Evaluating The Correlation Between Survivin Serum Content And The Severity Level Of Acne Vulgaris

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Abstract:
Background: Survivin is one of the apoptosis inhibitory protein family and is considered as a crucial protein that regulates mitosis and apoptosis inhibition. Moreover, this protein has a pivotal role in physiology and pathology such as cell/organ carcinogenesis in humans. A recent study showed that survivin is involved in acne vulgaris (AV) pathogenesis and acne scarring caused by inflammation.
Aims: This study aimed to evaluate the correlation between survivin serum level and the severity level of AV.
Methods: Blood samples were collected from 20 low-level AV patients, 20 moderate level AV patients and 20 severe level AV patients. After that, survivin serum level was measured by using ELISA.
Results: The results showed that the survivin serum level promoted with the increasing severity level of AV.
Limitations: This study was not designed to evaluate the inflammatory parameter caused by acne vulgaris.
Conclusions: Thus, the survivin serum content and the severity level of AV has the significant correlation.

Key-words: Acne vulgaris, survivin, severity level

Introduction:

Acne vulgaris (AV) is a common chronic inflammation-related disease found in the pilosebaceous follicle of skin tissue. Interestingly, about 650 million people worldwide suffer from this disease. Furthermore, the Global Burden of Diseases demonstrates that AV placed in the top eight as one of the most common diseases globally in 2010.[1] The other findings also revealed that AV is the most clinical case in the United States. Interestingly, AV has been known as one of the top 30 diseases that was diagnosed by non-dermatologists and the second most commonly diagnosed by a dermatologist.[2] In Australia, about 320 out of 3197 (10%) dermatologist' patients are AV survivors. While in Asia, around 19.6% of newly hospitalized patients are AV patients.[3]

AV is a long-term chronic disease and sometimes this disease has a relapse.[4] Although AV is not a life-threatening disease, it can cause adverse psychological and social effects in the patient's daily life.[5] The study showed that AV patients suffered from more mental issues than the others chronic diseases such as epilepsy and diabetes.[6] Another research group has been reported that AV patients under emotional disorders, social and psychological symptoms similar to asthma, epilepsy, diabetes, and arthritis patients showed several psychological signs such as felt shy (70%), anxious (63%), uncomfortable (67%), had social contact disorder (57%) and under unemployed situation.[7] The residual effects such as hypertrophic scars, hyperpigmentation and post inflammation can be severe on the skin that influencing the patients' quality of life.[4]

Based on the patient visit report of the Cosmetics Dermatology Division, Department of Dermatology and Venereology, Faculty of Medicine, University of Indonesia/Cipto Mangunkusumo Hospital (RSCM) Jakarta, the number of AV patient visitation in 2010 reached 2,489 visits with the number of new cases reaching 756 patients (30.37%).[8] A retrospective study at the H. Adam Malik Central General Hospital in Medan demonstrated that the proportion of AV patients in the period from January 2010 to December 2012 was about 1.10%.[9] Moreover, the data from the medical records of the Dermatology and Venereology Polyclinic of the Raden Mattaher General Hospital, Jambi found that AV cases in 2014 were about 140 out of 3,213 visits, in 2015 there were 58 out of 2,119 visits, and in 2016 there were approximately 32 out of 1,217 visits.

The multifactor pathogenesis of AV consists of four primary mechanisms that are interconnected and involve hormonal and immune influences, namely hyperproliferation of the follicular epidermis, excessive sebum production, inflammation, and *Propionibacterium acnes*. Importantly, the exact and specific order of development of AV is unknown. The current postulate describes the formation of the microcomedo in the initial stage which is known as a precursor to the occurrence of blackheads, papules, and pustules.[9] Increased production of sebum drives the microcomedo formation due to stimuli in the sebaceous or follicular glands of keratinocytes and androgens during puberty.[11] Androgen activity can be increased by insulin-like growth factor-1 (IGF-1).[12]

The study conducted by Assaf et al.[13] showed that serum IGF-1 and survivin levels as hallmarks in active AV patients and scar tissue post-AV inflammation. The results gained by evaluating the expression of in both lesions and healthy persons. The study showed that IGF-1 and survivin play a crucial role in the pathogenesis of active AV and scar tissue post-AV inflammation with a significant positive correlation between serum IGF-1 and survivin levels. On the other hand, this phenomenon has been followed by a decreased expression of FoxO via IGF-1/PI3K/Akt signaling pathway which in turn increased the survivin expression.

