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Original Article

Effect of bifidobacterium tetravaccine tablets-assisted therapy on T lymphocyte subpopulation of children with rotavirus enteritis

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Abstract: This study aimed to explore the effect of bifidobacterium tetravaccine tablets-assisted therapy on T lymphocyte subpopulation of children with rotavirus enteritis. A total of 86 cases of children with rotavirus enteritis in our hospital were randomly divided into the control group and observation group. The control group were given conventional treatments, such as antiviral, fluid infusion, correction of fluid and electrolyte imbalance and acid-base imbalance, etc., while the observation group were given additional bifidobacterium tetravaccine tablets besides conventional treatments. Flow cell method (fcm) was adopted to analyze proportions of CD3+, CD4+ and CD8+ in peripheral blood lymphocyte subpopulations as well as CD4+/CD8+ ratio before and after treatment. Curative effect, antidiarrhea time, fever abatement time, extinction time of emesis and length of stay in the hospital (LOS) of two groups were compared. Compared to the control group, the observation group showed significantly higher proportions of CD3+ and CD4+ cells and CD4+/CD8+ ratio ($P<0.05$), but far lower proportion of CD8+ cells ($P<0.05$). The observation group achieved significantly higher overall response rate than the control group ($P<0.05$); antidiarrhea time, fever abatement time, dehydration correction time and LOS of the observation group were significantly shorter than those of the control group ($P<0.05$). In conclusion, the proposed bifidobacterium tetravaccine tablets-assisted therapy can prominently improve immuneal functions of children with rotavirus enteritis, clinical symptoms and curative effect and shorten curative time.

Keywords: Bifidobacterium tetravaccine tablets, rotavirus enteritis, T lymphocyte subpopulation, curative effect

Introduction

Rotavirus (RV), the main pathogen of severe gastroenteritis in the world, can replicate in differentiated small intestinal mucosa, and its morbidity and severity rank the first in infantile diarrhea, especially in autumn and winter. Nearly all children younger than 5 years old in developing and developed countries will be infected with RV [1, 2]. RV enteritis, featured by high morbidity and strong infectivity, has always been the key of prevention and control in paediatric diseases. According to epidemiological investigations, there are 111 million infant gastroenteritis cases caused by RV every year, including 350,000-680,000 deaths. RV infection influences children's health significantly [3, 4]. At present, abundant researches on etiology, epidemiology, clinics and prevention related to RV infection have been reported. Immunology mechanism is attracting more and more research attentions. Children diarrhea is easy to turn into a chronic disease because of poor functions and stability of intestinal mucosal barrier. Moreover, pathogene will enter into lymph and blood through the intestinal mucosal barrier upon serious infectious diarrhea, thus resulting in general infection. Clinical test of CD3+, CD4+ and CD8+ is a common way to understand T cells. CD4+/CD8+ ratio plays an important role in immuneal regulation. At immunodeficiency, CD4+ cells and CD4+/CD8+ will decline sharply, thus influencing immune functions, restricting B lymphocyte and resulting in low level of immune globulin [5]. No reliable anti-RV drug is available yet. Despite of symptomatic treatment, immune functions upon diarrhea shall be fully considered and corre-

