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Original Article

Neutrophil to lymphocyte ratio as a predictor biomarker for asthma control

Pulmonology

Mariam M. Elshony[1], Taghreed S. Farag[1], Heba H. Eltrawy[1], Abeer M. Abdul-Muhaymen[2]

[1] Chest Diseases Department, Faculty of Medicine for Girls, Cairo, Al-Azhar University, Egypt.

[2] Clinical Pathology Department, Faculty of Medicine for Girls, Cairo, Al-Azhar University, Egypt

ABSTRACT

Background: Neutrophils have been studied by several authors as an important cell that plays a major role in many inflammatory disorders such as asthma. It is known that neutrophil to lymphocyte ratio (NLR) is an important prognostic biomarker for many disorders especially bronchial asthma.

Objective: To assess the relation of NLR and bronchial asthma severity and to determine NLR discriminative performance between controlled and uncontrolled asthma.

Methodology: This case-control study was performed upon 80 asthmatic patients and 80 healthy controls. The asthma diagnosis was established according to GINA guideline. Asthma control test (ACT), spirometry test, total and differential leucocytic count were done and NLR was calculated for all studied subjects.

Results: A statistically significant differences were identified between asthmatics and healthy subjects regarding age, sex and history of other allergies($p<0.001$). NLR was statistically significantly higher in asthmatic patients compared to controls ($p<0.001$). According to Receiver Operating Characteristic [ROC] curve, NLR could be used to predict asthma control at cutoff level > 1.32, with 92.5% sensitivity, 93.8% specificity, 93.7% positive predictive value and 92.6% negative predictive value.

Conclusions: Neutrophil to lymphocyte ratio could be an important predictor biomarker for asthma control.

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Keywords: Asthma, neutrophils, lymphocytes, biomarkers.

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Corresponding author: Heba H Eltrawy, Chest department, faculty of medicine for girls, Cairo, Al-Azhar university, Egypt. **Tel:** +201006381297. **E-mail:** hebahamed.18@azhar.edu.eg, hebahamed182@gmail.com

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INTRODUCTION

Neutrophilic, eosinophilic, paucigranulocytic or mixed granulocytic types are four various endotypes of asthma [1] depending on cell counts of peripheral blood, sputum or bronchoalveolar fluid [2].

Higher autophagy of neutrophils has been described in many pulmonary disorders as well as neutrophil extracellular traps and exosomes that has been newly studied as deriving from neutrophil [3]. So neutrophils role in pulmonary diseases have been established. Despite pulmonary diseases pathogeneses are being studied widely, there is still more and more to get clarification of the discrepancy and complexity, particularly the contribution of different immune mechanisms in the progress of pulmonary disorders [4].

Neutrophilic inflammations have been studied to be associated with a poor asthma control [5]. In addition to persistent asthma symptoms and atopy, they have been reported to be associated with the presence of eosinophilic inflammation in asthmatic patients [6]. Accordingly, NLR increase in asthmatics was considered. However, data related to NLR in ~~A~~sthmatic patients is inadequate [7] [8], [9]. Though association between asthma and NLR, [8] was discovered by earlier researches in adults, recent researches did not study NLR relation to neutrophilic asthma [9]. So, we aimed to assess the relation of NLR and bronchial asthma severity and to determine NLR discriminative performance between controlled and uncontrolled asthma.

SUBJECTS AND METHODS

This case-control study was conducted at chest diseases department, faculty of medicine for girls, Cairo, Al-Azhar University, Egypt, during the period from May 2021 to October 2021.

Inclusion criteria: 80 known bronchial asthma patients, they were more than 18 years old and presented with symptoms and signs of bronchial asthma and 80 healthy control volunteers.

Exclusion criteria: patients with other chest disease, infection and comorbidities or chronic disease e.g. renal diseases, liver diseases, cardiovascular diseases, and autoimmune diseases were excluded from the study.

Written consent was obtained from participants before including into the study. The study protocol was accepted by institutional review board of Faculty of Medicine for Girls, Al-Azhar University, Cairo, Egypt. Every participant can refuse participation or withdraw from the study at any time without any clarification of the reason and without troubling their right of medical service. Moreover, information was nameless and coded to guarantee privacy of patients.

