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Reflectance Confocal Microscopy of Rosacea: Disease Features and Microscopic Changes During Vitamin A and Vitamin E Supplementation Therapy

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ABSTRACT

Background: Rosacea is a chronic inflammatory skin disorder affecting the central face, often presenting with erythema, papules, pustules, and telangiectasia. Its pathogenesis involves dysregulation of the innate immune system, increased antimicrobial peptide (cathelicidin) activity, and microbial colonization, particularly by *Demodex folliculorum* and associated bacteria. Reflectance confocal microscopy (RCM) is a noninvasive imaging tool that enables high-resolution visualization of skin structures, offering insights into the microscopic features of rosacea.

Objective: This study aimed to analyze the morphological features of rosacea-affected skin using RCM and assess the clinical and microscopic effects of oral Vitamin A and E supplementation in patients with papulopustular rosacea (PPR).

Methods: Fifteen patients with PPR (IGA score 2-3) were treated with oral Vitamin A (50,000 IU) and Vitamin E (50 mg) daily for a minimum of 12 weeks. Clinical evaluations included lesion counts, erythema, IGA and RASI score, and burning sensation. RCM parameters assessed included inflammatory cell types, vascular changes, and infundibular morphology. Paired *t*-tests, Wilcoxon signed rank tests, and McNemar's Chi-squared test were used to compare baseline and posttreatment findings.

Results: Significant reductions were observed in papules, pustules, erythema, and burning sensation scores. RCM analysis showed a reduction in dermal inflammatory cells and Demodex-infested infundibula.

Discussion: Vitamin A and E supplementation was associated with clinical improvement and reduction in inflammatory and Demodex-related RCM features, suggesting a beneficial role in rosacea management. The findings highlight the potential of RCM as a noninvasive tool for monitoring rosacea treatment response. Further studies with larger sample sizes are needed to confirm these results.

1 | Introduction

Rosacea is a chronic, inflammatory cutaneous disorder that primarily affects the central face, including the cheeks, nose, chin, and forehead. Its clinical features typically include persistent erythema, papules, pustules, and telangiectasia. Rosacea affects approximately 5% of the adult population; it occurs in both men and women with onset usually after the third decade of life [1]. The typical patient is a middle-aged woman with a fair skin type [2]. The clinical presentation of rosacea varies widely, and its course is typically irregular, characterized by alternating periods of exacerbation and remission [3]. Rosacea can be classified into

four distinct clinical subtypes, which can overlap or progress from one form to another [4]: erythematotelangiectatic rosacea (ETR), papulopustular rosacea (PPR), phymatous rosacea, and ocular rosacea [5]. Although recent advancements have provided substantial insights into the mechanisms underlying the disease, the pathogenesis of rosacea continues to be a subject of investigation [3, 6]. Multiple factors are thought to contribute to its development, including dysregulation of the innate immune response and neuropeptide activity, microbial involvement, environmental factors, dietary triggers, and skin barrier dysfunction [4, 7]. Despite these advances, critical gaps remain in understanding the histopathological features of rosacea, particularly with regard to its subtypes and the age-dependent evolution of lesions [8].

Innate immune system dysregulation plays a central role in the pathogenesis of rosacea, characterized by the overproduction of antimicrobial peptides (AMPs) such as cathelicidins and proinflammatory cytokines [6]. The production of cathelicidins is induced by the activation of TLR-2, which are innate immune system receptors that are overexpressed on the skin of patients with rosacea [6]. Patients with rosacea also exhibit abnormally high levels of Kallikrein 5 (KLK5), a serine protease that processes CAMP into bioactive fragments such as LL-37, thereby amplifying the inflammatory cascade [9]. Elevated LL-37 levels stimulate the production of reactive oxygen species (ROS), proinflammatory cytokines (e.g., IL-8), and angiogenic factors (e.g., VEGF) via the epidermal growth factor receptor (EGFR) pathway. These processes underlie the chronic inflammation and vascular dysfunction characteristic of rosacea [6, 10].

