



KEMENTERIAN PENDIDIKAN DAN KEBUDAYAAN
UNIVERSITAS HASANUDDIN FAKULTAS KEDOKTERAN
KOMITE ETIK PENELITIAN KESEHATAN
RSPTN UNIVERSITAS HASANUDDIN



RSUP Dr. WAHIDIN SUDIROHUSODO MAKASSAR
Sekretariat : Lantai 2 Gedung Laboratorium Terpadu
JL.PERINTIS KEMERDEKAAN KAMPUS TAMALANREA KM.10 MAKASSAR 90245.

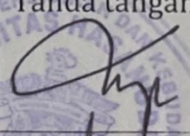

Contact Person: dr. Agussalim Bukhari.,M.Med.,Ph.D., SpGK TELP. 081241850858, 0411 5780103, Fax : 0411-581431

REKOMENDASI PERSETUJUAN ETIK

Nomor : 1142/UN4.6.4.5.31/ PP36/ 2019

Tanggal: 29 Nopember 2019

Dengan ini Menyatakan bahwa Protokol dan Dokumen yang Berhubungan Dengan Protokol berikut ini telah mendapatkan Persetujuan Etik :

No Protokol	UH19090685	No Sponsor Protokol	
Peneliti Utama	dr. Muhlis, SpKK, M.Kes	Sponsor	
Judul Peneliti	Efek Serum Autologous Terhadap Perubahan Manifestasi Klinik Penderita Akne Vulgaris		
No Versi Protokol	2	Tanggal Versi	13 Nopember 2019
No Versi PSP	2	Tanggal Versi	13 Nopember 2019
Tempat Penelitian	RSUP Dr Wahidin Sudirohusodo, RS Universitas Hasanuddin, RS Jejaring, BP Kulit dan Kelamin Veteran Makassar		
Jenis Review	<input type="checkbox"/> Exempted <input checked="" type="checkbox"/> Expedited <input type="checkbox"/> Fullboard Tanggal	Masa Berlaku 29 Nopember 2019 sampai 29 Nopember 2020	Frekuensi review lanjutan
Ketua Komisi Etik Penelitian Kesehatan FKUH	Nama Prof.Dr.dr. Suryani As'ad, M.Sc.,Sp.GK (K)	Tanda tangan 	
Sekretaris Komisi Etik Penelitian Kesehatan FKUH	Nama dr. Agussalim Bukhari, M.Med.,Ph.D.,Sp.GK (K)	Tanda tangan 	

Kewajiban Peneliti Utama:

- Menyerahkan Amandemen Protokol untuk persetujuan sebelum di implementasikan
- Menyerahkan Laporan SAE ke Komisi Etik dalam 24 Jam dan dilengkapi dalam 7 hari dan Laporan SUSAR dalam 72 Jam setelah Peneliti Utama menerima laporan
- Menyerahkan Laporan Kemajuan (progress report) setiap 6 bulan untuk penelitian resiko tinggi dan setiap setahun untuk penelitian resiko rendah
- Menyerahkan laporan akhir setelah Penelitian berakhir
- Melaporkan penyimpangan dari prokol yang disetujui (protocol deviation / violation)
- Mematuhi semua peraturan yang ditentukan



Effectiveness of topical autologous serum and 0.05% topical tretinoin cream in acne vulgaris: Analysis of Interleukin-17A and serum Vitamin A level

Muhlis Yunus ^{1*}, Muhammad Nasrum Massi ², Anis Irawan Anwar ¹,
Khairuddin Djawad ¹, Siswanto Wahab ¹, Rahmawati Minhajat ³, Uleng Bahrun ⁴,
Ilhamjaya Patellongi ⁵, Gemini Alam ⁶, Ni Nyoman Sri Budayanti ⁷

¹ Dermatology and Venereology Department, Faculty of Medicine, Hasanuddin University, Makassar, INDONESIA

