

**EFFECTS OF ENTERAL GLUTAMINE SUPPLEMENTATION ON  
REDUCTION OF INFECTION AND MORTALITY IN ADULT PATIENTS  
WITH SEVERE THERMAL BURNS IN KENYATTA NATIONAL  
HOSPITAL: A double blind randomized clinical trial.**

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**DECLARATION**

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**DEDICATION**

This work is dedicated to:

- My parents and siblings who have been supportive and source of encouragement throughout my career development.
- My best friend Marylyn for her love, unconditional support and understanding.

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### Abbreviations (in alphabetical order)

ABSI .....	Abbreviated Burns Severity Index
ABA.....	American Burns Association
CFU.....	Colony Forming Units
DM.....	Diabetes Mellitus
CRF.....	Chronic Renal Failure
ETI.....	Endotracheal Intubation
GIT.....	Gastrointestinal Tract
IL-2 .....	Interleukin 2
INF-Y.....	Gamma Interferon
KNH.....	Kenyatta National Hospital
MODS.....	Multiple Organ Dysfunction Syndrome
MOF.....	Multiple Organ Failure
NGT.....	Nasogastric tube
STSG.....	Split Thickness Skin Graft
SIDS.....	Systemic Inflammatory Dysfunction Syndrome

TBC ..... Total Blood Count

VAP..... Ventilator Associated Pneumonia

## ABSTRACT

**Background:** Post burn infection is a major cause of morbidity and mortality in patients with severe burns in developing countries, Kenya included. Glutamine is a non essential amino acid which has been found to reduce the incidence of burn wound infection and mortality in patients with severe burns. There is no published local prospective data on the positive effects of glutamine supplementation on reduction of postburn infection and mortality in severely burned patients.

**Objective:** This study sought to determine the effect of enteral glutamine in reducing the incidence of postburn infections and mortality in patients with severe burns.

**Study design:** A double blind randomized clinical trial.

**Setting:** Burns unit and ward 4D of Kenyatta National Hospital, Kenya

**Methods:** This was a double blind randomized clinical trial running for a period of 6 months from September 2010 to February 2011, involving 60 patients with severe burns who were randomized to 2 arms of treatment: (1) the glutamine group arm and the (2) isonitrogenous arm acting as the control. The patients randomized to the intervention group received glutamine in



form of B-immune. 5 g of glutamine was provided every 8 hours to make a total of 15 g every day. Nasogastric tube feedings or patient-assisted feedings of a mixture of B-immune and milk were initiated on day 1 postburns and continued for a period of 28 days. Patients in the control arm received Fresubin Original, which is isonitrogenous to B-immune. Samples for blood cultures and wound surface swab cultures were taken aseptically from each recruited patient on week 1, 2, 3 and 4 respectively. Specimen analysis involved gram staining, culture and biochemical tests in accordance to the standard microbiological procedures.

In this study, a postburn infection was defined as a positive culture obtained on pus swab sample or blood culture.

**Results:** Patients' demographic and baseline clinical characteristics were similar in both arms of treatment. For the entire 4-week treatment period, the odds ratio of a positive blood culture was almost three-fold higher (2.7 (1.3-5.6)) among patients in the control group compared to those in the Glutamine group ( $p=0.04$ ). In this particular study, glutamine reduced the incidence of

*Pseudomonas spp* by 3.3-4.5 times. There were no significant differences in the incidence of *Staphylococcus aureus* in the two groups for the entire treatment period at  $p<0.05$ . The odds ratio of death was approximately 6-fold higher in the control group than in the glutamine group. Patients receiving glutamine had a statistically significant shorter length of stay compared to those in the control group ( $p=0.001$ ).

**Conclusions:** Enteral glutamine supplementation in severely burned adult patients reduces blood infection by a factor of three, prevents bacteremia with *Pseudomonas aeruginosa*, decreases mortality rate and shortens duration of hospital stay.

## **INTRODUCTION**

Incidence of burns-related infection has remained high despite different preventive modalities currently in use. These modalities include among others; topical antibacterial agents, early excision of eschar, and timely closure of burn wound and all have been demonstrated to significantly reduce the occurrence of invasive burn wound infection and its related mortality <sup>1</sup>. However, severe thermal burns has an intrinsic systemic immunosuppressive effect and this could possibly explain the high incidence of burns related infection and mortality despite the aforementioned preventive modalities in use <sup>1</sup>. In this regard, the concept of immunomodulation

(in the prevention and management of burns related infections) has been introduced. This is exemplified by glutamine supplementation.

The incidence of burns-related infections is similar to that of trauma patients, with 50% being due to primary endogenous infections. The isolated pathogens are predominantly gram positive cocci <sup>2</sup>. In a retrospective study done in a Teaching Hospital in Syria by Dayoub A <sup>3</sup> and colleagues, 71 % of hospitalized burn patients' deaths were due to sepsis, septicaemia and complications of septicaemia. Incidence of burns-related infections and mortality rates among patients with moderate and severe burns in Kenyatta National hospital (KNH) is 13.3% as reported by Nthumba <sup>4</sup> in a prospective analytical study of 2003.

Enteral glutamine has been demonstrated to be effective in decreasing infections in several clinical settings. For instance, in a prospective Randomized Controlled Trial done by Ziegler TR, et al <sup>40</sup>, infection rate in Bone-marrow transplant patients who received glutamine supplemented nutrition was 12% as compared to 43% in non-supplemented group.

Similarly, infection rate was 3 times lower in glutamine group as opposed to control group in patients with multiple trauma <sup>5</sup>.

Many of the clinical trials on enteral glutamine supplementation have focused on critically ill patients (with mixed diagnoses) such as trauma patients, oncological and haematological patients demonstrating positive effects on reduction of infection rates. However, there is limited published data focused on effect of enteral glutamine on infection rate in patients with severe

burns. Results from recently published randomized clinical trials on effects of enteral glutamine, show a trend of an overall reduction in incidence of bacteremia, lower antibiotic usage and lower mortality rates in patients with severe burns <sup>6, 7, 8</sup>. These results are particularly from developed countries. Based on these studies majority of burn centres in Europe and United States of America routinely put their patients with severe burns on glutamine-rich diet. In our local setting, the supply of glutamine is erratic and is only given to critically ill patients but not to severely burned patients. There was therefore a need for a local study that illustrates the efficacy of enteral glutamine on reduction of infectious morbidity and mortality in patients with severe burns to justify routine supplementation of the same to this subset of patient population.

## **DEFINATION OF TERMS**

### **1) Severe burns (According to American Burns Association)**

Severe burn injury is defined as partial-thickness burns involving more than 25% of TBSA in adults or

20% of TBSA in children younger than 10 years or adults older than 50 years or

Full-thickness burns involving more than 10% of TBSA or

Burns in special areas such the face, eyes, ears or perineum that may result in functional or cosmetic impairment or

Burns complicated by inhalation injury or major trauma or

In the context of this study, severe burns is defined as partial thickness burns >25% but <60%. This is because previous studies in KNH have demonstrated better survival of patients with burns of TBSA <60%.

## **2) Burns related infections**

In the context of this study, postburn infection is defined as a positive growth of microbes on pus swab and blood culture.

# **LITERATURE REVIEW**

## **1. Epidemiology of Burn Wound Infections**

Burn wound infections are one of the most important and potentially life-threatening complications that occur in the acute period following injury <sup>9,11</sup>.

Thermal burns have an intrinsic systemic immunosuppressive effect and as subsequent manifestation of this, infection still remains a common cause of morbidity and mortality despite medical advances in various preventive modalities used to control post burn infections <sup>1</sup>. Broadly, the incidence of burn wound infection range from 40-50% as reported by Lorante <sup>2</sup>. Locally, the incidence of burn wound infections in patients specifically with moderate to severe burns as reported by Nthumba <sup>4</sup> was 13.3%.

The important risk factors that influence morbidity and mortality in patients with burns include:

(i) Patient demographics

Very young children and the elderly have an increased risk of being burned and have worse clinical outcomes than patients in other age groups <sup>11</sup>.

(ii) Self-inflicted burns

Individuals with deliberate self-inflicted burn injuries and the disabled have been shown to have more severe injuries and longer hospital stays than those with accidental injuries <sup>9</sup>.

(iii) Medical comorbidity

Patients with medical comorbidities such as Diabetes Mellitus, renal failure, HIV/AIDS have been shown to have higher morbidity and mortality <sup>10</sup>.

(iv) Impact of changes in Burn wound care

A steady decline in burn wound infection and subsequent tissue invasion and sepsis and associated mortality has been realized in the last 50 years. This is attributed to the substantial advances in burn wound care, particularly early eschar excision and Split Thickness Skin Graft (STSG) <sup>12, 13, 14</sup>. In one burn centre in 1978, there was a substantial reduction in the incidence of both burn wound infection and sepsis after the advent of early excision therapy. During this study period, the incidence of burn wound sepsis fell from 6% to 1% and the mortality rate for burn-related complications decreased from 40% to 18% <sup>15</sup>. Adan et al reproduced the study locally and reported similar outcomes <sup>21</sup>.

(v) Burn depth and Total Burns Surface Area (TBSA)

A descriptive prospective study done by Lorente and colleagues showed a direct correlation of burn depth and the risk of wound infection. In this study, infection occurred in 20% of cases with superficial and 2<sup>nd</sup> degree burns, whereas in deep 2<sup>nd</sup> and 3<sup>rd</sup> degree burns, the incidence of infection increased to 97% <sup>2</sup>.

In the same study, a direct correlation was also noted between TBSA and the incidence of burn wound infection <sup>2</sup>.

Abbreviated Burns Severity Index (ABSI) is a validated scoring system used worldwide in predicting clinical outcome and survival rate among patients with moderate and severe burns. It has 5 variables (age, sex, presence/absence of inhalation injury, burn depth, TBSA) with a minimum aggregate of 5 and maximum of 18 (see appendix 111). Randomisation based on ABSI gives reproducible results as the above confounding variables are controlled and in effect selection bias is minimized.

