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RELATIONSHIP BETWEEN SERUM SUBSTANCE P LEVELS AND ACNE VULGARIS

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ABSTRACT

This investigation aimed to see if serum substance P levels and acne vulgaris were related. This study applies the case-control method to an observational analytical investigation. Samples were collected at KSM Dermatology's Cosmetic Division Polyclinic. Blood samples were evaluated at the UI Faculty of Medicine, Integrated Laboratory, and venereology at UI Hospital, Medan. Each group in this study included a minimum of 18 samples. The study indicates a relationship between serum substance P levels and acne vulgaris. Acne vulgaris is 5.4 times more likely to occur in people with high serum substance P levels. greatest in males. The average serum substance P level in acne vulgaris is 62.6 ± 32.4 pg/ml, and the highest levels can be found in the age group of 28 to 32 years. The average serum substance P level of acne vulgaris based on gender is highest in men.

Keyword : Cosmetics, Substance Level, P Serum, Acne Vulgaris

INTRODUCTION

Inflammation of the skin, known as acne vulgaris, frequently affects the pilosebaceous unit, particularly in teenagers. Comedones, papules, pustules, nodules, and cysts are examples of polymorphic lesions found in acne vulgaris predilection locations (Goh et al., 2019; Wasitaatmadja, 2018; Kraft, 2011; Dawson AL and Dellavale, 2013). Acne vulgaris, one of the three most common skin conditions, affects 85% of those between 12 and 25 (Ghodsi et al., 2009). Ninety per cent of teenagers suffer from acne vulgaris, and over fifty per cent of those cases persist throughout adulthood (Kraft, 2011). As per the Indonesian Cosmetic Dermatology Study Group (KSDKI) (2015), acne vulgaris ranks as the third most prevalent ailment in dermatology clinics and hospitals. A study conducted by Anggrenni et al. (2014) in Medan found that the proportion of acne vulgaris cases at the H. Adam Malik Medan General Hospital (RSUP) was 1.10% of 2,138 visits from January 2010 to December 2012 (Anggrenni et al., 2014). According to Fachry et al. (2014), the population most often experiencing acne vulgaris is those under 21 (53.8%).

The aetiology of acne vulgaris consists of four elements. The colonisation of Cutibacterium acnes (C. acnes), a bacterium formerly known as Propionibacterium acnes, increased sebum production, and inflammatory processes are these (Zaenglein and Thiboutot, 2018; Dreno et al., 2019). Genetic factors, race, stress, diet, cosmetics, medications, pregnancy, menstrual cycle, alcohol, and smoking habits are some of the causes of acne vulgaris (Layton et al., 2016). One of the factors that cause acne vulgaris is stress. The production of hormones, cytokines, and neuropeptides is affected by stress, which causes the disease to persist and exacerbation (Rokowska-Waluch et al., 2016). The aetiology of acne vulgaris consists of four elements. The colonisation of Cutibacterium acnes (C. acnes), a bacterium formerly known as Propionibacterium acnes, increased sebum production and inflammatory processes (Zaenglein and Thiboutot, 2018; Dreno et al., 2019).

The undecapeptide substance P is formed from the amino acid sequence Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH2. Poids that share structural similarities

with "tachykinins" include neurokinin A, B, neuropeptide K, and neuropeptide γ (Suvas, 2017; Koh and Bhatia, 2011). Stress causes substance P to be released by peripheral nerves. Substance P may cause the differentiation and proliferation of sebum glands as well as boost lipid production in sebocytes, as evidenced by its capacity to induce the proliferation of sebaceous precursor cells and increase the size of sebocytes (Zari and Alrahmani, 2017). Using the investigator's global assessment scale (IGA), the severity of acne vulgaris was evaluated. In addition, the level of stress was measured using Holmes and Rahe's social adaption change scale (SRRS). Using enzyme-linked immunosorbent assay (ELISA), the serum concentration of substance P was assessed. In this study, acne vulgaris patients showed higher serum concentrations of substance P than controls.

The idea that neurogenic factors contribute to the aetiology of acne vulgaris was examined by Toyoda et al. (2002). This study used immunohistochemistry to compare the expression of neutral endopeptidase in sebaceous acini and the distribution of substance P nerve fibres around sebaceous glands in the facial skin of acne vulgaris sufferers and control people. Neutral endopeptidase expression in sebaceous acini and increased substance P immunoreactive nerve fibres around sebaceous glands were seen in acne vulgaris patients. Acne vulgaris is linked to substance P, as demonstrated above. Research on this topic is relatively scarce, though. This led scientists to assess substance P's role and correlation with acne vulgaris.

