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The Effect Of Enteral Glutamine To Increase The Macrophage Count In Full-Thickness Burns Infected With Acinetobacter Baumannii Bacteria In White Rats (*Rattus norvegicus*)

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ABSTRACT

In this study, we explore the impact of hypermetabolic conditions on the immune function of the body, with a particular focus on the role of glutamine, an amino acid involved in protein synthesis and the regulation of metabolic processes within the immune system. Macrophages and monocytes play a crucial role in responding to A.Baumanii infection, being the first line of defense. Our investigation aims to analyze the augmentation of macrophage cell count activity against A. Baumanii through the administration of glutamine. Using an experimental study design, 45 rats were randomly assigned to three groups: a control group, a Glutamine treatment group, and a Glutamine against A. Baumanii group. The rats were evaluated on days 1, 5, and 7, with enteral administration of glutamine at a dose of 1 g/kg body weight/day. Specimens were taken from the peritoneum tissue, and anatomical pathology preparations were conducted to calculate the number of macrophage cells. Data collected were then input into a table and processed using SPSS 26 for Windows. Results revealed a significant increase in macrophage cell count on day 1 in the Glutamine against A.Baumanii group compared to the control group ($p < 0.05$). On day 5, the macrophage cell count in both the Glutamine group and the Glutamine against A.Baumanii group was higher than in the control group, with significant differences observed between groups ($p < 0.05$). On day 7, a significant difference ($p < 0.05$) in the number of macrophage cells was noted between the Glutamine groups. In conclusion, enteral glutamine feeding led to a notable increase in the number of macrophage cells, indicating a positive impact on the immune system. This rise in macrophages correlates with enhanced phagocytic activity against A.Baumanii infection.

Keywords- *macrophages, glutamine, acinetobacter baumannii*

Introduction :

Hypermetabolic states can lead to various effects, ranging from fever to septic shock in burn patients. This condition can worsen if the provided nutritional support is inadequate, exacerbating micronutrient deficiencies, causing endocrine disruption, increasing catabolism, and promoting gluconeogenesis, thereby affecting immune system function [1,2]. Therefore, nutrition is not solely focused on meeting the energy, macro-nutrient, and micro-nutrient needs for sustenance. In pathological conditions, nutritional support is crucial to prevent the deterioration of disease conditions and to accelerate healing [3,4].

The concept of nutritional support to modulate immune function is known as immunonutrition (immuno-enhancing diets or immuno-modulating diets). This approach aims to address pathological changes in adaptive and natural immunity, which may occur secondary to inflammation, infection, or surgery, by providing specific immunonutrients [5]. The interaction between nutrients and the immune response to inflammation, infection, and surgery involves the release of pro-inflammatory cytokines, followed by an acute phase response characterized by hypermetabolism, protein destruction, and disruption of micronutrients [6,7].

Glutamine is the non-essential amino acid most prevalent in muscle and plasma of the human body. This amino acid has an important role in protein synthesis, a process to regulate energy metabolism.[8,9]. Glutamine has many functions in the immune system [10], increasing proliferation and improving the cell function of macrophages [11,12]. These immunocompetent cells are able to phagocytose pathogens, kill fungi, and produce nitric oxide (NO), interleukins, tumor necrosis factor α (TNFα) and reactive oxygen species (ROS) [13]. Glutamine is able to stimulate macrophages to increase arginase secretion and provide Nicotinamide Adenine Dinucleotide Phosphate (NADP) thereby increasing NO secretion by macrophages [14]. Nitric oxide is important in the mechanism of bacterial killing [12,13]. In previous studies, the administration of glutamine in vivo was able to increase macrophage activity against Mycobacterium bovis [7].

Approximately 70% of nosocomial infections are now resistant to at least one type of antibiotic that was previously effective in killing pathogenic bacteria [15,16]. Bacteria known for their virulence and the ability to develop resistance include *Acinetobacter species*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus spp*, and *Enterobacteriaceae* [17].

Acinetobacter baumannii has become a concern for clinicians and hospital epidemiologists in the last two decades, leading to opportunistic infections in immunocompromised patients. It is resistant to various types of antimicrobials and some disinfectants, now demonstrating resistance to almost all commonly used antibiotics, contributing to high mortality rates globally [18]. A 2013 Centers for Disease Control and Prevention (CDC) report highlighted multidrug-resistant Acinetobacter as a serious threat, causing 7,000 cases of infection and 500 deaths each year in the United States. According to data from the Clinical Microbiology Unit of Dr. Soetomo Hospital in January to December 2019, Acinetobacter baumannii bacteria were reported as the most common bacteria found in the burn room, surgical ward, and High Care Unit (HCU) [19,20].