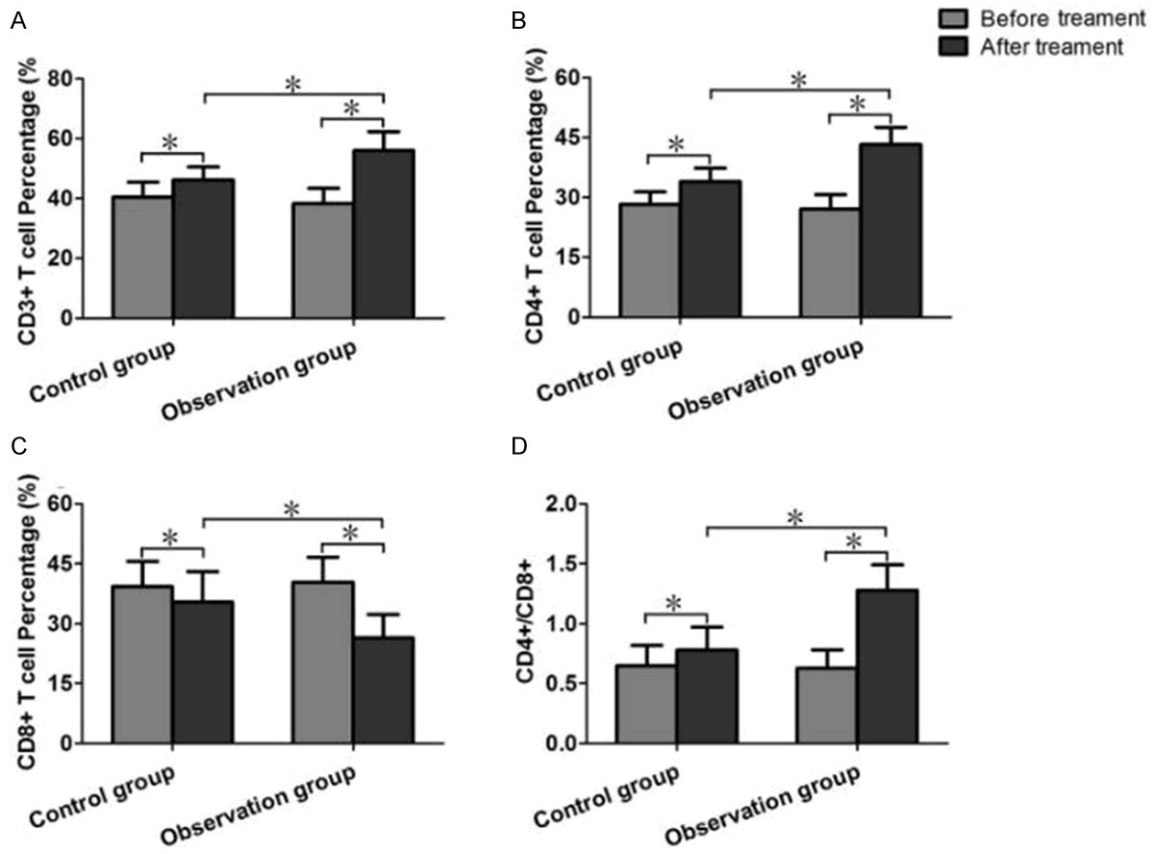

Figure 1. T lymphocyte subpopulations proportions of the control group and observation group before and after treatment. A. CD3+T lymphocyte subpopulations in two groups before and after treatment; B. CD4+T lymphocyte subpopulations in two groups before and after treatment; C. CD8+T lymphocyte subsets in two groups before and after treatment; D. CD4/CD8 ratio in two groups before and after treatment.

sponding treatment shall be adopted. Bifidobacterium is an important physiological bacterium in intestinal canal of human body, and its amount in the organism is one of health indexes. It can protect and regulate immunity, improve balance between bacterium and enzyme on surface of intestinal mucosa, and stimulate specific and non-specific immune functions [6-8]. In this paper, effect of bifidobacterium tetravaccine tablets-assisted therapy on T lymphocyte subpopulation of children with RV enteritis was discussed and its clinical curative effect was observed.

Subjects and methods

General data

A total of 86 cases of children with RV enteritis in Children's Hospital of Kaifeng City from December, 2013 to August, 2015 were chosen as test samples. They were randomly divided into the control group and observation group, 43 cases for each group. Stool RV test (+) and bacterial culture (-) of all cases were implemented using PCR method. Combining with typical clinical manifestation, they were all diagnosed having VR enteritis. The control group includes 25 males and 18 females aging from 6 months to 4 years old (1.9±0.7 years in average). The observation groups includes 23 males and 20 females aging from 5 months to 4 years old (2.1±0.8 years in average). There was no statistical significant differences between two groups in term of age, gender and dehydration degree ($P>0.05$). This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Children's Hospital of Kaifeng City. Written informed consent was obtained from all participants.

