The Effect of Forkhead Box Class O1, Mammalian Target of Rapamycin Complex 1, Survivin, and Interleukin-17 on the Degree of Acne Vulgaris Based on Serum Levels

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Abstract

BACKGROUND: Acne vulgaris (AV) is a health problem that is routinely found throughout the world and has an impact on the quality of life of sufferers. Several studies have shown that forkhead box Class O1 (FoxO1), the mammalian target of rapamycin complex 1 (mTORC1), survivin, and interleukin-17 (IL-17) play a role in the pathogenesis of multifactorial AV.

AIM: The study aims to determine the relationship between serum levels of FoxO1, mTORC1, survivin, and IL-17 with the severity of AV.

METHODS: This is observational research with cross-sectional design. Samples from venous blood sufferers of AV mild, moderate, and severe, each of 20 people were collected. Classification of AV was based on the criteria of Lehmann et al. Examination of serum levels of FoxO1, mTORC1, survivin, and IL-17 using the ELISA technique was done. The statistical test used was the ANOVA test and the Kruskal–Wallis test.

RESULTS: The results showed that average levels of FoxO1 decreased with increasing degree of severity of AV. An average increase in mTORC1, survivin, IL-17 levels with an increase in the severity of AV was found. Statistical test results showed a significant correlation of FoxO1, mTORC1, survivin levels, and the severity of AV (p < 0.05). IL-17 levels were not related to the severity of AV (p > 0.05).

CONCLUSION: This study concluded that FoxO1 levels decrease at increasing severity. Conversely, levels of mTORC1, survivin, and IL-17 increase with increasing degrees of the severity of AV.

Introduction

Acne vulgaris (AV) is a chronic inflammatory disease in folliculopilosebaceous units. AV is a skin disorder found in many parts of the world [1,2]. In general, AV sufferers are teenagers who enter puberty and AV has psychological and social effects for sufferers. There are four factors that influence the process of AV, namely, forkhead box Class O1 (FoxO1), mammalian target of rapamycin complex 1 (mTORC1), survivin, and interleukin-17 (IL-17). AV usually appears with increasing androgen hormones in the body. Androgen activity is triggered by insulin-like growth factor-1 (IGF-1) [3].

IGF-1 can suppress FoxO1 nuclei in SZ95 sebocytes which play a role in increasing lipogenesis [4]. FoxO1 functions as a regulator in the pathogenesis of AV where FoxO1 integrates external and internal growth factors signals at the level of gene regulation [5]. Decreased activity of FoxO1 in sebocytes of AV patients and increased activity of mTORC1 and survivin was published [6]. mTORC1 is a regulator of cellular growth and proliferation, lipid synthesis, and protein translation [7]. Survivin is an apoptotic inhibitor protein and regulates cell division, cell proliferation, and survival. IL-17 is a pro-inflammatory cytokine that can cause tissue damage and degeneration during chronic inflammation [8]. The role of Th17 cells in the pathogenesis of AV is still new and interesting, further research is needed regarding the interaction of IL-17 with the development of AV inflammation.

The key to the success of AV treatment is rational drug use based on type and degree of severity so that an increased understanding of the pathophysiology of AV will result in future anti-AV therapy ingredients and regimens. Until now, no research has been found on the relationship between levels of FoxO1, mTORC1, survivin, and IL-17 with clinical AV severity, especially in Jambi, Indonesia. Therefore, we have conducted research on the
The relationship of levels of FoxO1, mTORC1, survivin, and IL-17 with the severity of AV. The study aims to determine the relationship between serum levels of FoxO1, mTORC1, survivin, and IL-17 with the severity of AV.

Materials and Methods

The study was conducted at the Dermatovenereology Polyclinic of Raden Mattaher Regional General Hospital, Jambi, Indonesia.

Sample size

The study population was all AV sufferers who came to the Dermatovenereology Polyclinic of Raden Mattaher Regional General Hospital, Jambi, Indonesia. The sample selection was based on inclusion and exclusion criteria with a total sample of AV patients 60 people, consisting of 20 mild AV people, 20 moderate AV people, and 20 severe AV people.

Blood samples

Median cubital vein blood sampling was taken from 08.00 am to 11.00 am. Four milliliters venous blood samples were taken from each AV sufferer. Samples taken were included in two Vacutainer tubes. The first Vacutainer tube for survivin and IL-17 examination, left at room temperature for 30 min, centrifuged, and separated the insert serum into two cup samples. The second Vacutainer tube for an examination of FoxO1 and mTORC1, left at room temperature for 2 h until the blood is frozen, centrifuged, and separated the insertion serum into two cup samples and stored at −20°C.

