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Association of Elevated Neutrophil-Lymphocyte Ratio with Myocardial Infarction

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ABSTRACT

Background: Cardiovascular disease is a widespread health condition affecting millions worldwide. Acute myocardial infarction is the most prevalent type of acute cardiac disease that is encountered by many populations, including the asymptomatic population. MI can have significant damage to heart tissue. Study was to evaluate the relationship between the neutrophil-to-lymphocyte ratio (NLR) and the occurrence of myocardial infarction within the population of Hyderabad old city area, Sindh, Pakistan.

Methods: This cross-sectional study was conducted from July to September 2022 at Sindh University Jamshoro and Liaquat University Hospital. The sample size of 200 participants with a convenience sampling technique was used for data collection from the population of Hyderabad, Sindh, selected after excluding 150 patients with various inflammatory conditions. The target population of 5 lakh of main Hyderabad city area. The data analysis was done by using descriptive statistics, t-tests, and Pearson correlation using GraphPad Prism 5 with a significance threshold of $P \leq 0.05$.

Results: The descriptive results show that Males without Myocardial Infarction (MI) had a mean NLR of 2.7 with SD of 0.22, and females without MI had a mean NLR of 3.3 with Sd of 0.20, both within normal limits and inferential results show Males with MI showed a significantly higher NLR (mean = 4.1 with SD of 0.06 p = 0.0001, OR = 9), and females with MI had a mean NLR of 3.9 with SD of 0.07 (p = 0.0046, OR = 3.5), indicating a strong association between elevated NLR and MI.

Conclusion: This study concludes that there is a relationship between raised NLR and AMI patients with raised neutrophils after acute myocardial injury.

Keywords: Neutrophil Lymphocyte, Acute Myocardial Infarction, Coronary Heart Disease.

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INTRODUCTION

The most frequent category of acute cardiac problem encountered by people with previously asymptomatic heart conditions is acute myocardial infarction[1]. Cardiovascular disease is the most prevalent disease worldwide, affecting more developed countries in comparison to developing countries, with 3 million people developing MI per year. A recent study with a sample size of 19,781 patients affected by CAD shows that the prevalence of MI among those patients was up to 23.3%. This increase in the burden of disease is a result of many risk factors, including alcohol consumption, smoking, etc., but now the prevalence is decreasing in developed countries due to their early adaptation of preventive measures and early treatment plans, which decreases their rate of mortality annually[2,3]. Myocardial infarction has a major impact on blood cells in circulation. It generally initiates the myelopoiesis process within the body, increasing the number of white blood cells (WBCs), particularly neutrophils. That leads to severe damage to the heart tissue and acute presentations of infarctions. The following shift in WBC levels in the blood is brought on by certain alarming molecules the fact that are stimulated in the body, such as S100A9 and S100A8[4]. An ischemic insult to the heart cells, causes neutrophils to accumulate at the site of injury within hours, clearing the pathogens and debris from the vicinity. However, because the neutrophils stay at the site over an extended period, they cause more damage to the heart cells and worsen the disease[5].The neutrophils cause damage to the cardiomyocytes with the help of oxidase and protease enzymes present inside neutrophils that are present at the site of injury under certain inflammatory signals[6]. The role of neutrophils is not only in causing the development of MI, but it also plays a pivotal role in causing many cardiac arrhythmias, specifically ventricular tachycardia (VT). Many studies have shown that neutrophils use the Lipocalin-2 (Lcn2) pathway to increase levels of reactive oxygen species (ROS) and cause damage at the site of injury. Through this pathway, they also damage the normal cardiomyocytes, which ultimately leads to disturbance in the normal physiological ion channels that are present in the cardiomyocytes. This will contribute to the development of VT in the heart[7,8,9]. There is another pathway called neutrophil extracellular trap formation, or NETs, that can contribute to the myocardial injury by aggravating the local inflammation and tissue damage at the infarct site.[10] Previous study shows the role of neutrophils as pro-inflammatory and reparative agents that play a pivotal role in wound repair at the infarct site, but when they become dysregulated, it will lead to excessive formation of scars at the infarct site and lead to compromised heart function[11]. The elevated neutrophil count at the infarct site could potentially lead to increased risk of heart failure by modulating the fibroblast activity at the infarct site, yet further contributing to pathological scar formation. [13] There are some studies that show the molecular mechanism of neutrophil production after MI; for example, the study showed that the S100A8/A9 molecules and the NLRP3 inflammasome play a pivotal role in the production of neutrophils, which in turn is essential for granulocytosis, repair, and platelet activation that further exacerbates the inflammatory response and causes many thrombotic complications[14,15]. The neutrophil-to-lymphocyte ratio (NLR) is one of the most important serum inflammatory markers that is widely used worldwide for detecting prognosis and post-disease outcomes in many diseases, including coronary heart disease. The high levels of NLR ratio predict a worse prognosis for CAD due to long infarct size, low compliance, low fibrinolysis, high ischemia-reperfusion injury, and instant restenosis[16].This study aimed to investigate the possible association between a raised Neutrophil-Lymphocyte Ratio and Myocardial Infarction.

