The Difference of Serum Vitamin E Levels between Adolescent Patients with and without Acne Vulgaris

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ABSTRACT

Background: Acne vulgaris (AV) is a chronic inflammatory disease of the pilosebaceous unit, particularly among adolescents. The pathogenesis of AV is multifactorial, developing research studies the role of free radicals and antioxidants imbalance that cause oxidative stress in AV. The main antioxidant found in the skin is vitamin E, which functions as a protector against lipid peroxide. **Purpose**: To compare serum vitamin E levels in adolescents with AV and healthy adolescents without AV (controls). **Methods**: This is a cross-sectional observational analytic study that involved 17 adolescents with AV and 17 controls in Dermatology and Venereology Outpatient Clinic Dr. Soetomo General Academic Hospital Surabaya. The subjects have met the inclusion and exclusion criterias. **Result**: The mean of vitamin E level in adolescent patients with AV was 7.8 ± 1.07 mg/mL and 10 ± 1.06 mg/mL in controls with the p-value in this study was p = 0.0001. **Conclusion**: It was found that serum vitamin E levels in adolescent AV patients were significantly lower than the controls. Further research is required to find out more about the role of antioxidants in the pathogenesis of AV.

Key words: acne vulgaris, adolescent, vitamin E, antioxidant.

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INTRODUCTION

Acne vulgaris (AV) is a chronic inflammatory disease of the pilosebaceous unit, particularly among adolescents. Most cases of AV are accompanied by a pleomorphic variation of the lesion, such as comedones, papules, pustules, and nodules.[1] Acne vulgaris affects 80% of people at various ages between 11-30 years, prevalence in adolescents aged 10-19 years according to World Health Organization (WHO) ranges from 35-90%. Acne vulgaris occurs from infants to adults with a peak age of 16-19 years in males adolescent or 14 -17 years in female adolescents. As much as 67.2% AV cases in the Dermatology and Venereology Outpatient Clinic Dr. Soetomo General Academic Hospital Surabaya in 2009 were adolescents with a female to male ratio of 3:1. Acne vulgaris is one of the top 10 most common diseases in the Dermatology and Venereology Outpatient Clinic Dr. Soetomo General Academic Hospital Surabaya, with the prevalence of cases in the year of 2015, 2016 and 2017, respectively at 14.54%, 13.14%, and 9.54%; and respectively 29.2%, 35.4% and 37.9% of the cases were adolescents. Acne vulgaris can last for years and cause permanent disability and scarring which can effect on psychosocial development.[2,3]

Acne vulgaris is a common dermatological condition characterized by excess production of sebum due to hormones, follicular hyperkeratinization, and chronic inflammation in the pilosebaceous unit.[4,5] Acne vulgaris in puberty occurs because of an increase in androgen hormones. Increasing androgen hormones will cause an increase in the size of the sebaceous glands that stimulates sebum production. Sebum clogging in the hair follicles will trigger the formation of comedones and provide a growth medium for *Propionibacterium acnes (P.acnes)*.

Research on the etiopathogenesis of AV especially the role of free radicals and antioxidants is currently being developed. The skin is constantly exposed to oxidative stress induced by reactive oxygen species (ROS) produced both from endogenous sources (oxygen metabolism) and external pro-oxidants (radiation exposure, air pollution, oxygen intoxication, cigarettes, and alcohol). Reactive oxygen species mediates oxidative stress through interactions of free radicals with cellular molecules such as lipids, carbohydrates, proteins, and nucleic acids which then cause inflammatory reactions. Over the past few years, it has been known that patients with AV experience increasing oxidative stress both systemically and cutaneously.[6,7] A research conducted by Hani et al. in

2013 about biochemical markers of oxidative and nitrosative stress in AV found the role of biochemical markers of protein oxidation, lipid peroxide, or nitrosative stress on the development of AV.[8]

