CYP 1A1 GENE POLYMORPHISM IN ACNE VULGARIS PATIENTS IN MAKASSAR: A DESCRIPTIVE STUDY

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ABSTRACT
Cyp 1a1 Gene Polymorphism In Acne Vulgaris Patients In Makassar: A Descriptive Study

Acne vulgaris is a multifactorial disease caused by the presence of chronic inflammation in the pilosebaceous unit of the skin. Although it is considered to be an inherited disease, there are limited data supporting the involvement of genetic factor in its etiology. CYP 1A1 is by far the most active form and its natural metabolites are morphogenic for sebaceous gland. Forty two patients with acne vulgaris were included in the study. One microliter of blood sample were collected from each. Sequencing polymerase chain reaction (PCR-sequencing) assay was used for detection of the CYP 1A1 gene. The genomic collection observed with an explorative method and compared the genotype frequencies to its allele frequencies. The genotype distribution of this study was compared the genotype distribution of our study to other previous studies. The frequency of GG genotype was 33,3%, GA genotype was 40,5% and AA genotype was 26,2%. There was a significant relationship in genotype distribution between our study and the previous studies in German. There is a significant correlation between CYP 1A1 gen e polymorphism and acne vulgaris.

Key words: Polymorphisms, CYP 1A1, Acne Vulgaris

ABSTRAK
Polimorfisme Gen Cyp 1A1 pada Penderita Akne Vulgaris di Makassar: Studi Deskriptif

Akne vulgaris merupakan penyakit multifaktorial yang disebabkan oleh adanya inflamasi kronis pada unit pilosebasea. Walaupun diduga sebagai penyakit turunan, terdapat beberapa data yang mendukung peran genetik sebagai salah satu penyebabnya. sejauh ini CYP1A1 merupakan bentuk yang paling aktif dan bersifat morfogenik bagi kelenjar sebasea. Pada penelitian ini jumlah sampel sebanyak 42 akne vulgaris yang masing-masing diambil sampel darahnya sebanyak satu µL. Polymerase Chain Reaction-Sekuensing (PCR-sequencing) digunakan untuk mendeteksi adanya polimorfisme gen CYP 1A1, dalam penelitian ini mengamati kelompok genom dengan metode eksploratif dan membandingkan hasil frekuensi genotif dan frekuensi alelnya serta membandingkan kelompok sebaran genotif dengan penelitian sebelumnya. Pada kasus ditemukan alel GG sebanyak 33,3% alel GA sebanyak 40,5% dan alel AA sebanyak 26,2%. Terdapat hubungan signifikan pada kelompok sebaran genotif penelitian ini dibandingkan dengan kelompok penelitian sebelumnya di Jerman. Pada penelitian ini ditemukan hubungan yang bermakna pada polimorfisme gen CYP 1A1 pada kasus akne vulgaris.

Kata-kata Kunci : polimorfisme , CYP 1A1, akne vulgaris

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BACKGROUND

Acne vulgaris (AV) is a chronic inflammatory disease of pilosebaceous follicle typically affecting adolescents and adults.[1] Acne vulgaris can be characterized by the presence of comedone, papule, pustule, nodule and scar.[2-4] According to Combined Acne Severity Classification by Lehmann et.al. (2002), the severity of acne vulgaris can be classified into mild, moderate, and severe. It is considered mild when the number of comedone is less than 20, or inflammation lesions less than 15 or the total lesions less than 30 lesions, and considered severe when the number of comedone is more than 100, or the inflammation lesions is more than 50, or the total lesions is more than 125, or there are more than 5 cysts.[5] Acne vulgaris is a multifactorial disease. Some of the factors contributing to acne vulgaris development are the excessive sebum, abnormal follicular keratinization, proliferation of Propionibacterium acnes, inflammation, external and genetic factors.[1,2,4,6]