Data regarding age, gender, smoking history and history of other concomitant allergic diseases were reported. Asthma control test (ACT) was used, it involves five questions created to assess nighttime and daytime presenting symptoms, rate of beta 2 agonists usage as reliever medications, and restricting daily activities. Twenty-five points score reveals "complete control", while 20–24 points suggest "partial control", while less than 20 points indicate "uncontrolled" asthma [10]. Ventilatory functions was performed on (FUDUKA Spiro sift 5000). Spirometry was done before and repeated after the inhalation of short-acting β2-agonist (SABA). Heavy meals, physical exercise, and smoking were avoided 6 hours before test. Patients wear light cloths. Tests procedure was explained in full details. The following measurements were recorded: vital capacity (VC %), forced vital capacity (FVC %), forced expiratory volume in the first second (FEV1%), FEV1\FVC ratio and forced expiratory flow rate 25-75%. A post-bronchodilator spirometry was performed 10-15 minutes after the inhalation of 400µg Salbutamol. An increase in forced expiratory volume in 1st second >200 ml and ⁄ or 12% above the pre-bronchodilator FEV1 at time of assessment was considered diagnostic [11]. Spirometric evidence of airway obstruction in bronchial asthma includes FEV1/FVC ratio less than 80%, FEV1 % less than 80 percent predicted [12] and a positive finding of reversibility with a bronchodilator [11]. Ventilatory tests-parameters were measured using the best out of three satisfactory results in agreement in accordance with the European Respiratory Society [13].
Venous blood samples were drawn in early morning between 8.00- 9.00 a.m. after an 8- hour overnight fasting. Venous blood samples were collected from cubital vein and immediately putted in ethylene diaminetetraacetic acid- holding tubes (Becton Dickinson Vacuum) and mixed gently. Total leucocytic count (TLC), lymphocytes absolute percentage and count, and eosinophils absolute count and percentage were measured within 1–2 h of blood specimen by using a hematological (Sysmex XE-21N, Kobe, Japan) and (Neutrophil to lymphocyte ratio (NLR) was calculated for all studied subjects.

Analysis of Statistics
Statistical Program for Social Science (SPSS) version 24 was used to analyses collected data. Based on ACT-score the asthmatic patients were divided into well controlled, partially controlled and uncontrolled asthma subgroups. However, for ROC curve analysis for discrimination between controlled and uncontrolled asthma the partially controlled and uncontrolled asthma subgroups were grouped together and considered as one group. Quantitative data were stated as mean ±SD (for normally distributed data) and median (IQR) (for abnormally distributed data). Qualitative data were stated as rate and percentage. Independent-samples t-test of significance was used for comparison between two means (for normally distributed data). Mann–Whitney U test was used for comparison between two means (for abnormally distributed data). Kruskal Willis test (KW): for comparison between more than two means (for abnormally distributed data). Chi-square test was used for comparison between non-parametric data. Pearson's correlation coefficient (r) test was used for correlating two quantitative data in asthma group. ROC curve (Receiver Operating Characteristic Curve) was used to detect, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of NLR in discrimination between controlled and uncontrolled asthma. The confidence interval was set to 95%, and the margin of error accepted was set to 5%. So, the significance of the test was determined based on probability (P) values: p value ≤ 0.05 was considered significant, while p value > 0.05 was considered non-significant.

RESULTS

Table (1) shows significant difference (p-value = 0.001) between asthmatic patients and controls regarding age as there was increase in age and other allergies in patients group compared to controls (p-value < 0.001). There was statistically significant difference regarding sex, as 11 males (13.8%) and 69 females (83.7%) in patients' group while there were 30 males (37.5%) and 50 females (62.5%) in control group. Also, there was no significant difference (p-value > 0.05) between asthma and control groups as regard smoking.

Table (2) demonstrates statistically significant (p-value < 0.001) decreased spirometric parameters (FEF 25-75 % VC%, FVC%, FEV_1%, and FEV_1/FVC ratio) in patients group compared to control group. There was statistically significant (p-value = 0.035) increased TLC in patients' group (median = 7.9, IQR = 6.4 – 9.5) when compared to control group (median = 7, IQR = 5.3 – 8.9), and increased absolute lymphocytes in patients' group

(median = 2.7, IQR = 2.3 – 3.3) when compared to control group (median = 2.5, IQR = 2 – 3) and statistically significant (p-value < 0.001) increased eosinophil in patients' group (median = 2.9, IQR = 2.1 – 4) when compared to control group (median = 1.5, IQR = 1.2 – 2.1). There was no statistically significant difference (p-value > 0.05) between studied groups as regard neutrophil (% & absolute count) and lymphocytes (%).

