Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/paf3s690

"A COMPARATIVE ANALYSIS OF PRESERVATION OF GUT FUNCTION AND IMPROVEMENT IN OUTCOMES WITH GLUTAMINE AND PROBIOTIC SUPPLEMENTATION IN TREATMENT OF MAJOR BURNS"

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ABSTRACT

Introduction: Burn injuries produce extensive skin barrier disruption, which creates novel sites for bacterial colonization and contributes to an immunosuppressive state, making the burn patients vulnerable to infectious complications. The dramatic increase of gut permeability with disturbance of the intestinal flora and translocation of the microorganisms and/or their products from gastrointestinal tract to extra-intestinal sites seems to contribute to systemic sepsis and associated multiple organ failure after severe burns. In this study, we have used a comparison of Urinary lactulose mannitol ratio (L/M ratio), Sequential organ failure assessment (SOFA) scores and the duration required for wound healing as parameters to assess the outcomes with glutamine and probiotic supplementation in treatment of major burns.

Material and Methods: The study included 88 patients with more than 30% TBSA burns at admission and aged between 18 and 60 years. The subjects were randomly allocated to three groups. The control group received standard nutritional supplementation, and the Glutamine test group received an additional 0.3g/kg/day of enteral glutamine supplement and probiotic test group received probiotic preparation twice daily. L/M ratio were assessed at admission and at day 14; SOFA scores were assessed at admission and on day 3, and delta SOFA (3-0) were calculated. Results were tabulated and compared statistically.

Results: The average age among the Glutamine, Probiotic groups and Control groups were [35.9 +/- 11.07] years, [36.7 +/- 9.07] years and [38.3 +/- 10.6] years respectively, with the average percentage of burns were [44.8 +/- 8.09] %TBSA, [46.9 +/- 9.5] % TBSA and [45.7 +/- 8.7] % TBSA in the three

groups respectively. Significant reduction in the L/M ratio at day 14 is noted in both Glutamine and Probiotic groups when compared to Control group; however, no significant difference noted when compared with each other. Significant reduction in the duration of wound healing is noted in both Glutamine and Probiotic groups when compared to Control; however, no significant difference noted when compared with each other. The delta SOFA (3-0) score averaged [0.06 +/- 1.5], [0.3 +/- 1.3], [0.9 +/- 1.3] in the Glutamine, Probiotic and Control groups respectively.
Conclusion: Prophylactic administration of glutamine or probiotic appears to preserve gut function and improve wound healing time and overall outcome, with the former showing higher benefit as compared to the latter.

Keywords: Glutamine, major burns, Probiotics, Lactulose/Mannitol ratio, SOFA, delta SOFA, gut function.

INTRODUCTION:

Burn injuries produce extensive skin barrier disruption, which creates novel sites for bacterial colonization [1] and contributes to an immunosuppressive state, making the burn patients vulnerable to infectious complications [2]. Moreover, the dramatic increase of gut permeability with disturbance of the intestinal flora and translocation of the microorganisms and/or their products from gastrointestinal tract to extra-intestinal sites seems to contribute to systemic sepsis and associated multiple organ failure after severe burns [3]. Various treatment modalities were investigated to prevent the occurrence of bacterial translocation and enhance immune function after thermal injury [4]. Administration of an enteral diet enriched with immunomodulatory compounds, such as glutamine has been shown protective effect on gut mucosal barrier and to reduce wound infection rates and length of hospital stay in critically ill patients [5,6]. Another such treatment involves enteral supplement of probiotics which has similar protective effect on gut mucosal barrier[7].In this study, we have used a comparison of Urinary lactulose mannitol ratio, Sequential organ failure assessment (SOFA) scores, positive wound and blood cultures, and the duration required for wound healing as parameters to assess immune dysregulation, sepsis, risk of multi-organ dysfunction, gut permeability thereby derive an overall idea of the outcomes with glutamine and probiotic supplementation in treatment of major burns.

