Buchanan and Adix (9) was used to assess bleeding. The following four domains were assessed: i) Overall bleeding tendency; ii) bleeding from the oral cavity; iii) bleeding from the nose; and iv) cutaneous hemorrhage. The bleeding severity in each of the four domains was graded between 0 and 4.

Subsequently, the grades from each domain were added together to obtain the Buchanan score, which indicated the following: 0, no evidence of bleeding; 1, minor bleeding; 2, mild bleeding; 3, moderate bleeding; and 4, severe bleeding.

Children presenting ITP with rotavirus infection demonstrated a significantly higher frequency of a bleeding score of 3 when compared with those without rotavirus infection ($P<0.05$), as determined using the Fisher's exact test. However, there was no significant difference in bleeding scores 0-2 or 4 between the ITP children with or without rotavirus infection, according to the results of Fisher's exact test.

Response to therapy. Children with platelet counts $<50 \times 10^9/l$ received pharmacological therapy, which included intravenous immunoglobulin (IVIG), high-dose steroids and conventional-dose steroids (10). They were evaluated for their response to therapy at day 7. By contrast, children with platelet counts of $>50 \times 10^9/l$ did not receive any pharmacological therapy. The response to therapy was defined as complete (CR) if the platelet count was $>150 \times 10^9/l$ following treatment and partial (PR) if the platelet count was between $50-150 \times 10^9/l$ following treatment. All other children were considered to have no-response (NR) to treatment.

IVIG therapy was administered to 3 ITP children with rotavirus infection and to 7 without rotavirus infection. CR was achieved in 2 ITP children with rotavirus infection and 5 ITP children without rotavirus infection, while PR was achieved in 1 ITP child with rotavirus infection and 1 ITP child without rotavirus infection. In addition, 1 child without rotavirus infection showed NR to treatment.

High-dose steroid therapy (methylprednisolone or dexamethasone) was administered to 2 ITP children with rotavirus infection and to 5 without rotavirus infection. The treatment resulted in CR in 1 ITP child with rotavirus infection and 3 ITP children without rotavirus infection, as well as in PR in 1 ITP child with rotavirus infection and 1 ITP children without rotavirus infection. NR was observed in 1 children without rotavirus infection.

Furthermore, conventional-dose steroid therapy (prednisolone or dexamethasone) was administered to 17 ITP children with rotavirus infection and 16 without rotavirus infection. CR was achieved in 14 ITP children with rotavirus infection and 12 without rotavirus infection, while PR was achieved in 2 ITP children with rotavirus infection and 3 ITP children without rotavirus infection. NR was observed in 1 ITP child with rotavirus infection and 1 ITP children without rotavirus infection.

A total of 21 children with ITP received no treatment, and CR was achieved in all these children.

Discussion

Approximately 66% of children with ITP show a history of infectious illness a few days or a week prior to the onset of thrombocytopenia. A viral infection (such as varicella zoster, rubella, Epstein-Barr, influenza or human immunodeficiency virus-1) have been identified in a subset of these children, which indicates an etiological role of a preceding viral infection that leads to ITP (11). A previous case study indicated the existence of an association between rotavirus infection

Table I. Clinical features and laboratory findings in ITP.

Parameter	ITP with RV (n=21)	ITP without RV (n=33)	P-value
Median age, months	18.42	35.85	<0.001
Age range, months	4-49	5-98	
Gender ratio (M/F)	2.2:1	1.5:1	
Season of onset, n			
Spring (March-May)	1	7	
Summer (June-August)	6	10	
Autumn (September-November)	1	8	
Winter (December-February)	13	8	
Untreated platelet count, $\times 10^9/l$	23.51	22.69	0.722
Mean platelet volume, fl	7.88	9.98	0.032
Bleeding grades			
0	1	0	0.400
1	12	10	0.160
2	4	2	0.191
3	4	20	0.044
4	0	1	0.618

ITP, immune thrombocytopenic purpura; RV, rotavirus infection; M, male; F, female.

and ITP (12). The data presented in the current study demonstrated a higher prevalence of ITP in children presenting diarrhea with rotavirus infection compared with children without rotavirus infection. This suggests that an association exists between the clinical manifestation of diarrhea (9).

The mechanism of rotavirus-associated ITP could be elucidated by analyzing the kinetics of the immune response during rotavirus infection. Rotavirus antibodies can be initially detected in the serum as early as 2 days after diarrhea onset, which indicates a causative role of rotavirus infection in producing the diarrhea (13). However, in the present study, only 2 children developed ITP 2-4 days after diarrhea onset. Consequently, the kinetics of the immune response during rotavirus infection may not be the main mechanism of rotavirus-associated ITP.

In the present study, the majority of ITP cases (61.9%) in children with rotavirus infection occurred during the winter season. However, the incidence of ITP in children without rotavirus infection demonstrated no seasonal variation. In addition, the majority of subjects included in the current study dwelled in Tianjin in northern China. Notably, a previous large-scale epidemic study of rotavirus infection in the neighboring city of Beijing showed a peak number of cases in winter (14). By contrast, another study of new ITP cases from the Beijing Children's Hospital showed no seasonal variation (15).

In the current study, it is speculated that thrombocytopenia is not the only bleeding risk factor in ITP children with rotavirus infection, since the untreated platelet count showed no significant difference between the two groups. However, a significant decrease in MPV was observed in ITP children with rotavirus infection when compared with those without rotavirus infection. Similarly, Mete *et al* observed a

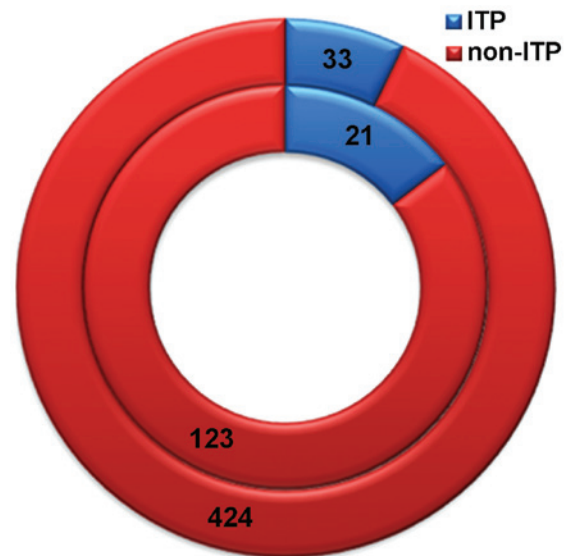


Figure 1. Donut chart showing the incidence of rotavirus infection in children with or without immune thrombocytopenic purpura.

significant decrease in MPV in children with acute rotavirus gastroenteritis (16). Since MPV is a marker of platelet function, we suggest that rotavirus infection may decrease platelet function.

However, the present study had a number of inherent limitations. Firstly, a large prospective cohort study was precluded due to the relatively small number of ITP children with rotavirus infection. Secondly, an observation bias is acknowledged since the study was not blinded. Finally, more sensitive detection methods for rotavirus have emerged over the past years, other than colloidal.

In conclusion, the present retrospective study investigated the association of rotavirus infection with the incidence of ITP in children. The results suggested that rotavirus may serve a causative role in ITP. Further prospective studies are required to determine how rotavirus affects platelets.

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