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Original Research Article **Pathology**

Correlation of Severity of Depression with a Novel Inflammatory Marker: Neutrophil to Lymphocyte Ratio

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Abstract

Introduction: Many new inflammatory markers are being extensively used recently for assessing the severity and prognosis of chronic conditions. Neutrophil-Lymphocyte ratio (NLR) because of its simplicity and affordability stands out. Its applicability to psychiatric disorders specifically Depression is an eye-opener. Hamilton Rating Scale for Depression (HAM-D), a clinician-based scoring system is better among other self-scoring systems where the study population is semi-illiterate. ***Materials and Method*:** Total of 80 patients over a period of 1 year presenting to psychiatric OPD with depressive symptoms were evaluated for the severity of Major Depressive Disorder (MDD) using HAM-D scale. A score of 7-17, 18-24 and >25 was categorised under mild, moderate, and severe degrees respectively. NLR was calculated and was correlated with their severity. Statistical analysis was done using PSPP software and p value of <0.05 was considered significant. ***Results:*** Out of the 80 patients 45 were females and 35 were males. The average age was 35.41 years ± 10.13 SD. 23 patients categorised under mild depression had mean NLR of 1.56 ± 0.46 SD, 40 patients with moderate depression and 17 patients with severe depression had mean NLR of 2.70 ± 1.08 SD and 4.62 ± 1.31 SD respectively. The mean value of NLR for patients of severe depression lied in the Mild-Moderate Inflammation zone (3-7). P-value was <0.05 and was considered significant. ***Discussion:*** A novel and easy biomarker like NLR can be as useful as assessing Inflammatory Cytokines like IL-6, IL-1 and TNF-α for assessing the severity of MDD. Rise of cortisol in persistent stress favours an increase in neutrophils and a reduction in lymphocytes, and thus, increased NLR. Thus, a rise of NLR with increasing severity of Depression could become a robust tool in future for predicting mortality and severity in patients suffering from MDD.

Keywords: Neutrophil to lymphocyte ratio (NLR), Severe depression, Hamilton Rating Scale for Depression (HAM-D), Major depressive disorder (MDD), Inflammtion.

INTRODUCTION

In the last 20 years, many new parameters of immune-inflammatory reactions and neuro-endocrine stresses like procalcitonin, neopterin, hs-CRP, IL-1, IL-6 etc have been well established in various diseases [1]. A novel inflammatory marker which stands out is the Neutrophil to Lymphocyte ratio (NLR), which is not only inexpensive but is also available at primary health care centres as well as requires much less expertise to interpret the result [1, 2]. Although initially NLR was used as an index in SIRS and other infectious disorders but today it is widely used across all disciplines of scientific research as well as in clinical practice [3]. Presently it is being used as a diagnostic as well prognostic marker in various pathological conditions. Inflammation secondary to cancers and risk stratification and assessment of Cardio-vascular disorders among many other disorders are the two well established conditions where NLR holds an honourable position. Many studies have been conducted in the past regarding the normal reference values for the NLR. Most significant among them was a study conducted by Azab et al (2014) in 9427 patients and revealed the average NLR to be 2.15. Based on the various values of NLR, the intensity of inflammation is often correlated [3]. Neutrophils represent the first defence of the body

i.e., innate immunity whereas lymphocytes can be linked to the regulatory or the adaptive component of the immunity. Dowlati *et al*., (2010) established that Major Depressive disorder is linked to low grade inflammation by evaluating several cytokine and Interleukin levels in patients diagnosed with MDD. So, NLR, a novel marker which also predicts the immune-inflammatory response of the body could be helpful in stratifying a heterogenous disorder like MDD and could also aid in diagnosis and add prognostic value to the disease [6]. Many self-rating scales for MDD have been used in various studies and are also popular among the clinicians because of the ease of administration. Such scales are sometimes are unreliable in semi-illiterate or illiterate patients and do not have much use in seriously ill patients [7, 8]. Whereas clinician-based scales like Hamilton Rating Scale for Depression (HAM-D) are more reliable and based on the 17 variables a score can be given to each patient. We in this study used a 17 version HAM-D and based on the scores patients were categorised into mild depression (7-17), moderate depression (18-24) and severe depression (>25). We in this study aim to correlate the NLR with increasing severity of Depression.

