Diagnostic Value of Neutrophil–Lymphocyte Ratios and Mean Platelet Volumes in the Activation of Recurrent Aphthous Stomatitis

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Abstract Recurrent aphthous stomatitis (RAS) is associated with endothelial dysfunction and chronic inflammation. The neutrophil-to-lymphocyte ratio (NLR) and mean platelet volume (MPV) are markers of inflammation and endothelial dysfunction, respectively. In the present report, we discuss the NLR and MPV values of patients with active and inactive RAS. In total, 42 patients (24 females and 18 males) with inactive RAS, 19 patients (12 females and 7 males) with active RAS and 40 healthy controls (24 females and 16 males) were enrolled. MPVs were measured and NLRs calculated. We sought correlations among the MPV and NLR findings in the active and inactive RAS groups and compared them with those of healthy controls. The MPV and NLR values were significantly higher in patients with active than inactive RAS (MPV, 10.6 ± 2.9 vs. 7.1 ± 2.4 fL, $p < 0.001$; NLR, 3.74 ± 1.9 vs. 2.1 ± 1.43 , $p = 0.015$). In addition, both MPV and NLR values in patients with inactive RAS didn't differ significantly compared to values observed in the controls (MPV, 7.1 ± 2.4 vs. 6.9 ± 2.1 fL, $p = 0.126$; NLR, 2.1 ± 1.43 vs. 2.07 ± 0.96 , $p = 0.525$). Both the NLR and MPV were significantly higher in patients with active RAS, emphasising the importance of inflammation and endothelial dysfunction in the pathophysiology of RAS activation.

Keywords Inflammation · Mean platelet volume · Neutrophil · Recurrent aphthous stomatitis

Introduction

Recurrent aphthous stomatitis (RAS) is chronic inflammation of the oral mucosa characterised by recurrent ulcerations; it affects approximately 20% of the general population [1]. The characteristic aphthae are painful, round or ovoid ulcers, developing most frequently on the buccal mucosa, vestibule, undersurface of the tongue, and floor of the mouth; the ulcers may be single or multiple and heal without scarring. Minor aphthae are usually <5 mm in diameter and heal within 7–10 days. A few patients present with much larger and more persistent ulcers, which sometimes affect the dorsum of the tongue or palate as well as other sites, and may scar upon healing (major aphthae) [2, 3]. The underlying aetiology remains unclear, though several predisposing factors are known, including autoimmune status, vascular abnormalities, genetic predisposition, viral and bacterial infections, food allergies, vitamin and microelement deficiencies, systemic diseases (e.g., celiac disease, Crohn's disease, and/or AIDS), increased oxidative stress, hormonal defects, mechanical injuries, and anxiety [3–6].

Mean platelet volume (MPV) is an indicator of platelet function, reflecting platelet production, stimulation, and activity [7]. Platelets have been suggested to play important roles in immune and/or inflammatory processes [8]. MPVs increase during vascular events, including atherosclerosis, the development of acute syndromes, and venous and/or arterial thrombosis or thromboembolism [9].

The neutrophil–lymphocyte ratio (NLR) is measured by dividing the neutrophil count by the lymphocyte count. The

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NLR serves as an indicator of systemic inflammation in patients with various conditions, including cardiovascular diseases [10, 11], and ulcerative colitis [12]; it is also a prognostic marker for many types of cancer [13–15]. Recently, the NLR has been widely used to determine the severity of inflammation.

RAS is associated with endothelial dysfunction and chronic inflammation [2, 3]. The NLR and MPV are markers of inflammation and vascular risk, respectively. Therefore, in the current study, we compared the NLRs and MPVs of patients with active and inactive RAS.

Materials and Methods

In this study 115 consecutive patients with the diagnosis of RAS who admitted to outpatient otolaryngology clinic of a tertiary referral center between November 2015 and July 2016 were identified retrospectively. The inclusion criteria were as follows: minor or major RAS, non-smoker, and a minimum of 2 years of RAS history. This ruled out other acute recurrent ulcers such as recurrent herpes. Patients with a history of Behçet's disease (BD); any systemic disease including a cardiovascular, endocrine, or metabolic disease; a hematinic deficiency; a history of steroid or oral contraceptive pill use; who were pregnant; who were diagnosed with cancer; and who smoked, were excluded.

RAS was diagnosed by reference to medical records, medical histories, and clinical examination. As a result, 42 patients (24 females and 18 males) with inactive RAS, 19 patients (12 females and 7 males) with active RAS and 40 healthy controls without any aphthae complaint (24 females and 16 males) were recruited consecutively for the study.

Blood samples were taken from the antecubital vein at 8 a.m. after an overnight fast. NLRs and MPVs were calculated (in duplicate) with the aid of an automated blood cell counter (Sysmex XN-1000, Sysmex Corporation, Kobe, Japan). The lower and upper MPV limits were 6.5 and 12 fL, respectively. Each NLR was calculated as the simple ratio between the absolute neutrophil and lymphocyte counts.

Measurements were compared statistically using the SPSS software (ver. 23.0 for Windows; SPSS Inc., Chicago, IL, USA). Continuous variables are presented as mean $\pm$ standard deviation and categorical variables as % ratios. Qualitative values were compared using the χ^2 test, and quantitative values were compared using a two-tailed *t* test. Correlations among the NLR and MPV data of the active, inactive RAS groups and controls were compared with analysis of variance (ANOVA). Tukey's test was applied to pairwise comparisons of each study group among NLR and MPV values. A *p* value <0.05 was considered to indicate statistical significance.

