

**CLINICAL SIGNIFICANCE OF NEUTROPHIL-TO-LYMPHOCYTE RATIO AS
AN EARLY PREDICTIVE MARKER OF PROGNOSIS IN PATIENTS WITH
BURN INJURY**



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By

(FAISAL LATIF)

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ABSTRACT

Introduction: Severe burn injury is followed by several systematic inflammatory changes as a part of body's initial response to the physiological stress. These changes reflect in Complete Blood Count measurements and can help predict the prognosis in the early stages. One of these measurements is the neutrophil-to-lymphocyte ratio (NLR), which reflects the imbalance between the inflammatory response (neutrophils) and the immune response (lymphocytes).

Objectives: The link between the neutrophil-to-lymphocyte ratio and burn prognosis has not been studied in Pakistan yet. The objective of our study is to assess the neutrophil-to-lymphocyte ratio as a poor prognostic factor in severely burned patients. This early identification of systemic inflammatory indicators can provide significant information about the burn prognosis.

Methodology: We enrolled adult patients with burn injuries at burn units as per the study's selection criteria. Their demographic information, burn characteristics, and CBC results were collected within the first 3 days post-burn injury. Patients' prognosis was evaluated after 6 weeks of data collection by investigating the total length of hospitalization, total ICU length, and mortality. Data analysis was performed using the Statistical Package for Social Sciences version 25.

Results: Out of 75 patients, 49.3% were male and 50.7% were female, with a mean age of 31.94 ± 10.87 . Pearson's chi-squared and the independent sample t-test were applied to check the association and compare the means of the study variables. Age, burn degree, TBSA%, total ICU length, and NLR value were found to be associated with mortality ($P < 0.05$). Using linear logistic regression, the elevated NLR value was found to be an independent predictor of burn-related mortality.

Conclusion: Elevated NLR value is associated with a poor prognosis in severe injury burn patients. Our study results can help medical professionals spot patients who are more likely to have bad consequences, guide management decisions, and thus reduce burn-related deaths in Pakistan.

Keywords: Blood Cell Count; Mortality; Neutrophil-to-Lymphocyte Ratio; Severe Burn Injury

CHAPTER-I

INTRODUCTION

Burn injuries are a serious trauma that can be described as skin or other organic tissue damage primarily caused by heat, chemicals, electricity, or radiations. The damage can range from mild redness of epidermis and pain in minor burns to the deep destruction of skin layers, blistering, or scarring in severe burns. The majority of burn injuries are brought on by heat from hot liquids, solids, or fire. Lesser, the trauma can also result from friction, radiation, cold, electric, or chemical sources. Despite the fact that transfer of energy leads to tissue damage in all kind of burn injuries, a different source of injury can result in different physiologic and pathophysiologic consequences (1). For instance, a burn resulting from fire contact can instantly cause a deep burn, while burns caused by boiling liquids or steam initially have a tendency to look superficial due to the quick dilution of energy and the source. Likewise, burns from the electric source result in deeper tissue damage than the visible skin injuries. Numerous factors, such as the crystallization of water in skin tissues and indirect harm from reperfusion and ischemia, contribute to the development of frostbite. These mechanisms cause deep tissue injuries in addition to skin necrosis (2).

It is essential to define a burn injury as per its degree of severity, including its size and depth apart from burn etiology (3). There are three main types of burns according to the severity of damaged skin and other tissues (4): first-degree burns, second-degree burns and third-degree burns.

The first-degree burns are the mildest that only affect the epidermis. These burns typically cause redness, pain, and mild swelling. Treatment of first-degree burns includes over-the-counter pain relievers, cool compresses, and ointments (1, 3, 5).

The second-degree burns are more severe, affecting the outer layer of skin as well as the underlying layer (the dermis). These burns cause more severe pain, redness, and swelling, and may blister. They can also be treated with over-the-counter pain relievers, cool compresses, and ointments, but in certain cases may require medical treatment or a surgery (3, 5).

The third-degree burns are the most severe and cause the most damage, reaching the deeper layers of skin as well as muscles and bones. They can be white, black or brown, appear dry and leathery, and often cause little or no pain due to nerve damage. These burns will typically require skin grafting, surgery and long term recovery (3, 6).

The method of treatment for burn damage depends on its specific cause and the severity of the burn. For example, despite deep thermal burns being operated very early, the same strategy would be incorrect for frostbite, for which potential thrombolysis, moist rewarming, and watchful waiting are the preferred treatments. The majority of first-degree and second-degree burns heal without the need for surgery, but more serious burns require careful management, which may involve topical antimicrobial dressings or surgery (6). When it comes to treatment, burns are also classified as either major or minor according to the total body surface area (TBSA) covered. Minor burns are superficial and typically cover less than 10% of the TBSA. On the other hand, the amount of a burn that qualifies as a major burn is not typically well-defined. Some guidelines for classifying severe burn injuries include: >10% of TBSA in elderly individuals, >20% of TBSA in adults, and >30% of TBSA in children. Burns may sometimes be accompanied by physical trauma that impact other organs of the body or smoke inhalation in addition to skin injury (7). Infection and other complications can also slow the healing process in burn injury. Because of these complex differences in etiology and severity, burns are highly difficult to cure and their treatment require a high level of good judgment throughout the entire procedure (8).

The body responses to a major or severe burn injury are also make it unique for the treatment. Burn damage causes both local and systemic stress reactions in the affected body. Locally, the burned area experiences swelling, inflammation, and pain as the immune system of the body mobilizes to fight against infection and encourage recovery. In systematic response, the body releases stress hormones like cortisol and adrenaline, which can raise blood pressure, blood sugar, and heart rate. This reaction aids in mobilizing resources to promote recovery and ward against complications. Medical experts must keep an eye on and control these reactions to ensure that burn patients heal as well as possible (9, 10).

The prognosis for burn patients depends on a number of variables. The size, depth, and placement of the burn on the body can all significantly affect the outcome. The patient's age and general health, including any pre-existing medical disorders, can also have an impact on how well they recover (1, 11). Additionally important considerations are the efficiency and quality of medical care, including early diagnosis, efficient wound care, and infection control (5, 6, 12). The prognosis may also be impacted by the existence of comorbidities such as infections, organ malfunction, or inhalation damage. Long-term recovery depends on rehabilitation and support services including physical and occupational therapy (9, 13, 14).

For patients with minor burn injuries, the prognosis is generally favorable. These burns typically affect only the outermost layer of the skin, known as the epidermis, and are often referred to as superficial or first-degree burns. With proper care, such as cool water application, over-the-counter pain relief, and keeping the area clean and protected, minor burn injuries usually heal within a week or two without leaving any lasting complications (15). However, it's important to monitor for signs of infection and ensure proper wound care to promote healing and minimize scarring. Major degree burns, such as second-degree and third-degree burns, have a more guarded prognosis compared to minor burns. These burns can extend deeper into the layers of the skin and may cause significant tissue damage. The healing process for major burns is more complex and often requires specialized medical care, including wound debridement, skin grafting, and intensive monitoring for complications such as infections and scarring (15, 16).

In addition to the extent, size, and location of the burn, lab tests are also crucial in figuring out how burn patients would survive. Complete blood counts (CBCs) are routinely performed laboratory tests to look for anemia or infections, electrolyte levels to determine the fluid balance in the body, liver function tests to look for signs of the liver's health, tests for kidney function to for the kidneys' function and coagulation profiles to investigate blood clotting time. Procalcitonin and C-reactive protein (CRP) testing can also be used to assess the severity of the inflammatory reaction. These tests help medical practitioners determine burn patients' prognoses and provide therapeutic advice in conjunction with clinical examination (17-19).

Among the above-mentioned investigations, CBC is the most common, cheap, and readily available laboratory investigation that provides valuable information about the patient's overall health and helps monitor any potential complications. CBC measures various components of the blood, including red blood cells, white blood cells, and platelets. Changes in these levels in burn patients can indicate anemia, infection, or impaired immune function, which can impact the patient's prognosis. For example, a low white blood cell count may suggest a weakened immune system, making the patient more susceptible to infections (20, 21).

Furthermore, some values extracted from CBC can help predict the prognosis of burn patients. For example:

1. **RCC (Red Cell Count):** The term "red cell count" (RCC) refers to the quantity of red blood cells in a specific amount of blood. It is a gauge of the blood's ability to carry oxygen. The burn injuries severity can be estimated using the red blood cell count. A lower count may indicate a more serious burn and can aid in prognosis prediction (22).
2. **RDW (Red Cell Distribution Width):** RDW counts the variations in red blood cell size. Increased cell size variability, which might be an indication of underlying diseases including anemia or inflammation, is indicated by an elevated RDW value. The patient's reaction to specific therapy and overall prognosis can be learned through monitoring RDW (22, 23).
3. **PLT (Platelet Count):** PLT measures the level of platelets in the blood. The clotting of blood depends heavily on platelets. A decreased platelet count may signal a poor prognosis in burn patients due to the increased risk of bleeding and other complications (23).
4. **PDW (Platelet Distribution Width):** PDW calculates the range of platelet sizes. The capacity to clot may be impacted by platelet malfunction or destruction,

which may be indicated by an elevated PDW score. Assessing PDW can assist in determining the likelihood of thrombotic or bleeding events in burn patients (24).

5. **NLR (Neutrophil-to-Lymphocyte Ratio):** NLR is a simple laboratory test that can be used as a prognostic factor in various medical conditions, including cancer. The NLR is calculated as the ratio of the number of neutrophils (a type of white blood cell) to the number of lymphocytes (another type of white blood cell) in the blood (25-27).

NLR has been studied as a potential prognostic tool in a variety of diseases. Some studies have found that an elevation in NLR value is linked with a poor prognosis in various diseases such as Cancer including breast, lung, colon, and gastric cancer. Cardiovascular disease. It has also been linked with an increased risk of cardiovascular events (28), Chronic Obstructive Pulmonary Disease (COPD (29), Rheumatoid Arthritis (30) ,Acute pancreatitis (31) , and kidney disease (32).

Thus, the utility of NLR as a prognostic tool in these diseases has been proven in several studies. In relation to these investigations, the current study focused on the **“Assessment of NLR as a poor prognostic factor in burn patients”**. The Neutrophil-to-Lymphocyte Ratio significance in anticipating physiological stress in the human body lies in immune response of the body under stress. The immune system reacts to stress on the body by producing numerous cells and substances to fight the threat, regardless the stress is brought on by an illness, an injury, or other circumstances (33).

Two categories of white blood cells, lymphocytes and neutrophils, work crucially to this immune response of the body. As the body's initial defense against infection or damage, neutrophils aid in pathogen elimination by engulfing and killing germs. Neutrophils do this as the first line of defense for the host immune system towards pathogenic invaders through a variety of mechanisms including chemotaxis, phagocytosis, granular proteins, the release of reactive oxygen species (ROS), and the generation and release of cytokines (33, 34). Neutrophils also represent as the primary effector cells that arise during the systemic inflammation response (SIRS) and serve a crucial regulatory function in adaptive immunity. As innate immunity regulators, neutrophils attract, activate, and

direct other immune cells. They do this by secreting a variety of immunomodulatory cytokines, pro-inflammatory, and chemokines that can improve the attraction and effector capabilities of other immune cells, including dendritic cells (DCs), NK cells, B cells, CD4 lymphocytes, CD8 lymphocytes, T cells, and the mesenchymal cells. These phenomena result in an increase in neutrophil count into the blood stream in physiological stress (35).

On the other hand, Lymphocytes, such as T cells and B cells, have responsibility for more focused immune reactions. These cells generate antibodies, direct immune cell activity, and identify and focus on particular antigens. The number of lymphocytes may decrease during physiological stress as a result of things like the death of cells (apoptosis) or redistribution to inflammatory areas. This lymphocyte redistribution is a typical immune system reaction to fight off the stressor and target the areas that need an immunological response. The drop in lymphocyte counts might also be attributed to the release of hormones associated with stress like cortisol. The sum of these elements causes a brief drop in lymphocyte count under physiological stress (36).

The combination of an increased number of neutrophils and a decreased number of lymphocytes leads to a high neutrophil to lymphocyte ratio (NLR). This increased ratio suggests an immune response that is unbalanced, with neutrophils predominating over lymphocytes. It implies an elevated inflammatory state and possible immune system dysfunction. As a result, the NLR is regarded as a physiological stress signal since it demonstrates the body's efforts to deal with the stressor and reestablish homeostasis.

