

The immunogenetic analysis of acne vulgaris

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Abstract: Polymorphisms that occur in the CYP1A1, CYP17 and TNF- α genes affects hyperkeratinization process, sebum production and inflammation in acne vulgaris. Polymorphisms of CYP1A1, CYP17 and TNF- α genes can be identified by using PCR and sequencing techniques. The aim of this study is to demonstrate the role of polymorphisms of CYP1A1, CYP17, and TNF- α genes and the interaction polymorphisms of CYP1A1, CYP17 and TNF- α genes to severe acne vulgaris. This study was conducted as an observational study with case-control method, in 64 patients with severe acne, and as controls 73 patients with mild acne and healthy people. Criteria based on Combined Acne Severity Classification. Biospesimen blood 1 ml taken from vena mediana cubiti then performed PCR and sequencing to determine the sequence of nucleotides in DNA fragments. The conclusion from the study shows that polymorphisms of CYP1A1, CYP17, TNF- α genes and the interaction polymorphisms of CYP1A1, CYP 17 and TNF- α genes is not a risk factor for severe acne vulgaris, but polymorphism of CYP1A1 gene is a risk factor for acne vulgaris.

Keywords: Severe Acne Vulgaris, Polymorphisms of CYP1A1, CYP17, and TNF- α genes, Interactions polymorphisms of CYP1A1, CYP 17 and TNF- α genes

1. Introduction

Acne vulgaris (AV) is a common skin disease, as well as on our society in Indonesia ⁽¹⁾. Acne does not threaten life but it often become a cosmetic problem in the form of severe AV resulting scar formation, and often leads to psychological complains, and even lead to lack of confidence in a person ^(1,2). In a study conducted by Abdel-Hafez et al (2009) demonstrated psychiatric symptoms such as anxiety, depression, paranoia, and psychosis related to AV and showed negative effect on the quality of life of patients ⁽³⁾.

Acne vulgaris is a chronic inflammatory pilosebaceous follicle disease, characterized by comedones, papules, pustules, nodules, and sometimes scars. Acne primarily affects the face, neck, upper trunk and upper arms ⁽⁴⁾. Acne vulgaris is mainly found in adolescence and adulthood, and often at a younger age or older ⁽¹⁾. In the United States this disease affects 40-50 million people each year ⁽⁵⁾, and the number of visits to the dermatologist are at 20% of all visits ⁽⁶⁾. From the medical record at several hospitals in Indonesia in 2008, obtained a high number of AV patients visits in some hospitals in Indonesia, among others, Dr Wahidin

Sudirohusodo Hospital there were 22 visits (21% of all AV patient visit), in Dr. Moh. Hoesin Palembang Hospital there were 20 visits (6% of all AV patients visit), and Dr. Sardjito Yogyakarta Hospital there were 97 visits (9% of all AV patient visit)

Acne can be caused by several factors, such as excess sebum production, follicular hyperkeratinization, proliferation of *Propionibacterium acnes* (*P. acnes*), inflammation, and genetics ⁽⁷⁾.

Several genes that may influence the occurrence of AV including gene CYP 1A1, CYP 17 and TNF- α . Research conducted in Germany showed that CYP 1A1 gene polymorphisms causing the lack of an active natural retinoids resulting in follicular hiperkeratinization resulting AV ⁽⁷⁾. CYP17 gene that encodes cytochrome P450c17 α is an enzyme in androgen biosynthesis that mediates the activity of steroid 17 α -hydroxylase and 17, 20-lyase. The presence of CYP 17 gene polymorphism affects the activity of the enzyme CYP 17 that will change the levels of androgen, progesterone and estrogen hormones. Androgens increase sebum production and follicular keratosis which plays an important role in the development of acne ⁽⁸⁾. Tumor necro-

sis factor-alpha (TNF- α) is one of the proinflammatory cytokines that initiate and regulate the flow of cytokines during the inflammatory response. The factors that cause changes in the production of TNF- α may influence the degree of inflammation. The existence of polymorphism in the TNF- α gene can increase the production of TNF- α and aggravate AV⁽⁹⁾. Expression of TLR2 and TNF- α by keratinocytes induced by *P. acnes* in acne inflammatory phase⁽¹⁰⁾.

The results of CYP 1A1, CYP 17 and TNF- α gene polymorphisms in AV is still very limited, and the results of research that has been done in Makassar who get results are not statistically significant between CYP 17 gene polymorphisms and progression of severe acne, prompted us to perform research on gene polymorphisms CYP 1A1, CYP genes 17 and TNF- α genes and their interactions in AV.

