



Increased Vitamin A Level is associated with Clinical Improvement in Mild Acne Vulgaris: A Pre-test and Post-test Clinical Trial

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Abstract

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BACKGROUND: Acne vulgaris (AV) is a global cutaneous disorder that although is benign, can significantly affect patient's quality of life. The pathognomonic feature of the disease is the emergence of comedones. There are four known pathophysiology of AV, increased sebum production, ductal cornification, colonization of the bacteria *P. acnes* in the pilosebaceous unit, and inflammation. Furthermore, vitamin A although already a well-established acne treatment that can affect all four of these factors, there are still limited data regarding the quantitative measurement of vitamin A itself in AV cases.

AIM: This study seeks to review the association between vitamin A levels measured quantitatively from open and closed comedones.

METHODS: We used Enzyme Linked Immunosorbent Assay (ELISA) to measure vitamin A levels from open and closed comedones with the outcome of inflamed lesions and comedones prior and after treatment with topical tretinoin 0.025% cream in patients with mild AV

RESULT: There was a 45.9% decrease of inflamed lesions from a mean of 9.15 to 4.2 lesions ($p=0.03$), and a 44.8% decrease in comedone count from a mean of 17.15 to 8.05 lesions ($p<0.01$) Subsequently there was also a 23.03% increase of vitamin A level from 272.5mg/dL to 335.28mg/dL ($p<0.01$).

CONCLUSION: Our study found that increased level of vitamin A is associated with significant clinical improvements in both inflamed and non-inflamed AV lesions.

Introduction

Acne vulgaris is one of the most common skin disorders and has long been a global burden. According to the Global Burden of Diseases, Injuries, and Risk Factors Study (GBD), a systematic scientific assessment study in 2019 found that acne vulgaris ranked as the 19th most common disease, rising from 27th in 1990 among people aged 10–24 years old [1].

The pathognomonic feature of AV is the presence of comedones. There are two types of comedones, open, and closed. Open comedones (blackheads) are caused due to the clogging of follicles with an opening, while closed comedones are clogged follicles without an opening [2]. Even though the disease is mostly confined in adolescents and teenagers, AV can persist into adulthood and in severe cases can cause disfiguring permanent scars [2], [3].

Vitamin A is an important micronutrient that serves various functions in the human body such as ocular function and bone development, as well as having protective effects in both skin and mucosa [4]. The use of Vitamin A both topically and orally was a breakthrough in the management of acne vulgaris for both superficial inflammatory lesions such as papules

and pustules, as well as deeper inflammatory lesions such as nodules and cysts [5].

Vitamin A, also called retinols, is a soluble vitamin that is closely related to tissue growth and differentiation [6]. 13-cis-retinoic acid is a form of Vitamin A that has been known to affect all four of AV pathophysiologies. The compound can reduce the activity of sebaceous glands that results in the decrease of sebum production and subsequently the number of *P. acnes* preventing the inflammation process. In addition, it can also inhibit the comedogenesis process by influencing the keratin production and epidermal differentiation as well as preventing progression of non-inflammatory comedones to inflammatory ones [7]. Tretinoin (all-trans retinoic acid) has immunomodulating properties that can influence the inflammatory cascade caused by the overgrowth of *P. acne* such as IL-5, IL-6, and IL-12. In addition, it is also a potent TNF-alpha inhibitor [8].

The key factor in inflammation in acne vulgaris lies on the activity of *P. acne*. Excessive colonization of this bacteria can lead to increased level of toxic oxygen metabolites as well accumulation of neutrophil tissue as part of the human's inflammatory immune response. Hence, the measurement of Vitamin A levels in AV should be considered as a risk factor for AV [7]. A clinical trial by Veraldi *et al.* evaluated the use of

combination of two topical Vitamin A derivatives, retinol, and hydroxyphenyl retinoate and found that it can reduce both inflammatory and non-inflammatory AV lesions to 70% and 60%, respectively, further proving the anti-inflammatory effects of Vitamin A and its derivatives [9].

To the best of our knowledge, there are still limited publications regarding the measurement of Vitamin A levels in acne vulgaris. One study by Cengiz, *et al.* in Turkey measured the levels of Vitamin A and E in patients with mild and moderate AV, while another study evaluated low-dose Vitamin A as treatment for AV [5], [10]. This study seeks to review the association between Vitamin A levels measured quantitatively using enzyme linked immunosorbent assay (ELISA) with the outcome of inflamed lesions (IL) and comedones before and after the treatment with Tretinoin 0.025% in patients with mild AV.

Methods

Study design

This clinical trial was performed at Hasanuddin University Hospital, Makassar, Indonesia for 6 weeks between October and November 2021.