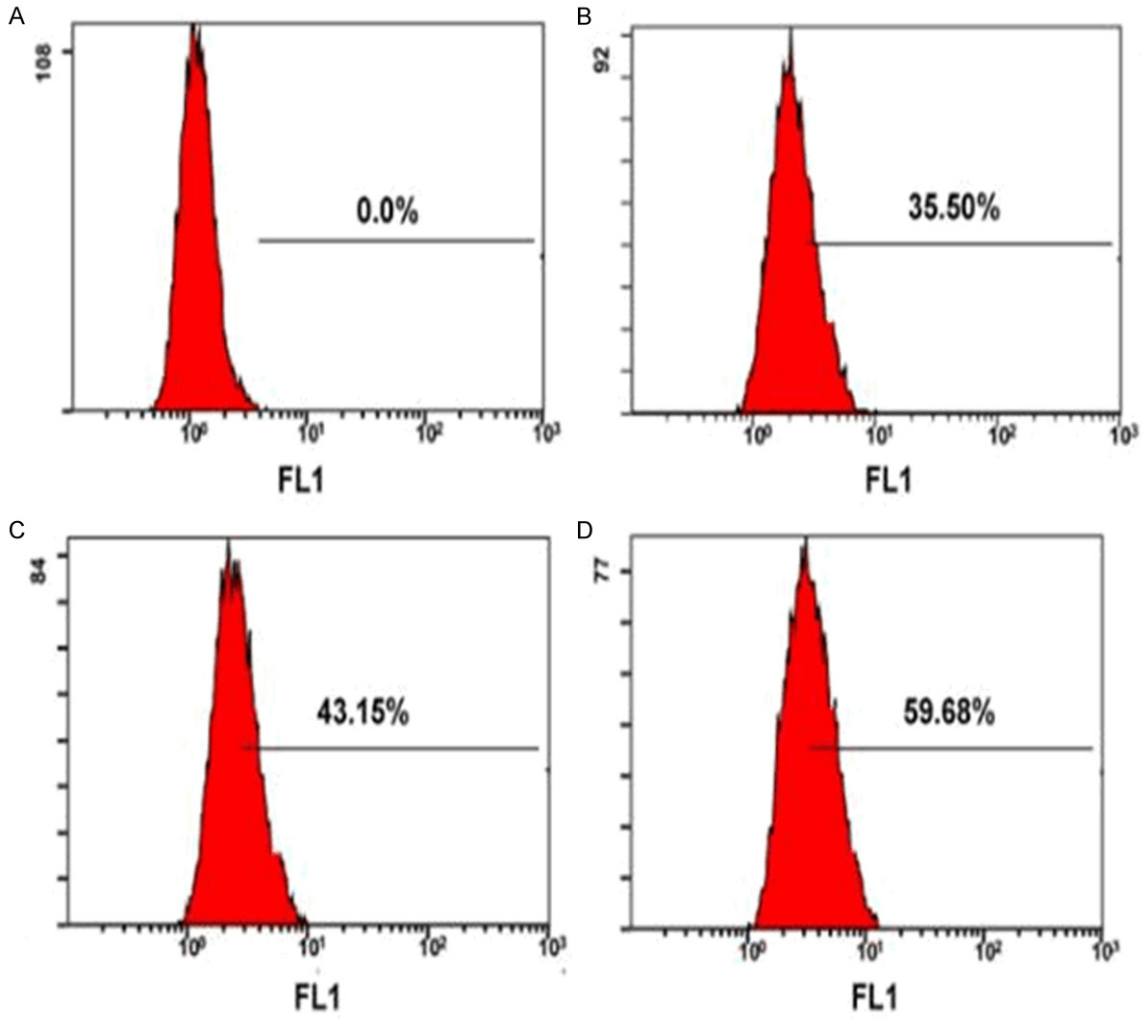

Figure 2. CD3 T lymphocyte cells of the control group and observation group. A. Negative control; B. CD3 T lymphocyte cells in control group and observation group before treatment; C. CD3 T lymphocyte cells in control group after treatment; D. CD3 T lymphocyte cells in observation group after treatment.