A comprehensive understanding of these phenomena makes it easy to acknowledge why the neutrophil-to-lymphocyte ratio (NLR) is high in burn patients who are under physiological stress. likewise in other illnesses, the Immune cells are activated and inflammatory mediators are released by the body in response to burn damage. This imbalance of neutrophils and lymphocytes that is indicated by a high NLR in burn patients points to an inflammatory response that is overly active. Multiple organ dysfunction syndrome (MODS), sepsis, and other consequences like systemic inflammatory response syndrome (SIRS) and sepsis can also result from this ongoing inflammation. In consequence, the NLR value can assist predict the likelihood of

complications and mortality in burn patients by acting as a marker for the intensity of the inflammatory response and reduce the global burden of burn deaths (37, 38).

Burn-related mortality from major degree burns has a significant global burden. It is declared as a major public health issue by World Health Organization that leads to an estimated 180,000 annual fatalities. The poor prognosis and outcomes in burn cases can be related to a number of things, such as insufficient resources for burn management, restricted access to high-quality healthcare, and a lack of knowledge and preventative measures. There may not be enough specialized burn centers and qualified medical personnel in many low- and middle-income nations, where burn injuries are more common. Receiving proper medical attention may take longer as a result, raising the danger of complications and fatality. The socioeconomic variables, such as inadequate education and poverty, might contribute to risky living arrangements and behaviors that raise the risk of burn injuries. Moreover, if post-burn patients have access to specialized burn care, the potential complications like systemic inflammatory reactions and altered immunological state are frequently present, which place a considerable stress on clinical care and nursing (39).

It is critical to give preference to burn prevention measures, increase access to high-quality healthcare, and improve burn management capacities in order to alleviate this global burden. This entails funding burn prevention and education initiatives, assuring the availability of burn treatment facilities with the necessary facilities, educating medical personnel on how to treat burn victims, and raising public knowledge of burn safety precautions. Moreover, Systemic inflammatory markers and other prognostic indicators can provide important information regarding the severity of the burned injury and how well the individual responded to treatment, which can help lower the worldwide burden of burn deaths (39, 40). Healthcare professionals can identify individuals who are more likely to experience difficulties by tracking these markers and modifying their treatment regimens accordingly. Early identification of systemic inflammation can lead to rapid therapies, such as giving the right antibiotics or putting anti-inflammatory management measures in place. This proactive approach can eventually lower the number of burn-related deaths worldwide by delaying the progression of complications, enhancing patient

outcomes, and improving patient care. overall, if these those factors are addressed properly at global level, it may be simple to lower the number of burn-related fatalities worldwide and enhance the general prognosis for burn victims (24).

In Pakistan, like other underdeveloped countries, burn injuries are a significant but understudied subject. According to the Global Burden of Disease 2010 report, Pakistan has an age-standardized death rate of 5.8 per 100,000 people for injuries brought on by heat, fire, and boiling substances. The burn-associated death rate among individuals between the ages of 15 and 55 was estimated by a burn facility-based investigation to be considerably higher, at 10.2 per 100,000 people. In a recent national study, the burn fatality rate in Pakistan was found to be 6.5%, with a Karachi burns center having an abnormally high death rate of 55.9% (41). There are several factors that contribute to such a high ratio of burn related deaths. Due to a number of circumstances, burn-related deaths are considerable in Pakistan. Firstly, there is a significant prevalence of injuries caused by burns in the nation, which frequently come from mishaps at home, in the office, or as a result of risky procedures. Secondly, a delay in receiving the right medical care for burn injuries may be caused by a lack of access to high-quality healthcare facilities, particularly in remote locations. The high incidence of fatalities is also attributed to inadequate knowledge of burn prevention and first aid techniques. The difficulties in giving burn victims the best care is further exacerbated by the Lack of specialist burn facilities and skilled medical personnel (42, 43).

In addition to these socioeconomical issues, lack of information on systemic inflammatory indicators might make it more difficult for medical professionals to determine how serious a patient's condition is, which can increase the risk of burn-related mortality. Without this vital information, it is difficult to identify individuals who are more likely to experience difficulties and to administer prompt interventions(40, 42). Delays in diagnosis and treatment of systemic inflammation may cause complications to worsen and perhaps result in fatalities. Having access to detailed information on systemic inflammatory indicators can assist guide treatment choices, enhance patient outcomes, and eventually lower the number of fatalities caused by burns. Therefore, the purpose of this study “**Assessment of NLR as a poor prognostic factor in burn patients**” was to

assess NLR's predictive relevance in patients with burns injuries. This early identification of systemic inflammatory indicators can give significant information for reduce the burn-related deaths in Pakistan. Early intervention and focused treatment of these patients can help stop the progression of problems; improve patient outcomes by allowing for prompt detection of systemic inflammation and eventually lower the risk of poor prognosis.

1. Objectives

- ✓ To determine the association of NLR with poor prognosis in patients with burn injury.
- ✓ To check association among the dependent variable (prognosis: survivors vs. non-survivors) and other demographic and hematological variables of the study.
- ✓ To determine whether the NLR value is an independent predictor of mortality.

CHAPTER-II

LITERATURE REVIEW

Burns injury is a life-threatening condition. All age groups can experience significant rates of morbidity and mortality from major burn injuries (44). According to the annual report of burn related mortality rates in the United States of America from 1990 to 2016, over the 26-year study period, the relative casualties from burn injuries remained constant. In both 1990 and 2016, Alaska experienced the highest percentage of burn incidence, closely surpassed by southeastern states. When incidence was considered, Mississippi and Alabama had the greatest relative mortality in 1990, and their mortality-incidence ratio went up in 2016. Additionally, 35 states showed a rise in the relative fatality of burn injuries during the course of the study (45). Regarding the trends of burn injuries in Europe, Brusselaer *et al.* (46) conducted a systemic review of the burn etiology, incidence, mortality, and morbidity. In this systematic review, they did literature research for studies on major burn injuries incidents in Europe from year 1986 to 2009. 76 studies with almost 186,500 patients were discovered. The annual incidence of fatal burns had declined over time, ranging from 0.2 to 2.9 per 10,000 individuals. 60% of patients were male, and almost 50% of them were patients under the age of 16. Scalds, Flame and contact burns were the most frequent causes of burns. Scalds were the most frequent injury in youngsters. The mortality rate has been dropping and ranges from 1.4% to 18% in this region. Major risk factors for death included advanced age, a bigger burn area, and chronic illnesses. Sepsis and organ failure were common causes of death. The two leading factors in early death were burn shock and inhalation injury.

These statistics are about the first world countries; in contrast, the incidence of burn related deaths in middle income countries are alarming. Among all burn related deaths worldwide, 90% of burn-related fatalities occur in low- and middle-income nations, which is quite alarming. Over 9 million people experience burns from fire, heat, and hot substances every year. Although the financial toll of these injuries is still not fully recognized, it is crucial to calculate it in order to devote resources for better access to care (39). A recent study conducted by Opriessnig *et al.* (2) in 2023 aimed to get a deep understanding of burn injuries worldwide. The authors looked at data from different

continents to learn about demographics, the prevalence of burns, infection rates, fatalities, and treatment modalities like dressing changes. The purpose of this thorough investigation was to find any significant variations or parallels in clinical practices that could be used to improve burn care internationally. The patient and hospital demographics, as well as aspects relating to burn injuries and their treatment, were examined in this study using statistical analysis. Additionally, they examined outcomes including infections, mortality, the length of ICU stays, and crucial aspects of burn dressings. It's interesting that healthcare professionals from all around the world have similar opinions and needs about burn care management and the best burn dressing. Significant disparities between continents were discovered by the study, with Australia having the highest rates of hospitalization and ICU stays. Africa has the highest rates of burn infections, death, and occurrences among youngsters due to its higher concentration of low-income nations. In conclusion, they found pronounced epidemiological variations in burn injuries and treatment among continents, which are probably caused by different infrastructure and conditions.

Gerstl *et al.* (47) examined the Global Burden of Disease Study 2019 which provided information on burn cases, fatalities, and disability-adjusted life years (DALYs). The authors multiplied the GDP per capita and DALYs for 190 nations to determine the economic burden. They also looked at patterns from 2009 to 2019, with an emphasis on South Asia and sub-Saharan Africa. This study stated that the prevalence of burns was higher than in the United States in South Asia. In comparison to the United States (0.7 per 100 patients), South Asia and sub-Saharan Africa had greater mortality rates (2.6 and 1.8, respectively). DALYs were a significant part of the burden of burn injuries. The GDP was significantly impacted by the significant economic losses brought on by burns. The authors of the study bring attention towards doing more work in the field of burn treatment, despite the advances.

In low-middle-income countries, one of the dilemmas is that the advantages of early excision in burn surgery may be lost with few resources, despite the benefits being obvious. In order to address this, Wong (48) did a systematic review and meta-analysis were performed in order to identify the surgical outcomes, including mortality, length of

stay, and sepsis to compare them with high-income nations. The purpose of the study was to examine the timing of excision in LICs and gauge how it affects patient outcomes. Researchers aimed to acquire a thorough knowledge of the variations in burn surgery prognosis between these two settings by looking at the data from both LICs and HICs. Researchers chose 41 out of 278 citations in both high- and low-income nations for full-text analysis. They found 16 trials that qualified as such through this technique. According to the results, both LICs and HICs have lower lengths of stay (LOS) following early excision. Intriguingly, death rates following late excision are lower than those following early excision in both HICs and LIC. Furthermore, the researchers found that mortality is reduced with late excision, and this pattern holds true even when the elderly are not included, when looking at a subgroup of elderly patients in HICs. Early excision also works well at lowering sepsis cases in both LICs and HICs. These results underline how crucial it is to take into account the time of excision in burn surgery for the best results in various socioeconomic contexts. However, it is difficult to evaluate the results of this study due to the different definitions of age, timing of excision, kind of comparing% TBSA, inclusion of inhalation damage, co-morbidities, and unquantified resource availability. That is why, it is important to draw reliable findings about how early excision for burns affects low- and middle-income nations.

Glaser *et al.* (49) conducted a registry study to objectively assess the existing practice of early burn tissue excision, its determinants, and its effects on patient outcomes across all German(a high-income-nation) burn clinics In this registry trial, the subjects were arranged into groups based on whether their initial surgical procedure was done soon after the burn (Early excision, done within 72 hours) or far later (Late excision, done after 72 hours). Apart from descriptive statistics, regressions analysis was used to determine the proportion of EE vs. LE and assess variables like inhalation injury, age, total body surface area (TBSA), burns etiology, obesity, and admission time. All patients who underwent at least one surgical procedure were questioned three years in a row for this study. Key patient outcomes, including mortality, length of hospital stay, and number of procedures, were examined for each group. Regression analyses were conducted to estimate the effect of EE on mortality, with a significance threshold set at $p < 0.05$. The results of the study highlighted that factor like age, burn etiology, inhalation injury, and

weight did not affect the difference between EE and LE. Patients with EE experienced considerably shorter median hospital stays (18 days vs. 21 days, $p = 0.01$) than patients with LE. For both groups, the average number of surgeries was comparable. Gross mortality in the EE group turned out to be comparable to LE. Moreover, this study showed the association between EE and TBSA burned, which is clinically sound and anticipated.

After a burn injury, early excision and skin graft continue to be the norm. Surgical competence, however, frequently restricts patient access to surgical intervention in a low-middle-income country. For this purpose, Gallaher (50) conducted a registry trial and described the adult burn patients' access to excision and graft and its association to burn-associated mortality in a sub-Saharan burn center. From 2011 to 2019, patients listed in the Lilongwe, Malawi's Kamuzu National Hospital Burn Registry were studied. The authors looked at patient traits, treatments, and results for people over the age of 16. To find the risk factors for death, updated Poisson regression analysis was utilized. There was a total of 573 patients who took part in the study. Among them, around 63% were males, with an average age of 30 years (median age range of 23-40). The total body surface area (%TBSA) averaged at 15% (with a median range of 8-26). It was found that flames were responsible for approximately 68% of all burns. Out of the patients, about 27% (154 individuals) undergo burn excision along with skin graft, and the usual waiting period for this procedure was around 18 days. After considering factors like age, %TBSA, and admission time, it was observed that operative intervention provided a significant advantage for the survival patients with fire burn injuries, with a relative risk (RR) of 0.16 (95% CI). While surgical intervention is crucial for improving outcomes in flame burn cases, many scald burn patients can be effectively managed with proper wound care. However, the study anticipated that due to limited resources, getting access to the operating room can be challenging, and burn victims may not always receive priority.

