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Original Article

Efficacy of montmorillonite and vitamin AD combined with zinc preparation in children with diarrheal disease and its effect on inflammatory factors

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Abstract: Objective: To investigate the effect of montmorillonite and vitamin AD combined with zinc preparation in children with diarrheal disease and its effect on inflammatory factors. Methods: A total of 156 children with diarrheal diseases admitted to our hospital from January 2018 to January 2020 were enrolled and divided into two groups (n=78 for each) using random number table. The control group (CG) was treated with montmorillonite, and the observation group (OG) was additionally treated with vitamin AD and zinc. Both groups completed 7 d of treatment and 3 months of follow-up to compare the efficacy rate, time to onset of symptom relief, inflammatory factor levels, T-lymphocyte levels, adverse drug reactions, and relapse rate. Results: The efficacy rate of 7 d treatment in the OG was 94.87%, higher than that of 56.41% in the CG ($P < 0.05$). After intervention, the OG had normal frequency of defecation, shorter time for stool to return to normal appearance, shorter duration of antiemetic, antipyretic, antidiarrheal treatment and shorter hospital stay than those of the CG ($P < 0.05$). The OG exhibited lower levels of CRP, TNF-α, NO, and PCT and higher levels of SOD than the CG ($P < 0.05$). The OG had lower levels $CD3^+$ and $CD4^+$ at 7 d after treatment, lower $CD8^+$ levels and higher $CD4^+/CD8^+$ levels than the CG ($P < 0.05$). The relapse rate at 6 months after treatment in the OG was lower than that in the CG ($P < 0.05$). Conclusion: The combination of montmorillonite, vitamin AD and zinc preparation can achieve higher short-time efficacy, shorten the time of disappearance of symptoms, reduce the level of inflammatory factors, and improve the level of T-lymphocytes, without increasing the incidence of adverse drug reactions.

Keywords: Montmorillonite, vitamin AD, Zinc preparations, pediatric diarrhea, inflammatory factors, T-lymphocyte levels, adverse reactions

Introduction

Diarrhea is a disease mainly caused by multiple pathogens and multiple factors in children, which is clinically characterized by increased frequency of stool and changes in stool shape, and some patients may be accompanied by fever, vomiting and abdominal pain [1]. Meanwhile, some patients may experience water, electrolyte and acid-base balance disorders after the onset of the disease. Evidence has shown [2] that the common pathogens of diarrhea in childhood include viruses, bacteria, parasites, fungi, *etc*. Extraintestinal disorders and misuse of antibiotics can cause imbalance of intestinal flora and affect the healthy development of children. Epidemiological results have shown [3] that diarrhea is a very common problem in children in China, second only to respiratory infections, and is also the cause of malnutrition and developmental disorders in children, as well as the main cause of death in children under 5 years old. Currently, the treatment options of pediatric diarrhea mainly focus on rehydration, antiretroviral therapy, and correction of acid-base changes. Although it can alleviate symptoms, the poor long-term prognosis and high recurrence rate make it difficult to achieve the expected therapeutic effect [4].

Montmorillonite is a common therapeutic drug used in the treatment of pediatric diarrhea. It

has a laminar structure and non-uniform charge distribution, which can immobilize and inhibit viruses, bacteria and toxins produced in the digestive tract [5]. Meanwhile, montmorillonite could cover the mucosa of the digestive tract and bind mucosal glycoproteins to repair and improve the defense of the mucosal barrier against external factors. Zinc preparations can enhance the regeneration of intestinal mucosal cells, improve the function of intestinal mucosa in sodium and water reabsorption, reduce the secretion of water electrolyte, improve the resistance and immunity of the body, delay the development of the disease, which is conducive to the recovery [6]. Vitamin AD is an essential substance for human development, which can ensure the integrity of epithelial tissue and is widely used in diseases such as rickets and chondromalacia [7]. A study has shown [8] that vitamin AD can reduce the incidence of infectious disease and the recurrence rate in patients with pediatric diarrhea. However, there are few clinical studies on the efficacy of montmorillonite and vitamin AD combined with zinc on inflammatory factors in children with diarrhea. This study was therefore conducted to investigate the effect of montmorillonite, vitamin AD combined with zinc in children with diarrheal disease.

Materials and methods

Clinical data

A total of 156 children with diarrheal diseases admitted to our hospital from January 2018 to January 2020 were divided into two groups using random number table, with 78 patients in each group. The study was approved by the Ethics Committee of the Anhui Children's Hospital and all procedures were done with patient/family's consent. The patients or their families signed informed consent form.