This case has correlation with other previous studies that demonstrated survivin improvement in proliferative and inflammatory keratinocytes. In this condition, the survivin is strictly involved in the pathogenesis of AV lesions. The survivin enhancement affects sebaceous glands (survival sebaceous) and perifollicular dermis tissue (scar formation).[14] Survivin expression has a positive correlation with the fibrosis pathogenesis in fibrosis disorder including scar generation and suppressing its appearance can be an option for repairing the lesion.[13]

Survivin is a member of the apoptotic inhibitor family, a protein molecule that has an essential role in the regulation of mitosis and the inhibition of apoptosis. This protein also plays a role in specific physiological processes and pathological conditions such as carcinogenesis in human cells/organs.[15] The survivin expression is depending on the stimulation of Akt kinase and mTORC1. The mTORC1 activation can increase the survivin expression. FoxO1 regulates the survivin promoter. The decreased level of FoxO and increased level of mTORC1 are the outcomes factor of the increased level of survivin in AV lesions.[13]

Besides the anti-apoptotic potency, the biological function of survivin in humans remains unclear. Several studies have explained its role in regulating cell function. Therefore, survivin disruption may have adverse consequences. Within the skin, survivin is involved in some pathological conditions such as psoriasis and tumors originating from melanocytes and epithelium. Survivin is also crucial for normal epidermal homeostasis, possibly supporting the self-renewal of epidermal stem cells. Interestingly, survivin is expressed in keratinocytes from the interfollicular epidermal and human sebocytes. Survivin acts as pro-survival in sebocytes and keratinocytes. Moreover, survivin regulates the balance of cell proliferation, differentiation, and apoptosis.[16]

Survivin is the smallest member of the apoptotic inhibitor protein family, which has a pivotal role in the regulation of cell division and apoptosis blockage through inhibition of caspase activation. Apoptosis is

the process of physiological cell death which plays a role in the development and maintenance of cell and tissue homeostasis — the abnormality of the apoptotic pathway causing several health problems such as cancer, autoimmune diseases, immunodeficiency, and degenerative diseases.[17] During terminal differentiation, sebocyte lipid droplets accumulate and show degeneration from the nucleus which results in programmed cell death. Epidermal keratinocytes undergo a unique form of terminal differentiation including programmed cell death, called cornification. Pro-survival signal balance and programmed cell death maintain sebocyte homeostasis, which is impaired in AV.[18]

Determination of AV severity level is crucial point to determine the characteristics and classification of patients used for therapeutic evaluation and epidemiological research. The classification for determining the type or grade of AV used in this study is according to the classification by Lehmann et al.[19], namely mild, moderate and severe grades. This method calculates the number of comedo lesions, inflammatory lesions, and total lesions.

Acne vulgaris incidence often following by scar tissue, resulting from the abnormal resolution or wound healing after tissue damage that occurs in sebaceous follicles during inflammation.[13] The rational use of drugs based on the type and severity of AV is the key to success in AV treatment, with an increasing understanding of AV pathophysiology to produce anti-AV therapy materials and regimens in the future.[20] Appropriate diagnosis and treatment must be done to prevent physical and psychological sequelae.[21]

Recently, there is no report about the association between the survivin expression and the clinical condition of AV severity. Based on the above explanation, the researchers wanted to conduct a study on the relationship between survivin levels and AV severity. Survivin is known to be a biomarker of carcinogenesis. Interestingly, the survivin is considered as a new AV biomarker. The role of survivin in AV pathogenesis needs further investigation, especially in Indonesia.

