Original article

No difference in inflammatory mediator expression between mast cell-rich and mast cell-poor rosacea lesions in Korean patients: a comparative study

Running title: Inflammatory mediator expression and mast cells in rosacea lesions

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Abstract

Objectives: This study aimed to evaluate the correlation between mast cell (MC) density in rosacea-affected skin and the expression of key inflammatory mediators, including interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and cathelicidin LL-37. By comparing lesions rich in MCs with those having fewer MCs, we sought to elucidate the role of MCs in the inflammatory mechanisms underlying rosacea pathogenesis.

Methods: Specimens were collected from 20 patients diagnosed with rosacea who attended the outpatient clinic between 2008 and 2013. Each specimen underwent staining using hematoxylin/eosin, Giemsa, IL-6, LL-37, and TNF-α for both histopathological and immunohistochemical analyses. The

number of stained cells was counted across 10 randomly selected dermal layers at a magnification of ×400 using light microscopy. The results were categorized based on the number of MCs counted: more than 10 MCs were classified as MC-rich, and 10 or fewer MCs as MC-poor.

Results: Among the 20 patients (10 MC-rich and 10 MC-poor), the MC-rich group demonstrated significantly higher MC counts than the MC-poor group (P<0.001). However, there were no significant differences in the expression levels of IL-6, LL-37, or TNF-α between the two groups. Additionally, MC density did not show any significant associations with patient demographics, clinical characteristics, or systemic comorbidities.

Conclusion: Increased MC density was not associated with differences in IL-6, TNF-α, or LL-37 expression in rosacea lesions. These findings suggest that mast cell infiltration may not directly influence the inflammatory mediator profile in rosacea. Further research is required to identify distinctive pathological features or markers that can elucidate the mechanisms of rosacea.

Keywords: Cathelicidins, Interleukin-6, Mast cells, Tumor necrosis factor-alpha, Rosacea

Introduction

Background

Rosacea is a chronic inflammatory disorder primarily affecting the central facial region, characterized by periods of exacerbation and remission. It manifests as four clinically recognized subtypes: erythematotelangiectatic, papulopustular, phymatous, and ocular rosacea, each with distinct clinical features [1]. Although the precise etiology of rosacea remains unclear, its pathogenesis is thought to involve an interplay of environmental triggers, vascular dysfunction, alterations in dermal matrix composition, and genetic predisposition.

Emerging studies have focused on the role of inflammatory responses in chronic skin conditions, with mast cells (MCs) receiving particular attention due to their involvement in various inflammatory skin diseases. MCs are known for their contribution to localized vasodilation and angiogenesis and are considered a key player in inflammatory cascades [2]. In rosacea, MCs are postulated to interact with

stimuli such as cathelicidin peptides and neuropeptides (NPs), potentially contributing to hallmark symptoms such as erythema and flushing [2,3]. MCs release a range of inflammatory mediators, including tumor necrosis factor (TNF), interleukin-6 (IL-6), and other pro-inflammatory cytokines, which may exacerbate the inflammatory state in rosacea [3]. Although previous studies have identified increased MC counts in rosacea-affected skin, the exact contribution of MCs to the condition's pathogenesis, particularly their influence on inflammatory mediator expression, warrants further exploration [4].

Objectives

This study aimed to assess the relationship between MC presence and the expression of specific inflammatory mediators in rosacea-affected skin. By analyzing these interactions, we sought to clarify further the potential role of MCs in the inflammatory processes underlying rosacea.

Methods

Ethics statement

This study received approval from the Institutional Review Board (IRB) of Ewha Womans University Mokdong Hospital (IRB No. EUMC 2024-11-001). A waiver of informed consent was granted, owing to the utilization of pre-existing databases and skin biopsy results.

Study design

This was a comparative study using biopsy samples from rosacea patients.

Setting

Rosacea patients who visited the outpatient clinic of the Department of Dermatology, Ewha Womans University Mokdong Hospital, and Ewha Womans University Seoul Hospital between 2008 and 2014 were included in this study. A 4-mm punch biopsy was taken from a facial lesion of these patients. The skin biopsy samples were used for mast cell quantification and immunohistochemical analysis of inflammatory mediators. Clinical data such as age, gender, duration of disease (in months), specific sites of facial involvement, clinical manifestations, and comorbidities were extracted from

electronic medical records. Systemic comorbidities were confirmed through medical records and documentation of current medications.