MATERIAL AND METHODS:

The study was prospective in design and was conducted from April 2022 to December 2023. Eighty-eight adult burn patients meeting the inclusion and exclusion criteria were randomized to three groups with 30 subjects each in Glutamine and Control group and 28 subjects in Probiotic group. The control group received standard enteral nutritional supplementation, while the glutamine test group received 0.3g/kg/day of glutamine supplement additionally and probiotic test group received probiotic preparation twice daily (BIFILAC GG , Allianz Biosciences, INDIA) as capsules containing 60 billion colony forming units (CFU) of Lactobacillus rhamnoses GG. The rest of treatment and management protocols were kept consistent among all subjects.
Intestinal permeability was determined with the lactulose-mannitol (L/M) absorption technique. After overnight fasting patients were given 2 g lactulose and 1g mannitol through their feeding tube. Urine was collected for the next 5 hours, and a 10-mL sample was taken and sent for analysis on the day of admission and at day 14.

INCLUSION CRITERIA:

1. Partial thickness thermal burns of >/=30% TBSA
2. Full thickness thermal burns of >/= 20% TBSA
3. Inhalational burns
4. Age >/= 18y and <60y

EXCLUSION CRITERIA:

1. Known renal or hepatic dysfunction (contraindication for additional glutamine supplementation
2. Presenting more than 48 hours of burn injury
3. Electrical burns and chemical burns

The demographic data and history of each patient was recorded before randomization. Lactulose/ Mannitol ratio was calculated at admission and again on day 14. SOFA score was assessed in all patients at admission (SOFA 0), and again on day 3 (SOFA 3), and delta SOFA (3-0) [difference in SOFA 3 from SOFA 0] was calculated. Additionally, bacterial cultures from burn wounds were sent on the 4th post burn day, and if positive, repeated weekly until negative culture was obtained. Blood cultures were also sent in the second post-burn week among patients that still required inpatient care. The results were tabulated and analysed using appropriate tests for statistical significance {ANOVA test for comparison of baseline data among all groups, student's t-test and Mann-Whitney U test for ordinal variables and Fisher's exact test for nominal variables} with the assumption of p-value of less than 0.05 to be statistically significant.

RESULTS:

Table 1: Demographic data and assessment parameters among the three groups (mean +/- SD). The age, percentage and baseline L/M ratios are comparable among all groups with no significant difference. L/M ratio- lactulose/mannitol ratio, delta SOFA (3-0)- difference in SOFA scores at day 3 and day 0.

Parameter	Glutamine	Probiotic	Control	P- value
Mean Age (years)	35.9667 +/- 11.0718	36.7143 +/- 9.0711	38.3 +/- 10.6808	0.6731
% TBSA burnt	44.8 +/- 8.096	46.9643 +/- 9.5703	45.7 +/- 8.7971	0.6466
L/M ratio day 0	0.0145 +/- 0.0082	0.0147 +/- 0.0072	0.0155 +/- 0.0079	0.87154
L/M ratio day 14	0.02183 +/- 0.02081	0.03696 +/- 0.05042	0.16527 +/- 0.12553	N/A
Delta SOFA (3-0)	0.0667 +/- 1.5298	0.3214 +/- 1.3623	0.9667 +/- 1.3767	N/A
Positive wound cultures	0.48 +/- 0.69	0.63 +/- 0.74	1.10 +/- 0.94	N/A
No of subjects with positive blood cultures	4	5	13	N/A
Total days for wound healing	14.03 +/- 3.42	15.54 +/- 4.01	21.33 +/- 7.68	N/A

As described in Table 1; the average age of subjects in the Glutamine, Probiotic groups and control groups were [35.9 +/- 11.07] years, [36.7 +/- 9.07] years and [38.3 +/- 10.6] years respectively. The average percentage of burns were [44.8 +/- 8.09] % TBSA, [46.9+/- 9.5] % TBSA and [45.7 +/- 8.7] % TBSA in the three groups. The three groups show no significant variation in the demographic data and extent of burns and can be considered comparable to measure outcomes.
The average L/M ratio of Glutamine group was [0.01 +/- 0.008] and [0.02 +/- 0.02] on day 0 and day 14 respectively; Probiotic group was [0.01 +/- 0.007] and [0.03 +/- 0.05] on day 0 and day 14 respectively and Control group was [0.01 +/- 0.007] and [0.1 +/- 0.1] on day 0 and day 14 respectively. The delta SOFA (3-0) score averaged [0.06 +/- 1.5], [0.3 +/- 1.3], [0.9 +/- 1.3] in the Glutamine, Probiotic and Control groups respectively. Positive wound cultures averaged [0.4+/- 0.6], [0.6+/- 0.7], [1.1 +/- 0.9] were noted in Glutamine, Probiotic and Control groups respectively.