Methods:

The study was conducted at the Dermatology and Venereology Polyclinic of Raden Mattaher General Hospital, Jambi and a private clinics in Jambi for both sampling and clinical examination. Laboratory tests were carried out at the Biomedical Laboratory of the Faculty of Medicine Andalas University, Padang. The study population consisted of AV patients who visited the Dermatology and Venereology Polyclinic of Raden Mattaher General Hospital, Jambi and a private clinics in Jambi.

Inclusion and exclusion criteria

The inclusion criteria for patients are 1) The respondents' age between14-35 years old; 2) women; 3) the respondents should approved and signed the informed consent. Exclusion criteria are 1) AV patients who were pregnant and breastfeeding; 2) suffering from systemic diseases such as diabetes mellitus, thyroid dysfunction, polycystic ovary syndrome, asthma, pulmonary tuberculosis, liver cirrhosis, malignant disease, systemic lupus erythematosus, assessed through history taking (anamnesis); 3) suffering from other inflammatory skin diseases such as psoriasis, Morbus Hansen, atopic dermatitis, allergic contact dermatitis, assessed through history (anamnesis) and dermatological examination; 4) respondents under glucocorticoid drugs, insulin, oral hypoglycemic drugs, and oral contraceptives treatment; 5) cigarette smoking; 6) getting AV (topical/systemic) therapy about four weeks before the examination; 7) using anti-inflammatory and antibiotics within eight weeks before the test; 8) using isotretinoin orally about six months before examination.

Consent document setting

The consent documents were given to the research subject. The consent documents determined the purpose and benefits of the research and the reasons for the prospective subjects included in the study, given information that the issue is free to refuse to take part in this research, the time needed, the rewards earned, notification of research results, research risks and direct benefits. After the prospective research subjects stated their willingness to participate in the study, the research subjects signed informed consent. Ethics of research in humans based on the protocol of human use as the object of research by the provisions of the Declaration of Helsinki and endorsed by the National Research Ethics Commission. Medical records relating to research are confidential.

Blood sampling

Patients included in this study must meet the diagnostic criteria of the combined acne severity classification by Lehmann et al.[19]. The Lehmann's classification of AV severity level divided into mild, moderate, and severe grade. For the anamnesis, a dermatological examination was performed by the researcher. Blood sampling was carried out by experts working in private clinical laboratories. Blood was taken from the median cubital vein, this was carried out from 08.00 to 11.00 in the morning, at the General Hospital Raden Mattaher, Jambi, the private clinics, and clinical laboratories in Jambi. Before conducting the research, an ethical agreement was requested from the Research Ethics Committee of the Faculty of Medicine, Andalas University. This study has carried out moral review tests at the Faculty of Medicine of Andalas University, Padang and passed the Ethics Study on February 5, 2018 (No: 084/KEP/FK/2018).

About 4 mL of venous blood was taken from each patient with 20 patients with mild AV, 20 patients with moderate AV and 20 patients with severe AV. Furthermore, an ELISA examination was performed to evaluate survivin serum levels (Human survivin ELISA kit from Elabscience).

Data analysis

Because there are three independent groups (mild AV, moderate AV, and severe AV group) with varying positive values, a statistical test was performed. Data were entered into a computer and analyzed using SPSS software. Before the statistical testing was done, the effect of the independent variables on the dependent variable was assessed with a normality test and the Kolmogorov-Smirnov test to determine whether the data had a normal distribution. When the data was usually distributed, then parametric tests with ANOVA were carried out. If the distribution was abnormal, data transformation was carried out. The level of significance was accepted if p-value <0.05.

Results:

Characteristics of respondents

In this study, about 60 female patients with AV case were involved. Several characters including age, education and employment status were evaluated in this study. The distribution of the characteristics of the subjects shown in Table 1.