Instruments

The kit used for examining levels of FoxO1, mTORC1, survivin, and IL-17 is a Human FoxO1 ELISA kit from Elabscience (catalog no: E-EL-H1099), Human RPTOR ELISA kit from FineTest (catalog no: EH1810), Human survivin ELISA kit from Elabscience (catalog no: E-EL-H1584), and Human IL-17 Immunoassay Quantikine ELISA kit (catalog no: D1700, S1700, PD1700). The inspection procedure is carried out according to the instructions of the kit used.

Ethical approval

Before conducting the research, an ethical agreement was requested from the Research Ethics Committee of the Medical Faculty of Andalas University, Padang, Indonesia. This study was carried out ethical review tests at the Medical Faculty of Andalas University in Padang, Indonesia, and passed the Ethics Study on February 5, 2018 (No: 084/KEP/FK/2018).

Data analysis

Statistical analysis using SPSS software and normality tests using the Kolmogorov–Smirnov and Shapiro–Wilk tests. The results of data that are normally distributed, FoxO1, mTORC1, and survivin were analyzed by ANOVA parametric test. Data that are not normally distributed, namely, IL-17, are non-parametric tests using the Kruskal–Wallis test. Furthermore, the Bonferroni post hoc test was conducted to find out the groups that were significantly different and the level of significance received if \( p < 0.05 \).

Results and Discussion

Relationship between FoxO1 levels and severity of AV

Based on the results of the study in Table 1, it was found that the average level of FoxO1 decreased with an increase in the severity of AV. The lowest FoxO1 levels are found in the most severe AV degrees (Figure 1).

<table>
<thead>
<tr>
<th>Severity degree</th>
<th>n</th>
<th>FoxO1 Average ± SD (ng/ml)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>20</td>
<td>11.99 ± 12.37</td>
<td>0.006</td>
</tr>
<tr>
<td>Moderate</td>
<td>20</td>
<td>5.64 ± 3.87</td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>20</td>
<td>4.21 ± 3.87</td>
<td></td>
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</tbody>
</table>

FoxO1: Forkhead box class O1.

This is because the reduced FoxO1 causes the genes and nuclear receptors involved in the AV to be activated resulting in increased signal transduction mediated by androgen receptors (ARs), increased cell proliferation, induction of lipase sebaceous, and upregulation of inflammatory cytokines [5].

![Figure 1: Relationship between forkhead box class O1 levels and severity of acne vulgaris](image)

FoxO1 not only suppresses protein synthesis and cell growth but also lipid metabolism. FoxO1 regulates transcription factors for sterol response promoter element-binding protein 1 (SREBP1) lipid synthesis, suppresses the activity of peroxisomal proliferator-activated receptor \( \gamma \) (PPAR\( \gamma \)) and liver X receptor \( \alpha \) (LXRA\( \alpha \)), both of which help stimulate...
sebaceous gland lipogenesis and stimulate expression of p21 and p27 [9].

Growth factor stimuli and other stimuli activate the PI3K/Akt pathway, resulting in phosphorylation of the FoxO1 protein. Increased growth factors during puberty and a signal of growth factors that persist due to lifestyle stimulate FoxO1 release from the nucleus into the cytoplasm through the activation of the PI3K/Akt pathway. Signals mediated by growth factor receptors are integrated at the PI3K/Akt activation level which ultimately results in core FoxO1 deficiency. The active kinase act translocates into the nucleus and phosphorylates the nucleus FoxO1, which is released from the nucleus into the cytosol, where FoxO1 is broken down by 14-3-3 cytoplasmic proteins, in ubiquitinylation and then destroyed in proteasomes (proteasomal degradation) [5].

Decreased core O1 fork box class has an effect on AR activity, PPARγ, LXRα, and gene expression that controls the cell cycle (cyclin D1, D2, p21, and p27), matrix modulation matrix metalloproteinase, activity of the SREBP1c, regulation of insulin glucose transporter protein-4 sensitivity, innate and adaptive immune responses that regulate antimicrobial peptide synthesis, toll-like receptor-2 expression, and T-cell proliferation (total leukocytes count proliferation) [5,6].