The positive blood cultures were noted in 4,5 and 13 patients in Glutamine, Probiotic and Control groups respectively in the second post-burn week.
The average total number of days required for wound healing were [14.03 +/- 3.4], [15.5 +/- 4.01], [21.3 +/- 7.6] in Glutamine, Probiotic and Control groups respectively.

Table 2: Results of tests for statistical significance among test parameters in the glutamine and probiotic groups as compared to the control group and one another.

Parameter	p-value of Glutamine v/s control group	p-value of Probiotic v/s control group	p-value of Glutamine v/s probiotic group
L/M ratio day 14	< .00001	< .00001	0.27134
Delta SOFA (3-0)	0.03486	0.11184	0.6171
Positive wound cultures	0.0139	0.0784	0.4902
No of subjects with positive blood cultures	0.0204	0.047	- - -
Total days for wound healing	< .00001	0.0008	0.1297

Table 2 elaborates the measurements of the outcome parameters and their comparative analysis.
Significant reduction in the L/M ratio at day 14 is noted in both Glutamine [p < 0.00001] and Probiotic [p < 0.00001] groups when compared to Control group; however, no significant difference noted when compared with each other[p-0.27].
Significant reduction in the duration of wound healing is noted in both Glutamine [p<0.00001]and Probiotic [p-0.0008] groups when compared to Control; however, no significant difference noted when compared with each other[p-0.12].
There was a significant reduction noted in the delta SOFA values of the glutamine group (p = 0.03486) as compared to the control group, whereas it was non-significant when probiotic group (p=0.11184) compared to the control group and when Glutamine group (0.6171) compared to the probiotic group indicating lesser risk of progressive organ dysfunction in patients after glutamine administration.
A significant reduction in number of positive wound cultures noted in Glutamine (0.0139) and Probiotic (0.0784) group when compared to Control group with better outcome in Glutamine group whereas no significant outcome noted between glutamine and probiotic groups (0.4902).
A significant reduction in number of positive wound cultures were noted in both Glutamine (0.0204) and Probiotic (0.047) groups each compared against control group with better outcome in Glutamine group.
The total number of days required for healing was significantly reduced with both the interventional groups when compared against control group with Glutamine (<0.0001) group has improved healing time than the probiotic group (0.0008).