Table 1: Characteristics of respondents

Characteristics of respondents	Mild AV		Moderate AV		Severe AV		Table 1		Table 1
	f	%	f	%	f	%	f	%	
				Age					
15-19 years old	5	25	6	30	13	65	24	40	
20-24 years old	8	40	10	50	5	25	23	38.3	
25-30 years old	7	35	4	20	2	10	13	21.7	0.053
Total		100	20	100	20	100	60	100	
				Education level					
Junior High	2	10	2	10	5	25	9	15	
Senior High	9	45	13	65	12	60	34	56.7	
Collage	9	45	5	25	3	15	17	28.3	0.195
Total	20	100	20	100	20	100	60	100	
				Occupation					
Jobless	0	0	1	5	0	0	1	1.7	
Housewife	1	5	0	0	1	5	2	3.3	
Student	3	15	4	20	6	30	13	21.7	
Bachelorate student	7	35	9	45	10	50	26	43.3	0.472
Private	9	45	6	30	3	15	18	30.0	
Total	20	100	20	100	20	100	60	100	

About 24 patients were found in the age group of 15-19 years (40%). Interestingly, the patient with severe AV were mostly in the age group of 15-19 years [Table 1]. Moreover, about 13 patients (65%) and moderate AV and mild AV patients group were found in the age group of 20-24 years, approximately ten patients with moderate AV (50 %) and eight patients with mild AV (40%). When looking at the education level, senior high school students were found mostly in all of the stages of AV, namely mild AV with nine patients (45%), moderate AV with 13 patients (65%) and severe AV with 12 patients (60%). While college students were mostly found in the medium and mild AV stages, approximately nine patients (45%) and ten patients (50%) respectively. Also, private sector workers were mostly found in the mild AV stage accounting for nine patients (45%).

The relationship of survivin serum contents with acne vulgaris severity level

Normality testing and correlation test between the independent and dependent variables were applied in this study. The normality test aimed to evaluate the data distribution. Based on the normality test, the survivin serum contents were abnormally distributed, therefore the data needed to be transformed. By using Log 10 function for transformation, survivin data turned into a normal distribution. After that, the survivin contents were analyzed by using a parametric test. The results of the study show that the relationship between survivin levels and the severity of AV was shown in Table 2.

Table 2: The relationship of survivin serum contents with acne vulgaris severity level

Variable	Severity level	n	Average	SD	p-value
Survivin (pg/ml)	Mild	20	72.65	33.61	0.016
	Moderate	20	99.19	38.22	
	Severe	20	124.64	81.03	

The average survivin level decreased while the level of the AV severity increased [Table 2]. The highest survivin level is the severe AV severity accounting for 124.64 ± 81.03 pg/ml, moderate AV accounting for 99.19± 38.22 pg/ml and mild AV accounting for 72.65 ± 33.61 pg/ml. The results of the statistical test showed a value of $p< 0.05$, meaning that there is significant correlation between the average level of survivin and the severity of AV.

Discussion:

Characteristics of research subjects

In the study, about 60 female AV patients consisting of three groups, namely 20 mild AV patients, 20 moderate AV patients, and 20 severe AV patients were evaluated. Patients were treated at the Polyclinic of Dermatology and Venereology General Hospital of Raden Mattaher and the private clinics in Jambi. Based on the results, the majority of the patients were in the age group of 15-19 years old consisting of 24 patients (40%). The patients with severe AV were mostly in the age group of 15-19 years consisting of 13 patients (65%) and both moderate AV and mild AV patients were found to be the highest in the age group of 20-24 years, approximately 10 patients had moderate AV (50 %), and 8 patients had mild AV (40%).

