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ORIGINAL RESEARCH

# Relationship of the Specific Inflammatory Cytokines KLK5, IL-8, and IL-22 with *Demodex* Density and Risk Factors in Rosacea

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**Objective:** This study aimed to analyze the differences in serum KLK5, IL-8, and IL-22 levels between patients with rosacea and healthy controls, compare the severity of *Demodex* mite infection between the two groups, explore the risk factors for the occurrence of rosacea, and to further explore the relationship between the severity of *Demodex* mite infection, risk factors and the levels of inflammatory cytokines.

**Methods:** A total of 27 rosacea patients and 10 volunteers without any skin diseases were enrolled to assess *Demodex* mite infestation using reflectance confocal microscopy (RCM) and to evaluate rosacea severity by clinician erythema assessment (CEA) and investigator global assessment (IGA). Serum inflammatory cytokines were measured by ELISA. Additionally, lifestyle factors were surveyed via questionnaire in 30 rosacea patients and 30 volunteers.

**Results:** Elevated levels of KLK5, IL-8, and IL-22 were observed in the rosacea group compared with healthy controls ($P < 0.05$). Dermoscopy revealed a higher density of *Demodex* mites in rosacea patients. Questionnaire analysis identified nine factors (age, gender, rest frequency, anxiety, sleep quality, smoking, drinking, allergy history, and family history) significantly associated with rosacea risk. After multivariate adjustment, age and poor sleep quality remained independent risk factors. Furthermore, severe *Demodex* infection correlated with significantly higher KLK5 and IL-8 levels than mild infection ($P < 0.05$). Among patients, anxiety was linked to increased KLK5 and IL-22, while poor sleep quality was associated with elevated KLK5 and IL-8.

**Conclusion:** We demonstrated a strong association between *Demodex* mite density and rosacea development. Importantly, significant elevations in serum KLK5, IL-8, and IL-22 were identified, supporting the role of *Demodex* mites in activating specific inflammatory pathways. The study further identified age and sleep quality as independent risk factors, underscoring the influence of psychological and lifestyle factors.

**Keywords:** rosacea, inflammatory cytokines, reflectance confocal microscope, questionnaire

## Introduction

Numerous variables contribute to the pathophysiology of rosacea, a chronic and complex disease. Rosacea has been connected to genetic variables, immunological dysregulation, microbial elements, UV exposure, nutrition, neurovascular factors, and stress, among other triggering factors.[1] Since the 1930s, *Demodex* mites have been thought to be the cause of facial rosacea because of their ability to activate these pathways and the fact that they typically cause positive infections in rosacea patients[2,3] Numerous symptoms have been linked to the presence of *Demodex* mites in rosacea sufferers, which may make the condition worse. Research has indicated a positive relationship between the density of *Demodex* mites and the intensity of rosacea symptoms, such as pustules, papules, and facial redness. Dryness, itching, and skin irritation were more common in those with higher *Demodex* numbers.[4] Additionally, *Demodex* mites use a variety of

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ways to elicit an immunological response in humans. This reaction is influenced by their proteins, waste materials (detritus), and skin irritation caused by chemicals or mechanical means.[5] The *Demodex* mites are microscopic commensal creatures that live in or close to sebaceous glands and hair follicles.[6] While the exact pathophysiology remains unclear, *Demodex* mites are implicated in the development of rosacea. *Demodex* mite density was considerably greater in rosacea patients than in healthy controls, according to a meta-analysis of 23 case-control studies.[3] Rosacea primarily presents in four clinical subtypes: erythematotelangiectatic rosacea (ETR), papulopustular rosacea (PPR), phymatous rosacea (PhR), and ocular rosacea (OR). Through comprehensive research, Forton et al verified that *Demodex* mites were the source of pulo-pustular rosacea (PPR) and proposed that erythematotelangiectatic rosacea (ETR) could be linked to the subclinical stage of *Demodex* mite disease.[7–9] According to clinical research, the facial *Demodex* mite density of both ETR and PPR patients was higher than that of the general population,[9] and 35 out of 39 PPR patients had facial *Demodex* mite density $\geq 5D/cm^2$ (normal value $<5D/cm^2$).[10] A new diagnostic method called reflectance confocal microscopy verified that patients' facial *Demodex* mite density was noticeably greater than controls.[11–14] These findings may indicate that the density of *Demodex* mite infection, some life factors in patients, influence the degree of rosacea development, and serum levels of inflammatory factors.