Yen (51) collected data from the National Health Insurance database to evaluate association of burn related death with burn epidemiology and associated risk factors in a population-based study in Taiwan (recently recognized as a high-income-country by

world bank 2022). The hospitalized burn patients between 2003 and 2013 were included (n=73,774). Age, the Charlson Comorbidity Index (CCI) and the R-Baux Score were observed to rise over time. There were also shorter hospital stays and a decline in the incidence of burns. The report demonstrates continued advancements in burn treatment provided in hospitals. There was no appreciable difference between the in-hospital mortality rates of 17.5/1000 in 2003 and 12.2/1000 in 2013. However, mortality was predicted by the following factors: male gender, older age, higher CCI score, inhalation injury, total burn surface area, and higher R-Baux score.

The recent literature on burn incidence identified that burn related mortality can be caused due to limited information of burn epidemiology and identification of associated risk factors. Accurate information about the epidemiology and etiologic burn patterns in low- and middle-income countries is inadequate and dispersed, owing to the substantial incidence of burn injuries in these regions. Gurbuz and Demir (52) sought to describe the epidemiologic traits and outcomes of burn injuries at the Burn Center of Turkey in a descriptive analysis. Retrospectively analyzing medical records, researchers divided patients into 10 sub-age groups in addition to the pediatric and adult age categories. A mortality rate of 2.5 percent was achieved with 24 fatalities out of the 946 individuals that were a part of the trial. The sub-age groups of 60 to 69, 80+, 18 to 29, 10 to 17, and 5 had mortality rates of 13.0%, 7.8 percent, 7.1 percent, 2.3%, and 0.5 percent, respectively. The 70 to 79 age group had the highest mortality rate at 33.3%. Inhalation damage, deep partially/full-thickness burns, age 18 years, total body surface area burned 20% (TBSA 20%), and fire-flame related burns were significant predictive variables for mortality in all age categories, according to multivariate regression analysis. The TBSA 60% section showed the highest connection, with a 25.9 times increased risk of death (P .0001). The 60+ sub-age group within the age groups also showed a significant connection with a 5.84 times higher risk of passing away. Additionally, the odds ratios for the sub-age categories of 18 to 29 and 29 to 59 climbed to 2.12 and 2.08, respectively. Surprisingly, there was no statistically significant difference in death prediction between the 0 to 17 sub-age group.

LM, NN (53) conducted a retrospective study with an emphasis on burn mortality; the study sought to evaluate gender-specific variations in burn patients in low-middle-income-nations. 5061 adult burn patients (16-64 years old) were the subject of a retrospective investigation from 2016 to 2018 at the Vietnam National Burn Hospital. Male patients were admitted earlier and more frequently (72.8%). While females were more likely to sustain scald injuries, males were more likely to sustain electrical and flame/heat injuries. In addition to having more profound burn injuries, more surgeries, and longer hospital stays, males also had greater burn extent. Although there were no appreciable differences in complications or overall mortality, the female group had a greater death rate for burn extents under 50% TBSA. In conclusion, there were differences in the burn results and features of male and female patients.

Bailey *et al.* (54) done a study in Dhaka, Bangladesh in June 2016, where information from 66 hospitalized burn patients and 88 burn patients who had passed away was gathered. The study looked at the patients' clinical data and demographic profile. Young adult males with lower socioeconomic level and children under the age of eight were high-risk categories for non-fatal burns. Flame, electrical, and scald burns were the most frequent types of non-fatal burns. Half of the patients who were discharged required surgical intervention and had mean 30 days of hospital stay. Injury severity, the requirement for surgery, and lack of earlier treatment were all factors linked to prolonged hospital admissions. High-risk populations included adult men and children under 8 years in death instances. The majority of deaths were caused by complications from flame burns, with the average total body surface area (TBSA) impacted being 46.4%. This study highlighted the incidence and seriousness of burn injuries, identify susceptible demographic categories, and identify prevalent causes of burns in Bangladesh. However, these results might also be pertinent to the prognosis and epidemiology of burns in other comparable low- and middle-income nations.

Khadem *et al.* (55) conducted a cross-sectional study in aim to accurately describe the geographic and demographic distribution of hospitalized burn cases in Iran's northeast from 2010 to 2015. The authors enrolled patients admitted to burn centers with acute burn injuries in this cross-sectional study. There were 3352 admissions in total, with

roughly 73.3% (2458). 62% (2065) of the patients were male, with an average age of 26.5 ± 19.3 years. The total death rate (717 patients) was 21%. Patients sent at this time had higher mean total body surface area (TBSA) ($50 \pm 26\%$), mortality rates (41.3%), length of hospitalization (20.1 ± 16.7 days), and burn severity index (8.7 ± 3.2) (all $p < 0.001$). The death rate in high TBSA group was 10 times higher than in patients with burn involving less than 20% TBSA (63% of patients had burnt TBSAs equal to or greater than 20%). Individuals with poorer abilities and competences were more likely to sustain burn injuries. This study emphasized on addressing the mechanisms and practices that cause severe burn injuries, as it is critical to give safety measures and preventive policies top priority in developing nations

Burn injuries are a major source of illness and mortality in developing nations. These are rather typical, frequently unintentional and unavoidable. Likewise in Pakistan, burn complications are a common problem. The 6.5% burn fatality rate in Pakistan leaves many survivors with long-term difficulties as a result of insufficient rehabilitation ⁽⁴¹⁾. B. Ali *et al.* (43) conducted a review aiming to draw attention to the physical and mental consequences of burn injuries patients in Pakistan. From March to May 2020, the authors looked for relevant papers using MEDLINE®, Google Scholar, and PubMed. Depression, PTSD, insomnia issues, anxiety, phobias, suicide thoughts, and personality changes are a few examples of psychological side effects in them. Scarring, discomfort, muscle atrophy, contractures, hypothermia, and infections were among a few of the physiological consequences. However, there is a lack of research on the occurrence and effects of long-term problems in post-burn patients. Pakistan also has a dearth of rehabilitation services and a high incidence of complications resulting from burns. In order to lower morbidity and enhance QOL, screening of burn patients for associated complications and their possible rehabilitation must be given top priority from the time of admission in Pakistan.

Better results and lower morbidity from burn injuries also depend on proper first aid care. However, there seems to be a global knowledge gap about burn control. Riaz *et al.* (42) purposed a study in objective to evaluate undergraduate students' knowledge and awareness of burn first aid treatment (BFAT) as well as the effect of training programs on that knowledge. The researchers conducted a cross-sectional survey in Karachi, Pakistan

with both medical and non-medical students. They used convenience sampling to send online surveys to 400 students. Using SPSS version 23.0 to examine the data, t-tests were used to determine relationships. To assess participants' understanding of BFAT, the study also computed a mean knowledge score. In this study, participants' knowledge scores were higher for medical students (5.81.6 vs. 3.61.5, P0.01) than for non-medical students. Both groups, however, still lacked a general understanding of burn first aid treatment (BFAT). When compared to students who did not receive formal training, students who did had a considerably higher mean knowledge score (P 0.01). The majority of pupils showed poor understanding of BFAT, highlighting the necessity of including this subject in the curriculum. To raise awareness and stop burn-related injuries (BRIs), the study also emphasized on educating the public through multimedia tools and to offer formal training programs.

In another cross-sectional survey, Binte Ali *et al.* (40) assessed the understanding and attitudes of medical students about burn consequences. Students from public medical institutes in Karachi, Pakistan, were asked to fill out a survey. The knowledge of burn complications among the 353 respondents varied depending on the condition. For instance, 86% knew what depression was, 53.3% knew what fear was, and 40.8% knew what post-traumatic stress disorder was. There were, however, understanding gaps, such as the 68% of students who believed scarring only happened in third-degree burns. Furthermore, 19.3% of respondents said it was false that all burn patients need surgery in order to recuperate. This study showed how medical students need better instruction on the physiological and psychological effects of burn injuries.

The prognosis of the severity of the victims has no agreement on the generally accepted rules. However, there are numerous grading systems that have been created to predict mortality and evaluate the clinical course. Turhan *et al.* (56) recently evaluated a generic scoring method frequently used in intensive care units along with four generally accepted burn-specific rating systems. The main goal of the study was to assess these models' prognostic skills and choose the best one for the patients in our Turkish patient population. In order to assess their link with mortality, the authors looked at a number of variables in their research, including gender, age, burn nature, total damaged surface area

(TBSA), and more. Same like the previous research, this study discovered that age, TBSA, full thickness burn %, burn kind, and inhalation injury were statistically significant factors linked to mortality. It's interesting to note that the study also found that the gender of the person was a significant risk factor for death. Furthermore, patient ABSI scores closely mirrored their actual fatality rate when comparing other burn mortality scoring systems. Thus, the results of the study implies that Abbreviated Burn Severity Index (ABSI) should takes into account all pertinent mortality-related characteristics.

Cheng *et al.* (57) also did their investigation in perspective to analyze a large sample of burn patients' characteristics and evaluated their burn index (BI) and other variables that can affect the prognosis. The authors acquired information from 106 burn facilities on the Chinese mainland, concentrating on patients who were admitted in 2014 and had burns that involve 30% of their entire body surface area. In order to conduct a retrospective analysis, they gathered data on epidemiological characteristics and outcomes. There were a total of 2483 individuals with severe burns with average age of 49 ± 16.77 years, and the male: female ratio of 2.25:1. The mean burn index (BI) was 39.5 ± 21.9 and the average total body surface area (TBSA) damaged by burns was $55.3 \pm 21.9\%$. The study documented that child experienced scald injuries in 81.07% of instances, whereas adults and seniors experienced flame injuries more frequently (66.89% and 74.31%, respectively). Nearly 46.48% of patients tried home remedies to treat their wounds, and 17.76% were admitted to the hospital more than six hours after the incident. and 46.48% of the patients treated their wounds with home treatments. The Burn index was found to be an unbiased prognosticator with an ROC curve of 0.941 and a mortality rate of 9.79%. In various age groups, higher BI levels were linked to a significantly higher mortality rate. Patients over the age of 60, those admitted more than six hours after the event, and those who sustained inhalation injuries were also more likely to pass away. For prevention, it's crucial to take into account the unique traits of various age groups, and BI can be a useful tool in determining the prognosis for individuals who have suffered severe burns.

The prognosis of burn patients is also challenging due to body's the non-infectious systemic inflammatory response, which is characteristic of thermal injuries. Over the past few decades, it is a topic of interest among the healthcare professionals to find biomarkers that help practice medicine in burn injury (58). The ability to recognize sepsis in burn patients using the well-known biomarker for infections, procalcitonin, has been demonstrated in literature. On the other hand, C-reactive protein didn't suppose improve the precision of infection detection in this population. Additionally, several cytokines had found as potential indicators in burn victims. Interleukin-6 as well as interleukin-8 have also revealed predictive usefulness for a variety of outcomes among these (59). However, it is difficult at times to have comprehensive clinical and blood monitoring plus the cost of these test on larger scale does not favor the low -middle-income countries. As a result, it is still a challenge to evaluate the clinical prognosis of burn patient in developing countries like Pakistan.

To address this problem, the researchers are putting emphasis on that issue and tried to find out some convenient and cheap methods that can predict the prognosis in severe burn injuries. One of the laboratory tests that is used the most frequently is the complete blood count (CBC). It is proved from the literature that in several medical disorders, inflammatory state and prognosis have been linked to various CBC components (32). The parameters generated from whole blood count have become attractive tools as researchers have come to understand the importance of readily accessible biomarkers in routine clinical practice. RDW has found to be linked with mortality in sepsis, cancer, hepatitis, and cardiovascular illnesses (60, 61). Likewise, the neutrophil-to-lymphocyte ratio (NLR) is a sign of systemic inflammation. In numerous illnesses, including cancer, heart failure, sepsis, and respiratory distress syndrome, the NLR has been linked to disease severity and survival (26-29, 32). In recent studies, patients with burns have showed benefit from the RDW-to-platelet ratio (RPR), the ratio of platelets to lymphocytes (PLR), and neutrophil-to-lymphocyte ratio (NLR). The dynamic profile of these biomarkers shows clear differences between disease survivors and disease non-survivors. So, the information from these biomarkers could be combined with clinical scores to help doctors understand and treat burn victims more effectively.

It has been identified that diverse modifications in CBC are brought on by severe burn damage (23). In this regard, Guldogan *et al.*, (24) analyzed the factors that are associated with mortality in extensive burn injuries. The study included 224 burn victims who have burns covering $\geq 30\%$ of TBSA. The parameter like age, gender, other comorbidities, and CBC measurements like hemoglobin count, WBC count, and PLT count were examined. All these factors had association with burn mortality rate ($P < 0.05$). Another most researched one is undoubtedly the onset of thrombocytopenia and its relationship to unfavorable outcomes. Hang *et al.*, 2019 and Lin *et al.*, 2022 (62, 63) evaluated the values of PDW and PPR as the independent predictors for the mortality in extensive burn injuries. RDW variations between heat injury survivors and non-survivors have also been explored more recently. Lai *et al.*, 2022 established that admission RDW value can be significant in predicting early mortality in extensive burn patients (64). However, a little is known about the condition of the inflammatory indices determined from CBC in burn patients. Adults with extensive burn injuries had higher fatality rates when their RPR was high (65). Additionally, greater NLR was linked to a higher chance of developing AKI following burn surgery (66). Procalcitonin is one specific inflammatory biomarker that has been used to predict survival of burn patients, although the findings have been inconsistent (67). While new inflammatory and prognostic indicators are still being sought for, it is imperative to identify more practical measures.

