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Evaluation of Systemic Inflammation in Lichen Planus Using Neutrophil CD64

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Abstract

Lichen Planus (LP) is a chronic inflammatory condition caused by T-cell-mediated immune responses, primarily affecting the skin and oral mucosa. The inflammatory marker CD64, which is expressed on neutrophils in response to inflammation, has demonstrated promise as a biomarker in several inflammatory disorders; however, its function in LP and its applicability for diagnosis and prognosis remain unknown. This study aims to evaluate systemic inflammation of LP patients using neutrophil CD64 as a potential biomarker. A total of five patients with LP diagnoses and five healthy controls were included in this study. nCD64 expression was measured by flow cytometric analysis from the peripheral blood samples of patients and healthy controls. The mean fluorescence intensity of nCD64 was noted and the data of patients and healthy controls were compared. A robust systemic inflammatory response was seen in LP patients, as evidenced by a substantially higher average nCD64 mean fluorescence intensity of 208.4 in comparison to 45.6 in healthy controls (p=0.05). Patients with LP exhibited a range of clinical features, including gingival desquamation, erosive lesions, and white striae. The clinical severity of LP lesions was correlated with the nCD64 mean fluorescence intensity whereas the severity of the disease was not correlated with the existing marker, erythrocyte sedimentation rate values. Thus nCD64 may be a useful biomarker for identifying systemic inflammation in patients with Lichen Planus and this may lead to better diagnosis and treatment in such patients. A large cohort study is warranted to confirm these preliminary findings.

Keywords: *Biomarker, Chronic Inflammatory Condition, Flow Cytometry, Systemic Inflammation, T Cell Mediated Immune Response.*

Introduction

Lichen Planus (LP) is a chronic papulosquamous condition that often impacts individuals in their middle age. It commonly appears as itchy, purple bumps, mainly on the skin and inside the mouth, but can also affect nails, scalp, throat, and genital areas [1]. Oral lichen planus (OLP) is a type of LP in the mouth that is known as a T-cell mediated chronic inflammatory condition, featuring white striations, plaques [2], and redness, primarily impacting the buccal mucosa [3,4], tongue, and gingiva. Currently, the lesions of oral LP are being treated with topical betamethasone 0.5mg mouth rinses and topical triamcinolone paste application [5,6].

CD64, or Fc gamma receptor I (FcγRI), is a receptor with a strong affinity for

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immunoglobulin G (IgG) found mainly on neutrophils when the body is responding to infections or pro-inflammatory cytokines like interferon-gamma (IFN-γ) and granulocyte colony-stimulating factor (G-CSF) [7,8]. Oral disease patients have different blood parameters [9,10]. Increased levels of neutrophil CD64 (nCD64) have been linked to systemic inflammation and sepsis, suggesting it could serve as a marker for inflammatory conditions [8,11,12]. Inflammation plays a main role in different pathological conditions [13,14].

Several studies in OLP have pointed out abnormal patterns of expression of different cytokines like interleukins (ILs), transforming growth factor-beta (TGF-β), IFN-γ, and tumour necrosis factor-alpha (TNF-α) in lesions, saliva, serum, and peripheral blood mononuclear cells (PBMCs) derived from patients [15,16]. This highlights the major inflammation related to the condition. This study aimed to assess nCD64 levels through flow cytometry to evaluate systemic inflammation in lichen planus. This study sought to investigate the hidden inflammatory mechanisms of LP and its possible systemic consequences. Improved knowledge of nCD64 levels may improve the early detection and management of LP and other inflammatory conditions, offering insights into the wider utility of flow cytometry for early disease diagnosis [5,17]. This study addresses a gap in the existing research by offering information on the systemic inflammatory response in lichen planus, with the possible impact on future treatment approaches and patient outcomes.

Materials and Methods

The methodology used in this study aimed to investigate the immune response in lichen planus by analyzing blood samples from 5 patients and 5 healthy controls. The study employed flow cytometry to assess specific immune cell markers (nCD64) in the blood samples.

Study Participants and Blood Sample Collection

A total of ten participants were enrolled in this study. The participants were divided into two groups:

1. Group A consisted of five patients diagnosed with lichen planus,
2. Group B included five healthy individuals as controls.

Patients with lichen planus were recruited based on specific diagnostic criteria (based on clinical history, presentation, and histopathological diagnosis). Approximately 500 microliters of peripheral blood were collected from each participant in ethylenediaminetetraacetic acid (EDTA) vacutainers. The blood samples were collected to assess systemic inflammation in lichen planus by assessing the mean fluorescence intensity of nCD64.

Staining Process

Upon collection, the blood samples were divided into two separate vacutainers, referred to as T1 and T2.