Participants

This study included twenty patients, each diagnosed with rosacea by dermatologists. However, the analysis did not categorize or differentiate between specific rosacea subtypes, and no patients were excluded.

Variables

The outcome variables included MC counts, clinical data, and the expression of inflammatory mediators such as IL-6, TNF-α, and LL-37.

Data sources/measurement

Mast cell quantification

Tissue specimens were fixed in 10% buffered formalin, embedded in paraffin, and sectioned at a thickness of 4 μm. The sections were stained with hematoxylin and eosin for routine histological examination and with Giemsa stain for MC visualization. Two independent observers, who were blinded to the clinical data, counted the MCs using an Olympus light microscope at 400× magnification. In each specimen, MCs were counted in 10 high-power fields (HPFs) that were randomly selected and distributed across different dermal layers: four fields in the papillary dermis, three in the mid-reticular dermis, and three in the bottom reticular dermis. Only cells displaying both a distinct nucleus and metachromatic granules were included in the count. The final MC count was determined by averaging the counts from both observers.

The compartments were divided into 22 sections to ensure a systematic and reproducible method for MC counting. This approach facilitated a comprehensive assessment of MC distribution throughout the tissue, minimizing bias due to regional variations in cell density. By dividing the area into smaller sections, the counting process was standardized, leading to more accurate and consistent results across various samples.

Specimens were categorized based on MC density, using well-defined criteria for different density groups. A threshold of 10 MCs per HPF was established from the MC counts, indicating that 10 MCs

per HPF is an appropriate cutoff to differentiate areas of high mast cell density from those with low density. This threshold allowed us to classify the samples into MC-rich and MC-poor groups, reflecting a significant variation in mast cell abundance. Images were captured with a Jenoptik ProgRes GRYPHAX Subra Microscope HD Camera.

Immunohistochemical analysis for inflammatory mediators

Immunohistochemical staining was conducted following standard protocols. The process began with deparaffinization of the sections, followed by antigen retrieval using citrate buffer at a pH of 6.0. This was succeeded by blocking endogenous peroxidase activity with 3% hydrogen peroxide. Primary antibodies targeting IL-6, TNF-α, and LL-37, all at a dilution of 1:100, were then applied. The intensity of the staining was assessed by two observers who were blinded to the sample identities. They used a semi-quantitative scoring system with the following scale: 0 (negative), 1 (weak), 2 (moderate), and 3 (strong). To ensure consistency, the scoring methodology was standardized across both observers, and an average score was calculated for each section. Both positive and negative controls were included to confirm the specificity of the staining.

Bias

There was no selection bias among the target subjects, as all cases were included that had histological confirmation.

Study size

No sample size estimation was done since all target subjects were included.

Statistical methods

Data analysis was conducted using IBM SPSS Statistics v.22.0. Continuous variables, such as MC counts, are presented as mean ± standard deviation, while categorical variables, including immune mediators, are reported as frequencies and percentages. Comparisons between groups were performed using the independent samples t-test. Correlations between MC counts and immunohistochemical markers were evaluated using Spearman's correlation coefficient. Statistical significance was established at $P<0.05$.

Results

Participants

This study encompassed 20 rosacea patients aged between 23 and 75 years, with an average age of 53.1 ± 13.2 years. The cohort was evenly divided between sexes, comprising 10 men and 10 women. The duration of disease among participants varied widely, ranging from 1 month to 15 years, with a median duration of 32.6 months. The most frequently affected areas were the forehead (45%), nose (35%), and cheeks (20%). Additional details on patient characteristics are provided in Table 1.

Clinicopathological and immunohistochemical results

Based on MC counts, patients were divided into two groups: the MC-rich group, which included individuals with 10 or more MCs per HPF (n=10), and the MC-poor group, which included individuals with fewer than 10 MCs per HPF (n=10). Representative high-magnification images of MC distribution for each group are displayed in Fig. 1A and 1B. There was a significant difference in the mean MC count between the groups, with the MC-poor group averaging 3.15 ± 0.87 MCs per HPF and the MC-rich group averaging 10.8 ± 2.58 MCs per HPF ($P < 0.001$) (Table 1).