Therapeutic method

The control group were given conventional treatments, such as antiviral, fluid infusion, correction of fluid and electrolyte imbalance and acid-base imbalance, etc. The observation group were given additional bifidobacterium tetravaccine tablets (commodity name: Siliankang; State Medical Permit No.: 060010; Longda New-Tech Bio-pharmacy Co., Ltd, Hangzhou, China) expect for conventional treatments. Half tablet each time for children younger than one year old and one tablet each time for children over one year old, 3 tims per day. Stool frequency and property, emesis symptom, dehydration state and adverse drug reaction of all cases during therapy were observed. Antidiarrehal time, fever abatement time, extinction time of emesis and LOS were recorded.

Detection method

For both groups, 1 ml anti-coagulative venous blood were collected before and after treatment. Next, 10 ul CD3+, CD4+, CD8+ McAb and 50 ul EDTA anti-coagulative blood were added into exclusive-use flow tubes, respectively. They were mixed fully and then incubated for 15 min in dark place. Later, 2 ml hemolysin was added in each tube and mixed completely, followed by 15 min incubation in dark place. Then, they were centrifugated for 5 min at a speed of 1000 r/min and supernatants were removed. Subsequently, 2 ml PBS was added into each tube and mixed evenly. They were centrifugated

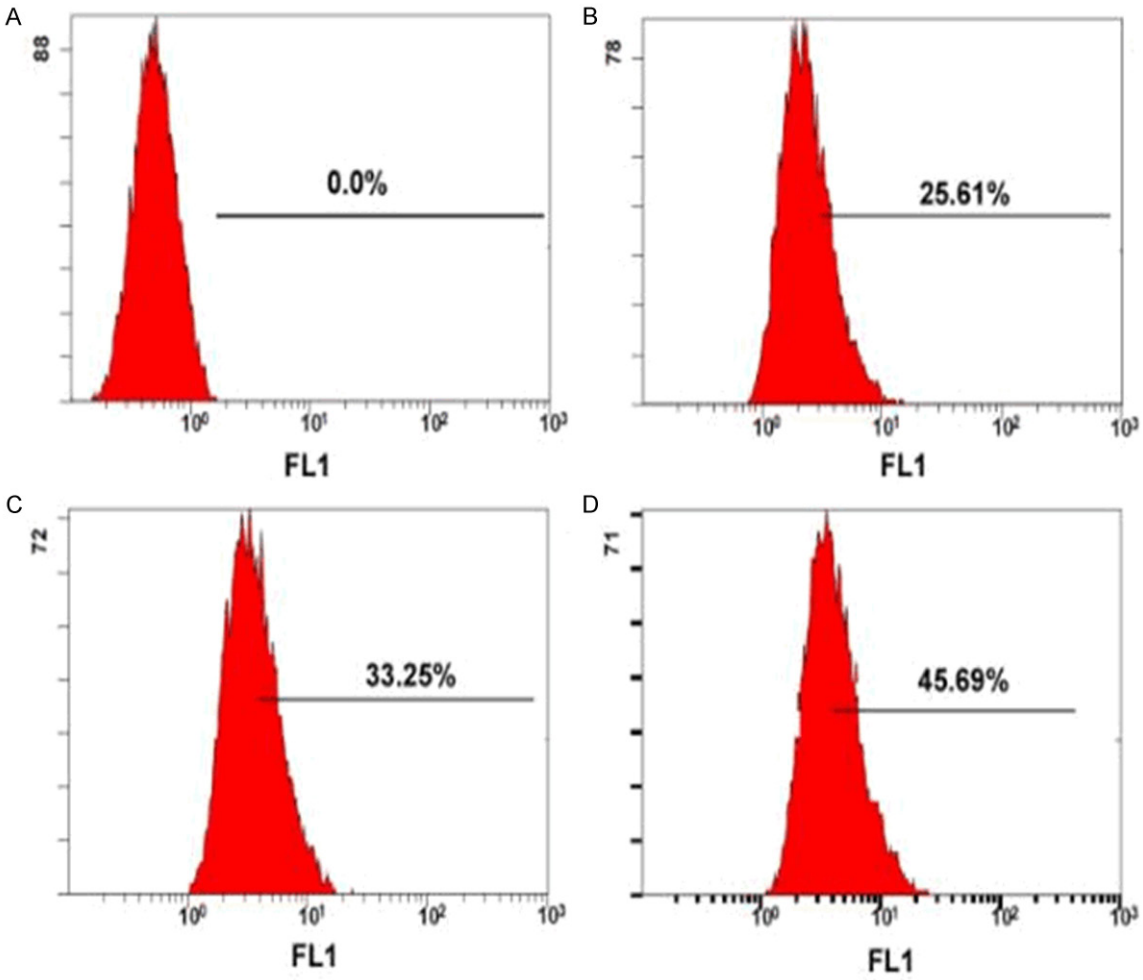