MATERIALS AND METHODS

- **Study Design:** An observational, hospital based cross sectional study.
- **Study Setting**: The present study was done in the Central Laboratory in MMIMSR, Mullana, Ambala in association with the Department of Psychiatry.
- **Study Period**: 1 year (August 2022- July 2023)
- **Study Population:** A total of 80 patients were included in this study based on the inclusion and exclusion criteria.

Inclusion criteria

1. All patients who presented to psychiatric OPD with depressive symptoms for the first time.

Exclusion criteria

1. Patients having any autoimmune, inflammatory, or other neuropsychiatric disorder.
2. Patients having disorders causing impaired cognition or history of any substance abuse.
3. Patients diagnosed with any chronic systemic disorders involving CVS, Kidney or Diabetes Mellitus.
4. Patients having any solid organ or hematological malignancies.
5. Patients on chronic medications or are currently on antidepressants.
6. Patients who suffered from fever in last 1 month
7. Pregnant or breastfeeding females.

Methodology

For evaluating the severity of depression HAM-D scale was used by the clinician. Based on the scores obtained, patients were categorised into three categories [7].

SCORE	SEVERITY
7-17	MILD
8-24	MODERATE
>25	SEVERE

For NLR- EDTA anticoagulated blood sample was used and was processed within 2 hours of collection using automated blood cell counter- Sysmex XN-550. Absolute Neutrophil count and Absolute Lymphocyte count were obtained and were divided to obtain the Neutrophil-Lymphocyte ratio.

$$NLR = \frac{Absolute\ Neutrophil\ count}{Absolute\ lymphocyte\ count}$$

Or

$$\frac{Relative\ Neutrophil\ count(\%)}{Relative\ lymphocyte\ count(\%)}$$

Based on the NLR values it was categorised into following categories [3].

NLR RANGE	INTENSITY OF IMMUNE REACTION
1-2	NORMAL
2-3	GREY ZONE
3-7	MILD-MODERATE
7-11	MODERATE-SEVERE
11-17	SEVERE
17-23	CRITICAL

Statistical Analysis

Statistical analyses were performed using PSPP software. Continuous data were expressed as Mean ± Standard Deviation while categorical data were presented as percentages. ANOVA test was used to measure the statistical difference between the population groups. A value of $p<0.05$ was considered statistically significant.

RESULTS

The study population consisted of 80 patients who presented to psychiatric OPD with depressive symptoms from August 2022 to July 2023. Out of 80 patients 45 were females and 35 were males. The average age of the study population was 35.41 years with SD of ± 10.13 years (Table 1). There were 23 cases who suffered from mild depression out of which

15 were females and 08 were males, out of 40 patients with moderate depression 26 were females and 14 were males and out of 17 cases who suffered from severe depression, 04 were females and 13 were males (Figure 1). The females were predominant in mild and moderate category, whereas the percentage of males was much more than females in severe category. The NLR among the study population ranged from 0.73 to 7.45 with a mean of 2.78 ± 1.47 SD (Table 2). Patients with mild depression had a mean NLR of 1.56 with SD of ± 0.46 (Table 3). Patients with moderate depression showed a mean NLR of 2.70 with ± 1.08 SD (Table 3). Patients suffering from severe depression showed a mean NLR of 4.62 with ± 1.31 SD (Table 3). The results revealed that mean NLR of mild depression (1.56), moderate depression (2.70) and severe depression (4.62) lied in the Normal (1-2), Grey zone (2-3) and mild-moderate inflammation zone (3-7) respectively. The ANOVA test to compare the means of the three groups revealed a p-value of <0.05 and was considered significant (Table 4).

