Original Article

Study of the changes in immune indexes, pathogenic characteristics and related risk factors in children with viral diarrhea

Di-Ying Wang, Feng Zhan, Hui-Ling Liu

Department of Clinical Laboratory, Changzhou Tumor Hospital Affiliated to Soochow University, Changzhou, China

Contributions: (I) Conception and design: DY Wang; (II) Administrative support: DY Wang; (III) Provision of study materials or patients: F Zhan; (IV) Collection and assembly of data: HL Liu; (V) Data analysis and interpretation: All authors; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Di-Ying Wang. Department of Clinical Laboratory, Changzhou Tumor Hospital Affiliated to Soochow University, Changzhou, China. Email: wdiying@163.com.

Background: Infectious diarrhea is a serious hazard to children under 5 years old. The causative microorganisms are mostly viruses and bacteria, with different treatment required for each. Currently, early clinical differential diagnosis is difficult with the available testing methods. Therefore, new and more sensitive indicators of viral infection reflect the early stage of infection are needed.

Methods: We collected blood samples and fresh fecal samples from 100 children diagnosed with viral diarrhea who were treated in the outpatient clinic at Changzhou Tumor Hospital Affiliated to Soochow University from January 2018 to December 2020. The levels of interleukin-2 (IL-2), IL-6, tumor necrosis factor-α (TNF-α), and C-reactive protein (CRP) in serum, the erythrocyte sedimentation rate (ESR) and enterovirus were measured and compared with those in a matched healthy control group. Patients' demographic and risk factor data were collected by interviewing parents.

Results: The mean levels of IL-2, IL-6, TNF-α, ESR, and CRP in the viral diarrhea group were higher than those in the healthy control group. Except for IL-6, the differences in inflammatory factors between groups were statistically significant ($P<0.05$). Human rotavirus (HRV) infection was the highest. Children's hand-washing habits, weekly disinfection of toys, and vaccination were protective factors for viral diarrhea, and indoor flies were an important risk factor ($P<0.05$).

Conclusions: The serum levels of markers were significantly increased in the children with viral diarrhea. HRV was the main pathogen, and mixed infections existed. Infection control requires hand washing, regular disinfection of toys, vaccination and preventing indoor flies.

Keywords: Children; viral diarrhea; inflammatory factors; pathogenic bacteria; risk factors

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Introduction

Infectious diarrhea is an intestinal disease worldwide, with high incidence rates and transmission among children (1). Diarrhea with a course of more than 2 weeks is generally called persistent diarrhea (PD) or chronic diarrhea diseases (CDD). The pathogenic microorganisms that cause diarrhea in children include bacteria, viruses, protozoa and fungi, among which viruses and bacteria are the main cause. Treatment differs, with bacterial diarrhea mainly treated with antibiotics, which are ineffective for viral diarrhea antibiotics (2). With improvements in sanitary conditions and the wide application of antibiotics, bacterial diarrhea has gradually decreased, and viruses are the most common pathogen causing diarrhea. Viral diarrhea

accounts for a small proportion in adult diarrhea, but it is the main cause of diarrhea in children. According to the World Health Organization, viral diarrhea is one of the top 10 causes of death in the world. Approximately 1.7 billion people suffer from diarrhea every year, with a fatality rate of ≈9%. Diarrhea is the second leading cause of death among children under 5 years old (3). Rational use of antibacterial agents and maintaining intestinal microecological balance are the key to the prevention and treatment of infantile diarrhea, at present, the differential diagnosis of infectious diarrhea mainly relies on stool culture and related inspections (4), but the slow process and the low positive rates of bacteriological and virus antigen testing do not meet the need for early clinical differential diagnosis (5). New and more sensitive indicators of viral infection, especially indicators that reflect the early stage of infection, are being investigated. Interleukin-2 (IL-2), IL-6, tumor necrosis factor-α (TNF-α), C-reactive protein (CRP) and the erythrocyte sedimentation rate (ESR) are all used as markers of early inflammation and are involved in the immune response (6-9), However, their application value in the management of viral diarrhea is unclear.

