



Correlation of intestinal flora disturbance with immune response and inflammatory response in patients with MODS

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ABSTRACT

Objective: To study the correlation of intestinal flora disturbance with immune response and inflammatory response in patients with MODS. **Methods:** 60 patients with MODS who were treated in our hospital between August 2013 and January 2017 were collected as the observation group, and 50 healthy subjects who received physical examination in our hospital during the same period were collected as normal control group. The differences in intestinal flora distribution in the feces samples, peripheral blood contents of T lymphocyte subsets and serum levels of immunoglobulin and inflammatory factors were compared between two groups of subjects. The relationship of intestinal flora distribution with immune response and inflammatory response was assessed by Pearson test. **Results:** Bifidobacterium and lactobacillus count as well as B/E value in feces of observation group were lower than those of normal control group while escherichia coli and enterococcus count were higher than those of normal control group; peripheral blood CD4⁺T lymphocyte proportion and CD4⁺/CD8⁺ratio were lower than those of normal control group while CD8⁺T lymphocyte proportion was higher than that of normal control group; serum immunoglobulin IgA, IgM and IgG levels were lower than those of normal control group; serum inflammatory factors PCT, IL-1, IL-6, IL-8 and TNF- α levels were higher than those of normal control group. Pearson test showed that the intestinal flora disturbance in patients with MODS is directly correlated with the immune response function and inflammatory response. **Conclusion:** There is obvious intestinal flora disturbance in patients with MODS, and it is one of the important causes of immune function injury and systemic inflammatory response in patients.

1. Introduction

Multiple organ dysfunction syndrome (MODS) is the acute function failure of more than one organs after severe infections, trauma and surgery, which generally first involves the lung, followed by heart, liver and kidney, central nervous system, gastrointestinal tract and immune system, etc[1,2]. The latest studies show that intestinal pathogens are the source that causes sepsis after stress stimulation, and are one of the origins of MODS[3,4]. Under physiological state, dominant bacterial community/subdominant

microflora are in balance in human intestinal tract, but when this balance is broken, and the intestinal barrier function is damaged, bacterial structure, function and space position can be changed, which will eventually cause endogenous infection and even MODS. In order to define the role that intestinal flora disorder played in the development of MODS, target flora count and proportion in feces specimens were compared between MODS patients and normal subjects in the study, and the correlation of flora balance with the body's immune function and systemic inflammatory response was further discussed, now reported as follows.

2. Information and methods

2.1 Case information

60 patients with MODS who were treated in our hospital between August 2013 and January 2017 were collected as the observation group, 50 healthy subjects who received physical examination

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Table 1.

Comparison of intestinal flora distribution in feces between two groups of subjects (logCFU/g wet feces).

Groups	n	Bifidobacterium	Lactobacillus	Escherichia coli	Enterococcus	B/E value
Control group	50	10.12±1.35	9.78±1.03	9.12±0.98	7.12±0.84	1.36±0.18
Observation group	60	8.54±0.96	8.04±0.93	11.63±1.74	8.86±0.94	0.92±0.14
T		8.121	7.093	9.162	7.384	6.492
P		<0.05	<0.05	<0.05	<0.05	<0.05

Table 2.

Comparison of peripheral blood T lymphocyte subset distribution between two groups of subjects.

Groups	n	CD4 ⁺	CD8 ⁺	CD4 ⁺ /CD8 ⁺
Control group	50	45.29±5.17	21.76±3.05	1.82±0.25
Observation group	60	36.88±4.03	29.58±3.41	1.17±0.16
T		10.293	9.283	7.268
P		<0.05	<0.05	<0.05

in our hospital during the same period were collected as normal control group, and the subjects themselves or their families signed the informed consent. Observation group included 31 men and 29 women that were 28-72 years old; normal control group included 26 men and 24 women that were 26-73 years old. The gender and age distribution of the two groups were not significantly different ($P>0.05$), and the hospital ethics committee approved the study.

2.2 Intestinal flora distribution

Feces specimens of two groups of patients were taken to detect flora distribution, including bifidobacteria, lactobacilli, escherichia coli and enterococcus, and the bifidobacterium/escherichia coli total number ratio (B/E value) was calculated.