² Clinical Microbiology Department, Faculty of Medicine, Hasanuddin University, Makassar, INDONESIA

³ Internal Medicine Department, Faculty of Medicine, Hasanuddin University, Makassar, INDONESIA

⁴ Clinical Pathology Department, Faculty of Medicine, Hasanuddin University, Makassar, INDONESIA

⁵ Physiology Department, Faculty of Medicine, Hasanuddin University, Makassar, INDONESIA

⁶ Pharmacy Department, Faculty of Pharmacy, Hasanuddin University, Makassar, INDONESIA

⁷ Clinical Microbiology Department, Faculty of Medicine, Udayana University, Denpasar, Bali, INDONESIA

*Corresponding author: muhlis.dv@gmail.com

Abstract

Introduction: Acne vulgaris is a chronic inflammatory disease of pilosebaceous unit caused by multifactorial which often occurs in adolescents and adults, yet it becomes a problem because it can cause scar acne due to acne vulgaris lesions, causing people to lack confidence. Treatment modality varies depend on the degree of acne vulgaris. In recent years, the study of blood serum use among dermatology field has begun because it has a unique component to be used as a therapy, including acne vulgaris therapy. This study aims to see the clinical changes in acne vulgaris by analyzing clinical lesion, lesional IL-17A, and serum vitamin A levels before and after the application of autologous serum and 0.05% tretinoin cream. **Material and Method:** This study was a pre and post treatment clinical trial involving 70 people with acne vulgaris who were divided into 2 groups, each with 35 people receiving 0.05% autologous serum and tretinoin cream application, respectively, for 28 days. **Result:** There was a difference in IL-17A level at the AV degree before and after autologous serum and 0.05% tretinoin cream application. Wilcoxon paired sample test indicated that there were differences in AV degrees before and after application of autologous serum or 0.05% topical tretinoin cream. **Conclusion:** Use of both autologous serum and 0.05% tretinoin cream improved acne vulgaris.

Keywords: autologous serum, tretinoin, IL-17A, serum vitamin A

Yunus M, Massi MN, Anwar AI, Djawad K, Wahab S, Minhajat R, Bahrun U, Patellongi I, Alam G, Sri Budayanti NN (2020) Effectiveness of topical autologous serum and 0.05% topical tretinoin cream in acne vulgaris: Analysis of Interleukin-17A and serum Vitamin A level. Eurasia J Biosci 14: 7585-7588.

© 2020 Yunus et al.

This is an open-access article distributed under the terms of the Creative Commons Attribution License.

INTRODUCTION

Acne vulgaris is a multifactorial chronic inflammatory disease of the pilosebaceous unit which often occurs in adolescents and adults. Inadequate treatment may lead to the development of acne scar with a negative impact on self-esteem and confidence level (Aydemir 2014). The pathogenesis of acne vulgaris is characterized by the involvement of androgens, follicular hyperkeratosis, increase in sebum excretion, and *Propionibacterium acnes* (*P. acnes*) colonization and inflammation (Kelh la et al. 2014; Bergler-Czop 2014). Th-17 cells are strong receptors in inflammatory tissues linked to a wide variety of inflammatory and autoimmune diseases.

Retinoids such as tretinoin target RXR (retinoid receptor X), the most common receptors for retinoid

signaling, which have been examined as a potential therapeutic target in several diseases including autoimmune and inflammation. Retinoids are considered the standard comedolytic agent used in the treatment of acne (Aydemir 2014; Tan et al. 2018; Anita et al. 2015).

In recent years, study of the use of blood serum in dermatology field has begun because it has a unique component to be used as a therapy, including acne vulgaris therapy.

Received: September 2019

Accepted: March 2020

Printed: December 2020

This study aimed to assess the effect of autologous serum and 0.05% tretinoin cream based on the clinical changes, IL-17A level, and vitamin A level.

METHODS

Study design

This randomized controlled clinical trial in patients with acne vulgaris took place from October 2019 to February 2020 in Department of Dermatology and Venereology, Universitas Hasanuddin, Makassar, South Sulawesi, Indonesia.

