

The association between acne vulgaris and Tumor Necrosis Factor- alpha gene promoter polymorphism at position-308

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الخلاصة

أجريت هذه الدراسة على (100) مريض من مرضى حب الشباب بمختلف درجات الشدة (64 إناث و 36 ذكور) من الفئة العمرية (11-40 سنة) من مراجعي قسم الأمراض الجلدية في مستشفى الديوانية التعليمي للفترة من كانون الأول 2011 حتى شباط 2012، إضافة إلى (50) شخص سالم ظاهرياً كمجموعة تحكم.

تم جمع عينات من الدم لاستخراج الحمض النووي من الكريات البيض لكلتا المجموعتين للكشف عن وجود أي ارتباط بين تعدد الأشكال الجينية لعامل نخر الورم-ألفا في الموقع 308 من المنطقة المروجة والاستعداد للإصابة بحب الشباب باستخدام تقاعلات البلمرة المتسلسلة وتقنية الأجزاء المتكسرة المتعدد باستخدام إنزيم NCOI والتي تعطي أجزاء ذات حجوم جزيئية مختلفة تعبر عن الأصناف الجينية المختلفة.

أظهرت تقنية الأجزاء المتكسرة المتعدد ثلاثة اصناف جينية لعامل نخر الورم-ألفا في الموقع 308 وهي: صنف متمثل للواقع GG، والصنف المتخالف GA، ونوع متحول متمثل للواقع AA وكان تردد هذه الأصناف الجينية الثلاثة عند المرضى كالاتي: (29% ، 67% و 4%) على التوالي، إما في مجموعة الاصحاء فقد كانت كالاتي: (58%، 30% و 15%) على التوالي.

وجدت الدراسة ان هناك فرق احصائي مهم بين الصنف الجيني من نوع GA بين مرضى حب الشباب مقارنة مع الاصحاء ($P < 0.001$). إضافة الى العثور على ارتباط مهم بين الاليل A عند الاناث المرضى (40.6%) مقارنة بالاناث الاصحاء (28.8%) إضافة الى ان جميع النساء المصابات بحب الشباب الشديد وجدن انهن حاملات للنوع الجيني GA.

نتيجة هذه الدراسة تشير الى ان تعدد الأشكال الجينية لعامل نخر الورم من الممكن ان تسهم في زيادة الاستعداد للإصابة بحب الشباب مع ملاحظة وجود ارتباط مهم بين الاليل A الطفيف وشدة المرض عند الاناث.

Abstract

Background ; This study was carried out on (100) patients (64 females & 36 males) with different severity of acne vulgaris with an age range (11-40) year, seen in AL-Diwaniya Teaching Hospital/ Department of Dermatology, from December 2011 to February 2012, another (50) apparently healthy subjects were taken as a control group.

Materials; Blood samples were collected from both groups, genomic DNA was extracted from peripheral blood leukocytes for further molecular study, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique was used .

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Results; genotyping of TNF- α revealed three genotypes; the wild homozygous GG type, the heterozygous GA & the mutant homozygous AA type, the genotype frequency for these three types in acne patients was (29%, 67% & 4%) respectively, in control group, however, it was (58%, 30% & 12%) respectively. The frequency for GA type was found statistically significantly increased in acne patients compared to the healthy controls ($P < 0.001$), significant association was found between the minor A allele and females patients (40.6%) compared to healthy females (28.8%), P value 0.001, also all the female that had severe and very severe acne carry the GA genotype.

Key words : Acne, Tumor Necrosis Factor-alpha, polymorphism.

Introduction

Acne vulgaris is one of the most common chronic skin diseases affecting adolescents, with a prevalence rate of 70-87 % ,However acne can remain throughout adulthood in 8 % of the patients (1). Clinically, adolescent acne consists of a combination of noninflammatory (open and closed comedons) and inflammatory (papules , pustules, and nodules) lesions (2). Although acne is not a life threatening disease, but still can lead to serious physical (permanent scarring and hyperpigmentation) and psychosocial difficulties (depression, anxiety, anger, impairment in self image)(3).The pathogenesis of acne is currently attributed to multiple factors such as increased sebum production and alteration in its quality , increased androgen activity, follicular hyperkeratinization ,proliferation of *Propionibacterium acnes*(*P.acnes*) and exhibition of pro- and anti-inflammatory properties(4), the affected persons' genetic background may also play an important role in acne predisposition, some studies showed that 81% of the variance of the disease was attributable to additive genetic effects and the remaining 19% was attributable to environmental factors(emotional stress, drugs, food ,menstruation, smocking) (5). Tumor necrosis factor-alpha (TNF- α) is one of the main proinflammatory cytokines that plays an important role in initiating and regulating the cytokines cascades in the inflammatory process of acne (6), the gene encoding it is located on the short arm of chromosome 6(6p21.3) in the major histocompatibility class III region , where a high degree of genetic polymorphism in the promoter region is a characteristic feature (7). Several single-nucleotide polymorphisms (SNPs) in the TNF gene promoter have been identified, some of which may regulate TNF-alpha expression and enhance its production in excess and therefore affecting the overall immune response. One of these polymorphisms represents a guanine (G) to adenine (A) transition at position -308 (*TNFA-308 G/A*) which has been examined in several inflammatory diseases, many studies focused on this subject to enhance the understanding of the etiology and pathology of certain diseases and to identify