2.3 Cellular immunity and humoral immunity

Immediately after admission (before treatment for MODS patients), 4.0 mL cubital venous blood was extracted from two groups of patients and anti-coagulated, 2 mL was used to detect T lymphocyte subset distribution by the flow cytometer (American BD Medical Equipment Co., Ltd., model BD FACS Calibur), including CD4⁺ and CD8⁺T lymphocytes, and the CD4⁺/CD8⁺ ratio was calculated; the other 2 mL was used to determine the contents of immunoglobulin (IgA), IgM and IgG by immunoturbidimetry.

2.4 Inflammation indexes

Immediately after admission (before treatment for MODS patients), peripheral blood serum was obtained from two groups of research subjects in the same way, and immunochromatography was used to detect inflammatory factor procalcitonin (PCT), and ELISA was used to detect interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-8 (IL-8) and tumor necrosis factor (TNF- α) content.

2.5 Statistical processing

All the data were processed by software SPSS 20.0, intestinal flora distribution, T lymphocyte subsets, immunoglobulin, inflammatory factor and other measurement data were in terms of mean \pm standard deviation and comparison between groups was by t test. Statistics $P<0.05$ was the standard of statistical significance in differences.

3. Results

3.1 Intestinal flora distribution

Comparison of intestinal flora distribution in feces between two groups of subjects was as follows: bifidobacterium and lactobacillus count as well as B/E value in feces of observation group were significantly lower than those of normal control group while escherichia coli and enterococcus count were higher than those of normal control group. Differences in bifidobacterium, lactobacillus, escherichia coli and enterococcus count as well as B/E value were statistically significant between two groups of subjects ($P<0.05$), shown in Table 1.

3.2 T lymphocyte subsets

Comparison of peripheral blood T lymphocyte subset distribution between two groups of subjects was as follows: peripheral blood CD4⁺T lymphocyte proportion and CD4⁺/CD8⁺ ratio of observation group was significantly lower than those of normal control group while CD8⁺T lymphocyte proportion was higher than that of normal control group. Differences in peripheral blood CD4⁺ and CD8⁺T lymphocyte proportion as well as CD4⁺/CD8⁺ ratio were statistically significant between two groups of subjects ($P<0.05$), shown in Table 2.

Table 3.

Comparison of serum immunoglobulin levels between two groups of subjects (g/L).

Groups	n	IgA	IgM	IgG
Control group	50	1.79±0.24	2.52±0.34	15.42±2.11
Observation group	60	0.48±0.06	0.71±0.08	7.23±0.89
T		7.192	9.832	12.372
P		<0.05	<0.05	<0.05

Table 4.

Comparison of serum inflammatory factor levels between two groups of subjects.

Groups	n	PCT	IL-1	IL-6	IL-8	TNF- α
Control group	50	0.21±0.04	8.25±0.97	6.12±0.75	30.29±4.51	0.91±0.12
Observation group	60	2.18±0.32	92.17±10.08	57.38±6.72	217.64±30.59	3.27±0.45
T		7.912	14.292	12.075	20.384	6.281
P		<0.05	<0.05	<0.05	<0.05	<0.05

3.3 Immunoglobulin

Comparison of serum immunoglobulin IgA, IgM and IgG levels between two groups of subjects was as follows: serum immunoglobulin IgA, IgM and IgG levels of observation group were significantly lower than those of normal control group. Differences in serum immunoglobulin IgA, IgM and IgG levels were statistically significant between two groups of subjects ($P<0.05$), shown in Table 3.

3.4 Inflammation indexes

Comparison of serum inflammatory factors PCT ($\mu\text{g/L}$), IL-1 (pg/mL), IL-6 (ng/mL), IL-8 (pg/mL) and TNF- α (ng/mL) levels between two groups of subjects was as follows: serum inflammatory factors PCT, IL-1, IL-6, IL-8 and TNF- α levels of observation group were significantly higher than those of normal control group. Differences in serum inflammatory factors PCT, IL-1, IL-6, IL-8 and TNF- α levels were statistically significant between two groups of subjects ($P<0.05$), shown in Table 4.

