Correlation between Intestinal Flora and Serum Inflammatory Factors in Post-stroke Depression in Ischemic Stroke

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ABSTRACT

Objective: To investigate the correlation between intestinal flora and serum inflammatory factors IL-1, IL-2, IL-6 and hs-CRP in post-stroke depression (PSD) in ischemic stroke patients.

Study Design: Observational study.

Study Place and Duration of Study: Jiulongpo District Hospital of Traditional Chinese Medicine, Chongqing City, China, from October 2018 to May 2020.

Methodology: One hundred and sixty-three patients with ischemic stroke were divided into Group A (PSD) and Group B (no PSD), according to whether they had PSD. Intestinal flora indexes (*Enterococcus faecalis, Escherichia coli* and *Bifidobacterium*) and serum IL-1, IL-2, IL-6 and hs-CRP were detected.

Results: Among 163 patients with ischemic stroke, 67 (41.10%) had PSD (Group A) and 96 (58.90%) had no PSD (Group B). Contents of *Enterococcus faecalis* and *Escherichia coli* in Group A were higher than those in Group B (both $p < 0.001$), and content of *Bifidobacterium* in Group A was lower than that in Group B ($p < 0.001$). Serum IL-1, IL-2, IL-6 and hs-CRP levels in Group A were higher than those in Group B (all $p < 0.001$). Pearson correlation test showed that contents of *Enterococcus faecalis* and *Escherichia coli* in Group A were positively correlated with IL-1, IL-2, IL-6 and hs-CRP, and content of *Bifidobacterium* was negatively correlated with IL-1, IL-2, IL-6 and hs-CRP.

Conclusion: There are intestinal flora imbalance and *Bifidobacterium* undergrowth in patients with PSD, which can lead to overexpression of serum inflammatory factors. Both may be involved in occurrence and progress of PSD in patients with ischemic stroke.

Key Words: *Ischemic stroke, Post-stroke depression (PSD), Intestinal flora, Inflammatory factors.*

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INTRODUCTION

Stroke is one of the most common neurological diseases in the world. Ischemic stroke is a type of stroke. Post-stroke depression (PSD) is one of the most common neuropsychological complications after ischemic stroke.[1] It has been reported that factors including obesity, diabetes, hyperlipidemia and hypertension are the risk factors for the occurrence and development of ischemic stroke.[2-4]

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Intestinal flora plays an important role in obesity, diabetes, hyperlipidemia, hypertension, and other diseases.[5,6] The main indexes of intestinal flora are *enterococcus, enterococcus faecalis, escherichia coli, bifidobacterium* and so on. Intestinal flora is involved in the host immune defense process. Intestinal flora disorder may result in a large amount of lipopolysaccharide produced by bacteria releasing into the blood, leading to peripheral immune activation and inflammatory response.[7] In the pathological state, the permeability of blood-brain barrier changes, IL-1, IL-2, IL-6, hs-CRP, and other inflammatory factors enter the centre nervous system (CNS), the inflammatory signal is transmitted to the CNS, and then glial cells are activated through NF-κB pathway, promoting the occurrence of depression.[8,9] A study found that dysbiosis of the gut microbiome might have a causal role in development of depressive-like behaviors through the host's metabolism pathway.[10]

Gut microbiota dysbiosis may be a newly identified high-risk factor for stroke.[11] Gut microbiota dysbiosis is associated with post-stroke comorbid cognitive impairment and depression.[12] At present, there are few reports on intestinal flora and PSD. At the same time, there are few reports on the correlation between intestinal flora and serum inflammatory factors IL-1, IL-2, IL-6, and hs-CRP in PSD in ischemic stroke patients.

The purpose of this study was to investigate the correlation between intestinal flora and serum inflammatory factors IL-1, IL-2, IL-6, and hs-CRP in PSD in ischemic stroke patients.