RESEARCH MATERIALS AND METHODS

This research employs a case-control methodology and is an observational analytical study. Samples were collected at KSM Dermatology's Cosmetic Division Polyclinic. Blood samples were evaluated at the UI Faculty of Medicine, Integrated Laboratory, and Venereology at UI Hospital, Medan. At the University of North Sumatra Hospital's Cosmetic Division Polyclinic of KSM Dermatology and Venereology in Medan, patients with acne vulgaris were treated as research subjects. Each group in this study included a minimum of 18 samples.

Material

The tools used include research status, a tourniquet, and a camera for documentation. All materials used include aprotinin, test tubes for standard and sample dilutions, vacutainers (sterile blood collection tubes), wound plasters, cotton, alcohol swabs, blood samples, one unit of substance P kit, microplate reader, pipettes, pipette tips, deionized or distilled water, spray bottles, manifold dispensers or automatic microplate dryers, 500-milliliter measuring cylinders, horizontal orbital microplate shakers, and Kontron.

Ways of working

Researchers recorded primary data such as the identity of the research subjects (age, gender, and address), anamnesis, physical examination, dermatological examination, and severity of acne vulgaris, according to Lehmann. Acne vulgaris is assessed by counting the number of lesions on blackheads, pimples, and cysts. There are three levels of acne vulgaris severity: mild, moderate, and severe. Continued by tracking and recording the results of the examination. Researchers and supervisors at the Dermatology and Venereology Polyclinic of the University of North Sumatra Hospital determined the clinical diagnosis of acne vulgaris.

Blood sample collection and storage

The patient completed an informed consent form before any blood was drawn. Survey respondents were invited to either sit or lie down. After applying the tourniquet, patients were instructed to clench their fists to view the veins in their upper arms. Using

gloves, the area near the puncture was cleansed with an alcohol swab before drying. The median cubital vein was then pierced at a 45-degree angle using the needle. The needle was turned facedown after the blood had entered it. The patient was asked to open his fist to allow the blood to circulate freely. The syringe was gradually withdrawn until the required blood volume was obtained after the toriquet was removed. The venous blood was then transferred to a vacutainer tube. Before centrifuging serum samples in a serum separator tube (SST) for fifteen minutes at 1000 rpm, let the sample clot for thirty minutes at room temperature. After that, take the serum out and either conduct the test immediately or store it below -20°C.

Blood sample processing

Give samples, reagents, and operational guidelines. The extra microplate strip is removed from the plate frame and rebuilt within the foil bag with the pack desiccant. Next, the non-specifically bound (NSB) well is filled with 100 µL of Calibrator Diluent RD5-45. Next, 50 microlitres of Calibrator Diluent RD5-45 are introduced into the B0 zero standard well. Next, fill the remaining wells with 50 µL of the sample, standard, or control. The layout board has a record of the standards and tested samples. Each well, except the NSB well, receives 50 µL of the Primary Antibody Solution. 50 millilitres of substance P conjugate will be added to each well, except the NSB well, and each well will turn blue. Put an adhesive strip over it. Using a 0.12" horizontal orbiting microplate shaker, the samples were incubated at room temperature at 500 rpm (plus or minus 50 rpm) for three hours.

After that, the process continues with aspiration and washing of each well, repeated three times, reaching four washes. Fill each well with 400 µL of Wash Buffer to wash the sample using an autowasher, manifold dispenser, or spray bottle. The liquid should be dumped at each phase for best results. Please aspirate or dump off any leftover desiccant following the last wash. Using a fresh paper towel, wipe the plate clean. Next, 200 µL of the substrate solution is added to each well. The sample is left on the bench at room temperature for half an hour while it is shielded from light. Next, each well received 50 millilitres of Stop Solution. The well's colour needs to shift from blue to yellow. Tap the plate lightly if the colour of the well seems green or if the colour change is inconsistent.

Using a microplate reader set at 450 nm, the optical density of each well is found in less than 30 minutes. If wavelength adjustment is available, set it to 540 or 570 nm. If not, lower the reading to 450 nm from 540 or 570 nm. This reduction makes up for the plate's optical flaws. Direct measurements at 450 nm could be higher and more accurate without any adjustments.

Data Processing and Analysis

The collected data were analyzed statistically. Data processing began with univariate analysis, which assesses the characteristics of one variable through descriptive tests. Then, bivariate analysis was used to see how the study variables interact; in this case, the Chi-square test was used to determine the relationship between serum substance P levels and acne vulgaris.