3.5 Correlation analysis

Pearson test showed that bifidobacterium and lactobacillus count as well as B/E value in feces of MOD patients were positively correlated with peripheral blood CD4⁺T lymphocyte proportion and CD4⁺/CD8⁺ ratio, and negatively correlated with CD8⁺T lymphocyte proportion; they were positively correlated with immunoglobulin IgA, IgM and IgG levels; they were negatively correlated with inflammatory factors PCT, IL-1, IL-6, IL-8 and TNF- α levels. Escherichia coli and enterococcus count in feces of MOD patients were negatively correlated with peripheral blood CD4⁺T lymphocyte proportion and CD4⁺/CD8⁺ ratio, and positively correlated with CD8⁺T lymphocyte proportion; they were negatively correlated with immunoglobulin IgA, IgM and IgG levels; they were positively correlated with inflammatory factors PCT, IL-1, IL-6, IL-8 and TNF- α levels.

4. Discussion

MODS can cause intestinal dysfunction, and the bacterial translocation caused by intestinal dysfunction can further aggravate the disease, and the two form a vicious cycle. The dominant bacterial community in healthy human mainly include bifidobacteria, Bacteroides and eubacterium, the subdominant microflora include escherichia coli and streptococcus, and lactobacilli belongs to subdominant microflora in number, and belongs to dominant bacterial community in function[5,6]. The normal intestinal barrier separates the bacteria and the internal environment in the intestinal tract, and the bacteria and endotoxin in the normal gut are not pathogenic. Intestinal function in patients with MODS is severely hit, the intestinal barrier function is damaged, so the flora number and space position change, and the dominant bacterial community/subdominant microflora balance is broken[7-9]. In the study, flora count in feces were compared at first between MOD patients and normal subjects, and it was found that bifidobacterium and lactobacillus count as well as B/E value in feces of observation group were significantly lower than those of normal control group while escherichia coli and enterococcus count were higher than those of normal control group. It indicates that there are the decrease in dominant bacterial community and the increase in opportunistic pathogen in the intestinal tract of patients with MODS, and confirms that there is intestinal flora disorder in patients with MODS.

There is significant immune dysfunction in patients with MODS and the intestinal flora disorder is directly involved in it. Under physiological state, the balance state of flora has immune protective effect, the cell wall of dominant bacterial community is stimulator of certain immune cells, and it can activate the immune cells, stimulate the secretion of antibody, improve the body's immunity and resist the attack of external flora[10-12]. Many studies have shown that the abnormal intestinal flora count or space position can lead to immune function damage, peripheral blood T lymphocyte subset levels were compared between the two groups in this study, and it was found that peripheral blood CD4⁺T lymphocyte proportion and CD4⁺/CD8⁺ratio of observation group were lower than those of normal control group while CD8⁺T lymphocyte proportion was higher than that of normal

control group, indicating that there is cellular immune dysfunction in patients with MODS. In terms of humoral immunity, serum immunoglobulin IgA, IgM and IgG levels of observation group were lower than those of normal control group, indicating that there is also humoral immune function damage in patients with MODS. Further Pearson test showed that the intestinal flora count and B/E value in patients with MODS were directly correlated with the above cellular immunity and humoral immunity indexes, directly indicating that the intestinal flora disorder is one of the direct causes of the immune suppression.

Systemic inflammatory response syndrome is the most basic pathological change of the MODS patients, and also the direct cause of multiple viscera and system damage^[13,14]. After pathogen infection, a large amount of pro-inflammatory mediators are released, they further activate mononuclear macrophages and neutrophils, lead to sustained secretion of inflammatory factors, thus form the inflammatory cascade reaction and even generate systemic inflammatory response syndrome (SIRS), and to detect the contents of inflammatory factors of MODS patients can objectively reflect the illness severity^[15]. PCT is a new type of inflammatory marker, and also known as a late inflammatory factor, but its specificity to reflect severe infection is extremely high^[16]. IL-1, IL-6, IL-8 and TNF- α are all classic pro-inflammatory agents, which are released into the blood early after pathogen infection and directly cause functional impairment of the individual viscera^[17,18]. In the study, serum inflammatory cytokine levels were compared between the two groups, and it was found that serum PCT, IL-1, IL-6, IL-8 and TNF- α levels of observation group were higher than those of normal control group, confirming that there is severe systemic inflammatory response in MODS patients. Further Pearson test showed that the intestinal flora count and B/E value in patients with MODS were directly correlated with inflammatory factor levels, confirming that the intestinal flora disorder is an important factor causing systemic inflammatory response.

To sum up, it can be concluded that there is intestinal flora disorder in MODS patients, and it is an important cause of immune response dysfunction and systemic inflammatory response.

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