Egypt, J. Plast. Reconstr. Surg., Vol. 32, No. 1, January: 91-97, 2008

The Role of Enteral Glutamine Supplementation in Modulation of the Immune Function and Outcome in Severely Burned Patients

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ABSTRACT

Glutamine is an important energy source for immune cells. It is a necessary nutrient for cell proliferation, and serves as specific fuel for lymphocytes, macrophages and enterocytes when it is present in appropriate concentrations. The main objectives of this study were to measure the impact of enteral glutamine supplementation on immunological function, infectious morbidity and length of hospital stay in severely burned patients.

Methods: Fifty patients were included in this study. Forty patients of them were severely burned. The remaining 10 were normal adults (control group). The burned patients were classified into 2 groups (20 patients each). Glutamine group (Ggp) in which patients received 0.5g/kg/day in three divided doses before meals for 21 days. The burned control group (Bgp) in which patients received placebo in form of glycine for 21 days. Measurement of plasma glutamine levels, Neutrophil phagocytosis index (NPI), Interleukin 2 (IL-2), IgA, IgG, IgM, C3 and C4 were done before treatment and after 3 weeks. Blood culture and wound swab culture were done for all patients every 3 days.

Results: Plasma glutamine levels were decreased in all burned patients compared with normal control group $p<0.01$. After supplementation by enteral glutamine granules for 3 weeks, plasma glutamine levels increased in glutamine (G) group than in burned control (B) group. The indices of cellular immune function NPI and IL-2 levels decreased in B group and increased after glutamine supply in G group $p<0.01$. The incidence of positive blood culture was three times higher in burned B group than in G group $p<0.01$. The hospital stay and mortality rate decreased after glutamine administration in G group.

Conclusion: Enteral glutamine granules replaced the reduction in plasma glutamine in severely burned patient, improve the cellular immune function, reduced blood culture positivity particularly with Gram-ve bacteria and may be a life saving intervention.

INTRODUCTION

Traumatic injuries results in a prolonged period of negative nitrogen balance. These changes become maximal in major thermal injury patients **[1]**. Patients suffering from severe burn injuries initially experience an ebb phase where there is a decrease in their metabolic rate and cardiac output **[2]**. Following successful fluid resuscitation, the cardiac output rapidly returns to normal and then achieves supernormal levels. Coincident with this, there is a marked increase in resting metabolic energy expenditure (RME) **[3]**. The degree of increase in metabolic rate appears to be directly proportional to the extent of burn injury. Metabolic rate reach up to 200% of normal in extensive burn injuries **[4]**. This increased RME contributes to malnutrition with severely body weight loss and negative nitrogen balance **[5]**.

In severe burned patients, the losses of body proteins may exceed 30-40g N/day, which is 10 fold higher than in a healthy person. Glutamine is a non essential amino acid with several biological properties that make it a good candidate for nutritional intervention in critical illness. It is an important energy substrate for immune cells **[6]**, and intestinal epithelium **[7]**.

Enteral nutrition has become the preferred method of feeding in severely burned patients because it is associated with fewer infections, preserves gut barrier function and prevents gut atrophy more effectively than parenteral nutrition **[8]**. Addition of enteral glutamine to nutritional regimens for critically ill patients may restore depleted plasma and muscle levels, improve nitrogen balance, prevent muscle wasting and ultimately improve outcome **[9]**.

The main objectives of this double blind randomized clinical trial were to measure the impact of enteral glutamine supplementation on infectious morbidity, length of hospital care, and immunological function in severely burned patients.

PATIENTS AND METHODS

This prospective, randomize double blind study was carried out in both burn unit and surgical intensive care unit in Mansoura Emergency Hospital after obtaining approval from the local Ethics and research Committee and written informed consent from all patients or their relatives. Forty patients with severe burn participated in this study, age ranged from 20-50 years. Total body burned surface area (TBSA%) ranged from 30-60%, full thickness burned area ranged 20-50%. All patients were admitted within 6 hours after injury.

Patients with 1st degree burn, pregnancy, Cardiovascular, hepatic or renal dysfunction or died within 72h of admission were excluded from the study.