The involvement of genetic factor in pathogenesis of the disease was evidenced in twins. However, studies dealing with the genetic element are rare.[1,2] Study by Goulden et. al. indicated that familial factor was important in vulnerability to persistent acne.[7] Goulden et. al. suggested that genetic factors regulate the abnormal follicular keratinization and response of sebaceous gland androgen in individual with persistent acne.[8]

Diet doesn't have direct role of pathogenesis acne vulgaris and has become agreement among dermatologist during 30 to 40 years. Research of diet at acne vulgaris during the year 1906 to 1972 cannot conclude the role of diet at the pathogenesis acne vulgaris, and Cordain et al anticipates that diet which can induce hiperinsulinemia, food-stuff containing high glicemic rate can increase insulin like growth factor 1 (IGF1) and degradation of insulin like growth factor binding protein 3 (IGFBP3) that results hiperandrogenism, sebore, and hyperkeratosis folikuler [9-11] but we do not make further research about diet at this research

Retinol (vitamin A) and its active retinoid are important in epithelial differentiation.

Retinoid influences the proliferation and differentiation of cells. Retinoid could affect the proliferation of epidermal keratinocyte and sebosit.[12] Follicular hyperkeratinization can occur due to lack of active natural retinoid.[13]

One of the enzymes involved in the metabolism of vitamin A (retinol) is cytochrome P-450. Among this enzyme families, CYP 1A1 is the most active isozyme subfamily. Study indicated that CYP 1A1 gene polymorphism was associated to acne.[13]

The visits associated to acne vulgaris in 2007 was about 5.5% from total visits in dermato-venereology polyclinic of Wahidin Sudirohusodo Hospital, Makassar and increased to 7.8% in 2008.[14]

It is the objective of this study to find out the features of CYP 1A1 gene polymorphism in relation to acne vulgaris vulnerability, spesifically in Makassar, South Sulawesi, Indonesia, a region from which such studies have not been reported.

METHODS

This study was conducted in Dermato-Venereology Polyclinic of Dr. Wahidin Sudirohusodo Hospital and Laboratory of Faculty Medical of Hasanuddin University, involving 42 acne vulgaris patients with explorative study design.

The inclusion criteria of acne vulgaris cases was based on Combined Acne Severity Classification as examined by a dermatologist and patients agreed to sign the informed consent. Whereas, the exclusion criteria included mild acne patient receiving antibiotic and anti-inflammatory treatment within last month, pregnant acne patient, and breast-feeding acne patient.

One milliliter of blood specimen was collected from 42 acne vulgaris patients. The specimens would be subjected to DNA isolation (sediment) for PCR test. The PCR method was prepared using DNA extract as positive control and sterile aquadest as negative control. DNA extract in 1,5 µL was added into PCR mix containing: PCR product/super mix 22.5 µL plus Forward primer (40 pM) 0.5 µL and Reverse primer (40 pM) 0.5 µL resulting in total PCR mix volume of 25 µL.

PCR results were ready to run in agarose gel 2.5%. The obtained results were run in agarose gel 2% (NuSieve GTG Agarose) and the vi-sible band at UV light was cut and entered into 1.5 mL effendorf tube followed by purification using QIAquick PCR purification kit with the sequencing PCR technique was the plain PCR with its composition: Primer Forward : 5'TAGGAGTCTT-GTCTCATGCCT '3, Primer Reverse: 5'AAGAGGTGTAGCCGCTGCACT'3 (TIB Molbiol) 1 pmol. The PCR results were then precipitated before entered into sequencing system to identify its nucleotide sequences.

RESULTS

This study was aimed to find out the features of CYP 1A1 gene polymorphism in acne vulgaris patients. Forty two acne vulgaris patients documented in medical record of Wahidin Sudirohusodo and Educational Network of Hasanudin University hospitals were recruited to this study. A molecular examination was performed using PCR method to observe the DNA band from case group, and then continued with PCR sequencing to determine the nucleotide sequences in a DNA fragment.

Electrophoresis of PCR products in case group of acne vulgaris indicated the positive dominance in band target of 355 bp.