Keratinocyte tryptic kinase releasing enzyme 5 (KLK5) is a serine protease that is expressed in the epidermis and is involved in cell renewal and maintenance of skin barrier function.[15] Through TLR2 and other signaling pathways, a variety of stimuli increase the expression of KLK5, which in turn causes the enzymatic breakdown of hCAP18 to produce an excess of Leucine-Leucine-37 (LL-37). This process attracts immune cells and inflammatory factors, mediates angiogenesis and inflammatory responses, and causes rosacea.[16,17] Compared to healthy participants, rosacea sufferers have much greater levels of LL-37 due to an abnormally increased KLK5 in their skin.[18,19] Toll-like receptors (TLRs) play a key role in inflammatory and innate immune responses.[20] Patient keratinocytes exhibit elevated expression of TLR2, which triggers KLK5 and influences antimicrobial peptide levels, hence intensifying symptoms.[21,22] Meanwhile, the density of *Demodex* mite on the face of patients was increased,[23,24] the substances released by them, such as chitin, induced the expression of KLK5 and promoted the production of inflammatory mediators, such as IL-1β and IL-8, by activating the TLR2 pathway.[25,26] One possible treatment approach is to inhibit KLK5 activity, which lowers LL-37 expression and blocks the inflammatory cascade response.[27]

Blocking IL-22, a cytokine that mediates immunological and tissue barrier communication, may be a viable therapeutic target because it plays a significant role in skin inflammation and disorders such as psoriasis and atopic dermatitis.[28] T cells and Innate Lymphoid Cells are the primary secretors of this factor,[29,30] and rosacea patients' lesion areas have significantly increased expression of IL-22, with a higher proportion of $CD4^+$T cells than $CD8^+$ T cells.[31,32] The CD4+ T cells then rapidly differentiate into helper T (Th) effector cells, which promote the activation of other immune cells by secreting various cytokines.[33] To improve Th17/Th2 responses, IL-22 suppresses C-C Motif Chemokine Ligand 22 (CCL22) and encourages neutrophil recruitment.[34–40] In the meantime, IL-22 controls keratinocyte and fibroblast activities via the IL-22R-JAK/STAT system and activates matrix metalloproteinases (MMP-1/3) to speed tissue repair.[34,39,41] The expression of IL-22 in rosacea serum has not yet been investigated, and exploring changes in its levels may fill mechanistic gaps and provide new directions for therapeutic strategies.

Elevated levels of IL-8, a crucial neutrophil chemokine, can be a sign of inflammatory activity and are implicated in immunomodulation and anti-infective activities in inflammatory disorders.[42] Research has demonstrated that rosacea patients' skin lesions, particularly those with the PPR subtype, have significantly greater levels of IL-8 and IL-8 gene expression than healthy people.[26,31] By inducing IL-8 release from sebocytes, an abnormally high density of *Demodex* mites may encourage neutrophil infiltration and a purulent inflammatory response. A high frequency of mite attacks (eg, 30) dramatically increased IL-8 expression.[6,43] However, there is no known association between IL-8 and serum levels in patients with rosacea.

This study aims to evaluate the levels of inflammatory cytokines in patients with rosacea, and to analyze the relationship between the levels of inflammatory cytokines and the severity and risk factors of *Demodex* mite infection.

# Materials and Methods

## General Information

The study cohort comprised 30 patients with confirmed rosacea diagnoses based on the Chinese Rosacea Diagnostic Criteria (2021 Revision),[44] who were prospectively enrolled as the rosacea group through systematic clinical evaluation. At the same time, 30 healthy individuals undergoing routine health check-ups in Yijishan Hospital of Wannan Medical College were selected as the control group. In the RCM assessment of *Demodex* mite density and serologic analysis, we included 27 patients with rosacea and 10 healthy individuals. 10 healthy individuals (4 men and 6 females) were chosen as the control group in this case-control study, while 27 patients (1 male and 26 females) with a clinical diagnosis of rosacea were chosen as the rosacea group over the same period. A comparison of the two groups' baseline data revealed that the patient group was 18–62 years old (28.92±10.04) and the control group was 22–36 years old (28.89±9.66). The age distribution difference between the two groups was not statistically significant ($P \geq 0.05$), but the sex ratio difference was ($P < 0.05$). The institutional review board gave its approval to the study's ethics. Patients with rosacea and healthy individuals all provided written informed consent.

## Diagnosis of Rosacea and Evaluation of Severity and Subtypes

The inclusion criteria comprised healthy and Chinese of both sexes with a persistent centrofacial erythema due to rosacea, who had not undergone any redness-relieving treatment for the past 3 months (no topical/systemic rosacea treatment or laser therapy).[45] Subjects meeting or experiencing any of the following conditions were excluded: under medication for rosacea; concomitant chronic skin diseases; pregnancy or breast-feeding; cancer or central nervous system disease.[46]

The severity was measured using Investigator's Global Assessment (IGA) and Clinician's Erythema Assessment (CEA).[47,48] And the CEA assessment referred to the VISIA® system, which is the most commonly used to quantify the severity of erythema in studies.[49]

## Enzyme-Linked Immunosorbent Assay (ELISA)

5 mL of fasting peripheral venous blood was collected from the rosacea group and control group, and the peripheral blood was placed in a centrifuge, centrifuged at 3000 rpm for 5 minutes, and 1 mL of serum from the top layer of the centrifuged serum was carefully pipetted into EP tubes, The tubes were labeled with the sample grouping and code and stored in the ultra-low temperature freezer at −80°C within 30 minutes, avoiding repeated freezing and thawing of all sera to be tested and awaiting further testing. The blood collection procedure was strictly sterilized according to the following regulations. The serum levels of KLK5, IL-8, and IL-22 in the rosacea group and healthy control group were determined by enzyme-linked immunosorbent assay (ELISA) according to the instructions manual of the instrument.