## **2. Microbiological analysis of burn wound infections**

Diagnosis of burn wound infection based on clinical signs and symptoms alone is difficult. Quantitative culture of tissue biopsy samples and histological verification of microbial invasion into viable unburned tissue has been the 'gold standard' method of confirming the presence of invasive burn wound infection particularly in unexcised areas of eschar. More recently, however, the value of this labour-intensive and costly quantitative burn wound tissue biopsy cultures has been challenged <sup>16</sup>. Many burn centres have adopted the more convenient practice of wound surface swabs for both qualitative and semi qualitative culture for infection surveillance since the advent of early eschar excision therapy. Steer and coworkers <sup>17</sup> have reported in the largest recent studies that compared the results of surface swabs versus biopsy cultures. In their study there was a significant positive correlation between the bacterial counts obtained by biopsy and pus swabs. Levine et al <sup>18</sup> additionally noted a linear numerical relationship between quantitative surface swab and biopsy sample counts of viable bacteria from burn wounds; counts of  $10^5$  bacteria per gram of biopsy sample were equated with counts of  $10^6$  bacteria obtained from surface swab samples. Infection surveillance using wound surface swabs is particularly important in resource-limited burn centres in developing countries like Kenya.

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Bharndiwas et al <sup>19</sup> also assessed the value of blood cultures in diagnosis of burn wound sepsis compared to burn wound cultures by either surface swab or tissue biopsy. Fifty patients with burns ranging from 30-50% TBSA were monitored for clinical signs of sepsis and only 62.5%



had positive burn wound cultures from surface swabs, compared to 87.5% who had significant bacterial count on biopsy cultures. Blood cultures were found to be only of prognostic value in this study <sup>20</sup>.

### **3 Prevention of burn wound infections**

#### **3.1 Topical antimicrobial agents**

Several studies have demonstrated the role of topical antimicrobials in decreasing infectious morbidity and mortality in patients with major burn injuries particularly before the era of early eschar excision and immediate STSG <sup>20</sup>.

#### **3.2 Early Eschar excision and grafting**

At one burn centre, it was noted that after 1973, when early excision and grafting were instituted, the incidence of documented systematic sepsis originating from burn wound decreased from 6% to 1%. During the same period, the author noted that the rate of death due to the wound sepsis decreased from 40% to 18% of all patient deaths <sup>15</sup>.

Locally, Adan in a prospective study demonstrated similar trend of reduced infection following early eschar excision and timely wound cover <sup>21</sup>. In this study, the incidence of infected skin graft in patients who underwent early excision and immediate STSG was 8% while the group who had delayed skin graft, the incidence was 14%.

#### **3.3 Early enteral feeding**

Recent studies have shown that early enteral feeding in combination with early excision of burn wound in severely burned children improved their clinical recovery and outcome. For instance,

Hart <sup>62</sup> found that early enteral feeding diminished the incidence of wound colonization and infection by bowel flora.

In a double Randomized Clinical Trial (RCT), Garrel <sup>7</sup> randomized 45 patients with severe burns into 2 treatment groups: One group received enteral glutamine and the other received conventional isonitrogenous mixture. Positive blood cultures were three times more frequent in the controls than in patients who received glutamine treatment. In the same study, *pseudomonas aeruginosa* was detected in 6 patients in the control group versus zero patients in the glutamine group.

Similarly, recently published data from a trial by Dechellote et al <sup>8</sup>, involving 114 patients with severe burns showed that enteral glutamine supplementation led to a statistically significant decrease in infectious comp

## **4 GLUTAMINE**

### **4.1 Physiology**

Knowledge on physiological and metabolic functions as well as the effect of glutamine on immune cells and the integrity of the gut mucosa, in the setting of both healthy and catabolic state is important in understanding the beneficial effects of glutamine supplementation.

Glutamine is the most abundant amino acid found in plasma and cells. The primary site of synthesis and storage is in the skeletal muscles. Glutamine is involved in gluconeogenesis, acid base system (as a substrate for renal synthesis of ammonia), and synthesis of other

amino acids. Glutamine is also a preferred oxidative fuel for rapidly dividing cells such as lymphocytes and mucosal cells in the Gastrointestinal Tract (GIT) <sup>22</sup>.

Under normal physiological conditions, glutamine is synthesized in large amounts by the human body and is therefore considered a non-essential amino acid. It has been hypothesized that glutamine may become a conditionally essential amino acid in patients with catabolic disease such as severe burns <sup>23</sup>. Several studies have shown that glutamine levels drop following extreme physical exercise <sup>26</sup> and stressful situations such as multiple trauma, major surgery <sup>(24,25)</sup> and critical illness like severe burns <sup>26</sup>. Low levels of glutamine have been associated with immune dysfunction and higher mortality in critically ill-patients<sup>(27,28)</sup>.

Tremel et al <sup>29</sup> reported that glutamine supplementation maintains gastrointestinal structure and is associated with decreased intestinal permeability as opposed to a standard Total Parenteral Nutrition which is glutamine free. They therefore suggested that translocation of gram – negative bacteria from the gut to burns wounds is diminished when glutamine is supplemented. Glutamine - supplemented formulas have resulted in greater preservation of skeletal muscles, improved nitrogen balance and immune cells function as reported by Tremel. During critical illness, a state of oxidative stress occurs due to an imbalance because there is increased production of reactive oxygen species and depletion of natural anti-oxidants. Glutamine is a precursor to glutathione (an important antioxidant). In light of this, it has been demonstrated that glutamine supplementation results in higher levels of glutathione and anti oxidative capacity <sup>30</sup>.

In the presence of glutathione peroxidase, glutathione is converted to its dimeric form and in effect, the oxygen radical is reduced to harmless water as shown below.



#### **4.2 Glutamine and functionality of immune system**

Macrophages, lymphocytes and neutrophils play important roles in immune responses to burns-related infections. Glutamine provides nitrogen for the synthesis of purine and pyrimidine nucleotides, which are necessary for the synthesis of new Deoxyribonucleic Acid (DNA) and Ribonucleic Acid (RNA) during proliferation, as well as for mRNA synthesis and DNA repair in macrophages <sup>31,32</sup>. Glutamine is an important source of energy for these white blood cells .

A recent study by Pithon-curi et al looked at the protective effect of glutamine supplementation against spontaneous apoptosis of human neutrophils <sup>33</sup>. The authors hypothesized that glutamine would provide protection against apoptosis because apoptotic neutrophils demand increased levels of glutamine. The results showed that glutamine delayed the process of apoptosis in human neutrophils. The authors speculated that the effect of glutamine in delaying apoptosis in neutrophils may be mediated by the antioxidant effect of glutathione.

Major thermal burns induce a state of immunosuppression that predisposes patients to infectious complications. This observation has been supported by the findings of

prolonged allograft survival energy and increased susceptibility to infection in severely burned patients.

The exact sequence of events that result in immunosuppression after burns injury remains unknown. However, biochemical changes that may affect immune system include those of the endocrine system, the arachidonic acid cascade, and the cytosine network. Following severe burn injury, there is an increase in the levels of catabolic hormones such as cortisol, glucagon and catecholamine<sup>34</sup>. Elevated levels of glucocorticoids observed in severe burns, inhibit production of Interferon- $\gamma$  (IFN- $\gamma$ ) and Interleukin-2 (IL-2)<sup>34</sup>.

Despite improvement in the early care of burns patients, Systemic Inflammatory Response Syndrome (SIRS), severe sepsis and consequent Multiple Organ Dysfunction Syndrome (MODS) remain major causes of morbidity and mortality in this subset of patient population. As a result, further efforts in the development and clinical trials of immune modulators such as glutamine may hold promise in reducing infectious morbidity and mortality in patients with severe burns.

#### **4.3 Glutamine and integrity of the gut structure**

Before a pathogen can establish an invasive infection within the host, it must break through the natural barriers of the skin or the mucosa. In severely burned patient, it has been shown that, the barrier function of the GIT is impaired<sup>34</sup>. This phenomena is thought to result from the following mechanisms.

1. Up-regulations of nitric oxide synthetase gene and consequent overproduction of nitric oxide<sup>36</sup>.

2. In postoperative patients, there is decreased intestinal motility and mucus secretion.

Increased exposure to endotoxin <sup>37</sup>.

3. Development of MODS in critically ill – patients has also been associated with derangement of their intestinal permeability <sup>38</sup>.

This loss of barrier function of GIT plays an important role in the translocation of gram – negative bacteria and endotoxin across the gut wall; subsequently resulting in sepsis <sup>40</sup>, prolonged systemic inflammatory response and eventually Multiple Organ Failure (MOF); a phenomena observed in patient with severe burns <sup>35</sup>.

Glutamine is utilized as a major fuel and nucleotide substrate by intestinal mucosal cells and the gut associated immune system <sup>39</sup> and so may prevent atrophy of the intestinal tract mucosa. Glutamine is therefore thought to have a positive effect on gut barrier function. Clark et al <sup>41</sup> recently tested in- vitro the hypothesis that glutamine availability plays a key role in modulation of bacteria translocation across the gut wall. In this study, Clark observed that glutamine prevents transcellular bacterial translocation under in-vitro stimulated stressful circumstances. Several studies show a role of glutamine in preventing bacterial translocation along the paracellular channels as well. For instance, Kouznetsova et al <sup>42</sup> showed in an in-vitro model that glutamine closes the paracellular channels. This effect was only observed when glutamine was supplied on the apical side of the cells rather the basolateral side. This may suggest that glutamine might be more effective when delivered by the enteral route rather than the parental route.

This is supported by two separate studies by Alverdy <sup>43</sup> and Panigrafin <sup>44</sup> who observed that glutamine provided by enteral route is superior to parenteral glutamine supplementation on reducing infectious morbidity and mortality in patients with severe burns.

#### **4.4 Glutamine and ischemia/reperfusion injury**

Gut ischemia / reperfusion secondary to splanchnic hypoperfusion is a common event after a variety of clinical conditions, e.g. hypovolemic shock in multiple trauma or severe burns. Anecdotal evidence shows that intestinal ischemia /reperfusion injury results in formation of reactive oxygen radicals <sup>30</sup>. In light of this, several studies have looked at consequences of ischemia/reperfusion injury such as loss of gut mucosal barrier, translocation of bacteria from the gut and prevention of the same with enteral glutamine supplementation <sup>52</sup>. Beneficial effects of glutamine in an event of ischemia/reperfusion injury has been observed in patients undergoing cardiopulmonary bypass during open heart surgery as documented by Hayashi et al <sup>59</sup>.