1. In T1, 5µl of nCD64PE (Phycoerythrin) (BD Biosciences, USA; Cat No. 558592) antibodies were added to detect nCD64 expression.
2. In T2, 5µl of IgG PE (Immunoglobulin G Phycoerythrin) (BD Biosciences, USA; Cat No. 555787) antibodies were added as an isotype control to establish the baseline fluorescence measurement for comparison.

The T1 and T2 vacutainers were then incubated at room temperature in the dark for 20 minutes. This incubation period allows the antibodies to bind specifically to nCD64 and IgG, respectively, on immune cells.

Lysis and Centrifugation

A lysis buffer was added to each vacutainer after the initial incubation with antibodies to help remove red blood cells and make it easier to analyse leukocytes expressing nCD64.

Samples were then incubated with lysis buffer for 15 minutes to effectively lyse the red blood cells. Subsequently, the T1 and T2 vacuum containers underwent a 5-minute centrifugation at 1500 rpm. The lysis buffer and the cellular components were separated by centrifugation.

Resuspension and Flow Cytometry

After centrifugation, the cellular pellet from each sample was resuspended in 500 mL of phosphate-buffered saline (PBS). This resuspension ensures that the leukocytes expressing nCD64 are in a suitable medium for flow cytometry analysis. Thus 500mL of samples were loaded into the flow cytometer (BD FACSLyric, USA) to acquire 10,000 events to access the MFI of nCD64 expression.

Data Analysis

The flow cytometry data was analysed using FACSuite 4.1 software. Data analysis involved quantifying and comparing the MFI levels of CD64 in the neutrophils between Group A (lichen planus patients) and Group B (healthy controls).

Ethical Considerations

Before commencing the study, ethical approval was obtained from the relevant institutional review board (IRB)/ethics committee. Informed consent was obtained from all participants, ensuring their voluntary participation and confidentiality.

Results

This study observed a group of five patients with different oral mucosal conditions, studying their demographics, clinical characteristics, comorbidities, nCD64 mean fluorescence intensity (MFI), and erythrocyte sedimentation rate (ESR) levels (Table 1; Figures 1 & 3). Patient P1, a 66-year-old woman, displayed erosive sores on the inner cheek and gum tissue, had diabetes in her medical history, and had a nCD64 MFI of 77 alongside a regular ESR of 17 mm/h. The 61-year-old female patient P2 exhibited gingival desquamation and reticular striae, and suffered from coronary artery disease and drug allergies, with an nCD64 MFI of 177 and an ESR of 30 mm/h. A 36-year-old female patient named P3 showed gingival peeling, redness, and a rough patch on the tongue, and suffered from hypothyroidism, with an nCD64 MFI of 472 and an ESR of 24 mm/h. Patient P4, a 47-year-old man, showed white striae on both sides of the buccal mucosa, without any other health issues, and had an nCD64 MFI of 225 along with a normal ESR of 13 mm/h. Patient P5, a 40-year-old woman, displayed redness in both mucosal areas and flaking in specific parts of the gums, had no other health conditions, with an nCD64 MFI of 91 and a standard ESR of 12 mm/h. These results highlight the range of clinical symptoms and features in individuals with oral mucosal conditions, showing an average nCD64 MFI of 208.4 for patients versus 45.6 for healthy controls, indicating statistical significance at a p-value of 0.05 (Table 1; Figure 2).

Table 1. Clinical and Biochemical Profile of Study Participants

Patients	Age/Sex	Clinical Observation	Clinical variant (Reticular/Erosive/ Annular/Papular/ Pigmented)	Co-morbidities	nCD64 MFI	ESR (mm/h)
P1	66/F	Right and left buccal mucosa, alveolar mucosa	Erosive	Diabetic	77	17 (normal)

P2	61/F	Gingival desquamation and reticular striae on gingiva	Erosive and reticular	Coronary artery disease, drug allergies	177	30 (normal)
P3	36/F	Gingival desquamation and erythema with reticulation and keratotic patch on the tongue	Erosive and anular	Hypothyroidism	472	24 (slightly elevated)
P4	47/M	Bilateral radiating white striae in the buccal mucosa	Reticular	Nil	225	13 (normal)
P5	40/F	Bilateral mucosal erythema, with localized gingival desquamation	Erosive	Nil	91	12 (normal)