FoxO1 links genetic, metabolic, and environmental factors in AV [7]. The concept of the core FoxO1 deficiency hypothesis in AV and the relationship of AV variants was first explained in AV that is exacerbated by the consumption of insulinotropic dairy products, carbohydrates that have a high glycemic index, smoking effects that stimulate AV, psychological stress, aneugenic effect of polyaromatic hydrocarbons, fibroblast growth effects mutant factor receptor-2 in Apert syndrome and nevus akneiformis, and AV-induced IL-1α in pyogenic arthritis, pyoderma gangrenosum, and acne syndrome [5].

The results of this study are in accordance with the research of Agamia et al. who used blood samples and tissue biopsy from lesions of patients with AV and normal skin in healthy controls found significant differences in the expression of FoxO1 and mTORC1 immunohistochemically between groups of AV patients and healthy controls. FoxO1 core expression in the healthy control group was higher than in the group of AV sufferers. Patients with high glycemic load consumption diets and sufferers with severe AV are significantly associated with lower FoxO1 core expression [7].

The results of this study prove the decline of FoxO1 in increasing AV degrees and provide insight into therapeutic strategies in the treatment of AV by increasing FoxO1 levels. AV trigger factors use the action by reducing FoxO1 levels. Retinoids, antibiotics, and dietary interventions will increase FoxO1 further normalize the increase in transcription of genes involved in AV [10].

**Relationship of mTORC1 levels with severity of AV**

Based on the results of the study in Table 2, it was found that the average mTORC1 level increased with an increase in the severity of AV (Figure 2). Increased mTORC1 levels will increase the severity of AV. This is because the increased activation of mTORC1 is able to activate lipogenesis and increase cell growth and proliferation. Mammalian target of rapamycin complex 1 positively regulates the activity of SREBP1, the main transcription factor of lipid biosynthesis. Mammalian target of rapamycin complex 1 regulates SREBP1 by controlling lipin 1, a phosphatidic acid phosphatase [9].

**Table 2: Relationship of mTORC1 levels with severity of acne vulgaris**

<table>
<thead>
<tr>
<th>Severity degree</th>
<th>n</th>
<th>mTORC1 Average ± SD (ng/ml)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>20</td>
<td>0.06 ± 0.02</td>
<td>0.002</td>
</tr>
<tr>
<td>Moderate</td>
<td>20</td>
<td>0.09 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>20</td>
<td>0.55 ± 0.80</td>
<td></td>
</tr>
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</table>

mTORC1: Mammalian target of rapamycin complex 1.

There are two mTORC1 activation pathways, namely, activation of Rheb GTPase through growth factor signals and high cellular energy levels, and translational inactive mTORC1 to active Rheb located in the endosome or lysosome compartment. Rheb activity is regulated by TSC1 and TSC2 proteins that form a functional heterodimer complex [10].

![Figure 2: Relationship of mammalian target of rapamycin complex 1 levels with severity of acne vulgaris](image)

mTORC1 is able to integrate various intra- and extra-cellular mediators such as growth factors (insulin, IGF-1) and energy signals (glucose, AMP/ATP ratio), control the adequate availability of amino acids, especially leucine, which is needed for its activation [6]. Insulin, IGF-1, long-chain amino acids, glutamine, and palmitate activate mTORC1. After the peak of puberty, the serum levels of IGF-1, the main growth hormone of puberty, decrease continuously. During puberty, mTORC1-mediated activation of nutrients overlaps with mTORC1 activation due to puberty (androgen/IGF-1), which triggers an AV epidemic [11].

Excessive activation of mTORC1 increases the secretion of androgen hormones and increases mTORC1 signaling from sebaceous follicles [12]. The results of this study are consistent with research by Agamia et al. who found significant differences in
FoxO1 and mTORC1 expression between groups of AV sufferers and healthy controls. mTORC1 is more expressed in the cytoplasm and nucleus in patients with high serum IGF-1. Patients with high glycemic load consumption and patients with severe AV were significantly associated with higher cytoplasmic mTORC1 expression [7]. However, it is different from Monfrecola et al. who found that mTORC1 increase was not related to AV severity [13].

Research by Monfrecola et al., on skin biopsy of AV lesions and non-lesions of AV patients and healthy controls, found that mTORC1 gene expression was significantly increased in AV patients in both lesions and non-lesions compared to healthy controls. However, the difference in increase in mTORC1 was not significant compared between AV lesions and non-lesions in AV patients. Ribosomal protein phospho-S6K1 is increased in both lesions and non-lesions of AV patients compared to healthy controls. Phospho-S6 ribosomal protein is one of the downstream regulators that are activated by mTORC1 and controls SREBP1 activity, which further increases AV lesions. Ribosomal protein Phospho-S6 increases in AV skin indirectly allowing the activation of mTORC1 and can increase lipogenesis [13].