## **5 EFFECTS OF GLUTAMINE IN THE CATABOLIC STATE**

### **5.1 Metabolic changes after major injury.**

Practical background knowledge on the pathophysiology and metabolic changes following major injury is essential in recognizing the need of starting early nutritional support. Major injuries such as severe burns induce metabolic changes which contribute to

systemic immune suppression in severely injured patients and increase the risk of infection and postinjury organ trauma <sup>45,46</sup>. Following major injury, metabolic changes that occur are characterized by hypermetabolism with increased energy expenditure, enhanced protein catabolism, insulin resistance associated with hyperglycemia and high insulin levels; the so called 'traumatic diabetes' <sup>45,46</sup>. The alteration of physiological pathway leads to development of hyperglycemia and metabolic acidosis <sup>47</sup>. The physical and psychological stimulation of neuroendocrine axis through fear, stress, pain, inflammation and shock increases the caloric turnover significantly above the baseline situation in healthy individuals and severely injured subjects <sup>47</sup>. This leads to increased level of catabolic hormones such as cortisol, glucagon and catecholamines and decreased levels of insulin resulting into the posttraumatic catabolic diabetic state <sup>47</sup>.

## **5.2 Metabolic control and immunonutrition**

As a consequent of hypermetabolism in severely traumatized patient, complication such as hyperglycemia, hypoproteinemia, lactate acidosis and immunosuppression set in <sup>45,47</sup>. Therefore the presence and clinical implication of these metabolic changes must be recognized early with the aim of instituting optimal therapeutic regimes to reduce morbidity and mortality. This posttraumatic catabolic state requires an adjusted energy balance with early protein substitution and hypercaloric nutrition <sup>45</sup>. Early enteral nutrition has been advocated as the concept of choice for nutrition of polytraumatized and severely ill patients. In this regard, prospective controlled trials have demonstrated the positive effect



of an early full enteral nutrition and these benefits include a decreased posttraumatic infection rate, a shorter duration of hospital stay and overall improved clinical outcome <sup>60</sup>. The concept of “immunonutrition” was coined in the recent years and is exemplified by the enteral supplementation of glutamine, as one of the most promising nutrient supplements that influence clinical outcomes in severely injured patients <sup>48</sup>. Glutamine is an important amino acid which exerts its metabolic benefits beyond its nutritional value by mediating immunological effects such as induction of neutrophil phagocytic activity and oxidative outburst. Glutamine has also been shown to protect neutrophils from undergoing apoptosis in vivo <sup>33</sup>. In addition, glutamine is a precursor to the reducing agent glutathione and thus contributes to antioxidant effects and cellular protection from ischemia/reperfusion mediated injury <sup>30,59</sup>.

Over the past decade, it has been established through clinical trials that specific nutritional supplements influence patients response to metabolic stress. The primary goal of these supplementation is directed to immunomodulation and enhancement of immune system, enforcement of the gut barrier as well as protection of body cells from oxidative stress. Many studies have centered on the provision of amino acid glutamine in achieving these goals in various patient population groups including critically-ill patients, polytrauma, haematological, oncological patients as well as patients with severe burns.

Numerous studies (as reported by Novak et al) <sup>50</sup> in their systematic review of aggregated randomized trials, have demonstrated that intravenous glutamine increases nitrogen balance, reduces the rate of infectious complications, shortens hospital stay reduces hospital costs

and improves survival of critically ill-patients including thermally injured patients. Prospective randomized single- blinded multicentre trial by Conejero et al <sup>51</sup>, looked at the effect of glutamine - enriched enteral diet compared to a control group diet without glutamine on intestinal permeability, infectious complications and mortality in 84 critically- ill patients with Systemic Inflammatory Response Dysfunction Syndrome (SIRS). The control group received 66.6 g per day of protein and glutamine group received 52.5g of protein per day, 14g of which was glutamine. In the glutamine group the rate of pneumonia was reduced as opposed to the control group. Consistent with this study, Houdijk et al <sup>5</sup> in a similar CRT observed that there was a significant preponderance of gram – negative bacteria in the control group as compared to the Glutamine group.

Infections are an important cause of morbidity and mortality in patients with multiple trauma. Houdijk et al in a RCT looked at the effect of Glutamine- supplemented enteral nutrition on severely injured patients <sup>5</sup>. In their study, patients with multiple trauma, and who had an injury severity score of 20 or more and APACHE 2 were randomly allocated to either glutamine supplemented enteral nutrition or glutamine – free diet. Both treatment groups received a balanced isonitrogenous isocaloric enteral feeding. The groups had similar demographic characteristics, injury severity scores, APACHE scores, types of injury and the type of operation. Furthermore there were no differences between the 2 groups in terms of amount of daily caloric intake and the number of days in enteral nutrition. In their study, houdijk et al found that glutamine enriched nutrition reduced the number of pneumonia,

bacteremia and septic events in severely injured patients to a level of clinical significance ( $p < 0.05$ ).

Many of the clinical trials on enteral glutamine supplementation have focused on critically ill patients (with mixed diagnoses) such as trauma patients, oncological and haematological patients demonstrating positive effects on reduction of infection rates. However, there is limited published data focused on effect of enteral glutamine on infectious morbidity in patients with severe burns. In a (RCT) study by Wishmeyer et al <sup>6</sup>, there was a significant reduction in the incidence of gram negative infection in the glutamine group compared to control group: 8% vs. 43% respectively. Garrel et al <sup>7</sup> recently studied the effect of enteral glutamine supplementation on patients with severe burns. He randomized the patients to two treatment groups; one receiving enteral glutamine-supplemented formulation and the other group received isonitrogenous isocaloric formulation without glutamine. They found that enteral glutamine supplementation reduced blood infection by a factor of three, prevented bacteremia with *Pseudomonas aeruginosa* and shortened length of hospital stay. However this study did not address the issue of mortality. Similarly, results from a clinical trial by Dechellote and colleagues, involving 114 patients with severe burns showed that enteral glutamine supplementation led to a statistically significant decrease in infectious complications in the glutamine treatment group as opposed to control group <sup>8</sup>. In a RCT study done in India by Vishwanath, treatment effect of enteral glutamine supplementation reached clinically significant levels <sup>61</sup>.

Another controlled, double-blinded randomized trial by Zhoe et al <sup>52</sup> focused on the effect of enteral glutamine on gut permeability in 40 patients recovering from severe burns injury. Glutamine was given as dipeptide of alanyl -Glutamine in a dose of 0.35g/kg/day. Nasogastric tube (NGT) feeding was initiated on postburn day I and the control group received isocaloric and isonitrogenous enteral feed without glutamine. The authors observed that glutamine reduced gut permeability and decreased plasma endotoxin concentration. These alterations were associated with less infection morbidity, shorter hospital stay and lower costs.

There are no local studies available that evaluates possible positive beneficial effects of enteral glutamine on infectious morbidity in patients with severe burns.

### **5.3 Enteral versus parental nutrition**

Total parental nutrition (TPN) was popular in the 1970s and 1980s when it was used indiscriminately to counteract the metabolic problem associated with critical illness <sup>46</sup>.

Enteral nutrition, on the other hand, has been advocated as a means of reducing mucosal atrophy and intestinal permeability with subsequent reduction of the incidence of bacterial translocation from the gut, and the consequent septic complications. In addition, enteral nutrition in general is popular because it is cheaper, more physiological and safer. Kudsk et al also reported a significant low incidence of septic morbidity in enterally-fed critically-ill patients as opposed to a similar cohort who were provided with TPN <sup>53</sup>.

However there is paucity of data and studies comparing enteral vs parenteral provision of glutamine particularly in patients with severe burns. The limited data available yielded

discordant results. Alveardy et al observed that glutamine supplemented diet is superior to parenterally provided glutamine on improving clinical outcomes such as reduction of infectious morbidity and mortality <sup>43</sup>. Griffith et al on the other hand reported that parenteral glutamine was superior to enteral glutamine in critically ill patients in terms of reducing infection rates <sup>54</sup>.

#### **5.4 Duration of glutamine supplementation to observe effect.**

Optimum duration of glutamine supplementation in critical illness is not yet defined. No enhanced protein synthesis is observed by use of short – term (48 hrs) enteral glutamine supplementation in burn patients. Plasma glutamine levels start to rise in critically ill patients from day 3 of administration of enteral glutamine. Clinical benefits such as reduction of infections rates and shortened duration of hospital stay together with cost savings is observed in patients who have received at least 5 days of glutamine administration. This 5 days- minimum of glutamine administration is supported by evidence in the RCT of Jones et al <sup>55</sup> and systematic review by Garcia <sup>56</sup>. No published trials have fully investigated the use of glutamine therapy in burns beyond 4 weeks. There may be no added benefit to routinely continue with glutamine supplementation after this period in burns as the inflammatory response returns to near baseline by then <sup>57</sup>.

#### **5.5 Optimum dose of glutamine.**

The optimum dose of glutamine remains undefined – however dose requirements may differ depending on the disease. In the overall, clinically important difference appear to commence at doses greater than 0.2 g/ kg/ day<sup>54</sup>. Most trials have used 15 – 20 g/d of glutamine.

## **5.6 Adverse effects of Glutamine.**

Metabolic safety of glutamine in terms of dose and duration have been studied in both healthy and catabolic subjects and no clinical evidence of toxicity nor generation of potentially neurotoxic metabolites of glutamine has been reported<sup>58</sup>.

## **STUDY JUSTIFICATION**

Unlike in developed countries where majority of deaths in patients with severe burns are attributed to inhalation injury, postburn septic complications contributes to a significantly high proportion of morbidity and deaths in KNH Burns Unit. Postburn sepsis is therefore a public health problem that can be prevented or minimized with early introduction of enteral glutamine supplementation. Previous clinical studies in severely ill-patients and polytraumatised patients suggest a positive effect of enteral glutamine on reduction of infectious morbidity but few clinical trials have focused on possible clinical benefits in severely burned patients. The limited data available on the beneficial effects of reduced burn wound infection by glutamine supplementation are results from clinical trials done in western countries whose population characteristics is different from the local population. In KNH Burns unit and ward 4D, patients are many and majority fail to get burn wound infection preventive modalities such as early

excision with STSG done on time. By the time escharectomy and STSG is done, invasive infection on the burn wound has already set in. There is therefore a need to introduce other adjunct preventive therapies like glutamine supplementation to reduce incidence of postburn infections. In our local setting glutamine is given only to critically ill-patients but not to severely burned patients. Therefore there was a need for local study that illustrates the efficacy of enteral glutamine supplementation on reduction of infectious morbidity and mortality in patients with severe burns to justify routine supplementation of the same to this particular subset of patient population.

Management of established burns-related infections poses a difficult challenge considering the resistance patterns of microbes to antibiotics and furthermore use of antibiotics is costly. On the other hand, combining preventive modalities geared to tackling burns-related infections such as early eschar excision and timely wound closure together with immunomodulation in form of early introduction of enteral glutamine is a cheap and convenient method particularly in resource-limited burns centre like KNH.