METHODOLOGY

This study was approved by the Ethics Committee of the Jiulongpo District Hospital of Traditional Chinese Medicine, Chongqing City, China, from October 2018 to May 2020. A total of 163 patients with ischemic stroke admitted to hospital were included. The inclusion criteria were that CT or MRI diagnosed as acute ischemic stroke; normal cognitive function; no communication disorder; and able to complete the evaluation of Hamilton depression scale (HAMD) and other depression rating scales; onset from 2 to 4 weeks; all patients had informed consent. The exclusion criteria were that patients diagnosed with depression and other mental diseases before the onset of ischemic stroke; with cardiac, respiratory or renal failure; with malignant tumors; complicated with infection; with cognitive impairment, aphasia, visual impairment, and unable to cooperate with the depression scale; taking antidepressants during the survey; not cooperate. The diagnostic criteria of PSD were according to the third edition of Chinese classification and diagnostic criteria for mental disorder (CCMD-3), Hamilton adulteration scale -24 (HAMD-24) scores ≥ 8, and self-rating depression scale (SDS) depression severity index ≥ 0.5. The patients were divided into Group A (PSD), and Group B (no PSD) according to whether they had PSD.

The intestinal flora indexes (*Enterococcus faecalis*, *Escherichia coli* and *Bifidobacterium*) and serum inflammatory factors IL-1, IL-2, IL-6 and hs-CRP were detected. The culture and detection of intestinal flora were carried out in an incubator at 37℃. *Bifidobacterium* was cultured in aerobic conditions for 3 days, and *Enterococcus faecalis* and *Escherichia coli* were cultured in aerobic conditions for 2-4 days. After cultivation, the contents of *Enterococcus faecalis*, *Escherichia coli* and *Bifidobacterium* in feces were detected by ATB-expression semi-automatic microbial detection system. Results were expressed as the logarithmic value of colony formation unit per gram of wet feces that is lg (CFU/g). The fasting venous blood was taken and centrifuged. The upper serum was left to detect the level of inflammatory factors IL-1, IL-2, IL-6 and hs-CRP by enzyme-linked immunosorbent assay (ELISA) method.

The data were analysed by SPSS version 25. The data satisfying normal distribution were expressed as mean ± standard deviation, and independent sample t-test was used to compare. Count data was represented by n (%). Pearson correlation analysis was used. $P < 0.05$ indicated that the difference was statistically significant.

Table I: Comparison of intestinal flora indexes between the two groups.

Parameter	Group A (n=67)	Group B (n=96)	p-value
Enterococcus faecalis (lg CFU/g)	8.94±1.45	7.55±0.86	<0.001
Escherichia coli (lg CFU/g)	7.51±1.22	6.79±0.77	<0.001
Bifidobacterium (lg CFU/g)	7.06±1.14	8.84±0.99	<0.001

Table II: Comparison of serum inflammatory factor levels between the two groups.

Parameter	Group A (n=67)	Group B (n=96)	p-value
IL-1 (ng/L)	36.31±5.88	24.86±2.79	<0.001
IL-2 (ng/L)	24.08±3.90	15.62±1.76	<0.001
IL-6 (ng/L)	23.75±3.85	18.04±2.03	<0.001
hs-CRP (mg/L)	6.19±1.00	4.75±0.54	<0.001

RESULTS

Among the 163 patients with ischemic stroke, 85 (52.15%) were males and 78 (47.85%) were females. The mean age was 55.91 ± 8.61 years. Sixty-seven (41.10%) had PSD (Group A) and 96 (58.90%) had no PSD (Group B). The contents of *Enterococcus faecalis* and *Escherichia coli* in Group A were higher than those in Group B (both $p < 0.001$, Table I), and the content of *Bifidobacterium* in Group A was lower than that in Group B ($p < 0.001$, Table I).

The levels of serum IL-1, IL-2, IL-6, and hs-CRP in Group A were higher than those in Group B (all $p < 0.001$, Table II). Pearson correlation test showed that the contents of *Enterococcus faecalis* and *Escherichia coli* in Group A were positively correlated with IL-1, IL-2, IL-6, and hs-CRP; and the content of *Bifidobacterium* was negatively correlated with IL-1, IL-2, IL-6 and hs-CRP, as shown in Table III.

DISCUSSION

PSD in ischemic stroke patients often shows passive and pessimistic emotions and have sleep disorders; and the negative emotions and sleep disorders can stimulate catecholamine secretion *in vivo*, promote platelet aggregation, and then increase the risk of the recurrence of ischemic stroke. Some studies have shown that depression has various effects on nerve, endocrine, immunity and gastrointestinal function.[13] Depression will affect the normal rhythmic movement of gastrointestinal tract and the secretion of mucous membrane.[14]

Enterococcus faecalis is an important opportunistic pathogen in gastrointestinal tract.[15] *Escherichia coli, (E. coli)* is a conditional pathogen that can induce gastrointestinal infection under certain conditions.[16] *Bifidobacterium* is considered as a kind of beneficial bacteria in the human intestine, and it can inhibit the proliferation of pathogenic bacteria in the body.