Subjects

Male and female patients who visited the dermatology and venereology clinic of Hasanuddin University hospital within the age 15–35 years old were eligible for this study. Inclusion criteria included patients with mild acne vulgaris based on the Acne Grading System by Lehman *et al.* [11]. The diagnosis was made by lesion counting; mild acne vulgaris was diagnosed when patients presented with <20 comedones, <15 inflamed lesions, or total lesions <30. All subjects had no prior history of acne treatment. All patients had signed informed consent forms and were not pregnant, lactating, nor on hormonal contraception. Exclusion criteria included patients who were currently on acne vulgaris treatment, both systemic and topical, including antibiotics, corticosteroids, or any hormonal therapy in the past 30 days before the start of the study.

Study protocol

The study was conducted in two sessions with an interval of 30 days. On the initial visit, subjects underwent history taking and physical examination, as well as having their photographs taken. Lesion counting based on Lehman's Criteria was performed to analyze the number of inflamed lesions (IL), which accounted for pustules, nodules, and cysts of the

subjects, and comedones. Samples for analysis were taken from ten open or closed comedones chosen randomly by applying gentle vertical pressure on the lesion using an extractor in cases of open comedones, or by initially widening the opening using a 27G needle followed by gentle pressure with extractor in case of closed comedones. All samples were acquired from the cheeks, stored in Eppendorf® tubes, and kept frozen at -20°C . When samples were ready to be measured, they were defrosted, homogenized, and centrifuged at 2000–3000 rpm. The supernatant produced was then mixed with buffer from the (ELISA) kit (Cat#1548hu Human Vitamin A, VA ELISA Kit, Bioassay Technology, China). Vitamin A levels were then assessed using an ELISA reader (Thermo Scientific™ Multiskan™ FC Filter-based Microplate Photometer, Thermo Fisher Scientific, Waltham, United States).

After the initial visit, subjects were instructed to apply one fingertip unit (FTU) of 0.025% tretinoin cream at night and one FTU of SPF 30 sunscreen at morning on the forehead, both cheeks, and nasal area which were then spread to cover the whole face for 30 days. On the second visit, photographs, and samples from comedones were acquired from the similar location and measured using the same protocol.

Ethical clearance

This study was done after ethical clearance had been obtained from the Faculty of Medicine Hasanuddin University Ethical Board Committee (529/UN4.6.4.5.31/PP36/2020). The protocols were done in line with the Declaration of Helsinki guideline.

Statistical analysis

Paired t-test was conducted to compare the Vitamin A level before and after therapy and analyzed using SPSS version 28.0 (SPSS Inc. Chicago, IL, USA). Normality of data distribution was assessed using Shapiro–Wilk test. $p < 0.05$ was considered to be statistically significant.

Results

A total of 20 subjects (11 females and nine males) with mild AV participated in this study. Demographic distribution found the youngest age of subject being 16 years old, and the oldest being 33 years old. The mean age of all participants was 25 years old. Before application of topical therapy, the mean number of IL was 9.15, while the mean number of comedones was 17.95. The mean Vitamin A level before the treatment was 272.5 mg/L. After 30 days of tretinoin 0.025%

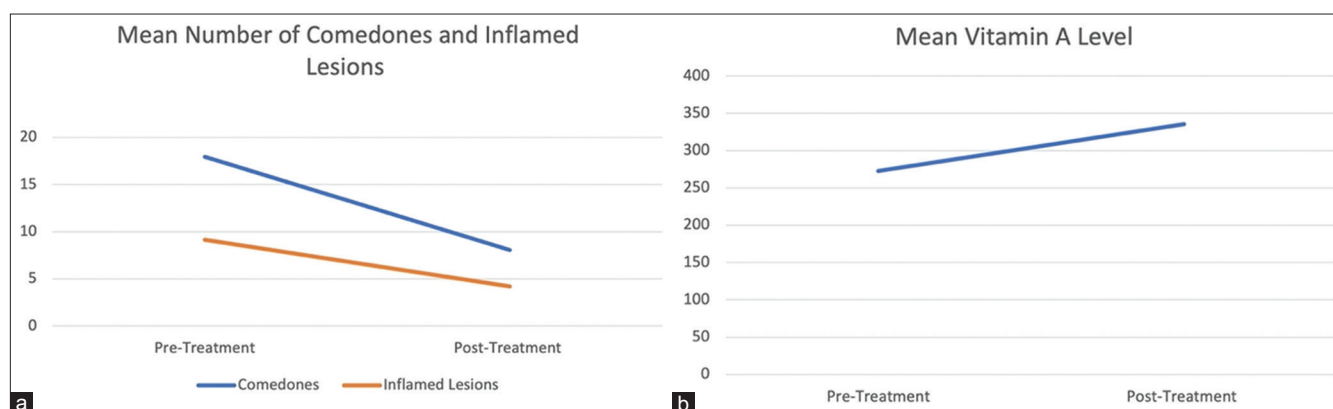


Figure 1: (a) (Left) Markedly decrease of Comedones and IL after 30 days of tretinoin treatment; (b) (Right) Conversely, a significant increase of Vitamin A level were found

cream treatment, a significant clinical improvement shown by the decrease of IL and comedones was found (Figure 1a), as well as an increase in the mean Vitamin A level (Figure 1b). There was a 45.9% decrease of inflamed lesions to a mean of 4.2 lesions ($p = 0.03$) and a 44.8% decrease in comedone count to 8.05 lesions ($p < 0.01$) Subsequently, there was a 23.03% increase of Vitamin A level from 272.5 mg/dL to 335.28 mg/dL ($p < 0.01$).