The purpose of this study was to determine the effect of enteral glutamine supplementation vs. isonitrogenous glutamine-free dietary regime (control) on the incidence of wound infection and mortality in adult patients with severe burns. Results obtained from this study will be used in formulating and improve on the management protocol for patients with severe burns geared to minimizing infectious morbidity and mortality.

Information derived from this study may also form a solid basis for other related studies in future.

## **HYPOTHESIS**

Null hypothesis

There is no difference in the incidence of postburn infection and mortality in patients receiving glutamine rich diet and those in the control group.

## **OBJECTIVES**

**Primary objectives**



To determine the role of enteral glutamine in the reduction of infections and mortality in patients with severe burns.

### **Secondary objectives**

1. To determine the effect of glutamine supplementation on the incidence of post burn infections in patients with severe burns.
2. To compare the mortality trends in patients with severe burns receiving glutamine enriched nutrition vs those receiving nutrition formulation without glutamine
3. To determine the relationship of glutamine enriched nutrition and duration of hospital stay.

## **METHODOLOGY**

### **STUDY POPULATION**

The study population was patients with severe burns admitted in the Burns Unit or Ward 4D of the Kenyatta National Hospital.

## STUDY DESIGN AND BLINDING

This was a double blinded randomized clinical trial running for a period of 6 months from September 2010 to February 2011. There were 2 arms of treatment in the study: the glutamine (1) arm and the isonitrogenous (conventional) (2) arm acting as control. Randomization was concealed so that the principal study investigator and the recruited patients were not aware in advance to which group the patients would be assigned. The research assistant in charge of patient allocation was reached by phone when a patient was admitted.

Informed consent was obtained from all the patients.

## SAMPLE SIZE

Sample size calculation was done to detect a reduction of 35% of infectious morbidity and mortality of patients receiving glutamine compared to the control group with alpha of 0.05 and power of 90%. Using the formula highlighted below, we got a sample size of 30 patients for each treatment arm and a total of 60 patients for the whole study.

$$n = \frac{(Z_{1-\alpha/2} + Z_{1-\beta})^2 \sigma^2}{\delta^2}$$

where  $\alpha = 0.05$  (i.e.  $Z_{1-\alpha/2}$  is 1.960)

$1 - \beta$  is the power of 90% (i.e.  $Z_{1-\beta}$  is 1.282)

$\sigma$  is standard deviation (estimated as 3.4\_ derived from previous similar study)<sup>61</sup>

$\delta$  is the desired reduction of burns related infection and mortality rate in the glutamine group (by a factor of 2).

$$\text{Thus } n = \frac{(1.960 + 1.282)^2 (3.4)^2}{\delta^2}$$

$$2^2$$

= 30 patients in each treatment group and 60 patients for the whole study

### **Variables evaluated**

1. Independent variables- patient demographic data
2. Dependent variables
  - Number of positive blood cultures in each treatment group
  - Number of positive burn wound cultures in each treatment group
  - Average duration of hospital stay in each treatment group
  - Number of deaths in each treatment group

### **INCLUSION CRITERIA**

The study included:-

- 1 Adult patients with TBSA >25% but <60%
- 2 Duration of burns less than 48 hrs at 1st contact.
- 3 Patients willing to consent to participate in the study
- 4 Patients with second degree deep burns and beyond

### **EXCLUSION CRITERIA**

The study excluded any patient:-

- 1 who decline or were severely ill to give consent to participate in the study
1. with comorbid medical conditions e.g. Diabetes Mellitus Chronic Renal Failure or on immunosuppressive therapy such as chemotherapy or on steroids.
2. with inhalation injury because of 2 reasons:
  - Several studies have shown such patients have a high mortality
  - Such patients may require prolonged intubation and may develop Ventilation Associated Pneumonia (VAP)-a confounding factor
- 4 with severe burns and are on TPN
- 5 Admissions or referral with burns injuries older than 48 hours duration

## **RECRUITMENT AND SAMPLING METHOD**

All eligible patients were recruited into the study. At first contact, patients were subjected to 2 levels of randomization.

Step 1: stratified randomization on the basis of Abbreviated Burns score Index (ABSI). ABSI is a validated scoring system used worldwide in predicting clinical outcome and survival rate among patients with moderate and severe burns. It has a minimum aggregate of 5 and maximum of 18 (see appendix 111). The 4 strata for each treatment group were:

5-8 Total Burn Score (TBS)

9-12 Total Burn Score

13-15 Total Burn Score

16-18 Total Burn Score

Step 2: Within each stratum patients were then randomly assigned to either glutamine treatment group (1) or the conventional isonitrogenous/control group (2). This was done randomly by assigning the patients with numbers from the table of random numbers generated using Microsoft Excel software.

Burns-related infection risk factors were randomized to achieve a balance of risk factors (associated with postburn morbidity and mortality) between the two groups. The group assignment was not known to both patients and the principal investigator, but known by the research assistant who did not take part in the final data collection and analysis.

The recruitment period ran for a period of 6 months. Each patient was followed up for 4 weeks

## **PATIENT CARE**

Initial patient management including fluid resuscitation and stabilization was carried out irrespective to treatment group the patient was allocated. Parkland formula was used for fluid resuscitation with Ringer's lactate solution and targeted to achieve 40 mL/hr urinary output. Topical dressing was done routinely whereas early excision and grafting were performed within 7 days postadmission whenever it was possible. Pain was managed with morphine intravenously: Continuous infusion was accompanied by bolus administration during the therapeutic procedures such as wound dressing. Energy requirements were calculated using the Curreri formula.

## **MATERIALS AND METHODS**

The patients were randomized to either treatment groups at the first contact, and then were evaluated by principal investigator by taking a clinical history and performing physical examination. The following clinical characteristics were evaluated and recorded.

1. TBSA using Lund and Browder chart- appendix V
2. Clinical estimation of burn depth

The lab investigations ordered on first contact included total blood count, urea creatinine and electrolytes (U/E/C), and all were processed in University of Nairobi Department of Pediatrics laboratory.

### **Glutamine Group (1)**

The thirty patients randomized to this group received glutamine in form of B-immune. This formulation provides 0.20g /kg/day of glutamine. B- Immune is in powder form and each 40 mg sachet contains 17.03 grammes of protein, 5g of which is glutamine. 5 g of glutamine was provided every 8 hours to make a total of 15 g every day. Contents of B- immune were reconstituted by mixing with milk or porridge and nasogastric tube feedings or patient –assisted feeding initiated on post burn day 1 and continued for a period of 28 days.

#### **1) Collection and Transportation of surface swab specimen**

Samples for blood cultures and wound surface swabs cultures were taken on week 1, 2, 3 and 4 in that order.

Copan Swab (Eswab) was utilized in collection and transport of samples from a burn wound. Copan Eswabs is a commercially prepared product with a modified transporting medium which can sustain the viability of organism that includes clinically important aerobes and,

anaerobes and fastidious bacteria. Before use, this product was stored at temperature 5-25<sup>0</sup>c. Specimen collection began after removal of dressings and topical antibacterial agents. In order to obtain adequate cellular material for culture, the end of a sterile swab was moved over minimum of 1 cm area of burn wound.

The following stepwise specimen collection procedure was strictly followed (keeping with the standard procedure):

1. Using the swab applicator, the specimen was collected from multiple sites of wound to get a representative sample.
2. Swab was then placed into the tube and the cap replaced and secured tightly
3. Patient information (age ,sex, and the study number) was then entered on patient identification label

In order to maintain optimum organism viability, the specimen was taken to the microbiology laboratory immediately, within two hours of collection. In the event of delays, the specimens were refrigerated at 4-8<sup>0</sup> c in the microbiology laboratory and processed within 48 hours.

The collection and all microbiological tests were all done by the research assistant who also works as the microbiology laboratory technician at the University of Nairobi microbiology laboratory. The microbiological tests were all done at University of Nairobi microbiology laboratory.

Culture investigation of swab specimen started with inoculation onto solid agar culture medium in Petri dish plate. The objective of this was to obtain a primary inoculum which was then subjected to further subcultures in order to isolate and obtain specific micro-organisms.

## **2) Analysis of swab specimen**

Swabs were inoculated on the Blood Agar and MacConkey's Agar and direct smears were prepared. Smears were stained by the Gram's staining method and microorganisms were subsequently identified using standard biochemical tests <sup>68</sup>.

To achieve reliable and valid microbiological results, stipulated quality control measures were adhered to the latter. For instance Copan Eswab, a commercially prepared product, was used in collection and transport of burn wound swab samples. This product contains a transport medium which maintains viability of these organisms until processing is done. Also all the culture media used in this study were commercially prepared culture. Of primary importance is that each batch of commercially prepared culture media must be checked for contamination before passing for use in the laboratory. If contamination is more than 10%, the whole batch is discarded. Finally, analysis of specimen to arrive at accurate identification of microorganism followed standard microbiological procedures.

## **Blood cultures**



Upto 10 mls of blood was collected aseptically in glucose phosphate broth before administration of antibiotics and was then incubated at 37<sup>0</sup> centigrade for 48 hours. It was then sub-cultured on Blood Agar and MacConkey's Agar and finally, Gram-staining plus standard biochemical tests done.

The number of positive blood culture and wound surface swabs cultures were recorded.

In the context of this study an infection was defined as a positive culture obtained on pus sample or blood.

Duration of hospital stay for each patient was recorded.

### **Control/standard enteral formulation group (2)**

The thirty patients randomly assigned to this group received Fresubin Original a formulation which is isonitrogenous to B- Immune and is routinely provided to patients with severe burns in KNH burn unit. Fresubin Original has amino acids similar to that of B-Immune; however, unlike B- Immune it is glutamine-free. Collection, transport and laboratory processing of samples for blood culture and burn wound surface swabs were done in the same manner and sequence as for patients in the glutamine group.

The number of positive blood cultures, and wound surface swab cultures were recorded.

