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Original Article

Effects of Enteral Glutamine and Vitamin C Supplementation on Cytokines and Outcomes in Surgical Intensive Care Unit Patients: A Randomized Clinical Trial

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Background: The increased requirement for exogenous glutamine in hypermetabolic states which depletes endogenous glutamine, and supplemental glutamine may be needed in critically ill patients. Vitamin C is an antioxidant that has been suggested to have synergistic effect with glutamine to improve the clinical outcomes. This study aimed to investigate the effects of combined enteral glutamine and vitamin C supplementation on proinflammatory cytokines and clinical outcomes in surgical intensive care unit (SICU) patients.

Materials and Methods: This prospective double-blind randomized study enrolled SICU patients who were able to sustain enteral feeding. The treatment (GA) group received glutamine and vitamin C; the placebo (C) group received maltodextrin of equivalent calories. Pre- and posttreatment plasma glutamine, interleukin-6 (IL-6), IL-10, and clinical data were collected and analyzed.

Results: Thirty-one patients were enrolled in the GA group, and 10 patients in the C group. The nutritional supplements were equal in calories in both groups, but the GA group had more nitrogen (glutamine) and vitamin C. After intervention, the GA group showed significantly increased plasma glutamine levels and decreased IL-6. The mean c-reactive protein (CRP) level was lower in the GA group. The lengths of SICU stay, APACHE II scores, and infectious complications were less in the GA group. While the mortality of SICU patients was associated inversely with the plasma glutamine level, increased pre-treatment IL-6 was correlated with higher levels of IL-10, CRP and longer stays in the ICU. Higher post-treatment IL-6 levels were observed in patients with infectious complications, SICU and hospital mortality.

Conclusions: Early administration of combined enteral glutamine and vitamin C supplementation increases plasma glutamine level and is associated with decreased serum IL-6 levels, reduced APACHE II scores, and infectious complications in SICU patients. Whether an appropriate dosage of glutamine/vitamin C can improve clinical outcomes remains to be determined.

Keywords: Ascorbic acid (Vitamin C); Glutamine; Critical care; Enteral nutrition

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Introduction

Glutamine, the most abundant free amino acid in the body, is an important energy source and is essential for intestinal and immune cell growth and differentiation, as well as modulation of inflammatory and oxidative stress responses.[1,2] Low plasma glutamine is associated with severe illness, poor clinical outcomes, and high mortality in critical care patients.[3] Low glutamine on admission to the intensive care unit (ICU) was an independent risk factor for post-ICU mortality.[4] In patients undergoing cardiac surgery, low preoperative plasma glutamine levels were even lower after surgery and were associated with postoperative infection.[5] Because the increasing requirement of exogenous glutamine during hypermetabolic states significantly depletes endogenous glutamine, critically ill patients may require glutamine supplementation.[6] Glutamine supplementation in critical care patients has been shown to have beneficial effects, including improved nitrogen balance, shortened ICU and hospital stays, and reduced risk of infection and mortality.[7] In surgical intensive care (SICU) patients with severe brain injuries and head and neck surgeries, immune-nutrition with glutamine supplementation reduced septic complications, accelerated wound healing, and shortened SICU and hospital stays.[8]

However, not all studies have found that glutamine supplementation improves outcomes. Although few studies have investigated the effects of combining glutamine and antioxidant supplementation, clinical trials stemming from the proposed design for the "REducing Deaths due to OXidative Stress" (REDOXS) study[9] evaluated the combined approach in critical care patients in the United States, Canada, and Europe.[10,11] Results showed that early provision of high-dose glutamine was not beneficial when administered separately from artificial nutrition, especially in patients with multi-organ failure or those who had renal dysfunction at entry into the study.[10] In fact, glutamine supplementation was associated with increased mortality in these patients.[11] The REDOXS trials used parenteral and enteral administration, noting that previous randomized trials provided nutrients enterally without showing a treatment effect, and a larger treatment effect was associated with parenteral delivery of glutamine and other nutrients.[9-11] Parenteral nutrition can be implemented more easily and earlier, while enteral nutrition can only be administered to hemodynamically stable critically ill patients.