Objectives :

The experimental study aimed to assess the potential of glutamine in increasing the number of macrophages in full-thickness burns infected with Acinetobacter baumannii. If the administration of glutamine is proven to enhance the body's immune response, it can be further discussed as a supplementation to support the treatment of infections in full-thickness burns caused by Acinetobacter baumannii.

Methodology :

Male Wistar rats (200–300g, 12 weeks of age) were obtained from the Animal Lab of the Biochemical Department, Faculty of Medicine, Universitas Airlangga, and were maintained under a 12-hour light/dark cycle. Food and water were available ad libitum. The rats were divided into three groups (Group 1 was the control group; Group 2 was the glutamine group; and Group 3 was the glutamine and infected *A. baumannii* group). The sample size was five rats per group, and observations were made on days 1, 5, and 7. With enteral administration of glutamine at 1g/kg/day, specimens were taken from the peritoneal tissue, anatomical

pathology preparations were made, and the number of macrophage cells was counted.

Findings :

Forty-five white rats were randomized into nine groups as the experimental animals in this study. Divided into three groups, Group I underwent pharmacological intervention with A. baumannii, Group II underwent pharmacological intervention, and Group III served as the control group. In the treatment group, 1 cm^2 full-thickness thermal contact burns were induced. Prior to this, the rats were anesthetized, and the area was antisepticized with 10% povidine iodine and Savlon 1:30. Thermal contact burns were generated using a steel metal disk with a diameter of 10 mm, previously heated in boiling water at a temperature reaching 100°C. The heated steel was applied to the proximal dorsal region for 15 seconds.

In the treated group, the wound surface was inoculated with A. baumannii bacteria using a sterile cotton bud. Subsequently, each group received oral glutamine treatment for 7 days at a dose of 1 g/kg BW/day, administered enterally in a rounded form. Evaluations were conducted on days 1, 5, and 7. Specimens from the peritoneal tissue were obtained and stained with Hematoxylin and Eosin. The stained tissue sections were examined under a microscope at the Anatomical Pathology Laboratory of the Faculty of Medicine, Universitas Airlangga. The number of macrophage cells was counted under a microscope.

The calculation of macrophage numbers in the observational group on days 1, 5, and 7 after treatment was conducted uniformly in each control and pharmacological intervention group of glutamine administration. Statistical analysis revealed a significant difference in the number of macrophage cells in each group (p = 0.000).

Table 1: Analysis of macrophage count comparison on day -1

	Mean ± SD	**P-value**	**C-Glutamine**	**C-Glutamine+ *A.Baumanii***	**Glutamine-Glutamine+ *A.Baumanii***
Control	82.60 ± 8.87	0.002			
Glutamine	105.80 ± 7.56		0.009	0.009	0.009
Glutamine+ *A.Baumanii*	119.60 ± 2.96				

*** = analysed using** *Mann- Whitney test*

**** = significant (p <0.05)**

Table 1 presents the results of statistical analysis for the mean macrophage cell counts on day-1, comparing the control group, pharmacological intervention with glutamine administration, and treatment with glutamine administration and inoculation of A. baumannii germs. Significant differences were observed (p < 0.002). Between-group statistical analysis revealed significant differences in macrophage cell counts between the control group and glutamine (p < 0.009), control with glutamine treatment and inoculation of A. baumannii germs (p < 0.009), and the group receiving glutamine and glutamine with inoculation of A. baumannii germs (p < 0.009).

Table 2: Analysis of macrophage count comparison on day -5

	Mean ± SD	**P-value**	**C-Glutamine**	**C-Glutamine+ *A.Baumanii***	**Glutamine-Glutamine+ *A.Baumanii***
Control	109.20 ± 2.490	0.000			
Glutamine	116.40 ± 4.827		0.007	0.005	0.000
Glutamine+ *A.Baumanii*	101.60 ± 2.73				

*** = analysed using** *Mann- Whitney test*

**** = significant (p <0.05)**

Table 2 shows the results of statistical analysis of the mean number of macrophage cells on day 5 in the control group, pharmacological intervention of glutamine and treatment of glutamine administration and inoculation of *A. Baumanii* germs where significant differences were obtained. (p = 0.000). Statistical analysis of between groups showed a significant difference in the macrophage cell count between the control group with glutamine (p < 0.007), control with glutamine treatment and inoculation of *A.Baumanii* germs (p