Results

The active RAS group included 19 patients (12 females and 7 males) and a mean age of 43.8 ± 8.6 years (range 17–55 years), the inactive RAS group included 42 patients (24 females and 18 males) and a mean age of 44.2 ± 9.2 years (range 21–60 years) and the control group included 40 healthy subjects (24 females and 16 males) with a mean age of 41.9 ± 10.76 years (range 20–58 years). The age and sex distribution of the participants did not differ significantly between groups (all *p* > 0.05).

The MPV and NLR values were significantly higher in patients with active than inactive RAS (MPV, 10.6 ± 2.9 vs. 7.1 ± 2.4 fL, *p* < 0.001 ; NLR, 3.74 ± 1.9 vs. 2.1 ± 1.43 , *p* = 0.015). Both values were statistically higher in patients with active RAS compared to the controls (MPV, 10.6 ± 2.9 vs. 6.9 ± 2.1 fL, *p* < 0.001 ; NLR, 3.74 ± 1.9 vs. 2.07 ± 0.96 , *p* = 0.01). In addition, the MPV and NLR values in patients with inactive RAS didn't differ significantly compared to values observed in the controls (MPV, 7.1 ± 2.4 vs. 6.9 ± 2.1 fL, *p* = 0.126; NLR, 2.1 ± 1.43 vs. 2.07 ± 0.96 , *p* = 0.525). Table 1 summarizes the MPV and NLR values.

Discussion

The process culminating in thrombosis in patients with RAS or BD has not yet been elucidated. Also, the precise pathogenic mechanism triggering the formation of RAS vascular lesions remains unclear. Endothelial and platelet dysfunction are thought to play important roles in thrombosis development [16–18]. Platelet dysfunction can be identified by calculating the MPV, which measures platelet size and activity. Correlations between an increased MPV and certain thrombotic diseases such as deep-vein thrombosis, acute myocardial infarction, and acute ischaemic cerebrovascular events have been reported. Such clinical consequences of a high MPV are explained by the fact that larger platelets are more reactive and more prone to aggregate, consequently triggering endothelial dysfunction [19]. NLR is an emerging marker of inflammation and plays a role in endothelial dysfunction [20, 21]. BD patients exhibit problems with neutrophil chemotaxis [22]. Therefore, in the present study, we explored the relationships between the NLRs and MPVs of patients with active and inactive RAS.

The literature lacks any comparison of MPVs and NLRs during active and inactive RAS periods. Most studies have dealt with BD. Ekiz et al. [23] measured the MPV in patients with BD or RAS, and in healthy controls. The MPVs of BD and RAS patients were significantly higher

Table 1 MPV and NLR values

	Active RAS group (n = 19)	Inactive RAS group (n = 42)	<i>p</i> *	Control group (n = 40)	<i>p</i> **	<i>p</i> ***
MPV (fL)	10.6 ± 2.9 (6.3–14)	7.1 ± 2.4 (4.1–11.3)	<0.001	6.9 ± 2.1 (4.3–9.9)	<0.001	0.126
NLR	3.74 ± 1.9 (1.3–5.2)	2.1 ± 1.43 (0.3–4.6)	0.015	2.07 ± 0.96 (0.4–4.9)	0.01	0.525

Values in parentheses are ranges

MPV mean platelet volume, NLR neutrophil–lymphocyte ratio, RAS recurrent aphthous stomatitis

* Analysis between active and inactive RAS groups

** Analysis between active RAS and control groups

*** Analysis between inactive RAS and control groups

than those of the controls, suggesting that MPV can be used as an inexpensive and simple diagnostic marker for BD and RAS patients. Karagoz and Tanoglu [24] emphasised that MPV might be useful in the diagnosis of BD and RAS. Turkcu et al. [25] measured the MPV in patients with ocular BD; the figures differed significantly between the BD and control groups. Recently, the NLR has become a commonly studied inflammatory marker. Rifaioglu et al. [26] retrospectively reviewed data from 65 patients with active and inactive BD; the NLR was higher in BD patients than in healthy subjects, and an increase in the NLR correlated with a rise in disease activity. Similar findings were reported by Ozturk et al. [21]. The cited authors found a significant relationship between the NLR and BD. Our data are similar to these previously published findings.

The strength of our study is the assessment of the association between NLR, MPV and activation of RAS for the first time. The results of the present study confirm the crucial role of the vascular, thrombotic, and inflammatory processes in the activation of RAS. Discovering the real etiopathogenetic factors in the activation of RAS may help to predict the occurrence of disease and to develop the causative, effective management. This study included a relatively small number of patients that limits the interpretation and generalizability. Additional larger, population-based studies with long-term clinical follow-up data are needed to accurately verify the preliminary results of the present study.

Conclusions

The significant associations evident between an elevated NLR and MPV, and active RAS, indicates that vascular, thrombotic, and inflammatory processes are important in the pathophysiology of RAS activation. We speculate that both the NLR and MPV may be used to predict RAS activation. However, increased platelet reactivity or sub-clinical inflammation alone does not explain aphtous lesion

formation; the pathogenesis is more complex (being multifactorial). Thus, additional studies are required to verify our preliminary results.

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Compliance with Ethical Standards

Conflict of interest The authors have no conflicts of interest or financial ties to disclose in regard to this study.

Ethical standards All procedures performed in studies involving human participants were in accordance with the 1964 Helsinki declaration, its later amendments or comparable ethical standards and was approved by the Research Ethics Committee of a Tertiary Referral Center. Written informed consent was obtained from all participants prior to the study.

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