## RCM Assessment of Demodex Mites Density

Mite density was determined using an RCM hand instrument (VivaScope® 3000, Lucid, Rochester, NY, USA). Ten 1 $mm^2$ (1000 × 1000 μm) non-overlapping pictures were taken from the areas mentioned in the sebum measurement. In order to best visualize and count mites, sections were obtained from the epidermis to the dermis. For every photograph, the quantity of mites, follicles, and infested follicles was tallied. The number of mites per follicle and the number of mites per infested follicle were determined using these numbers.[50] Any stage of worms and the *Demodex* mite detected under a microscope was deemed positive. Every specimen that tested positive was inspected and counted in complete slices. The number of *Demodex* mites was 1 to 5, considered mildly infected, 6 to 10 moderately infected, and more than 10 severely infected. A density of ≥ 5 mites per $cm^2$ is defined as severe, while < 5 mites per $cm^2$ is defined as mild.[13]

## Questionnaire

General information about the rosacea group and the control group was gathered retrospectively from the hospital's medical record system. This information included gender, age, style of facial cleansing, frequency of daily cleansing, frequency of weekly makeup, preference for spicy foods, regularity of rest, sleep quality, frequent anxiety, smoking,

alcohol use, family history, and allergy history. Multivariate logistic regression analysis was used to examine the risk factors for the development of rosacea, and components that demonstrated statistically significant differences in the univariate study's findings were added to the multivariate analysis.

## Statistical Analysis

The questionnaire differences in lifestyle habits among patients with rosacea and healthy controls were examined using the statistical software SPSS 26.0. The chi-square test was used to evaluate the differences between groups. First, candidate variables with statistical significance were screened out ($P<0.05$). Multivariate logistic regression analysis was used to further study the risk variables of rosacea occurrence. $P<0.05$ was statistically significant.

The results of RCM assessment and serological detection were statistically evaluated by using GraphPad Prism 8.0 software. Subgroup comparisons were made based on the number of subgroups. One-way analysis of variance was used to compare three or more groups. Non-parametric tests are used for non-compliance. If the data between the two groups conform to the normal distribution and the chi-square variance, the independent sample *t*-test is used. Non-parametric tests are used for non-compliance.

# Results

## Levels of Pro-Inflammatory Cytokines in the Serum of Rosacea

The mean level of KLK5, IL-8, and IL-22 in the rosacea group was 143.06 ± 100.64 pg/mL, 475.50 ± 302.91 pg/mL, and 19.40 ± 9.21 pg/mL, respectively. And the mean level of KLK5, IL-8, and IL-22 in the control group was 52.64 ± 15.16 pg/mL, 341.12 ± 71.60 pg/mL, and 11.81 ± 4.48 pg/mL, respectively. Rosacea patients had considerably greater serum levels of KLK5, IL-8, and IL-22 than the control group, and the difference was statistically significant ($P < 0.001$ and $P < 0.05$), as shown in Figure 1.

## RCM Evaluation Results of Demodex Mite Density

The specimens with positive *Demodex* mite infection were examined and counted (Figure 2). The mean density of *Demodex* mites infection in the rosacea group was 6.67 ± 4.89, and the density of *Demodex* mites infection in the control group was 0.60 ± 0.80, and the difference between the two groups was statistically significant ($P < 0.05$); The severity and subtype of Demodex infestation in the rosacea group were statistically different from those in the control group ($P < 0.05$). There was no statistically significant difference in the rate of microscopic detection of *Demodex* mites in rosacea, as shown in Table 1.