Participant

Subjects were equally divided into 2 groups with one group receiving autologous serum and another receiving 0.05% tretinoin cream; both treatments were applied for 28 days. Males and females aged 15-35 years old with no recent or current acne therapy and not pregnant (for women) were eligible to participate in this study.

Study protocol

A total of 50 cc blood from the median cubital vein was drawn into a tube without coagulant. The blood was centrifuged at a speed of 3000 rpm for 10-15 minutes and the resulting serum and blood clot were separated in separate containers. A total of 2 cc of the serum was used to examine vitamin A level and around 2 cc of serum was applied to acne lesions at night for 28 days. The serum was stored in the freezer of a household refrigerator. The other group was given 0.05% tretinoin cream which was applied on the acne lesions at night for 28 days. In this group, 10 cc venous blood drawn by the same method as the other group was centrifuged and examined for the vitamin A level. Both groups were instructed not to use facial wash, sunscreen, and any cosmetic products to avoid bias.

Measurement

The number of lesions (comedo, pustule, papule, and nodule) in each patient before and after treatment was assessed using zigzag method and by taking standardized clinical photography. The lesion count before and after treatment was then compared to assess for any clinical improvement.

The IL-17A and vitamin A levels were examined from the clinically most severe lesion. A 26G needle was used to puncture the lesion and the materials were taken using sterile cotton and put into a tube filled with distilled water. The IL-17A level was examined using ELISA (Enzyme-Linked Immunosorbent Assay) technique.

Statistical analysis

All statistical analyses were performed using SPSS version 22 (SPSS Inc., Chicago, IL, USA). Wilcoxon test was used to compare the IL-17A, vitamin A level, and lesion count before and after treatment in both groups. A p-value of <0.05 was considered as statistically significant.

RESULTS

A total of 70 people (20 males and 50 females) who fulfilled the inclusion criteria participated in this study.

Serum Vitamin A Level

No difference in serum vitamin A level between the topical autologous treatment groups before and after topical autologous serum and topical 0.05% tretinoin cream applications for 28 days.

IL-17A Level

A significant difference in lesional IL-17A level before and after application of autologous serum and 0.05% tretinoin cream ($P < 0.05$).

Acne Vulgaris Degree

The Wilcoxon paired sample test indicated that there were significant differences in lesion count before and after the application of autologous serum or topical 0.05% tretinoin cream from 30.73 ± 13.24 to 14.54 ± 9.00 and 16.70 ± 7.00 to 8.05 ± 6.00 , respectively ($p < 0.05$).

DISCUSSION

The degree of acne vulgaris severity in this study was assessed by quantitating the number of comedones, papules, pustules, and nodules in a zigzag manner from the front, right and left sides of face. Acne gradation is an important clinical feature to determine the type and evaluate treatment result. Although, there are many acne gradation assessment systems, to date, there is yet a universally accepted gold standard. There are many different methods of determining the acne severity, based on grading, lesion counting or photography, but they cannot be performed easily in daily practice (Zaenglein et al. 2016; Pugashetti and Shinkai 2013). In 1975 a classification system was created, Kligman and Plewig also created numerical grading (1975) and in 2008, Hayashi et al. in Japan created grading criteria for the severity by classifying the used number of acne assessment method by means of photography and counting inflammatory lesions on one side faces with photographs, and rated as low (0-5), moderate (6-20), severe (21-50), very severe (> 50) (Adityan et al. 2019; Hayashi et al. 2018).

Clinical manifestations changes of assessed acne vulgaris in this study were lesions in the form of blackheads, papules, pustules, cysts, and nodules when the first patient arrived and after received autologous serum or 0.05% tretinoin cream applications for 28 days every night. Then the researchers assessed occurring clinical manifestation changes, or even improving if the acne vulgaris lesions were reduced, or there was a change after received topical autologous serum or topical 0.05% tretinoin cream. It was compared before and after application.