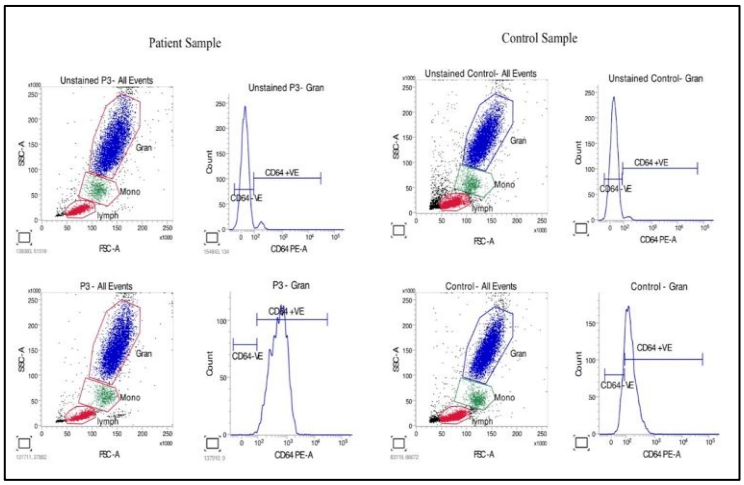

Figure 1. Representative Dot Plot and Histogram showing nCD64 Expression of a Patient and a Healthy Control

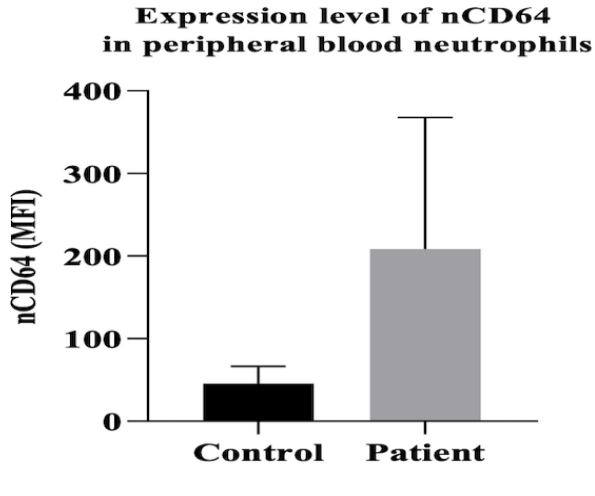

Figure 2. Expression Level of nCD64

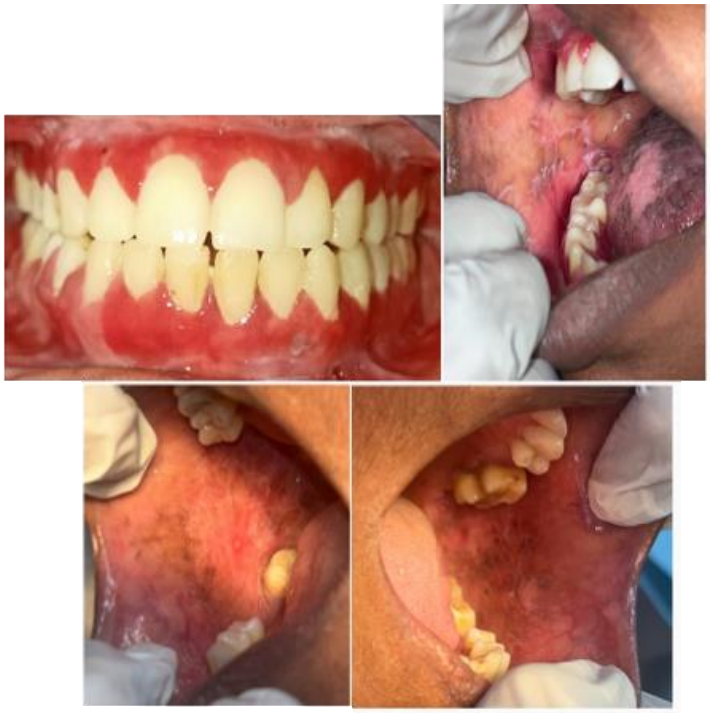

Figure 3. Clinical Presentation of Oral Lichen Planus

Discussion

The pathophysiology of Lichen Planus is immune-mediated. dermatological research has placed a great deal of emphasis on finding reliable biomarkers for its diagnosis and prognosis. This study examines the index potential of neutrophil CD64 (nCD64) as an LP biomarker thus investigating its influence on prognosis in both short-term and long-term cases. nCD64 index could provide information about the inflammatory mechanisms relevant to LP owing to its stability and quick reaction to bacterial infections [18,19]. Several biomarkers have been studied previously, yet, only a few have shown promising results for both short- and long-term prognosis. The findings of this study suggest that nCD64 has the potential for such practical applications. nCD64 expression rises around an hour after microbial invasion and stays constant for more than 24 hours, serving as a marker of the host immune response to bacterial infection [18]. This characteristic of nCD64 has been seen in different illnesses, including acute pancreatitis and early-onset sepsis. Although nCD64's value as a prognostic marker in viral disorders is well established, less is known about how it functions in non-infectious inflammatory conditions like LP [20].