Table (3) shows statistically significant increase in NLR in patients' group (median = 2.5, IQR = 1.8 – 3.5) when compared to control group (median = 1.02, IQR = 0.89 – 1.2). Table (4) shows statistically significant (p-value < 0.001) increased NLR in uncontrolled patients (Median = 3.5, IQR = 3.4 – 3.7) when compared to partially controlled patients (Median = 2.5, IQR = 2.2 – 3.5) and well controlled patients (Median = 1.62, IQR = 1.32 – 1.82). Table (5) showed significant (p-value < 0.001) positive correlation (r = 0.53) between NLR and disease duration (r = 0.53), while there was significant negative correlation between NLR and FEV1/FVC ratio (r = - 0.66) and FEV1 (r = - 0.77) in patients' group. Based on ROC curve, it was shown that NLR can be used to predict asthma control at a cutoff level of > 1.32, by sensitivity of 92.5%, specificity of 93.8%, 93.7% PPV and 92.6% NPV (AUC = 0.97) (table 6, figure 1).

Table (1): Comparison of demographic data between the studied groups

Item		Asthma group (n = 80)	Control group (n = 80)	Statistical test	p-value
Age (years)	Median (IQR)	49 (34 – 54)	30 (25 – 44)	MW = 745	0.001*
Sex	Male	11 (13.8%)	30 (37.5%)	X^2 = 11.8	0.001*
	Female	69 (86.2%)	50 (62.5%)		
Smoking	No	58 (72.5%)	52 (65%)	X^2 = 1.05	0.306
	Yes	22 (27.5%)	28 (35%)		
Other allergies	No	47 (58.8%)	80 (100%)	X^2 = 41.5	0.001*
	Yes	33 (41.2%)	0 (0%)		

MW: Mann Whitney U test, X^2: Chi-square test, *: Significant p-value.

Table (2): Comparison of PFTs and complete blood count indices between the studied groups

Item		Asthma group (n = 80)	Control group (n = 80)	MW	p-value
VC%	Median (IQR)	50 (42-59)	63 (55-76)	1455	0.001*
FVC%	Median (IQR)	42 (32-52)	60 (51-68)	1454	0.001*
FEV_1/FVC ratio	Median (IQR)	74 (60-82)	95 (87-99)	367.5	0.001*
FEF 25 – 75%	Median (IQR)	34 (21-47)	61 (55-65)	427.5	0.001*
FEV_1%	Median (IQR)	54 (36- 67)	88 (84-95)	85	0.001*
TLC ($\times 10^3$/ml)	Median (IQR)	7.9 (6.4-9.5)	7 (5.3-8.9)	2584	0.035*
Neutrophils %	Median (IQR)	54 (45-63)	57 (45-62)	3017	0.533
Lymphocytes %	Median (IQR)	37 (30-45)	36 (28-44)	2946	0.462
Absolute neutrophils	Median (IQR)	4.3 (3.2-5.4)	3.7 (3-5.2)	2896	0.3
Absolute lymphocytes	Median (IQR)	2.7 (2.3-3.3)	2.5 (2-3)	2508	0.018 *
Eosinophils %	Median (IQR)	2.9 (2.1-4)	1.5 (1.2-2.1)	961	0.001*

MW: Mann Whitney U test, PFTs: pulmonary function tests. FVC: forced vital capacity, FEV_1: forced expiratory volume in 1st second, FEF25-75%: forced expiratory flow rate 25-75%. TLC: total leucocytic Count, (abs): absolute count, *: Significant p-value.

Table (3): Comparison of NLR between studied groups

NLR	Asthma group (n = 80)	Control group (n = 80)	MW	P-value
Median (IQR)	2.5 (1.8-3.5)	1.02 (0.89- 1.2)	172	0.001*

MW: Mann Whitney U test, NLR: neutrophil to lymphocyte ratio, *: Significant p-value.

Table (4): Comparisons of NLR regarding asthma control level

		ACT			KW	P-value
		Well controlled (n = 27)	Partially controlled (n = 27)	Uncontrolled (n = 26)		
NLR	Median (IQR)	1.62 (1.32-1.82)	2.5 (2.2-3.5)	3.5 (3.4-3.7)	59.1	0.001*

KW: Kruskal Willis test, NLR: neutrophil to lymphocyte ratio, *: Significant p-value.