The microbiome, particularly the presence of *Demodex folliculorum* mites and their associated bacteria, such as *Bacillus oleronius*, plays an important role in rosacea pathogenesis [11]. This microbial colonization has the potential to activate toll-like Receptor 2 (TLR2) and consequently exacerbate the inflammatory processes characteristic of the disease [7, 12].

Reflectance confocal microscopy (RCM) allows the visualization of superficial layers of the skin at an almost histological resolution in vivo [13, 14]. In contrast to other diagnostic approaches such as the standardized superficial skin biopsy (SSSB), skin scraping, or regular biopsy, RCM leaves the examined site unaltered and allows repeated examinations of the same skin area. Recently, the utilization of RCM in diagnosis and therapeutic monitoring of rosacea has been reported, especially focusing on the analysis of the variations of *Demodex folliculorum* concentration and presence [15–18].

The aim of our study was to systematically characterize the morphological aspects of rosacea-affected skin using RCM. We analyzed not only Demodex's presence and concentration but also other key RCM features, including infundibular hair follicles morphology, the type of inflammatory pattern, and vascular features [19, 20]. To further explore whether Demodex plays a causative role in rosacea pathogenesis, we evaluated patients receiving oral Vitamin A and E supplementation, which does not exert direct toxic effects on Demodex mites after 12 weeks of treatment. This allowed us to assess whether clinical or microscopic skin changes occurred independently of Demodex concentration. The average number of weeks of oral Vitamin A and E supplementation was also assessed during the index period.

2 | Methods

We retrospectively retrieved the medical records of 15 patients diagnosed with facial PPR and an Investigator Global Assessment (IGA) grade of 2-3, between January 1, 2023, and October 1, 2024. These patients were treated as part of routine clinical practice, and the treatment was prescribed as standard care. These patients received daily oral supplementation of Vitamin A and Vitamin E supplementation, 50,000 IU + 50 mg (Tocalfa, Cantabria Labs, Madrid, Spain) daily, for a minimum of 12 weeks. For each patient, demographic data, duration of PPR, comorbidities, and previous therapies were evaluated. Patients were included in the study if a complete set of clinical and RCM images was available both at baseline (T0) and at the 12-week follow-up visit (T1). The average number of weeks of oral Vitamin A and E supplementation was also assessed during the index period. Patients previously diagnosed with acne vulgaris, endocrinologic disorders, or who had undergone any invasive facial procedure were excluded from the study. Adverse events were assessed retrospectively by reviewing the clinical notes from follow-up visits for any patient-reported or clinician-observed side effects. Because the therapeutic regimen consisted of 50,000 IU of Vitamin A daily for a limited duration of 12 weeks, which is generally below the threshold for chronic toxicity, routine laboratory monitoring (i.e., blood exams for liver enzymes or lipid profiles) was neither required nor performed. The study was conducted in accordance with the ethical principles originating from the Declaration of Helsinki and Good Clinical Practices and in compliance with local regulatory requirements. The study was reviewed and approved by an institutional review board (EC: 454/2024/OSS).

Standardized assessment of facial rosacea manifestations was performed using a digital photography analysis system of automated facial features (VISIA, Canfield Scientific, Inc.). RCM images were obtained through a commercial handheld Confocal Microscope (VivaScope 3000, Caliber I.D., Rochester, NY, USA) at four different points on the right cheek. At each randomly selected point, an automatic software protocol (VivaStack Caliber I.D., Rochester, NY, USA) was used to acquire a series of consecutive RCM images starting from the skin surface down to 150 μm in depth (corresponding to the papillary dermis), separated by 2-μm steps.

2.1 | Measurements

Clinical evaluations included assessment of the IGA score, Rosacea Area and Severity Index (RASI) assessment, the total number of rosacea lesions (TLC), the number of papules (Pa) and pustules (Pu), and the grade of erythema (E) and telangiectasia (T) [4, 21, 22]. To assess the Burning Numeric Rating Scale (NRS) value, patients were asked to select a number from 0 ("*no burning sensation*") to 10 ("*worst burning sensation imaginable*") that best described the worst level of burning sensation over the past 24 h [23, 24].