To deal with the harmful effects of ROS, skin is equipped with antioxidant defense mechanisms to prevent the formation of ROS in the form of enzymatic antioxidants and non-enzymatic antioxidants.[9] Among these antioxidants, α-tocopherol is the main form of vitamin E and an important fat-soluble antioxidant. In AV, low level of vitamin E can cause the formation of oxidative stress in the pilosebaceous unit, giving rise to an ideal micro-aerophilic environment that will increase the colonization of *P. acnes*. In addition, α-tocopherol is a fat-soluble vitamin that works to break the chains during the lipid peroxidation process. Between the skin surface lipids, there is squalene, a triterpenoid molecule specific to human sebum. Its oxidation produces squalene peroxide which is proven to be comedogenic and increases sebum production. The presence of vitamin E in the skin plays a role in limiting the potentially harmful effects of squalene peroxide. In addition to releasing inflammatory mediators, squalene peroxide can also be comedogenic.[10-12]

El-Akawi et al. in 2006 conducted plasma vitamin E level examination in 100 patients with severe, moderate, and mild AV and 100 control subjects. The severity of AV is determined based on the Global Acne Grading System (GAGS). The results showed that vitamin E levels were significantly lower in patients with severe AV compared with moderate AV, mild AV, and controls.[13] Ozuguz et al. in 2013 conducted studies on vitamin A and E levels examination in 94 patients with mild, moderate, and severe AV compared with 56 controls who did not suffer from AV. The study found that vitamin E levels were significantly lower in patients with AV than in controls.[14]

This study aims to compare the serum vitamin E levels in adolescent AV patients with adolescent without AV in the Dermatology and Venereology Outpatient Clinic Dr. Soetomo General Academic Hospital Surabaya. It is expected that this research can provide scientific contributions and can be used as a basis for further research on serum vitamin E levels in adolescent patients with AV.

METHODS

This is a cross-sectional observational analytic study that aims to find out serum vitamin E levels in adolescents with AV and adolescents without AV. This study was conducted by consecutive sampling from November 2018 until January 2019. The samples were adolescent patients with AV and adolescents without AV who visited the Medical Cosmetic Division of Dermatology and Venereology Outpatient Clinic Dr. Soetomo General Academic Hospital Surabaya; and met the inclusion and exclusion criterias. The inclusion criterias of samples were adolescent patients aged 10-19 years with a diagnosis of AV, not taking drugs containing vitamin E 3 days before examination, and willing to sign the informed consent. The inclusion criterias for the control group were healthy adolescent patients, no history of AV, no history of AV exacerbations, no history of AV scars at the time of the inspection, and willing to participate and to sign the informed consent. The exclusion criterias of samples were patients with severe systemic disease (cardiovascular disease, asthma, psoriasis vulgaris, diabetes mellitus, malignancy, liver dysfunction), Human Immunodefficiency Virus-related immunodeficiency, autoimmune diseases (systemic lupus erythematosus, rheumatoid arthritis), chronic diseases (tuberculosis infection), and polycystic ovary syndrome based on history and clinical examination, pregnant and lactating women, have a history of smoking and drinking alcohol. All research samples underwent historytaking, physical examination, and examination of serum vitamin E level using the High-Performance Liquid Chromatography (HPLC) method. The data were collected and arranged in tables and graphs.

RESULT

The number of male subjects in AV group and control group were more than female subjects. In the AV group, there were 13 male subjects (76.5%) and 4 female subjects (23.5%). In the control group, there were 11 male subjects (64.7%) and 6 female subjects (35.3%). There were 34 subjects with median age 17 years old (15-19 years old) in the AV group and 18 years old (16-19 years old) in the control group (Table 1).

Fifteen subjects in the AV group and 17 subjects in the control group were unemployed. In the AV group, there were 6 high schools graduates, 1 junior high school graduate, and 10 vocasional school graduates. In the control group, there were 2 high school graduates and 15 vocational school graduates. All subjects (100%) were unmarried. Most subjects have AV onset for 2 years (41.2%). No comorbidities observed in all subjects (100%). There were 11 subjects (64.7%) that confirmed they have a family member with AV history, and 6 subjects (35.3%) claimed that they do not have a family member with AV history. Twelve subjects (70.6%) in the AV group

claimed that the AV was stress triggered. Eleven subjects (64.7%) in the AV group had never received any forms of therapy, either oral or topical antibiotics, soap for acne, sunscreen, and other topical therapies. All subjects (100%) were found often consume steamed rice, 9 subjects in the AV group (52.9%) often consume fried chicken, and 8 subjects (47.1%) in the control group often consume fresh spinach. The basic data of this research subjects can be seen in Table 2.