The purpose of this study was to analyze the changes in the serum levels of IL-2, IL-6, TNF-α, and in the ESR in children with viral diarrhea, as well as the etiological characteristics and disease-related risk factors, to provide references for the diagnosis, prevention and control of pediatric viral diarrhea.

We present the following article in accordance with the STROBE reporting checklist (available at https://dx.doi.org/10.21037/tp-21-433).

Methods

Research subjects

From January 2018 to December 2020, children with diarrhea aged 40 days to 60 months were selected from the outpatient department of Changzhou Tumor Hospital Affiliated to Soochow University. Among them, 100 children diagnosed with viral diarrhea by laboratory tests were selected, and 100 healthy children of the same sex and age in the hospital during the same time period were selected as the controls using 1:1 pairing. Viral diarrhea was clinically defined as defecation ≥3 times per day with a change in stool characteristics, and was confirmed by laboratory tests as human rotavirus (HRV), norovirus (Nov), human astrovirus (HAstV), human adenovirus (HAdV) or mixed infection. Informed consent was given by parents or guardians of the children and clinical data were provided (*Figure 1*). All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of Changzhou Tumor Hospital Affiliated to Soochow University (No.: 2017332).

Detection of inflammatory factors

Fasting venous blood was collected from the children in both groups in the morning, and serum was separated. IL-2, IL-6 and TNF-α were detected by ELISA kits (Shanghai Kaibo Biological Co., Ltd.). CRP was detected by Hitachi 7060 automatic biochemical analyzer using the appropriate detection reagent (Shanghai Rongsheng Biotechnology Co., Ltd.). ESR was measured by automatic analyzer (Shanghai Xunda Medical Instrument Company). All measurements are carried out in strict accordance with the manufacturers' instructions. The differences in the serum levels of inflammatory factors were compared between the two groups.

Detection of enteroviruses

A diarrhea virus joint detection system (Beijing Bohui) was used. Following the manufacturer's instructions.

Risk factor investigation

The risk factors in the case and control groups were investigated by a face-to-face questionnaire that included (I) baseline clinical information (child's name, sex, date of birth, place of residence, whether received HRV, etc.); (II) family history: parents' education level, home environment (type of drinking water, pet keeping, toy disinfection, indoor flies), and hygiene habits (disinfection of tableware, kitchen knives and boards; cooking hygiene, hand-washing, etc.).

Statistical analysis

SPSS 22.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Measurement data with a normal distribution are expressed as $\bar{x}\pm s$. The *t*-test of independent samples was used for comparison between groups. The Chi-square test was used for comparison of count data between groups. The risk factors of viral diarrhea were analyzed by univariate analysis and logistic regression model. P<0.05

