Original article

The Effects of Early Enteral Nutrition when Combined with Probiotics in Patient with TBI

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Summary. *Objective:* To explore the effects of early enteral nutrition (EEN) when combined with probiotics. Especially the effects on neurological function, nutrition, inflammation, immune function, and serum vascular endothelial growth factor (VEGF) expression in patients with traumatic brain injury (TBI). *Methods:* A total of 136 TBI patients treated in our hospital participated in the study and were randomly divided into control (n=68) and treatment group (n=68). Only patients who were in the treatment group were treated with Bifid Triple Viable Enteric-Coated Capsules (BTVECC), while control group was treated with placebo. Neurological function, nutrition status and immune function were assessed by Glasgow coma scale (GCS) score, the levels of D-lactic acid, serum inflammatory factors, T lymphocyte subsets, procalcitonin (PCT) and VEGF respectively. The results were compared between both groups before and after EEN treatment. Any incidence of complications was also recorded. *Results:* By comparing with those before EEN treatment, the levels of serum transferrin (TRF), prealbumin (PA), albumin (ALB), immunoglobulin A (IgA), IgG, IgM, cluster of differentiation 3$^+$ (CD3$^+$), CD4$^+$, CD4$^+$/CD8$^+$ and GCS score significantly increased. While the levels of serum interleukin-6 (IL-6), IL-8, tumor necrosis factor-α (TNF-α), D-lactic acid, PCT, VEGF and CD8$^+$ remarkably declined in both groups after treatment. Although both groups showed notable changes, more significant changes were observed in BTVECC-treated group compare to control (p<0.05). In addition, the incidence rate of complications was evidently lower in treatment group than that in control group (p<0.05). *Conclusion:* EEN combined with probiotics significantly improved the nutritional status and serum VEGF expression, while reduce inflammatory response, complications, and enhance immune function in TBI patients, which has important clinical application value.

Key words: early enteral nutrition, probiotics, traumatic brain injury, inflammatory factors, nutritional status

Introduction

Traumatic brain injury (TBI) is one of the most common presentations in neurosurgery. It was reported that there is around 2.4 million TBI-related hospital visits every year in U.S.A, and according to WHO, TBI will be the third leading cause of death and disability worldwide by 2020 [1]. TBI patients usually have eating disorders since most of them are accompanied by hypoperfusion of gastrointestinal mucosa while gastrointestinal dysfunction, insufficient nutrient absorption, and reducing body immunity occur leading to a series of complications [2]. Previous study has demonstrated that TBI patients could suffer from systemic inflammatory response under long-term pathological stress, aggravating the condition of disease [3]. Vascular endothelial growth factor (VEGF) is a trophic factor playing a vital role in the survival of newborn neuronal precursors and implicating in neurogenesis after cerebral ischemia and neurite outgrowth. Thus, promoting the expression of vascular endothelial growth factor (VEGF), could ameliorate blood circulation in brain tissue and alleviate cerebral edema [4].

Neuroprotective strategies aimed at preventing cellular death in the affected brain have been largely disappointing, thus, to seek other potential therapies with low toxicity and less adverse effects is a pressing challenge [5]. TBI patients are mainly treated with enteral nutrition in the clinic, but nutrients cannot be absorbed normally due to gastrointestinal dysfunction [6]. Some probiotics such as the multispecies probiotic (Ecologic®Barrier) has been demonstrated to reduce symptoms of stress, anxiety, and depression in randomized controlled trials—three very common symptoms following a TBI [7]. According to clinical studies, the probiotic L. plantarum can effectively enhance the body's immunity while protect the gastrointestinal mucosal barrier and hence could correct the gastrointestinal flora disorder caused by TBI and promote the absorption of enteral nutrition [8]. Therefore, this study was developed to explore the treatment effects of early enteral nutrition (EEN) combined with an established multi-strain probiotic (bifid triple viable) on the nutrition, inflammation, and immune function in TBI patients, as to provide instructions to clinical diagnosis and treatment.

Materials and Methods

General data

A total of 136 TBI patients who were treated in our hospital from Feb. 2018 to Dec. 2019 participated in the study. All patients included in this study met our selection criteria, which will be described later and were divided into control (n=68) and treatment (n=68) group randomly. All patients understood and accepted the whole process in our study, no patient dropped out during research. There were no significant differences in demographic characteristics between both groups, such as age, gender, duration from injury to admission, cause of injury, and type of diseases ($p>0.05$, Table 1).