Fuss *et al.*, 2019 (68) found that those with higher NLR values had a higher risk of developing sepsis, a severe complication of burn injury characterized by inflammation throughout the body. It was a survey study which include clinical materials from 188 patients. The location of injury, kind, and depth of the burns were assessed on each subject. The findings of laboratory tests were also employed, including neutrophil-lymphocyte ratio, C-reactive protein, and ESR rate. According to the findings of the investigation, PCT and NLR were found to be more reliable markers for distinguishing SIRS from sepsis (systemic inflammatory response syndrome) as compared to ERS, WBC count, or CRP. The study also found that patients with higher NLR values had a greater risk of death

The findings from previous international studies suggest that NLR can be useful in predicting the risk of sepsis and death and may also be useful in monitoring treatment response in burn patients. Still, in Pakistan, the study on NLR as a prognostic indicator in severe burns has not been conducted. Other local studies found the NLR value to be a significant prognostic indicator in several chronic diseases like COVID-19, ST-segment elevation myocardial infraction, sepsis, cancer, and COPD (69-72). Murtaza *et al.* (73) did research on the clinico-pathological profile of burn patients through other CBC measurements such as TLC, Hb, and PLT, and the authors found these measurements to be important indicators of burn survival. But NLR value is not addressed specifically in the case of burn related mortality. So, to the best of our knowledge, we are pioneer at conducting the **“Assessment of NLR as a poor prognostic factor in burn patients”** study. The results of our research can assist in understanding the utility of NLR as a prognostic factor, and it can be used in combination with other clinical and laboratory parameters to guide patient management. This early identification of systemic inflammatory indicators can provide significant information about the prognosis. Keeping an eye on such signals can help medical professionals spot patients who are more likely to have bad consequences, guide management decisions, and thus reduce burn-related deaths in Pakistan.

1. Rationale of the study

The rationale of the study is to highlight significance of Neutrophil to Lymphocyte Ratio as a prognostic indicator in severe injury burn patients and hence help in the management of hospitalization, intensive care unit, prevention of complications, mortality prediction and counselling in hospitalized burn patients in local population.

2. Operational Definitions

Complete blood count is a blood test that measures various components of the blood, including red blood cells, white blood cells, hemoglobin, hematocrit, and platelets. CBC is measured by taking a blood sample and analyzing it using specialized laboratory equipment called “Hematology Analyzer”. The sample is typically obtained from a vein

in arm (commonly the median cubital vein), and the machine counts and measures the different components of the blood automatically.

Neutrophil-to-lymphocyte ratio It's a straightforward calculation that helps assess the balance between these two types of white blood cells. A simple division between the number of neutrophils and the number of lymphocytes in a given blood sample is done to calculate the neutrophil-to-lymphocyte count ratio.

Total body surface area It is a measurement used to estimate the extent of a burn injury. It is typically calculated using a specific formula, such as the Rule of Nines or the Lund-Browder chart, which take into account the different body regions and their corresponding percentages.

Rule-of-nine It is a method used to estimate the percentage of total body surface area (TBSA) affected by a burn. According to this rule, the body is divided into different regions, each representing a specific percentage of TBSA. For adults, each arm accounts for 9% of TBSA, the head and neck together account for 9%, each leg accounts for 18%, the front and back of the torso each account for 18%, and the groin area accounts for 1%. The assigned percentages are then added up to calculate the total body surface area.

3. Hypothesis

- **Alternate hypothesis:** Elevated admission NLR value is associated with the poor prognosis in severe injury burn patients.
- **Null hypothesis:** Elevated admission NLR value is not associated with the poor prognosis in severe injury burn patients.

CHAPTER-III

METHODOLOGY

1. Research Design

Analytical cross-sectional study

2. Clinical Settings

Burn units

3. Sample Size 75

A sample size of ~ 73 was calculated by using following formula. Keeping in view similar studies, 80 burn patients will be enrolled in this study.

$$n = \frac{Z^2 \times P (1 - P)}{e^2}$$

Where: $z = 1.96$ for a confidence level (α) of 95%, p = proportion (expressed as a decimal), e = margin of error.

$$z = 1.96, p = 0.05, e = 0.05$$

$$n = 1.96^2 \times 0.05 \times (1 - 0.05) / 0.05^2$$

$$n = 0.1825 / 0.0025 = 72.99$$

$$n \approx 73$$

The sample size is equal to 73

Online Sample Size Calculator

Confidence Level (α): 95% ▼

Margin of Error (e): 5 %

Population Proportion (p): 5 %

Population Size (N) (optional):

Calculate

Reset

Results

Your recommended sample size is: **73**

The sample size (n) is calculated according to the formula: $n = z^2 * p * (1 - p) / e^2$

4. Sampling Technique

Convenience sampling technique

5. Duration of Study

The duration of the study was 6 months after the approval of synopsis.

6. Selection Criteria

6.1. Inclusion Criteria

The study will include the patients who meet one or more following criteria:

- Adult patients of both genders presenting with burn injuries involving $\geq$ 20 of TBSA for second degree or $\geq$ 10% for third degree burns (74).
- Age between 15 to 60 years.

6.2. Exclusion Criteria

- Patients who arrive at burn unit more than 72 hours after burn injury.
- Patients having insufficient clinical data.

7. Ethical Consideration

Ethical approval of the project had taken from Ethical Review Board, Superior University, Lahore before its commencement.

- Informed written consent will be obtained from every participant.
- Secrecy of participants' personal information will be ensured.
- Dignity of participants will be of utmost important while collecting samples

8. Data Collection Procedure

A total of 75 adult patients presenting with burn injuries at burn units were enrolled for the study as per selection criteria. Patients' demographic information (age and sex), TBSA, burn depth, etiology, and CBC investigation within the first 3 days was collected. The NLR value was calculated by dividing the absolute values of neutrophil count and lymphocyte count with one another. The prognosis was evaluated after 6 weeks of data collection from the patients to investigate total length of hospitalization, total intensive care unit length, and mortality.

Sampling and Parameters:

Total Body Surface Area (TBSA%)

Evaluation of the total body surface area in burn patients was done using the Wallace Rule of Nines.

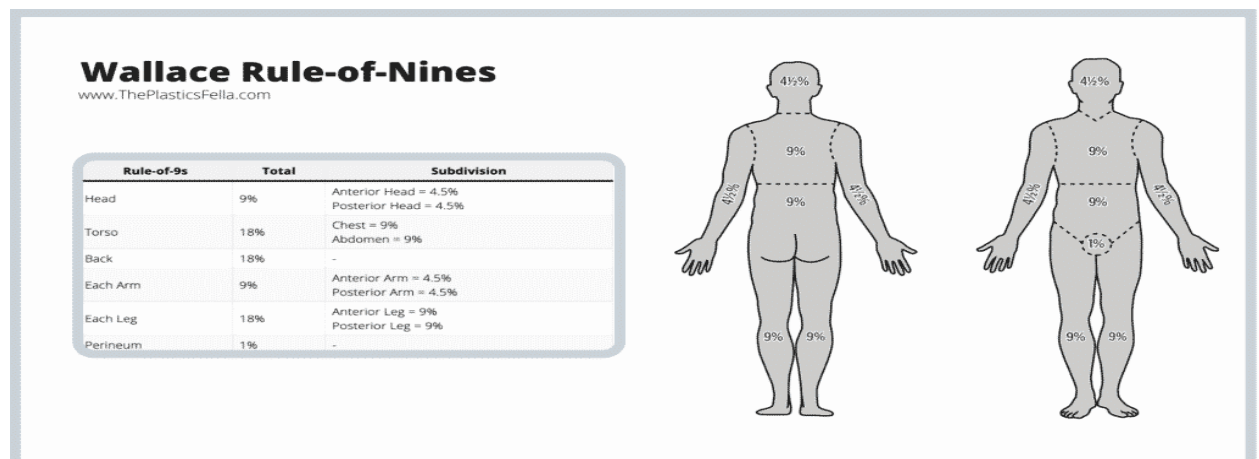


Figure 1- Percentage of total body surface area (TBSA) affected by a burn.

Burn depth

Burn depth and TBSA are the two essential elements of a burn assessment. One of the most crucial responsibilities of the clinical examiner is the evaluation of burn depth, which leads to the prognosis of whether a burn site is predicted to heal by itself within 21 days.

The estimation of burn depth included 5 key assessment parameters:

- ✓ There are three levels of burn depth: first degree, second degree, and third degree.
- ✓ The evaluation involves color, blanching, blisters, sensation, and Pain.
- ✓ First degree burns resemble a sunburn in that they are red, hot, painful, and lack blisters.
- ✓ Blisters appear on second degree burns because the dermis is affected.
- ✓ Charred, insensate, and leathery skin can occur in third degree burns.

Complete blood count (CBC)

Complete blood count (CBC) data show considerable hematologic alterations that follow a severe burn damage. We assessed trends in the CBC components in severely burned patients throughout the first three days following the injury and examine CBC component variations of the survivors and non-survivors. The time period from the date of hospital admission to the date of the final clinical follow-up or mortality was referred as overall survival. The main CBC measurements that we assessed included:

1. Hemoglobin levels (Hb Gm%)

It refers to the amount/concentration of hemoglobin in grams per deciliter. Normal values range between 12.0 and 15.5 in females and 13.5 and 17.5 grams per deciliter in males.

2. Red Blood Cell Count (RBC/mm³)

It refers to the amount/concentration of red blood cells per cubic millimeter of the blood. Normal values range between 4.2 and 5.4 million in females and 4.7 and 6.1 million/ cubic millimeter in males.

3. Total Leukocyte Count (TLC/mm³)

It refers to the amount/concentration of leukocytes (white blood cells) per cubic millimeter of the blood. Normal values range between 4000 and 11000/ cubic millimeter.

4. Total Platelet Count (PLT $\times 10^3/\mu\text{l}$)

It refers to the amount/concentration of platelets per microliter of the blood. Normal values range between 150 and 400 $\times 10^9$ per liter.

5. Neutrophil to Lymphocyte ratio (NLR %)

The NLR ratio was obtained by dividing the Absolute Neutrophil Count by the Absolute Lymphocyte Count. The normal range is between 1-2; NLR levels in adults that are higher than 3.0 and lower than 0.7 are considered abnormal and indicate a pathological state.

9. Data Analysis

The collected data was analyzed using SPSS (statistical package for social sciences) version 25.

- Normality of data was checked using skewness and Kurtosis.
- Continuous variables are expressed as Mean $\pm$ S.D.
- Categorical variables are expressed as percentage and frequency.
- Graphical representations have constructed as required.
- Pearson's chi-square test was applied to check the association among the categorical study variables.
- Independent sample t-test was applied to compare means between two study groups: survivors and non-survivor.
- Linear Logistic Regression Analysis was applied to check whether the NLR value is an independent predictor of Mortality.
- Level of confidence was set at 95 % and P-value ≤ 0.05 was considered as statistically significant.

Plan of Work

The plan of work of this study titled “Assessment of NLR as a poor prognostic factor in burn patients” is as follows:

Activity (Months)	1	2	3	4	5	6	7	8	9	10	11	12
Synopsis Approval												
Sample Collection												
Sample Processing and lab work												
Data entry and analysis												
Thesis writing												
Thesis submission												

CHAPTER-IV

RESULTS

Initially, we gathered the hematologic and demographic information of 89 individuals who fit the study's inclusion requirements. The prognosis has been assessed following a 6-week data collection period in order to inquire about mortality, the length of the intensive care unit, and the overall length of hospital stay. We were unable to get follow-up information from 11 patients for some reason. Additionally, three of the individuals were died due to other complications or comorbidities. Thus, 75 patients in total have been the subject of statistical analysis. Below is a flow chart that shows the total number of participants involved in the study.