RESULTS AND DISCUSSION

In this investigation, thirty individuals with acne vulgaris and thirty more patients with the same diagnosis were evaluated for substance P. At the Cosmetic Division Polyclinic of KSM Dermatology and Venereology, University of North Sumatra

Hospital, Medan, the supervisor and patients had a physical examination and an amnesia to confirm the diagnosis of acne vulgaris. Additionally, blood samples were tested at the University of North Sumatra's Integrated Laboratory of the Faculty of Medicine.

Characteristics based on age

Acne vulgaris is a chronic inflammation of the pilosebaceous unit commonly found in ages 12 to 25 years, with a prevalence of 85% (Goh et al., 2019; Wasitaatmadja, 2018; Kraft, 2011). Acne vulgaris complaints most often appear during puberty and peak during mid to late adolescence. The following table shows the distribution of study subjects based on age found in the study.

Table 1. Distribution of research subjects by age

Age	Acne vulgaris group		Control group	
	n	%	n	%
18-22 Year	6	20	6	20
23-27 Year	13	43,3	13	43,3
28-32 Year	5	16,7	5	16,7
33-37 Year	4	13,3	4	13,3
≥ 38 Year	2	6,7	2	6,7
Total	30	100	30	100

Table 1 shows that in this study, most subjects in the acne vulgaris and control groups were aged 23-27 (43.3%). The next group was 18-22 years (20%), 28-32 years (16.7%), 33-37 years (13.3%), and the rest were over 38 years old (6.7%).

In a study conducted by Skroza et al. (2018) in Italy, most acne vulgaris patients were in the age range of 12-25 years (58.7%) (Skroza et al., 2018). In another study in Medan, most acne vulgaris patients were aged 17-25 years (96.8%) (Rahmayani et al., 2019). Likewise, a survey conducted by Sutrisno et al. (2020) in Medan found that the highest age range of acne vulgaris patients was 21-25 years (40%).

In contrast to Okoro et al.'s (2016) research in Nigeria, which found 71.7% acne vulgaris in female students aged 15-19. Vilar et al.'s (2015) research in Brazil found 89.3% of acne vulgaris at an average age of 16. Fachry et al. (2014) studied USU FK female students from the 2011 class who suffered from acne vulgaris and found that the highest age was 21 years (53.8%). According to Yutrishia et al.'s (2016) research, those aged 15 to 21 experienced the majority of acne vulgaris (68%).

The majority of the study subjects were adolescents and young adults. Acne vulgaris is one of the main problems for adolescents during puberty, and the highest prevalence occurs in middle and late adolescence. The hormone DHEAS regulates sebaceous gland activity that increases during puberty. Androgen hormones also increase sebum production so that comedones can form due to hypercornification of the sebaceous ducts (Lucky et al., 1994).

Characteristics based on gender

Acne vulgaris can occur in both men and women and in some studies, the prevalence of this disease is related to different genders. The following table shows the distribution of study subjects by gender.

Table 2. Distribution of research subjects by gender

Gender	Acne vulgaris group		Control group	
	n	%	n	%
Male	7	23,3	7	23,3

Female	23	76,7	23	76,7
Total	30	100	30	100

In this study, the research subjects were more female, namely 23 women (76.7%), compared to 7 men (23.3%) in the control and acne vulgaris groups. A similar thing was found in a study conducted by Eyuboglu et al. (2018) in Turkey, which examined 164 acne vulgaris sufferers, where 64% were women and 36% were men (Eyuboglu et al., 2018). In addition, Rahmayani et al. (2019), in their research in Medan, found that most acne vulgaris sufferers were women (60.6%). Research by Sutrisno et al. (2020) also found that the % of acne vulgaris sufferers were women, 68%.

In contrast to the aforementioned results, men had a 39.7% prevalence of acne vulgaris and women a 35.7% prevalence in a meta-analysis study on acne vulgaris patients carried out in China by Li et al. (2017). However, gender had no bearing on the prevalence of acne vulgaris in a study done in Saudi Arabia by Robaee et al. (2005). Researchers claim that variables specific to each study account for the diversity in the prevalence of acne vulgaris by gender. The higher frequency of acne vulgaris in women than in men may be due in part to hormonal reasons. This is because female acne vulgaris tends to be more chronic and that female puberty occurs earlier than male puberty (Baumann et al., 2009). The use of cosmetics on the face and the propensity of women to seek treatment right away when having cosmetic problems are additional factors that impact the occurrence of acne vulgaris in women (Khunger and Kumar, 2012).

Mean Serum Substance P Levels in Research Subjects

The average levels of substance P were seen in the acne vulgaris and control groups. The following is a table of the average levels of substance P in the research subjects found in this study.