Inclusion and exclusion criteria

Inclusion criteria: 1) Patients who had a sustained TBI that was lasted at least three months prior to draw medical attention, 2) The time duration from injury to admission was less than 24h, 3) patients without intracranial active bleeding confirmed by head CT examination, 4) patients with severe TBI and Glasgow Coma

Table 1. Comparison of baseline data between the two groups (n=68)

	Control group	Observation group	t/χ^2	p
Age (Y)	41.25±5.28	41.54±5.19	0.323	0.374
Male/female (n)	38/30	40/28	0.120	0.729
Duration from injury to admission (min)	78.69±9.87	78.05±9.06	0.394	0.347
Cause of injury [n (%)]				
Traffic accident	25 (36.76)	27 (39.71)	0.300	0.990
Weight crushing	16 (23.53)	15 (22.06)		
Violent impact	14 (20.59)	12 (17.65)		
Others	13 (19.12)	14 (20.59)		
Open brain injury	21 (30.88)	19 (27.94)	0.142	0.707
Closed brain injury	47 (69.12)	49 (72.06)		
Type of disease [n (%)]				
Subdural hematoma	15 (22.06)	13 (19.12)		
Epidural hematoma	17 (25.00)	16 (23.53)		
Brain-stem injury	15 (22.06)	17 (25.00)	0.321	0.988
Intracerebral hematoma	12 (17.65)	11 (16.18)		
Skull fracture	9 (13.24)	11 (16.18)		

Scale scores between 5 to 8, and 5) patients and families who agreed to participate and signed the informed consent. Potential participants were excluded if they had: 1) complications causing severe dysfunction in the heart, liver, or kidney, 2) hypoproteinemia, diabetic ketosis, or protein metabolism disorders, 3) thyroid disease, hematological disease, or malignant tumor(s), 4) a history of abdominal trauma, severe organic lesion, or gastrointestinal disease, 5) congenital metabolic disorders or allergy to nutrients, 6) pregnant or lactating, or 7) severe infectious diseases at the time of admission.

Methods

A total of 68 patients in the control group and 68 patients in the probiotic group participated in our study. The patients in both groups underwent basic treatments including increased dietary fruit and vegetable intake to promote basic gastrointestinal health, regular exercise, and sufficient rest, such as a 20-minute nap during the middle of the day. One gram acetazolamide per day was given to all patients to reduce intracranial pressure. Above all, all patients underwent early enteral nutrition therapy. At the early stage of admission, patients who remained intubated with a naso-gastric tube and Peptison (NMPN H20010285, NUTRICIA, Wuxi) was perfused transnasally at a constant speed; the amount of medication was increased from an initial dose of 500 mL/d gradually to about 2000 L/d. The main ingredients of Peptison are water, maltodextrin, whey protein hydrolysate, vegetable oil, vitamins, and minerals. Patients who were in treatment group took Bifid Triple Viable Enteric-Coated Capsules (NMPN S19993065, Jincheng Haisi Pharmaceutical Co., Ltd.) (BTVECC) 2 capsules/time orally twice a day, while control patients were treated with placebo. Patients in both groups were treated for 2 weeks. Each gram of BTVECC contained long bifidobacterium (≥1.0×106 CFU), Lactobacillus acidophilus (≥1.0×106CFU), and Enterococcus faecalis (≥1.0×106 CFU).

Observation indexes

1) Levels of serum inflammatory factors: 5 mL of fasting venous blood was collected the second day post treatment at 8am daily during the study. Blood samples were then centrifuged, and serum was separated and stored at -75°C. The levels of serum interleukin-6 (IL-6), IL-8 and tumor necrosis factor-α (TNF-α) were analyzed via enzyme-linked immunosorbent assay using kits (Wuhan Boster Biological Technology Co., Ltd.) strictly according to the instructions. 2) Immune function indexes: Patients' immune system status was measured by monitoring the level of immunoglobulin A (IgA), IgG and IgM by a commercial nephelometry assay using a BN-II device (Dade Behring, Germany). The Manufacturer indicates the following reference intervals for healthy adults: IgA 70-400 mg/dl, IgG 700-1600 mg/dl and IgM 40-230 mg/dl. 3) Levels of serum T-lymphocyte subsets: Before and after 2-weeks' treatment, 5 mL of fasting venous blood was drawn at 8 am daily. Blood samples were centrifuged, and serum was stored in a refrigerator at -75°C before use. The levels of serum cluster of differentiation 3^+ ($CD3^+$), $CD4^+$, $CD8^+$ and $CD4^+/CD8^+$ were measured using a flow cytometer. 4) Glasgow coma scale (GCS) score [8]: It consists of evaluations of the movement, activity, language, and eye opening, low GCS score corresponds to severer coma. 5) Nutritional status indexes: the levels of serum transferrin (TRF), prealbumin (PA) and albumin (ALB) were detected using a Roche 2010 full-automatic electrochemiluminescence immunoassay analyzer. 6) The level of serum D-lactic acid was determined via improved enzymatic spectrophotometry, and the levels of serum procalcitonin (PCT) and VEGF were tested via enzyme-linked immunosorbent assay using kits (BRAHMS, Germany). 7) Incidence of complications: The incidence of upper gastrointestinal bleeding, pulmonary infection, diarrhea, and electrolyte disturbance was recorded in both groups according to Physician's assessment.