The current research shows that increasing mTORC1 plays a role in the pathogenesis and increasing severity of AV so that the mTORC1 inhibitors are expected to be effective for the treatment of AV. In AV therapy, the pathway that stimulates mTORC1 activation needs to be passed down, either through drugs or dietary interventions. The recommended dietary interventions are reducing the total energy of glucose and fat; limiting insulin/IGF-1 signals from protein consumption of dairy products; and limiting total leucine [10]. FoxO1 is a negative regulator of mTORC1 activity. Increased Akt/mTORC1 signal increases sebocyte survival, growth, and lipogenesis which will increase the growth of Propionibacterium acnes and the formation of biofilms, hyperseborrhea, and dysseborrhea by increasing the release of free fatty acids which will stimulate inflammation [6].

**Relationship between survivin levels and the severity of AV**

Based on the results of the study in Table 3, it was found that survivin levels increased with increasing severity of AV. Increased survivin levels also increase the severity of AV (Figure 3). This is because excessive survivin will inhibit apoptosis, both in keratinocyte cells and sebocyte cells resulting in cell hyperproliferation. Increased keratinocyte cell proliferation is needed for the growth and maintenance of AV lesions. Increased survivin also inhibits sebocyte apoptosis so that it will increase the total amount of sebum, which is an important energy source for P. acnes growth. Increased P. acnes growth will increase P. acnes triglyceride lipase and free palmitate formation from sebum. Free P. acnes and palmitate are important signals that activate inflammatory NLRP3 so that they release cytokines such as IL-1α, IL-1β, IL-6, IL-8, and tumor necrosis factor-α (TNF-α) that cause the development of inflammatory lesions [6].

**Table 3: Relationship between survivin levels and the severity of acne vulgaris**

<table>
<thead>
<tr>
<th>Severity degree</th>
<th>n</th>
<th>Survivin Average ± SD (pg/ml)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>20</td>
<td>72.55 ± 33.61</td>
<td>0.016</td>
</tr>
<tr>
<td>Moderate</td>
<td>20</td>
<td>99.19 ± 38.22</td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>20</td>
<td>124.64 ± 81.03</td>
<td></td>
</tr>
</tbody>
</table>

Survivin acts as a pro-survival of sebocytes and keratinocytes. Survivin is expressed in keratinocytes from the basal cell layers of the interfollicular epidemis and human sebocytes [14]. Survivin plays a role in homeostasis by inhibiting apoptosis and regulating cell division, proliferation, and cell survival [15]. Excessive expression of survivin inhibits apoptosis. Apoptosis is a counterweight to proliferation, and decreased apoptosis is associated with epidermal hyperproliferation. Increased keratinocyte proliferation is a consistent picture of AV lesions and is needed for growth and maintenance of AV lesions [16].

**Figure 3: Relationship between survivin levels and the severity of acne vulgaris**

The results of this study are consistent with the research of Assaf et al. who examined serum IGF-1 and survivin levels, and their expression in skin tissue in 15 patients with active lesions and 15 patients with post-inflammatory scar tissue compared with 15 healthy control individuals showed that serum survivin levels were significantly higher in active AV and AV scarring compared to healthy controls. Serum survivin levels were significantly higher in the AV scar group than in the active AV group. Increased survivin can affect the sebaceous glands and perifollicular dermal tissue in the formation of scar tissue. Survivin expression is related to the pathogenesis of fibrosis in fibrotic diseases including the process of AV scarring. However, this study did not compare survivin levels with the severity of AV [15]. Sebocytes undergoing apoptosis reduce the total amount of sebum which is an energy source for the growth of P. acnes so that the growth of P. acnes is reduced. Decreased P. acnes decreases the amount of triglyceride lipase and the formation of free palmitic acid so that it will reduce inflammation activation which is ultimately expected to reduce the severity of AV. The development of new drugs with low-molecular-weight survivin inhibitors is expected to lead to new treatment options for AV with survivin regulation strategies.
**Relationship between IL-17 levels and the severity of AV**

Based on the results of the study in Table 4 and Figure 4, it was found a tendency to increase the average level of IL-17 with an increase in the severity of AV. In AV, IL-17 secretion from Th17 cells is obtained through differentiation of CD4 T cells with the help of cytokines induced by P. acnes, namely, IL-1β, IL-6, and tumor growth factor-β (TGF-β) [17], where cytokines are cytokines this pro-inflammation results from early inflammatory lesions of AV [18].