RCM images were evaluated in accordance with the findings of the previous RCM studies on acne and rosacea, focusing in particular on the presence of spindle cells, roundish bright particles, corresponding to inflammatory cells in the epidermis, dermal inflammatory cell, spongiform edema, and dilated vessels in the dermis. Furthermore, investigators counted the number of hair follicles showing normal infundibular morphology, the

TABLE 1 | Patient characteristics at baseline.

Patient characteristics at baseline		Mean (SD)
Age		45.6 (19.8)
Duration of disease		12.2 (8.9)
Burning sensation (NRS 1–10)		5.7 (1.6)
Patient characteristics at baseline (dichotomic)		**Frequencies (%)**
Sex (male)		46.7%
Comorbidities	Arterial hypertension	20.0%
	Anxiety or depressive disorder	26.7%
	Dyslipidemia	33.3%
	Inflammatory bowel disease	0.0%
	Previously treated *Helicobacter pylori* infection	6.7%
Previous rosacea therapies	Doxycycline 40–100 mg	80.0%
	Ivermectin (topical)	100.0%
	Isotretinoin (systemic)	6.7%
	Metronidazole (topical)	100.0%
	Laser or light-based therapies	40.0%
	Brimonidine tartrate gel	13.3%
Ocular manifestations		13.3%
Presence of rhinophyma		6.7%

number of infundibula presenting a thickened bright border, and the number of dilated infundibula (> 150 μm) infested or non-infested by Demodex mites [15, 25–27]. The investigators assessing the clinical and RCM images were not blinded to the treatment time points (T0 vs. T1).

2.2 | Statistical Analysis

Descriptive analysis was used to summarize baseline demographic and clinical characteristics, treatment details, and study outcomes. Means and standard deviations (SD) were calculated for continuous variables, while counts and percentages were used for categorical variables. The normality of continuous paired data differences was assessed using the Shapiro–Wilk test. Normally distributed data were analyzed using the paired Student's *t*-test, while non-normally distributed data were evaluated using the Wilcoxon signed-rank test. Categorical data, such as the presence or absence of specific microscopic features, were evaluated using generalized linear mixed models (GLMMs) to account for within-patient clustering, with "Patient ID" included as a random effect. Poisson GLMMs were utilized for count data, and binomial GLMMs were used for categorical data. To rigorously control for multiple testing in this analysis, p values were adjusted using the Benjamini–Hochberg false discovery rate (FDR) method, with significance set at an adjusted $p < 0.05$. The analysis was performed with SPSS Statistics, Version 20.0 (IBM, Armonk, NY) and R software 4.4.0 (R Foundation for Statistical Computing, Vienna, Austria, https://www.r-project.org/).

3 | Results

Complete demographic characteristics and clinical features of all patients at baseline are reported in Table 1. The mean age of the patients was 45.6 years (SD 19.81), and 46.6% were males. The average duration of rosacea history was 12.13 years (SD 8.92), and the mean number of papules and pustules at baseline were 10.8 (SD 6.1) and 1.3 (SD 1.2), respectively. Overall erythema, teleangiectasia, and IGA were 2.3 (SD 0.5), 2.1(SD 0.6), and 2.3 (SD 0.5), respectively. Baseline RASI and burning NRS were 17.0 (SD 2.2) and 5.3 (SD 1.7). While all patients were evaluated for the study at the 12-week mark (T1), the average total number of weeks of oral Vitamin A and E supplementation prescribed was 19.2 (SD 5.8).

The analysis of clinical features, patient-reported outcomes, and RCM parameters revealed significant improvements from baseline (T0) to posttreatment (T1) (Table 2). Unless otherwise specified, all reported p values have been adjusted for multiple comparisons using the Benjamini–Hochberg FDR method. Papule and pustule counts, as well as RASI scores, significantly decreased from a mean of 10.80, 1.33, and 17.03 to 3.73, 0.47, and 7.20, respectively. Erythema and IGA scores also improved significantly (median reduction, $p < 0.001$, Wilcoxon signed rank test) (Figure 1).