METHODS

This cross-sectional study was conducted from July 2022 to September 2022 at Department of Physiology of Sindh University Jamshoro in collaboration with Liaquat University Hospital in Hyderabad, with approval from the university's ethical and review board with MR no; Physiol/27.

This study was conducted to investigate any possible association between raised NLR in patients with acute myocardial infarction. The sample size for this study was calculated using the formula names Raosoft Sample Size Calculator[8.350] participants visited the hospital's cardiac unit; among them, 150 patients were excluded from the study who had any inflammatory condition, including chronic cholecystitis, gout, rheumatoid arthritis, pharyngitis, nephritis, and bronchitis. In the final, 200 participants were selected using a convenience sampling technique for this study; among them, 100 males and 100 females were present. For convenience, the participants were divided into two groups: a control group of 120 participants (M = 70, F = 50), including participants who had no history of acute myocardial infarction, and a study group of 80 participants (M = 30, F = 50), including patients who had a history of myocardial infarction. After giving all details of this study to participants in a very understandable manner, written consent was obtained after ensuring that all participants were voluntary, had the right to withdraw at any time without affecting their medical care, provided information regarding any potential risk or discomfort associated with the study such as minor discomfort during sample collection, assured confidentiality and the anonymity of their data, addressed any concerns raised by participants regarding this study and gave satisfactory answers to their queries. This study included all male and female participants within the age range of 30

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to 70. All participants who had a diagnosis of acute myocardial infarction (AMI) confirmed by clinical symptoms including chest pain, palpitation, shortness of breath, electrocardiographic changes including the prolonged QRS complex and T-wave inversion, and elevated cardiac biomarkers (troponin levels) were included in this study. All those were excluded with a history of chronic infection, hematological disease (leukemia, lymphoma), autoimmune disease (rheumatoid arthritis), or any congenital disorder that affected the study results. The samples were taken from all participants after taking all precautionary measures, and laboratory tests were performed for blood levels of triglyceride, total cholesterol, LDL, HDL, fasting and postprandial blood sugar, urea, creatinine, WBC count, platelet count, and NLR. The NLR values were measured with an automated cell counter at the diagnostic and research laboratory at Liaquat University Hospital in Hyderabad.

Data analysis was performed using GraphPad Prism5. The data analysis plan follows A; for continuous demographic variables, e.g., age, and for categorical clinical variables (e.g., gender, smoking status), frequency distribution and percentages of categorical variables were used. B, for visualizing the distribution of NLR among the various patients, histograms were used. The inferential analysis also followed as the independent sample t-test was used to compare males and females of Control and study group participants for NLR. The significance threshold is set at $P \leq 0.05$.

RESULTS

The Table 1 showing the population's demographic data shows the male population's mean age was 51.90 years (SD 12.52) compared to 59.10 years (SD 8.18) in females. The mean systolic BP in males was 175.1 mmHg (SD 38.85), while 143.9 mmHg (SD 11.67) in females. The creatinine kinase levels were higher in males with a mean of 124.4 U/L (SD 34.75) than in females (mean 84.70 U/L, SD 35.76). Lymphocyte counts were also higher in males (mean 1.77 × 10^9/L) in comparison with females (mean 1.51 × 10^9/L). Platelet counts were similar between genders, with males at 201.7 ×10^9/L and females at 207.9 ×10^9/L. Raised neutrophil levels were higher in males (mean 6.42 ×10^9/L) compared to females (mean 5.46 ×10^9/L).

Table 1: Demographic Characteristics of the Participants

Parameters	Mean	SD	Min-Max
Age In Male Population	51.90	12.52	30.00 70.00
Age In Female Population	59.10	8.183	47.00 70.00
Creatine Kinase in Males	124.4	34.75	70.00 168.0
Creatine Kinase In Females	84.70	35.76	30.00 135.0
Systolic Bp in Males	175.1	38.85	130.0 265.0
Systolic Bp in Female	143.9	11.67	125.0 160.0

Lymphocytes In Make	1.770	0.1802	1.500 2.000
Lymphocytes In Female	1.510	0.1586	1.300 1.800
Platelets In Male	201.7	4.63	197.0 211.0
Platelets In Female	207.9	6.823	198.0 217.0
Raised Neutrophils Of Male	6.420	0.5610	5.500 7.000
Raised Neutrophils in Female	5.460	0.4718	4.700 6.200

Table 2: Comparison of Blood Parameters among Male Participants.