2. Material and Methods

This study is an observational study with case-control method, in which the cases were severe acne patients and the controls were patients with mild acne and healthy people. The study was conducted in the Department of Dermatology and Venereology Hasanuddin University, Wahidin Sudirohusodo Hospital and network hospitals, laboratory of Microbiology, Faculty of Medicine Hasanuddin University in Makassar, and laboratory Eijkman in Jakarta in March 2010-September 2011.

The study population was divided into two characters, namely the case with the following inclusion criteria: patients with severe acne based on Combined Acne Severity Classification criteria, those are assessed by a dermatologist and patients signed the informed consent. While the excluded case criteria were: patients with severe acne who received antibiotics and anti-inflammatory medication for 1 month, patients with severe acne who use hormonal contraceptives, patient with severe acne who smoke, patients with severe acne who are pregnant and breastfeeding. Inclusion criteria for the control group were patients with mild acne and healthy people on the same age with the case and agreed to and signed informed consent. As for the exclusion criteria for the control group were mild acne patients who received antibiotics and anti-inflammatory medication for 1 month, patients with mild acne who use hormonal contraceptives, patients with mild acne who are smokers, and patients with mild acne who are pregnant and breastfeeding. Determination of the sample based on the table of Roach et al, with OR = 3.5, the value of $\alpha = 0.05$, $\beta = 0.1$, and $f = 0.30$ is 56. In this study, severe acne cases collected amount 64 people and control 73 people.

Blood specimens were taken 1 mL of 64 patients with severe acne as cases and 73 patients with mild acne and healthy controls. The specimen will then be treated DNA isolation (sediment) for PCR testing. PCR method is prepared using DNA extract as a positive control and sterile distilled water as a negative control.

3. Results

The number of samples in this study were 137 people consisting of 53 males (38.7%) and 84 females (61.3%). The sample consisted of 64 patients with severe AV, 31 males (48.4%) and 33 females (51.6%) and 73 patients with mild AV and healthy individuals, 22 males (31.1%) and 51 females (69.9%).

Table 1 shows that from 64 people with severe AV, there were 31 (51%) who had a GG genotype, compared to 30 (50%) with mild AV and healthy individuals. GA genotype in severe AV are at 30% compared to 70% in mild AV and healthy individuals. While the AA genotype in severe AV 58.3% compared to 41.7% in light AV and healthy individuals (statistical test $p = 0.032$).

Table 1. Distribution of CYP1A1 genotype on the severe AV group and mild AV and healthy individuals group.

Groups	Genotype CYP 1A1			Total (%)
	GG (%)	GA (%)	AA (%)	
Severe AV	31 (50,8)	12 (30,0)	21 (58,3)	64 (46,7)
Mild AV and healthy individual	30 (49,2)	28 (70,0)	15 (41,7)	73 (53,3)
Total	61 (100,0)	40 (100,0)	36 (100,0)	137 (100,0)

Note. Pearson Chi-Square $p = 0,032$

Tabel 2 shows that from 64 people with severe AV, there were 20 (46,5%) who had CC genotype, compared to 23 (53,5%) with mild AV and healthy individual. TC genotype exist in 44,7% severe acne compared to 55,3% in mild AV and healthy individual. While the TT genotype 48.9% in severe AV compared to 51.1% of mild AV and healthy individuals (statistical test $p = 0.918$).

Table 2. Distribution of CYP17 genotype on the severe AV group and mild AV and healthy individuals group.

Groups	CYP 17 genotype			Total (%)
	CC (%)	TC (%)	TT (%)	
Severe AV	20 (46,5)	21 (44,7)	23 (48,9)	64 (46,7)
Mild AV and healthy individual	23 (53,5)	26 (55,3)	24 (51,1)	73 (53,3)
Total	43 (100,0)	47 (100,0)	47 (100,0)	137 (100,0)

Note: Pearson Chi-Square $p = 0,918$

Tabel 3 shows that from 64 people with severe AV, there were 3 (37,5%) who had AA genotype, compared to 5 (62,5%) with mild AV and healthy individual. GA genotype exist in 55,6% severe acne compared to 44,4% in mild AV and healthy individual. While the GG genotype 46.7% in severe AV compared to 53.3% of mild AV and healthy individuals (statistical test $p = 0.757$).

The results of analysis of the interaction between genotype, whether it is in the CYP 1A1, CYP 17, and TNF- α gene in severe AV, showed that the difference was not sta-

tistically significant.

Table 3. Distribution of TNF- α genotype on the severe AV group and mild AV and healthy individuals group.