Discussion

Acne vulgaris (AV) is the most common skin disease. It is a chronic inflammatory disease of the pilosebaceous unit that most commonly affects teenagers (80%) [3]. AV can manifest in both inflammatory and non-inflammatory lesions. Inflammatory lesions include papules, pustules, and nodules, while non-inflammatory lesions can manifest as either open or closed comedones [12]. In addition, AV lesions can also cause various degrees of scarring that can severely impact patient's quality of life. There are various factors that influence the pathophysiology of acne, such as increased sebum production, ductal cornification, colonization of the bacteria *P. acnes* in the pilosebaceous unit, and inflammation [10].

ELISA is one of the quantitative tests available to measure Vitamin A level concentration. It is a sensitive and specific analytic biochemistry that can use various samples, in our case, tissues samples acquired from either closed/open comedones were homogenized and mixed with the buffer from the ELISA kit. Reagents produced were then incubated and provide optical change such as color change and were then be able to be indirectly quantify Vitamin A levels [13]. A study by Cengiz *et al.* found that Vitamin A levels are higher (461.78 ± 89.78 mg/dL) in moderate AV compared to mild AV (437.09 ± 79.93 mg/dL) [5]. There are previous studies regarding the measurement

of Vitamin A levels from various human tissues such as the skin, comedones, surface lipids, bacteria, and serum, with the study method using high-pressure liquid chromatography (HPLC). Comedones, which are the pathognomonic feature of AV, consist of a wide array of substances such as sebum lipids, microorganisms, and epidermal cell debris, with high concentration of various forms of Vitamin A such as carotenes and retinoids, making it an ideal sample to measure Vitamin A levels [14].

To the best of our knowledge, even though Vitamin A and its derivatives have long been used for the treatment of AV, there are still limited data on the measurement of Vitamin A level itself especially using objective measurement tools such as ELISA on AV patients. Our study found that there was a significant increase of Vitamin A level of 23.03% after 30 days of tretinoin 0.025% application that directly corresponds to almost a 50% decrease in the number of both IL and comedones in patients with mild AV. A study by Ozuguz *et al.* found that there is a correlation between Vitamin A levels and severity of AV. In addition, patients with AV have a lower Vitamin A level compared to normal patients (0.49 ± 0.24 compared to 0.40 ± 0.26) [15]. Another recent study also supported the notion that lower levels of Vitamin A are correlated to the severity of AV, where when measure between mild, moderate, and severe AV, the mean levels of Vitamin A were 62.39 ± 12.91 , 54.81 ± 11.92 , and 51.78 ± 9.7 , respectively. Another recent study also supported the notion that lower levels of vitamin A is correlated to the severity of AV, where when measure between mild, moderate, and severe AV, the mean levels of vitamin A were 62.39 ± 12.91 , 54.81 ± 11.92 , and 51.78 ± 9.7 respectively, lower than the mean vitamin A level of 66.34 ± 17.66 in the control group [16]. This lower level of Vitamin A is suggested to be caused due to its increase use by the body to suppress the inflammation process that plays an integral role in the pathogenesis of AV. The low level of Vitamin A can then possibly aggravate AV, by increasing the activity as well as size of sebaceous glands that leads to an increase in *P. acne* colonization

and subsequent inflammation process. In addition, the low levels of Vitamin A are also associated with increased hyperkeratinization [7].

In our study, the increased levels of Vitamin A after topical tretinoin can be attributed to the absorption of Vitamin A and its derivatives by the stratum corneum, as they are a fat-soluble compound that to some extent can penetrate as deep as the dermis. In addition, there are also certain retinoid receptors on the skin such as the cytosolic retinoid binding protein that showed high affinity toward retinol [17]. The previous studies have evaluated Vitamin A levels in acne patients with normal control group and have compared the Vitamin A levels in mild and moderate acne. However, there is yet any study that directly compares the levels of Vitamin A in patients with AV before and after tretinoin treatment. Limitations in our study include the relatively low numbers of subjects and limitation to patients with only mild AV.

Conclusion

Increased level of Vitamin A is associated with significant clinical improvements in both inflamed and non-inflamed mild AV lesions. Further research is needed especially in moderate-severe AV.

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