Table (5): Correlation study between NLR and other studied data in bronchial asthma group

Bronchial asthma group	NLR	
	r	p-value
Age	-0.14	0.217
Asthma duration (years)	0.53	0.001*
VC%	0.03	0.803
FVC%	-0.06	0.602
FEV_1/FVC ratio	-0.66	0.001*
FEF 25-75%	-0.17	0.143
FEV_1%	-0.77	0.001*
TLC ($\times 10^3$/ml)	0.02	0.876
Neutrophils %	0.03	0.77
Lymphocytes %	-0.05	0.673
Absolute neutrophils	0.00	0.989
Absolute lymphocytes	0.03	0.786
Eosinophils %	-0.16	0.163

NLR: neutrophil to lymphocyte ratio, FVC: forced vital capacity), BD: bronchodilator FEV1: forced expiratory volume in 1st second, FEF25-75%: forced expiratory flow rate 25-75%. TLC: total leucocytic Count, (abs): absolute, *: Significant p-value.

Table (6): Diagnostic performance of NLR in prediction of asthma control

NLR	Cutoff	AUC	Sensitivity	Specificity	PPV	NPV
	> 1.32	0.97	92.5%	93.8%	93.7%	92.6%

PPV: positive predictive value, AUC: Area under curve, NPV: negative predictive value, NLR: neutrophil to lymphocyte ratio

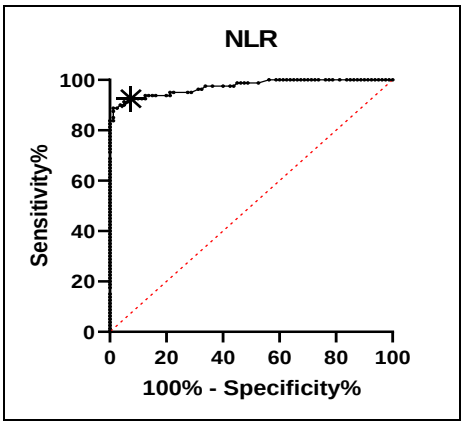

Figure (1): ROC curve of neutrophil-lymphocyte ratio in prediction of bronchial asthma control

DISCUSSION

Cornerstone in asthma control is regular updating in asthma managing strategy. Both ACT and measurement of NLR as an inflammatory marker are used to evaluate and follow up management in asthmatics patients [14].

Neutrophils contribute to inflammation of airways and are triggered in inflammatory pulmonary diseases like asthma [15]. NLR is expected to be a biomarker for systemic and airways inflammation [16], and it may be altered in asthmatics and healthy adults. three studies showed that NLR was significantly differ between control subjects and asthmatics patients [17, 18 ,19]. In contrast, other two studies revealed no significant difference between asthma and healthy controls [10, 21].

Current study revealed that the NLR was increased significantly in patients with asthma compared to controls (p<0.001), this is in agreement with Gungen and Aydemir study in Turkey [22] who reported that the mean NLR was significantly higher in asthmatics compared to controls. It was reported higher NLRs could be associated with Asthma severity, inflammation, long hospitalization, and mortality [23]-[24]. Increased cytokines levels usually accompanied with increased neutrophils [25]. Pro-inflammatory mediators like tumor necrosis factors and interleukin-6 are increased in asthma and subsequently increases immune cells as natural killer cells and neutrophils [25]. Additionally, Chinese Liu et al [26] research concluded that NLR level is clearly increased in severe asthma. New Japanese research by Mochimaru et al [27] recorded that NLR level is raised in acute exacerbations of asthma in adults. Shi et al [21] founded that elevated NLR are involved in diagnostic and prognostic features of asthma. Raised NLR is clarified as one of inflammatory markers that are significant in

asthma progress. Many researchers investigated the relationship between neutrophilic inflammation and level of asthma control. The relation between neutrophils and uncontrolled asthma might be observed in eosinophilic and non-eosinophilic asthma. The TH-2 helper cells initiate the neutrophilic inflammation [28].

Current study reported that white blood cells count elevation in asthmatic patients compared to controls (p=0.035). Zhang et al [9] concluded that hematological indices such as leukocytic count are useful in follow up of poorly controlled asthma. Current study showed eosinophils and lymphocyte absolute count elevation in asthmatic patients compared to controls which in agreement with Ramirez-Velazquez et al [29] that found higher percentage of peripheral blood CD4+ and CD8 +T lymphocytes in asthmatics compared to healthy controls and concluded that T cells and eosinophils, involved in bronchial inflammation in allergic asthma. Our study showed that mean NLR of asthmatic group was significantly higher in uncontrolled group than well controlled asthma (p<0.001). This finding is agreed with Egyptian study results of Hendy et al [30] which stated that NLR predicts precisely poor controlled asthma according to ACT classes among patients with bronchial asthma.