Groups	TNF- α genotype			Total (%)
	AA (%)	GA (%)	GG (%)	
Severe AV	3 (37,5)	5 (55,6)	56 (46,7)	64 (46,7)
Mild AV and healthy individual	5 (62,5)	4 (44,4)	64 (53,3)	73 (53,3)
Total	8 (100,0)	9 (100,0)	120 (100,0)	137 (100,0)

Ket: Pearson Chi-Square $p = 0,757$

4. Discussion

In this study found that the group with the genotype GG, GA and AA give a substantial proportion of the occurrence of cases of acne vulgaris (Table 1), but the proportion of cases with genotype AA higher than genotypes GG and GA. GG and GA genotype at position that gives the proportion of controls more than the AA genotype, this is a question to the results in Table 1.

Combined genotypes GG and GA show properties AV protection against the occurrence of severe cases with ER of 0.85 (95% CI: 0.69 to 1.04). The fact shows the nature of which is not in line with expectations (GG and GA genotypes as risk factors). The answer may lie in the selection of mild AV were included in the control group, it may be the genotypes GG and GA as the initial trigger pain (mild AV) should be grouped together with severe AV. Further analysis of the patients with grouping mild and severe AV as cases and healthy individuals as controls. The results of the analysis between GG and AA genotype of CYP1A1 gene showed that individuals who have the GG genotype of CYP1A1 genes have ER 1.04 times (95% CI: 0.69 to 1.55) than individuals who have genotype AA CYP1A1 gene. The results of the analysis between GA and AA genotype of CYP1A1 gene showed that individuals who had GA genotype of CYP1A1 gene have ER 0.71 times (95% CI: 0.47 to 1.07) than individuals who have genotype AA CYP1A1 gene. The Further analysis showed true GG genotype as a risk factor and genotyping GA can not be proven as a risk factor for acne vulgaris.

Theoretically, groups with genotypes GG and GA CYP 1A1 gene has a natural retinoid levels were lower than in the group with AA genotype. This occurrence due to the increased activity of the enzyme in the metabolism and inactivation of ATRA to 4-oxo-retinoic acid, so that active natural retinoids will be reduced. Deficiency of active natural retinoids on the skin can cause abnormal differentiation and hiperkeratinization sebositis follicular canal (7). It is well known that vitamin A deficiency resulted in a variety of epithelial metaplasia is accompanied by an increase in cell proliferation and hyperkeratosis of the skin. From several studies in animals and infants with vitamin A deficiency, suggesting that vitamin A deficiency causes a disease re-

sembling follicular keratosis Darier and pityriasis rubra pilaris (11). Hiperkeratinization follicular epithelium is one important factor in the pathogenesis of acne (12).

Study conducted by Paraskevaidis et al in Germany about the role of CYP 1A1 gene polymorphisms in acne showed normal frequency of m2 mutations from adenine to guanine substitution (A to G) result a change of isoleucine to valine amino acid which increases the activity of the enzyme. Another study focused on the development of cancer bronchogenic also found the effect of the polymorphism of CYP 1A1 gene by increasing the activity of the enzyme (7).

Cytochrome P450c17 is an enzyme in the synthesis of sexual steroids. This enzyme catalyzes the 17 α -hydroxylation of pregnenolone and progesterone. Enzyme activity is required in the synthesis of androgens and estrogens. CYP17 gene is a gene that codes for this enzyme (13). Varian of polymorphic P450c17 gene (CYP17) encode this enzyme can lead to changes in the activity of CYP17 (14).

The role of androgens in the pathogenesis of acne has been known. (8). There is evidence that androgenic stimulation of sebaceous glands important role play in the occurrence of acne (15). However, the actual genetic reasons, how androgen effect on acne is still not clear (8).

CYP17 gene in this research is done as study by He et al, in which the results obtained homozygous frequency C allele CYP17 gene in a group of severe AV in men was significantly higher than the control group. this study can be concluded that the homozigosis CYP17-34C / C may increase the risk of severe acne in men (8).

In this study, mutations of CYP17 gene occur with nucleotide substitution T / C at the 5' untranslated region (UTR) CYP17 promoter. Alleged that the substitution T / C will increase the rate of transcription of this enzyme in the CC genotype due to increased activity of promoters (14) and the consequence is an excessive production of glucocorticoids (13). Fiegelson and Haiman et al get estrogen levels were higher in women with the A2 allele (allele C) CYP17 gene (16, 17).