In addition, the other follow up evaluation and investigations done included;

- 1 Total blood count of patients of both treatment groups on day 14 and 28
- 2 Duration of hospital stay.
- 3 Final out come i.e. mortality or discharged

## **DATA COLLECTION AND ANALYSIS**

Data was collected by the principal investigator using pre-designed questionnaire. Coded data were entered into Microsoft Excel © and data cleaning done before doing analysis. Data was analyzed using the STATA version 9.2

The descriptive analysis of demographic and clinical characteristics is presented in form of tables and charts. The proportion of the number of positive blood cultures and pus swab cultures in both arms of treatment were compared. The summary of the proportions of mortality rate and length of hospital stay were presented for both groups. Statistical comparisons were made using Student's t-test for continuous variables and chi-square or Fisher's exact test when categorical variables were compared. P value of 0.05 or less was considered significant

Mantel-Haenszel method was utilized in testing the significance of treatment effect

### **Ethical considerations**

1. This study began after full approval of the ethical and research committees of University of Nairobi, Department of Surgery and KNH
2. This procedure was part of evaluation and surgical treatment of patients and never interfered with the patient management even after withdrawal from the study.
3. It was done after the patient signed informed consent.
4. All information gathered was utilized for the purpose of this research and was kept confidential throughout the study period.

5. Results of positive pus swab and blood cultures and abnormal baseline investigations like decreased hemoglobin level was passed on to the care givers as part of continued patient management

## **RESULTS**

A total of 60 adult patients with severe thermal burns who met the inclusion criteria were recruited into the study and randomly assigned to receive glutamine (n = 30) or fresubin (n = 30). The recruitment of the study participants ran for duration of 6 months between September, 2010, and February 2011 at KNH. A total of 240 wound surface cultures and another 240 blood cultures were done in the entire treatment period. All the sixty patients were included in the intention-to-treat analysis.

The ages of the patients ranged from 17 to 74 years with a mean of 29.5 years (SD 10.7).

Table 1: t-test comparing mean age of participants by treatment group

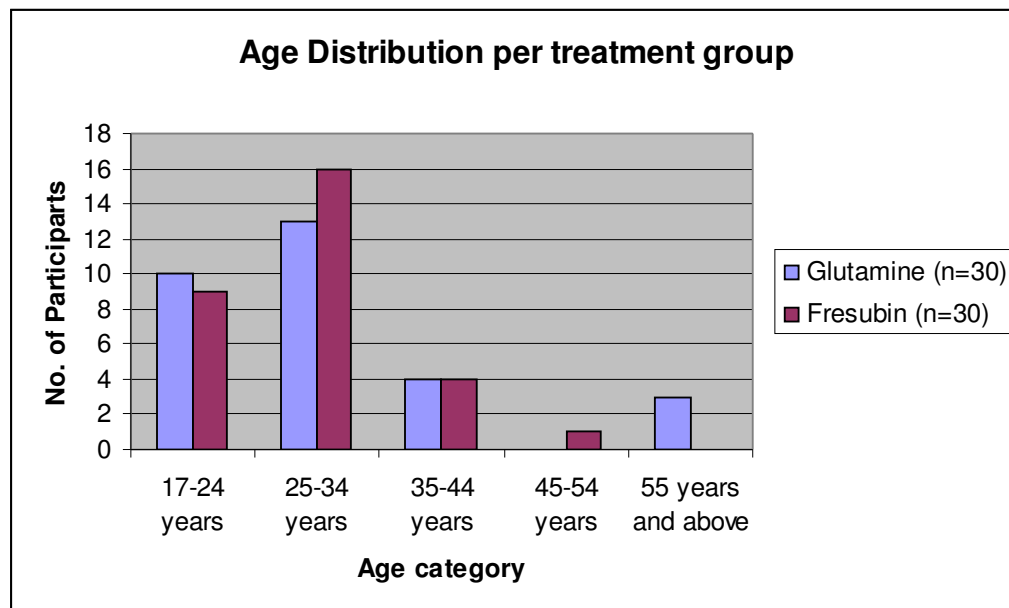
Group	N	Mean age (SD)	95% CI	P value
Glutamine	30	30.7 (13.5)	25.7-35.8	0.35
Fresubin	30	28.2 (6.8)	20.6-30.7	

The mean age for patients receiving glutamine was 30.7 years (SD 13.5) compared to a mean age of 28.1 years (SD 6.8) for patients receiving Fresubin. There was no significant differences in the age of patients assigned to intervention or control arm (p=0.35).

Table 2: Age group distribution per treatment group

Age categories in years	Glutamine (n=30)	Fresubin (n=30)
17-24	10(33.3%)	9(30%)
25-34	13(43.3%)	16(53.3%)
35-44	4(13.3%)	4(13.3%)
45-54	-	1(3.3%)
55 years and above	3(10%)	-

As shown in Table 2 above, the most common age group was 25 to 34 years and this age group comprised 43.3% and 53.3% of patients receiving Glutamine and Fresubin, respectively. In addition, majority of patients in both groups were aged less than 44 years.



**Figure 1:** Age group distribution per treatment group

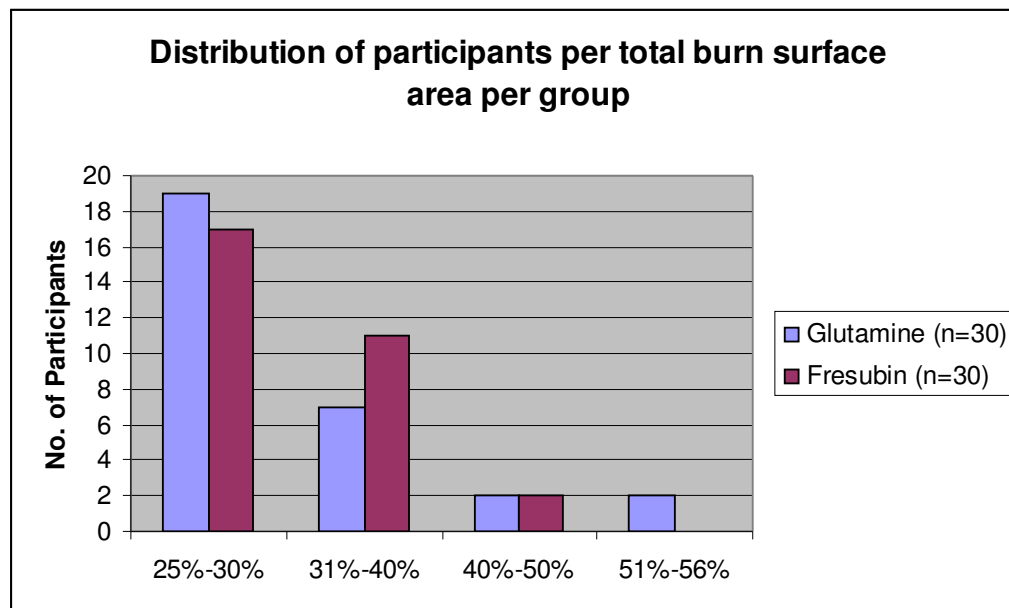
Table 3: Gender distribution per treatment group

	Glutamine (n=30)	Fresubin (n=30)
Gender		
Male	16(53.3%)	13(43.3%)
Female	14(46.7%)	17(56.7%)

There was no significant difference in male/female ratio in both arms of treatment.

Table 4: TBSA strata per treatment group

Total burnt surface area	Glutamine (n=30)	Fresubin (n=30)	Chi square
25%-30%	19(63.3%)	17(56.7%)	$\chi^2=3$ ; d.f=3
31%-40%	7(23.3%)	11(36.7%)	
40%-50%	2(6.7%)	2(6.7%)	P = 0.49
51%-56%	2(6.7%)	0	

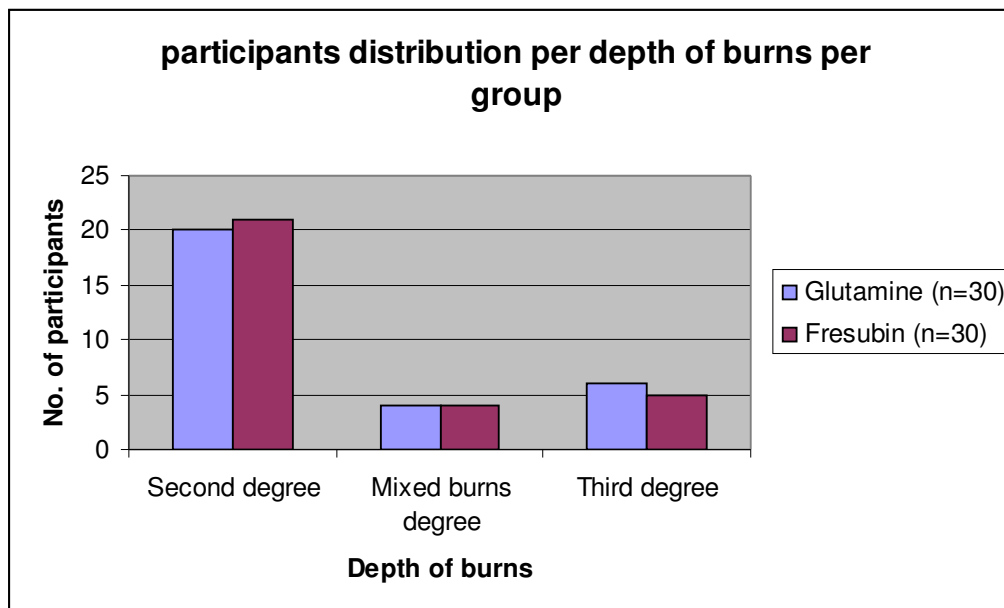


**Figure 2.** Distribution of participants versus TBSA per treatment group

Most patients in both groups had between TBSA ranging from 25%-30%. Sixty three percent of patients receiving glutamine had 25%-30% burns while 56.7% of patients in the control group had the same TBSA. The TBSA of patients in both arms of treatment did not differ significantly. (chi square [d.f] = 3.0[3],  $p = 0.49$ ) as demonstrated in table 4.

Table 5 participants' distribution versus depth of burns per treatment group

	Glutamine (n=30)	Fresubin (n=30)
Depth of burns		
Second degree deep	20 (66.6%)	21(70%)
Mixed burns	4(20%)	4(13.3%)
Third degree	6(20%)	5(16.7%)



**Figure 3.** Participants' distribution versus depth of burns per treatment group

The depth of burns of patients in both arms of treatment did not differ significantly (chi square [d.f] =2.3[4],  $p = 0.88$ ). Second degree burns were most commonly reported degree of burns occurring in 63.7% of patients assigned to glutamine and 70% of patients assigned to Fresubin group.

Table 6 Abbreviated Burns severity Score Index per treatment group

	Glutamine (n=30)	Fresubin (n=30)
Abbreviated Burns Severity	$9 \pm 3$	$8 \pm 2$
Score Index (mean $\pm$ SD)		

The severity index was almost similar in both arms of treatment:  $9 \pm 3$  and  $8 \pm 2$  for glutamine and control group respectively. The number of patients with a severity index equal to or more than 10 was 2 and 1 in glutamine and control groups respectively.