Studies have indicated that nCD64 can differentiate bacterial and viral infections, showing increased expression in bacterial infections [18]. Another study conducted by Lu et al. (2019) [20] found that the combination of nCD64 and CD35 levels aids in distinguishing between bacterial and viral infections. On the other hand, the research concentrated on LP, which is not infectious and did not determine if nCD64 levels vary between infectious and non-infectious patients or between bacterial and viral flare-ups. The increased levels of nCD64 in LP patients indicate an initial immune reaction, but more studies are required to establish its accuracy in distinguishing various exacerbation types. This study showed that the nCD64 MFI average was notably higher (208.4) in LP patients in contrast to healthy controls (45.6), with a p-value of 0.05. This discovery aligns with the function of nCD64 as an indicator of overall inflammation. The relationship between nCD64 and the severity of LP lesions has not been definitively established. In a study conducted by Patnaik et al. (2020) [7], nCD64 was highlighted as a diagnostic and

prognostic indicator in sepsis, indicating that its application in inflammatory diseases could go beyond just sepsis.

Moreover, the ESR levels in this research did not show a connection with the severity of localized mucosal lesions, which is consistent with the results of Ataş et al. (2016) [19], who indicated that the neutrophil-lymphocyte ratio is more useful in evaluating systemic inflammation in LP. Similarly, Chandrasekar et al. (2023) [16] emphasized the significance of biomarkers rather than broad inflammatory markers for evaluating LP. Age and type of infection can also impact immune responses, contributing to the variation in nCD64 levels. Liu et al. (2022) [21], found that alterations in nCD64 expression are mainly associated with the presence of microbes and the severity of infection, rather than age or gender. The effectiveness of nCD64 as a diagnostic and prognostic indicator has been extensively recorded in sepsis and other inflammatory conditions [11,17]. For example, research has demonstrated that levels of nCD64 can efficiently detect bacterial infections and track disease advancement [22,23]. Yet, measuring nCD64 levels usually depends on intricate flow cytometry methods and is usually done infrequently in clinical environments [24]. Utilizing advanced technologies like microfluidic biochips has shown the promise of increased frequency and precision in measurements [18].

Previous research by Dimoula et al., (2014) [22] has recognized the practical difficulties of quantifying nCD64, including the requirement for flow cytometry and the limitations of performing tests only once a day. The study utilized a microfluidic biochip to consistently and effectively measure nCD64 in patients, demonstrating its potential for enhanced prognosis accuracy through real-time monitoring. Deng et al. (2023) [12] have highlighted the importance of conducting more research to differentiate between bacterial and viral infections, which may improve the accuracy of nCD64 diagnosis in LP patients. Rassol HJ et al. (2023) [25] highlighted the importance of T-cell subsets, particularly CD4 and CD8, in comprehending oral lichen planus, which reinforces the requirement for thorough biomarker analysis. Also, studies are highlighting the role of nCD64 in inflammatory situations of various other disease conditions [26-29]. The clinical utility of mineralized artificial materials, including calcium carbonate, PRF, and nano-hydroxyapatite, spans various specialties [30-33]. To ascertain how nCD64 levels vary among LP subtypes and to evaluate its function in distinguishing between various illnesses, more research is necessary. Future research ought to focus on improving nCD64's prognostic and diagnostic utility in LP. There are different in silico methods to determine these conditions. Further we can move on it.

Therefore, the current study highlights the need for more research even as it confirms the usefulness of nCD64 as a prospective biomarker for LP. Future research should concentrate on differentiating between infectious and non-infectious exacerbations and investigate the role of nCD64 in combination with other biomarkers to improve prognostic and diagnostic skills in inflammatory diseases associated to LP. According to the results, nCD64 may play a crucial role in the clinical management of LP, improving patient outcomes and enabling more focused treatment strategies.

Conclusion

The results of this research emphasized the possible usefulness of the neutrophil CD64 (nCD64) index as a biomarker for Lichen Planus (LP), specifically in its capacity to provide information on both immediate and future outcomes. The nCD64 index has a quick and consistent reaction to bacterial infections, making it useful for promptly and accurately tracking inflammatory conditions such as LP. This marker is highly useful in clinical

environments that require regular monitoring due to its stability for 24 hours. Nevertheless, the research faced various constraints, such as the challenge of distinguishing between infectious and non-infectious LP patients, as well as differentiating between bacterial and viral exacerbations. Moreover, using intricate flow cytometry techniques for measuring nCD64 levels presents practical hurdles in everyday clinical settings. Despite these constraints, the research emphasized the importance of further investigating trustworthy biomarkers for LP. The nCD64 index has the potential to be combined into dental practice after more validation, to improve the management of LP by distinguishing between various types of inflammatory responses. Future research should concentrate on improving our knowledge of nCD64's specificity and sensitivity, investigating its connection with other biomarkers, and creating easier quantification techniques to increase its clinical usefulness in dentistry and other medical fields.

Conflict of Interest

The authors declare no conflict of interest

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