Patient self-reported burning sensation was also significantly reduced, with mean scores decreasing from 5.33 (SD: 1.72) to 3.27 (SD: 1.22) (mean difference: 2.07, 95% CI [0.89, 3.24], $p < 0.001$).

Of the 60 total RCM acquisitions obtained at baseline (T0), 59 were analyzed, and one was discarded due to image artifacts. The same number of acquisitions was obtained and analyzed at T1. Analysis using GLMM demonstrated highly significant reductions in Demodex infestation (FDR-adjusted $p < 0.01$) and infested dilated infundibula (FDR-adjusted $p = 0.02$) following treatment, alongside significant improvements in noninfested dilated infundibula (FDR-adjusted $p < 0.01$), while the increase in follicles showing normal infundibular morphology showed a trend toward improvement (FDR-adjusted $p = 0.06$). Changes in inflammatory markers, specifically spindle cells and roundish bright particles, did not reach statistical significance after FDR adjustment, whereas dermal cells demonstrated a significant reduction (FDR-adjusted $p = 0.04$) (Figures 2 and 3).

TABLE 2 | Clinical features and RCM parameters.

	T0	T1	Test	FDR adjusted *p* value	Mean difference	95% CI
Clinical Features						
Papules, mean (SD)	10.80 (6.18)	3.73 (1.91)	Paired *t*-test	< 0.01*	7.07	[4.26, 9.87]
Pustules, mean (SD)	1.33 (1.18)	0.47 (0.52)	Paired *t*-test	0.02*	0.87	[0.24, 1.49]
Erythema, mean (SD)	2.33 (0.49)	1.73 (0.46)	Wilcoxon signed rank	< 0.01*	—	—
Telangiectasia, mean (SD)	2.07 (0.59)	2.00 (0.53)	Wilcoxon signed rank	0.77	—	—
IGA, mean (SD)	2.33 (0.49)	1.13 (0.35)	Wilcoxon signed rank	< 0.01*	—	—
RASI, mean (SD)	17.03 (2.23)	7.20 (3.41)	Wilcoxon signed rank	< 0.01*	—	—
Patient Self-Reported Parameters						
Burning sensation (NRS), mean (SD)	5.33 (1.72)	3.27 (1.22)	Paired *t*-test	< 0.01*	2.07	[0.89, 3.24]
RCM Features						
Presence of spindle cells (%)	27.12	30.51	GLMM	0.64	—	—
Presence of roundish bright particles in the epidermis (%)	38.98	30.51	GLMM	0.76	—	—
Presence of dermal inflammatory cells (%)	42.37	22.03	GLMM	0.04*	—	—
Presence of spongiform edema (%)	6.78	1.69	GLMM	0.33	—	—
Presence of dilated vessels in the dermis (%)	57.63	47.46	GLMM	0.33	—	—
Number of hair follicles presenting normal infundibula, mean (SD)	1.08 (0.70)	1.53 (0.84)	GLMM	0.06	—	—
Number of infundibula presenting a thickened bright border, mean (SD)	0.17 (0.38)	0.15 (0.36)	GLMM	0.80	—	—
Number of dilated infundibula (> 150 μm) infested by Demodex, mean (SD)	1.12 (0.62)	0.68 (0.63)	GLMM	0.02*	—	—
Presence of at least one dilated infundibula infested by Demodex mites (%)	86.44	59.32	GLMM	< 0.01*		
Number of dilated infundibula (> 150 μm) noninfested by Demodex, containing amorphous material, mean (SD)	0.41 (0.59)	0.95 (0.75)	GLMM	< 0.01*	—	—

*$p < 0.05$.