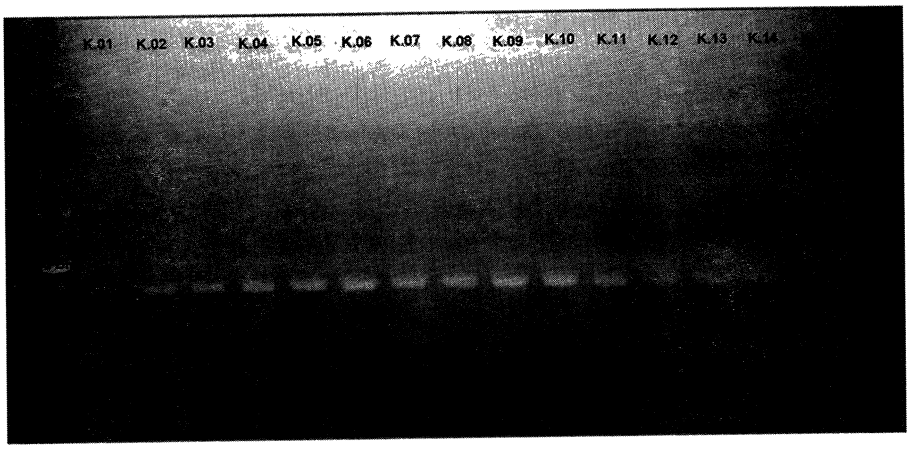

Figure: 1. Electrophoresis results of PCR product in acne vulgaris cases.

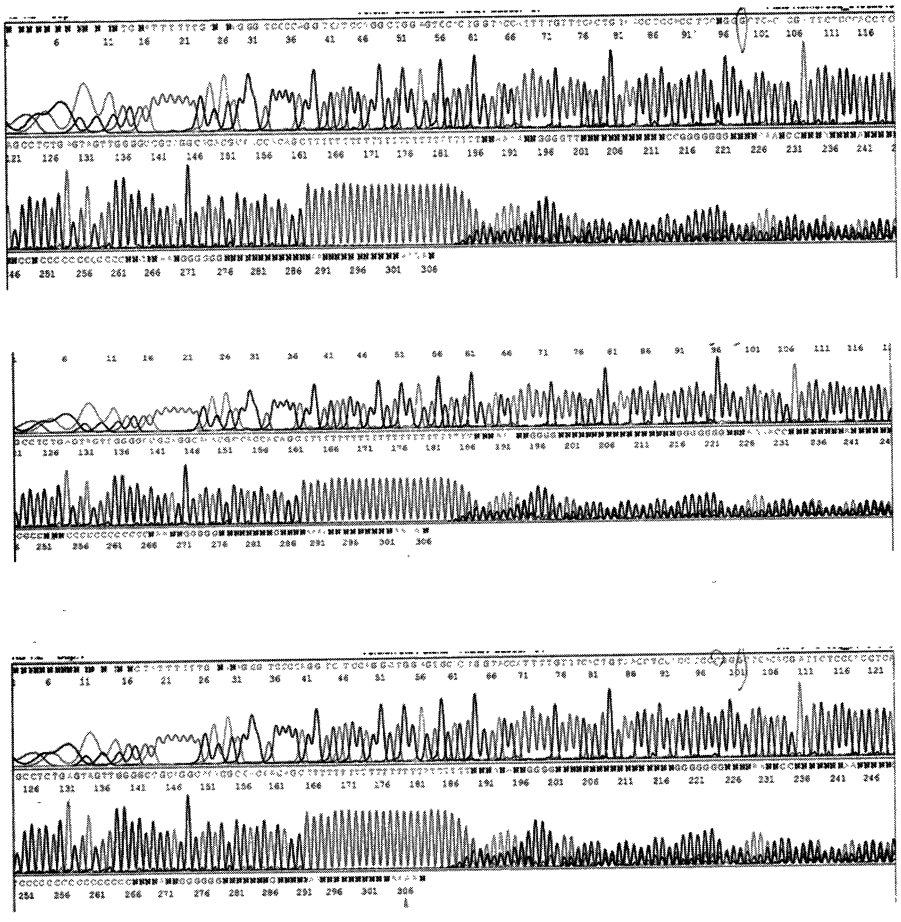

Fig: 2. Direct sequencing of PCR, derived from CYP 1A1 gene. a. Homozigous carriers of the polymorphic G allele b. Heterozigous carriers of the poly-

The number of G allele in the sample was 73.8%, dominant compared to allele A, thus A allele do not always reflect a healthy individual and G allele is not necessarily always a reflection of mild acne individual.