Table 1: Statistical analysis of Age of patients

		age
N	Valid	80
	Missing	0
Mean		35.41
Median		33.00
Std Dev		10.13
Range		44.00
Minimum		19
Maximum		63

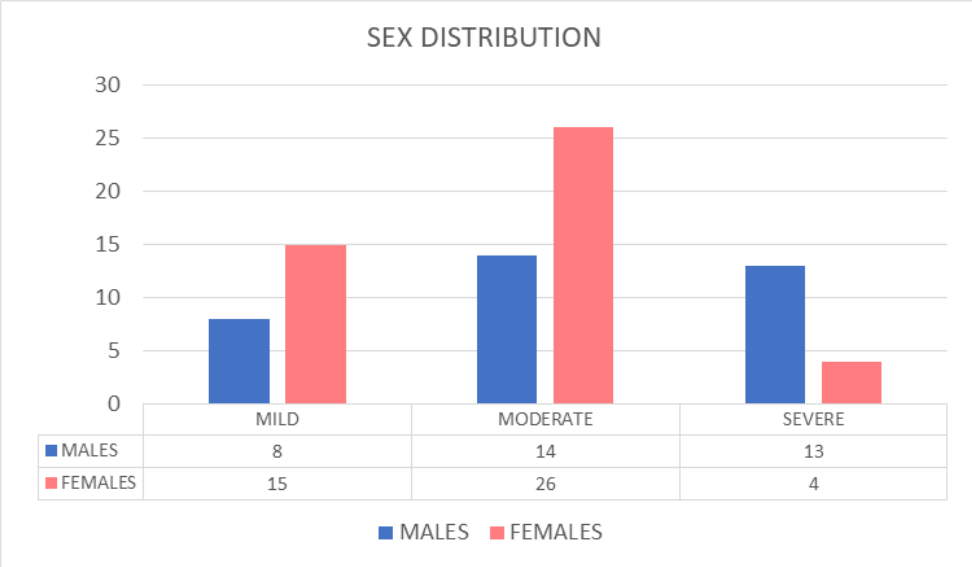

Figure 1: Sex Distribution based on severity of Depression

Table 2: Statistical Analysis of NLR in patients

		NLR
N	Valid	80
	Missing	0
Mean		2.78
Median		2.49
Std Dev		1.47
Range		6.73
Minimum		.73
Maximum		7.45

Table 3: Statistical analysis of NLR with severity of Depression

	severity	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
NLR	mild	23	1.56	.46	.10	1.36	1.76	.73	2.50
	moderate	40	2.70	1.08	.17	2.36	3.04	1.17	5.57
	severe	17	4.62	1.31	.32	3.94	5.29	2.60	7.45
	Total	80	2.78	1.47	.16	2.45	3.10	.73	7.45

Table 4: ANOVA test analysis of the three Groups based on severity of Depression

		Sum of Squares	df	Mean Square	F	Sig.
NLR	Between Groups	92.13	2	46.06	45.70	.000
	Within Groups	77.60	77	1.01		
	Total	169.73	79			