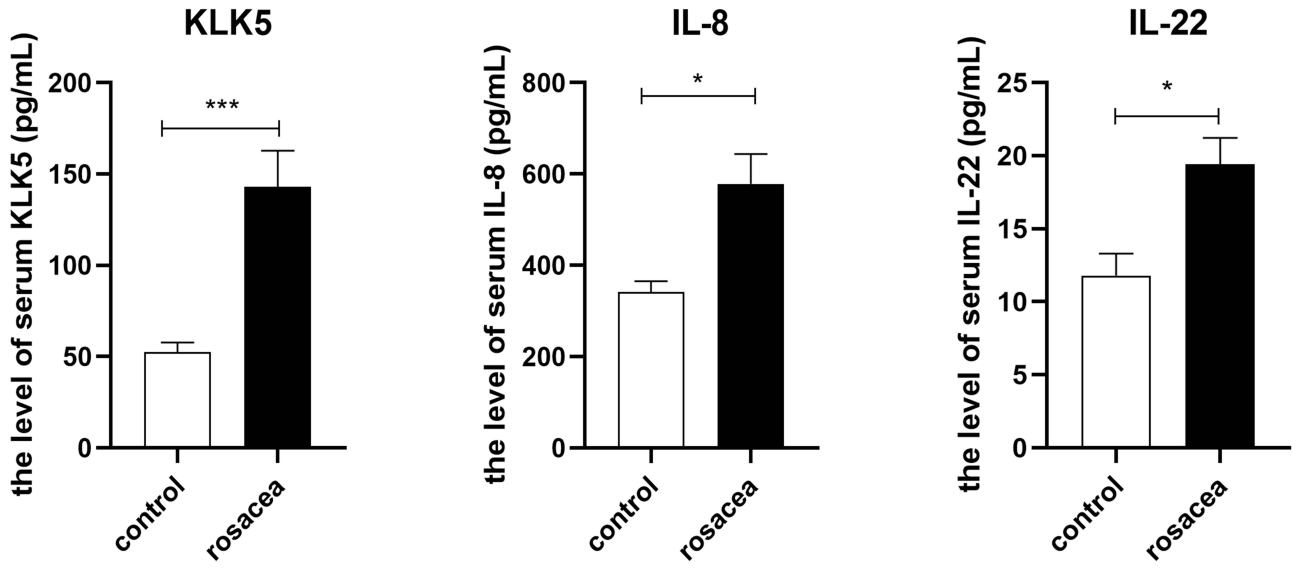


**Figure 1** The Levels of inflammatory cytokines (KLK5, IL-8, IL-22) in serum between the rosacea group and control group by ELISA. Data are expressed as mean ± SEM, *indicates $P < 0.05$, ***indicates $P < 0.001$.

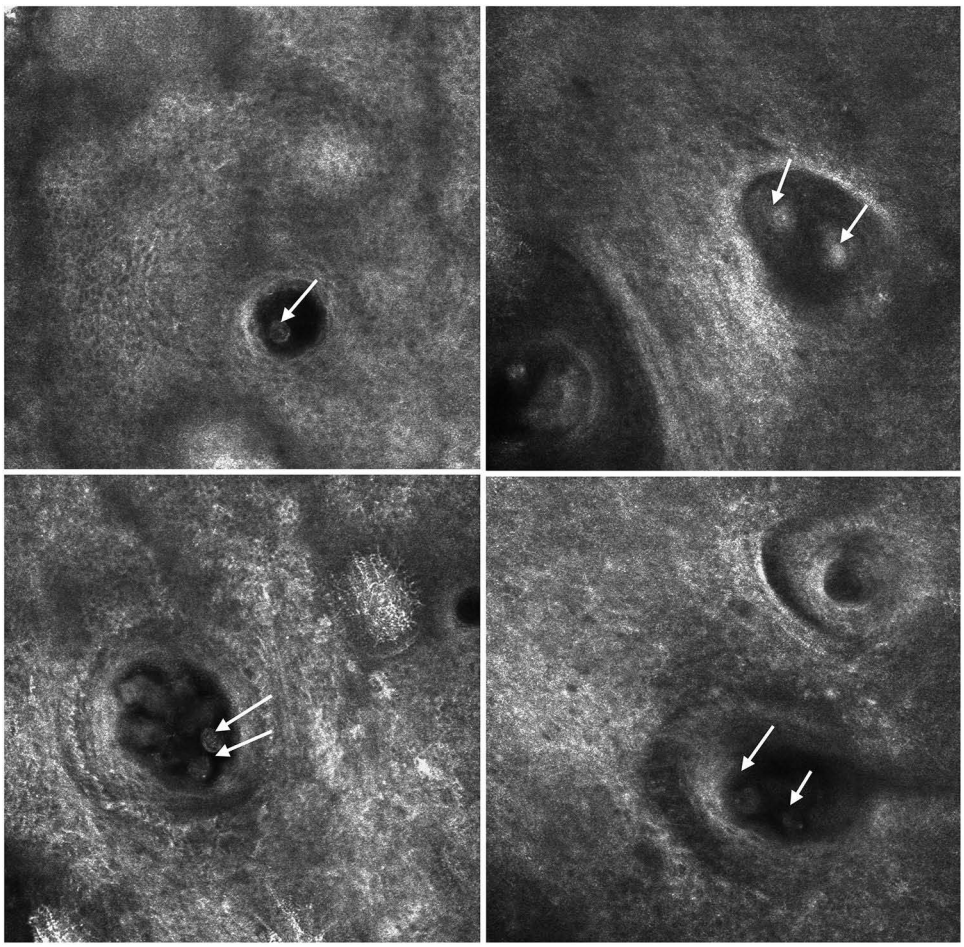

**Figure 2** Confocal images of the cheek (0.5×0.5 $mm^2$). *Demodex* mites appear as clusters of bright, roundish structures when reflectance confocal microscopy images are taken perpendicularly. The white arrow indicates the parasitic *Demodex* mites.