Table 3. Mean serum substance P levels in research subjects

Group	Serum substance P levels (pg/ml)		
	n	Mean	SD
Akne vulgaris	30	62,6	32,4
Control	30	40,3	17,0

In Table 3 above, the average value of serum substance P levels was 62.6 ± 32.4 pg/ml in 30 people in the acne vulgaris group, while the average value of serum substance P levels in 30 people in the control group was 40.3 ± 17.0 pg/ml. Until now, research on serum substance P levels in acne vulgaris patients is minimal. The study was conducted in Poland by Rokowska-Waluch et al. (2016), with the results obtained in the form of an average value of serum substance P levels of 0.6 ± 0.09 pg/ml in 40 people in the acne vulgaris group who were examined. Meanwhile, the control group had an average value of 0.49 ± 0.12 pg/ml. This aligns with this study because the acne vulgaris group had higher serum substance P levels than the control group.

Salomon et al. (2007) conducted a plasma substance P-level examination in 49 atopic dermatitis patients in Poland. Plasma substance P-level examination was performed in 49 atopic dermatitis patients. During an exacerbation, the plasma substance P level was examined, and the average result was 195.25 ± 96.04 pg/ml.

Distribution of Serum Substance P Levels by Age

The following table shows the distribution of serum substance P in the acne vulgaris group based on age.

Table 4. Distribution of substance P levels by age

Age	Serum substance P levels (pg/ml)			
	Acne vulgaris group		Control group	
	n	Mean ± SD	n	Mean ± SD
18-22 Year	6	48,5 ± 19,8	6	47,9 ± 18,3
23-27 Year	13	64,9 ± 29,0	3	38,9 ±17,5
28-32 Year	5	87,7 ± 46,6	5	35,5 ± 11,4
33-37 Year	4	57,0 ± 33,4	4	41,3 ± 21,7
≥ 38 Year	2	38,2 ± 25,8	2	36,6 ± 23,5

Based on Table 4 above, in the acne vulgaris group, the highest mean serum substance P levels were found in the 28-32 year age group, which was 87.7 ± 46.6 pg/ml, followed by the 23-27 year age group with an average P substance level of 64.9 ± 29.0 pg/ml, the 33-37 year age group with an average P substance level of 57.0 ± 33.4 pg/ml, the 18-22 year age group with an average P substance level of 48.5 ± 19.8 pg/ml, and the ≥ 38 year age group with an average P substance level of 38.2 ± 25.8 pg/ml. Meanwhile, in the control group, the highest mean serum substance P levels were found in the 18-22 year age group, which was 47.9 ± 18.3 pg/ml, followed by the 33-37 year age group with an average P substance level of 41.3 ± 21.7 pg/ml, the 23-27 year age group with an average P substance level of 38.9 ± 17.5 pg/ml, the ≥ 38 year age group with an average P substance level of 36.6 ± 23.5 pg/ml, and the 28-32 year age group with an average P substance level of 35.5 ± 11.4 pg/ml.

To date, researchers have not found any research determining the value of serum substance P levels in acne vulgaris based on age. However, a study conducted by Yamada et al. (2002) on 49 healthy people in Japan aged between 17 and 80 years found no significant correlation between serum substance P levels and its metabolites in human tears.

Distribution of Serum Substance P Levels by Gender

Acne vulgaris can occur in both men and women. The following is a table of the distribution of serum substance P based on gender in acne vulgaris patients found in this study.

Table 5. Distribution of serum substance P levels by gender

Gender	Serum substance P levels (pg/ml)			
	Acne vulgaris group		Control group	
	n	Mean ± SD	n	Mean ± SD
Male	7	63,0 ± 27,5	7	47,8 ± 20,3
Female	23	62,5 ± 34,3	23	38,0 ± 15,7

In Table 5 above, the average value of serum substance P levels in the male acne vulgaris group was 63.0 ± 27.5 pg/ml, while the average serum substance P levels in the female acne vulgaris group were 62.5 ± 34.3 pg/ml. Meanwhile, the average value of serum substance P levels in the male control group was 47.8 ± 20.3 pg/ml, while the average serum substance P levels in the female control group were 38.0 ± 15.7 pg/ml. Researchers have not found any studies looking for differences in serum substance P levels in humans associated with gender. Until now, researchers have only seen one study by Yamada et al. (2002) in Japan that looked for levels of substance P and its metabolites in human tears and found an average of 306 ± 96.5 pg/ml. Based on this

research, there was no significant difference in substance P levels based on gender, but the average substance P levels in women were higher than in men.