Vitamin C is an important component in endogenous antioxidant defense, and was the main antioxidant used in combination with glutamine in the REDOXS trials.[10,11] Supplementation of vitamin C has been studied in critically ill patients with injuries,[12] severe sepsis,[13] and after cardiac surgery.[14] Vitamin C has been found to have immunomodulatory effects on cytokine production.[15] High-dose vitamin C improves the impairment of microcirculatory flow by inhibiting tumor necrosis factor (TNF)-induced expression of intracellular adhesion molecules,[16] and inhibits the release of proinflammatory interleukin (IL)-6 from muscle tissue.[17] In addition, the response of healthy athletes to vitamin C supplementation includes increases in circulating cortisol and enhancement of the acute phase protein response.[18] Critically ill patients, however, may have severe vitamin C deficiency as a result of low socio-economic status, alcoholism, and severe psychiatric illness that has led to poor nutrition.[19]

Elevated inflammatory indicators such as C-reactive protein (CRP) and cytokines such as interleukins are found in critically ill patients with hypercatabolism.[20,21] IL-6 is a proinflammatory cytokine produced by immune competent cells and released at the early stage of the inflammatory process. Elevation of plasma IL-6 is associated with adverse outcomes, including organ dysfunction and failure, and high mortality rates.[3] The severity and outcomes of chronic obstructive pulmonary disease (COPD),[20] acute pancreatitis (AP),[22] systemic inflammatory response syndrome (SIRS),[23] and sepsis, are associated with increased levels of IL-6 and IL-10. Acute Physiology and Chronic Health Evaluation (APACHE) II (and III) scores are also reliable prognostic indicators in severe illness and extensive surgery outcomes, and a link between APACHE II (and III) scores and cytokines has been demonstrated; increases of IL-6 and other inflammatory cytokines in serious illnesses are

associated with an increase in APACHE II or III scores.[24] We hypothesized that enteral glutamine and vitamin C supplementation would decrease inflammation in critically ill SICU patients. The aim of this study was to investigate the effects of combined enteral glutamine and vitamin C supplementation on proinflammatory cytokines and clinical outcomes in SICU patients who were able to sustain enteral feeding.

Materials and Methods

Subjects

This study enrolled surgical patients from the SICU of Chung Shan Medical University Hospital. All participants were between the ages 20 and 85 years, were able to sustain enteral feeding, and were not enrolled in other clinical trials in the previous 1 month and during the study period. Only SICU patients cared by the 9 participating surgeons were enrolled. Exclusion criteria were as follows: patients older than age 85 years, pregnant, patients with an abnormal liver function (receiving drugs or clinical therapies), abnormal renal function (under hemodialysis), and multi-organ failure (more than 2 organs), and expected SICU stay of less than 72 hours. Ethics approval was obtained from the Institutional Review Board of Chung Shan Medical University Hospital and signed informed consent was obtained for all study subjects.

Study design

In this prospective double-blind randomized study, subjects were randomly assigned to a group receiving glutamine and vitamin C (GA) or a placebo group (C) receiving maltodextrin, according to a sampling ratio of 3:1. The 3:1 ratio is a standard ratio for testing a hypothesis. Subjects in the GA group received 10 g L-glutamine, 3.2 g maltodextrin, 90 mg vitamin C, and 15 mg sodium per serving provided by Nutritec-Enjoy Nutrition Inc. (Taipei, Taiwan); subjects in the C group received isocaloric maltodextrin as placebo. Patients were enrolled in this study only after enteral feeding was well tolerated (defined as 60% of the total caloric goal achieved by enteral feeding). A total of 0.3-0.5 g/kg/day of glutamine or isocaloric maltodextrin was administered to the patients in the GA and placebo groups, respectively, during their stay in the SICU. The intended duration for GA/C supplementation was 14 days. Blood samples were collected for biochemical and hematological analyses on the day enteral feeding began, and every 7 days or on the day of discharge from SICU (Figure 1). The data of post-intervention was that at 14 days or the day discharged from ICU if <14 days.

Both the GA and C group would be provided with glutamine supplements at the completion of the study according to the patient's preference.

Assessments

Body height and weight were measured, and body mass index (BMI) was calculated at baseline. APACHE II score was calculated to classify disease severity. Nutritional requirements and glutamine supplementation were evaluated by doctors and dietitians. Dietary intakes were recorded daily during the study period to calculate total calories, protein, glutamine, and vitamin C intake.

Blood samples were tested for albumin, red blood cell (RBC), hemoglobin (Hb), white blood cell (WBC), CRP, glutamine, IL-6 and IL-10 levels.

Clinical outcomes examined were hospital and ICU length of stay, duration of ventilator use, SICU infectious complications, and SICU and hospital mortality.[22] SICU infectious complications were defined as culture-proved infection occurring within 72 hours after entering the SICU. Patients with deep site infection caused by anastomosis leakage was excluded.[9]

Detections of glutamine and interleukins

For the analysis of glutamine concentrations, plasma was deproteinized with 5% (v/v) $HClO_4$ and then neutralized with 0.5 M triethanolamine/2 M KOH. A universal pH indicator was added to ensure that the deproteinized samples were properly neutralized. Glutamine concentrations were analyzed enzymatically with asparaginase/glutamine dehydrogenase as described by Windmueller and Spieth.[25] The dilution factor during the deproteinization procedure was adjusted for the glutamine concentration of each sample.