Table 1. Sosiodemography characteristic of age and sex of the subjects

Variabel	Acne vulgaris	Control	p Value
Sex n(%)			
Male	13 (76.5)	11 (64.7)	0.707
Female	4 (23.5)	6 (35.3)	
Age			
Median	17	19	0.013
Range Age	15-19	16-19	

Table 2. Basic data of research subjects

Variables	Acne Vulgaris, n= 17 (%)	Control, n= 17 (%)
Age	17 (15-19 years old)	18 (16-19 years old)
Sex		
Male	13 (76.5)	11 (64.7)
Female	4 (23.5)	6 (35.3)
Formal Education		
Middle School	1 (5.8)	0 (0)
High School	6 (35.3)	2 (11.7)
Vocational School	10 (58.8)	15 (88.2)
Employment Status		
Employed	2 (11.8)	0 (0)
Unemployed	15 (88.2)	17 (100)
Marital Status		
Married	0 (0)	0 (0)
Not Married	17 (100)	17 (100)
Onset (years)		
1	5 (29.4)	0 (0)
2	7 (41.2)	0 (0)
3	4 (23.5)	0 (0)
4	1 (5.9)	0 (0)
Comorbidity		
Yes	0 (0)	0 (0)
No	17 (100)	0 (0)
Family History		
Yes	11 (64.7)	0 (0)
No	6 (35.3)	0 (0)
Stress		
Yes	12 (70.6)	0 (0)
No	5 (29.4)	0 (0)
Therapy		
Yes	6 (35.3)	0 (0)
No	11 (64.7)	0 (0)
Consumed foods with Vitamin E		
Steamed Rice	17 (100)	17 (100)
Fried Chicken	9 (52.9)	4 (23.5)
Fresh Spinach	4 (23.5)	8 (47.1)

The results of serum vitamin E levels in adolescent patients ranged from 7.82 ± 1.07 mg/L in the AV group and 10 ± 1.06 mg/L in the control group, p = 0.0001 (p <0.05). Serum vitamin E levels are shown in Table 3 and Figure 1.

Table 3. Serum vitamin E levels in adolescent patients with acne vulgaris and controls

Description	AV, n = 17	Control, n = 17	p Value
Mean	7.82	10.00	0.0001
Standard Deviation	1.07	1.06	
Minimum	6.00	8.00	
Maximum	10.00	12.00	