Figure 3. CD4+ T lymphocyte cells of the control group and observation group. A. Negative control; B. CD4+ T lymphocyte cells in control group and observation group before treatment; C. CD4+ T lymphocyte cells in control group after treatment; D. CD4+ T lymphocyte cells in observation group after treatment.

for 5 min at a speed of 1000 r/min supernatants were eliminated again. Remains were washed with PBS twice and supernatants were eliminated. Finally, 2 ml PBS were employed and mixed completely for following test on upflow machine.

Curative effect assessment

Evaluation criterion of curative effect referred to China's Judgment Criterion of Curative Effect on Diarrhea [9]. Remarkable effective: 72 h later, diarrhea frequency reduced to less than twice per day, no obvious abnormity but shaped and soft stool was detected in stool routine examination, and clinical symptoms totally disappeared. Effective: 72 h later, diarrhea frequency reduced to less than three times per day, stool properties were improved greatly (without stool abnormality), and clinical symptoms basically disappeared. Ineffective: 72 h later, improvements of stool property, diarrhea frequency and clinical symptoms were not satisfying or even exacerbated compared to those before the treatment. Overall response rate = (remarkable effective+effective)/total cases * 100%.

Statistical analysis

All data were analyzed using SPSS 17.0 software (SPSS Inc, Chicago, IL, USA). Effective comparison used Chi-square test. Measurement data were expressed in x±s. Inter-group