Patient's classification:

Patients were randomly assigned (sealed closed envelope) to one of two group (20 patients each). Glutamine group G (n=20) in which patients supplied with 0.5g/kg/day glutamine granules on three divided meals and burn control group B (n=20) in which patients supplied with placebo (0.5g/kg/day glycine) on three divided meals. The test and control solutions were packed at the same size and the packaging materials have the same appearance. Neither the patient nor the investigator knew if the applied enteral nutrition regimen were with or without glutamine.

The normal control group C (n=10) were selected from the workers in the institute of burn unit (20-50) years. All control subjects were healthy, without any history of recent or remote gastrointestinal tract, cardiovascular, hepatic or renal diseases.

Patient care nutritional support:

The Parkland formula was used for fluid resuscitation with Ringer's lactate solution and targeted to achieves 40mL/h urine output. Debridement and grafting were performed within 21 days postadmission. Inhalation injury was assessed by bronchoscopy on admission. Some patients with inhalation burn needed tracheal intubation and artificial ventilation (Table 1). Pain was managed with morphine intravenously specially during therapeutic procedures. The study lasted from admission to the complete healing of burn wounds and donor sites. Enteral nutrition in all patients was started within 24h of admission either by mouth or through a nasogastric tube according to patients general condition. The initial rate of 30mL/h was increased every 12h up to 120mL/h depending on patient's tolerance. All patients received the same formula (15% of energy is derived from fat, 20% from proteins, and 65% from carbohydrates).

There were no added immunonutrients. Parenteral nutrition was started when necessary to meet the nutritional requirements of the patients as soon as possible. This parenteral nutrition commenced within 48h of admission. Criteria for starting parenteral nutrition were failure to reach the goal for caloric intake within 24h post admission by enteral route or calculated energy requirements at entry >2900Kcal/day. Dextrose 25% with amino acid 6.5% and intralipid 20% was delivered. The aminoacid solution does not contain glutamine. Energy requirements were calculated with the Curreri formula **[10]** and adjusted for the measurement of energy expenditure by indirect calorimetry twice a week, as described previously **[11]**. Vitamins and trace elements were added to the dextrose aminoacid mixture. Enteral glutamine in glutamine group was given for 3 weeks even when enteral nutrition was interrupted for surgical procedures or parenteral nutrition.

Sample collection and biochemistry:

1- *Urine analysis:*

Twenty-four hour urine was collected throughout the study. Its volume was determined. Total nitrogen and cortisol were measured in all samples.

2- *Preparation of blood samples:*

Peripheral blood samples were obtained from patients at 1st day of study before glutamine treatment and after 3 weeks of treatment. About 40mL of peripheral blood was collected in a test tube containing 8mL of 6% dextran and 1.2mL of heparin sodium (1000u/mL). The test tube was placed up right for 45-60min to allow the separation of red cells and plasma. The plasma layer was then removed to centrifuge tube to be centrifuged at 2000rpm for 5min and frozen at –30ºC for future batch analysis of plasma glutamine contents, immunoglobulines, complement and IL-2 level. These measurements were done at admission and after 3 weeks. Neutrophils contaminating erythrocytes was resuspended. The erythrocytes were removed by hypotonic lysis, and the neutrophils were >95% pure. The cells were washed two times with Hanks balanced salt solution (HBSS) and suspended in a known volume of 1x HBSS with calcium and magnesium. The neutrophils were counted and adjusted to a concentration of $1x10^7$ cells/mL **[12]**.

Neutrophil phagocytosis index (NPI):

Neutrophils ($5x10^6$) were incubated in HBSS with $1x10^7$ staphylococcus aureus and 100mL of

pooled normal serum in a final volume of 1mL. After 2h, incubation with rocking at 37ºC was done. The NPI was determined by microscopic observation after staining with Gram's stain under a light microscope, NPI cells of phagocytosis bacteria/total cells x 100%.

The plasma glutamine concentration:

Plasma glutamine concentration was quantitated by high pressure liquid chromatography. The detailed methods were described previously **[13]**.