4.1- Skewness and kurtosis for normality distribution

We have applied skewness and kurtosis to check the normal distribution of our study variables. Using the descriptive statistics, the results showed that our data had a near-normal distribution. Since the values for skewness and kurtosis are considered acceptable when between -2 and +2, we assumed that our data is normally distributed. George and Mallery (75) stated that the normal distribution of the data should be considered normal if the skewness ranges from -2 to +2 and the kurtosis ranges from -7 to +7. Also, when the sample size is larger than 30 or 40, the sample distribution is considered normal, and a violation of the normal distribution is not a major concern. This suggests that we can apply the parametric tests to our study variables (76).

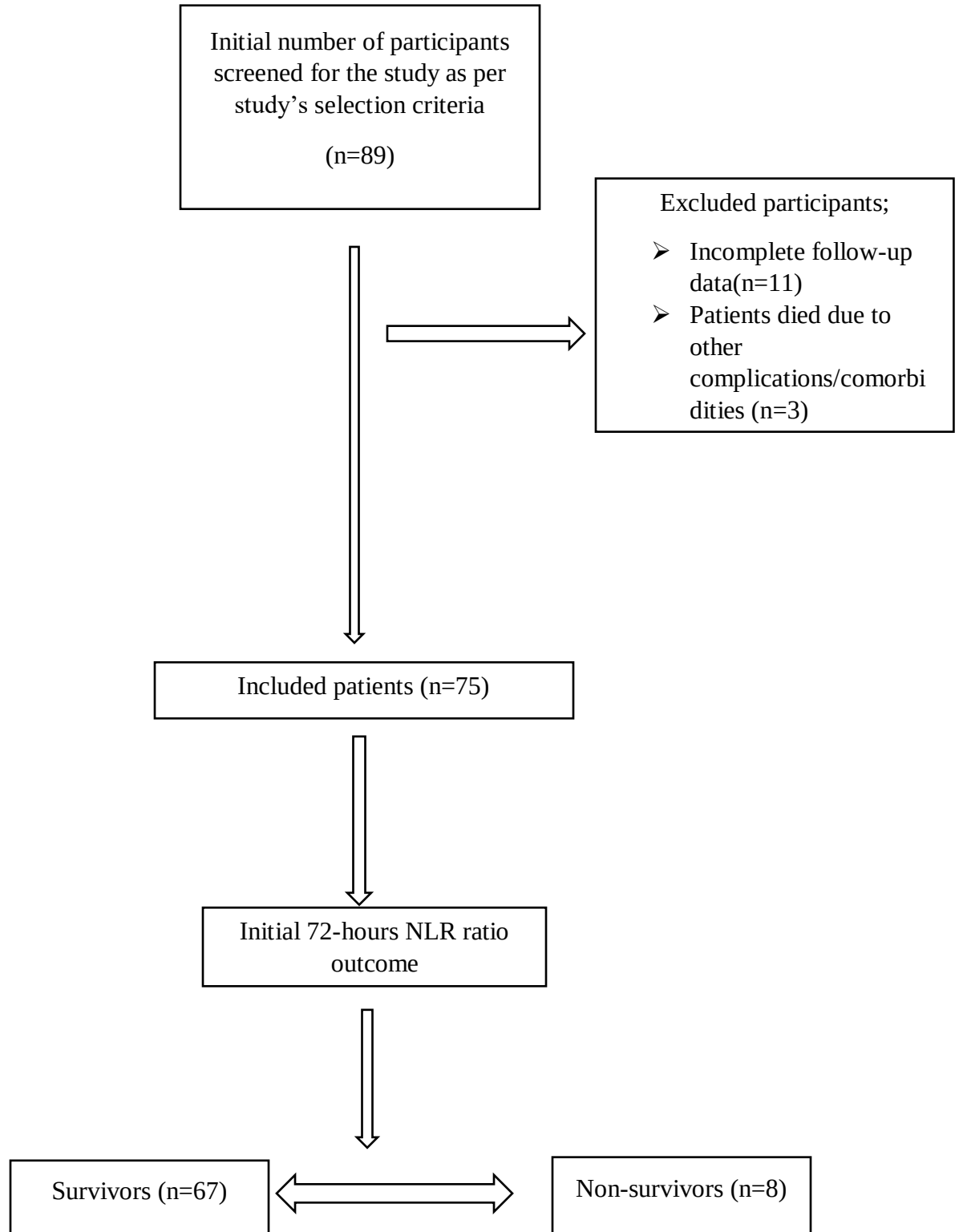


Figure 2- Flow chart depicting the participants of the study (n=75).

Table 4.1- Demographic characteristics of total study participants (n=75)

Study variables	Mean±SD	Frequency(%age)	Minimum value	Maximum value
Age	31.94±10.87	-	15	58
Male	-	37(49.3%)	-	-
Female	-	38(50.7%)	-	-
TBSA (%)	28.73±7.73	-	16	58
Second Degree of burn	-	51(68%)	-	-
Third degree of burn	-	24(32%)	-	-
ICU length(days)	9.53±5.84	-	2	34
Hospital stay (days)	24.2±5.87	789	13	38
Survivors	-	67(89.3%)	-	-
Non-survivors	-	8(10.7%)	-	-

The demographic information for 75 patients that were included in the statistical analysis is shown in Table 4.1. For continuous data, descriptive analysis was used to get the Mean±SD, whereas percentages and frequency were determined for categorical variables. With a mean age of 31.94 ± 10.87 , there were 37 (49.3%) male and 38 (50.7%) female patients among the admitted patients. The TBSA (%) mean for them was 28.73 ± 7.73 . fifty-one patients (68%) had second-degree burns when they were first seen, while 24 patients (32%) had third-degree burns. With an average of 9.53 ± 5.84 ICU days, the total number of hospital days were 24.2 ± 5.87 . Of the patients with burn injuries, sixty-seven (89.3%) had recovered, while eight (10.7%) had died.

Table 4.2 - Comparison between the Demographic characteristics of study groups: survivor (n=67) vs. non-survivors (n=8)

	Survivors	Non-survivors	
Study variables	(n=67)	(n=8)	P value
Age	30.61±10.04	43.12±11.77	0.02
Male	32	5	.431
Female	35	3	
TBSA	26.91±5.49	43.75±6.58	<0.001
Second Degree of burn	50	1	<0.001
Third degree of burn	17	7	
ICU length(days)	8±4	22±6	<0.001
Hospital stay (days)	24±5	23±6	.545

Table 4.2 shows the comparison of the study groups as per their demographic characteristics. The research participants were divided into two groups: survivors (n = 67) and non-survivors (n = 8). Their means were compared using an independent sample t-test. The Pearson's chi-square test was used to check association among categorical variables. The significance level was set at $p \leq 0.05$. There were 32 men and 35 women in the survival group and 5 men and 3 women in the non-survival group ($p=.431$). The age difference between survivors (30.61±10.04) and non-survivors (43.12±11.77) was statistically significant at the $P=0.02$ level. In comparison to the survival group, their TBSA (%) was likewise significantly higher ($P<0.001$) (26.91±5.49 vs. 43.75±6.58). The difference in degree of burns was also statistically significant ($P<0.001$): 50 patients who survived the burn injury had second-degree burns and 17 of them had third-degree burns. Among the non-survival group, only one patient presented with a second-degree burn, while 7 patients suffered from significant third-degree burns. The non-survivor group had a greater mean number of total ICU length days (22±6) compared to the survivors (8±4) ($P<0.001$). however, no statistical difference ($P=.545$) was found in mean hospital stay days (23±6 vs. 24±5) between the groups.

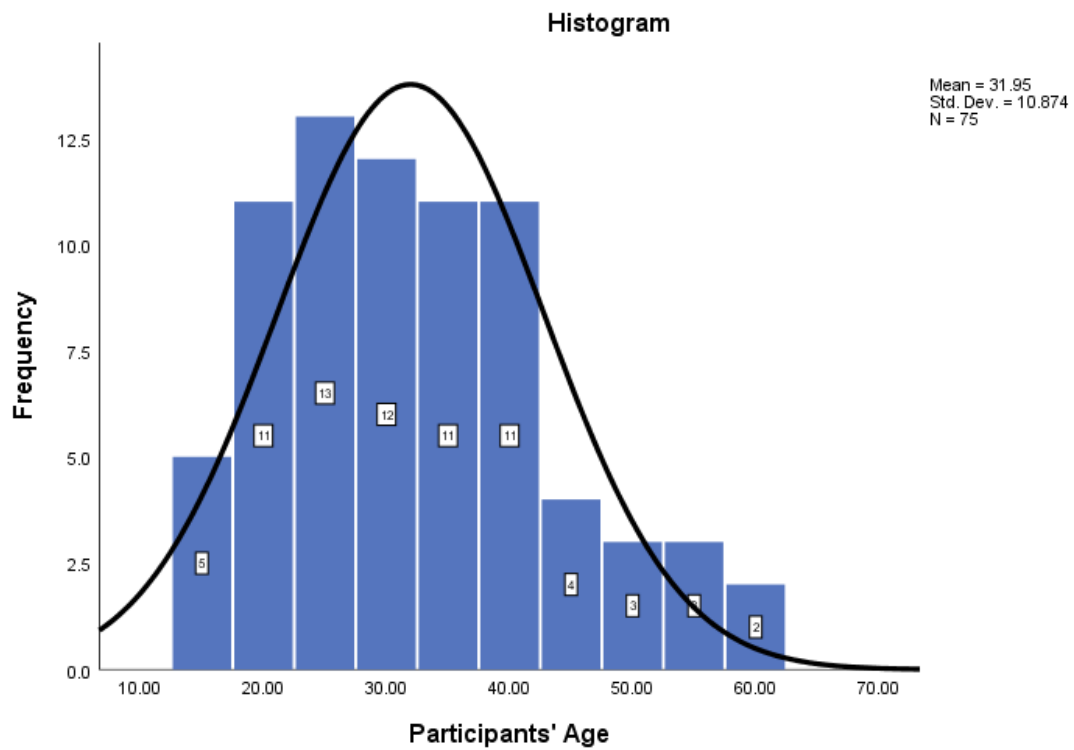


Figure 2- Histogram representing the mean age of study participants (N=75)

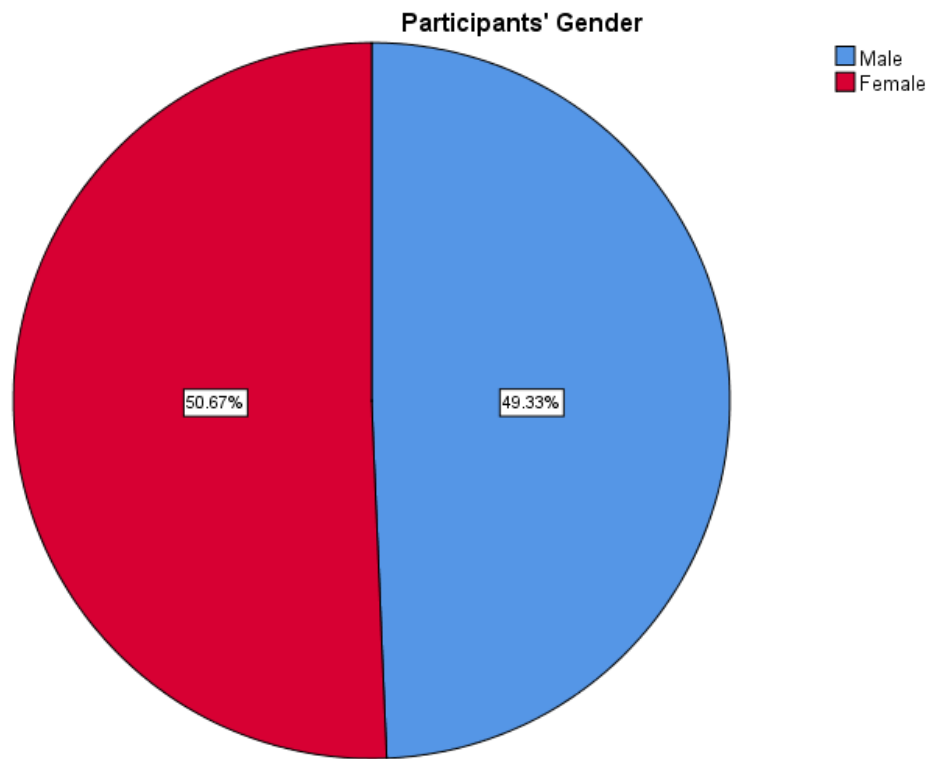


Figure 3- Bar Chart representing the percentage of males and females in study participants (N=75)

Table 4.3- comparison of the study groups according to their hematological variations

Study variables	Survivors (n=67)	Non-survivors (n=8)	P value
Hb Gm%	10.88±2.55	10.26±2.39	0.51
RBC/mm ³	4.45±.75	4.36±.71	0.74
TLC/mm ³	13500±11136	15000±6210	0.04
PLT×10 ³ / μL	232.46±48.85	168.25±62.28	0.001
Neutrophil count (%)	83.74±8.1	93.10±8.31	<0.001
Lymphocyte count (%)	14.56±4.07	7.37±1.30	<0.001
NLR ratio (%)	6.12±1.58	12.90±2.09	<0.001
Hospital stay (days)	24±5	23±6	.545

Table 4.3 represents the hematological variation between the survivors (n = 67) and non-survivors (n = 8). Their means were compared using an independent sample t-test. The Pearson's chi-square test was used to check association among categorical variables. The significance level was set at $p \leq 0.05$. There was a significant difference ($P < 0.05$) found between the means of these groups for PLT×10³/ μL (232.46±48.85 vs 168.25±62.28), TLC/mm³ (13500±11136 vs. 15000±6210), neutrophil count (83.74±8.1 vs. 93.10±8.31), lymphocyte count (14.56±4.07 vs. 7.37±1.30), and the NLR ratio (6.12±1.58 vs. 12.90±2.09). No significant difference ($P > 0.05$) was found between the RBC/mm³ 4.45±.75 vs. 4.36±.71 and Hb Gm% (10.88±2.55 vs. 10.26±2.39).