DISCUSSION

Most of the studies in the literature and evidences that link inflammation to MDD are predominantly based on the three changes that occur in our immune system when suffering from MDD [6, 9, 10]. Firstly, MDD is almost always associated with raised levels of inflammatory markers like TNF-α, IL-6, IL-1, CRP etc. Secondly patients suffering from various chronic inflammatory disorders are at a higher risk of encountering MDD, having a 5-10 times increased risk [6, 10, 11]. Lastly, whenever therapeutic cytokines are given, there is a higher risk of suffering Depression. Various pathophysiological processes have been suggested in various articles across the literature suggesting an inflammatory origin to depression. These include mechanisms like insensitivity to glucocorticoid inhibitory feedback, reduced parasympathetic signalling, increased anterior cingulated cortex activity, reduced production of brain derived neurotrophic factor and reduced hippocampal volume [12-14]. The neutrophil-lymphocyte ratio (NLR) is a novel and a simple biomarker of inflammation and is being used in various medical diseases and neuropsychiatric disorders as prognostic as well as a diagnostic marker [4, 15]. Increased NLR is also interconnected with oxidative stress and increased cytokine productions. NLR is inexpensive and easily available parameter which reflects the immune-inflammatory response of the body as a reaction between innate and adaptive cell immunity during various pathological conditions including psychiatric and behavioural disorders. R. Zahorec in year 2021 had published the meta-analysis of data from about 200 studies that were conducted over the last 20 years, on the usefulness of NLR for not only screening but also prognosis in different pathologies and risk stratification in cardiovascular disorders [3]. In last 20 years NLR is being widely used as a useful marker in different types of cancer, SIRS, sepsis, behavioural and psychiatric pathologies, MI and different autoimmune disorders [16]. But what stands out is the direct correlation of NLR with the severity of depression which was seen in the present study. In this study we aimed to associate NLR with increasing severity of depression and it was seen that there was a significant rise in NLR with rising HAM-D score i.e., the severity of depression. Hamilton Rating Scale for Depression (HAM-D), a clinician-based scoring system for assessing the severity of depression, has significant advantage over other self-rating scoring systems like Beck Depression Inventory (BDI) especially where the study population is semi-illiterate. As the study was carried in a rural tertiary Health Care centre, using HAM-D over BDI helped in increasing the authenticity of the present study. Based on the scores obtained the population was divided on the basis of severity into three categories. A score of 7-17 was associated mild depression whereas the scores 18-24 and >25 were categorised under moderate and severe degrees of depression respectively. The results in this study revealed that mean NLR of mild depression (1.56), moderate depression (2.70) and severe depression (4.62) lied in the Normal (1-2), Grey zone (2-3) and mild-moderate inflammation zone (3-7) respectively. NLR, a handy and convenient marker reflects the association and complex relations among the three suprasystems: vegetative nervous system, neuroendocrine and immune systems and hence suggesting an interdependency among the fields of Psychiatry, Immunopathology and Hematology [3]. A study conducted by Arabska, *et al*., also observed that NLR is higher in patients with more severe symptoms compared to the group with milder symptoms, before medication use (2.10 ± 2.13 vs. 2.01 ± 0.75, $p = 0.004$), which is in analogous to the present study [17]. A similar study was carried out in adolescents by Gonca_Özyurt et al. which also showed a positive correlation between the severity of depression and neutrophil-lymphocyte ratios. There have been many clinical and experimental studies which have concluded that Immune system is altered during the process of clinical depression [1, 3, 5, 10, 18]. Although acute stress stimulates the immune functions of the body but persistent stress is associated with suppressed immune system. It is observed that cortisol is raised in chronic stress, which favours an increase in the population of neutrophils and a reduction in lymphocytes, and thus, increases the NLR index. Altered levels of various inflammatory cytokines like tumor necrosis factor alpha

(TNF-α) and interleukins (IL-1, IL-6) play the major role in pathophysiology of depression. Although Inflammatory cytokines are salient biomarkers in the course of disease starting right from the diagnosis to treatment selection and prognosis to long-term follow-up but the increased cost and limited attainability of the immunoassays are some of the limitations to their practical use [11, 19]. Thus, a cheap and easy to obtain biomarker like Neutrophil-Lymphocyte ratio could become a sturdy and powerful biomarker in assessing the severity and predicting the mortality in chronic diseases like Major Depressive Disorder.