## Results of Questionnaire Analysis

Through a comparative analysis of the general demographic data between the rosacea group and the control group. There were statistically significant differences between the two groups in terms of age over 40 years old, gender, rest frequency,

**Table 1** RCM Evaluation Results of *Demodex* Mite Density

| | Rosacea Group | Control Group | P |
|---|---|---|---|
| *Demodex* density (IQR) | 8 | 0 | |
| *Demodex* detection rate | 100.00% (27/27) | 40.00% (4/10) | P>0.05 |
| *Demodex* infection severity | | | P<0.05 |
| None | 0 | 60.00% (6/10) | |
| Mild | 44.44% (12/27) | 40.00% (4/10) | |
| Moderate | 25.93% (7/27) | 0 | |
| Severe | 29.63% (8/27) | 0 | |
| Assessment | | | P<0.05 |
| None | 0 | 100% (10/10) | |
| IGA | 40.74% (11/27) | 0 | |
| CEA | 37.04% (10/37) | 0 | |
| IGA scores = CEA scores | 22.22% (6/27) | 0 | |

**Note**: Data are presented as Inter Quartile Range (IQR).
**Abbreviations**: CEA, clinician erythema assessment; IGA, investigator global assessment.


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sleep quality (good/poor), anxiety (yes/no), smoking history, drinking history, allergy history, and family history ($P < 0.05$). However, for the methods of face washing, the frequency of face washing > 2, the frequency of makeup application per week >2, and the preference for spicy foods, the differences between the two groups were not statistically significant ($P > 0.05$), as shown in Table 2.

By employing multivariate regression analysis and conducting comparative assessments between the rosacea group and the control group, the results indicate that sleep quality and gender are two independent risk factors for rosacea development, as shown in Table 3.

**Table 2** Comparison of General Information for Questionnaire Analysis

| Influencing Factors | Rosacea Group (n) | Control Group (n) | $\chi^2$ | P |
|---|---|---|---|---|
| Gender | | | 16.484 | P<0.05 |
| Male | 3 | 18 | | |
| Female | 27 | 12 | | |
| Age | | | 9.317 | P<0.05 |
| ≤40 | 28 | 18 | | |
| >40 | 2 | 12 | | |
| Method of washing face | | | 0.268 | P>0.05 |
| Water | 15 | 17 | | |
| Soap or cleanser | 15 | 13 | | |
| Daily cleansing frequency | | | 3.750 | P>0.05 |
| ≤2 times | 27 | 21 | | |
| >2 times | 3 | 9 | | |
| Weekly makeup frequency | | | 0.800 | P>0.05 |
| ≤2 times | 24 | 21 | | |
| >2 times | 6 | 9 | | |
| Spicy food preference | | | 2.500 | P>0.05 |
| Yes | 21 | 15 | | |
| No | 9 | 5 | | |
| Daily routine regular | | | 9.643 | P<0.05 |
| Yes | 8 | 20 | | |
| No | 22 | 10 | | |
| Sleep quality | | | 5.711 | P<0.05 |
| Good | 7 | 16 | | |
| Poor | 23 | 14 | | |
| Frequently anxious | | | 5.455 | P<0.05 |
| Yes | 18 | 4 | | |
| No | 12 | 26 | | |
| Smoking | | | 7.680 | P<0.05 |
| Yes | 29 | 21 | | |
| No | 1 | 9 | | |
| Alcohol Drinker | | | 4.800 | P<0.05 |
| Yes | 6 | 14 | | |
| No | 24 | 16 | | |
| Allergy history | | | 6.648 | P<0.05 |
| Yes | 13 | 4 | | |
| No | 17 | 26 | | |
| Family history | | | 4.320 | P<0.05 |
| Yes | 8 | 2 | | |
| No | 22 | 28 | | |

**Table 3** Results of Binary Logistic Regression Analysis

| Variable | β | SE | Wald | P | OR | 95% CI |
|---|---|---|---|---|---|---|
| Age | −3.212 | 1.472 | 4.759 | P<0.05 | 0.040 | 0.002~0.722 |
| Gender | 1.140 | 1.273 | 0.801 | P>0.05 | 3.125 | 0.258~37.888 |
| The work and rest situation | −0.128 | 1.009 | 0.016 | P>0.05 | 0.880 | 0.122~6.357 |
| Sleep quality | −1.888 | 0.940 | 4.032 | P<0.05 | 0.151 | 0.024~0.956 |
| Anxiety | −1.611 | 0.896 | 3.233 | P>0.05 | 0.200 | 0.035~1.156 |
| Smoking | 1.494 | 2.223 | 0.452 | P>0.05 | 4.454 | 0.057~347.428 |
| Alcohol drinker | −0.523 | 1.206 | 0.188 | P>0.05 | 0.593 | 0.056~6.308 |
| Allergy history | −1.088 | 0.981 | 1.230 | P>0.05 | 0.337 | 0.049~2.305 |
| Family history | −2.207 | 1.739 | 1.611 | P>0.05 | 0.110 | 0.004~3.323 |

**Abbreviations**: β, beta coefficient; SE, standard error; OR, odds ratio; CI, confidence interval.

## Correlation Among the Severity of Demodex Mite Infection, Pathogenic Factors, and Levels of Inflammatory Factor in Patients with Rosacea