Present study revealed that NLR was positively correlated with duration of asthma, while it was negatively correlated with FEV_1% and FEV_1\FVC ratio. That may explain that the NLR ratio was increased with severe asthma. Current study reported that NLR can be used to predict asthma at a cutoff level of > 1.32, with 92.5% sensitivity, 93.8% specificity, 93.7% PPV and 92.6% NPV (AUC = 0.97 & p-value < 0.001). which is similar to results of Nacaroglu et al [31] who found that NLR is an actual predictor of asthma in children. Also, these results in concordance with Netherland's study of Bruijnzeel et al [32] who reported that neutrophils phenotypes are predictor of severity in severe neutrophilic asthma and NLR play a major role in disease course prediction and echoes the control level of asthma with its positive association with ACT.

CONCLUSIONS

Neutrophil to lymphocyte ratio could be considered a valid marker in follow up of asthma management. Neutrophil to lymphocyte ratio is correlated with asthma duration and severity. NLR could be an important predictor biomarker for asthma control.

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الملخص العربي

نسبة العدلات إلى الخلايا الليمفاوية كمؤشر حيوي للتنبؤ بمستوى التحكم في الربو

مريم مسعد الشوني[1]، تغريد سعيد فرج[1]، هبة حامد الطراوي[1]، عبير محمد عبد المهيمن[2]

[1] قسم الأمراض الصدرية، كلية طب بنات، القاهرة، جامعة الأزهر، جمهورية مصر العربية

[2] قسم الباثولوجيا الإكلينيكية، كلية طب بنات، القاهرة، جامعة الأزهر، جمهورية مصر العربية

ملخص البحث

الخلفية: تمت دراسة العدلات من قبل العديد من المؤلفين كخلية مهمة تلعب دورًا رئيسيًا في العديد من الاضطرابات الالتهابية مثل الربو. من المعروف أن نسبة العدلات إلى الخلايا الليمفاوية هي علامة بيولوجية تنبؤية مهمة للعديد من الاضطرابات وخاصة الربو الشعبي.

الهدف: تقييم العلاقة بين نسبة العدلات إلى الخلايا الليمفاوية وشدة الربو الشعبي وتحديد الأداء التمييزي لنسبة العدلات إلى الخلايا الليمفاوية بين الربو المنضبط وغير المنضبط.

الطرق: تم إجراء هذه الدراسة (الحالات والشواهد) على 80 مريضًا بالربو و 80 شخصًا من الأصحاء. تم تحديد تشخيص الربو وفقًا لإرشادات جينا تم إجراء اختبار التحكم في الربو، واختبار قياس التنفس، وعدد الكريات البيض الكلي والنوعي ، وتم حساب نسبة العدلات إلى الخلايا الليمفاوية لجميع الأشخاص الذين خضعوا للدراسة.

النتائج: تم تحديد فروق ذات دلالة إحصائية بين المصابين بالربو والأشخاص الأصحاء فيما يتعلق بالعمر والجنس وتاريخ الحساسية الأخرى. كان نسبة العدلات إلى الخلايا الليمفاوية أعلى من الناحية الإحصائية في مرضى الربو مقارنة بالمجموعة الضابطة. وفقًا لمنحنى روك ، يمكن استخدام للتنبؤ بالتحكم في الربو عند مستوى القطع> 1.32، مع حساسية 92.5٪ وخصوصية 93.8٪ وقيمة تنبؤية إيجابية 93.7٪ وقيمة تنبؤية سلبية 92.6٪.

الاستنتاجات: يمكن أن تكون نسبة العدلات إلى الخلايا الليمفاوية علامة حيوية تنبؤية مهمة للسيطرة على الربو.

الكلمات المفتاحية: الربو ، العدلات ، الخلايا الليمفاوية ، المؤشرات الحيوية.

الباحث الرئيسى

الأسم: هبة حامد الطراوي, قسم الامراض الصدرية، كلية طب بنات, القاهرة, جامعة الأزهر, جمهورية مصر العربية.

الهاتف: 01006381297

البريد الالكترونى: hebahamed182@gmail.com -hebahamed.18@azhar.edu.eg