REFERENCE

1. Menzel, A., Samouda, H., Dohet, F., Loap, S., Ellulu, M. S., & Bohn, T. (2021). Common and novel markers for measuring inflammation and oxidative stress ex vivo in research and clinical practice—which to use regarding disease outcomes?. *Antioxidants*, *10*(3), 414.
2. Buonacera, A., Stancanelli, B., Colaci, M., & Malatino, L. (2022). Neutrophil to lymphocyte ratio: an emerging marker of the relationships between the immune system and diseases. *International journal of molecular sciences*, *23*(7), 3636.
3. Zahorec, R. (2021). Neutrophil-to-lymphocyte ratio, past, present and future perspectives. *Bratisl Lek Listy*, *122*(7), 474-488.
4. Azab, B., Camacho-Rivera, M., & Taioli, E. (2014). Average values and racial differences of neutrophil lymphocyte ratio among a nationally representative sample of United States subjects. *PloS one*, *9*(11), e112361. doi: 10.1371/journal.pone.0112361.
5. Dowlati, Y., Herrmann, N., Swardfager, W., Liu, H., Sham, L., Reim, E. K., & Lanctôt, K. L. (2010). A meta-analysis of cytokines in major depression. *Biological psychiatry*, *67*(5), 446-457. doi: 10.1016/j.biopsych.2009.09.033
6. Krishnadas, R., & Cavanagh, J. (2012). Depression: an inflammatory illness?. *Journal of Neurology, Neurosurgery & Psychiatry*, *83*(5), 495-502.
7. Hamilton, M. (1960). A rating scale for depression. *J Neurol Neurosurg Psychiatry*, *23*(1), 56-62. doi:10.1136/jnnp.23.1.56.
8. Sousa, R. B. N., Alves, L. H., Carmo, V. C., Manso, C. S., & Alves, F. M. (2022). Applicability of the Neutrophil/Lymphocyte Ratio in Behavioral Studies. *Int J Blood Res Disord*, *9*, 075. doi.org/10.23937/2469-5696/1410075
9. Karg, K., Burmeister, M., Shedden, K., & Sen, S. (2011). The serotonin transporter promoter variant (5-HTTLPR), stress, and depression meta-analysis revisited: evidence of genetic moderation. *Archives of general psychiatry*, *68*(5), 444-454.
10. Pascoe, M. C., Crewther, S. G., Carey, L. M., & Crewther, D. P. (2011). Inflammation and depression: why poststroke depression may be the norm and not the exception. *International Journal of Stroke*, *6*(2), 128-135.
11. Liu, Y., Ho, R. C. M., & Mak, A. (2012). Interleukin (IL)-6, tumour necrosis factor alpha (TNF-α) and soluble interleukin-2 receptors (sIL-2R) are elevated in patients with major depressive disorder: a meta-analysis and meta-regression. *Journal of affective disorders*, *139*(3), 230-239. doi: 10.1016/j.jad.2011.08.003
12. McEwen, B. S. (1998). Stress, adaptation, and disease: Allostasis and allostatic load. *Annals of the New York academy of sciences*, *840*(1), 33-44.
13. O'Brien, S. M., Scully, P., Fitzgerald, P., Scott, L. V., & Dinan, T. G. (2007). Plasma cytokine profiles in depressed patients who fail to respond to selective serotonin reuptake inhibitor therapy. *Journal of psychiatric research*, *41*(3-4), 326-331.
14. Torun, D., Ozelsancak, R., Yiğit, F., & Micozkadıoğlu, H. (2012). Increased inflammatory markers are associated with obesity and not with target organ damage in newly diagnosed untreated essential hypertensive patients. *Clinical and Experimental Hypertension*, *34*(3), 171-175.
15. Man, M. A., Rajnoveanu, R. M., Motoc, N. S., Bondor, C. I., Chis, A. F., Lesan, A., ... & Fira-Mladinescu, O. (2021). Neutrophil-to-lymphocyte ratio, platelets-to-lymphocyte ratio, and eosinophils correlation with high-resolution computer tomography severity score in COVID-19 patients. *PloS one*, *16*(6), e0252599.
16. Özyurt, G., & Binici, N. C. (2018). Increased neutrophil-lymphocyte ratios in depressive adolescents is correlated with the severity of depression. *Psychiatry research*, *268*, 426-431.
17. Arabska, J., Łucka, A., Magierski, R., Sobów, T., & Wysokiński, A. (2018). Neutrophil-lymphocyte ratio is increased in elderly patients with first episode depression, but not in recurrent depression. *Psychiatry research*, *263*, 35-40.
18. Sunbul, E. A., Sunbul, M., Yanartas, O., Cengiz, F., Bozbay, M., Sari, I., & Gulec, H. (2016). Increased neutrophil/lymphocyte ratio in patients with depression is correlated with the severity of depression and cardiovascular risk factors. *Psychiatry investigation*, *13*(1), 121-126.
19. Mazza, M. G., Lucchi, S., Tringali, A. G. M., Rossetti, A., Botti, E. R., & Clerici, M. (2018). Neutrophil/lymphocyte ratio and platelet/lymphocyte ratio in mood disorders: a meta-analysis. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *84*, 229-236. doi: 10.1016/j.pnpbp.2018.03.012.