targets for therapeutic intervention (8), regarding acne vulgaris there is three studies worldwid concerned in this subject focusing on Turkish, Central Europeans and Polish populations only, similar study not present locally(9)(10)(11).

The aim of this study was to reveal any association between acne vulgaris and tumor necrosis factor α gene G/A polymorphism at site -308 in the promoter region.

Materials and methods

The current study was conducted on 100 patients (36 males ,64 females) seen in Al-Diwaniya Teaching Hospital /Department of Dermatology during December 2011 to February 2012. The patients were diagnosed clinically by a dermatologist as having acne vulgaris and according to the Global Acne Grading System (12),They were divided into: mild, moderate ,severe and very severe acne. Venous blood samples were collected in ethylenodiaminotetra acetic acid (EDTA) containing tubes. DNA was extracted from whole blood using a genomic DNA extraction mini kit(Genaid /USA) and Polymerase chain reaction–restriction fragment length polymorphism(PCR–RFLP) assay was used to determine *TNFA* -308 G/A polymorphism. AccuPower™ PCR PreMix Kit used to amplify the TNF-alpha gene , The primers used to determine this polymorphism were forward 5'-AGGCAATAGGTTTTGAGGGCCAT-3', reverse 5'-TCCTCCCTGCTCCGATTCCG- 3'(13).PCR thermocycling conditions were 2 min for initial denaturation at 95°C; 35 cycles at 95°C for 45 s for denaturation, 1 min at 60°C for annealing and 90 s at 72°C for extension, followed by 7 min at 72°C for final extension. The PCR products were 107 bp. After amplification, PCR products were digested by restriction endonuclease 10U *NcoI* (US Biological/USA) for 14 h at 37°C. The genotyping of the TNF-alpha gene was determined by fragment separation at 75 V for 50 min on a 2.5% agarose gel .A 100bp marker(Promega/USA)was used as a size standard for each gel lane. The gel was visualized under UV light using a gel electrophoresis visualizing system.

Results

The genotypes frequency in acne patients were as follow ; GG(29%), GA(67%) & AA(4%) ; while in the healthy subjects ;GG (58%) ,GA (30%), AA (12%),figure(1).

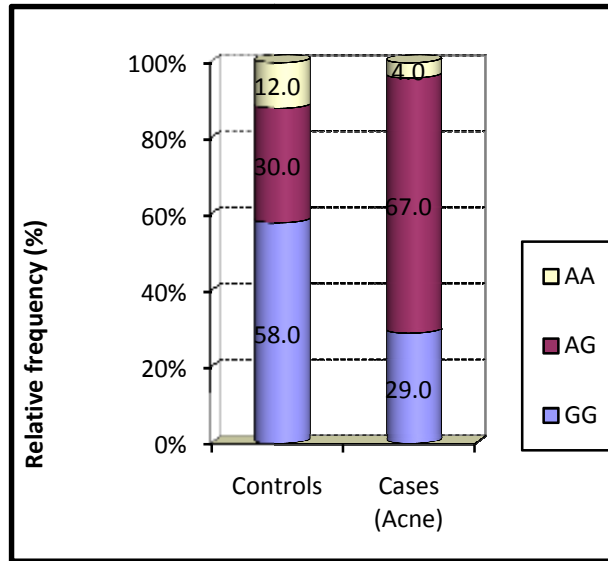


Figure (1): Component bar chart showing a case- control comparison in relative frequency of 3 selected genotype.

The GA genotype frequency was higher in acne patients (67%) compared to healthy controls (30%),the difference in distribution of TNF- α genotypes between patient and control groups were statistically significant, ($P < 0.001$, OR 4.74, 95% CI 2.3-9.9), as shown in table (1).

Table(1): Genotype distribution of TNF- α in acne and control subjects.