Statistical analysis

SPSS19.0 software (SPSS Inc., Chicago, IL, USA) was used for data processing. Numerical data was expressed as mean ± standard deviation ($\overline{x}\pm s$), and *t* test was performed. Enumeration data were expressed as [n (%)], and chi-square test was performed. $p<0.05$ suggested the statistically significant difference.

Results

Levels of inflammatory factors in both groups before and after treatment

Compared with those before treatment, the levels of serum IL-6, IL-8 and TNF-α declined in both groups after treatment, more obviously in BTVECC-treated group (p<0.05) (Figure 1).

Comparison of nutritional status between the two groups before and after treatment

Compared with those before treatment, the levels of serum TRF, PA and ALB rose in both groups after treatment, more significantly in BTVECC-treated group (p<0.05) (Figure 2).

Comparison of immune function between the two groups before and after treatment

The levels of serum IgA, IgG and IgM were higher in both groups after treatment than those before treatment, more evidently in BTVECC-treated group (p<0.05) (Figure 3).

Levels of T lymphocyte subsets in both groups before and after treatment

Compared with those before treatment, the levels of $CD3^+$, $CD4^+$ and $CD4^+/CD8^+$ rose, while the level of $CD8^+$ declined in both groups after treatment, and the changes were more apparent in BTVECC-treated group (p<0.05) (Figure 4).

Comparisons of serum VEGF expression and GCS score between the two groups before and after treatment.

The patients in the two groups had a lower serum VEGF expression and a higher GCS score after treatment than those before treatment, and the BTVECC-treated group exhibited more evident improvement (p<0.05) (Figure 5).

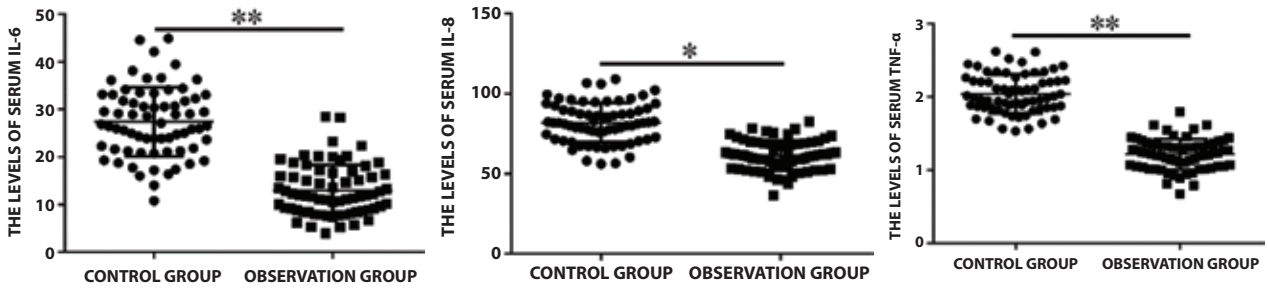

Figure 1. Comparison of levels of serum inflammatory factors between control group and treatment group after treatment (n=68)