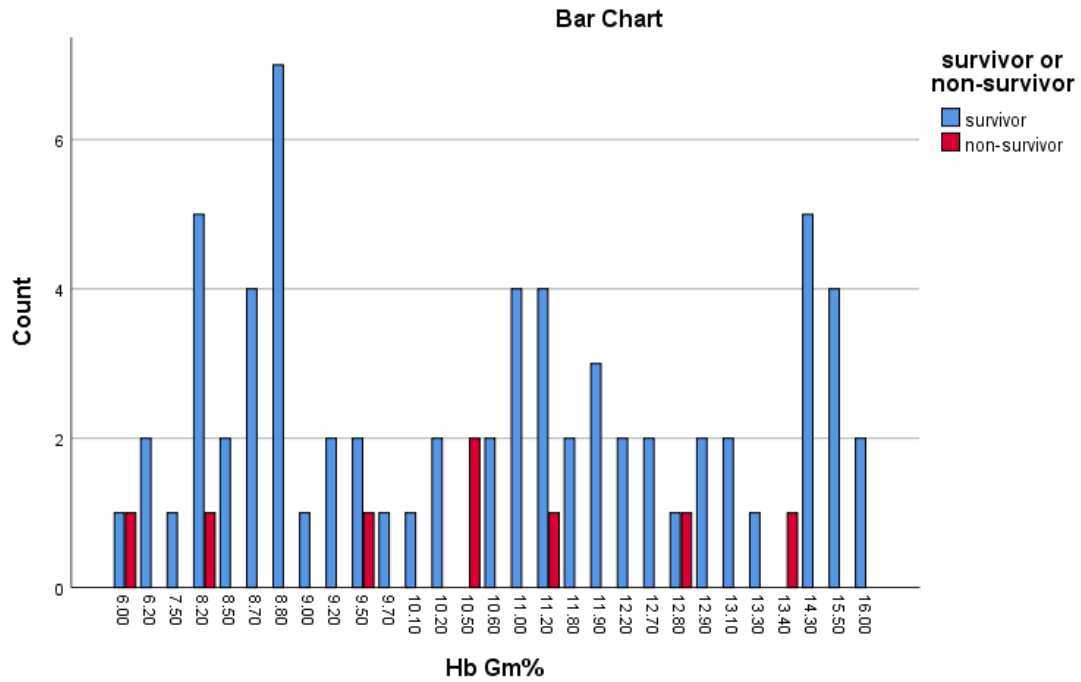


Figure 4- Clustered Bar Chart representing the Hb Gm% (hemoglobin in grams per deciliter) distribution among the survivors and the non-survivors group.

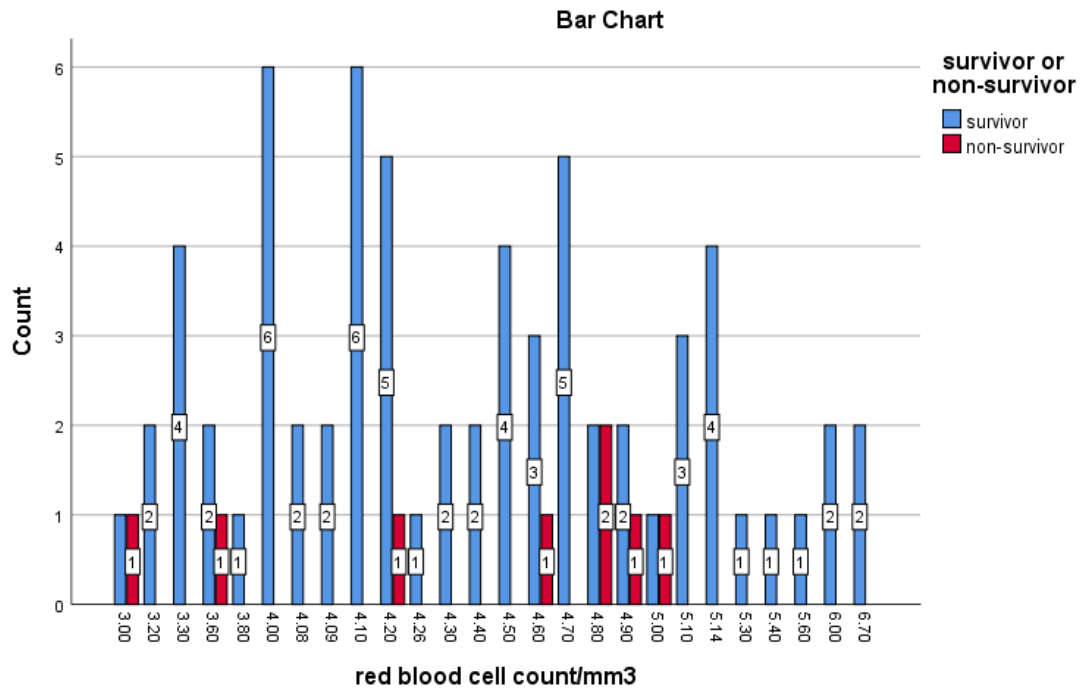


Figure 5- Clustered Bar Chart representing Red Blood Cell Count/ cubic millimeter distribution among the survivors and the non-survivors group.

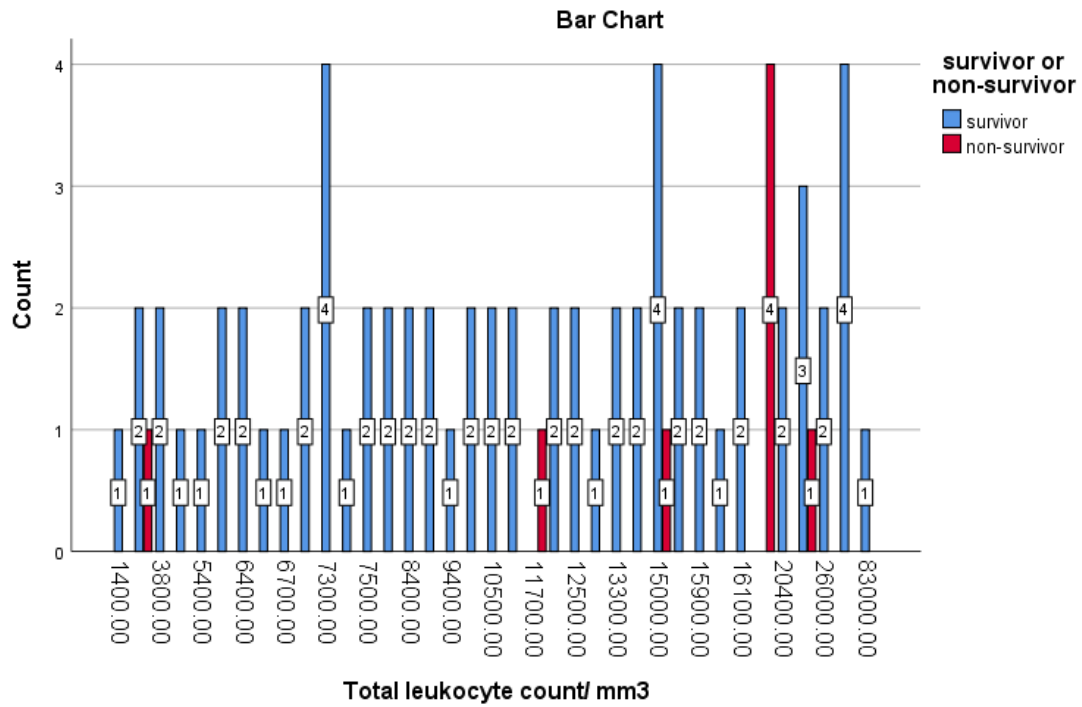


Figure 6- Clustered Bar Chart representing white Blood Cell Count/ cubic millimeter distribution among the survivors and the non-survivors group.

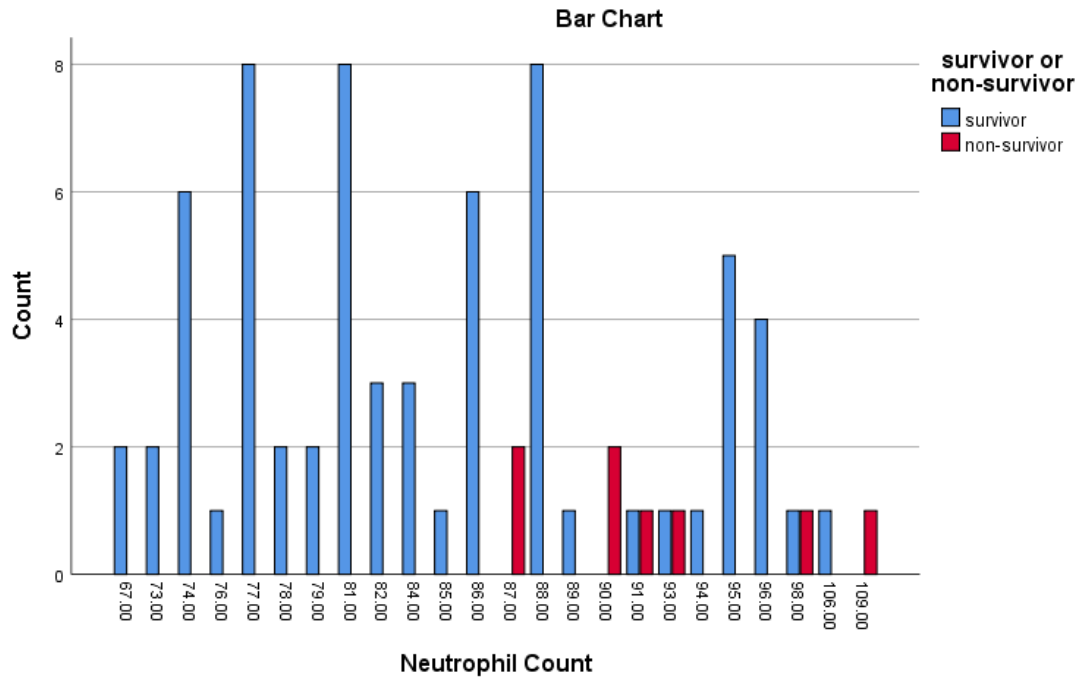


Figure 7- Clustered Bar Chart representing Neutrophil Count distribution among the survivors and the non-survivors group.