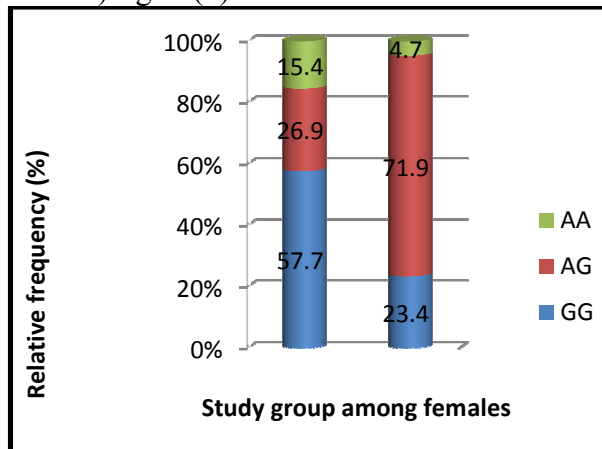
Genotypes	Controls		Cases (Acne)		OR	Chi	P
	N	%	N	%			
GG	29	58.0	29	29.0	0.30	11.356	.002
GA	15	30.0	67	67.0	4.74	17.225	0.001
AA	6	12.0	4	4.0	0.31	3.125	
Total	50	100.	100	100.0			

Regarding the GA genotype distributions in acne patients ,no gender-specific differences were detected, (P value=0.169) as shown in table (2).

Table (2):The risk of having a specific genotype in male gender compared to female among Cases (Acne) .

Genotype	Gender				Statistical analysis				
	Female		Male		OR	95% CI of OR	Chi	P value	
	N	%	N	%					
GG	15	23.4	14	38.9	2.08	(0.9 to 5)	2.626	0.105[NS]	
AG	46	71.9	21	58.3	0.55	(0.2 to 1.3)	1.890	0.169[NS]	
AA	3	4.7	1	2.8	0.58	(0.1 to 5.8)	0.214	0.644[NS]	
Total	64	100.0	36	100.0					

Comparisons between the study groups regarding the same gender, the data showed that there was a positive association between the minor-308 A allele and females with acne in which the number and frequency of this allele [52(40.6%)] in comparison with healthy females [11(28.8%)] (P value =0.001 ,OR= 6.94) in which 100% of the female with severe and very severe acne carrying the GA genotype, figure(2) ,where this was not the case in males (p value =0.061, OR=2.8) figure(3).

**Figure (2):Component bar chart showing a case –control comparison in relative frequency of the 3 selected genotypes in females.**

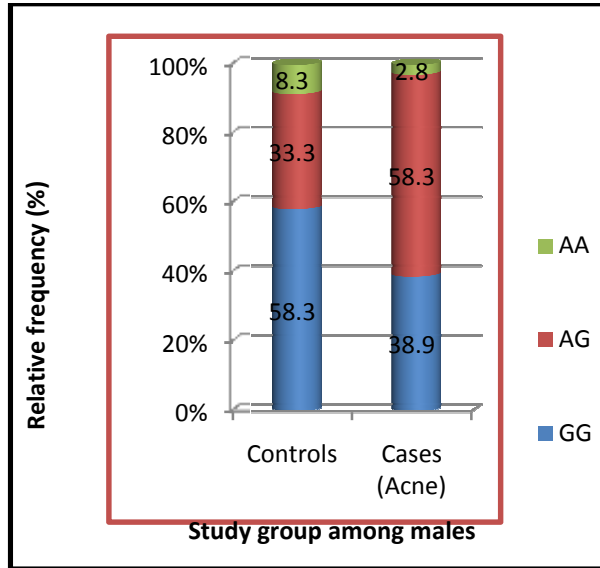


Figure (3):Component bar chart showing a case –control comparison in relative frequency of 3 selected genotypes in males

In this study no association was detected between the severity of acne and distribution of TNF- α GA genotype in acne patients in which 60% of mild cases, 76.2% of the moderate cases and 61.1% of the severe cases carried the GA genotype (P value=0.33) as shown in table(3).

Table(3) : The association between severity of Acne and genotype.

Genotype	Severity of Acne					
	Mild		Moderate		Severe/Very severe	
	N	%	N	%	N	%
GG	13	32.5	10	23.8	6	33.3
AG	24	60.0	32	76.2	11	61.1
AA	3	7.5	0	0.0	1	5.6
Total	40	100.0	42	100.0	18	100.0

P (Chi-square) = 0.33[Not significant]