Inclusion and exclusion criteria

Inclusion criteria: (1) Patients who met the diagnostic criteria for pediatric diarrhea in "Zhu Futang Practical Pediatrics (7th edition)" [9]; (2) those treated with montmorillonite, vitamin AD and zinc, which were tolerated by the patients; (3) Those with complete baseline and follow-up data; (4) And those who had watery or lose stools.

Exclusion criteria: (1) Patients combined with immune deficiency or severe malnutrition and hereditary metabolic diseases; (2) Patients with severe infections, food allergies, inflammatory bowel disease and chronic gastrointestinal diseases; (3) Patients who had undergone thoracic and abdominal surgery three months prior to participation and had severe liver and kidney dysfunction; (4) Patients with severe organ disorders and severe malnutrition.

Methods

Both groups were given routine examinations after admission, and patients were treated with conventional methods such as rehydration, anti-infection, and regulation of intestinal flora according to the degree and nature of dehydration. The dietary management was strengthened, and the balance of acid-base and water-electrolyte was regulated [10]. In the control group (CG), montmorillonite treatment was used. The montmorillonite powder (Hunan Zhonghe Pharmaceutical Co., Ltd., H20094210) was administrated according to the age of the child. For children aged 1-2 years, 1.0 g of montmorillonite was taken orally; for children aged 2-3 years, 1.5 g of montmorillonite was taken orally; for children aged > 4 years, 2.0 g of montmorillonite was taken three times a day for 7 consecutive days. In the observation group (OG), vitamin AD and zinc were added to the regimen. One capsule of vitamin AD (Anhui Wildman Pharmaceutical Co., Ltd., H20084481) was taken orally twice a day for 7 d (1 course of treatment); 10 mL of zinc preparation (Hainan Huigu Pharmaceutical Co., Ltd., H20083017) was taken orally after meals twice a day for 7 d (1 course of treatment). All patients were followed up for 3 months after completion of treatments.

Outcome measurement

(1) Response rate. The efficacy was evaluated as markedly effective, improved, and ineffective at 7 d after treatment in the two groups, respectively. Markedly effective: stool shape, color and frequency of defecation were normal after the completion of the treatment course, and there was no abdominal distension, or abdominal pain. Improved: stool features and frequency of defecation were improved after the completion of the treatment, accompanied by mild abdominal pain and abdominal disten-

Table 1. Comparison of clinical data between the two groups

Clinical data		Observation group (n=78)	Control group (n=78)	χ^2/t	*P*
Gender	Male	54 (69.23)	52 (66.67)	0.618	0.338
	Female	24 (30.77)	26 (33.33)		
Age (years)		1.59±0.43	1.60±0.44	1.423	0.714
Duration of disease		1.32±0.26	1.34±0.29	1.936	0.632
Stool frequency (times/day)		8.51±0.69	8.52±0.71	1.025	0.825
Degree of dehydration	Mild	52 (66.67)	48 (61.54)	0.982	0.428
	Moderate	20 (25.64)	22 (28.21)		
	Severe	6 (7.69)	8 (10.26)		

Table 2. Comparison of response rate

Group	Markedly effective	Improved	Ineffective	Response rate
Observation group (n=78)	50 (6.410)	24 (30.77)	4 (5.13)	64 (82.05)
Control group (n=78)	28 (35.90)	16 (20.51)	34 (43.59)	44 (56.41)
χ^2				6.392
P				0.026

sion. Ineffective: the treatment option was ineffective or needed to be adjusted [11].

(2) Time to onset of symptom relief. Normal stool frequency, time to return of normal stool shape and color, duration of antiemetic, antipyretic, and antidiarrheal treatment as well as mean length of hospital stay [12] were recorded in both groups.

(3) Inflammatory factor levels. In both groups, 3 mL of peripheral blood was collected before and at 7 days after treatment, and the patients' C-reactive protein (CRP) levels were measured by immunoturbidimetric assay after centrifugation; tumor necrosis factor-α (TNF-α), nitric oxide (NO), and superoxide dismutase (SOD) levels were measured by enzyme-linked immunosorbent assay [13]; calcitonin (PCT) levels were measured by semi-quantitative solid-phase immunoassay [14].

(4) T-lymphocyte levels were measured as follows. The above isolated serum samples were obtained and the $CD3^+$, $CD4^+$, $CD8^+$ and $CD4^+/CD8^+$ levels were measured by flow cytometry [15].

(5) Adverse drug reactions and relapse rates. The incidence of mild constipation, liver and kidney impairment, and atopic dermatitis during treatment was recorded. Both groups were followed up for 6 months, and the relapse rate at 1, 3, and 6 months after treatment was recorded.