Other studies demonstrated that the highest prevalence of AV incidence were the age group of 15-19 years old (38%), followed by 20-24 years old (36%) and only 1.4% of acne patients were found at 40-44 years old.[22] AV prevalence occurs in various countries; it generally occurs in adolescents with a percentage of more than 80%.[23] The onset of AV can vary in each age group but usually begins in adolescence, reaching a maximum peak at the age of 14-19 years old and continuing into adulthood.[24]

The research on the epidemiology of AV in female adolescents and young adults in Riyadh revealed more than one third (38%) of participants were 15-20 years old, followed by 20-25-year-olds (35.5%), 25-30 years old (10.2%), only 9.4% were aged ≤ 15 years, and 6.9% were over 30 years old.[24] Moderate-to-severe AV affects about 20% of young people, and the severity of AV is associated with puberty. The AV affects almost all adolescence between the ages of 15 and 17 years old, and about 15%-20% have moderate to severe AV grade.[23] Youth is a period in which physical, emotional and social changes occur. This period is a transition period, where adolescents form new relationships, friendships, and romance, they to get a new status in society.[25] Numerous skin care products are sold freely in pharmacies and promoted to the public. However, without consulting a dermatologist, patients will choose free cosmetic or treatments that may harm their acne.[26]

High school students have the highest prevalence of acne case which consisted of mild AV counting for nine patients (45%), moderate AV accounting for 13 patients (65%) and severe AV accounting for 12 patients (60%). Research carried out in Germany showed that AV prevalence in high school students was generally about 93.3%.[27] Moreover, research on the epidemiology of AV in female adolescents and young adults in Riyadh found that more than a third of participants were female students (37.5%), 29.8% had a high school education, and only 6% had an elementary school education.[24] A study conducted by Zari and Turkistani[26] in Jeddah showed that 35.8% of students received AV treatment without a prescription. Hormonal factors and increased use of cosmetics play a pivotal role in improving the occurrence of AV cases in women, the use of cosmetics has been discussed as an essential cause of mild and moderate AV in the female population.[28]

The major occupation of the AV patients was mainly college students, in the severe AV degree group which consisted of ten patients (50%) and the moderate AV group which included of nine patients (45%). While the occupation of the mild AV degree group was private sector workers, who were consisting of nine patients (45%). According to the study conducted in Saudi Arabia found approximately 56.2% of female college students experienced AV.[24] Moreover, the retrospective study

in Surabaya found that the distribution of employment for new AV patients was mainly students or college students that reach about 39.1%. Interestingly, the latest AV patients were students or college students. The demand for having smooth and beautiful facial skin is increasing, along with interest in the opposite sex and competition among others to get attention causing a high level of awareness of patients, especially students and college students to immediately looking for treatment.[29] AV has a negative impact on personal relationships, sports activities and job opportunities in adolescents and young adults.[30]

The relationship of survivin serum contents with acne vulgaris severity level

Survivin is a apoptotic inhibitor protein that plays a crucial role in homeostasis by inhibiting apoptosis and regulating cell division, proliferation, and cell survival. Various studies have shown survivin expression in the subpopulation of keratinocytes in the normal epidermal basal cell layer of humans. Furthermore, survivin is also found in the normal sebocytes of the human sebaceous glands.[13] There are two mechanisms which cause programmed cell death namely extrinsic and intrinsic factor. Both of these factors are regulated by some caspase proteins, especially caspase-8 and caspase-9. External factors can act through the initiation of cell death receptor ligase which causes caspase-8 activation. Intrinsic factors or intracellular stimuli, such as DNA damage, can occur through the mitochondrial apoptosis pathway initiated by the release of a second mitochondria-derived activator of caspase (Smac). The Smac is a mitochondrial protein that support the occurrence of apoptosis by inhibiting survivin. Second mitochondria-derived activator of caspase activates caspase-9 initiator which causes the formation of apoptosomes. Excessive survivin expression inhibits apoptosis. In general, survivin in mammals prevents apoptosis through direct inhibition of the caspase-9 initiator.[31]