In the case group, the GG alleles were observed in 14 samples, GA alleles in 17 samples and AA alleles in 11 samples. There was a difference in

genotype frequency between homozigous group (GG) and heterozigous group (GA). Genotype frequency rate in homozigous group (GG) was 33.3% and in heterozigous grup (GA) was 40.47%, and the homozigous group (AA) was 26.1%.

In this study, all sequencing results were compared to full sequencing of 355 bp CYP 1A1 gene as a reference from the bank gen using BLAST program (basic local alignment search tool) (alert-2) from webside: NBCI.

DISCUSSION

CYP 1A1 gene was used in this study according to Paraskevaidis *et al.* (1998) study on 96 Germanic acne patients. The study showed normal frequency (*odds ratio* 1,02, *confidence limit* 0,41-2,52, p=0,96) of m2 allele (m2 mutation) in the form of adenine substitution by guanine (A to G). M2 mutation resulted in more active enzyme form. Previous studies focused on bronchogenic cancer also indicated that CYP 1A1 polymorphism resulted in higher enzyme activity.[10,12] In the current study, the frequency of GG genotype was 33.3%, GA genotype was 40.47%, and AA genotype was 26.1%. This increased GA and GG genotypes and decreased AA genotype indicated a significant difference. Statistical test was not performed in this study for it was a descriptive study.

CYP 1A1 enzyme is the most active from of cytochrome P-450 enzyme family oxidizing retinal into atRA. In addition, this enzyme is also involved in metabolism and inactivation of atRA into a more polar metabolite, 4-hydroxy and 4-oxo-retinoic acid. This 4-hydroxy and 4-oxo retinoic acid are more hydrophylic so the turnover and excretion of retinoid are higher. Therefore, there is a natural retinoid deficiency resulting in hyperkeratinization of follicular channel in acne development.[13]

Other studies have indicated the presence of CYP 1A1 polymorphism and a mutation in the exon 7 of CYP 1A1 gene, both were attributable to lung cancer vulnerability increase.[14]

Other studies which has been done by Wiwiek et al, has compared the habit of consuming risk diet between case and control group. It was observed that individuals with the habit of consuming risk diet in the case group was 60.6% and those without this habit was 12.5%, that the frequency of individuals with habit of consuming risk diet in the case group was 4,85-fold higher than those without this habit (p = 0.015). This indicated that the habit of consuming risk diet is a risk factor for the case group.[15]

Chocolate, oily and fatty diet, and high glucose diet are considered the inducing factor for acne development. The effect of fatty diet and insulin resistance are still controversial. Studies in animals almost virtually indicated insulin resistance in high fat diet, whereas in human it was less conclusive. Most studies indicated to relationship between fatty diet and insulin resistance. It has been reported that chocolate was not a significant inducing factor for acne; however the methods used in these studies were not adequate.[17]

Assumption that acne severity correlates to sebum secretion results in a hypothesis that diet with high fat and carbohydrate could induce the development of acne due to more comedogenic sebum production (serum fat level increased) that in turn block the pilosebaceuos follicle to produce follicle rupture and inflammation.[16]

To our knowledge, this research have got approval from the ethic com-

mittee of Hasanuddin University in Makassar and this is the first study reporting the CYP 1A1 gene polymorphism in acne vulgaris patients in Indonesia, particularly in Makassar, South Sulawesi province. Further studies will be needed to provide more informations in this field.