Statistical analysis

Data were processed by SPSS18.0 software. Count data were expressed as n (%) and compared by χ^2 test. The measurement data were expressed as mean ± standard deviation ($\bar{x}$ ± sd) and examined by *t* test. $P < 0.05$ means difference is statistically significant.

Results

Comparison of the baseline data

There was no significant difference in terms of the baseline data including gender, age, duration of disease, stool frequency and degree of dehydration between the two groups ($P > 0.05$, **Table 1**).

Comparison of response rate

The response rate at 7 d after treatment with montmorillonite, vitamin AD and zinc was 94.87% (including 50 cases markedly effective and 24 cases with improvement) in the OG, which was higher than 56.41% (including 28 cases markedly effective and 16 cases with improvement) in the CG ($P < 0.05$, **Table 2**).

Comparison of symptom relief time

Symptoms were significantly improved in both groups. There was no significant difference in time to stop vomiting and time to bring down the fever between the two groups ($P > 0.05$). The normal stool frequency, time to return of the stool characteristic, time to stop vomiting, bring down fever, stop diarrhea and the average

Table 3. Comparison of symptom relief time ($\bar{x}$ ± sd)

Grouping	Number of cases	Normal stool frequency	Time to return of normal stool	Duration of antiemetic treatment	Duration of antidiarrheal therapy	Duration of fever-reducing therapy	Average length of hospital stay
Observation group	78	7.12±0.62	7.36±0.81	2.39±0.51	2.32±0.41	1.49±0.42	4.58±0.81
Control group	78	12.53±1.61	12.49±0.94	2.40±0.52	4.11±0.48	1.51±0.43	6.72±0.89
t	-	6.392	5.335	1.481	6.735	1.223	5.791
P	-	0.000	0.000	0.326	0.000	0.778	0.000

Table 4. Comparison of inflammatory factors ($\bar{x}$ ± sd)

Grouping		CRP (mg/L)	TNF-α (pg/mL)	NO (umol/L)	SOD (IU/L)	PCT (ng/mL)
Observation group (n=78)	Pre-treatment	9.43±1.04	43.29±5.36	15.79±1.67	25.39±3.25	2.41±0.85
	7 d after treatment	2.41±0.69[a,b]	18.49±3.21[a,b]	10.23±1.12[a,b]		
Control group (n=78)	Pre-treatment	9.42±1.02	43.40±5.37	15.80±1.68	25.40±3.26	2.40±0.84
	7 d after treatment	4.75±0.78[b]	25.37±4.61[b]	13.51±1.41[b]	41.23±4.69[b]	1.41±0.43[b]

Compared with the control group, [a]$P < 0.05$; compared with the pre-treatment within same group, [b]$P < 0.05$.

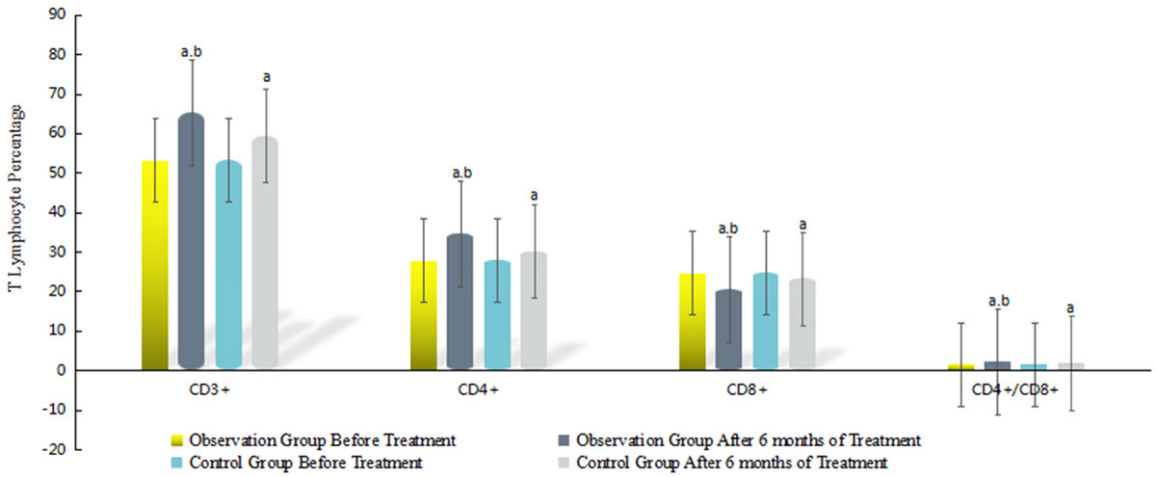

Figure 1. T-lymphocyte levels in the two groups. Note: compared with the pre-treatment within the same group, [a]$P < 0.05$; compared with the control group, [b]$P < 0.05$.

length of hospital stay in the OG were shorter than those in the CG after intervention ($P < 0.05$, **Table 3**).