The concentration of plasma IL-6 and IL-10

were measured spectrophotometrically by using the Bio Legend ELISA MAX Deluxe Sets (Bio Legend, San Diego, CA). Each sample was analyzed in duplicate and the average was obtained.

Statistical analysis

Due to the small sample size, continuous variables were presented as median and IQR (interquartile range). For continuous variables, the Mann-Whitney U test was performed to compare differences between the placebo group and GA group, and the Wilcoxon signed rank test was performed to compare pre- and post-treatment biochemical indicators and cytokines. Categorical variables are presented as counts and percentages; the chi-square test or Fisher's exact test was performed to compare correlations between the placebo group and GA group, and the McNemar test was performed to compare correlations between pre- and posttreatment glutamine < 420 μmol/L. The Pearson's and Spearman's correlation tests were performed to identify factors correlated with differences in IL-6 and glutamine between pre- and post-treatment among clinical factors and serum data correspondingly. Statistical analyses were performed with IBM SPSS statistical software version 22 for Windows (IBM Corp., Armonk, NY, USA). Two-tailed value of $p < 0.05$ indicated statistical significance.

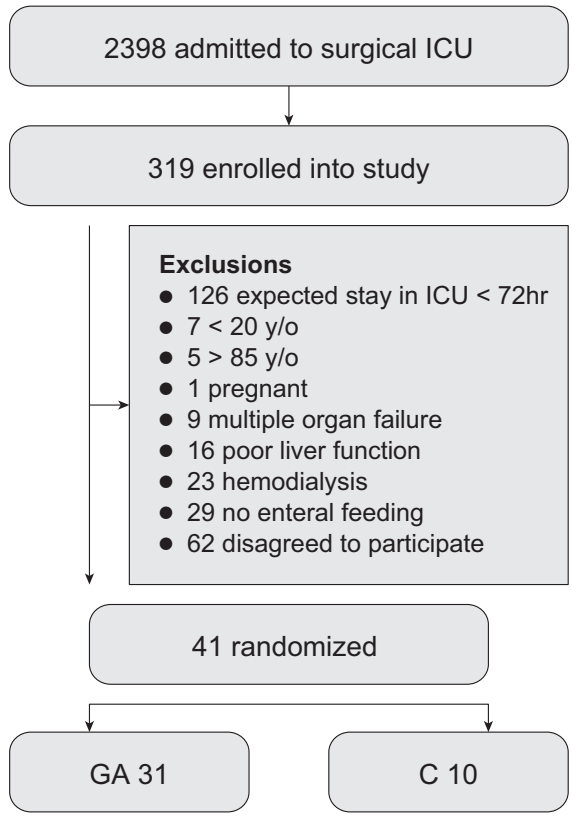
Results

Baseline characteristics of the study population

A total of 2398 patients were admitted to the SICU of Chung Shan Medical University Hospital between January 2013 and June 2014. Figure 1 shows the flow chart of patient recruitment, and final analytic sample. A total of 41 patients were enrolled and were randomly assigned to the GA group (n=31) or C group (n=10). The most common reasons for exclusion were patients could not sustain enteral feeding, patients or their family did not provide signed informed consent to participate in the study, and patients were expected to be transferred out of SICU within 72 hours.

Patient baseline characteristics, biochemical indicators and cytokines levels were presented in Table 1. For the GA group, 9 patients were

Figure 1. Flow chart of patient recruitment.



admitted to ICU due to head and neck surgery, 3 empyema surgery, 3 lung lobectomy, 1 lung bullectomy, 1 esophagectomy, 1 common bile duct lithotomy, 1 jejunum perforation, 3 peptic ulcer, 1 heart lung surgery, 1 mump, 2 neutropenia, 2 multiple trauma, 1 septic shock, 1 spine surgery, 1 tracheal surgery; for the C group, there were 2 head and neck cancer surgery, 1 empyema surgery, 1 lung lobectomy, 1 esophagectomy, 2 peptic ulcer, 1 heart valve replacement, 1 pneumonia, 1 septic shock. No significant differences were found between the GA group and the placebo group in age, sex, height, weight, BMI, and test period (all, $p > 0.05$). During the study period, total protein (including amino acids), glutamine, and vitamin C intake were significantly higher in the GA group compared to the placebo group, while total caloric intakes were similar between the 2 groups (Figure 2; Table 1). The GA group received an additional 0.35 g/kg/day glutamine on average more than the control group ($p<0.001$). The intake of vitamin C

Figure 2. The study protocol.