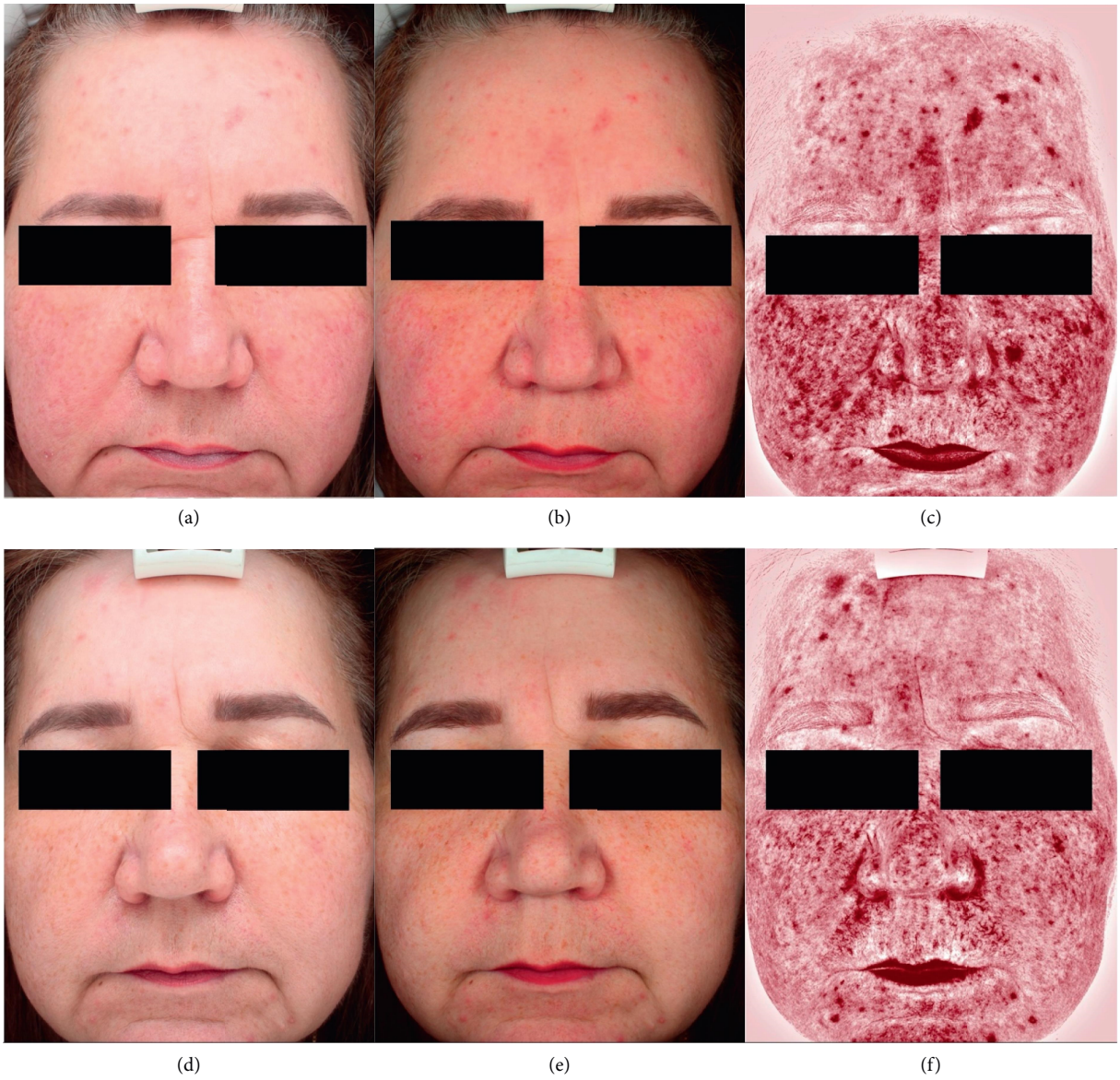

FIGURE 1 | Nonpolarized, polarized, and VISIA red area images of a female patient affected by rosacea before (a–c) and after 12 weeks of Vitamin A and Vitamin E supplementation therapy (d–f).

Regarding safety, no patient discontinued the medication or reported relevant adverse events.