AV = Acne vulgaris

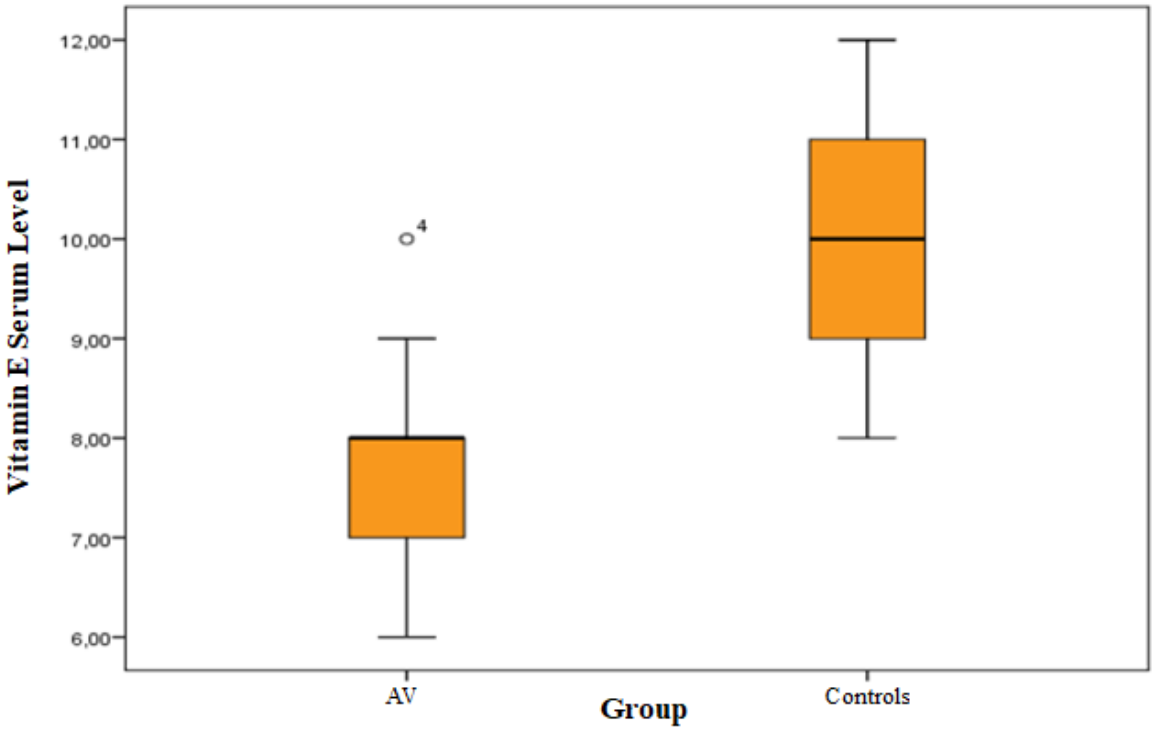

Figure 1. Boxplot graph of vitamin E levels in adolescent patients with Acne vulgaris and controls.

DISCUSSION

In this study, 13 subjects (76.5%) were male. This data showed that the number of male AV patients is more than female AV patients. Acne vulgaris can affect both men and women. However, several studies found that AV is more common in male adolescents. Yahya in 2009 conducted a study at a school in Kaduna, Nigeria, reported that there were 379 students with AV, and 198 (52.2%) of them were male students.[15] There is research that claimed AV in females emerges sooner, in more severe forms compared to AV cases in males during puberty. Acne vulgaris is found more in male adolescents, but more severe form of AV is more common in males during puberty, and AV in male adolescents is one of the most visible markers when there is an excess of androgens.[16,17]

This study involved 34 AV subjects with an age range of 10-19 years. The age range was determined by WHO definition on adolescent, which is 10-19 years old. The average age in the AV group was 17 years old (15-19) and 18 years old (16-19) in the control group. This was concordance with a research of Layton AM and Mawson RL, confirming the age range of AV is 15-20 years old.[18]

There were 15 unemployed subjects (88.2%) in the AV group and 17 unemployed subjects (100%) in the control group. There were only 2 employed subjects (11.8%) in the AV group. All subjects, were unmarried. Acne vulgaris is a multifactorial disease with external factors such as stress on working environment.[19]

The onset of AV varies in each age group, more common in puberty, and can continue into young

adulthood. The results of this study were in line with the research conducted in Saudi Arabia by Darwish in 2013. In his study, 37.8% of the total sample visited the doctor within 1 year after the symptoms appear. Poli et al. in 2011 discovered the same result; the average AV patients who seek treatment had suffered from AV for more than 12 months (49.6%). A study conducted by Al Robaee in 2005 on 717 patients found that 40.3% of patients had been suffered from AV for 3 months. Tallab's study in 2004 found that 76.2% of patients had suffered more than one year from AV.[20,21]

Most subjects, which were 11 subjects (64.7%), had a family history of AV. This is in accordance with the research conducted by Ebede et al. in 2009 involving 200 individuals with AV who stated if they had a family member suffering from AV.[22] A research by Mariana in 2016 also found supporting results. There were more patients with a family history of AV than without AV. Bagatin et al. found that about half of the adolescent population reported there was father or mother with AV.[23] A study conducted by Di Landro in 2009 found that AV was influenced by family history, and the prevalence of AV was higher in maternal family history compared to paternal or another family history. The more family members with AV history, the higher chance that other family member to suffer from AV. Genetic influence on AV has been reported in twin studies and several genealogical studies, but only a few of them described the relationship between the two.[24] Research by Sobjanek suggested that there was a relationship between AV and Human leucocyte antigen (HLA), CYPA1, CYP17, CYP21, MUCI, AR, and MCR-5 genes, with further explanation required.[25]