CONCLUSIONS

The frequencies of the CYP 1A1 homozygotes and heterozygotes G allele in the acne vulgaris group were significantly higher than that in the control group, suggesting that CYP 1A1 homozygosity and heterozygosity may increase the risk of developing severe acne. The polymorphism of the CYP 1A1 gene is one of the most likely loci in developing acne vulgaris in Makassar.

REFERENCES

1. Baz, K, Erdal, M, Yazici, A, Soymelez, F, Guvenec, U, Tasdelen, B, Ikizoglu, G. Association between tumor necrosis factor-alpha gene promoter Polymorphism at-308 and acne in Turkish patients. Arch Dermatol Res. 2008;300:371-6.
2. Zanglein, AL, Graber EM. Thiboutot DM, Strauss JS. Acne vulgaris and acneiform eruptions. In Wolf, K., Goldsmith, L. A., Katz, S. I. & Gilchrest, B. A. (Eds.) Fitzpatrick's Textbook of Dermatology. 7th ed. New York, Mc Graw Hill 2008
3. James WD, Berger TG, Elston, D. M. Andrews' disease of the skin clinical dermatology, Philadelphia, Saunders Elsevier. 2006a
4. Simpson N, Cunliffe W. Disorders of the sebaceous glands. In Breathnach, T., Cox, S. & Griffiths, C. (Eds.) Rook's textbook of dermatology. Massachusetts, Blackwell Science. 2007
5. Lehmann HP, Robinson KA, Andrews JS, Holloway V, Goodman SN. Acne therapy: a methodologic review. J Am Acad Dermatol, 2002;47: 231-40.
6. He L, Yang Z, Yu H, Cheng B, Tang W. The relationship between CYP17- 34T/C polymorphysm and acne in chinese subjects revealed by sequencing. Dermatology, 2006;212:338-42
7. Goulden, H. & Cunliffe, W. 1999a The familial risk of adult acne: a comparison Between first-degree relatives of affected and unaffected individuals. British Journal of Dermatology. 141: 297-300.
8. Ballanger F, Baudry P, N'Guyen JM, Khammari A, Dreno B. Heredity: A Prognostic Factor for Acne. Dermatology. 2006;212: 145-49
9. Cordain L, Lindeberg S, Hurtado M, Hill K, Eaton S, Brand-MillerJ. Acne vulgaris a disease of western civilization. Arch Dermatol. 2002;138: 1584-90.
10. Cordain L. Implications for the role of diet in acne. Semin Cutan Med Surg. 200524: 84-91.
11. Spencer E, Ferdowsian H. Barnard N. Diet and acne: a review of the evidence. Int J Dermatol. 2009;48: 339-47.
12. Gollnick H, Cunliffe W. Management of acne a report from a global alliance to improve outcomes in acne. J Am Acad Dermatol. 2003; 49: S1-38.
13. Paraskevaidis A, Drakoulis N, Roots I, Orfanos CE. Zouboulis C C. polymorphism in the human cytochrome P-450 1A1 Gene (CYP 1A1) as a factor developing acne. Dermatology, 1998;196:171-75.
14. CosmaG, Crofts, Currie D,Toniolo P. Racial differences in restriction fragment length polymorphism and messenger RNA inducibility of the human CYP 1A1 gene. Cancer Epidemiol., Biomarkers & Prev. 1993;2:53-7.

15. Dewiyanti W, Anwar AI, Tabri F, Massi N, Budu Bahar B. Peran polimorfisme gen CYP 1A1 pada akne berat. Makassar, Program Pascasarjana Universitas Hasanuddin. 2010
16. Batya B, Davidovici M, Wolf R. The role of diet in acne: facts and controversies. Clin Dermatol. 2010;28: 12-6.
17. Layton A. Disorders of the sebaceous glands. dalam Burns, T., Breathnach, S., Cox, N. & Griffiths, C. (Eds.) Rook's textbook of dermatology. 8th ed. Massachusetts, Wiley-Blackwell. 2010.