The plasma immunoglobulin complement and IL-2 level:

The concentration of plasma immunoglobulin including IgA, IgG and IgM were measured by immuno-electrophoresis and complement including C_3, C_4 were measured with immunodiffusion. The plasma IL-2 level was measured by radio immunoassay **[14]**.

3- *Blood and wound culture:*

Blood was cultured in duplicate when body temperature was >38.5ºC or <36.5ºC. Samples were obtained from a central catheter in most instances. Wound infection was monitored by swab sampling of the wounds in all burned patients (in both groups).

Length of care was the time from admission to the complete healing of grafted and ungrafted wounds, including donor sites, as determined by the surgeon.

St*atistical analysis:*

All values were expressed as means (SD). Parametric variables were analyzed using student *t* test. Analysis of variance (ANOVA) was used to compare between more than two groups followed by Tukey Kramer multiple comparison tests. The frequency of surface of burn, age and sex were evaluated using X^2 test. Values of $p<0.05$ was considered statistically significant.

RESULTS

There was no significant differences between the corresponding clinical characteristics of the patients in both groups (Table 1). Nutritional data on energy and protein intake, resting energy expenditure, nitrogen balance blood insuline given and urinary cortisol excretion were presented in Table (2). There were no significant differences in these variables between both groups.

On admission plasma glutamine levels were decreased in all burned patients compared with normal control group ($p<0.01$). On the other hand, plasma glutamine level in glutamine group (Ggp) was significantly higher than burn control group (Bgp) after 3 weeks of treatment $p<0.01$ (Table 3). Cellular immune function was significantly suppressed after burn including neutrophils phagocytosis index (NPI) and IL-2 levels. Compared with B group these two indices in G group were significantly increased after 3 weeks of treatment $p<0.01$ (Table 4). So, glutamine supplementation appeared to improve the cellular immune function after severe burn.

Table (1): Patients' clinical characteristics in both groups of the study.Values are expressed as mean (SD) or percentage of patients.

	Glutamine group (Ggp) n=20	Burn group (Bgp) n=20
Age (yrs)	36±7	38±9
Body weight (Kg)	84±19	86±13
Gender (MIF)	6/14	5/15
Total body burned area (%)	42±19	40±18
Full thickness burned area (%)	28±14	27±12
Inhalation injury (n)	4	3

Table (2): Energy and protein intake, resting energy expenditure (REE), nitrogen balance and metabolic characteristics of both groups values are presented as mean (SD).

	Burn control group (Bgp) n=20	Glutamine group (Ggp) n=20
Energy intake (Kcal/day)	2867±1273	2763±1265
Resting energy expenditure (Kcal/day)	2296±1012	2265±1123
Protein intake (g/day)	183±87	186±96
Nitrogen balance	–5.1±3	–4.9±6
Insulin given (u/day)	39±22	40±62
Blood glucose (m mol/l)	8.8±1.6	8.6±1.5
Urinary cortisol (n mol/day)	1675±1432	1718±1537

Table (3): Plasma glutamine concentration of all groups of patients (μ mol/l). Values are presented as mean (SD).

	Control group (Cgp) n=10	Burn control group (Bgp) n=20	Glutamine group (Ggp) n=20
Before treatment	615.6 (90.1)	399.41 (172.34)•	405.42 (132.76)•
After treatment By 3 weeks	615.6 (90.1)	412.41 (162.21)	609.86 (147.38)*

• Significantly significant compared to the control groups.
* Significantly significant compared to B group.

Table (4): Changes in neutrophils phagocytosis index (NPI), immunoglobulins and complements (C3, C4) in both groups of patients. Values are expressed as mean (SD).