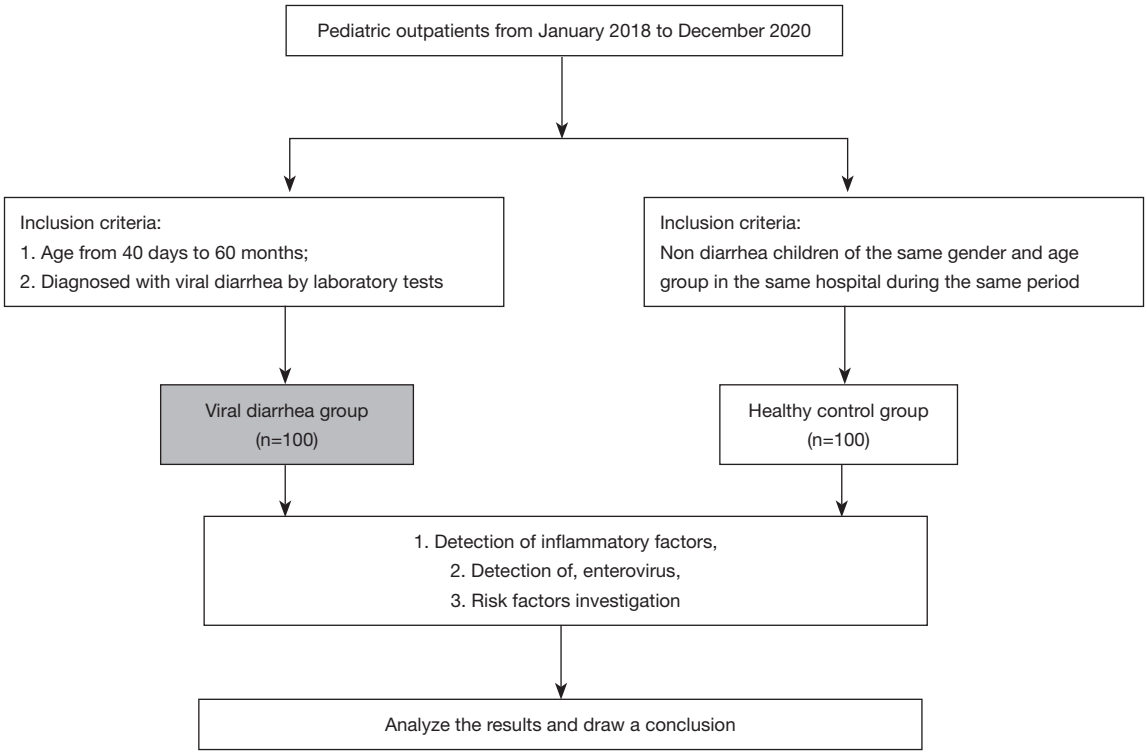

Figure 1 Study flowchart.

was considered a statistically significant difference.

Results

Levels of inflammatory factors

The levels of IL-2, IL-6, TNF-α, ESR, and CRP in the viral diarrhea group were respectively 50.38±16.45 pg/mL, 11.80±6.31 pg/mL, 67.55±14.08 pg/mL, 22.11 ±10.44 mm/h, and 11.28±7.22 mg/L versus 25.20±8.75 pg/mL, 10.33±4.61 pg/mL, 56.24±10.42 pg/mL, 7.02±3.11 mm/h, and 5.01±2.44 mg/L in the healthy control group. Except for IL-6, the differences in the levels of inflammatory factors were statistically significant between the two groups (P<0.05) (*Table 1*).

Types and proportions of viral infections

Among the 100 fecal samples, 92 were single virus-positive samples (92%), and 8 were mixed infections (8%); among the single-virus infections, HRV infection was the highest, with a total of 63 cases (63%). Mixed infections of HRV+ NoV (3 cases) and HRV+ HastV (3 cases) were the most common (*Tables 2*,*3*).

Risk factor analysis

Univariate analysis

Univariate analysis was performed for the factors related to viral diarrhea in children, and the results are shown in *Table 4*. Incidence was related to both the children's and the cooks' hand-washing habits, daily disinfection of tableware, weekly disinfection of toys, vaccination, whether separate cutting boards were used for raw and cooked food, and the presence of flies indoors (P<0.05).

Multivariate analysis

The results of multivariate logistic regression analysis are shown in *Table 5*. Children's hand-washing habits, weekly disinfection of toys, and vaccinations were protective factors (P<0.05). Indoor flies were an important risk factor for childhood viral diarrhea (P<0.05).

Discussion

The human immune system includes immune organs

Table 1 Comparison of immune indexes between the two groups

Group	n	IL-2 (pg/mL)	IL-6 (pg/mL)	TNF-α (pg/mL)	ESR (mm/h)	CRP (mg/L)
Viral diarrhea group	100	50.38±16.45	11.80±6.31	67.55±14.08	22.11±10.44	11.28±7.22
Healthy control group	100	25.20±8.75	10.33±4.61	56.24±10.42	7.02±3.11	5.01±2.44
F		27.089	14.568	7.875	90.605	96.034
P value		0.001	0.061	0.001	0.001	0.001

IL-2, interleukin-2; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; ESR, the erythrocyte sedimentation rate; CRP, C-reactive protein.

Table 2 Fecal enterovirus detection

Pathogen	No. of positive cases	Constituent ratio* (%)
HRV	63	63
NoV	25	25
HAstV	12	12
HAdV	8	8

*, because of mixed infection, the total positive number is not equal to 100%. HRV, human rotavirus; NoV, norovirus; HAstV, human astrovirus; HAdV, human adenovirus.