Stress plays a role in causing AV. There were 12 subjects (70.6%) who claimed that stress was the triggering factor of AV. This was accordant with Purwanidyah's study in 2013 on profiles of AV patients in Medan. Stress was reported as a psychological factor that caused AV in 90% of subjects.[26] Psychological stress can exacerbate AV. This was consistent with research conducted by Chiu et al. in 2003 on AV severity in students during exam.[27] Similar results were obtained by Halvorsen et al. in 2009. The research found that AV become worse before and during exams, which was triggered by increased stress experienced by the students.[28] Elsaie et al. mentioned that corticotropin-releasing hormone (CRH), also known as the stress hormone and CRH-R receptors have been found in sebaceous glands. Corticotropin-releasing hormone directly induces lipid production and increases the conversion of dehydroepiandrosterones (DHEAS) to testosterone in sebocytes. This process is suspected of playing an important role in the relationship between stress and sebum production. The sebaceous gland also has receptors for substance P, which is a neuromediator that is released in response to stress. In vitro, substance P stimulates sebaceous secretion. It is postulated that substance P plays a role in AV as a response to stress.[29]

Steamed rice was the consumed food in all with total 34 subjects (100%), followed by fried chicken in 9 subjects (52.9%) in AV group, and fresh spinach in 8 subjects (47.1%) in the control group. White rice is widely consumed because it is a staple food in Indonesia. The vitamin E content in chickens is 0.86 mg lower than the vitamin E content in fresh spinach which is 2.67 mg, it is supported by several observational studies that have been conducted to assess the relationship between diet and acne.[30,31] A cross-sectional study by Cordian et al. in 2002 (level of evidence 2) observed two non-westernized populations in the Kitavan islands in Papua New Guinea (n = 1200) and acne hunter-gatherers in Paraguay (n = 112) who consumed a low-fat diet and low glycemic index. In both groups, there was no AV observed.[32] Cross-sectional study by Ikaraoha et al. in 2005 (level of evidence 2) examined 174 Nigerian students, and 75% of participants believed that oil and fat affected acne.[33]

There are at least 4 important factors that play a role in the pathogenesis of AV, namely follicular epidermal hyperproliferation, increased sebum production, *P. acnes*, and inflammatory reactions. Microbes, genetic, and various environmental factors are also associated with the pathogenesis of AV.[1,4,19] Over the past few years, AV patients experience increasing oxidative stress both systemically and cutaneously. Oxidative stress is an imbalance condition where there are more oxidants than antioxidants. Reactive oxygen species mediates oxidative stress through interactions of free radicals with cellular molecules such as lipids, carbohydrates, proteins, and nucleic acids. Of all these components, lipids are the most sensitive, in which polyunsaturated fatty acids in cell membranes react with ROS to form peroxidation products.

To resolve the harmful effects of ROS, the skin is equipped with antioxidant defense mechanisms in the form of enzymatic antioxidants such as glutathione peroxidase (GSH-Px), catalase (CAT), superoxide dismutase (SOD), and non-enzymatic antioxidants. Non-enzymatic antioxidants found in cells are α-tocopherol, ubiquinone, β-carotene, ascorbate, and glutathione. Among these antioxidants, α-tocopherol and β-carotene are concentrated in the

cell membrane, in vivo functioning as a protector against lipid peroxide. Inadequate antioxidant protection or increasing production of ROS creates a condition called oxidative stress, which contributes to the emergence of inflammatory skin diseases. Oxidative stress can be found in acne and can play a role in its pathogenesis.[34] Research conducted in 2013 by Hani et al. on biochemical markers of oxidative and nitrosative stress in AV found the role of biochemical markers of protein oxidation, lipid peroxide, or nitrosative stress on the development of AV.[8] Previous research also supports the effect of ROS in the etiopathogenesis of acne. Decreasing activity of SOD and GSH-Px enzymes, in which these enzymes are responsible for the defense of antioxidative reactions, were found in papulopustular acne. The finding was in accordance with the role of ROS in acne.[35]

Vitamin E is the leading lipophilic antioxidant found in plasma, membranes, and tissues. It helps to protect important cell structures, especially cell membranes, from damage caused by free radicals. Antioxidants that can donate hydrogen atoms are called hydrogen donors. Vitamin E is located in membranes and lipoproteins, as a hydrogen donor, that can stop the radical chain reaction of lipid peroxidase. Therefore, vitamin E is called as a chain-breaking antioxidant. Vitamin E (TocH) always donates hydrogen atoms to peroxyl lipid radicals to propagate the chain reaction of lipid peroxide.[34,36]