Table 7 Duration in hours post burns prior to admission

	Glutamine (n=30)	Fresubin (n=30)
Hours post burns (mean $\pm$ SD)	15.4 $\pm$ 8.5	16.5 $\pm$ 8.0

The mean duration postburns prior to admission among all patients was 16 hours (SD 8.2). This duration did not differ significantly per treatment group as presented in Table 4 (Fresubin =16.5 hours [SD 8.0] versus glutamine 15.4 hours [SD 8.5],  $p=0.62$ ). This implies the difference in the results observed in the two groups is not attributed to this parameter.

## Incidence of infection

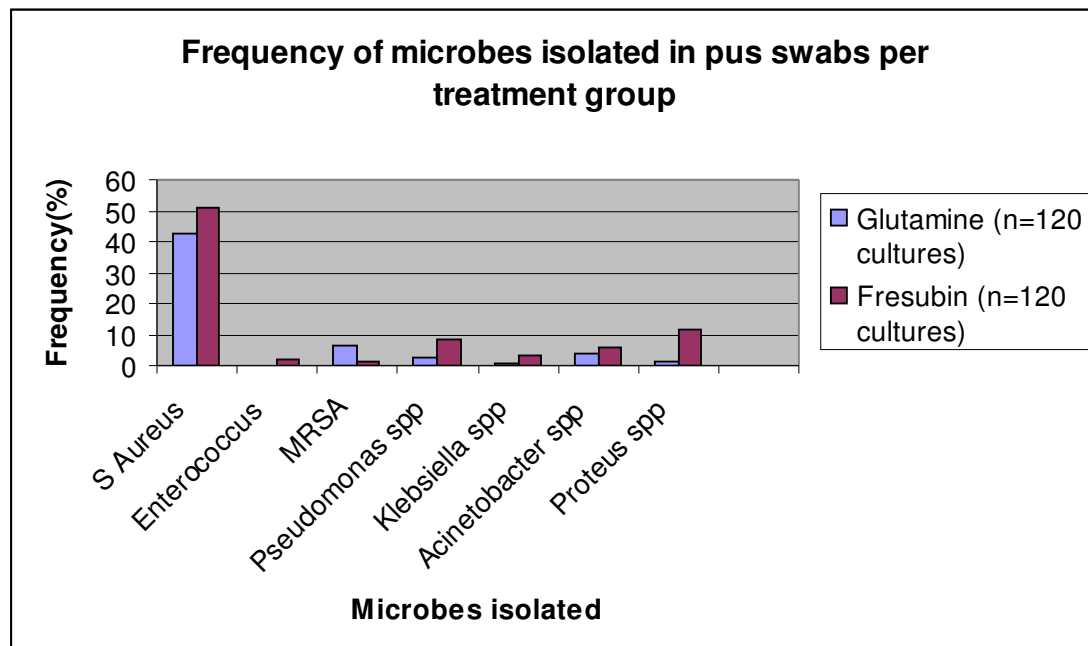
### a) Surface swabs

A total of 240 surface swabs cultures were conducted during the entire treatment period.

Table 8 Frequency of microbes isolated in pus swabs per treatment group

Bacterium isolated	Glutamine (n=120 cultures)	Fresubin (n=120 cultures)	Odds ratio (95% CI)	P
<i>S Aureus</i>	51/120(42.5%)	61/120(50.8%)	1.4(0.8-2.3)	0.2
<i>Enterococcus</i>	0/120(0)	2/120(1.7%)	-	0.16
<i>MRSA</i>	8/120(6.7%)	2/120(1.6%)	0.23 (0.05-1.2)	0.05
<i>Pseudomonas spp</i>	3/120(2.5%)	10/120(8.3%)	4.5(1.0-20)	0.03
<i>Klebsiella spp</i>	1/120(0.8%)	4/120(3.3%)	0.2(0.03-2.2)	0.17
<i>Acinetobacter spp</i>	5/120(4.1%)	7/120(5.8%)	1.4(0.4-4.6)	0.55
<i>Proteus spp</i>	2/120(1.6 %)	14/120(11.7%)	7.8(1.7-36.3)	0.002





**Figure 4:** Frequency of microbes isolated in pus swab culture per treatment group.

The most common organism in both arms of treatment was *Staphylococcus aureus*; 42.5% and 50.8% in glutamine and fresubin respectively. Other common pathogens isolated in patients receiving glutamine- free diet were *proteus spp* (11.7%) and *pseudomonas spp* (8.3%)

**Table 9:** Bacterial organisms isolated from surface swabs weekly in each treatment groups

	Week 1		Week 2		Week 3		Week 4	
	Glutamine	Fresubin	Glutamine	Fresubin	Glutamine	Fresubin	Glutamine	Fresubin
<i>S aureus</i>	19	17	19	19	9	15	4	10
<i>Enterococcus</i>	-	-	-	-	-	1	1	-
<i>MRSA</i>	-	-	5	2	2	-	1	-
<i>Pseudomonas</i>	1	1	2	2	0	3	0	4
<i>Klebsiella spp</i>	-	-	1	-	-	2	-	2
<i>Proteus spp</i>	1	-	1	3	-	6	-	5

*Staphylococcus aureus* remained the predominant organism isolated in both arms of treatment in the entire period of treatment. Gram negative bacteria such as *pseudomonas spp* started colonizing the burn wound from day 4 henceforth and its incidence rose sharply in glutamine-free group in the last 2 weeks of treatment.

Table 10 positive swab cultures per week per treatment group

<b>All patients (n = 60)</b>				
	Glutamine (n = 120 swab cultures)	Fresubin (n = 120 swab cultures)	Odds ratio (95% CI)	P
<b>Positive swab</b>				
<b>Total (week 1-week 4)</b>	56/120(46.6%)	75/120(62.5%)	1.9(1.1-3.2)	0.014
Week 1	21/120(17.5%)	19/120(15.8%)	0.7(0.2-2.2)	0.58
Week 2	21/120(17.5%)	25/120(20.8%)	2.1 (0.6-7.6)	0.23
Week 3	9/120(7.5%)	19/120(15.8%)	4.0(1.2-12.8)	0.01
Week 4	5/120(4.1%)	12/120(10%)	3.3(0.9-11.7)	0.04

In overall, glutamine supplementation has a strong association with the reduction of incidence of burn wound infection P value=0.014 as shown on table 9.

There were no significant differences in the incidence of positive swabs during week 1 or week 2 in the two groups (Table 10). During the third and fourth weeks the odds of positive culture were significantly higher in the fresubin group, with OR = 4.0 (1.2-12.8) and OR = 3.3 (0.9-11.7), respectively. The effect of glutamine on incidence of infection reached a significant level in week 3 and 4 with p value less than 0.05.

#### **b) Blood culture**

Out of the 240 blood culture conducted among 60 patients over 4 weeks, a total of 54 bacterial pathogens were grown from 35 (58.3%) patients. The most common pathogen grown from blood cultures was *staphylococcus aureus*: 15.8% and 9.1% in control and glutamine group respectively. The least isolated microbe was *proteus*: 0% and 1.6% in the glutamine and the control group respectively. In respect to the other gram negative bacteria, it is evident from table 11 that glutamine has a strong correlation with reduction in the incidence of infection caused by *klebsiella spp* and *pseudomonas spp* at a p value of 0.02 and 0.04 respectively.

Table 11: frequency of microbes isolated in blood culture per treatment group

Bacterium isolated	Glutamine (n=120 cultures)	Fresubin (n=120 cultures)	Odds ratio (95% CI)	P
<i>S Aureus</i>	11/120(9.1%)	19/120(15.8%)	1.9(0.8-4.1)	0.12
<i>Proteus spp</i>	0/30(0)	2/120(1.6 %)	-	0.15
<i>Enterococcus</i>	3/120(2.5%)	8/120(6.7%)	2.8(0.7-10.8)	0.12
<i>MRSA</i>	3/120(2.5%)	2/120(1.6%)	0.6(0.1-4.0)	0.65
<i>Klebsiella spp</i>	0/120	5/120(4.1%)	-	0.02
<i>Pseudomonas spp</i>	5/120(4.1%)	12/120(10%)	3.3(0.9-11.7)	0.04

**Figure 5** frequency of microbes isolated in blood culture per treatment group

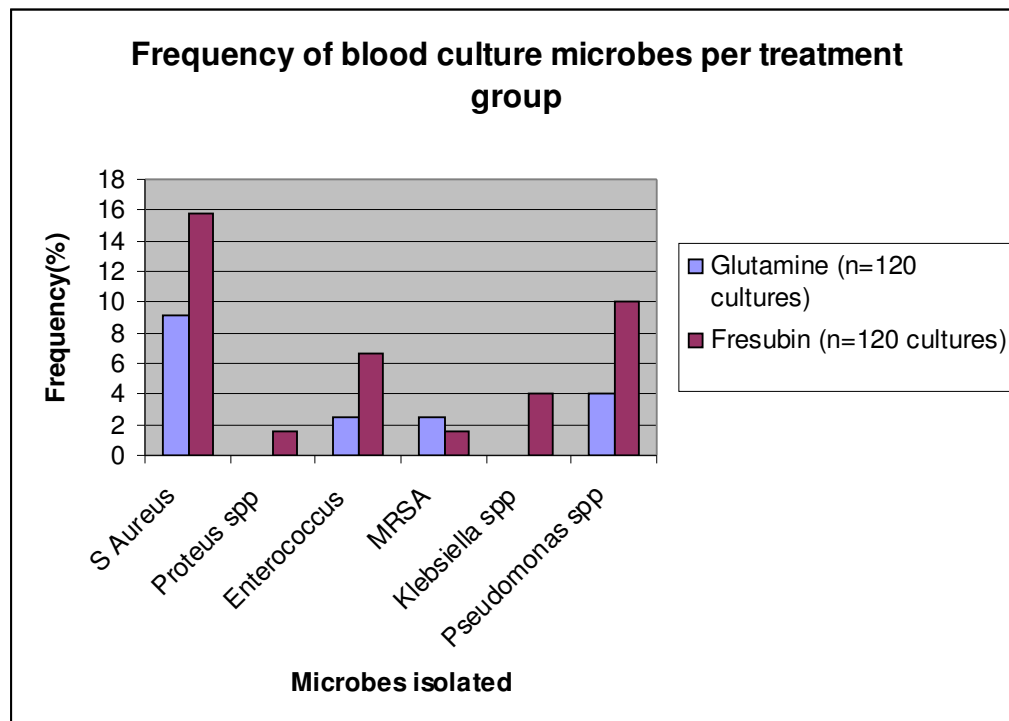


Table 12. frequency of positive blood culture per treatment group at different time points

All patients ( n = 60)				
	Glutamine (n=120 blood cultures)	Fresubin (n=120 blood cultures)	Odds ratio (95% CI)	P
<b>Positive blood culture</b>				
<b>Total (week 1-week 4)</b>	13/120(10.8%)	30/120(25%)	2.7 (1.3-5.6)	0.004
<b>Weekly positive blood cultures</b>				
Week 1	1/120(0.83)	1/120(0.83%)	1.0(0.06-17.2)	0.99
Week 2	8/120(6.7%)	7/120(5.8%)	0.8(0.25-2.7)	0.76
Week 3	2/120(1.6 %)	14/120(11.7%)	7.8(1.7-36.3)	0.002
Week 4	3/120(2.5%)	10/120(8.3%)	4.5(1.0-20)	0.03

Table 12 above demonstrates a statistically significant increase in infection in the control group in week 3 and 4. Also the total for the 4 weeks show a significant difference in the 2 groups.