The use of autologous serum for the treatment of acne vulgaris by Muhlis et al in 2016 indicated an

improvement in the clinical manifestation of acne vulgaris after the application for 28 days. In that study, patients' blood was drawn for checking after they were given a single dose of 100 mg of systemic doxycycline, then again the patients' blood was drawn for next 2 hours which was then centrifuged. However, that study did not give clarity whether the improvement in acne vulgaris lesions was induced by autologous serum only or because of 100 mg single dose doxycycline administration (Yunus et al. 2017). Nurhayati et al. in 2016 also conducted a study on the use of autologous serum in acne vulgaris sufferers indicated clinical improvement after 28 days application. In that study, patients' blood was drawn while they were in the preovulatory phase and then the blood was centrifuged. This study assessed estrogen level in the preovulatory phase because in that phase the estrogen level reached its peak. As it was known that estrogen inhibited androgens, thereby reducing sebum production, as a factor in the formation of acne lesions (Edison et al. 2016).

In the field of dermatology, as far as the researchers' knowledge based on reviewed articles in the last 3 years starting from 2017 – 2019, the use of autologous serum in the field of dermatology was in skin rejuvenation, wound healing and scar acne.

Back to the study, in the group that received 0.05% topical tretinoin cream application, there was an improvement in clinical manifestation when it was compared before and after application. Lawrence et al. 2019 used a new lotion formula of 0.05% tretinoin as an effective and well-tolerated topical treatment for moderate to severe comedonal acne vulgaris among adolescents after daily application for 12 weeks (Eichenfield et al. 2019; Draelos et al. 2019).

In addition, Mukherjee et al compared the concentration of tretinoin preparation of 0.025%, 0.05% and 0.1%. The incidence of side effect was significantly higher in 0.1% tretinoin group compared to 0.025% tretinoin group. Low strength tretinoin might become a good choice for patients who could not tolerate standard therapy by 0.05% (Mukherjee et al. 2006; Schmidt and Gans 2011; Chandrashekhar et al. 2015). The result indicated that, there was a level of vitamin A in the serum, indicating that there was no difference in serum of vitamin A level among the two treatment groups by using topical autologous serum or topical vitamin A cream. Serum of vitamin A level in the sample of this study varied from the lowest by 0.16 ug/dl and the highest by 0.72 ug/dl. In the field of dermatology, serum was used as a skin rejuvenation therapy, wound healing and scar repair by measuring growth factor (Thielitz et al. 2006; Du and Lei 2020).

There were differences in IL-17A level at the AV degree before and after topical autologous serum application for 28 days. Likewise, there were differences in the level of IL-17A at AV degrees before and after

topical 0.05% tretinoin cream application for 28 days. Vitamin A (all-trans retinoic acid) and vitamin D (1,25-dihydroxyvitamin D3) inhibited *P. acnes*-induced Th17 differentiation. Both suggested that IL-17 was induced by *P. acnes* and expressed in acne lesions, and that vitamin A and vitamin D became effective tools to modulate Th17-mediated diseases such as acne (Anitua et al. 2015; Hayashi et al. 2018; Mannello et al. 2008).

The study conducted by Hanna et al. demonstrated that as in psoriasis, the Th17 pathway was significantly regulated both RNA and protein levels in acne vulgaris lesions. The results suggested a new pathomechanism in inflamed acne and opened the possibility of new therapies targeting the Th17 system in severe acne. The characteristic of human Th17 cells was IL-17 expression, *P. acnes* induced expression of IL-17 gene (28-fold), and retinoid receptor expression 10-fold (Kelh  la et al. 2014; Sardana and Verma 2017).