Table 3 Fecal enterovirus mixed infection

Mixed infection	N	Constituent ratio (%)
HRV + NoV	3	3
HRV + HAstV	3	3
NoV + HAstV	1	1
HRV + HAdV	1	1

HRV, human rotavirus; NoV, norovirus; HAstV, human astrovirus; HAdV, human adenovirus.

such as bone marrow and the thymus, immune cells such as lymphocytes, and immune active molecules such as antibodies and immunoglobulins. It is the basis for the body to recognize and resist foreign pathogens and maintain physiological functions and homeostasis (10). The development of both the immune system and digestive system of infants is not mature, and the level of immune antibody from mothers gradually declines (11), leading to increased occurrence of infectious diarrhea. Of the variety of causative pathogens, bacterial and viral infections are most common, especially viruses. It is currently believed that HRV is the most common cause of both viral diarrhea and acute gastroenteritis in children. Approximately 140 million children worldwide have HRV diarrhea each year, of which ≈25 million are outpatients and 2 million are visits. Due to the lack of effective symptomatic treatment, the number of deaths of children under 5 caused by HRV each year is about 527,000, mainly in developing countries (12,13). NoV is the second most common pathogen causing acute gastroenteritis in children, but with increasing HRV vaccination rates in some countries and regions, NoV is becoming the main cause of viral diarrhea in children in some regions (14,15). HAstV infection is widespread around the world. Due to different geographic locations and detection methods, the detection rate of HAstV varies between 2% and 9%. For example, the detection rate of HAstV viral diarrhea in South Korea is only 1.3% (16), 0.6% in Ho Chi Minh City, Vietnam (17), and 13.5% in Brazil (18). In the present study, 92 single virus-positive samples (92%) were detected, and 8 cases of mixed infection (8%). HRV infection was the highest single-virus infection, with a total of 63 cases (63%), followed by NoV infection, with a total of 25 cases (25%), consistent with previous domestic research (19,20).

In this study, analysis of serum indicators showed that IL-2 in the viral diarrhea group was significantly increased, indicating immune dysfunction. This is because the virus invades the jejunal villi epithelial cells and stimulates their large secretion T lymphocytes, and T lymphocyte activation can trigger the activation of the NF-κB pathway and upregulate the expression of IL-2. As a cytotoxic inflammatory factor, the increased level of IL-2 not only means autoimmunity is restricted, but also causes enhanced local inflammation and destruction of the intestinal barrier system (21,22).

TNF-α is an important member of the inflammatory cytokine network. It has strong inflammatory activity and can directly act on vascular endothelial cells to increase their permeability, leading to a large amount of intestinal wall exudation and excessive secretion of TNF-α, which can enter the blood circulation and cause systemic reactions, such as fever, because TNF-α is an endogenous pyrogen and

Table 4 Single-factor analysis results of children with viral diarrhea

Factor	Viral diarrhea group (n=100)	Healthy control group (n=100)	χ^2/t	P value
Sex, n [%]			2.040	0.199
Male	62 [62]	52 [52]		
Female	38 [38]	48 [48]		
Age (months)	21.74±15.54	22.06±15.42	0.146	0.884
Residence, n [%]			0.842	0.359
City	66 [66]	72 [72]		
Rural	34 [34]	22 [22]		
Education level of parents, n [%]			2.741	0.098
Junior college or above	81 [81]	29 [29]		
Senior high school and below	19 [19]	71 [71]		
Hand-washing habits of children, n [%]			109.033	0.001
Qualified	21 [21]	94 [94]		
Unqualified	79 [79]	6 [6]		
Hand-washing habits of cooks, n [%]			11.971	0.001
Qualified	82 [82]	97 [97]		
Unqualified	18 [18]	3 [3]		
Disinfect tableware every day, n [%]			22.154	0.001
Yes	27 [27]	60 [60]		
No	73 [73]	40 [40]		
Flies indoors, n [%]			77.003	0.001
Yes	83 [83]	79 [79]		
No	17 [17]	21 [21]		
Virus vaccination, n [%]			14.101	0.001
Yes	6 [6]	25 [25]		
Unknown	11 [11]	11 [11]		
No	83 [83]	64 [64]		
Disinfect toys weekly, n [%]			17.151	0.001
Yes	72 [72]	94 [94]		
No	28 [28]	6 [6]		
Drinking habit, n [%]			0.385	0.825
Bottled water	51 [51]	50 [50]		
Boiled tap water	47 [47]	49 [49]		
Other	2 [2]	1 [1]		
Use of cutting boards, n [%]			5.556	0.018
Separate raw from cooked	56 [56]	72 [72]		
Undivided	44 [44]	28 [28]		
Pet keeping, n [%]			0.209	0.648
Yes	33 [33]	30 [30]		
No	67 [67]	70 [70]		