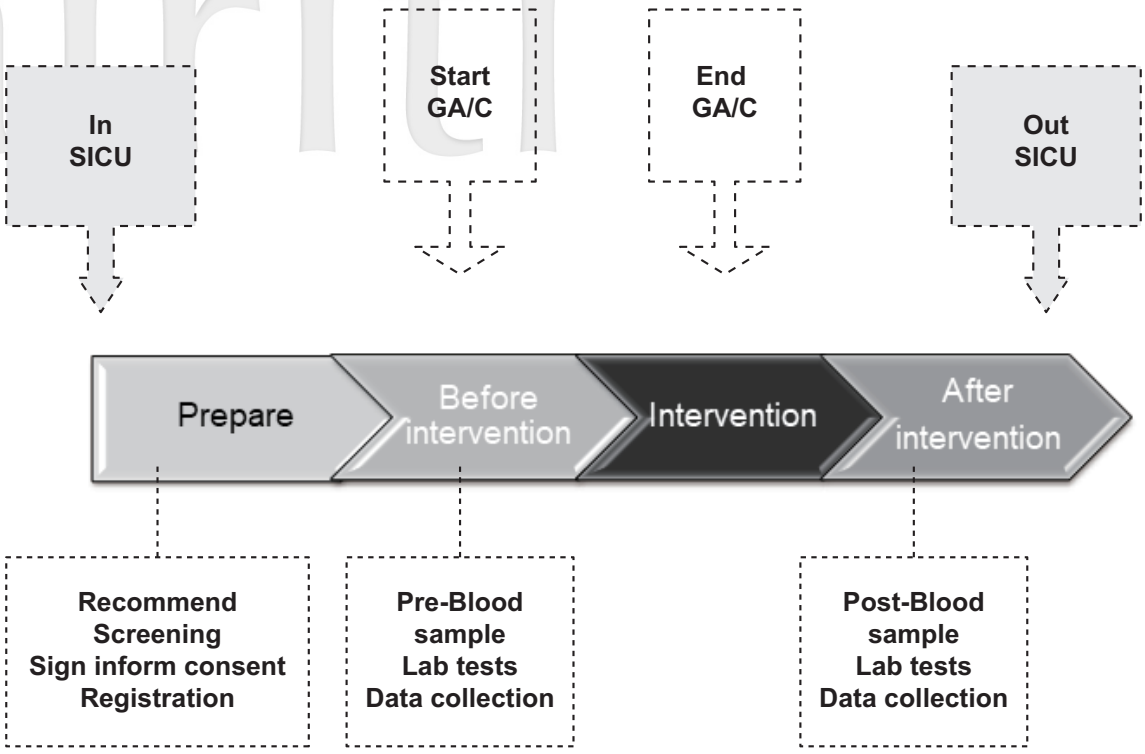


Table 1. Baseline characteristics of placebo group and GA group

	Placebo (n=10)	GA (n=31)	p-value
Baseline characteristics			
Age (years)	70.5 (60-83)	60 (43-74)	0.058
Sex			0.914
Female	4 (40%)	13 (41.94%)	
Male	6 (60%)	18 (58.06%)	
Height (cm)	168 (160-172)	165 (155-170)	0.200
Weight (kg)	51 (41-65)	54 (48-63)	0.533
BMI (kg/m^2)	19.29 (14.88-26.22)	21.82 (18.14-25.63)	0.439
Test period (days)	7 (6-10)	6.5 (4-9)	0.362
Nutrition supply			
Calories (kcal/kg/day)	24.88 (21.75-28.31)	27 (22.01-32.27)	0.529
Protein (g/kg/day)	0.86 (0.7-1.25)	1.4 (1.06-1.62)	0.003*
Glutamine (g/day)	0 (0-3.05)	20.63 (16.67-24.29)	0.001*
Vitamin C (mg/kg/day)	286.96 (204.1-358.2)	447.56 (394.5-506.7)	0.001*

* $p < 0.05$, represents significant difference between placebo group and GA group

Table 2. Biochemical indices, cytokines, and APACHE II scores between placebo group and GA group at pre- and post-treatment