4 | Discussion

In this study, we analyzed the subclinical alterations characterizing the skin of rosacea patients using RCM imaging. RCM is a noninvasive imaging technique that enables the visualization of microscopic architectural features and individual cells in the epidermis and upper dermis with near-histologic resolution. The histologic correlation of structures observed through both dermoscopy and in vivo RCM has been widely demonstrated [28, 29]. The evaluation of several RCM parameters, including both inflammatory features and hair follicle morphologies, enabled us to differentiate classic rosacea features from those of other facial dermatoses, particularly acne vulgaris. The definition of the RCM characteristics of rosacea may be relevant for the assessment of rosacea responses to specific treatments. A few studies have already investigated whether RCM could be used as a noninvasive, objective method to assess and monitor rosacea by measuring the presence and concentration of Demodex mites [11, 15, 16]. To the best of our knowledge, only two studies have focused on RCM features of rosacea-affected skin beyond Demodex concentration [15, 27]. In one of these studies, the authors compared rosacea with sensitive skin and concluded that the detection of parakeratosis, enhanced honeycomb pattern, spongiform edema, and invisible dermal papillae was not sufficient to clearly distinguish between the two conditions [27]. Unlike Ma et al., we demonstrated significant variability in the microscopic aspects of rosacea-affected skin regarding the inflammatory features [27]. At baseline, the most frequently observed RCM parameter was the presence of at least one dilated infundibulum infested by Demodex mites (detected in 86.4% of acquisitions) followed by the presence of dilated vessels in the dermis (detected in 58% of acquisitions). Other mild–moderate inflammatory features were less frequent, with at least spindle

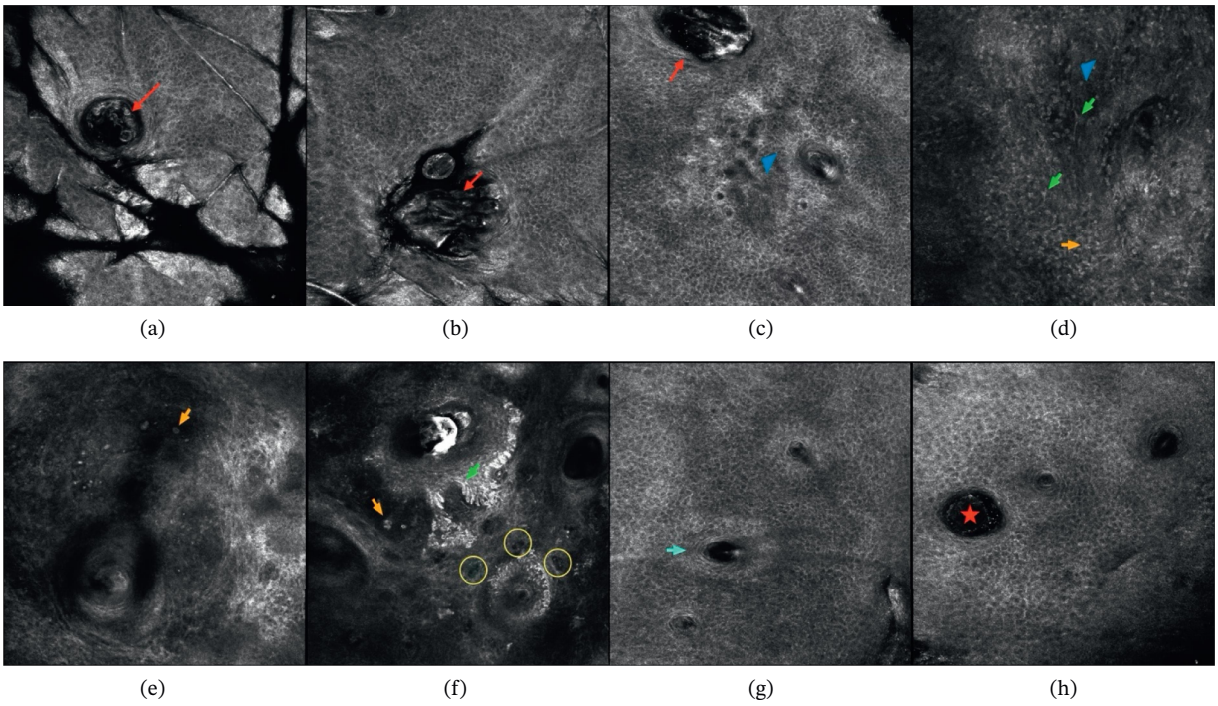

FIGURE 2 | RCM images, showing Demodex mites inside the infundibulum of a hair follicle (red arrow) (a–c). Interkeratinocyte edema and spongiosis (blue triangle) (c, d). Spindle cells (green arrow) and roundish bright particles (orange arrow) (d–f). Dilated vessels (yellow circles) (f). Normal follicles (light blue arrow) (g). Demodex debris and remnants inside the infundibulum of a hair follicle (red star) (h).