Table 5 Results of multivariate logistic regression analysis of children with viral diarrhea

Related factor	β	SE	Ward	OR	95% CI	P value
Hand-washing habits of children	4.131	0.705	34.329	62.240	15.629–247.864	0.001
Hand-washing habits of cooks	2.048	1.360	2.269	7.754	0.539–111.442	0.132
Disinfect tableware every day	1.000	0.575	3.031	2.720	0.882–8.388	0.082
Flies indoors	–3.130	0.637	24.181	0.044	0.013–0.152	0.001
Virus vaccination	2.656	1.246	4.541	14.240	1.237–163.871	0.033
Disinfect toys weekly	1.868	0.884	4.465	6.475	1.145–36.618	0.035
Use of separate cutting boards	1.216	0.614	1.792	3.375	0.860–13.252	0.181

acts on the hypothalamus. It can also cause monocytes and vascular endothelial cells to secrete large amounts of IL-1 and IL-6, which enter the blood circulation and enhance the cytokine-mediated inflammatory response. In this study, the level of serum TNF-α in the viral diarrhea group was significantly higher than in the healthy control group. A study found that the level of TNF-α in severe diarrhea was significantly higher than that in the mild group, indicating that TNF-α is the main pro-inflammatory factor in viral infections without antiviral effect (23).

CRP is a normal protein component in human plasma at very low levels, but is also an acute-phase protein, with levels closely related to inflammation and tissue damage. When injuries or inflammation occur, the synthesis and decomposition rate of CRP in the liver increases. CRP is not affected by factors such as sex, age, etc. (24), Moreover, detection is convenient and results can be obtained quickly. Therefore, CRP can be used as a measure of the acute stage of infection, and our test results showed that the difference between the viral diarrhea and healthy control groups was highly significant (P<0.001).

ESR is a non-specific marker of tissue inflammation or destruction, especially potential (latent) disease, which is used in the diagnosis of malignant tumor. Monitoring the ESR can also be used to understand the progress of a disease, guide prognosis, and to evaluate a person's health status. It reflects the aggregation of fibrinogen and immunoglobulin. In acute inflammation, acute-phase substances (i.e., ESR, fibrinogen, etc.) increase rapidly, and their components can promote cell aggregation more or less, so ESR can be accelerated 2–3 days after inflammation (25). Our test results showed that the difference between the viral diarrhea and healthy control groups was highly significant (P<0.001).

Study limitations

There was no analysis of the changes in inflammatory factors during remission, no research on the influence of the levels of inflammatory factor and pathogen distribution on clinical symptoms, and no virus testing for patients in different seasons and of different ages. We will carry out supplementary research on these aspects in the future.

Conclusions

Based on our analysis, the factors associated with viral diarrhea can be summarized into three aspects: personal hygiene, family environmental hygiene and vaccination. As the main pathogens of viral diarrhea (e.g., HRV), mainly enter patients through contaminated food or utensils, good environmental and hygiene habits are effective measures to reduce the occurrence of viral diarrhea, which is consistent with previous research results of diarrhea risk factors (26). Vaccination is also a protective for viral diarrhea (27).

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Footnote

Reporting Checklist: The authors have completed the STROBE reporting checklist. Available at https://dx.doi.org/10.21037/tp-21-433

Data Sharing Statement: Available at https://dx.doi.org/10.21037/tp-21-433

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://dx.doi.org/10.21037/tp-21-433). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of Changzhou Tumor Hospital Affiliated to Soochow University (NO.:2017332) Informed consent was given by parents or guardians of the children.

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