	Placebo (n=10)	GA (n=31)	p-value‡
Biochemical indices			
Albumin (g/dl)			
Before treatment	3.05 (2.6-3.2)	2.55 (2.2-2.7)	0.028*
After treatment	2.8 (2.6-3)	2.65 (2.3-3.05)	0.406
p-value§	0.495	0.122	
RBC (10^4/µl)			
Before treatment	378 (333-430)	362 (320-390)	0.457
After treatment	355.5 (323-388)	362.5 (301-388)	0.988
p-value§	0.169	0.94	
Hemoglobin (g/dl)			
Before treatment	10.55 (10-12)	11 (10-11.8)	0.796
After treatment	10.55 (9.5-11.3)	11 (9.5-12)	0.457
p-value§	0.107	0.805	
WBC (µl)			
Before treatment	9875 (8600-11080)	10720 (7470-15280)	0.988
After treatment	9540 (8600-11760)	8990 (6240-11360)	0.379
p-value§	0.959	0.035†	
CRP (mg/dl)			
Before treatment	7.54 (5.61-10.5)	6.92 (2.7-11.7)	0.806
After treatment	6.34 (1.93-14.2)	1.45 (0.86-6.04)	0.111
p-value§	1	0.109	
Glutamine (µmol/L)			
Before treatment	463.15 (229.72-668.66)	467.25 (132.48-639.22)	0.685
After treatment	673.97 (283.82-1029.4)	754.15 (360.26-883.64)	0.94
p-value§	0.333	0.019†	
Glutamine <420µmol/L			
Before treatment	5 (50%)	13 (43.33%)	0.714
After treatment	3 (30%)	7 (29.17%)	0.961
p-value§	0.625	0.227	
Cytokines			
Il-6 (pg/ml)			
Before treatment	140.2 (76.04-378.44)	208.34 (94.55-344.38)	0.302

After treatment	94.17 (38.86-146.61)	64.94 (16.21-132.67)	0.597
p-value§	0.285	0.021†	
IL-10 (pg/ml)			
Before treatment	32.9 (16.25-55.45)	30.02 (13.38-45.85)	0.903
After treatment	27.2 (10.84-55.35)	12.77 (4.57-43.41)	0.273
p-value§	0.575	0.511	
APACHE II score			
Before treatment	14.5 (13-23)	19 (14-23)	0.512
After treatment	16 (14-17)	8.5 (6-16.5)	0.075
p-value§	0.715	0.003†	

‡ p-value shows comparison between placebo group vs. GA group
§ p-value shows comparison between pre- and post-treatment within the same group
* $p < 0.05$ represents significant difference between placebo group and GA group
† $p < 0.05$ represents significant difference between pre- and post-treatment within the same group

was between 167 and 660 mg/day, all within the range of recommended dietary allowance (RDA) in Taiwan. The vitamin C intake of the GA group was double that of the control group ($p<0.001$).

Biochemical indices, cytokines, and APACHE II scores before and after treatment

The pre- and post-treatment levels of biochemical indices and cytokines and APACHE II scores are presented in Table 2. Pre-treatment albumin was significantly lower in the GA group than in the placebo group (2.55 vs. 3.05 g/dl, $p = 0.028$). Within the GA group, posttreatment WBC count was significantly decreased ($p = 0.035$), and post-treatment glutamine level was significantly increased ($p = 0.019$) compared to pre-treatment values. No significant intergroup or intragroup differences were found in RBC, hemoglobin, and CRP level. No significant intergroup differences were found between pre- and post-treatment cytokine levels. However, IL-6 was significantly decreased after treatment in the GA group ($p = 0.021$), while no significant intragroup differences were found in IL-10 level in either group (all, $p > 0.05$). No significant differences were found in pre- or post-treatment APACHE II scores between the GA group and the

Table 3. Clinical outcomes

	Placebo (n=10)	GA (n=31)	p-value
Length of stay			
Hospital (days)	33 (29-56)	33 (27-53)	0.773
SICU (days)	21 (18-28)	16 (9-27)	0.316
Ventilator use (days)	6.5 (5-10)	3 (2-7)	0.096
Infectious complications [a]	3 (50%)	6 (20.69%)	0.162
Mortality			
In-hospital mortality (n, %) [a]	2 (20%)	3 (9.68%)	0.580
SICU mortality (n, %) [a]	0 (0%)	2 (6.67%)	0.999

[a] Analysis by Fisher's exact test.

placebo group. APACHE II scores were significantly decreased in the GA group after treatment ($p = 0.003$, Table 2).

Clinical outcomes

No significant differences were found between the GA group and the placebo group in hospital length of stay, SICU length of stay, and duration of ventilator use. There were no significant differences between the groups in infectious complications, in-hospital mortality, and SICU mortality (all, $p > 0.05$, Table 3).