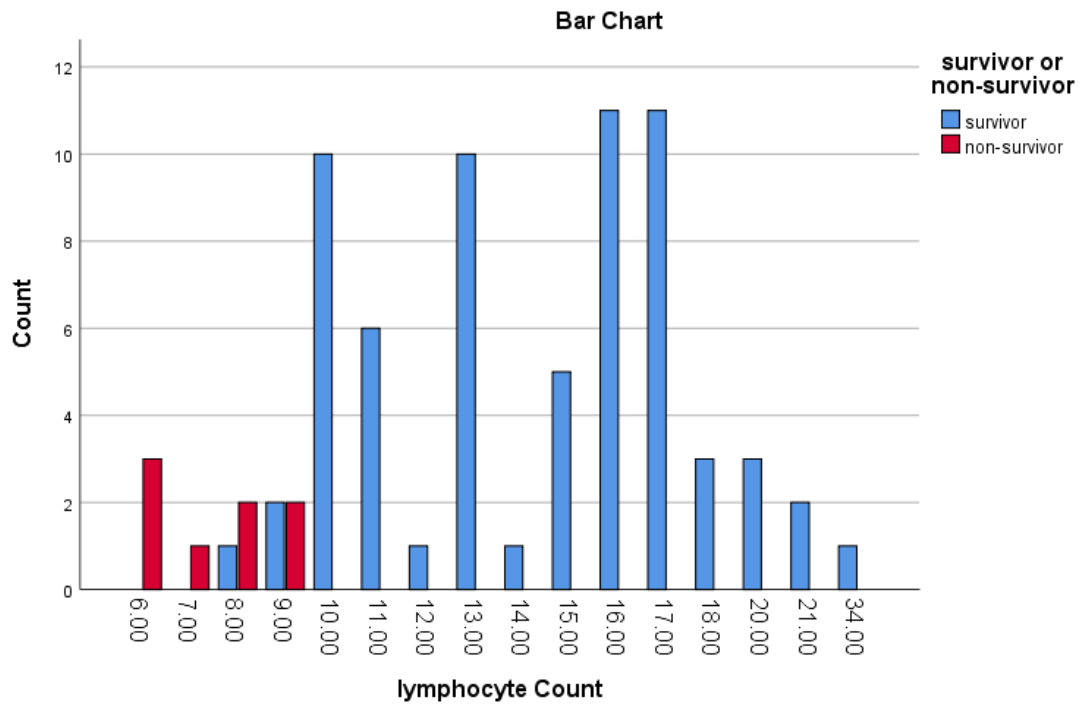


Figure 8- Clustered Bar Chart representing Lymphocyte Count distribution among the survivors and the non-survivors group.

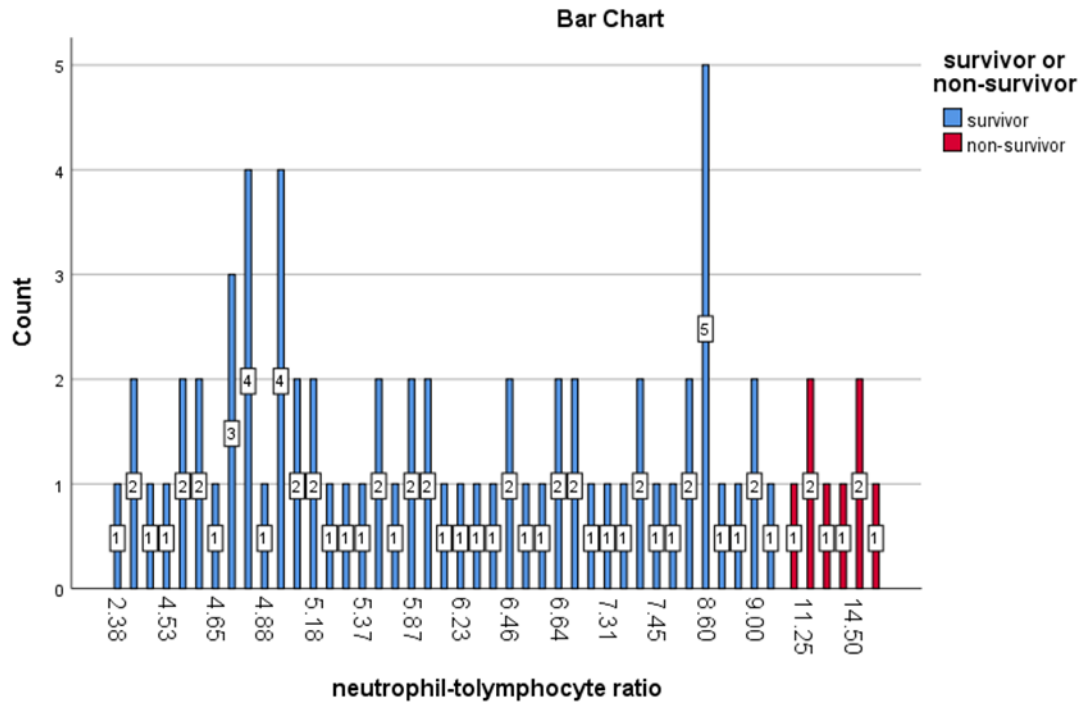


Figure 9- Clustered Bar Chart representing the Neutrophil-to-lymphocyte ratio distribution among the survivors and the non-survivors group.

4.4. Statistical analysis for primary outcome of the study

Our study intended to test the hypothesis “Is the elevated admission NLR value associated with a poor prognosis for severe burn patients?”. The null hypothesis and the alternate hypothesis are as follow:

- **Alternate hypothesis:** Elevated admission NLR value is associated with the poor prognosis in severe injury burn patients.
- **Null hypothesis:** Elevated admission NLR value is not associated with the poor prognosis in severe injury burn patients.

4.4.1- Independent sample t-test for testing the study’s hypothesis.

To test this hypothesis, we first applied an independent sample t-test to compare the mean NLR value of the survivors and the non-survivors. The independent sample t-test compared the mean admission NLR values between survivors and non-survivors of severe burn injuries to determine if elevated NLR is significantly associated with mortality. There was a statistically significant difference ($P < 0.001$) between their means values (6.12 ± 1.58 vs. 12.90 ± 2.09). The results imply that the mean NLR values of the two groups are statistically significant. On these bases, we accept the alternate hypothesis stating that the “elevated admission NLR value is associated with the poor prognosis in severe injury burn patients.

Table 4.4.1.- Group Statistics

survivor or non-survivor		N	Mean	Std. Deviation	Std. Error Mean
neutrophil-to- lymphocyte ratio	Survivor	67	6.1231	1.58067	.19311
	non-survivor	8	12.9069	2.09907	.74213

Table 4.4.1 - independent sample t-test for the equality of means

		Independent Samples Test								
		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
neutrophil-to-lymphocyte ratio	Equal variances assumed	1.596	.210	-11.075	73	.000	-6.78389	.61253	-8.00467	-5.56311
	Equal variances not assumed			-8.846	7.976	.000	-6.78389	.76685	-8.55316	-5.01462

4.4.2- Crosstabulation analysis for testing the study hypothesis

In the next step, we applied the crosstabulation analysis to check the association between the NLR value and prognosis (survivor vs. non-survivor group). The Pearson Chi-Square test determined if there is a significant association between elevated admission NLR levels (categorized) and mortality outcomes (survivors vs non-survivors) in severe burn patients. We changed the NLR value variable from continuous to categorical form by dividing the NLR into two categories: NLR < 8 and NLR >8. The results of the statistical analysis between these two categorical variables showed that there were 58 survivors in the NLR<8 category and 0 non-survivors in the same category. Nine survivors had NLR> 8, while all the non-survivors (n = 8) had NLR> 8. The Pearson Chi-square and Spearman's correlation analysis P values were less than 0.05, which shows a significant association between the NLR value and the prognosis. Based on these results, we reject the null hypothesis of our study and accept the alternate hypothesis stating that "an elevated admission NLR value is associated with poor prognosis in severe injury burn patients."

Table 4.4.2 (a)- NLR category * survivor or non-survivor Crosstabulation

Count

		survivor or non-survivor		Total
		survivor	non-survivor	
NLR category	NLR< 8	58	0	58
	NLR> 8	9	8	17
Total		67	8	75

Table 4.4.2 (b)- Pearson Chi-Square Test to association between NLR-category and study groups

	Value	Df	Asymptotic Significance (2- sided)	Exact Sig. (2- sided)	Exact Sig. (1- sided)
Pearson Chi-Square	30.553 ^a	1	.000		
Continuity Correction ^b	25.814	1	.000		
Likelihood Ratio	27.415	1	.000		
Fisher's Exact Test				.000	.000
Linear-by-Linear Association	30.146	1	.000		
N of Valid Cases	75				

a. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 1.81.

b. Computed only for a 2x2 table

Table 4.4.2 (c)- Symmetric Measures

		Value	Asymptotic Standard Error ^a	Approximate T ^b	Approximate Significance
Interval by Interval	Pearson's R	.638	.093	7.084	.000 ^c
Ordinal by Ordinal	Spearman Correlation	.638	.093	7.084	.000 ^c
N of Valid Cases		75			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

c. Based on normal approximation.

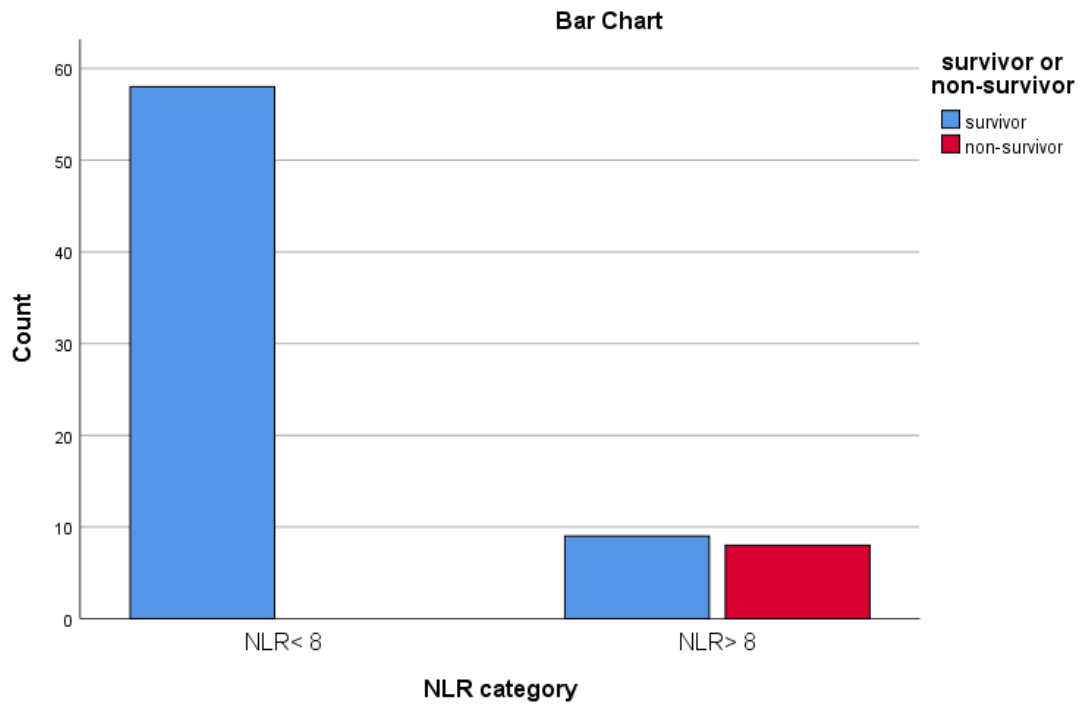


Figure 10- Clustered Bar Chart representing the NLR category distribution among the survivors and the non-survivor groups

4.4.3. Regression analysis for testing the study's hypothesis

In the final step, we applied linear regression analysis to check whether the neutrophil-to-lymphocyte ratio is an independent predictor of survival in burn patients. Regression analysis determined the strength and nature of the relationship between elevated admission NLR values and mortality in severe burn patients. By modeling NLR as an independent variable and mortality (survivors vs non-survivors) as the dependent variable, regression analysis quantified how changes in NLR levels affect the likelihood of poor prognosis, allowing for predictions and assessing statistical significance. The table provided the descriptive statistics of these two study variables. The next table shows correlation, which shows that both variables are significantly related. The ANOVA table reports how well the equation of the regression fits between these variables. The significance value of <0.001 demonstrates that the neutrophil-to-lymphocyte ratio is an independent predictor of survival. The negative coefficient value in the coefficient table (-.56) shows that as the value of the independent variable increases, it leads to poor prognosis (mortality). Based on these results, we reject the null hypothesis of our study and accept the alternate hypothesis stating that "an elevated admission NLR value is associated with a poor prognosis in severe injury burn patients."