We analyzed the correlation of the severity of *Demodex* mite Infections with levels of Inflammatory factors. The invasion of *Demodex* mites can cause changes in cytokine levels.[51] For KLK5 inflammatory factor, the mean level was 189.37 ±109.20 pg/mL in the severe group, 120.51±28.85 pg/mL in the moderate group and 125.34±110.96 pg/mL in the mild group; For IL-8 inflammatory factor, the mean level was 733.32±280.92 pg/mL in the severe group, 474.52±318.93 pg/mL in the moderate group and 304.19±144.37 pg/mL in the mild group; For IL-22 inflammatory factor, the mean level was 23.03±7.13 pg/mL in the severe group, 16.17±9.11 pg/mL in the moderate group and 18.87±9.67 pg/mL in the mild group. There was no statistically significant difference between any of the IL-22 groups, while the KLK5 and IL-8 levels varied statistically significantly between the severe and mild *Demodex* mite infection levels, as shown in Figure 3.

For the correlation of anxiety and sleep quality with levels of Inflammatory factors, rosacea can affect rosacea patients through physical and psychological factors, and anxiety and sleep quality were statistically analyzed about inflammatory factor levels.[52,53] The mean KLK5 level in the anxious group was 174.87±119.11 pg/mL, the mean IL-8 level was 422.09 ±263.54 pg/mL, and the mean IL-22 level was 24.12±8.27 pg/mL; in the non-anxious group the mean KLK5 level was 96.81 ±24.69 pg/mL, the mean IL-8 level was 531.88 ± 357.01 pg/mL, and the mean level of IL-22 was 12.54 ± 5.40 pg/mL. Among rosacea patients, the levels of KLK5 and IL-22 in those with anxiety were significantly higher than those without anxiety, and the levels of KLK5 and IL-8 in those with poor sleep quality were significantly higher than those with good sleep quality, as shown in Figure 4.

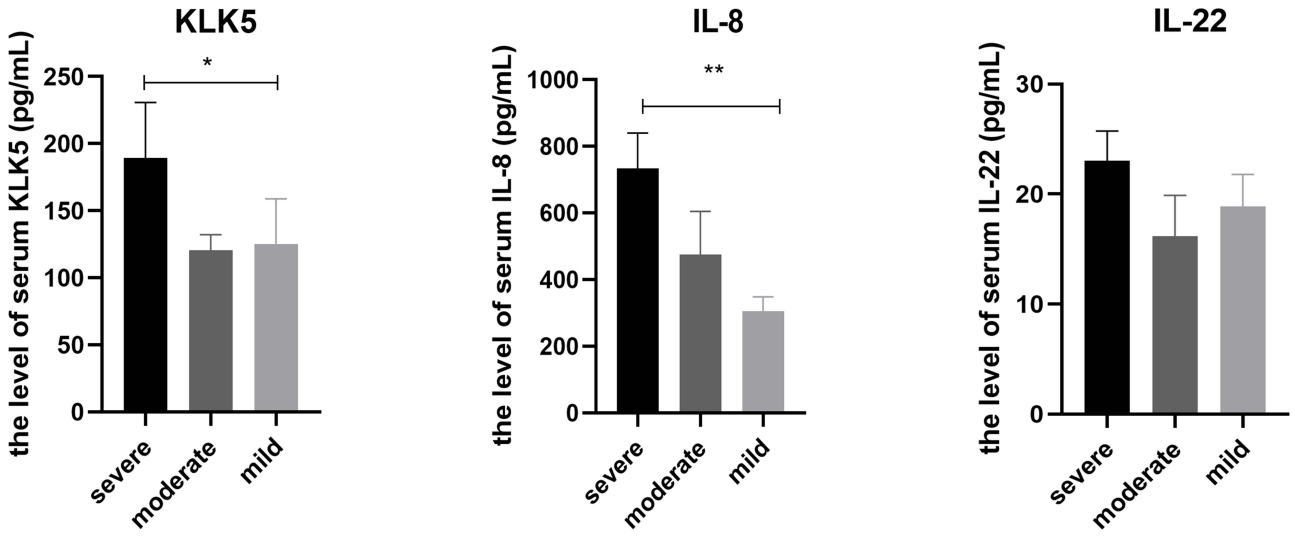


**Figure 3** The correlation between the infestation severity of *Demodex* mites and Inflammatory cytokine (KLK5, IL-8, IL-22) levels. Data are expressed as mean ± SEM, *indicates P < 0.05, **indicates P<0.01.