Associations between pre- and post-treatment plasma glutamine, cytokines, and clinical outcomes

At pretreatments, the glutamine levels were represented negatively associated with IL-6 ($\beta = -0.363$, $p = 0.021$, Table 4). The IL-6 levels were positively associated with IL-10 levels and CRP (IL-10: $\beta = 0.415$, $p = 0.007$; CRP: $\beta = 0.768$, $p = 0.001$). After treatments, the glutamine levels were negatively associated with the ICU mortality ($\beta = -491.325$, $p = 0.044$). The IL-6 levels were significantly decreased when RBC and albumin levels were increased (RBC: $\beta = -0.487$, $p = 0.004$; albumin: $\beta = -0.585$, $p = 0.001$). And the IL-6 levels were also significantly increased when pre-treatment IL-10 and CRP levels were increased (IL-10: $\beta = 0.415$, $p = 0.007$; CRP: $\beta = 0.768$, $p = 0.001$). IL-6 levels increased significantly as SICU length of stay increased ($\beta = 4.342$, $p < 0.001$). Additionally, IL-6 levels increased significantly as SICU and in-hospital mortality increased (ICU: $\beta = 345.093$, $p \leq 0.001$; hospital: $\beta = 249.469$, $p < 0.001$) (Table 4).

The association of changes in IL-6 and glutamine were also evaluated with age, BMI, vitamin C, the change from pre-to-post treatments in cytokines and clinical outcomes. Similarly, the univariate analysis also represented changes of IL-6 and glutamine was negatively associated ($\beta = -0.402$, $p = 0.028$, Table 5). Moreover, the change of IL-6 level might be also increased when the change of IL-10 increased, or mortality in SICU or in hospital occurred (change in IL-10: $\beta = 0.557$, $p < 0.001$; ICU: $\beta = 407.37$, $p = 0.034$; in-hospital: $\beta = 429.49$, $p = 0.034$). The change of glutamine level was shown increased significantly when CRP levels decreased ($\beta = -0.602$, $p = 0.015$, Table 5).

Discussion

The current study showed that enteral administration of glutamine and vitamin C to SICU patients resulted in a decrease of WBC count, CRP, IL-6 level, and APACHE II score, effects not seen in patients that received placebo. However, there were no significant differences in clinical outcomes (SICU and in-hospital length of stay, infectious complications, duration of ventilator use, as well as SICU and in-hospital mortality) between the 2 groups.

The vital role of IL-6 in regulation of inflammation and clinical outcomes was evident in the results of the Nutrition Risk in the Critically Ill Score (NUTRIS) trial, which showed that incorporation of IL-6 in a scoring algorithm significantly increased the predictive ability for mortality rate and duration of mechanical ventilation.[26] IL-6 was also a recommended nutritional assessment marker for ICU patients in the ASPEN 2016 study,[27] where it was shown to be a sensitive and consistent predictor of clinical outcomes in critically ill patients. Plasma glutamine concentration is often low during hypermetabolic and hypercatabolic states seen commonly in ICU and SICU patients. Typically, as a patient recovers plasma glutamine levels will increase. Results of 2 studies showed that plasma glutamine levels increased significantly in pediatric and adult ICU patients after nutrition therapy with glutamine supplementation.[28,29] Results of our study also demonstrated this benefit glutamine supplementation.

Significantly lower APACHE II scores were seen in the treatment group after the intervention. These findings are consistent with the conclusions of a meta-analysis that evaluated the efficacy of glutamine-enriched parenteral nutrition in patients with severe acute pancreatitis,[30] and a systematic review that investigated the effects of parenteral glutamine supplementation in critical illness.[31] After reviewing 26 studies that examined glutamine supplementation in critical

Table 4. Associations between plasma glutamine, cytokines and clinical outcomes at pre- and post-treatment

Parameters	Glutamine				IL-6			
	Pre-treatment		Post-treatment		Pre-treatment		Post-treatment	
	β (SE)	*p*	β (SE)	*p*	β (SE)	*p*	β (SE)	*p*
Age	-0.216 (2.793)	0.939	4.826 (2.892)	0.939	-0.191 (1.977)	0.923	0.271 (1.155)	0.816
BMI	-16.445 (10.235)	0.994	-8.464 (13.265)	0.994	0.583 (7.816)	0.941	3.392 (5.081)	0.509
Serum Data								
Glutamine	1	-	1	-	-0.363(0.145)	0.021*	-0.402(0.160)	0.018*
Vit C	-0.294 (0.407)	0.474	0.204 (0.514)	0.694	-0.076 (0.306)	0.805	-0.045 (0.198)	0.820
IL-6	-0.363(0.145)	0.021*	-0.402(0.160)	0.018*	1	-	1	-
IL-10	-0.131(0.170)	0.421	-0.017 (0.198)	0.926	0.415 (0.149)	0.007*	0.466 (0.145)	0.005*
WBC	-0.029 (0.154)	0.861	0.179 (0.177)	0.312	0.175 (0.158)	0.273	0.103 (0.224)	0.462
RBC	-0.370 (0.165)	0.823	0.116 (0.190)	0.512	-0.183 (0.143)	0.253	-0.487 (0.148)	0.004*
Albumin	0.140 (0.165)	0.395	0.242(0.174)	0.207	-0.116 (0.174)	0.457	-0.585(0.167)	0.001*
CRP	-0.450 (0.234)	0.092	-0.259 (0.279)	0.333	0.768 (0.136)	0.001*	0.679 (0.159)	0.004*
Clinical Outcomes								
APACHE II	4.850 (7.845)	0.541	4.371 (11.817)	0.717	-2.748 (6.057)	0.653	8.375 (4.038)	0.056
Ventilator use (days)	-11.720 (11.502)	0.315	-4.445 (13.009)	0.735	10.322 (8.293)	0.221	7.037 (4.837)	0.156
Length of stay								
Hospital length of stay (days)	-0.189 (0.622)	0.763	-1.091 (0.907)	0.238	-0.266 (0.457)	0.563	0.109 (0.355)	0.761
SICU length of stay (days)	-0.839 (2.727)	0.760	-4.230 (3.038)	0.173	-1.107 (1.997)	0.583	4.342 (0.921)	<0.001*
Infectious complications (ref : no infection)	-14.981 (118.383)	0.900	-7.554 (143.04)	0.958	38.083 (86.407)	0.662	85.679 (55.977)	0.138
SICU mortality (ref: survival)	-74.394 (212.733)	0.729	-491.325 (233.788)	0.044*	-66.450 (163.015)	0.686	345.093 (72.617)	<0.001*
In-hospital mortality (ref: survival)	-177.373 (145.417)	0.230	-266.446 (174.451)	0.137	-151.673 (106.728)	0.163	249.469 (53.398)	<0.001*