Comparison of inflammatory factors

The OG exhibited lower levels of CRP, TNF-α, NO and PCT and higher levels of SOD than the CG ($P < 0.05$, **Table 4**).

Comparison of T-lymphocyte levels

There was no significant difference in the levels of T lymphocytes between the two groups before treatment ($P > 0.05$). The OG showed higher levels of $CD3^+$, $CD4^+$, $CD4^+/CD8^+$ and lower levels of $CD8^+$ than the CG ($P < 0.05$, **Figure 1**).

Comparison of adverse drug reactions and relapse rates

The incidence of adverse drug reactions and the recurrence rate at 1 and 3 months after treatment exhibited no significant difference between the two groups ($P > 0.05$). The recurrence rate at 6 months after the course of treatment in the OG was lower than that in the CG ($P < 0.05$, **Table 5**).

Discussions

Diarrhea is common in the pediatric populations. With the progression of the disease, some children may have symptoms such as vomiting, low appetite (the stool frequency per

Table 5. Comparison of adverse drug reactions and relapse rates [n (%)]

Grouping	Number of cases	Adverse reactions			Relapse rate		
		Mild constipation	Liver and kidney damage	Rash and allergy	1 month	3 month	6 month
Observation group	78	2 (2.56)	2 (2.56)	2 (2.56)	0 (0.00)	0 (0.00)	2 (2.56)
Control group	78	2 (2.56)	0 (0.000)	2 (2.56)	0 (0.00)	2 (2.56)	14 (17.95)
χ^2	-		0.214		0.000	1.013	5.014
P	-		0.645		1.000	0.314	0.025

day can be more than 10 times, mostly characterized by watery stool or loose stool) [16]. A study has shown [17] that pediatric diarrhea is closely related to intestinal/external infections, improper dietary care, primary or secondary disaccharidase deficiency, *etc*. The growth and health of the children will be affected if no effective therapeutic interventions are provided. Symptomatic treatment was often used for pediatric diarrhea, which can improve the symptoms and delay the development, but the long-term prognosis is poor and the recurrence rate is high after treatment [18].

In recent years, montmorillonite and vitamin AD combined with zinc preparations has been used in children with diarrhea, achieving positive results [19]. In the present study, the response rate of zinc combination with montmorillonite and vitamin AD was 94.87% in the OG, which was higher than that of 56.41% in the CG ($P < 0.05$), suggesting that zinc combined regimen can achieve higher efficacy in pediatric diarrhea and is beneficial to the recovery of children. Montmorillonite is a commonly used medicine for children with diarrhea, especially for acute and chronic diarrhea in adults and children. It can also be used as an adjunctive treatment for pain symptoms caused by esophageal, gastric and duodenal diseases. Modern pharmacological results [20] have shown that montmorillonite has a layered structure and a non-uniform charge distribution, which can fix viruses, bacteria and toxins in the digestive tract. At the same time, montmorillonite has a covering effect on the mucous membrane of digestive tract, and can repair and improve the mucous membrane barrier from both qualitative and quantitative aspects through the interaction with mucous protein and avoid the defense ability of attacking factors. Research of domestic scholars [21] shows that montmorillonite will not enter the blood circulation during administration and can be excreted out of the body with the peristalsis of the digestive tract together with the fixed attacking factors. Another domestic study [22] has shown that montmorillonite can act on the surface of mucus and bind to glycoprotein in children with diarrhea, which is helpful to enhance the defense ability of intestinal mucosa, thereby maintaining normal intestinal flora and exerting a good therapeutic effect. Meanwhile, montmorillonite dispersion can promote the formation of stool around montmorillonite, improve stool morphology as well as the dehydration of children caused by diarrhea [23]. At present, there is a lack of uniform standards for the administration of montmorillonite. A study has found that enema with montmorillonite could ensure that the medicine acts directly on the digestive tract, reduces adverse effects, and promotes rapid repair of damaged cells [24]. However, the children showed poor compliance with the enema. Therefore, in the present study, the patients were orally administrated. In this study, the normal stool frequency, the time to return of stool properties, duration of related treatment and average hospital stay in the OG were shorter than those in the CG ($P < 0.05$), suggesting that montmorillonite, vitamin AD combined with zinc can shorten the time of symptom improvement as well as the duration of the disease, and promote the recovery of the children.