Vitamin A (ATRA) and vitamin D (1,25D3) shared the retinoid X receptor (RXR) as a common receptor that encoded and inhibited Th17. All-trans retinoic acid (ATRA) and 1,25D3 were commonly used for therapy in dermatologists because of their ability to modulate immune responses (Iwata et al. 2003). Therefore, Sigmundsdottir et al., 2007 compared the role of ATRA and 1,25D3 in the Th17 generation induced by *P. acnes*. *P. acnes* stimulation in the absence of ATRA and 1,25D3 led to IL-17 mRNA expression and protein expression. The addition of ATRA, 1,25D3, or a combination of two prior on the activation with *P. acnes* downregulated IL-17 mRNA induction and protein expression. Our findings strongly suggested that ATRA and 1,25D3 effectively inhibited the required genes expression for the development and differentiation of *P. acnes*-related Th17 cells (Iwata et al. 2003; Sigmundsdottir et al. 2007).

In this study, both autologous serum and tretinoin 0.05% reduced IL-17 A level in acne vulgaris lesions, this was relevant with Kinda et al., 2014; Chang et al., 2010; Ikeda et al., 2010; Mucida et al., 2007 that ATRA and 1,25D3 inhibited IL-17 mRNA and protein expression as a response on the stimulation with *P. acnes* and IL-17 expression occurred in skin biopsy of acne patients (Mucida et al. 2007).

CONCLUSION

Interleukin-17A level decreases after topical autologous serum and topical 0.05% tretinoin cream application and the use of autologous serum and 0.05% tretinoin cream improved the lesions in acne vulgaris

What is already known on this topic

- IL-17A is involved in the pathogenesis of acne vulgaris
- Autologous serum is beneficial in skin rejuvenation, wound healing and treating scar acne.

What this study adds

- The administration of topical autologous serum resulted in acne vulgaris improvement
- The administration of autologous serum decreased lesional IL-17A level in patients with acne vulgaris.

ACKNOWLEDGEMENTS

The authors would like to thank Ivan Kurniadi, MD, for his kind assistance in layout and editing.