*p < 0.05 indicates statistical significance.

Table 5. Univariate analysis of factors associated with changes in IL-6 and glutamine between pre- and post-treatment

	IL-6 changes		Glutamine changes	
	β±SE	p-value	β±SE	p-value
Age	-0.71±2.82	0.804	3.49±4.38	0.434
BMI	3.26±18.03	0.858	-6.21±27.15	0.821
Vit C	0.04±0.41	0.930	0.60±0.62	0.344
Pre- to post-treatment changes				
Glutamine change	-0.402±0.131	0.028*		
IL-6 change			-0.402±0.131	0.028*
IL-10 change	0.557±0.150	<0.001*	-0.030±0.203	0.864
WBC change	-0.055±0.01	0.755	0.046±0.199	0.795
RBC change	-0.122±0.06	0.49	0.080±0.163	0.654
Albumin change	-0.024±0.186	0.289	0.003±0.200	0.985
CRP change	0.429±0.567	0.397	-0.602±0.132	0.015*
Clinical Outcomes				
APACHE II change	-9.24±10.23	0.387	-17.16±18.16	0.367
Ventilator (days)	-10.50±12.64	0.415	5.53±19.74	0.782
Length of hospital stay change (days)	-0.54±0.77	0.49	0.12±1.17	0.921
Length of SICU stay change (days)	-7.24±4.69	0.137	8.13±7.82	0.311
Infectious Complications (ref : no infect)	59.24±149.92	0.697	154.7±218.7	0.488
Mortality in SICU (ref: survival)	407.37±179.22	0.034*	-429.14±310.82	0.183
Mortality in Hospital (ref: survival)	429.49±190.11	0.034*	-434.31±303.15	0.167

* $p < 0.05$ indicates significant association with IL-6 or glutamine changes after treatment.
Change refers to differences in levels of a particular cytokine or biochemical index between pre- and post-treatment.

care patients, Wischmeyer et al.[31] concluded that parenteral glutamine supplementation in addition to nutritional support was associated with significant reductions in hospital length of stay and mortality. However, the results of the REDOXS trials [10,11] suggest that early provision of high-dose glutamine to shock patients with multiple organ failure may have paradoxical harmful effects, such as alterations in kidney and liver function due to possible neurotoxic effects of glutamate, a metabolite of glutamine. This discrepancy may be because the total calories and protein given in the REDOXS studies were at levels that were too low for glutamine to have an effect, and because the REDOXS studies only included very severely ill patients. In a study evaluating the ability of glutamine supplementation to modify oxidative stress and inflammation in critically ill children,[28] IL-6 was not reduced significantly and IL-10 was not affected at all; however, heat shock protein-70 was significantly reduced for a longer period in children who received glutamine supplementation, but remained high in those who did not receive supplementation.