Variable	NLR less than 3	NLR more than 3	Mean of NLR	P-value	Odd Ratio
Male with No MI (N=70)	45(64.29%)	25(35.71%)	2.7	0.0001	9.000
Male with **MI** (N=30)	5(16.67%)	25(83.33%)	4.1		
Total	50	50			

Table 2 shows the comparison of blood parameters among male participants of control and study groups based on the neutrophil-lymphocyte ratio. In male participants without myocardial infarction, 64.29% had NLR less than 3, while 35.71 had NLR more than 3, with a mean of NLR about 2.7. Among the male participants with myocardial infarction, 16.67% had NLR less than 3, while 83.33% had NLR more than 3, with a mean NLR of 4.1. The P-value of comparison is 0.0001 and the odds ratio is 9.0, showing the strong statistical relation and association between these two entities.

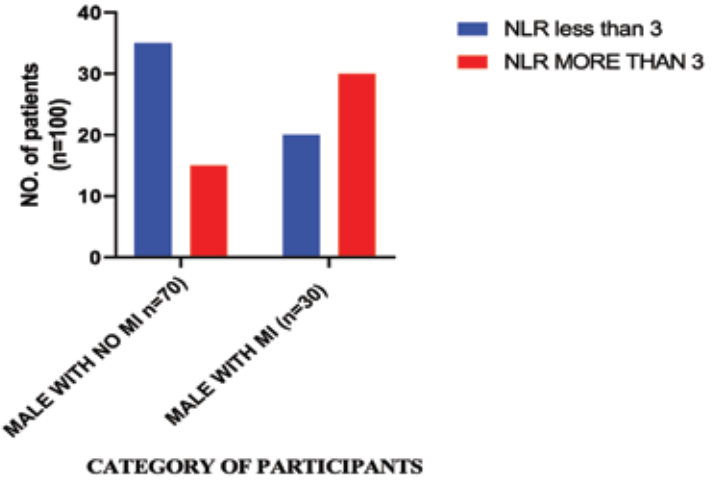

Figure: 1 Distribution of Male Participants by Neutrophil-to-Lymphocyte Ratio (NLR) and Myocardial Infarction (MI) Status

Figure: 1 shows the distribution of patients with NLR more than and less than 3 across Male participants of the study and case group. Normal males predominately have NLR less than 3, while males who had MI were predominately associated with NLR more than 3. This indicates the statistically significant relationship between these two variables.

Table 3: Comparison of Blood Parameters Among Female Participants

Variable	NLR less than 3	NLR more than 3	Mean of NLR	P-value	Odd Ratio/ Risk Ratio
Female with No MI(n=50)	35	15	3.3	0.0046	3.500/1.750
Female with MI (n=50)	20	30	3.9		
Total	50	50			

Table 3 shows the comparison of blood parameters among male participants of control and study groups based on the neutrophil-lymphocyte ratio. In female participants without myocardial infarction, 70% participants had an NLR less than 3, while 30% participants had an NLR of more than 3, with a mean NLR of 3.3, while on the other hand Female participants with MI: 40% had an NLR less than 3, and 60% had an NLR more than 3, with a mean NLR of 3.9, while NLR = 3 is considered a threshold and it does not have an impact on the overall results. The P-value of comparison is 0.0046 with an odds ratio of 3.5, showing a strong statistical relation and association between these two entities.

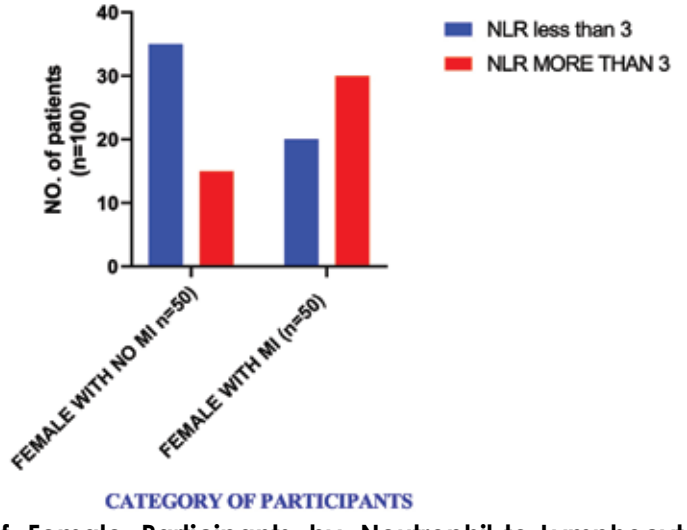

Figure: 2 Distribution of Female Participants by Neutrophil-to-Lymphocyte Ratio (NLR) and Myocardial Infarction (MI) Status.