Low vitamin E levels can lead to the formation of oxidative stress in the pilosebaceous unit, creating an ideal micro-aerophilic environment that will increase the colonization and the proliferation of *P. acnes*. *Propionibacterium acnes* has an important role in the inflammatory process of AV by producing chemotactic factors for neutrophils. Neutrophils produce free radicals in the form of superoxide anion radicals, hydrogen peroxide, and hydroxyl radicals, which will cause cell damage and worsen the inflammatory process. Inadequate antioxidant protection and increasing production of ROS produce a condition called oxidative stress, which contributes to the emergence of inflammatory skin diseases. Vitamin E is an important fat-soluble antioxidant. Low vitamin E levels will also increase ROS production that will increase oxidative stress and inflammatory processes which are one of the pathogenesis of AV.[34,36] In addition, there is squalene between skin surface lipids, a triterpenoid molecule specific to human sebum, that bind with singlet oxygen, protecting the skin from lipid peroxidase, which sometimes produces oxidation of squalene peroxide that is proven to be comedogenic. The supply of vitamin E to the skin plays a role in limiting squalene peroxide.[34,36]

The mean serum vitamin E levels obtained were 7.8 mg/L in the AV group and 10 mg/L in the control group. The normal level of serum Vitamin E is 3-14 mg/L, therefore, it appeared that vitamin E levels in AV patients were within normal limits. The statistical test used was the t-test, a comparative study to assess the difference between certain values with the average of the group population. The test results showed that there was a significant difference in serum vitamin E levels between the AV and control group with lower scores on AV group.

The results were in accordance with the study of El-Akawi et al. in 2006 on vitamin E levels in plasma involving 100 subjects with AV and 100 control subjects. The severity of AV is determined based on the GAGS. The research results showed that the vitamin E levels in patients with severe AV were significantly lower than moderate AV, mild AV, and control subjects. The vitamin E levels in mild and moderate AV were lower than control subjects but still within normal limits. This might be due to the vitamin E accumulation in cellular membranes, preventing serum vitamin E levels to decrease rapidly. Insufficient of fruits and vegetable diet causes low vitamin E levels, creating depletion of antioxidants to combat ROS or reactive oxygen radicals produced in the inflammatory phase and promoting the lipid peroxidase process in the patient's skin.[13] Abulnaja conducted a study in 2009 on oxidant/antioxidant status in obese adult women with AV. The research showed that vitamin E levels significantly lower in non-obese subjects. It might be caused by psychological stress, such as depression and anxiety. Vitamin E contributes to preventive activities against the formation of free radicals such as singlet oxygen, superoxide, and hydroxyl radicals from the formation of AV.[37] Ozuguz et al. conducted a study comparing vitamin A and vitamin E levels in 94 subjects with mild, moderate, and severe AV with 56 control subjects without AV. It was found that the vitamin E levels of AV subjects were significantly lower than control subjects, yet still under the normal limit than those without AV. It might be due to a lack of vitamin E intake in AV patients. It was also found that the AV patients consumed more fast foods, increasing vitamin E requirement as the body also needs vitamin E for fat metabolism.[14]

Karen E, et al. in 2000 conducted a study on mice with normal vitamin E levels and exposed the mice to ultraviolet B (UVB) to cause acute chronic damage to the skin (such as pigmentation, burns, and even skin cancer). Vitamin E concentration levels

were measured in the skin, liver, and fat tissues. Afterward, topical and oral vitamin E were given. Vitamin E concentration in the skin, liver, and fat tissues increased, and significant improvements from UV-induced skin damage such as inflammatory lesions and pigmentation were observed.[38]

Based on research conducted in 17 adolescent patients with AV and 17 adolescent without AV who came to Dermatology and Venereology Outpatient Clinic Dr. Soetomo General Academic Hospital Surabaya could be concluded that the average level of vitamin E serum in adolescent patients with AV is 7.8 mg/L, and the average of vitamin E serum level in control subjects is 10 mg/L and vitamin E serum level in adolescent with AV is lower than control subjects. The results of this study are expected to give contributions to further research in determining the role of vitamin E in the AV pathogenesis and understanding the effectiveness of vitamin E for AV treatment.

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