Zinc is a trace element required for human growth, and can be directly involved in the synthesis of protein, nucleic acid and many vitamins, which plays a key role in the development and repair of the integrity of the gastrointestinal tract mucosa and plays an anti-infective role as well as maintains normal immune function [25]. However, for children with diarrhea, the rapid passage of nutrients through the intestinal tract could cause damage to the ab-

sorption function of the gastrointestinal tract, causing damage to the intestinal mucosa and leading to malnutrition. In addition, diarrhea can increase the loss of zinc in the body and exacerbate the occurrence and development of the disease, leading to a decrease in levels of T-lymphocytes [26]. Therefore, oral preparations can not only improve the absorption of zinc, allowing it to come into direct contact with the intestinal tract, but also promote the repair of the intestinal mucosa, increase thymosin and T-lymphocyte activity, and improve the immunity of children [27]. When vitamin A is deficient or insufficient, the visual, immune and hematopoietic systems of children will be affected. A study [8] has shown that vitamin A could help fight infections and maintain normal immune function, and that vitamin A deficiency can cause damage to the integrity of mucous membranes, making it difficult to maintain normal immune function and aggravating the occurrence and development of diseases. Appropriate vitamin A supplementation can enhance the defense function of children and shorten the course of diarrhea. In the present study, the levels of $CD3^+$, $CD4^+$, $CD4^+/CD8^+$ in the OG were higher than those in the CG ($P < 0.05$), while $CD8^+$ levels in the OG were lower than those in the CG ($P < 0.05$), suggesting that the combination of montmorillonite, vitamin AD and zinc could improve the T-lymphocyte levels of children with diarrhea, enhance the immunity and achieve a good prognosis.

The occurrence and development of diarrhea in children is a multifactorial process, often accompanied by release of inflammatory factors. CRP is an acute phase response protein and is the main and most sensitive nonspecific response marker; it begins to rise 6-8 h after the onset of acute infection and peaks at 24-48 h. CRP expression is also a marker of inflammation. Therefore, the level of CRP expression is correlated with the severity of disease [28]. TNF-α is mainly produced by activated monocytes/macrophages, which can promote the phagocytosis of neutrophils, induce acute phase protein synthesis, and promote cell proliferation and differentiation. TNF-α can also change the normal morphology and structure of intestinal epithelial cells via IFN-γ, which can increase the permeability of intestinal mucosa, induce the necrosis and apoptosis of colonic epithelial cells and promote the occurrence of diarrhea. PCT is a glycoprotein secreted by the thyroid follicle, and its expression level is low or absent in normal human body. However, infectious diseases and pediatric diarrhea will increase its expression level. NO is a common oxygen free radical, which can affect the release and secretion of various immune active substances, directly participate in the inflammatory response, and produce waterfall cascade reaction, aggravating the occurrence of diarrhea. Clinically, montmorillonite and vitamin AD in combination with zinc in pediatric diarrhea can exert the advantages of different therapeutic approaches, reduce the level of inflammatory factors and fundamentally control the occurrence and development of the disease [29]. In the present study, the levels of CRP, TNF-α, NO and PCT in the OG were lower while the SOD levels were higher than those in the CG ($P < 0.05$), suggesting that the combination of montmorillonite, vitamin AD and zinc in pediatric diarrhea can reduce the inflammatory response and oxidative stress. The combined regimen in children with diarrhea has a high safety profile, does not increase the incidence of adverse drug reactions, and can obtain a good long-term prognosis. In this study, the incidence of adverse reactions and the recurrence rate at 1 and 3 months after treatment were not significantly different between the two groups ($P > 0.05$), and the recurrence rate in the OG at 6 months after treatment was lower than that in the CG ($P < 0.05$), suggesting that the combined therapy is safe and can reduce the long-term recurrence rate of diarrhea in children.

However, there are also some limitations in this study. On the one hand, this study only included a small number of cases, which still needs to be verified with a large sample size. On the other hand, there are many limitations in data processing in the experiment, which need further study and discussion.

In summary, montmorillonite, vitamin AD and zinc can be prescribed for children with diarrhea, which have high response rate, shorten the time of disappearance of symptoms, reduce the level of inflammatory factors, improve the level of T-lymphocytes, without increasing the incidence of adverse drug reactions, and reduce the long-term recurrence rate.

Disclosure of conflict of interest

None.

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