In terms of clinical characteristics, there were no significant demographic differences between the MC-poor and MC-rich groups. Both groups had similar ages (52.8 ± 14.18 years vs. 53.3 ± 12.89 years, $P = 0.935$) and an equal distribution of genders. Additionally, there were no significant differences in the severity of clinical features, including affected sites and the incidence of pruritus, although pruritus was more commonly reported in the MC-poor group, though not to a statistically significant extent. Regarding systemic comorbidities, prevalent conditions included diabetes mellitus, hypertension, dyslipidemia, liver disease, gastritis, and peptic ulcer. While these comorbidities appeared more frequently in the MC-rich group, the difference was not statistically significant. Furthermore, no meaningful correlation was found between MC counts and systemic comorbidities (Table 1).

Table 1. Clinicopathological and immunohistochemical results of two groups

	Mast cell-rich group	Mast cell-poor group	**P value**
Demographics			

Sex (female)	6 (60)	4 (40)	0.328
Age*	52.8±14.18	53.3±12.89	0.935
Past history	7 (63.64)	4 (36.36)	0.370
Diabetes mellitus	3 (75)	1 (25)	0.582
Hypertension	4 (57.14)	3 (42.86)	1.000
Dyslipidemia	1 (50)	1 (50)	1.000
Liver disease	1 (50)	1 (50)	1.000
Gastritis, peptic ulcer	2 (66.67)	1 (33.33)	1.000
Site			
Forehead, glabella	7 (46.67)	8 (53.33)	1.000
Periorbital	1 (33.33)	2 (66.67)	1.000
Cheeks	6 (46.15)	7 (53.85)	1.000
Nose, perinasal	6 (42.86)	8 (57.14)	0.628
Chin	4 (33.33)	8 (66.67)	0.170
Perioral, lip	3 (100)	0 (0)	0.211
Signs and symptoms			
Erythematous papules	5 (38.46)	8 (61.54)	0.350
Erythematous patches	6 (46.15)	7 (53.85)	1.000
Telangiectasia	3 (75)	1 (25)	0.582
Itching	2 (25)	6 (75)	0.170
Immunohistochemical parameters*			
Mast cells	**10.8±2.58**	**3.15±0.87**	**<0.001**
IL-6	1.32±0.71	1.56±0.91	0.519
LL37	0.8±0.55	1±0.59	0.443
TNF-α	0.84±0.65	1.04±0.32	0.400

Abbreviations: TNF-α, tumor necrosis factor; IL-6, interleukin-6; LL-37, cathelicidin LL-37; Mast cell-rich grou: the number of counted mast cells (MCs) was 10 or more; Mast cell-poor group: the number of counted MCs was fewer than 10.
*Data are presented as mean ± standard deviation, while other data are expressed as number (%). Bold font indicates statistically significant differences (P<0.05).

Distribution patterns of inflammatory mediators within various skin compartments

Immunohistochemical analysis was performed to evaluate the expression of inflammatory markers in the epidermal and dermal layers, as well as in the skin appendages. The semi-quantitative analysis revealed no statistically significant differences in marker expression between the two groups. Specifically, IL-6 levels were 1.32 ± 0.71 in the MC-rich group and 1.56 ± 0.91 in the MC-poor group (P=0.519); LL-37 levels were 0.8 ± 0.55 in the MC-rich group and 1.0 ± 0.59 in the MC-poor group (P=0.443); TNF-α levels were 0.84 ± 0.65 in the MC-rich group and 1.04 ± 0.32 in the MC-poor group (P=0.400). Table 2 provides additional details on the distribution patterns of these markers across different skin compartments.

Table 2. Comparison between mast cell-rich group (mast cell-rich group) and mast cell-poor group (mast cell-poor group) in the number of mast cells, IL-6, LL-37, TNF-α (number/HPF [×400])

	Slide number	Mast cells	IL-6	LL37	TNF-α
Mast cell-rich group	1	12.93	1	0.4	0.2
	2	8.45	0.8	0.8	0.6
	3	12.53	1	0.4	0.6
	4	16.2	1.8	0.2	0.2
	5	10.4	0.4	0.4	0.8
	6	11.55	0.8	0.6	0.4
	7	8	0.8	0.6	0.4
	8	9.13	2.2	1.2	2
	9	10.5	2.4	1.6	1.6
	10	8.3	2	1.8	1.6
Mast cell-poor group	1	3.1	1.2	0.4	0.6
	2	1.1	0.4	0.6	1.4
	3	4.35	0.6	1.2	1
	4	2.9	2.4	0.8	1
	5	4.05	1.2	2.4	1.6
	6	2.95	0.6	0.8	0.8
	7	3.45	1.4	1.4	0.8
	8	3.15	2.8	0.4	0.8
	9	3	2.6	1	1
	10	3.45	2.4	1	1.4

Abbreviations: TNF-α, tumor necrosis factor; IL-6, interleukin-6; LL-37, cathelicidin LL-37.