Microcomedo formation requires complex interactions among the disorders of follicular keratinization, sebaceous gland hyperplasia, and excessive colonization of the sebaceous glands by *P. acnes*. The process of scar tissue can occur at each stage of the acne. Furthermore, survivin enhancement can affect the sebaceous glands and perifollicular dermis tissue in the formation of scar tissue. Survivin expression has a mechanical relationship with the pathogenesis of fibrosis in fibrotic diseases including the process of AV scarring. Survivin has the crucial role in AV pathogenesis and the IGF-1 regulation. Another study showed significant level of survivin serum and IGF-1 in AV patients.[13]

IGF-1 signal enhancement is associated with an increase in survivin expression through the decreased expression of FoxO proteins, including FoxO1 which is mediated by IGF-1/PI3K/Akt. Survivin promoter is regulated by FoxO1 and FoxO3a.[13] Survivin disorders have adverse consequences. AV is a pro-survival disease of the sebaceous glands and follicles due to insufficient cell death signaling.[32] Sebocyte apoptosis decreases the total amount of sebum, which is an important energy source for *P. acne* growth. Reduced number of *P. acnes* also decreases the lipase triglyceride of *P. acnes* and free palmitate formation from sebum. Propionibacterium acnes and free palmitate are essential signals that activate inflammation of NLRP3.[18] In AV there is an increase in survivin anti-apoptotic protein expression mediated by Akt.[33]

In this study, from the 60 subjects in the study sample, survivin serum levels increased followed by an increasing AV severity level. The mean level of survivin serum at mild AV severity level was 72.65±33.61 pg/ml, the moderate AV severity level was 99.19±38.22 pg/ml, and the severe AV severity level was 124.64±81.03 pg/ml.

In this study, all patients experienced active AV lesions whose survivin levels increased followed by an increased AV severity. Mostly, the survivin is expressed keratinocyte hyperproliferative lesions. Despite its association with mitotic progression and antiapoptotic activity, its expression does not appear to be related to proliferative status and apoptosis. Increased keratinocyte proliferation is a consistent hallmark of AV lesions and may be needed for their growth and maintenance, so it is possible that there are other factors involved in the development of AV. Apoptosis and proliferation are the biological processes that

play critical function in homeostasis. The down regulation of apoptosis activity is generally thought to be associated with hyperproliferation of the epidermis.[34]

This study is accordance with the results of Assaf et al.[13] who examined serum IGF-1 and survivin levels in 15 AV patients with active lesions and 15 patients with post-inflammatory scar tissue compared with 15 healthy control patients, they found that serum survivin levels were significantly higher in active AV and AV scar tissue compared to healthy controls. The survivin serum level was significantly higher in the AV scar tissue group than the active AV group. However, this study did not compare survivin levels with AV severity.

Under physiological conditions, survivin is unable to inhibit apoptosis through direct binding to a caspase. Survivin not only inhibits apoptosis but also regulates cell division. The survivin has functions that related to differences in compartments of the proteins in cells including the nucleus (nuclear), cytoplasm (cytoplasmic) and mitochondria (mitochondrial). Localization in the nucleus is related to the capacity of the survivin to regulate cell division; mitochondrial survivin is associated with the inhibition of apoptosis. The apoptosis begin when the cytoplasmic survivin exits the mitochondria and unable to protect the cell from apoptosis due to post translation modification. In this condition, the cells will loses its cytoprotective ability. The last finding showed that survivin is detected extracellularly. It has antiapoptotic and proliferative activity.[35]

It has been known that survivin promoted anti-apoptotic and cell cycle regulation. In human keratinocytes, although overexpression of survivin does not change in keratinocyte proliferation or differentiation, survivin downregulation damages the abilities of cells to proliferate and form the colonies in vitro. This study suggest that survivin is essential for the continuity of cell cycle development and the proliferation of the abilities in keratinocytes.[35]

This study was not designed to evaluate the inflammatory parameter caused by acne vulgaris.

In conclusion, there is significant correlation between the survivin serum content with the severity level of acne vulgaris in this study.

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