Table 4.4.3 (a)- Descriptive Statistics

	Mean	Std. Deviation	N
survivor or non-survivor	.1067	.31077	75
neutrophil-to-lymphocyte ratio	6.8467	2.66266	75

Table 4.4.3 (b)- Correlations

		survivor or non-survivor	neutrophil-to-lymphocyte ratio
Pearson Correlation	survivor or non-survivor	1.000	.792
	neutrophil-to-lymphocyte ratio	.792	1.000
Sig. (1-tailed)	survivor or non-survivor	.	.000

	neutrophil-to-lymphocyte ratio	.000	.
N	survivor or non-survivor	75	75
	neutrophil-to-lymphocyte ratio	75	75

Table 4.4.3 (c)- ANOVA^a

Model		Sum of Squares	Df	Mean Square	F	Sig.
1	Regression	4.480	1	4.480	122.658	.000 ^b
	Residual	2.666	73	.037		
	Total	7.147	74			

a. Dependent Variable: survivor or non-survivor

b. Predictors: (Constant), neutrophil-to-lymphocyte ratio

Table 4.4.3 (d)- Coefficients

Coefficients ^a										
		Unstandardized Coefficients		Standardized Coefficients			95.0% Confidence Interval for B		Collinearity Statistics	
Model		B	Std. Error	Beta	t	Sig.	Lower Bound	Upper Bound	Tolerance	VIF
1	(Constant)	-.526	.061		-8.589	.000	-.648	-.404		
	neutrophil-to-lymphocyte ratio	.092	.008	.792	11.075	.000	.076	.109	1.000	1.000

a. Dependent Variable: survivor or non-survivor

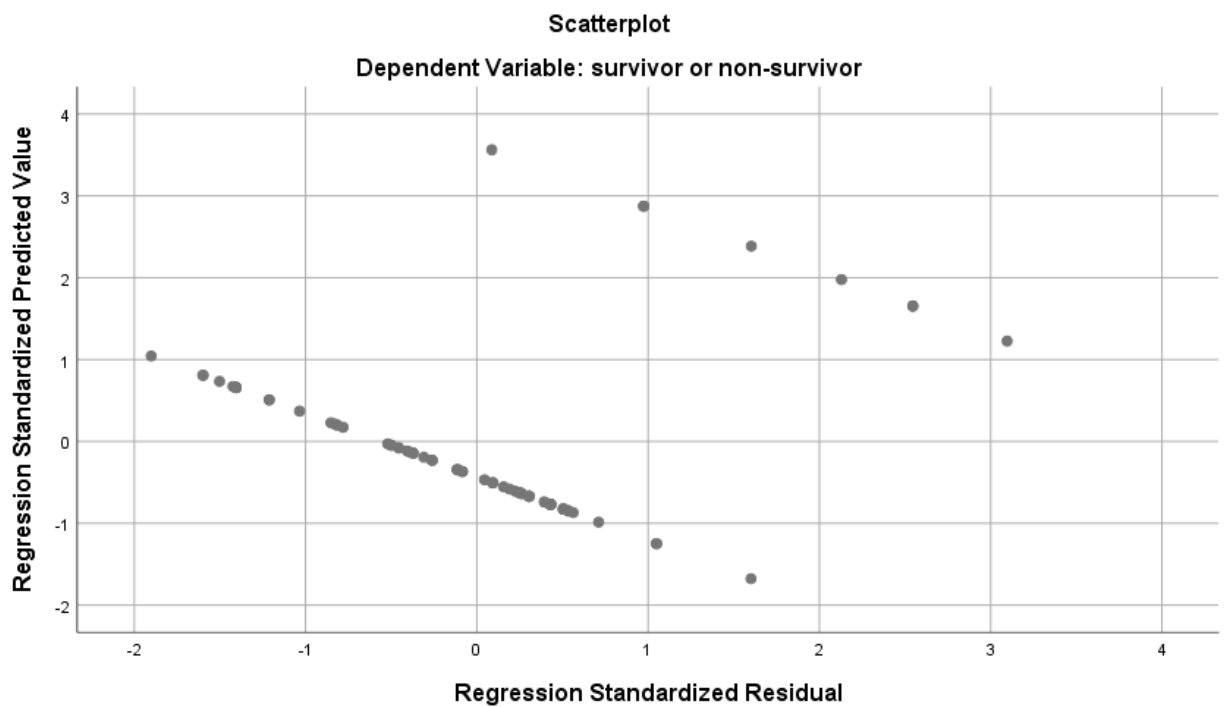


Figure 11- Regression lines in scatter plot represents that the regression lines have negative slope. It indicates that the independent predictor (NLR ratio) and the dependent variable prognosis (survivor vs. non-survivors) have a negative linear relationship.

CHAPTER-V

DISCUSSION

Severe burn injury is followed by several systematic inflammatory changes as a part of the body's initial response to the physiological stress. These changes reflect in complete blood count measurements and can help predict the prognosis in the early stages. One of these measurements is the neutrophil-to-lymphocyte (NLR) ratio, which reflects the imbalance between the inflammatory response (neutrophils) and the immune response (lymphocytes) (5). The NLR value can assist in predicting the likelihood of complications and mortality in burn patients by acting as a marker for the intensity of the inflammatory response. Having access to detailed information on systemic inflammatory indicators can help guide treatment choices, enhance patient outcomes, and eventually lower the number of fatalities caused by burns.

Our study aimed to assess the neutrophil-to-lymphocyte ratio as a poor prognostic factor in severely burned patients, and in our opinion, we are pioneers in conducting this study in Pakistan. In addition to the NLR ratio, we also evaluated the relationship between burn-related mortality and patient demographic variables like age, gender, TBSA, degree of burn, total ICU length, and total days of hospital stay.

In our study, we found a significant age difference ($p = 0.02$) between survivors (30.61 ± 10.04) and non-survivors (43.12 ± 11.77), indicating that an older age can be related to a poor prognosis (mortality). The difference between the TBSA (%) was likewise significantly higher (26.91 ± 5.49 vs. 43.75 ± 6.58) in the non-survivor group, and the difference in burn degree was also statistically significant ($P < 0.001$). However, no statistical difference was found between gender and burn-related mortality ($P = .431$). Several studies from previous literature go in favor of our study results. a previous study by Yen (51) demonstrated that mortality in burn patients can be predicted by older age, TBSA, and burn depth. Similarly, Gurbuz and Demir (52) found in their study that the 70–79 age group had the highest mortality rate. Tiryaki *et al.*, (77) in their study also

concluded that older age is a major risk factor for burn-related mortality in patients with electrical burns. Some of the factors that were found by Brusselaers et al. (2010) to affect the outcome of burn included; age, gender, and the socio-economic status of the patient (78). Regarding the relationship between the burn degree and mortality, the results from studies by Forbinake *et al.* (79) and Le Qiu *et al.* (80) are consistent with our study results, which reported that the second-degree burns and the third-degree burns had a greater risk of mortality. Hussain et al. (2017) gave a detailed description, showing that there is a higher mortality rate especially where the burns are deep, including full thickness burns which destroy the skin layers. This is due to the heightened inflammatory response, which hampers the body's potential for coagulation, increases the risk of infection, and slows healing from trauma, thus causing sepsis and multi-organ dysfunction. Specifically, the study pointed out that patients with deeper burns, as the aforementioned factors indicate, need more critical care and longer hospitalization, which again underlines the dramatic effect of burn depth on survival (78). Likewise, concerning the severity of burns and their expected outcomes, Chipp et al. (2018) explored burn injuries in patients and found out that the depth of burns is a key determinant of mortality. Accordingly, in their study, they revealed that patient with deeper burns seemed to be at a much higher risk of severe complications and even had longer periods of recovery. The wound depth is said to determine the degree of the inflammatory response and the suppression of the immune system that leads to high mortality (78). In a recent study by Turhan *et al.* (56), the authors linked burn-related mortality with burn degree and total damaged surface area (TBSA). It's interesting to note that this study also found that the gender of the person was a significant risk factor for death. However, in Ederer's 20 years long single center study, no significant relationship between gender and burn-related mortality was found (81). Similar to the Ederer study, our study also found no statistically significant difference concerning gender and burn-related mortality.

Our results showed a significant difference ($P < 0.05$) between the means of survivors vs. non-survivors for $PLT \times 10^3$ (232.46 ± 48.85 vs. 168.25 ± 62.28), TLC/mm^3 (13500 ± 11136 vs. 15000 ± 6210), neutrophil count (83.74 ± 8.1 vs. 93.10 ± 8.31), lymphocyte count (14.56 ± 4.07 vs. 7.37 ± 1.30), and the NLR ratio (6.12 ± 1.58 vs. 12.90 ± 2.09). These

changes in CBC brought on by severe burn damage have been identified in previously published literature. An initial increase in WBC count in both non-survivors and survivors is an indication of the body's systematic inflammatory response leading to the mobilization of leukocytes. Likewise, the combination of an increased number of neutrophils and a decreased number of lymphocytes leads to a high neutrophil-to-lymphocyte ratio (NLR), which suggests an immune response that is unbalanced. It implies an elevated inflammatory state and possible immune system dysfunction. As a result, a higher NLR value in the non-survivor group is regarded as greater physiological stress in their body's as it requires more effort to deal with the stressor and reestablish homeostasis (10, 82).

Our study results determined that certain variables of CBC are indeed related to burn-associated mortality as supported by other scientific works, too. For example, Pham et al. concluded that higher WBC of the first days after the burn increases mortality rate significantly. High WBCs are usually associated with systemic inflammation or infection and it results in complications including sepsis and multi-organ dysfunction thus, enhancing the mortality rate (83). Similarly, Greenhalgh et al. pointed out that platelet count was deemed as having an impact on the prognosis in burn patients. In their study, they found that thrombocytopenia is associated with higher mortality because low platelet values usually indicate severe SIRS and coagulopathy, which has been previously associated with worse outcome (84). Guldogan et al. (24) also confirmed that CBC measurements like hemoglobin count, WBC count, and PLT are associated with burn-related mortality and found it statistically significant. Another study by Le Qiu et al. (80) found a significant difference in 3rd day values of $TLC \times 10^9/L$ (11 ± 5 vs. 12 ± 9), $PLT \times 10^9/L$ (132 ± 70 vs. 93 ± 85), neutrophil count, lymphocyte count, and the NLR value ($9.01 \pm 5.1c$ vs. 12.01 ± 2.11).

Our study findings indicate that elevated admission NLR values are significantly associated with burn-related mortality in severe burn patients. First, we applied an independent sample t-test to compare the mean NLR value of the survivors and the non-survivors, and a statistically significant difference ($P < 0.001$) was found between (6.12 ± 1.58 vs. 12.90 ± 2.09) these groups. The purpose of performing this statistical test

was to compare the sample means between two groups to determine whether the mean NLR values of these groups are statistically significant. In the next step, we applied the crosstabulation analysis to check the association between the NLR value and prognosis (survivor vs. non-survivor group). It is a test that sums up the relationship between two categorical variables by recording their frequency. In the final step, we applied linear regression analysis to check whether the neutrophil-to-lymphocyte ratio is an independent predictor of survival in burn patients. It is a test that determines the strength of the relationship between an independent variable(s) and a dependent variable. All these tests favored accepting our alternate hypothesis.

In line with the finding of our study, the results of the Le Qiu study indicated that a high 3rd-day NLR value is associated with 90-day burn mortality in severe post-burn injuries. Furthermore, Fuss *et al.* (68) found that those with higher NLR values had a higher risk of developing sepsis in burn injuries. In this investigation, PCT and NLR were found to be more reliable markers for distinguishing SIRS from sepsis (systemic inflammatory response syndrome) as compared to ERS, WBC count, or CRP. The study also found that patients with higher NLR values had a greater risk of death. Additionally, greater NLR was found to be linked to a higher chance of developing AKI following burn surgery (66). Additionally, findings from the study by Hu *et al.* further favor the NLR value as a prognostic indicator in severe burn injuries. Their study demonstrated that a higher NLR value >14 is clearly linked to mortality in those patients (85).

Our study was the first in Pakistan to explore and anticipate the association of high NLR values at admission with burn-related mortality. It provides strong evidence supporting NLR as a reliable early prognostic marker for mortality. Our research findings can facilitate enhanced clinical decision-making through the early detection of high-risk patients, thus optimizing their management and outcomes in burn care.

Strength of the study

- The strength of our study lies in the fact that we have collected prospective data by addressing the patients in real time.
- We recorded their prognosis from time-to-time whenever possible.
- In addition to our study's primary outcome, we recorded several other demographic and hematological variables that can predict mortality in burn patients.
- Lastly, a rigorous analysis to test our study's hypothesis ensures the authenticity of our study findings.

Limitations of the study

The limitations of our study include:

- A small sample size ($n = 75$), which questions the generalization of our study's results.
- The sample was collected from a limited number of burn centers.
- Our study did not compare the data in terms of different treatment approaches that can predict patient outcome (survival).

CHAPTER-VI

CONCLUSION

In conclusion, this study highlights the clinical significance of the admission neutrophil-to-lymphocyte ratio as a predictive marker of survival in patients with severe burn injuries. A higher NLR value was found to be associated with increased mortality in burn patients. These findings emphasize the importance of considering NLR value as a potential marker for risk stratification and treatment decisions in burn care. However, it is important to acknowledge the limitations of our study, such as the small sample size. Future researchers should explore the significance of admission NLR in burn patients by enrolling a larger population. Additionally, further research is needed to explore the underlying mechanisms and different treatment approaches that can predict patient outcome (survival) and validate the clinical utility of the NLR value.