Previous studies have proved that *Bifidobacterium* has an obvious antidepressant effect and microbial community regulation. *Bifidobacterium* has provided a new treatment strategy for the treatment of depression.[17]

Table III: Correlation between intestinal flora and serum inflammatory factors in patients with PSD in Group A (n = 67).

Parameter	IL-1		IL-2		IL-6		hs-CRP	
	r	p-value	r	p-value	r	p-value	r	p-value
Enterococcus faecalis	0.680	<0.001	0.439	<0.001	0.538	<0.001	0.357	0.003
Escherichia coli	0.306	0.012	0.263	0.032	0.325	0.007	0.284	0.020
Bifidobacterium	-0.263	0.031	-0.289	0.018	-0.286	0.019	-0.333	0.006

The results of this study showed that the contents of *Enterococcus faecalis* and *Escherichia coli* in Group A were higher than those in Group B, and the content of *Bifidobacterium* in Group A was lower than that in Group B. The results showed that the number of beneficial bacteria in feces of patients with PSD decreased and the number of harmful bacteria increased. This indicates that there is microecological disorder in intestinal flora in patients with post-ischemic stroke depression.Intestinal flora disorder can further aggravate the damage of intestinal barrier function, resulting in excessive release of local inflammatory factors in gastrointestinal tract.[18]

The increase of inflammatory factors is closely related to the emergence and aggravation of depressive symptoms.[19,20] The levels of IL-1, IL-2, IL-6, hs-CRP and other inflammatory factors were highly expressed in depression population,[21,22] indicating that inflammatory cytokines are involved in the pathogenesis of depression as network neurotransmitters. This results showed that the levels of serum IL-1, IL-2, IL-6, and hs-CRP in Group A were higher than those in Group B, indicating that the levels of serum IL-1, IL-2, IL-6, and hs-CRP in patients with PSD are higher than those in patients with post-ischemic stroke. It is suggested that inflammatory cytokines IL-1, IL-2, IL-6, and hs-CRP may be involved in the occurrence and development of depression and inflammatory pathological mechanism after ischemic stroke. To date, only a handful of studies have sought to explore the relationship between stroke-induced inflammation and PSD.[23] A study found whether serum inflammatory cytokines levels 72 hours post-stroke could predict long-term PSD.[24]

Pearson correlation test showed that the contents of *Enterococcus faecalis* and *Escherichia coli* were positively correlated with IL-1, IL-2, IL-6 and hs-CRP, and the content of *Bifidobacterium* was negatively correlated with IL-1, IL-2, IL-6 and hs-CRP in Group A with post-ischemic stroke depression. It is suggested that intestinal flora disorder in patients with post-ischemic stroke may further promote the occurrence and development of depression after ischemic stroke by increasing intestinal permeability, promoting bacterial displacement in intestinal tract, activating downstream signal immune response and aggravating inflammatory response.[25]

It should be pointed out that this study was a correlation study. The results of the study could only illustrate intestinal flora and the levels of serum inflammatory factors IL-1, IL-2, IL-6, and hs-CRP were closely related to PSD in ischemical stroke patients. However, it could not explain the cause and effect of the changes in the internal flora and levels of serum inflammatory factors of ischemical stroke patients in the occurrence of PSD. Meanwhile, limited to the current technical conditions and patients' compliance, there is no long-term dynamic monitoring of intestinal microecological environment in patients with post-ischemic stroke depression.

CONCLUSION

There are intestinal flora imbalance and *Bifidobacterium* undergrowth in patients with PSD, which can lead to the overexpression of serum inflammatory factors. Both may be involved in occurrence and progress of PSD in patients with ischemic stroke.

ETHICAL APPROVAL:
Approval for this study was obtained from the Institutional Review Board (IRB) of the Jiulongpo District Hospital of Traditional Chinese Medicine, Chongqing City, China

PATIENTS' CONSENT:
Informed consents were taken from all patients.

CONFLICT OF INTEREST:
The authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:
YK: Study concept, design, data maintenance and critical review of first draft.
YY and YM: Data acquisition, checking and statistics.
JW: Study design and compilation of results.
HC: Study design, statistical analysis.
DW: Interpretation of results and final approval of the manuscript.

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