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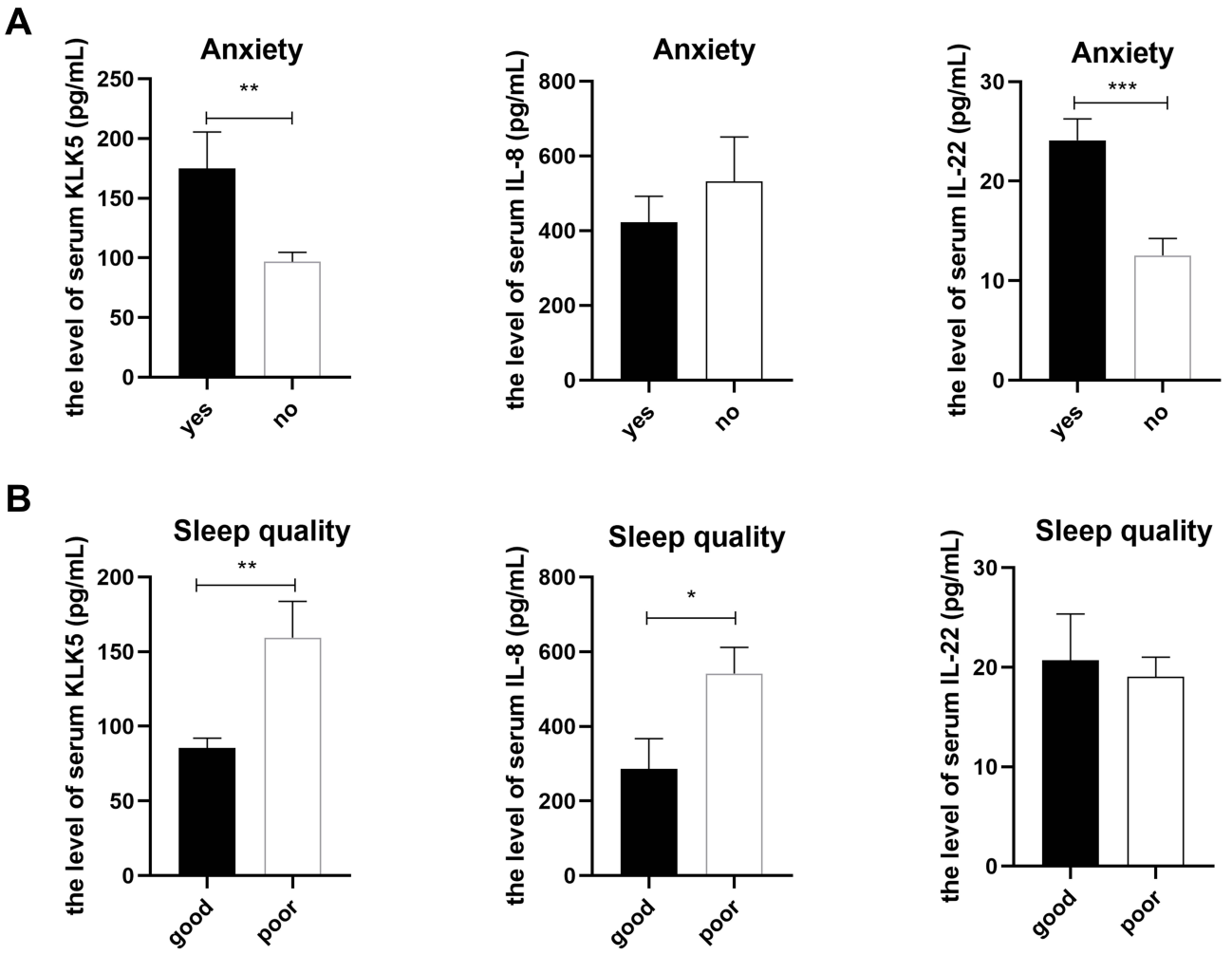


**Figure 4** The correlation between anxiety, sleep quality, and the Inflammatory cytokine (KLK5, IL-8, IL-22) levels. (**A**) The correlation between anxiety and inflammatory cytokine levels: "yes" indicates anxiety, and "no" indicates no anxiety. (**B**) The correlation between sleep quality and inflammatory cytokine levels, "good" indicates good sleep, and "no" indicates poor sleep. Data are expressed as mean ± SEM, *indicates P < 0.05, **indicates P<0.01,***indicates P <0.001.

## Discussion

Rosacea is a common chronic inflammatory disease that manifests as erythema, papules, or pustules on the nose, chin, cheeks, and forehead, as well as frequent flushing of the face. *Demodex* mites may be related to the occurrence of rosacea.[54] One of the markers of rosacea etiology is elevated LL-37 levels.[55] More LL-37 is produced as KLK5 bioactivity and quantity increase, and this protein is crucial for starting the inflammatory response in rosacea.[18] Our results show that the level of KLK5 has increased, which directly indicates that the process of cleaving the inactive hCAP18 precursor to generate the bioactive LL-37 has been significantly enhanced. It is known that LL-37 forms pro-inflammatory oligomers in patients with rosacea, not only recruiting inflammatory cells such as neutrophils, but also directly inducing vasodilation and abnormal keratinization, which is highly consistent with the clinical manifestations of papules, pustules, and erythema in rosacea.[56] The skin of patients with rosacea has noticeably greater concentrations of IL-22, a cytokine generated by adaptive T cells.[29] This study found that the level of the inflammatory cytokine IL-22 in the peripheral blood of patients with rosacea was significantly elevated. This finding is of great significance, as IL-22 is a key effective factor for Th17 and Th22 cells. Its main target is keratinocytes, which can drive their proliferation and inhibit differentiation, thereby causing epidermal hyperplasia and impaired barrier function - this is closely related to the common skin barrier disruption and inflammatory response observed in rosacea.[31] This discovery creates new opportunities for research on rosacea treatment. Specifically, genes encoding the inflammatory cytokine IL-8 are expressed at

higher levels in patients with papulopustular rosacea (PPR).[26] This study demonstrated a significant upregulation of the inflammatory cytokine IL-8 in the serum of patients with rosacea, which is consistent with the view reported that a pro-inflammatory microenvironment exists in this disease.[57]