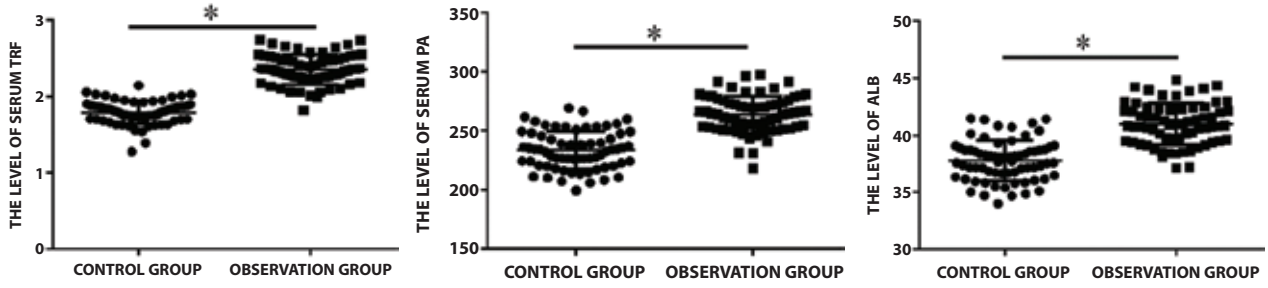

Figure 2. Comparison of nutritional status between control group and treatment group after treatment (n=68)

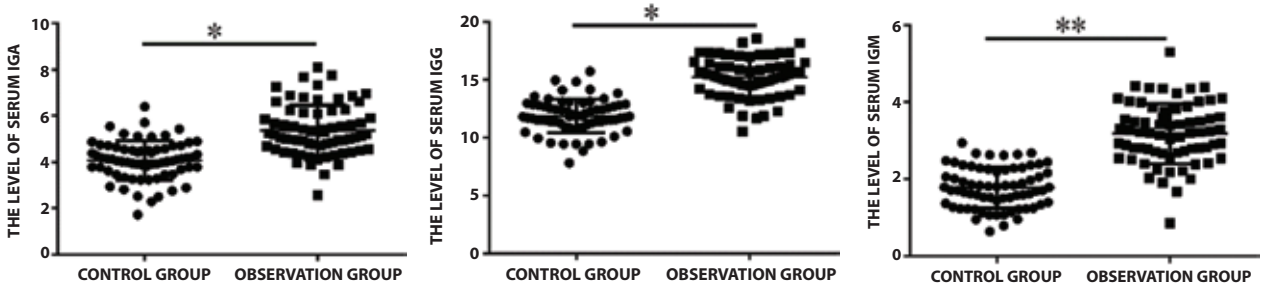

Figure 3. Comparison of levels of serum T lymphocyte subsets between control group and treatment group after treatment (n=68)