**Table 4: Relationship between IL-17 levels and the severity of acne vulgaris**

<table>
<thead>
<tr>
<th>Severity degree</th>
<th>n</th>
<th>IL-17</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>20</td>
<td>6.45 ± 1.71</td>
<td>0.708</td>
</tr>
<tr>
<td>Moderate</td>
<td>20</td>
<td>6.78 ± 1.99</td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>20</td>
<td>7.01 ± 3.36</td>
<td></td>
</tr>
</tbody>
</table>

IL-17: Interleukin-17.

Statistical test results showed no significant difference from the average IL-17 level based on the severity of AV (p > 0.05). These results indicate that inflammation has occurred in every degree of AV, even since the beginning of the AV process. Inflammation is one of the important factors that play a role in the pathogenesis of AV. Inflammation that occurs in AV is mainly induced by immunological reactions to P. acnes and these bacteria can increase the immune response in the sebaceous glands. P. acnes can trigger inflammatory responses and induce monocytes to secrete pro-inflammatory cytokines that play a role in initiating the formation of inflammatory AV lesions, such as IL-17, IL-1β, IL-6, TGF-β, IL-1α, IL-8, and TNF-α [17].

**Figure 4: Relationship between interleukin-17 levels and the severity of acne vulgaris**

The inflammatory process is often referred to as a secondary process that occurs in AV. Inflammatory lesions in the form of papules, pustules originate from lesions that are clinically referred to as non-inflammatory lesions, namely, comedones. Blackheads are clinically non-inflammatory lesions but, in fact, blackhead lesions have occurred microscopic inflammation, as evidenced by the discovery of IL-1α pro-inflammatory cytokines in comedones lesions [19].

In the development of AV lesions, follicular rupture occurs resulting in the release of P. acnes, sebum, and cellular debris into the dermis which increases inflammation. P. acnes or secreted cytokines can interact with immune cells in the dermis also affect the development of Th17. P. acnes can also initiate specific immune responses through the recruitment of T-cell populations, depending on the type of lesions and cytokines in their environment, the type of P. acnes, and the immune status of individuals [20].

In determining the severity of AV according to Lehmann criteria based on the calculation of the number of non-inflammatory lesions and the number of inflammatory lesions and total lesions so that in each group, mild, moderate, and severe AV degrees can contain both inflammatory and non-inflammatory lesions. In patients with AV, P. acnes was significantly increased compared to non-AVs. P. acnes can be found in inflammatory and non-inflammatory lesions. The number of P. acnes bacteria found is more in inflammatory lesions when compared with non-inflammatory lesions and an inflammatory lesion can provide a more suitable environment and more nutrients for P. acnes colonization so as to enhance the inflammatory process [21]. Clinically, blackheads are non-inflammatory lesions, but microscopically are inflammatory lesions as evidenced by IL-1α pro-inflammatory cytokines found in comedones lesions [22]. In blackheads, lesions can be found other cytokines such as TNF-α and CD + T-cells in large numbers [23]. Inflammatory cells (CD3 and CD4 T-cells) are also found to be increased in microcutaneous lesions with a number that is not much different when compared with pustular lesions [24].

These results are consistent with the study of Maulinda (2016) who used blood samples of patients with AV comedonal lesions and papulopustular showed that serum IL-17 levels of papulopustular type AV patients were no different compared with comedonal type AVs. Research by Kistowska et al., using biopsies and blood samples of moderate AV patients, showed that Th17 and Th17/Th1 cells induced by P. acnes can be found in peripheral blood cells of AV patients and in low concentrations in healthy individuals [25]. However, the research of Kistowska et al. did not compare with the degree of severe AV and mild AV. Pathogenesis of AV associated with various systemic and environmental risk factors shows that AV is not a simple disease. AV is influenced by various intrinsic, extrinsic, and genetic backgrounds. The possibility of polymorphism as a genetic factor was not investigated [25].

**Conclusion**

This study concluded that FoxO1 levels decrease at increasing severity. Conversely, levels of mTORC1, survivin, and IL-17 increase with increasing degrees of severity of AV.
Acknowledgment

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References

PMid:28249066

PMid:28919117

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PMid:26257240

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PMid:23731728

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