REFERENCES

- Aydemir EH. Acne vulgaris. *Turkish Archives of Pediatrics/Türk Pediatri Arşivi*. 2014;49(1):13.
- Kelhälä H-L, Palatsi R, Fyhrquist N, Lehtimäki S, Väyrynen JP, Kallioinen M, et al. IL-17/Th17 pathway is activated in acne lesions. *PLoS one*. 2014;9(8):e105238.
- Bergler-Czop B. The aetiopathogenesis of acne vulgaris—what's new? *International journal of cosmetic science*. 2014;36(3):187-94.
- Tan A, Schlosser BJ, Paller A. A review of diagnosis and treatment of acne in adult female patients. *International journal of women's dermatology*. 2018;4(2):56-71.
- Anitua E, Muruzabal F, Tayebba A, Riestra A, Perez VL, Merayo-Llodes J, et al. Autologous serum and plasma rich in growth factors in ophthalmology: preclinical and clinical studies. *Acta ophthalmologica*. 2015;93(8):e605-e14.
- Zaenglein AL, Pathy AL, Schlosser BJ, Alikhan A, Baldwin HE, Berson DS, et al. Guidelines of care for the management of acne vulgaris. *Journal of the American Academy of Dermatology*. 2016;74(5):945-73. e33.
- Pugashetti R, Shinkai K. Treatment of acne vulgaris in pregnant patients. *Dermatologic therapy*. 2013;26(4):302-11.
- Adityan B, Kumari R, Thappa DM. Scoring systems in acne vulgaris. *Indian Journal of Dermatology, Venereology, and Leprology*. 2009;75(3):323.
- Hayashi N, Kurokawa I, Siakpere O, Endo A, Hatanaka T, Yamada M, et al. Clindamycin phosphate 1.2%/benzoyl peroxide 3% fixed-dose combination gel versus topical combination therapy of adapalene 0.1% gel and clindamycin phosphate 1.2% gel in the treatment of acne vulgaris in Japanese patients: A multicenter, randomized, investigator-blind, parallel-group study. *The Journal of dermatology*. 2018;45(8):951-62.
- Yunus M, Anwar AI, Wahab S, Tabri F, Patellongi I, Widodo J. Effect of Doxycycline in Autologous Serum to Improvement Clinical Manifestation of Acne Vulgaris. *American Journal of Clinical and Experimental Medicine*. 2017;5(2):30-5.
- Edison N, Amin S, Djawad K, Satriono R, Widodo Y, Manggau MA. Effectivity of Preovulation Phase Autologous Serum Application on Moderate-Severe Acne Vulgaris Patients. *American Journal of Clinical and Experimental Medicine*. 2016;4(6):170-78.
- Eichenfield LF, Sugarman JL, Guenin E, Harris S, Bhatt V. Novel tretinoin 0.05% lotion for the once-daily treatment of moderate-to-severe acne vulgaris in a preadolescent population. *Pediatric dermatology*. 2019;36(2):193-9.
- Draeos ZD, Rheins LA, Wootten S, Kellar RS, Diller RB. Pilot study: Autologous platelet-rich plasma used in a topical cream for facial rejuvenation. *Journal of cosmetic dermatology*. 2019;18(5):1348-52.
- Mukherjee S, Date A, Patravale V, Korting HC, Roeder A, Weindl G. Retinoids in the treatment of skin aging: an overview of clinical efficacy and safety. *Clinical interventions in aging*. 2006;1(4):327.
- Schmidt N, Gans EH. Clindamycin 1.2% tretinoin 0.025% gel versus clindamycin gel treatment in acne patients: A Focus on Fitzpatrick skin types. *The Journal of clinical and aesthetic dermatology*. 2011;4(6):31.
- Chandrashekar B, Anitha M, Ruparelia M, Vaidya P, Aamir R, Shah S, et al. Tretinoin nanogel 0.025% versus conventional gel 0.025% in patients with acne vulgaris: A randomized, active controlled, multicentre, parallel group, phase iv clinical trial. *Journal of clinical and diagnostic research: JCDR*. 2015;9(1):WC04.
- Thielitz A, Krauthem A, Gollnick H. Update in retinoid therapy of acne. *Dermatologic therapy*. 2006;19(5):272-9.
18. Du R, Lei T. Effects of autologous platelet-rich plasma injections on facial skin rejuvenation. *Experimental and therapeutic medicine*. 2020;19(4):3024-30.
- Agak GW, Qin M, Nobe J, Kim M-H, Krutzik SR, Tristan GR, et al. Propionibacterium acnes induces an IL-17 response in acne vulgaris that is regulated by vitamin A and vitamin D. *Journal of Investigative Dermatology*. 2014;134(2):366-73.
- Mannello F, Tanus-Santos JE, Meschiari CA, Tonti GA. Differences in both matrix metalloproteinase 9 concentration and zymographic profile between plasma and serum with clot activators are due to the presence of amorphous silica or silicate salts in blood collection devices. *Analytical biochemistry*. 2008;374(1):56-63.
- Sardana K, Verma G. Propionibacterium acnes and the Th1/Th17 axis, implications in acne pathogenesis and treatment. *Indian Journal of Dermatology*. 2017;62(4):392.
- Iwata M, Eshima Y, Kagechika H. Retinoic acids exert direct effects on T cells to suppress Th1 development and enhance Th2 development via retinoic acid receptors. *International immunology*. 2003;15(8):1017-25.
- Sigmundsdottir H, Pan J, Debes GF, Alt C, Habtezion A, Soler D, et al. DCs metabolize sunlight-induced vitamin D3 to program T cell attraction to the epidermal chemokine CCL27. *Nature immunology*. 2007;8(3):285-93.
- Mucida D, Park Y, Kim G, Turovskaya O, Scott I, Kronenberg M, et al. Reciprocal TH17 and regulatory T cell differentiation mediated by retinoic acid. *science*. 2007;317(5835):256-60.