< 0.005) and the group giving glutamine and glutamine with inoculation of *A.Baumanii* germs (p = 0.000)

Table 3: Analysis of macrophage count comparison on day -7

	Mean ± SD	**P-value**	**C-Glutamine**	**C-Glutamine+ *A.Baumanii***	**Glutamine-Glutamine+ *A.Baumanii***
Control	94.20 ± 3.421	0.000			
Glutamine	109.40 ± 4.450		0.000	0.053	0.000
Glutamine+ *A.Baumanii*	89.60 ± 1.673				

*** = analysed using** *Mann- Whitney test*

**** = significant (p <0.05)**

Table 3 shows the results of statistical analysis of the mean macrophage cell count on day 7 in the control group, pharmacological intervention of glutamine administration and treatment of glutamine and inoculation of *A. Baumanii* germs where significant differences were obtained. (p = 0.000). Statistical analysis of between groups showed significant differences in the macrophage cell counts between the control group with glutamine (p = 0.000), control with glutamine treatment and inoculation of *A.Baumanii* germs (p < 0.05) and groups providing glutamine and glutamine with inoculation of *A.Baumanii* germs (p = 0.000).

Table 4: A analysis of the comparison of the macrophage count in the control

	Mean ± SD	**P-value**	**D-1/D-5**	**D-1/D-7**	**D-5/D-7**
Control					
D-1	82.60 ± 8.877				
D-5	109.20 ± 2.490	0.007	0.042	0.042	0.043
D-7	94.20 ± 3.421				

*** = analysed using** *Mann- Whitney test*

**** = significant (p <0.05)**

In Table 4 shows the results of the analysis of the number of macrophage cells in the control group which was assessed from the time of administration where in the control group there was a significant difference p < 0.007 in the number of macrophage cells. The analysis test comparing the administration time of day -1 and day-5 obtained p < 0.042. day-1 and day -7 p < 0.042 and day -5 and day-7 p < 0.043.

Table 5: A analysis of the comparison of the macrophage count in the Glutamine

	Mean ± SD	**P-value**	**D-1/D-5**	**D-1/D-7**	**D-5/D-7**
Glutamine					
D-1	105.80 ± 7.563				
D-5	116.40 ± 4.827	0.02	0.034	0.3	0.004
D-7	109.40 ± 4.450				

*** = analysed using** *Mann- Whitney test*

**** = significant (p <0.05)**

The results of the analysis of the macrophage cell count in the Glutamine group where assessed from the time of administration where there was a significant difference p < 0.02 in the macrophage cell count. The analysis test comparing the time of administration of day -1 and day-5 obtained p < 0.034. day-1 and day -7 p < 0.3 and day -5 and day-7 p < 0.004.

Table 6: A analysis of the comparison of the macrophage count in the Glutamine+ *A.Baumanii*

	Mean ± SD	**P-value**	**D-1/D-5**	**D-1/D-7**	**D-5/D-7**
Glutamine+*A.Baumanii*					
D-1	119.60 ± 2.968				
D-5	101.60 ± 2.793	0.000	0.000	0.000	0.000
D-7	89.60 ± 1.673				

*** = analysed using** *Mann- Whitney test*

**** = significant (p <0.05)**

The results table 6 of the analysis of the macrophage cell count in the Glutamine group and the inoculation of *A.Baumanii* germs where assessed from the time of administration obtained a significant difference p = 0.000

in the number of macrophage cells. The analysis test comparing the time of administration of day -1 and day-5 obtained p = 0.000. day-1 and day -7 p = 0.000 and day -5 and day-7 p = 0.000.

Discussion :

This study aims to prove that the administration of glutamine can increase the number of macrophage cells. The increase in macrophage proliferation can occur because the amino acid glutamine has a role in the metabolism of macrophage cells involved in the immunity process [21]. Glutamine is an important source of nutrients that have biological effects [22,23]. The formation of energy from glutamine consists of sequential steps starting with the deamination process of glutamine by the enzyme glutaminase to form glutamate [24]. Glutamate forms ketoglutarate which has undergone the process of hydrolysis. further enter the Krebs cycle to produce energy [25]. The amino acid can be directly into energy or converted into glucose through the process of gluconeogenesis process. According to in vivo and in vitro study, glutamine acts as the main energy source in immune cells such as lymphocytes, macrophages and fibroblasts [23,26,27].