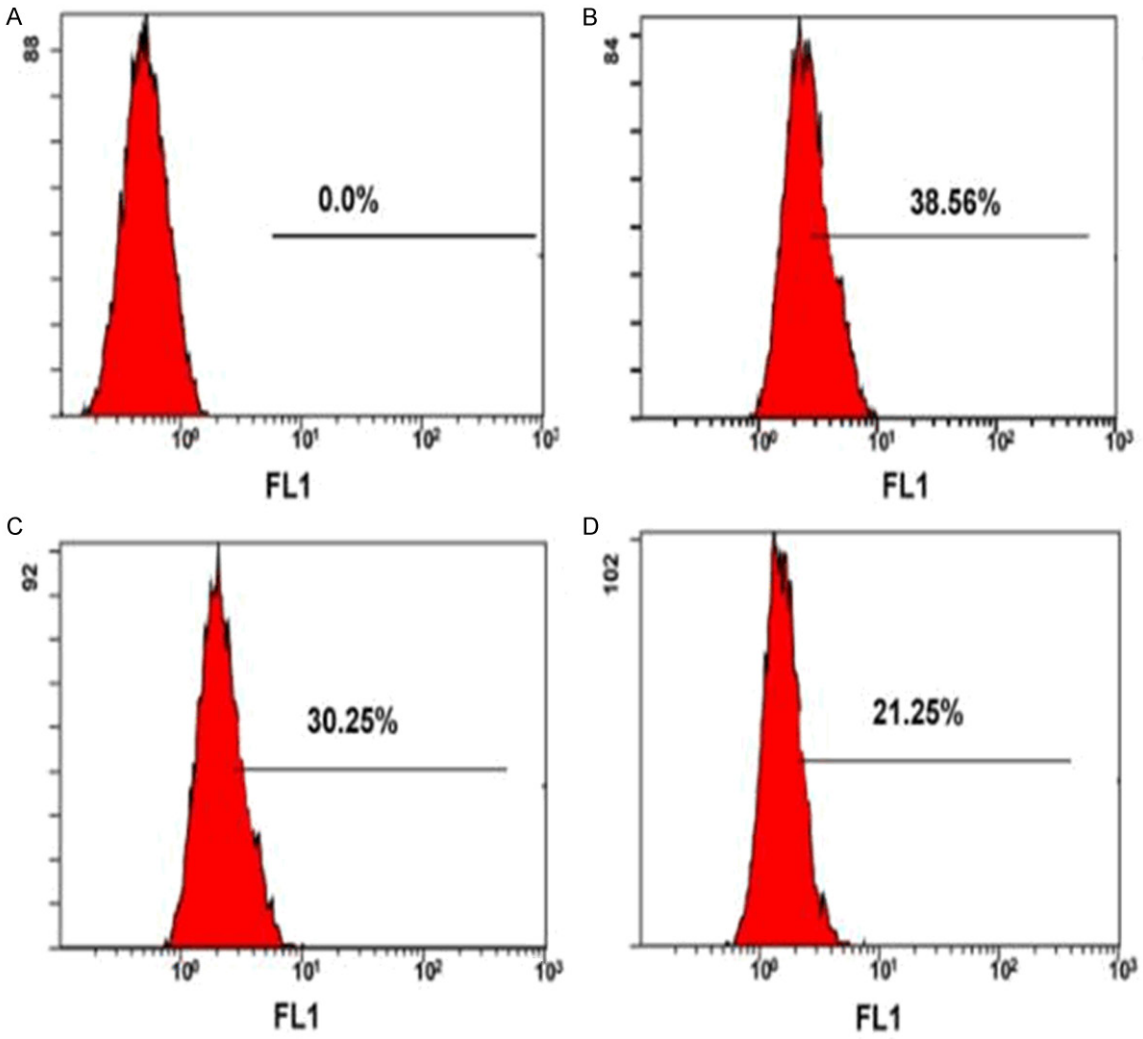

Figure 4. CD8+ T lymphocyte cells of the control group and observation group. A. Negative control; B. CD8+ T lymphocyte cells in control group and observation group before treatment; C. CD8+ T lymphocyte cells in control group after treatment; D. CD8+ T lymphocyte cells in observation group after treatment.

comparison was for t-test. $P<0.05$ indicated that the difference was statistically significant.

Results

Comparison of T lymphocyte subpopulation proportions after treatment

As shown in **Figures 1-4**. Before treatment, there is no statistical significant difference between the control group and observation group in proportions of CD3+, CD4+, CD8+ and CD4+/CD8+ ratio ($P>0.05$). But after treatment, proportions of CD3+, CD4+, CD8+ and CD4+/CD8+ ratio of the observation group are significantly higher than those of the control group ($P<0.05$), whereas the proportion of CD8+ cells is far lower ($P<0.05$).

Comparison of major symptoms improvement time after treatment

Compared to the control group, the observation group shows significantly shorter anti-diarrhea time, fever abatement time, extinction time of emesis and LOS after treatment. Difference of symptoms improvement time between two groups has statistical significance ($P<0.05$) (**Table 1**).

Comparison of curative effects

After treatment, the control group achieved 23 remarkable effective cases and 11 effective cases, showing an overall response rate of 81.40%. The observation group achieved 31 remarkable effective cases and 12 effective

Table 1. Comparison of clinical symptoms in two groups of children

Group	n	Anti-diarrhea time (d)	Fever abatement time (d)	Extinction time of emesis (d)	LOS (d)
Control group	43	4.12±0.63	3.85±0.54	2.85±0.41	6.55±2.06
Observation group	43	2.43±0.47	2.15±0.47	1.51±0.36	4.11±1.53
t		3.14	4.96	3.27	2.93
P		<0.01	<0.05	<0.05	<0.05

Note: LOS: length of stay in the hospital.