## Mortality

As shown in Table 13 below, a total of 11 (18.6%) deaths occurred among patients enrolled in the study. These deaths occurred more commonly in patients who received Fresubin (n = 9, 30%) than in those who received Glutamine (n = 2, 6.9%). The odds of death in the control group was approximately 6-fold higher (OR 5.8 [95% CI, 1.03 - 32.5] p = 0.024) than in the group of patients receiving Glutamin

Table 13: 4-week mortality rate per treatment group

<b>All patients=60</b>				
Deaths	Glutamine (n=30)	Fresubin (n=30)	Mantel-Haenszel Odds ratio (95% CI)	p
<b>Total deaths</b>	2/30(6.9%)	9/30(30%)	5.8(1.02-35.5)	0.024
<b>Gender</b>				
Male	0/9(0)	7/23(30.4%)	8.9(1.8-97.0)	0.03
Female	2/20(10%)	2/7(28.6%)		
<b>Age (years)</b>				
17-24	0/10(10%)	3/9(33.3%)	14.5(1.9-24.4)	0.014
25-34	0/12(0)	3/16(18.8%)		
35-44	1/4(25%)	2/4(50%)		
45-54	-	1/1(100%)		
55 and above	-	1/3(33.3%)		
<b>Depth of burns</b>				
Second degree	1/18(5.6%)	6/21(28.6%)		
Mixed degree	0/1(0)	-	6.0(1.9-38.1)	0.03
Third degree	1/6(16.7%)	2/3(66.6%)		

The Mantel-Haenszel Odds ratios (95% CI) presented in Table 13 above showed that the higher odds of death among patients receiving Fresubin compared to glutamine persisted even after adjusting for the effect of gender and age of the patients, TBSA and the depth of burns (all p values in these strata > 0.05).

### Length of hospital stay

The mean length of hospital stay for all the 60 patients was 38.6 days [SD 9.9]. Patients receiving glutamine enteral supplementation had a significantly shorter length of stay compared to those on Fresubin ( $p = 0.001$ , Table 14). On average, patients on glutamine stayed in hospital for 34.8 days [SD 7.8] and those receiving Fresubin stayed on for 43.8 days [SD 10.2].

Table 14: Length of hospital stay among adult patients admitted with thermal burns receiving glutamine and fresubin enteral supplementation

Characteristic	Glutamine (n=30) Mean [SD]	Fresubin (n=30) Mean [SD]	P
Length of stay (days)	34.8 [7.8]	43.8 [10.2]	0.001
<b>Depth of burns</b>			
Second degree	34.1[5.2]	43.3[10.6]	0.002
Mixed burns	39.6[3.2]	40.3[9.6]	0.91
Third degree	34[14.9]	50[9.5]	0.14

Further analysis stratified by depth of burns showed that patients with second degree burns receiving glutamine had significantly shorter lengths of stay ( $p=0.002$ , Table 14) while length of stay did not differ significantly per treatment group among patients with mixed burns ( $p = 0.91$ ) and third degree only burns ( $p = 0.14$ ).

## DISCUSSION

Post burn infection is a major cause of morbidity and mortality in patients with severe burns particularly in developing countries, Kenya included. This contrasts sharply to the pattern observed in developed countries where the majority of deaths in patients with severe burns are

attributed to inhalation injury<sup>1</sup>. The incidence of postburn infections is still high despite various preventive modalities and this is attributed to systemic immunosuppressive effect of severe burns<sup>1</sup>.

The objective of this 6 month RCT was to determine the effectiveness of enteral glutamine in reducing the incidence of postburns infections and mortality in adult patients with thermal burns above 25% TBSA. In overall, the results of this local study are equally comparable to those done in developed countries. Chi-test and percentages were computed to measure the relationship between various variables. Patients' demographic and baseline clinical characteristics were similar in both arms of treatment. The patients were put through similar management except in the aspect of nutritional supplementation. This is important since significant differences in the results could be attributed to glutamine supplementation. The respondents' ages ranged from 17 to 74 with a mean age of 29.5 and standard deviation of 10.7. The mean age for patients receiving glutamine was 30.7 years (SD 13.5) compared to a mean age of 28.1 years (SD 6.8) for patients receiving Fresubin. As shown in table 1 there was no significant differences in age of patients assigned to intervention or control arm ( $p=0.35$ ). Majority of patients in both groups were aged less than 44 years.

In respect to gender, there was no significant male/female ratio in both arms of treatment as illustrated in table 3. Majority of patients had sustained burns between 25-30%. Sixty three percent of these patients with TBSA 25-30% were in the intervention group and 56.7% were in

the control group (chi square [d.f] = 3.0[3], p = 0.49) table 4. This difference is not statistically significant and therefore does not influence the treatment outcome.

In addition, the depth of burns of patients in the two groups did not differ significantly (chi-square [d.f] = 2.3[4], p = 0.88) and therefore unlikely to influence the treatment outcome.

Second degree deep burns was the most commonly reported degree of burns occurring in 63.7% of patients assigned to glutamine and 70% of patients assigned to Fresubin (table 5).

The severity index was similar in both arms of treatment: 9 +/-3 and 10+/-2 for glutamine and control group respectively. The number of patients with a severity index >10 was 2 and 1 in glutamine and control groups respectively.

In this particular study, the most commonly isolated micro-organism in pus swab and blood culture was *Staphylococcus aureus* predominating in the first week and second week (table 8 and 9). This coincides with other studies done elsewhere<sup>(63, 64)</sup>. This is probably because

*Staphylococcus aureus* is a normal skin flora which causes invasive infection on a burn wound following loss of skin barrier and depressed systemic cellular/humoral immune responses<sup>37, 65, 66</sup>.

There were no significant differences in the incidence of positive cultures of *Staphylococcus aureus* in the two groups for the entire treatment period of 4 weeks (Table 8). Important to note is *Staphylococcus aureus* was the only gram positive bacteria isolated. This is perhaps because *streptococcus spp* has been eradicated following the advent of antibiotics. *Streptococcus spp* was the most predominant pathogen colonizing burn wound before the era of antibiotics<sup>2</sup>.



Gram negative bacteria such as *pseudomonas*, *proteus* and *klebsiella spp* were isolated from pus swab from day 4 onwards and the incidence rose sharply in the the last 2 weeks of treatment in the control group (table 9). This mirrors findings of a Syrian study done by Dayoub in 1995<sup>3</sup>. During the third and fourth weeks the odds ratio of positive culture were significantly higher in the fresubin group, with OR = 4.0(1.2-12.8) and OR = 3.3(0.9-11.7) respectively. The effect of glutamine on the incidence of infection reached a significant level in week 3 and 4 with p value less than 0.05(table 10).

Of the 35 patients with a positive blood culture during the entire 4 week period, 24 (80%) were receiving Fresubin while only 11(36.7%) of the patients were receiving Glutamine. The difference registered was of statistical significance ( $p=0.004$ , Table 12). For the entire 4-week treatment, the odds ratio of a positive blood culture were almost three-fold higher (2.7 (1.3-5.6) among patients on Fresubin compared to those on Glutamine (table 12). Effect of glutamine on the incidence of bacteremia reached significant levels in week 3 and 4 ( $p$  value of 0.002 and 0.03) respectively. On week 1 and 2 however, glutamine does not have significant impact on the incidence of bacteremic episodes ( $p=0.99$  table 12). An Indian study of 2009 reported similar results<sup>61</sup>.

In this particular study, glutamine reduced the incidence of gram negative bacteria such as *pseudomonas spp* by 3.3-4.5 times (table 8 and 11). This compares to results of RCT by Garrel in which glutamine reduced the incidence of infection by a factor of 3 and prevented bacteremia of *Pseudomonas aeruginosa*<sup>7</sup>. In this study therefore enteral glutamine appears to reduce incidence of gram negative sepsis more than that of gram positive bacteria. This is because enteral

glutamine has a tropic effect on GIT mucosa and therefore prevents translocation of GIT-derived gram negative bacteria to the burn wound. This clinical observation is supported by in-vitro studies by Clark<sup>41</sup> and Koutzernova<sup>42</sup>.

The overall mortality rate of patients with severe burns in both arms of treatment combined was 18.9%. A high mortality rate was noted in patients who received Fresubin (n = 9, 30%) than those who received Glutamine (n = 2, 6.9%). The odds ratio of death in the control group was approximately 6-fold higher (OR 5.9 [95% CI, 1.03 - 32.5] p = 0.024) than in the group of patients receiving Glutamine. The Mantel-Haenszel Odds ratios (95% CI) presented in Table 13 showed that the higher odds of death among patients receiving Fresubin compared to Glutamine persisted even after adjusting for the effect of gender and age of the patients, TBSA and the depth of burns (all p values > 0.05). Results from this study demonstrated a statistically significant effect of enteral glutamine supplementation on the reduction of mortality rate among patients with severe burns. Similar results were reported by Garrel<sup>7</sup> in 2003. This reduction in mortality could be the result of the improved condition of the patient after glutamine supplementation. Glutamine supplementation improves a patient's nutritional status and subsequently enhancing both humoral and cellular immunity. This enables him/her to cope with postburns infection more effectively and subsequent attenuation of SIRS. Progression to Multiple Organ Failure (MOF) is therefore arrested. MOF is commonly associated with high mortality rate.