Oral administration of glutamine at a dosage of 1g/kgBW/day has been shown to enhance both the quantity and phagocytic activity of macrophages against A. baumannii bacteria. According to Ardawi, a dose of 1g/kgBW/day is considered effective in significantly increasing macrophage numbers. The 7-day treatment duration aligns with the findings of Rogero's research, demonstrating an increase in macrophage count and function [7,28].

In this research, the mean macrophage cell count increase at H-1 was found to be 82.60 ± 8.87 in the control group, 105.80 ± 7.56 in the glutamine group, and 119.60 ± 2.96 in the glutamine against A. baumannii group. There were significant differences ($p < 0.05$) in the macrophage cell count increase on day -1.

Comparison of the mean macrophage cell count on day 5 revealed values of 109.20 ± 2.490 for the control group, 116.40 ± 4.827 for the glutamine group, and 101.60 ± 2.73 for the glutamine against A. baumannii group. In this comparison, the macrophage cell count was higher in the glutamine group than in the control group, and there was a significant difference between the groups ($p < 0.05$)

The mean macrophage cell counts on day 7 was 94.20 ± 3.420 in the control group, 109.40 ± 4.450 in the glutamine group, and 9.60 ± 1.673 in the glutamine against A. baumannii group. On day 7, there was a decrease in the macrophage cell count in the glutamine against A. baumannii group, and the count was lower than in the other groups

The primary energy source for immune cells under normal conditions is glucose. However, in inflammatory conditions such as burns, the demand for glutamine by the cells increases [29]. If this condition persists, the body's glutamine reserves will decrease, resulting in an imbalance between supply and demand. This ultimately hinders optimal immune cell functions [7,30] This theory was confirmed in the present study.

"The results of this study indicate a difference in the mean number of macrophage cells in the Glutamine treatment group against A. baumannii on day-1 (89.60 ± 1.673), day-5 (101.60 ± 2.793), and day-7 (89.60 ± 1.673). Statistical tests revealed significant differences ($p < 0.005$) between macrophage cells compared on these days. The findings suggest that glutamine has a positive effect on enhancing the phagocytic ability of macrophages, potentially by providing NADPH for enzymatic reactions in the phagocytosis process

Glutamine, serving as a precursor of arginine through conversion to citrulline, further transforms into arginine. The reaction of arginine with NADPH enhances the secretion of nitric oxide (NO) by macrophages. Consequently, the provision of glutamine results in a heightened phagocytosis ability.

In the study of glutamine against A. baumannii on day -1 and day-5, there was an increase in the mean macrophage cell count, but on day-7, the macrophage cell count was lower. The release of glutamine into the circulation depends on its availability within the organs in burn wounds during hypermetabolic/catabolic conditions. Additionally, in infectious conditions, it may cause hemostasis disorders in amino acid metabolism within tissues [6,7].

In response to *A. baumannii* infection, macrophages and monocytes are among the first to initiate an early response. In vivo phagocytosis of A. baumannii by macrophages can be observed as early as 4 hours after

infection [20]. Furthermore, macrophages produce high amounts of MIP-2, IL-6, and TNF-α as an early response to A. baumannii infection [29]. According to this study, there was an increase in the mean macrophage cell count on day 1 (119.60 ± 2.96). Other literature suggests that macrophages can phagocytize more than 80% of bacteria within the first 24 hours [2,8].

The glutamine treatment resulted in a significant difference in the number of macrophage cells between the control group and the glutamine group. This study suggests glutamine as a potential alternative nutrient for burn patients infected with A. baumannii, supporting macrophage cell activity as a vital component of the body's immune system. However, it is important to note that this study has limitations, as it only examines the effect of glutamine on macrophage cells. Nonetheless, providing glutamine can help support the immune cell needs during the treatment period, preventing a decline in the patient's immune response and subsequently reducing the risk of morbidity and mortality in burn patients. This, in turn, may enhance the patient's survival rate.

CONCLUSION

Increasing the macrophage count cell affects the function of the immune system so that it increases the phagocytic activity of macrophages against *A.Baumanii* infection. glutamine can support the needs of immune cells during the treatment period so that the patient's immune response does not decrease which further reduces the risk of morbidity and mortality in burn patients so that patient survival rates can increase.

Ethical Clearance
This research has been approved by the Research Ethics Committee, Faculty of Dental Medicine, Universitas Airlangga, with ethical clearance number 956/HRECC.FODM/XII/2022

Conflict Of Interest
There is no conflict of interest in writing this research report

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Author Contribution
All authors have the same contribution in writing the report on the results of this study, from the stage of proposal preparation, data search, and data analysis, to the interpretation of research data and presentation of the final report.

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