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Evaluation of Serum Ceramides C16, C24, and Sphingosine-1-Phosphate (S1P) in Patients with Acne Vulgaris

A protocol of thesis submitted for partial fulfillment of Master degree in Dermatology,Venereology and Andrology

by

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Introduction

Acne vulgaris (AV) is a chronic, inflammatory disease of the pilosebaceous unit that is globally prevalent and represents a great public health burden ***(Vasam et al., 2023)***. Acne affects approximately 9.4% of the world's population, ranking the eighth most prevalent skin condition worldwide ***(Reynolds et al., 2024)***. It affects more than 85% of adolescents and frequently persists into adulthood ***(Guguluș et al., 2025)***.Clinically, acne vulgaris is characterized by a spectrum of lesions, including non-inflammatory comedones (open and closed) and inflammatory papules, pustules, nodules, and cysts, often leading to post-inflammatory hyperpigmentation and scarring ***(Vasam et al., 2023)***.

Lesions predominantly occur on regions rich in sebaceous glands such as the face, chest and upper back. The severity and chronicity of the condition, along with its visible disfigurement, often result in significant psychosocial distress, including anxiety, depression, and poor self-esteem which negatively impacting patients' quality of life ***(Kazan, 2024)***.

The pathogenesis of acne is multifactorial, involving a complex interplay between genetic predisposition, environmental exposures (e.g., humidity, pollution, comedogenic cosmetics), hormonal fluctuations, and immune dysregulation. The four classical pathogenic mechanisms include increased sebum production, abnormal follicular keratinization, colonization by *Cutibacterium acnes* and activation of innate and adaptive inflammatory responses ***(Alsaadoon et al., 2024)***. Although inflammation was once thought to occur secondary to microbial colonization, recent studies demonstrate that inflammatory processes are activated in the early stages of lesion development, even in clinically non-inflamed comedones ***(Tanghetti, 2013)***.

Emerging evidence has highlighted the crucial role of lipids—particularly sphingolipids—in skin homeostasis, barrier function, and immune signaling. Among these lipids, ceramides (CERs) and sphingosine-1-phosphate (S1P) are of particular

interest due to their dual structural and signaling roles in the skin ***(Masuda-Kuroki et al., 2023)***. The outermost layer of the epidermis, the stratum corneum, consists of a lipid matrix comprising approximately equimolar concentrations of ceramides, free fatty acids, and cholesterol. This matrix is essential for maintaining skin barrier integrity and hydration, and any imbalance can contribute to dermatological disorders ***(Mijaljica et al., 2024)***.

Ceramides are sphingolipid molecules composed of sphingosine and fatty acid chains of varying lengths. Long-chain ceramides (C16–C20) and very-long-chain ceramides (C22–C24) have been shown to exert differential effects on keratinocyte behavior and cutaneous inflammation. Specifically, recent data indicate that certain ceramide species play a role in modulating inflammation and cellular proliferation in the skin ***(Ho et al., 2022)***. For instance, C16 ceramide is often associated with pro-apoptotic and pro-inflammatory effects, whereas very-long-chain ceramides like C24 may contribute to anti-inflammatory functions and promote cell survival. Dysregulation in the balance of these ceramide species can thereby influence the severity and progression of inflammatory skin diseases, including acne vulgaris ***(Custodia et al., 2022; Thakkar et al., 2025)***. Sphingomyelins (SMs), abundant in both circulation and cell membranes, serve as precursors to ceramides. These SMscan be hydrolyzed by sphingomyelinase (SMase) into ceramides, which in turn can be further metabolized into sphingosine and subsequently phosphorylated into Sphingosine-1-phosphate (**S1P**) by the action of sphingosine kinase . This conversion pathway is of great interest in inflammatory research ***(Thakkar et al., 2025)***.

Sphingosine-1-phosphate (S1P) is a potent bioactive lipid that plays diverse roles in immune regulation, angiogenesis, vascular integrity, and inflammatory signaling. In the skin, S1P regulates the behavior of keratinocytes, immune cells, and vascular endothelial cells. It promotes cellular proliferation and survival and can influence immune cell migration and cytokine production. Thus, alterations in serum

or tissue levels of S1P may reflect or contribute to the inflammatory milieu observed in acne vulgaris ***(Custodia et al., 2022; Su et al., 2024)***.

Although the roles of sphingolipids have been studied in dermatologic disorders such as atopic dermatitis, psoriasis, and ichthyosis, data on their involvement in acne vulgaris remain limited. Most previous studies have focused on skin tissue analyses ***(Alizadeh et al., 2023; Delcheva et al., 2024)***, whereas investigations into serum sphingolipid profiles—especially ceramides C16, C24, and S1P—in acne patients are scarce. Understanding systemic alterations in these bioactive lipids may provide insights into the inflammatory and metabolic underpinnings of acne vulgaris, potentially identifying novel biomarkers or therapeutic targets.

Aim of the work

1. To measure serum levels of ceramides C16 and C24 in patients with acne vulgaris.
2. To determine the serum level of sphingosine-1-phosphate (S1P) in the same patient group.