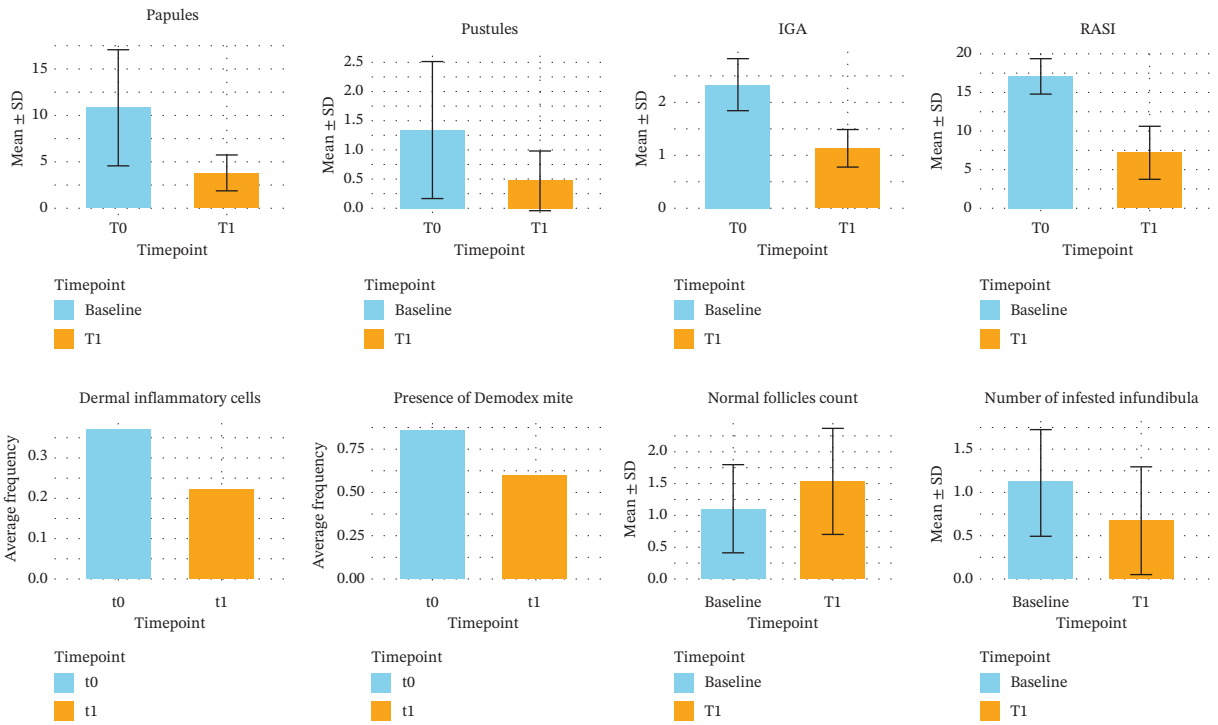

FIGURE 3 | Boxplots showing parameters' changes over time.

cells, roundish bright particles, and dermal inflammatory cells, detected in 27.1%, 39.0%, and 42.4% of baseline acquisitions, respectively. Notably, our data demonstrate that rosacea is characterized by significantly different RCM features compared to acne. These include frequent Demodex detection, distinct RCM inflammatory patterns, and lower rates of infundibular hyperkeratinization (6.13% of rosacea hair follicles compared to approximately 30% in acne hair follicles, as reported in the literature) [19, 25]. These findings support the notion that rosacea does not involve a fundamental follicular abnormality, unlike acne vulgaris [30, 31]. Importantly, our core RCM findings regarding the decrease in dermal inflammatory cells and the

increase in noninfested infundibula and the eradication of Demodex mites proved highly robust. These parameters remained statistically significant even after applying stringent mixed-model analyses designed to account for potential pseudoreplication bias and multiple testing penalties.