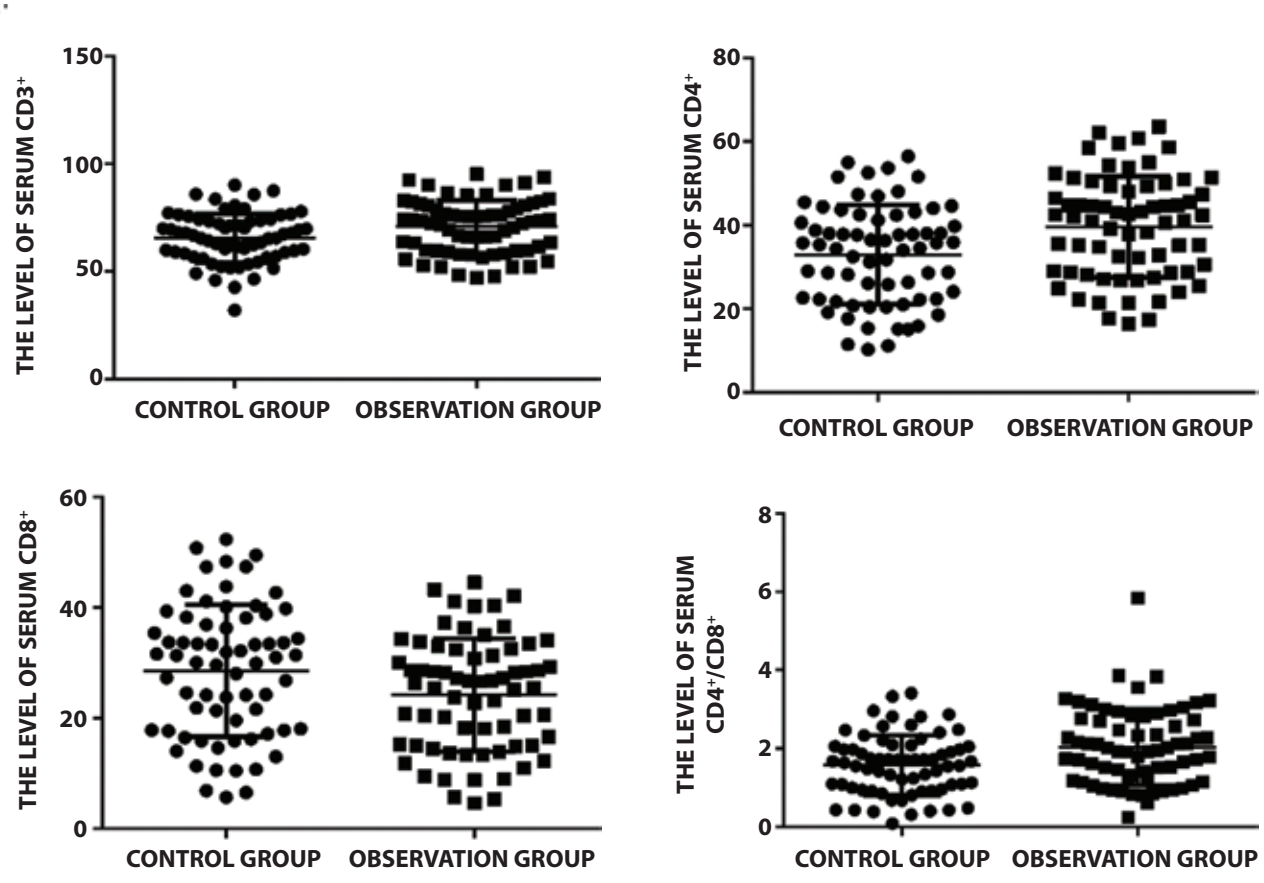

Figure 4. Comparison of levels of serum T lymphocyte subsets between control group and observation group after treatment (n=68)

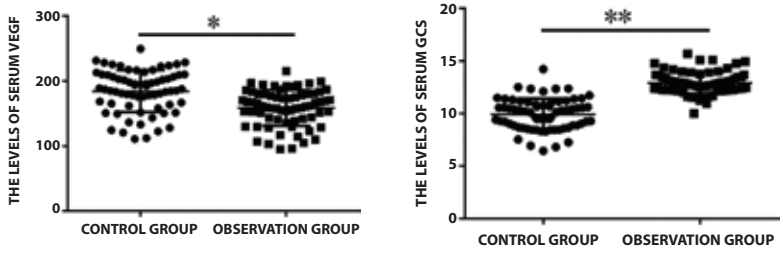

Figure 5. Comparisons of serum VEGF expression and GCS score between control group and treatment group after treatment (n=68)

Comparison of levels of serum D-lactic acid and PCT between the two groups before and after treatment

The levels of serum D-lactic acid and PCT declined in both groups after treatment compared with those before treatment, more remarkably in BTVECC-treated group (p<0.05) (Figure 6).

Complications in both groups

The total incidence rate of complications was lower in BTVECC-treated group than that in control group (p<0.05) (Table 2).

Discussion

Various degrees of "nerve-endocrine-immune function" disorders will occur in TBI patients under stress conditions [9]. On the one hand, it will inhibit the humoral and cellular immune functions in the body, lower the anti-infection ability and seriously affect the prognosis. On the other hand, it can induce the production of a large number of inflammatory factors to damage the nuclei. In addition, TBI patients are continually in a hypermetabolic state, with great energy requirements and consumption, and they are vulnerable to hypoproteinemia, hyperglycemia and negative nitrogen balance [10, 11]. If the above symptoms cannot be solved promptly, the duration required for wound healing would be prolonged, together with the decline in immunity and body mass, which would result in malnutrition that would further raise disability and even mortality rate. One study has shown that EEN can effectively improve gastrointestinal function and nutritional level in patients, thereby reducing the incidence rate of postoperative complications [12]. However, another study indicated that long-term enteral nutrition would damage the structure of gastrointestinal mucosa that leads to gastrointestinal flora imbalance and intestinal mucosal atrophy, which would further reduce immunity, and even affect the recovery of disease in severe cases [13]. In our study, the BTVECC used is a