Discussion

Key results

This study aimed to elucidate the role of MCs in the pathophysiology of rosacea and to investigate the relationship between MC density and mediator release in affected skin. We observed distinct differences in MC counts among patients, allowing us to categorize them into MC-poor and MC-rich groups. Although there were increased MC counts, these did not correlate with significant differences in clinical characteristics or biological markers, such as IL-6, LL-37, and TNF-α. These findings underscore the complexity of the cytokine network in rosacea and suggest that MC infiltration alone may not be sufficient to fully drive clinical manifestations.

Interpretation/comparison with previous studies

The pathogenesis of rosacea is not fully understood; however, MCs are known to enhance various inflammatory processes and are linked to angiogenesis, which is relevant to the pathophysiology of rosacea [1]. Cathelicidin, highly expressed in rosacea, releases its cleavage product LL-37. This, in

conjunction with heightened serine protease activity, triggers inflammatory cascades, including the activation of MCs [5, 6]. Additionally, dysregulated immune responses and neurovascular dysfunctions are implicated as contributing factors in rosacea [7].

MCs can be activated through various pathways. Beyond the IgE-mediated degranulation pathway [2], activation can also occur via pathogen- and pattern-recognition receptors, including Toll-like receptors. TNF-α is known to trigger the activation of the NLRP3 inflammasome pathway and also activates NF-κB in MCs [8]. Additionally, other receptors such as MRGPRX2, complement receptors, and NP and neurotransmitter receptors can influence MC activation in response to LL-37 and NPs [9, 10]. The diversity of these pathways highlights the complexity of MC activation mechanisms, which may explain why MC density alone did not correlate with specific clinical or inflammatory features in our cohort [11, 12].

Furthermore, rosacea is associated with a variety of comorbidities, including allergies, respiratory and gastrointestinal disorders, metabolic and cardiovascular diseases, and certain malignancies [4, 13]. It has been suggested that shared genetic and environmental factors, along with immune regulatory processes, may underlie these associations [13]. In our study, patients exhibited systemic comorbidities such as diabetes mellitus, hypertension, and dyslipidemia. However, no significant association was found between MC counts and the prevalence of systemic comorbidities, likely due to the small sample size.

Limitations

The limitations of this study include a small sample size and variability in disease duration and previous treatments among patients, which could influence MC density, cytokine expression, and clinical characteristics. Additionally, the absence of standardized laboratory data on systemic comorbidities limits our ability to interpret potential correlations between these comorbidities and MC count or activity. Moreover, variations in histopathological characteristics and types of rosacea lesions were not thoroughly analyzed, potentially affecting the intensities of MC and cytokine expression. Methodological constraints, such as possible recall and response biases, along with a lack of control for detection and measurement biases in MC counting, may also have impacted the results.

Suggestion for further studies

Future studies should consider using larger, well-characterized samples from multiple centers, along with standardized protocols for quantifying MC activity and inflammatory markers in different rosacea subtypes and lesion characteristics.

Conclusion

MCs play a significant role in the pathogenesis of rosacea through mechanisms involving innate immune responses, neurogenic inflammation, angiogenesis, and fibrosis [1]. However, this study found no significant association between MC count, cytokine levels, and the clinical features of rosacea. Our findings suggest that MC activity may be a more valuable indicator than MC count alone for evaluating the biological behavior of rosacea. Additionally, relying solely on IL-6, LL-37, and TNF-α staining may not adequately capture MC activity. It is possible that MCs impact the surrounding skin tissue indirectly, without directly influencing the specific biological markers evaluated in this study.

The pathology of rosacea likely involves multiple factors, indicating complex interrelationships among its contributing elements. This study failed to establish a clear correlation between the clinical characteristics of rosacea and either MC count or cytokine levels. Therefore, further research is crucial to identify distinct pathological markers that could elucidate the mechanisms underlying rosacea. Future studies should also concentrate on examining the complex relationship between MCs and the multifaceted nature of this condition to improve our understanding and management approaches.

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Conflicts of interest

No potential conflict of interest relevant to this article was reported.

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Data availability

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Supplementary materials

Not applicable.