In this study was found the group with CC and TC genotype are not a risk factor for severe AV if compared with the TT genotype, although the CC genotype had higher androgen levels. Research by Cibula et al did not show a positive relationship between androgen production and severity of acne in women over the age of 17 years (18). Another study examined the association between CYP17 gene polymorphism in women using PCR have also been carried out by Tian et al used 3 genotyping androgen-related gene CYP 17 and the results showed significant results between the genotypes of androgen is A2, A2 of the CYP17 gene in 34 bp where there is a single base polymorphism on changes from T to C in the untranslated region (UTR) of the CYP17 gene. The effect of increased risk of acne in women with higher levels of androgens (19).

This study found no statistical relationship polymorphism with severe AV because acne is a disease that is influenced by a variety of factors, mainly by environmental

factors.

In this study the use of TNF- α gene promoter at position -308 and the obtained results from 64 people with severe AV, there are 3 people (37.5%) who had AA genotype, compared with 5 (62.5%) with severe AV and healthy individuals. GA genotype present in 55.6% severe AV compared to 44.4% by mild AV and healthy individuals. While the GG genotype in severe AV compared with mild AV 46.7% and 53.3% of healthy individuals (statistical test $p = 0.757$). From these results it can be seen GG genotype frequency distributions predominantly found in the severe AV and mild groups and healthy individuals, and there was no significant difference in genotype distribution in both groups so that it can be interpreted that there is no relationship between genotype distribution of TNF- α with severe AV. This is consistent with research conducted Baz K et al (2008) in 113 patients with acne, 30 of them with severe AV and GG genotype distribution obtained in 16 (53.3%), genotype GA in 12 (40.0%) and genotype AA in 2 (6.7%) compared with 32 patients with mild AV GG genotype distribution of 16 (50.0%), genotype GA 15 (46.8%) and the AA genotype 1 (3.2%) with the results of statistical tests ($p = 0.463$) (9). Based on statistical tests, our study found no significant association between TNF- α gene polymorphisms with severe AV events ($p = 0.757$). This suggests that TNF- α gene polymorphism is not a risk factor for severe AV. In accordance with the results of the study in Turkey found the severity of acne vulgaris genotype was not associated with TNF- α . This can be caused by the expression of TNF- α genotype is associated with individual HLA (20) and is also influenced by the ethnic as found in the study by Baz K et al in several different ethnic, found also that the frequency of heterozygotes genotif G / A is very low on spare Asia (8-18%) when compared with research on European interest (27% -35.5%) (9).

In this study, all the results of sequencing compared to full sequencing TNF α gene promoter along the 605 base-pair (bp) referral of the gene bank using the BLAST program (blast-2) from the website: NCBI, and in our study with a sample of 64 patients with severe AV, we found 3 (4.7%) mutations at position -308. Thus the discovery of the G allele frequency in TNF- α gene promoter -308 dominant position when compared with the frequency of allele A. From the frequency distribution of genotypes, mutant AA genotype was found by 3 (4.7%) which is in line with research conducted in Turkey Baz K et al who found 4 (3.5%) of 113 patients with acne mutations and research conducted by Szabo K et al in Romania that found 4 (1.8%) of 229 patients with acne mutation (9, 21). Some research on the analysis of allele frequencies of TNF- α was reported by Szabo et al in position 1031 T> C, -857C> T, -863C> A, -308G> A and -238G> A suspected role in the development of the inflammatory reaction in acne vulgaris. Results obtained no significant differences in allele frequencies between cases and controls at the position -1031, -861, -238 SNPs, whereas at position -857 and -308 obtained significant results ($p = 0.010$) between allele C major and acne, as

well as the minor allele at position -308 increased in patients with acne. G nucleotide replacement in the TNF- α gene at position -308 are associated with increased transcriptional activity of TNF- α and increased levels of circulating TNF- α (22). Substitution of guanine (G) to adenine (A) is also associated with increased susceptibility suffering from chronic inflammatory diseases, including acne vulgaris (9). Some diseases are associated with polymorphism in the TNF- α gene at position -308 one of them is breast cancer (23), chronic hepatitis B (22) dan schizophrenia (24) have been published.

Various other related studies identified a single nucleotide polymorphism in the TNF- α gene promoter humans have been reported, and found an association between increased susceptibility to various diseases. Research in Chile by Cuenca J et al against TNF- α genotyping conclude that there is a difference in the spread of allele TNF- α and ethnic populations that have a high proportion of allele TNF- α is also likely to have increased the incidence of multiple metabolic diseases predisposing to chronic, degenerative diseases, inflammatory and autoimmune diseases. This indicates that there is a diversity of results of each minor alleles of several different tribes and populations and changes in one or two copies of chromosome 6 in the promoter region of the TNF gene cluster contained in the HLA class III genes may lead to increased risk of various diseases (25). From the results of our study and compared with some previous studies that have been published can be concluded that TNF- α gene polymorphism at position -308 in touch with a person suffering from acne vulgaris vulnerability, but not related to the degree of severity of acne vulgaris.