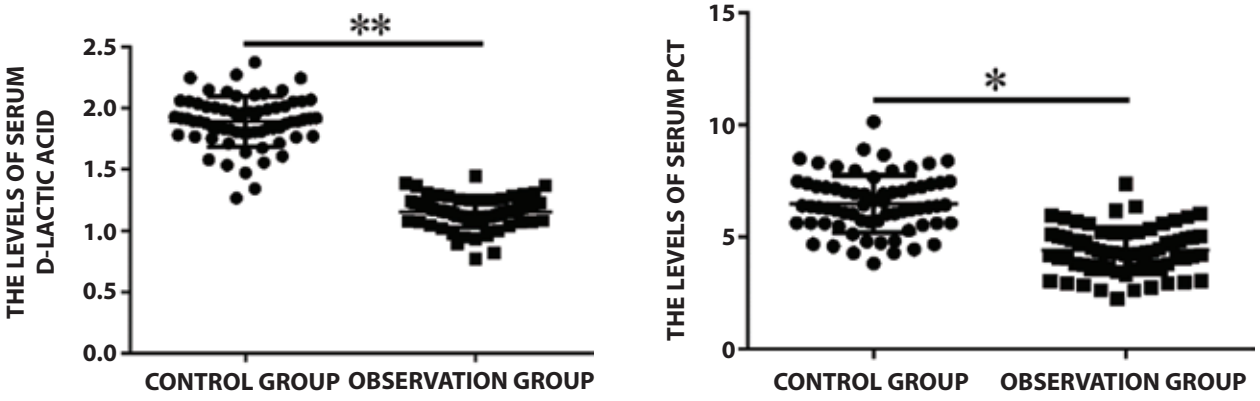

Figure 6. Comparison of levels of serum D-lactic acid and PCT between control group and treatment group after treatment (n=68)

Table 2. Comparison of incidence of complications between the two groups [n (%)]

Group	Upper gastrointestinal bleeding	Pulmonary infection	Diarrhea	Electrolyte disturbance	Total incidence rate
Control group	2 (2.94)	2 (2.94)	5 (7.35)	9 (13.24)	18 (26.47)
Observation group	1 (1.47)	0 (0.00)	2 (2.94)	2 (2.94)	5 (7.35)
χ^2					8.843
p					0.003

live microbial preparation with a complex mechanism of action. BTVECC is purported to help maintain the intestinal micro-ecological balance and inhibit pathogens from binding to the intestinal mucosa. In addition, it also supports the repairing of interstitial cells and epithelial cells of small intestine, helps restore gastrointestinal motility, and enhances the tolerance to enteral nutrition [13]. Moreover, probiotics can strengthen the intestinal flora function and reduce the incidence of gastrointestinal complications in patients.

The intestine is the largest lymphoid organ in human body, it is particularly important to keep and repair the local intestinal immunity of TBI patients [14]. The $CD4^+$ cells play a significant role in intestinal cellular immunity, and the imbalance of the $CD4^+$/$CD8^+$ ratio result in the decline in immune function [15]. In humoral immunity, serum IgG is the most important antibody component that protect the body from viruses and bacteria. In intestinal mucosal immunity, IgA is a key component that maintains the immune barrier function of the intestinal mucosa [16]. In our research, we found that EEN with probiotics could improve cellular and humoral immunity in TBI patients. A possible reason is that when probiotics is used together with EEN, it can effectively stimulate local intestinal immunity, and raise the Ig levels, thereby enhancing the immunity. Besides, the level of other important pro-inflammatory cytokines, such as IL-6, IL-8 and TNF-α [17-20], were significantly lower in the BTVECC-treated group. These findings are consistent with the notion that EEN combined with probiotics effectively alleviated the inflammatory response in the patients. As for VEGF, which is a growth factor that can promote angiogenesis and endothelial cell division, plays an important role in trauma. According to our results, the levels of serum VEGF, serum PCT and D-lactic acid significantly reduced in BTVECC-treated group, indicating the possible feature of repairing and protecting of BTVECC in gastrointestinal mucosal barrier. Also, the results also indicated improvements in the nutritional status of elderly patients with TBI, which is consistent with the research results from Warren M. Lastly, PA, which is an index for short-term nutritional status in the body, can significantly decline after protein loss or shortage of calories [21]. TRF is not only an important carrier for iron transport in the plasma, but also a key index for short-term nutritional status in the body [22]. To sum up, EEN combined with probiotics can greatly improve the nutritional status of TBI patients.

Conclusion

In conclusion, EEN combined with probiotics can significantly improve the nutritional status and serum VEGF expression. Also, it can reduce the inflammatory response and complications, and enhance the immune function in TBI patients, which has important clinical application value.

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