A significant correlation exists between rosacea and human mite infestation.[6] The population of human mites in the skin of patients with rosacea is significantly higher than in normal-skinned patients, suggesting their possible etiologic significance in this disease.[43] Chang et al reported that the average *Demodex* mite density among 1150 rosacea patients was 71.0 mites/cm$^2$, while the mean *Demodex* mite density for healthy control patients was 8.7 mites/cm$^2$. The density of *Demodex* mites in the rosacea group was significantly different from that in the control group.[3] In this study, we found that the density of *Demodex* mites was significantly higher in patients with rosacea than in normal subjects. Our research has confirmed the above viewpoint.

Rosacea has been linked to lifestyle factors and psychological factors.[58] Smoking is causally associated with reduced risk of rosacea.[59] The most common triggers associated with rosacea include alcohol, spicy foods.[60] Based on the analysis of the questionnaire data, nine factors were found to be significantly associated with the risk of developing rosacea, including age (≥40 years), gender, rest frequency, anxiety (yes/no), sleep quality (good/poor), history of smoking, drinking history, allergy history, and family history. After adjustment for multivariate regression modeling, gender and sleep quality are independent risk factors for the occurrence of rosacea.

According to Mehmet Salih Gurel et al, rosacea inflammation is primarily attributed not to the density of Demodex mites, but to the invasion of Demodex-derived allergens or pathogens into the dermis, ultimately leading to an aggravated inflammatory response.[50] The research provided serological support for the hypothesis that the pathophysiology of rosacea is linked to two inflammatory factors, KLK5 and IL-8, by correlating the severity of *Demodex* mites with the aforementioned three inflammatory cytokines in rosacea patients. The research also found that the levels of KLK5 and IL-8 in patients with rosacea suffering from severe Demodex infestation were significantly higher than those in patients with mild Demodex infestation. The levels of two inflammatory cytokines, KLK5 and IL-22, were significantly elevated in rosacea patients with anxiety symptoms. The levels of KLK5 and IL-8 were significantly elevated in rosacea patients with poor sleep quality. This finding provides serological support for the hypothesis that anxiety and poor sleep quality are related to the levels of inflammatory cytokines in rosacea patients.

## Conclusion

This study offers significant insights into the multifactorial pathogenesis of rosacea. We confirmed a strong correlation between increased dermal *Demodex* mite density and the occurrence of rosacea. More importantly, our research provides novel serological evidence demonstrating significantly elevated levels of serum KLK5, IL-8, and IL-22 in rosacea patients, supporting the hypothesis that *Demodex* mite infestation promotes inflammation in rosacea through these specific pathways. Furthermore, we observed that the levels of KLK5 and IL-8 increased significantly with rosacea severity, underscoring their crucial role in disease progression. Additionally, our study highlights the substantial impact of psychological and lifestyle factors. Specifically, we identified age and poor sleep quality as independent risk factors for rosacea. Notably, anxious patients exhibited markedly higher levels of KLK5 and IL-22, while those with poor sleep quality showed significant elevations in KLK5 and IL-8, suggesting the involvement of a potential psychoneuroimmune mechanism.

## Abbreviations

CEA, clinician erythema assessment; IGA, investigator global assessment; RCM, Reflectance Confocal Microscope.

## Data Sharing Statement

Upon reasonable request, the corresponding author will provide the datasets used and/or analyzed in this study.

## Ethics Statement

The human studies were reviewed and approved by the Medical Ethics Committee of Wannan Medical College (Approval Number:20240245). Written informed consent was given by every rosacea patient and health checkup patient. This study complies with the Declaration of Helsinki.

## Author Contributions

Jingang Xu, Data curation and Writing-original draft. Investigation Methodology, Validation, and Conceptualization. Yao Zhang, Data curation and Writing-original draft. Investigation, Visualization, Formal analysis. Chao Ci, Investigation, Data Curation, Supervision, Project administration and Resources. Xiaohong Lu, Writing-original draft, Investigation, Resources, Supervision, Resources and Formal analysis. Xing Hu, Writing-original draft, Investigation, Data Curation, Supervision, Resources and Software. Jinhong Zhao, Writing – review & editing, Funding acquisition, Methodology, Supervision, Project Administration, Data Curation. Yuanyuan Li: Writing – review & editing, Conceptualization, Validation. All authors took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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## Disclosure

The authors report no conflicts of interest in this work.

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