	Control group n=10	Burn control group n=20		Glutamine group n=20	
		Before ttt	Using placebo for 3 weeks	Before ttt	After ttt by 3 weeks
Neutrophil phagocytosis index (%)	37.80 (6.71)	27.70 (5.32)•	23.34 (4.61)	23.34 (4.67)•	33.65 (5.42)*
IL-2 (mg/L)	6.43 (1.60)	4.92 (1.70)	4.32 (1.43)	4.75 (1.82)	6.67 (2.61)
IgA (g/L)	2.71 (0.39)	2.04 (0.30)	2.26 (0.23)	2.05 (0.36)	2.32 (0.14)
Igm (g/L)	0.89 (0.32)	0.77 (0.21)	0.82 (0.13)	0.87 (0.23)	0.76 (0.43)
IgG (g/L)	10.41 (4.21)	9.32 (3.42)	8.93 (2.31)	8.87 (3.62)	9.56 (3.42)
C3 (g/L)	1.13 (0.79)	0.99 (0.63)	1.05 (0.78)	1.03 (0.54)	0.96 (0.76)
C4 (g/L)	0.36 (0.12)	0.31 (0.070	0.29 (0.09)	0.32 (0.08)	0.29 (0.11)

• Significantly significant when compared with control group.
* Significantly significant when compared with burned control group after treatment.

Indices of humoral immune function such as plasma immunoglobulins and complements (C_3, C_4) concentration were lower after severe burn than that in normal controls, but these changes were not significant $p>0.05$ (Table 4). Patients in glutamine group showed no significant changes as regarded the indices of humoral immunity compared to patients in burn group (Table 4). The incidence of positive blood cultures (PBc) was three times higher in burned patients (B) group than in glutamine group of patients (4.2±6.1 Vs 1.1±2.6 days/pt respectively $p<0.05$) (Table 5). In addition the number of patients with PBc for 2 days of study time was higher in Bgp than in Ggp (seven Vs zero) $p<0.01$. The type of bacteria found in blood cultures was not different between both groups: One patient with Gram –ve bacteria in Ggp versus eight in Bgp $p<0.01$. The number of patients with +ve wound culture was higher in Bgp than Ggp (nine versus three) $p<0.05$ (Table 5).

The average hospital stay in days was significantly shorter in Ggp than that in Bgp (42.68±12.75 Vs 52.61±13.36 respectively) $p<0.05$ (Table 5). The mortality rate was significantly decreased in Ggp compared to Bgp (Zero versus seven) $p<0.01$, Table (5).

Table (5): Values of blood culture results, antibiotics given, wound infections and number of deaths, hospital stay or number (%). Values are expressed as mean (SD).

	Burn control group n=20	Glutamine group n=20
No. of patients with positive blood culture (PBc) (%)	10 (50%)	4 (20%)*
No. of patients with gram –ve bacteria (%)	8 (40%)	1 (5%)*
No. of patients with >2 days of PBc (%)	7 (35%)	0 (0%)*
No. of PBc/patient (mean ± SD)	4.2 (6.1)	1.1 (2.6)*
Antibiotic use per a (mean ± SD)	4.6 (0.22)	1.20 (0.24)
No. patients with wound infection (%)	9 (45%)	3 (15%)*
Hospital stay (days)	52.61 (13.36)	42.68 (12.75)*
No. of death per protocol analysis	7 (35%)	0 (0%)*

PBc = Positive blood culture.
* Significantly significant compared to the other group.

DISCUSSION

The present study showed significant decrease in the plasma glutamine levels in two burned groups. Similarly Parry and his colleagues reported decreased plasma glutamine levels of burned patients by 58% and remain depressed for more than 21 days after injury **[15]**.

After severe burns, large amounts of glutamine are released from muscle tissue providing essential substrates for visceral organs for acute phase protein synthesis, and energy production. Despite the accelerated release of glutamine from skeletal muscle, blood glutamine may not be increased after burn **[16]**. Intestine, liver and the immune system significantly increase their uptake of glutamine by as much 5-8 fold in severe trauma or inflammation **[17]**. So, the level of glutamine consumption was far larger than glutamine synthesis and the plasma glutamine level was significantly decreased.

The results of our study demonstrated that enteral glutamine granules supplementation in a dose of 0.5g/kg/day on three divided doses for 21 days restored the plasma glutamine concentration to near normal levels. In agreement with our results Peng and colleagues showed that enteral glutamine supplementation 0.5g/kg/day for 14 days reversed the decreased plasma glutamine concentration in severe burned patients **[18]**.