References

1. Aroni K, Tsagroni E, Kavantzas N, Patsouris E, Ioannidis E. A study of the pathogenesis of rosacea: how angiogenesis and mast cells may participate in a complex multifactorial process. *Arch Dermatol Res* 2008;300:125-31. https://doi.org/10.1007/s00403-007-0816-z.

2. Muto Y, Wang Z, Vanderberghe M, Two A, Gallo RL, Di Nardo A. Mast cells are key mediators of cathelicidin-initiated skin inflammation in rosacea. *J Invest Dermatol* 2014;134(11):2728-36. https://doi.org/10.1038/jid.2014.222.

3. Aksoy B, Ekiz O, Unal E, Ozaydin Yavuz G, Gonul M, Kulcu Cakmak S, et al. Systemic comorbidities associated with rosacea: a multicentric retrospective observational study. *Int J Dermatol* 2019;58:722-8. https://doi.org/10.1111/ijd.14353.

4. Mascarenhas NL, Wang Z, Chang YL, Di Nardo A. TRPV4 Mediates Mast Cell Activation in Cathelicidin-Induced Rosacea Inflammation. *J Invest Dermatol* 2017;137:972-5. https://doi.org/10.1016/j.jid.2016.10.046.

5. Yamasaki K, Di Nardo A, Bardan A, Murakami M, Ohtake T, Coda A, Dorschner RA, Bonnart C, Descargues P, Hovnanian A, Morhenn VB, Gallo RL. Increased serine protease activity and cathelicidin promotes skin inflammation in rosacea. *Nat Med* 2007;13(8):975-80. https://doi.org/10.1038/nm1616.

6. Marchitto MC, Chien AL. Mast Cell Stabilizers in the Treatment of Rosacea: A Review of Existing and Emerging Therapies. *Dermatol Ther (Heidelb)* 2021;11:1541-9. https://doi.org/10.1007/s13555-021-00597-7.

7. Wang L, Wang YJ, Hao D, Wen X, Du D, He G, Jiang X. The Theranostics Role of Mast Cells in the Pathophysiology of Rosacea. *Front Med (Lausanne)* 2019;6:324. https://doi.org/10.3389/fmed.2019.00324.

8. Nakamura Y, Franchi L, Kambe N, Meng G, Strober W, Nunez G. Critical role for mast cells in interleukin-1beta-driven skin inflammation associated with an activating mutation in the nlrp3 protein. *Immunity* 2012;37:85–95. https://doi.org/10.1016/j.immuni.2012.04.013

9. Subramanian H, Gupta K, Ali H. Roles of Mas-related G protein-coupled receptor X2 on mast cell-mediated host defense, pseudoallergic drug reactions, and chronic inflammatory diseases. *J Allergy Clin Immunol* 2016;138:700–10. https://doi.org/10.1016/j.jaci.2016.04.051

10. Rosa AC, Fantozzi R. The role of histamine in neurogenic inflammation. *Br J Pharmacol* 2013;170:38–45. https://doi.org/10.1111/bph.12266

11. Gupta K, Subramanian H, Ali H. Modulation of host defense peptide-mediated human mast cell activation by LPS. *Innate Immun* 2016;22:21–30. https://doi.org/10.1177/1753425915610643

12. Mukai K, Tsai M, Saito H, Galli SJ. Mast cells as sources of cytokines, chemokines, and growth factors. *Immunol Rev* 2018;282(1):121-50. https://doi.org/10.1111/imr.12634.

13. Holmes AD, Spoendlin J, Chien AL, et al. Evidence-based update on rosacea comorbidities and their common physiologic pathways. *J Am Acad Dermatol* 2018;78: 156–66. https://doi. org/10.1016/j.jaad.2017.07.055.