Relationship between Serum Substance P Levels and Acne Vulgaris

The relationship between substance P levels and acne vulgaris can be seen in table 6 below :

Table 6. Relationship between serum substance P levels and acne vulgaris

Serum substance P levels	Group		Total	p
	Akne vulgaris n (%)	Controln (%)		
High	21 (70)	9 (30)	30 (50)	0,005
Low	9 (30)	21 (70)	30 (50)	
Total	30 (100)	30 (100)	60 (100)	

In Table 6 above, the results of the relationship between serum substance P levels and acne vulgaris are presented in a 2x2 table. The serum substance P category is divided into two, namely, high at ≥44.65 pg/ml and low at <44.64 pg/ml. The subject groups in the study consisted of the acne vulgaris group and the control group. The statistical analysis used in this study was the Chi-square test. Based on the Chi-square test, it can be seen that the p-value is 0.005, so it is concluded that there is a statistically significant relationship between serum substance P levels and acne vulgaris. Furthermore, to assess the effect of serum substance P levels on the incidence of acne vulgaris, the Odds Ratio (OR) value was calculated, namely 5.4 (95% IK 1.8-16.4). This shows that high serum substance P levels cause a 5.4-fold risk of acne vulgaris.

More research should be done on serum substance P levels in people with acne vulgaris. To determine the association between the severity of acne vulgaris, the level of emotional stress, and the serum concentration of substance P, Rokowska-Waluch et al. (2016) conducted research in Poland on 40 patients with acne vulgaris and 40 controls who were between the ages of 18 and 34. Serum substance P levels were observed to be greater in acne vulgaris patients than in controls. However, this difference was not statistically significant. This is predicated on the investigation's findings.

Substance P is known to be involved in the pathogenesis of acne vulgaris because it can cause adipogenesis by increasing PPAR-γ.72 Based on research in Korea by Lee et al. (2008), the addition of substance P to sebocyte cultures causes immunoreactivity and increased RNA amplification of IL-1, IL-6, TNF-α and PPAR-γ. This further supports that substance P is involved in the pathogenesis of acne vulgaris (Lee et al., 2008). In addition, Toyoda et al. (2002) in Japan found that nerve fibres closer to the sebaceous glands in acne vulgaris patients were more immunoreactive to substance P than controls. Several other studies have also linked substance P to different skin diseases. Salomon et al. (2007) from Poland studied 49 atopic dermatitis patients aged 17-56 years. This investigation measured substance P plasma levels in patients with atopic dermatitis during flare-ups, remissions, and control periods. According to the study, patients with atopic dermatitis had considerably greater plasma levels of substance P during flare-ups and remissions than the control group.

The advantage of this study is that the patient and control groups have the same or almost the same characteristics, thus reducing bias factors in the research results. In addition, diseases that can affect serum substance P levels were excluded. Substance P is the primary mediator of neurogenic inflammation and itching. Skin neuropeptides,

especially substance P, play a role in the pathogenesis of various skin diseases, such as psoriasis, atopic dermatitis, acne vulgaris and rosacea (Mijouin et al., 2013).

Stress is one of the factors that contributes to acne vulgaris. This is based on research Sutrisno et al. conducted in Medan (2020). This study involved 100 acne vulgaris patients who underwent observational analysis. Based on the Lehmann criteria, each patient's acne vulgaris severity was assessed using the Acne Grading Indonesian Acne Expert Meeting 2015. Next, a stress level questionnaire utilising the Holmes-Rahe stress scale was completed. The results of this study suggested a relationship between the stress scale and the severity of acne vulgaris. In Poland, the relationship between stress indicators and serum substance P levels was also documented by Rokowska-Waluch et al. (2016). The group with acne vulgaris and the control group had examinations. The Holmes-Rahe stress scale questionnaire was used to evaluate the stress scale. According to the study, the control and acne vulgaris groups differed statistically significantly. The group with acne vulgaris had greater serum substance P levels than the control group, according to the results. Stress causes substance P to be released by peripheral nerves. Substance P can increase sebocyte size and promote the growth of sebaceous precursor cells. This indicates that substance P can enhance lipid synthesis in sebocytes and cause the proliferation and differentiation of sebaceous glands (Yadav et al., 2013).

CONCLUSION

Research has been conducted on the relationship between serum substance P and acne vulgaris with the following conclusions:

1. A relationship between serum substance P levels and acne vulgaris exists. High serum substance P levels cause a 5.4-fold risk of acne vulgaris.
2. The average serum substance P level in acne vulgaris is 62.6 ± 32.4 pg/ml.
3. The highest average serum substance P level in acne vulgaris can be found in the age group 28-32 years.
4. The average serum substance P level in acne vulgaris based on gender is highest in men.

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