Glutamine has been reported to improve the immunologic function in trauma patients. In this study compared with the B group, glutamine administration improved the cellular immune function after burn. NPI and IL-2 levels were increased in G group $p<0.05$. The finding of this study are consistent with those previously obtained by Peng and colleagues who demonstrated significant suppression of the indices of cellular immune function such as NPI and IL-2 levels after severe burn. Glutamine administration for 14 days in their study improved the cellular immune function in severely burned patients **[18]**.

Glutamine is an important energy source for immune cells, particularly macrophages and lymphocytes. All rapidly proliferating cells, mainly those of the immune system strictly depend on the availability of glutamine as an energy (carbon, nitrogen) source **[19]**. Glutamine is also one of the major precursors when purines and pyrimidines are synthesized. It is utilized as a precursor for nucleotide biosynthesis which is of particular importance for the high cell proliferation rate in immune cells **[20]**.

Other authors reported that the D related human leukocyte antigen (HLA-DR) expression plays a critical role in the induction of the cellular immune response **[21]**. In trauma patients, parenteral glutamine supplementation restored HLA-DR expression in monocytes **[21]**. Glutamine also stimulated lymphocyte proliferation in mice **[22]**, and increased lymphocyte count during acute pancreatitis **[23]**. Thus, glutamine improve the cellular immune response during critical illness.

On the other hand, our results showed no significant difference in humoral immune function indices (Immunoglobulines and complement) between the group and B group. Peng and associates reported that these results may be due to intensive response of B lymphocytes to glutamine administration compared to T lymphocytes, neutrophils and macrophages **[18,24]**.

Contrary to our result Yeh and colleagues found enhanced humoral immunity and attenuated oxidative stress induced by burn injury in an experimental study on mice supplied by glutamine enriched diet **[25]**.

The frequency of Gram-negative bacteremia was 40% in the burn control group Vs 5% in the G group. These data are consistent with the results of a previous trial of glutamine administration in burned patients **[27]**. Those studies demonstrated a statistically significant reduction in the incidence of bacteremia, septic episodes, and preumonia in glutamine treated patients versus an isonitrogenous control.

The results of our study and the previous studies **[26,27]** support the hypothesis that glutamine may enchance gut barrier function and prevent bacterial translocation from the gut. In support of this hypothesis trials of glutamine supplementation in burned animals indicate that glutamine can prevent the translocation of radiolabeled bacteria across the gut barrier **[28]**. Furthermore, as a primary respiratory fuel for the enterocytes and colonocytes, glutamine supplementation has been shown to prevent increases in gut atrophy and permeability related to total parenteral nutrition **[29]**. Glutamine as a precursor of glutathione has major antioxidant properties particularly on gut mucosa, and this may be protective for immune function in the gut **[29]**.

Garrel and colleague hypothesized that pseudomonas aeruginosa which is the most infectious Gram negative bacteria in burned patients may be sensitive to the amount of glutamine in its environment and the lack of glutamine may trigger both

proliferation of the bacteria and crossing of the epithelial barrier **[29]**. Together with weakening of the gut immune system, related at least in part to glutamine deficiency, these phenomena may explain P. aeruginosa infection.

We also observe marked decrease in the average number of positive blood cultures per patient and in over all daily antibiotic used in glutamine supplemented patients. Similar to our observation was seen by Houdijk et al. and Garrel et al., who found reduction in blood infection and in average daily antibiotic used in G group **[29,30].**

The mortality rate and hospital stay reduced in G group compared with B group $p<0.01$. The mechanism of the glutamine effects on mortality rate and hospital stay in our patients can only be speculated upon the reduction of blood infection, improved wound healing and the trophic effects of glutamine on the cellular immune system. In patients who received placebo, blood infection and bacteremia was more frequent and more prolonged with delayed wound healing. So, the mortality rate and hospital stay were increased **[29,31]**.

Conclusion:

In conclusion, enteral glutamine granules replace the reduction in plasma glutamine in severely burned patient, improve the cellular immune function, reduce blood culture positivity particularly with Gram-ve bacteria and may be a life saving intervention.

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