Table 2. Comparison of overall response rate between two groups

Group	n	Remarkable effective(n)	Effective (n)	Ineffective (n)	Overall response rate (%)
Control group	43	31	11	1	97.67%
Observation group	43	23	12	8	81.40%

cases, presenting an overall response rate of 97.67%. Difference of overall response rate between two groups has statistical significance (P<0.05) (**Table 2**).

Discussion

RV enteritis is the most common pathogen of acute infantile diarrhea, especially in autumn and winter. Main clinical manifestations are emesis and diarrhea. Serious patients often have complications of dehydration, acidosis and electrolyte imbalance, and even suffer damages to various systems (e.g. respiratory tract, nervous system, heart and liver) [10-12]. Previous researches on RV infection mechanism mainly focus on humoral immunity. In recent years, effect of cellular immunity on RV infection attracts more and more research attentions. Immune cells are mainly lymphocytes, such as TB cell, K cell, NK cell, etc. except for direct involvement in immune response and immune regulation, they can generate all kinds of bioactive substances and exert different biological effects in immune response. Clinically, CD3+, CD4+ and CD8+ T lymphocytes test is a common way to understand cellular immune functions of the organism. Researches show that virus infections would affect proportions of CD3+, CD4+ and CD8+ T lymphocytes and decrease CD4+/CD8+ ratio. This causes imbalance of immune regulating functions of the organism and insufficient antibody of B lymphocytes, thus further influencing immune functions [13, 14]. Our research found that CD3+, CD4+ and CD8+ T lymphocytes and CD4/CD8 ratio in PB of children with RV are different from those of normal children, indicating that children with RV are suffering significantly abnormal immune functions. Hence, improving immune functions of patient children effectively is of great significance to enhance curative effect.

Intestinal flora regulation is an important component of therapeutic strategy of RV enteritis. Various micro-ecological agents are widely applied to clinical treatment. Bifidobacterium tetravaccine tablets are compound tetravaccine agents composed of bifidobacterium infantis, lactobacillus acidophilus, enterococcus faecalis and bacillus cereus. After taken orally, they will plant and multiply in intestinal tract and supplement tremendous beneficial bacterium for intestinal tract. They will form a biological barrier, inhibit growth and reproduction of enteropathogenic bacteria, and reduce generation of enterogenous toxin, which is beneficial for intestinal flora balance. Meanwhile, they can stimulate organism immune system to generate a large amount of cell factors, stimulate immunity of the organism and form immune barriers [15-17].

This study gives bifidobacterium tetravaccine tablets to children with RV enteritis except for conventional treatment. Variations of T lymphocyte subpopulation in PB before and after the treatment are observed. Research results show that compared to results before treatment, the control group has evidently higher proportions of CD3+ T lymphocyte and CD4+ T lymphocyte and CD4+/CD8+ ratio, but greatly lower proportion of CD8+ T lymphocyte. Proportions of CD3+ T lymphocyte and CD4+ T lymphocyte and CD4+/CD8+ ratio the observation group are far higher than those of the control group do, but the proportion of CD8+ T lymphocyte is

far lower. This reflects that bifidobacterium tetravaccine tablets can prominently improve T lymphocyte subpopulation in PB of children with RV and recover normal immune fcuntional region. Furthermore, we analyze clinical symptoms and curative effect improvement state of two groups and conclude that the observation group achieves far better curative effect than the control group. Additionally, all clinical indexes of the observation group, such as anti-diarrhea time, fever abatement time, extinction time of emesis and LOS, decrease greatly compared to those of the control group. This indicates that bifidobacterium tetravaccine tablets-assisted therapy for RV enteritis has certain curative effect. Such curative effect may be realized by improving immune functions of patient children.

In short, bifidobacterium tetravaccine tablets-assisted therapy for RV enteritis has remarkable curative effect and could relieve clinical symptoms quickly. This may be because bifidobacterium tetravaccine tablets improve T lymphocyte subpopulation of patient children and enhance their cellular immune functions.

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Disclosure of conflict of interest

None.

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