Research Protocol

15 February 2023

The Efficacy and Safety of Topical Vitamin D and Supplementation in Acne Vulgaris

The Study of VDR, IL-1β, IL-6, IL-10 and IL-17 Expression

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1 Introduction

This document is a clinical trial protocol. This research will be conducted based on the standards of the Good Clinical Trial Method and regulations from the relevant institutions and ethics committees.

1.1 Background

Acne vulgaris (AV) is a chronic inflammatory disease with multifactorial causes in the skin's pilosebaceous follicular units, with clinical manifestations in the form of comedones, papules, pustules, nodes, and pseudocysts. The following factors are considered important for the etiology of AV: increased rate of sebum excretion, endocrinological factors such as androgens, abnormal keratinization of the follicular infundibulum, the proliferation of Cutibacterium acnes (*C. acnes*), and inflammation. Recent studies at the molecular and cellular levels have clarified how these factors interact and the role of the innate immune system. Inflammatory processes have been demonstrated in all types of lesions – preclinical microcomedones, comedones, inflammatory lesions, 'post inflammatory' erythema or hyperpigmentation, and scarring. Inflammation localized to the pilosebaceous can be considered a hallmark of acne and should be managed through several therapeutic routes. Clinicians tend to think that oral antibiotics should be used to treat inflammation in acne. However, this treatment are associated with resistance and low outcome due to its adverse events such as erythema, desquamation, and dry skin. There is evidence of the use and opportunity of vitamin D as a novelty treatment influencing the immune system. 25OHD and 1,25(OH)2D are both catabolized by CYP24A1. 1,25(OH)2D is a ligand for the vitamin D receptor (VDR), a transcription factor that binds to sites in DNA called vitamin D response elements (VDRE). Thousands of these binding sites regulate hundreds of genes through several signaling pathways in different cell types, including their regulation in immune cells by toll-like receptors (TLRs), the primary signaling nucleus of *C. acnes* that interacts with the innate immune system, causing acute and chronic inflammation.

2 Study Objectives

2.1 Primary Objective

The primary objective of this study is to evaluate the efficacy and safety of combination topical vitamin D and supplementation as adjuvant therapy in acne vulgaris compared to placebo and topical vitamin D monotherapy.

2.2 Secondary Objective(s)

1. To assess vitamin D Receptor (VDR) expression on acne lesion and blood sample
2. To assess the effect of combination topical vitamin D and supplementation on IL-1β expression on acne lesion
3. To assess the effect of combination topical vitamin D and supplementation on IL-6 expression on acne lesion
4. To assess the effect of combination topical vitamin D and supplementation on IL-10 expression on acne lesion
5. To assess the effect of combination topical vitamin D and supplementation on IL-17 expression on acne lesion

3 Study Design

3.1 General Design

It is a randomized double-blinded controlled trial to assess the efficacy and safety of combination topical vitamin D and supplementation as adjuvant therapy in acne vulgaris compared to placebo and topical vitamin D monotherapy. In week 8^{th}, the patients will be observed to see if there is any recurrence of the lesion.

3.2 Settings and Conduct

This study was performed in Central Jakarta, Jakarta Capital Special Region, Indonesia. Patients were assigned into three groups. The first group was given the combination of topical vitamin D and supplementation also standard treatment with topical adapalene. In

comparison, the second group was given topical vitamin D monotherapy and standard treatment with topical adapalene. Last group was given only the standard treatment with topical adapalene as control group.

3.3 Primary Study Endpoints

- Clinical symptoms
  Changes in counts of inflammation and non-inflammation lesions (assessed in week 8th)
- Changes in VDR, IL-1β, IL-6, IL-10 and IL-17 expression on acne lesion (Time frame: change of expression from baseline to week 8th)

3.4 Primary Safety Endpoints

Adverse events will be assessed during study periods. Any adverse events including local/skin inflammation caused by either oral or topical vitamin D will also be monitored by dermatologist in charge.

4 Subject Selection and Withdrawal

4.1 Inclusion Criteria

a. Men and women diagnosed with moderate or severe acne vulgaris
b. Aged over 18-50 years.
c. During the study, were willing not to use skin care products either in oral and/or topical form on the face, as well as other treatments outside of standard AV treatment.
d. Willing to take part in the examination and treatment in accordance with the provisions of the study and follow the control time schedule in accordance with the predetermined research plan also willing to sign the informed consent form.

4.2 Exclusion Criteria

Patients will be excluded if

a. Women who are pregnant and breastfeeding.
b. Have history using topical antibiotics in the past 2 weeks.

c. Have history using topical corticosteroids in the past 2 weeks.
d. Have history taking vitamin D supplements in the last 1 month.
e. Have history taking oral antibiotics in the last 1 month.
f. Have history using oral corticosteroid use in the last 1 month.
g. Have history using oral and topical retinoid use in the last 3 months.
h. Have history using topical BPO in the last 1 month.
i. Using hormonal contraception for women.
j. Have history of drug allergies or skin disorders due to side effects of first-line therapy drugs for moderate/severe acne vulgaris.
k. Impaired liver and kidney function.