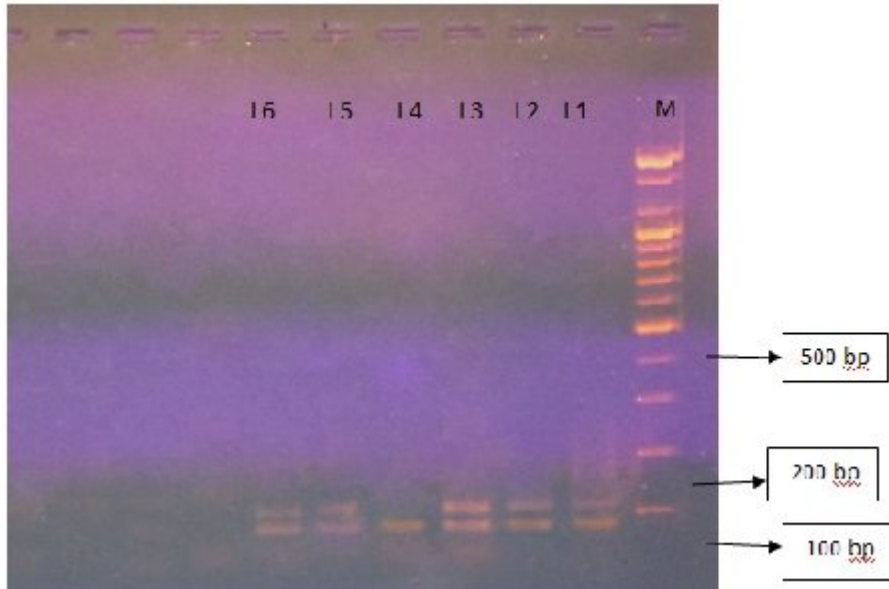


Figure (4): Ethidium bromide-stained agarose gel of PCR-RFLP amplified 107 bp region of TNF α gene. Lane (M) : DNA molecular size marker (100bp ladder) ,Lane 1,2,3,5,6 GA genotype when two DNA fragments seen 107 & 87 bp. Lane 4 GG genotype when only 87 bp fragment seen.

Discussion

The cytokine TNF- α is a key molecule in various biological processes, it has a dual role in acne pathogenesis, it is involved in the early stages in the initiation of lesion formation & regulation of the innate immune events, while in the later stages it has an important role in the control of inflammatory reaction & has been suggested to be associated with excessive inflammation and thus the immunopathology of acne vulgaris (11), also its misregulation can have deleterious effects on the tissue. In the current study direct comparison between TNF-genotypes and clinical features of the disease (susceptibility & severity) was investigated focusing on the biallelic single nucleotide polymorphism in TNF gene in the context of the promoter region. TNF α -308 GA genotype frequency was, statistically significantly increased in patients compared to controls (P value <0.001, odd ratio 4.74 & the etiologic fraction=0.529). This result was consistent with the results of Baz *et al.*(9) on acne vulgaris in Turkish population (p value <0.001 & OR=4.054), this increase in susceptibility to the disease may be due to the possibility that the A nucleotide containing allele may be responsible for a generalized increase in transcriptional activity compared to the -308 G allele and the synthesis of TNF α is known to be regulated, in part, at the transcriptional level (14)(15) There are also some studies which show an increased production of TNF- α

associated with TNFA-308 A allele and Patients with *TNFA-308 GA* heterozygosity have increased TNF-alpha production(15),but this result did not agreed with the study of Sabjanek *et al* .and Szabo *et al*.(10,11) that showed a lack of association between this SNP and acne susceptibility in polish and central European populations respectively, these differences may be explained by the difference in the linkage groups associated with TNF α -308 SNP in the geographically distal populations studied (11).

In the present study, no gender-specific differences was found in the distribution of genotypes in the patients group (p value =0.169) was found. These findings are compatible with findings of Baz *et al*.(9), that statistically, no difference exist between genders in the genotype frequency in patients'group (p value=0.518).However , significant association was discovered between the minor A allele and acne in female patients compared with females of the control group (p value=0.001, OR=6.94 & 95% CI 2.5 to 19.3). this was not the fact regarding the male (p value =0.061, OR=2.80, 95% CI, 1 to 8.2) moreover , the results showed that 100 % of the female patients that had severe and very severe acne carry the GA genotype ,these results compatible with result of Szabo *et al*.(11) in which significant association was found between the minor A allele and acne also a positive correlation was also detected between the degree of severity and the frequency of A allele in females in the Central European population. In the current study no statistical association was found between the GA genotype frequency and severity of the lesion, compared to the other genotypes (P value =0.33), this result agreed with the study of Baz *et al* .(9) .in view of acne is a multifactorial disease ,it is possible that the severity of acne may be readily influenced by other factors such as environmental and other genetic elements(16)(17).

Conclusions

The association between acne vulgaris susceptibility and Tumor Necrosis Factor-alpha (TNF- α)-308 G/A polymorphism can be demonstrated,a positive correlation was detected between the severity of the inflammatory symptoms and the frequency of the minor A allele in female patients.

Recommendations

1. Further studies on this subject with larger samples size are required.
2. Studying the effect of this biallelic base exchange polymorphism on TNF- α expression by measuring its level both locally within the black head comedon and in the serum of the patients may be helpful to clarify the disease.

3. TNF- α is one of the crucial pro-inflammatory cytokine involved in the pathogenesis of acne vulgaris ,extended studies to be proposed for the role of anti-TNF drugs as a novel treatment for those patients with chronic nodular acne who fail to respond to standard systemic therapies.

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