Patients and methods

Study approval: The study will be approved by Research and Ethical committee at Faculty of Medicine, Sohag University. Informed written consent will be obtained from all participants after explanation of nature of the study.

Study Design: This is a case-control, cross-sectional, analytical study

Site of the Study: The study will be conducted at the Dermatology Outpatient Clinic in Sohag University Hospitals.

Sample size calculation

The sample size was determined to detect both group differences in serum lipid mediators and correlations with acne severity. Previous work in acne patients (Kaya et al., 2019) included only 30 cases and 20 controls but demonstrated significant lipid alterations. Using standardized effect sizes (α=0.05, power 80%), a moderate correlation ($r \approx 0.40$) requires ~47 subjects, while a moderate between-group difference (Cohen's $d \approx 0.6$) requires ~44 per group. Accordingly, 40 patients and 40 matched controls (n=80) .

Study Population:

A. Inclusion Criteria:

For Case Group

This group will consist of (40) patients :

- Both males and females aged 15–40 years.
- Mild, Moderate, severe and very severe cases will be included.

For Control Group:

- This group will consist of age- and sex-matched (40) healthy volunteers.

B. <u>Exclusion Criteria (for both groups):</u>

- Presence of any dermatological condition other than acne vulgaris (for cases).
- Use of systemic or topical treatment such as isotretinoin or antibiotics within the last 3 months.
- Pregnancy or lactation.
- History of lipid-lowering therapy or supplements affecting ceramide or sphingolipid metabolism.

<u>All patients in this study will be subjected to:</u>

1. <u>Clinical Evaluation</u>

A. History including:

- **Demographic data:** Name, age, sex, residence and occupation.
- **History of present illness:**
  - Age of onset of acne.
  - Duration of acne symptoms.

- **Medical History:**
  - Presence of chronic diseases (e.g., diabetes, polycystic ovarian syndrome).
  - Menstrual and contraceptive history in females

B. Dermatological examination:

- **Lesion Assessment:** Documentation of acne lesion types (comedones, papules, pustules, nodules, cysts) Presence of erythema,edema or redness
- **Distribution:** Affected anatomical areas (face, chest, back, shoulders).
- **Scar Evaluation:** Presence and severity of post-acne scarring (atrophic, hypertrophic).

C. Acne Severity Assessment:

Global Acne Grading System (GAGS): It is a system which evaluates acne severity by dividing the face, chest, and back into six regions, each assigned a factor according to its size. The most severe lesion type in each region is graded (0–4), multiplied by the regional factor, and summed to yield a global score (0–44). Severity is then classified as mild, moderate, severe, or very severe. **(Doshi A, 1997)**

The final global score is interpreted as follows:

- 0 → No acne
- 1–18 → Mild acne
- 19–30 → Moderate acne
- 31–38 → Severe acne
- 39–44 → Very severe acne

2. Laboratory Investigation:

Evaluation of Serum Ceramides (C16, C24) and Sphingosine-1-Phosphate (S1P).

a. Sample Collection and Processing:

- **Timing:** Morning samples will be collected after overnight fasting to reduce variability due to dietary lipid intake.
- **Procedure:**
    - A volume of 5 mL of venous blood will be drawn using standard phlebotomy techniques.
    - Blood samples will be allowed to clot at room temperature and then centrifuged at 3000 rpm for 10 minutes.
    - The separated serum will be aliquoted into sterile cryovials and stored at −80°C until analysis to preserve lipid stability.

b. Quantitative Measurement of Lipid Mediators:

- **Method of Analysis:**
- **Quantitative determination of the target sphingolipids will be performed using liquid chromatography coupled with triple quadrupole mass spectrometry (LC-MS/MS)** ***(Agilent 6460 Triple Quadrupole LC-MS/MS, USA)*****.**

Procedure:

1. **Add 100 µL serum** into a glass vial.
2. Add **400 µL methanol** and **200 µL chloroform** (containing internal standards).
3. Vortex for 30 seconds.
4. Add **200 µL water** and **200 µL chloroform**.
5. Vortex and centrifuge at relative centrifugal force (1000_3000)g for 10 minutes to separate layers.

6. Carefully collect the **lower organic phase** (contains lipids).
7. Evaporate under nitrogen gas at room temperature or using a vacuum concentrator.

8. Reconstitute dried extract in **100 µL of methanol/water (9:1)** with 0.1% (optimized according to instrument condition) formic acid for LC-MS/MS injection .

Statistical Analysis

Data will be statistically described in terms of mean_standard deviation (SD), median and range, or frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups will be done using Kruskal Wallis test. Within group comparison of numerical variables will be done using Wilcoxon signed rank test for paired (matched) samples. For comparing categorical data, Chi square (x2) test will be performed. Exact test will be used instead when the expected frequency is less than 5. Correlation between various variables will be done using Spearman rank correlation equation for non-normal variables/non-linear monotonic relation. P values less than 0.05 will be considered statistically significant. All statistical calculations will be done using computer program SPSS

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