Therefore, further discussion is necessary to see the effects of retinoid therapy in severe AV group. Retinoid therapy is given in the form of topical tretinoin 0.05 to 0.1% and / or oral isotretinoin. The proportion of patients with severe AV who got improvement after retinoid therapy, with GG genotype of CYP1A1 gene 50%, while those with the AA genotype of CYP1A1 gene as much as 42.9%. This suggests that administration of retinoids in severe AV patients with CYP1A1 gene polymorphisms provide a better outcome than severe AV patients without CYP1A1 gene polymorphisms, which are consistent with the theory that the group with the GG and GA genotypes of CYP 1A1 gene has a lower natural retinoid levels than in group with the AA genotype, thus, giving retinoid provide improvements in severe AV patients with CYP1A1 gene polymorphism.

This study still remains questions that has not been completely answered. From the three gene polymorphisms studied, only further analysis of CYP1A1 gene polymorphisms showing the GG genotype of CYP1A1 gene as a risk factor for acne vulgaris. Theoretically, individuals with the GG genotype of CYP1A1 gene can be fall into active natural retinoid deficiency (7). Therefore, further discussion is necessary to see the effects of retinoid therapy in severe AV group. Retinoid therapy is given in the form of

topical tretinoin 0.05 to 0.1% and / or oral isotretinoin. The proportion of patients with severe AV who got improvement after retinoid therapy, with GG genotype of CYP1A1 gene 50%, while those with the AA genotype of CYP1A1 gene as much as 42.9%. This suggests that administration of retinoids in severe AV patients with CYP1A1 gene polymorphisms provide a better outcome than severe AV patients without CYP1A1 gene polymorphisms, which are consistent with the theory that the group with the GG and GA genotypes of CYP 1A1 gene has a lower natural retinoid levels than in group with the AA genotype, thus, giving retinoid provide improvements in severe AV patients with CYP1A1 gene polymorphism.

The proportion of patients who experienced severe AV have repair after receive retinoid therapy with CC genotype CYP17 gene by 60%, while those with the TT genotype CYP17 gene as much as 42.9%. That administration of retinoids in patients with severe AV CYP17 gene polymorphism provides better results. Notice, in the group of the CYP17 gene with the CC genotype have higher androgen levels (18). There is evidence to convince that androgenic stimulation of sebaceous have role play in acne (15). In vitro, isotretinoin and ATRA is a potent inhibitor of cell proliferation and lipid synthesis in human sebosit and retinoids that decrease the synthesis of triglycerides and free fatty acids in the culture sebosit (26), so that administration of retinoid is expected to provide improvements in patients with severe AV the CYP17 gene polymorphism.

The proportion of patients who experienced severe AV repair after receiving retinoid therapy with AA genotype of TNF- α gene as much as 33.3%, while those with GG genotype of TNF- α gene as much as 47.1%. That administration of retinoids in patients with severe AV with TNF- α gene polymorphism did not give better results than patients without severe AV TNF- α gene polymorphism. Theoretically, the G nucleotide substitution in the gene TNF- α -308 locations with nucleotide A in individuals with the TNF- α gene polymorphisms resulting in elevated levels of TNF- α can influence the degree of inflammation and severity of acne (9). The role of retinoids in the treatment of AV is through keratolytic activity and regulation of proliferation and differentiation to eliminate comedones (27) and inhibit the secretion of the sebaceous glands (28) thereby granting retinoids can not be expected to provide more improvement in patients with severe AV gene polymorphisms of TNF- α than patients without severe AV TNF- α gene polymorphism.

Overall the test results showed no significant probability value, however is still visible from the statistical test data showing the positive direction toward the severe AV.

5. Conclusion

CYP1A1 gene polymorphism is not a risk factor for severe AV but it is a risk factor for acne vulgaris. CYP17 gene and TNF- α polymorphism are not risks factor for severe AV. The interaction between gene polymorphisms of CYP1A1, CYP17 gene and TNF- α gene is also not a risk

factor for severe AV. It is need to perform a measurement of natural retinoids, the 4-oxo retinoic acid, the rate of sebum, the hormones androgen and TNF- α level in relation to the influence of CYP1A1 gene polymorphisms, gene CYP17 and TNF- α gene expression in the creation of severe AV. Should be examined other genes that may play a role in the creation of heavy AV.

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