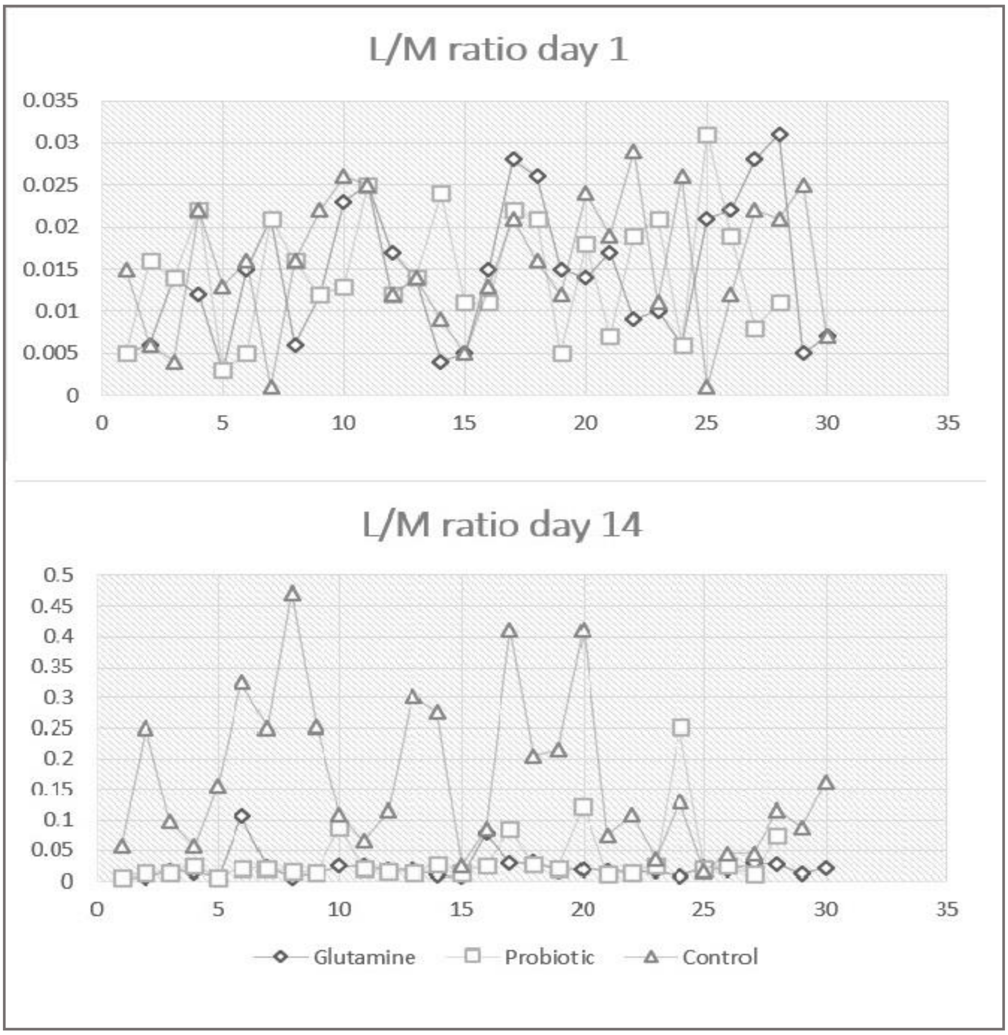

Image 1: Comparison of Lactulose/Mannitol ratios among control, glutamine, and probiotic groups at admission and at day 14.

At admission, baseline L/M ratios were noted to be similar among all 3 groups; at day 14, the L/M ratio in subjects of control group were raised significantly whereas those of Glutamine and Probiotic groups were comparable to baseline groups.

DISCUSSION:

Extensive burn injury results in increased infections associated with immunosuppression. Glutamine is a conditionally essential amino acid intimately related to the normal function of the immunologic system and intestinal tract. Plasma glutamine concentrations fall significantly after injury, and it has been suggested that this decrease may be related to the decreased host resistance to infection [8]. In addition, bowel permeability is increased in thermally injured patients [9,10] which may be causally related to the glutamine deficiency and the immunodeficient state [11,12] because glutamine is necessary to maintain normal cell turnover and gut integrity [13,14]

Glutamine participates in multiple vital pathways in the body. Not only is it crucial as a precursor of glutathione, the most important antioxidant in the system, but also helps maintain the immune system and cell turnover. Therefore, it is thought of to be beneficial in all conditions of severe stress, including major burns [15,16]. It is also protective towards damage due to inhalational injury and maintenance of gut mucosal barrier.

Glutamine can be supplemented by both enteral or parenteral routes, although the former is preferred. A major source for systemic sepsis in such conditions is the breakdown of the critical gut mucosal barrier, leading to bacterial translocation into the circulation,[17] and enteral preparations both reduce the incidence of ileus, as well as maximize delivery of glutamine to the gut mucosal cells. It has therefore been largely accepted that enteral glutamine preparations are preferable in the management of burns.

The intestinal barrier includes mechanical, immunological, biological, and chemical barriers;

Biological barrier- Lactobacillus and Bifidobacterium, which are the intestinal resident bacteria, mainly form the biological barrier. The resident bacteria form biofilm on the intestinal epithelial surfaces, resist the invasion of exogenous pathogenic bacteria, and provide the intestinal epithelial cells with nutrients by producing short-chain fatty acids, lactic acids, and others.