In the current study, there was a significant increase in plasma glutamine and a decrease in IL-6 level after enteral glutamine administration. Prior studies have also reported a decrease of plasma IL-6 after glutamine supplementation.[28,29] The improved clinical outcomes after enteral glutamine supplementation seen in some studies may be the result of decreased inflammation resulting from a decrease of proinflammatory cytokines such as IL-6.[29]

Vitamin C is an antioxidant, and associated with wound healing and control of infection.[32] Vitamin C plasma concentrations in plasma and in leukocytes fall within 24 hours of an acute illness despite supplementation through a daily recommended dose. [32,33] Supplementation with as much as 30 times the recommended daily dosage may be needed to restore normal plasma values in patients with inflammatory conditions.[16] Long et al.[12] reported that a super high dose of 3,000 mg/day of vitamin C is needed to correct low plasma levels in critically ill patients. Fowler et al.[13] reported that 50 and 200 mg/kg/day of vitamin C increased plasma levels by 200-fold in sepsis patients, and after supplementation

Sepsis-related Organ Failure Assessment (SOFA) scores and mortality rate decreased. Sadeghpour et al.[14] found that high-dose vitamin C reduced hospital length of stay in surgical patients. Although the role of vitamin C in decreasing IL-6 levels in ICU patients has not been studied previously, vitamin C had no suppressive effect on IL-6 in healthy athletes.[15,18] However, it was found to have a synergistic effect when used in combination with other antioxidants such as vitamin E.[17] The vitamin C supplied in the present study was within the normal dosage range, although the GA group received double the dosage of vitamin C compared with that provided to the placebo group.

The nutritional benefits of glutamine have been clarified in several studies, including that it increases plasma proteins and improves nitrogen balance.[34,35] When Koksai et al.[35] compared the effects of intravenous, enteral, and intravenous plus enteral supplemental glutamine on transferrin in malnourished septic patients, the combined route was associated with the most rapid and positive outcomes during the catabolic phase of their illness. The authors believed the superior outcomes were related to more efficient utilization of exogenous substrate for protein synthesis, which is not seen with either parenteral or enteral routes alone. Several randomized trials and meta-analyses support the use of glutamine in ICU, which has been incorporated into guidelines.[36] The REDOXS study was conducted by Dr. Heyland in 2006 for he suggested that high dose of glutamine would improve the survival for multi-organ failure patients.[9] The report published in 2013 showed that the patients included into the study suffered from more than 2 organ failures and were dependent on mechanical ventilator support.[10] Interestingly, both enteral and intravenous glutamine were used in amounts much more than the recommended dosage. In addition, the serum glutamine levels were not available in most of the patients and was not lower than normal mostly in the patients with data detected. Most of patients' energy and protein supplements were less than 60% of their requirements. The relative risk was 1.00~1.64 that just crossed the statistical significance with $p = 0.05$.[10] These findings taken together suggested that glutamine overdose for malnourished multi-organ failure patients with normal serum glutamine levels could be harmful. Further analyses showed that glutamine was harmful for patients with hepatic failure. Serum glutamine level <420 or >930 increased mortality, and hepatic failure impaired the metabolism of glutamine and raised the level higher than the normal.[3,4,37] Glutamine should not be provided for this group of patients. However, the patient number in the REDOXS study was huge that outweighed the following meta-analyses and guidelines. The ASPEN 2016 guidelines for ICU did not recommend routine intravenous glutamine for ICU patients, while the EPSEN 2018 guidelines suggested glutamine for burn and trauma patients, but not used for patients with hepatic and renal failure.[27,38] Our study carefully excluded the severe hepatic and renal failure patients, energy and protein was well provided, the serum glutamine levels were not too high, and glutamine was fed enteral only. Our results showed the GA group still had no clinical benefits, but the glutamine level was associated with the clinical outcomes. Further future

studies are warranted to analyze the subgroups that benefited from the glutamine and vitamin C supplements.

This study has several limitations, including its small sample size. The foci of this study were serum IL-6 and glutamine levels. After the intervention period, both GA and C group were provided glutamine supplements GA to facilitate patient recruitment after initial failure of recruitment at the beginning of the study. This compromise may influence the final clinical outcomes. Since the IL-6 levels were successfully decreased, further study design may aim at improving clinical outcomes. Although the ratio of experimental versus control subjects was 3:1, this should be satisfactory for evaluating our hypothesis. The main ingredient of the placebo was maltodextrin, which provided the same calories as the GA material. The study design called for the administration of isocaloric, but not isonitrogenous, diets to the 2 groups which is consistent with general protocols for the study of nutrition therapy.[39]

Conclusions

This is the first prospective randomized, double-blinded, and controlled clinical study for investigating the effect of enteral glutamine and vitamin C supplementation on proinflammatory cytokines in surgical ICU patients. Early administration of combined enteral glutamine and vitamin C supplementation increased plasma glutamine level, and was associated with decreased serum IL-6 levels and reduced APACHE II scores in SICU patients. However, in this study administration of glutamine/vitamin C was not associated with significant improved clinical outcomes. Whether an appropriate dosage of glutamine/vitamin C can improve clinical outcomes remains to be determined.

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