The progressive and prolonged reduction in clinical severity parameters, such as lesion count, IGA, and RASI, during Vitamin A and E supplementation, correlates closely with the improvement of several key RCM parameters that are associated with rosacea pathogenesis. Vitamin A supplementation and retinoid therapy positively impact rosacea by strengthening the skin's barrier function, reducing transepidermal water loss, stimulating collagen synthesis through the TGF-β pathway, and inhibiting matrix metalloproteinases (MMPs) to prevent collagen and elastin degradation [32–34]. They also promote elastin fiber production, reducing vasodilation. Furthermore, retinoids regulate sebaceous gland activity by decreasing lipogenesis and suppressing sebocyte proliferation and differentiation [32, 33]. Their anti-inflammatory properties successfully modulate cytokine and growth factor production. Because Vitamin A and E are not known to possess direct acaricidal properties, we hypothesize that their pleiotropic effects indirectly create a hostile environment for Demodex colonization. This is achieved through reduced sebum concentration and increased production of AMPs, including enhanced expression of KLK5, Kallikrein 7, and cathelicidin [9, 35]. Vitamin E is among the earliest recognized biologic antioxidants [36, 37]. Its major mechanism of action is arresting chain propagation by scavenging lipid peroxyl radicals [38, 39]. Several studies have demonstrated that, in human skin, Vitamin E has photoprotective activities, significantly reducing acute skin responses, such as erythema and edema, sunburn cell formation, deoxyribonucleic acid (DNA) adduct formation and immunosuppression [37, 39, 40].

Regarding the safety of this regimen, oral Vitamin A supplementation carries well-documented potential risks. While systemic adverse effects—such as hepatotoxicity, lipid profile alterations, musculoskeletal pain, and mucocutaneous symptoms—typically require dosages higher than the 50,000 IU daily utilized in our study, teratogenicity remains a critical exception [41, 42]. Therefore, strict avoidance of pregnancy is mandatory for female patients of childbearing potential. Consistent with this safety profile, our retrospective evaluation did not reveal any severe adverse events. Nonetheless, clinicians should remain vigilant regarding the patient's cumulative Vitamin A intake, including dietary sources and other supplements.

In accordance with the previous results reported by Logger et al., epidermal inflammatory cells, that are constituted by spindle cells and bright particles in the epidermis, showed relatively modest changes during the observation time [15]. However, in contrast to previous studies, we observed a notable and significant posttreatment reduction in the presence of dermal inflammatory cells, decreasing from 42.37% to 22.03% (GLMM FDR-adjusted $p = 0.04$), with important implications for potential therapeutic benefit. The significant decrease in the number of Demodex-infested infundibula must be interpreted cautiously. As previously noted, this decline likely reflects a secondary improvement driven by the reduction in overall inflammation and the altered follicular microenvironment rather than a direct acaricidal effect of the vitamins. Furthermore, natural variability and fluctuations in Demodex populations cannot be entirely excluded. Nevertheless, this observation corroborates the concept that Demodex mites play a crucial role in triggering the inflammatory events closely associated with rosacea exacerbations [15, 17].

The in vivo microscopic study of skin dynamics before and during treatment enhances our understanding of disease pathophysiology and provides valuable insights into optimal therapeutic modalities. However, the small sample size, retrospective design, the lack of blinding during RCM image assessment, and the absence of a control group limit the generalizability of these findings and preclude definitive conclusions regarding treatment efficacy. Because this is an uncontrolled observational study, causal inference regarding the treatment cannot be definitively established. Further studies involving larger sample sizes, randomized control groups, and comparisons of different therapies are needed to validate these preliminary results. Nevertheless, RCM shows promise as a useful tool for the objective evaluation of clinical management, mechanisms of action, and time-course.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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