Therapeutic strategies for the burn-induced intestinal barrier dysfunction:

It is well recognized that the treatment of post-burn intestinal barrier dysfunction is an important part of the burn treatment and directly related to the level of comprehensive treatment of severe burns.

Many clinical and experimental studies in the past have suggested that taking some positive and reasonable measures such as supplementation of Glutamine and Probiotics along with established protocols like adequate fluid resuscitation and early enteral feeds is beneficial to the intestinal barrier in the early stage of severe burns [18].

Probiotics are defined according to the World Health Organization (WHO) as “live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host” [7]. Major strains of probiotics include Lactobacillus and Bifidobacterium species [1].

These bacteria can maintain gut equilibrium and prevent bacterial translocation by several mechanisms, including:

1. Maintenance of the gut barrier function;
2. Protection of the sites of bacterial invasion from colonization by pathogenic agents;
3. Competition with pathogenic micro-organisms for nutritional requirements;
4. Increase of intestinal acidity, motility and mucin;
5. Inhibition of the growth of pathogenic bacteria through production of organic acids and bacteriocin-like substances [19].

Lactic acid bacteria (LAB) were reported to have a direct stimulatory effect on the cells of the innate immune system that exert adjuvant activity at the intestinal mucosal surface and improve phagocytosis by increasing the proportion of natural killer (NK) cells, macrophages and lymphocytes [20].

The action of glutamine can broadly be thought of as anti-inflammatory, immune regulatory and anticatabolic in nature. We have used two major parameters, SOFA scores and Lactulose/Mannitol ratio as determinants of the same. SOFA score is widely applied to predict prognosis in sepsis and critical illnesses, and is similarly considered to have good applicability in predicting mortality and outcomes in burns and burn-related sepsis. It has also been regarded that delta SOFA (3-0), i.e., the difference in SOFA score at day 3 from the score at day 0, offers a better predictive value of outcomes.

One of the widely used methods to measure small bowel permeability is the L:M test, in which, after a period of fasting, subjects are asked to drink a solute containing the two sugars lactulose and mannitol. Urinary excretion of both lactulose and mannitol are then measured several hours after ingestion of solute, and the lactulose: mannitol ratio (LMR) is calculated as an indicator of permeability [21].

The L:M test is useful as both sugars are passively absorbed from the intestine, not extensively metabolised, and excreted unchanged in urine in proportion to the quantities absorbed [21]. The smaller sugar alcohol molecule (mannitol) is assumed to permeate transcellularly through the water pores of the membrane, whereas the larger disaccharide molecule (lactulose) is assumed to permeate paracellularly through the tight junctions [22]. In states of increased gut permeability, lactulose would traverse through the paracellular spaces, cleared by glomerular filtration, not undergo selective reabsorption, and present itself in higher levels in urine, thus leading to an increased LMR.

The L:M test is thought to be a good representative of gut permeability as measurements using a single molecule do not account for confounding factors such as intestinal transit time, gastric emptying rate,

renal/ hepatic function, or total urinary excretion [23]. By taking the ratio of excretion of two molecules, the effects of these confounding factors can be eliminated[24].
Intestinal permeability- ratio between urinary lactulose and mannitol-was reduced in the Glutamine and probiotic group, whereas permeability in all patients of the control group increased. As derived from the results of our study, glutamine appears to confer significant protective effect against hemodynamic instability, vital organ dysfunction, immune dysregulation, and catabolism. We also noted significant reduction in positive wound and blood cultures with both groups. However, the finding might be of value in the context of long-term inflammatory sequalae in survivors, and further follow-up and analysis is required to ascertain the same and the power of study is limited by subject size and larger RCT's are required to establish the results.

CONCLUSION:
Prophylactic administration of glutamine or probiotic appears to preserve gut function and improve wound healing time and overall outcome, with the former showing higher benefit as compared to the latter.

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