Patients receiving glutamine enteral supplementation had a statistically significantly shorter length of hospital stay compared to those on Fresubin ( $p = 0.001$ , Table 14). On average, patients on glutamine stayed in hospital for 34.8 days [SD 7.8] and those receiving Fresubin stayed on for 43.8 days [SD 10.2]. These results are equally comparable to those reported in other studies<sup>(7, 52, 67)</sup>. Enteral glutamine improves general condition of the patient, is associated with positive nitrogen balance and improved nutritional status: septic complications which usually lengthens patients' hospital stay are therefore reduced<sup>29</sup>.

Further analysis stratified by the depth of burns showed that patients with second degree burns receiving glutamine had significantly shorter lengths of stay ( $p=0.002$ , Table 14). These results are similar to those reported in other studies<sup>(7, 52, 67)</sup>.

Length of hospital stay did not however differ significantly by treatment group among patients with mixed burns ( $p = 0.91$ ) and third degree burns ( $p = 0.14$ ). This sharply contrasts with studies done elsewhere<sup>(6, 7, 8, 52, 67)</sup>. The inter-centre variation in the relationship of glutamine and hospital stay in respect to the depth of burns may be attributed to subjective clinical assessment of the depth of burns. The clinical judgment of burn depth is subject to both intraobserver and interobserver variation. In addition, depth of burns might change with time and get worse when adequate hydration is not provided within the first 24 hours postburns or in a setting of invasive infection of burn wound. Decreased skin perfusion due to inadequate hydration to correct shock might result in conversion of a zone of stasis in a burn wound to a zone of coagulation, thereby increase in burn depth.

## **Conclusion**

Enteral glutamine supplementation in severely burned adult patients reduces blood infection by a factor of three, prevents bacteremia with *P. aeruginosa*, decreases mortality rate and shortens duration of hospital stay. In this study therefore, enteral glutamine appears to reduce the incidence of gram negative sepsis more than that of gram positive bacteria.

## **RECOMMENDATIONS**

- 1 Supplementation of enteral glutamine should be one of the core and integral patient management strategies of reducing postburn infection and mortality in patients with severe burns in poor resource Burn centres like that of KNH.
- 2 Further study to evaluate the role of glutamine supplementation in burns arising from other etiologies besides thermal such as chemical and electrical burns.
- 3 Strict infection control practices (i.e., physical isolation in a private room, use of gowns and gloves during patient contact, and handwashing before and after each patient visit) and appropriate empirical antimicrobial therapy are essential to help reduce the incidence of infections due to the antibiotic-resistant organisms.
- 4 KNH Burn Unit should routinely determine and track the specific pattern of burn wound microbial colonization, time-related changes in the predominant microbial flora of the burn wound in individual patients, the antimicrobial susceptibility profiles of microorganisms implicated in burn wound infections in a given time period, and trends in the nosocomial spread of these pathogens.

## **Study Limitations**

- 1 Lack of laboratory standardization in terms of intraobserver variation and calibration (considering lab work was processed in only one laboratory).
- 2 Some of necessary investigations were not done on time. This may have arisen due to shortage of reagents and supplies at KNH.
- 3 Only patients with thermal severe burns were included (chemical, and electrical burns were excluded).

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## APPENDIX I : DATA COLLECTION SHEET

Group 1 ☐ 2 ☐

Patient data

1. Study number
2. Age
3. Sex
4. Date of recruitment into study
5. No of hours post burns
6. TBSA ESTIMATION (%)
5. Estimated burns depth
6. Abbreviated Burns Severity Score Index
7. INFECTION

### A.SURFACE SWAB CULTURE OUTCOME

WEEK	RESULT (NEGATIVE OR POSITIVE)	MICROBE ISOLATED
WEEK 1		
WEEK2		
WEEK 3		
WEEK 4		

### B. BLOOD CULTURE OUTCOME

WEEK	RESULT(NEG POSITIVE)	OR MICROBES ISOLATED
WEEK 1		
WEEK 2		
WEEK 3		
WEEK 4		

7 DURATION OF HOSPITAL STAY----- DAYS

#### 8 FINAL OUTCOME

☐ Discharged home alive

☐ Died at the hospital

## **APPENDIX 11**

### **The Abbreviated Burn Severity Index**

<b>Variable</b>	<b>Patient Characteristic Score</b>
Sex	
Female	1
Male	0
Age in years	
0–20	1
21–40	2
41–60	3
61–80	4
81–100	5
Inhalation injury	1
Full-thickness burn	1
TBSA burned (%)	
1–10	1
11–20	2



21–30	3
31–40	4
41–50	5
51–60	6
61–70	7
71–80	8
81–90	9
91–100	10

## **Appendix 111**

### **CONSENT BY THE PARTICIPATING PATIENT**

**Study No.....**

**Hospital No.....**

#### **Purpose of the study**

The purpose of this study is to determine the effectiveness of enteral glutamine on reduction of infectious morbidity and mortality on adult patients with severe burns at Kenyatta National hospital. The information gathered will be used to improve the management of patients with severe burns.

#### **Risks and benefits**

This study will provide clinicians essential information on the positive effects of enteral glutamine supplementation in patients with severe burns and therefore aid them in improving clinical management of these particular patients. There is no harm or risk anticipated for

participating in this study. However, during the study if the researcher identifies a complication on you, he will recommend/ refer you appropriately. No additional tests outside the usual ones for treatment will be carried out and no extra cost to you will be incurred for participating in the study.

### **Voluntary participation**

Participation in this study is out of your own free will. Medical care will not be denied in case you decline to participate in the study. You may terminate participation at any time with no consequences whatsoever.

### **Confidentiality**

All information will be treated with confidentiality. Your identity will not be published whatsoever.

I the undersigned have been explained to and understand the above and voluntarily accept to participate in the study.

Signature/Thumb print:

(Patient/Next of kin) Tel 1 (patient):..... Tel 2 (Next of Kin):.....

DR. DAVID KIPKOECH KIBOR – TEL 0724536011

Chairman of KNH/ UON Ethics Research Committee- 0722708808

## **Appendix 1V**

### **KIBALI CHA RUHUSA**

**Nambari ya utafiti:..... Nambari ya Hospitali:.....**

### **Sababu ya utafiti**

Sababu ya utafiti huu ni kutibitisha manufaa chakula chenye amino acid ya glutamine kwa kupunguza infections na maafa kwa wagonja wenye wamechomeka sana Utafiti huu utafanyika

katika hospitali kuu ya Kenyatta na matokeo yake yatatumiwa kupendekeza njia za kuboresha matibabu kwa wagonjwa wenye wamechomeka sana.

### **Hatari na manufaa**

Utafiti huu utaimarisha ujuzi wa madaktari kwa matibabu kwa wagonjwa ambao wamechomeka sana. Hatutarajii hatari zozote kwako unaposhiriki kwenye utafiti huu. Iwapo wakati wa utafiti, mtafiti atagundua shida katika matibabu yako, atapendekeza au kukutuma kwa matibabu yanayofaa. Utafiti huu hautakugharimu fedha zaidi.

### **Uhusika Kwa hiari**

Kuhusika kwa utafiti huu ni kwa hiari yako mwenyewe na hauwezi kushurutishwa. Utahudumiwa ata kama ukikataa kuhusika kwa huu utafiti. Una uhuru kutamatisha kuhusika wakati wowote bila madhara yoyote ile.

### **Usiri**

Habari zozote utakazotoa zitawekwa kwa siri na jina lako halitachapishwa popote. Ninathibitisha yakuwa nimefahamu yale nimeelezwa na mtafiti na nimekubali kwa hiari yangu mwenyewe kuhusika katika utafiti huu.

Sahihi/Kidole cha Gumba: (Mhusika/next of kin)

Simu 1 (Mhusika):..... Simu

2 (next of kin):.....

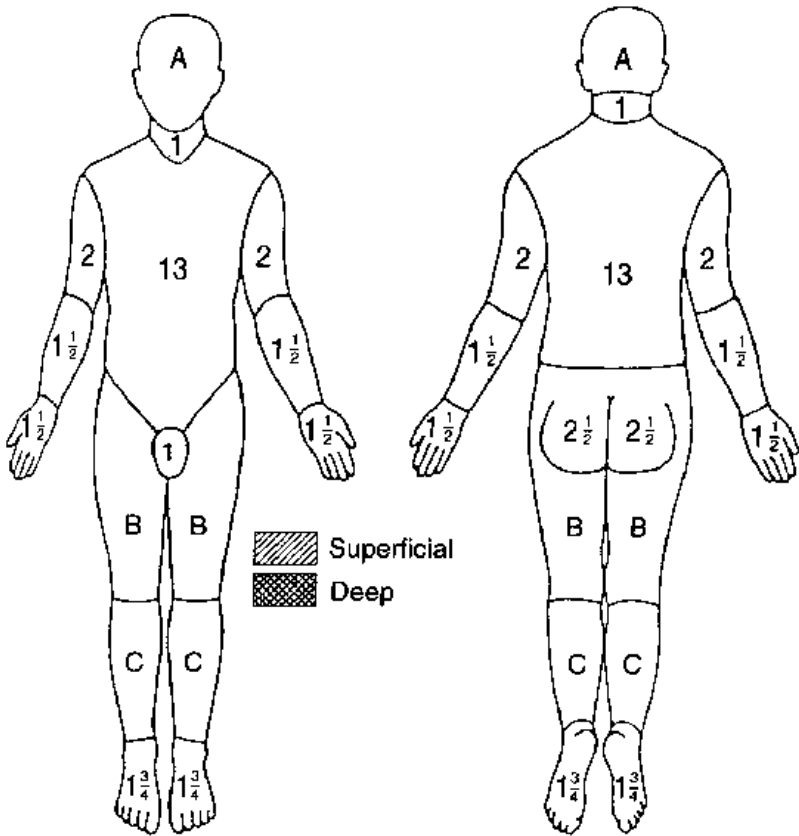
.DR. DAVID KIPKOECH KIBOR – TEL 0724536011 Nambari ya Simu mwenyekiti wa kamati ya utafiti

## **APPENDIX V**

### **ASSESMENT OF TOTAL BURNT SURFACE AREA.**

**Lund and Browder charts.**

The burn diagram table was completed to calculate the total area of burn involved (Reproduced with permission from Grabb and Smith’s Plastic Surgery 6<sup>th</sup> Edition page 154)



Region	%
Head	
Neck	
Ant. Trunk	
Post. Trunk	
Right arm	
Left arm	
Buttocks	
Genitalia	
Right leg	
Left leg	
Total burn	