Figure: 2 shows the distribution of patients with NLR more than and less than 3 across female participants of the study and case group. Normal female predominately has NLR less than 3, while females who had MI were predominately associated with NLR more than 3. This indicates the statistically significant relationship between these two variables.

DISCUSSION

This study aims to investigate the relationship between raised neutrophils in the blood of patients with acute myocardial infarction by using blood parameters including NLR, PIR, and other blood parameters (TG, Lipoproprotiens, C-reactive proteins, etc.). It was a cross-sectional study carried out from July 2022 to September 2022 at the Department of Physiology of Sindh University Jamshoro in collaboration with Liaquat University Hospital in Hyderabad after getting approval from the university's ethical board. The sample size of this study was comprised of 200 participants, and among them, the gender distribution was 100 male and 100 female participants. All participants were told about the purpose of this study and what procedures would be used. After informed consent, written consent was taken in their native languages, so every participant understood the point written in the consent form, and all participants were allowed to withdraw at any moment during the study without affecting their medical management outcome.

The study results show that among the sample size of 200 participants, 55 showed a raised NLR after acute myocardial injury, and their gender-wise distribution was male (n = 25) with mean value of NLR of 4.1 with standard deviation of 14.1 and female (n = 30) with mean NLR of 3.9 with standard deviation of 7.1 showing that the NLR is more elevated in the male population in comparison to the female population after acute myocardial infarction. This is statistically supported by a p value of 0.0001 for males and p value of 0.0046 for females with an odds ratio of 9.0 and 3.5 respectively showing that this relation is statistically significant, these research results are consistent with prior research conducted by Smith et al., which highlighted the potential influence of gender on post-AMI inflammatory responses[10]. The statistical analysis is further strengthened by the study results of Johnson R. et al., with a p-value of 0.03 and a hazard ratio of 1.45, showing that the raised NLR is more associated with AMI that occurs due to an increase in post-MI inflammation in these patients[11]. The gender-wise difference in raised NLR shows that there is a significant role for gender in the inflammatory response following AMI, with males potentially exhibiting a higher inflammatory reaction compared to females[12]. T The gender disparity in inflammatory responses is due to many genetic factors that affect their immune and hormonal responses to the injury[13]. Some previous studies have shown that this difference in raised NLR in genders is due to estrogen, which plays a significant role in protecting against the inflammatory response that causes the lower levels of NLR in AMI patients[14]. In contrast to it, another study shows that testosterone levels in males are more likely associated with inflammatory responses and oxidative stress in the male body, which leads to raised NLR in AMI patients[15].

However, it is essential to acknowledge the alternative view regarding the observed differences in the levels of NLR in post-AMI patients and the normal population. It's essential to consider the broader body of research in this area. Contrary to the results of this study, some studies have failed to identify any supporting evidence for this relationship. For instance, Rodriguez et al. conducted the same study with the same principles but failed to find any statistically significant differences in levels of NLR between normal participants and patients with AMI[16]. Furthermore, a meta-analysis conducted by Martinez et al. found inconclusive evidence that supports any association between raised NLR levels and the population affected by AMI[17]. Nguyen et al. also tried to investigate the role of different genders in inflammatory responses following AMI using data from a population-based cohort. Their study also did not find any significant differences in NLR elevation between genders[18].

Finally, our study suggests that disparities in NLR elevation in post-AMI and normal participants are still a conflicting topic for discussion, and these conflicting findings in the literature further complicate this. Further research is necessary to identify the mechanism leading to the change in results.

CONCLUSION

This study concludes to the point that patients with acute myocardial infarction are more likely to show a raised NLR in comparison to a normal, non-affected population, which is further supported by statistically significant values, including a p-value of less than 0.005 and an odds ratio greater than 1, showing that this relationship between two entities is statistically correct.

LIMITATIONS

The first limitation is that this study was conducted for only a short period, so long-term complications and their relation to NLR cannot be addressed. Secondly, the study design is cross-sectional. Thirdly, this study was conducted in one hospital with a limited sample size, so the results cannot be applied to the whole population. Fourthly, there are many novel

inflammatory biomarkers present that can be used to study NLR, but those were not used in this study.

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CONFLITS OF INTEREST
None

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AUTHORS' CONTRIBUTION
SFU: Contributed to Acquisition, Analysis, or Interpretation, **SA:** Contributed to Conception or Design, **HURC:** Literature search, **NN:** Critical reading and Revision, **SB:** Satiation, Data collection

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