Figure legends

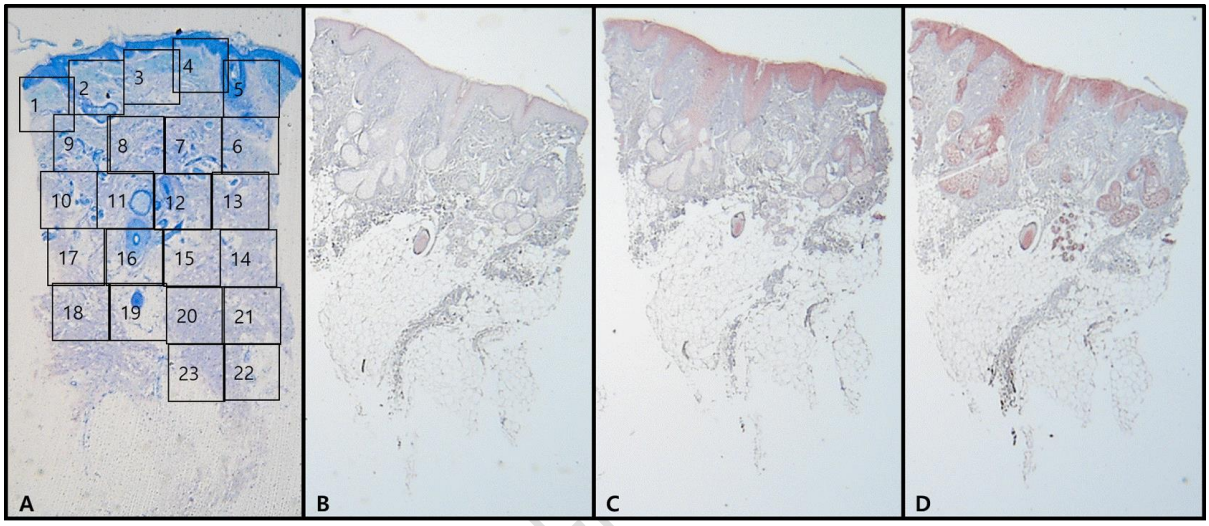

Fig. 1. (A) Histopathology of the lesional skin in mast cell-rich group (Mast cell-rich group) patient. Numbers show the counting methods in mast cell numbers. (Giemsa, ×40). (B, C, D) Immunohistochemical analysis (×40). (B) IL-6 staining, (C) LL-37 staining, (D) TNF-α staining.

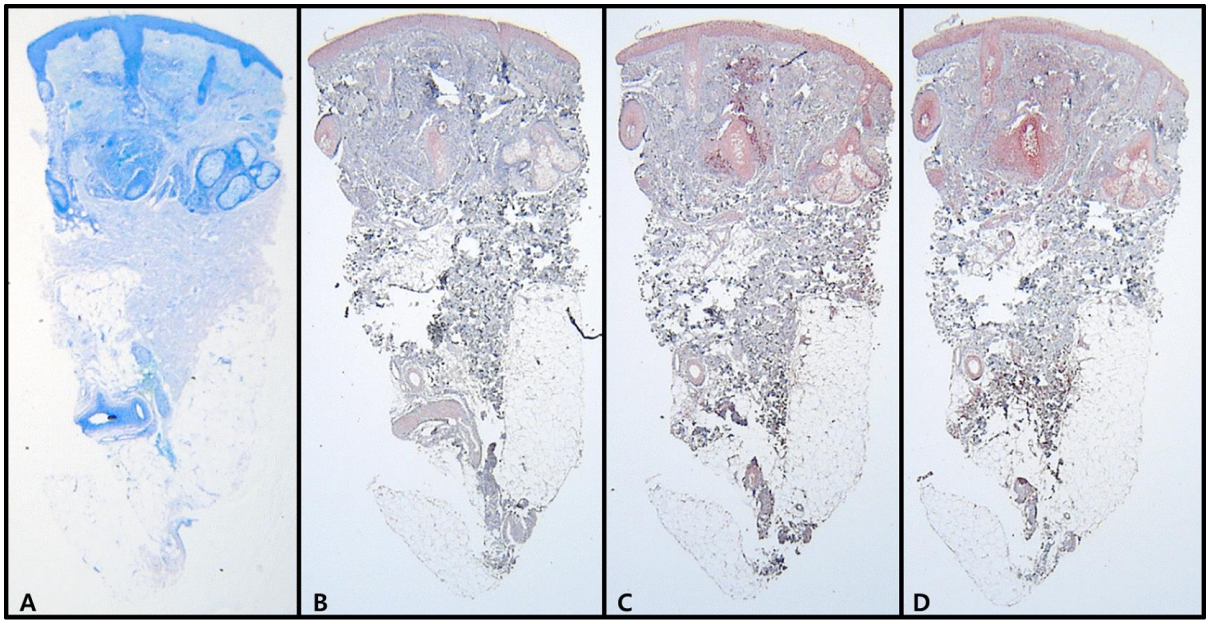

Fig. 2. (A) Histopathology of the lesional skin in the mast cell-poor group (Mast cell-poor group) patient. (Giemsa, ×40). (B, C, D) Immunohistochemical analysis (×40). (B) IL-6 staining, (C) LL-37 staining, (D) TNF-α staining.