4.3 Early Withdrawal of Subjects

The protocol treatment will be discontinued if patients

a. There are severe side effects in the subject in the form of gastrointestinal disorders, severe liver function disorders, and severe irritation, edema or bullous lesions at the site of topical drug application.
b. Subjects decided to withdraw from the study before the second week.
c. Subjects did not come for 2 consecutive follow-ups. The tolerance limit for follow-up is D+3 from the specified examination date.
d. Subject confirmed positive for COVID-19
e. Subjects did not follow established research protocols.

4.4 Sample Estimate

The sample size in this study can be calculated based on the ratio of two proportions, using the following formula:

$$N1 = N2 = \frac{(Z\alpha\sqrt{2PQ} + Z\beta\sqrt{P_1 Q_1 + P_2 Q_2})^2}{(P_1 - P_2)^2}$$

N1 = Control group sample size

N2 = N3 = The number of samples of the test group

α = Type 1 error, set at 0.10 (90% confidence level) with a one-way hypothesis, so Z α = 1,64

β = if it is set at 20%, then the power of this study is 1- β = 80%, from the table so that Z β = 0.84

P_2= The proportion of treatment effectiveness in the control group obtained from the previous research was [16] = 0.70

$Q_2 = 1 - P_2 = 1 - 0,70 = 0,30$

$P_1 - P_2$ = The minimum difference in the proportion of treatment effectiveness that is considered significant is set at 0.25.

$P_1 = P_2 + 0,20 = 0,70 + 0,25 = 0,95$

$Q_1 = 1 - P_1 = 1 - 0,95 = 0,05$

$P = \frac{P1+P2}{2} = 0,825$

$Q = 1 - P = 1 - 0,825 = 0,175$

Obtained results:

$$N1 = N2 = \frac{(1,64\sqrt{2 \text{ x } 0,825 \text{ x } 0,175} + 0,84\sqrt{0,95 \text{ x } 0,05 + 0,70 \text{ x } 0,30})^2}{(0,25)^2}$$

$$= 27,35$$

$$= 28$$

Assuming that the difference in the proportion of moderate/severe acne vulgaris improvement between the group with standard therapy + oral vitamin D and topical vitamin D and the control group was only 25%, so the sample size for each group was 28 people.

Anticipation of drop out by 20%:

$$n' = \frac{n}{(1-f)}$$

$$n' = \frac{28}{(1-0,2)} = 35 \approx n1 = n2 = n3 = 35 \text{ people.}$$

Information:

n'1 = n'2 = n'3	= the size of the research sample for each group
n'	= sample size after correction with anticipation of DO
n	= sample size based on previous estimates
f	= prediction of the percentage of sample drop out

The sample size after being corrected with anticipation of dropping out became n'1 = n'2 = n'3 = 35 people.

5. Study Procedures

Treatment phase:

The selected subjects were divided into three groups through a process of randomization and sample stratification per group. The first group (A) will receive a combination of oral vitamin D 2000 IU 1x1 and topical 7-Dehydrocholesterol 5000 mcg 2x1, the second group (B) will receive an oral placebo and topical 7-Dehydrocholesterol 5000 mcg 2x1 and the third group (C) will receive an oral placebo and basic ingredient placebo topical vitamin D similar to the ointment to be tested. All subjects were still given 0.1% adapalene cream as a standard topical treatment for AV. The three groups will receive intervention for 8 weeks (56 days). AV lesion extraction will be performed, peripheral venous blood sampling will be performed to examine serum 25(OH)D and serum VDR. Examination of serum VDR and tissue VDR were using flowcytometry. Examination of inflammatory cytokines IL-1β, IL-6, IL-10, and IL-17 in tissues were using the Luminex Kit. Random allocation is done by block randomization technique using a computer program (Randlist®). Capsule drugs will be recalculated and topical drugs will be weighed when the subject returns for follow-up. Research drugs when given to subjects or returned to researchers were included in sealed envelopes. Each subject received a diary sheet which included a statement column to drink/not drink/apply/not apply and have/no side effects and their type, which had to be filled in every day according to the diary attached.

Arms and intervention

Arm	Intervention/treatment
Experimental: combination topical vitamin D and supplementation Daily supplementation (1x/day, in the morning) and topical application (2x/day, in the morning and in the afternoon, minimum duration 4 hours on the skin) for 56 days	Drug: combination vitamin D 2000 IU 1x1 and topical 7-Dehydrocholesterol 5000 mcg 2x1
Active Comparator: oral placebo and topical vitamin D Daily supplementation (1x/day, in the morning) and topical application (2x/day, in the morning and in the afternoon, minimum duration 4 hours on the skin) for 56 days	Drug: oral placebo and topical 7-Dehydrocholesterol 5000 mcg 2x1
Placebo Comparator: Daily supplementation (1x/day, in the morning) and topical application (2x/day, in the morning and in the afternoon, minimum duration 4 hours on the skin) for 56 days	Drug: oral placebo and basic ingredient placebo topical vitamin D similar to the ointment

6 Sample Size

6.1 Target sample size

105 patients (35/arm)

7 Ethical Committees

- Name of ethics committee : Ethics committee of the Faculty of Medicine, University of Indonesia – Cipto Mangunkusumo Hospital

Medical Sciences

- City : Central Jakarta
- Province : Jakarta Capital Special Region
- Country : Indonesia
- Approval date : September, 5th 2022
- Ethics committee reference number : KET-922/UN2.F1/ETIK.PPM.00.02/2022