Investigation of Association Between Tlr2 And Tlr4 Gene Polymorphisms and Acne in Turkish Subjects.

Türk Toplumunda Tlr2 ve Tlr4 Gen Polimorfizmleri ile Akne Arasındaki İlişkinin Araştırılması

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Abstract

Background: In this study, it was aimed to evaluate the effects of growth pain on life quality in children and adolescents.

Methods: 30 patients diagnosed with growth pain and 30 healthy children as control group matched of age and gender were included in the study. Child Depression Inventory (CDI), State-Trait Anxiety Inventories for Children (STAI-C) and Pediatric Quality of Life Inventory Parent and Child Versions (PedQL-P and C) were applied to both patient and control groups.

Results: PedQL-P and C scores of children and adolescents with growth pain were statisticaly significance lower than control group ($p<0.01$). STAI-C and CDI scores of the patient were statistically significance higher than control group.

Conclusion: When compared to control group, lower life quality scale scores, increased anxiety levels tendency to depression and generally decreased life quality status were determined in growth pain group.

Key Words: Growth pain, child, adolescent, life quality

Özet

Amaç: Bu çalışmada büyüme ağrısı olan çocuk ve ergenlerde, rahatsızlığın yaşam kalitesine olan etkisinin değerlendirilmesi amaçlandı.

Metaryal ve metod: Büyüme ağrısı tanısı alan 30 hasta ile birlikte yaş ve cinsiyetleri eşleşmiş 30 sağlam çocuk kontrol grubu olarak çalışmaya dahil edildi. Gruplara, Çocuklar için Depresyon Ölçeği (ÇDÖ), Çocuklar için Durumluk-Süreklilik Kaygı Ölçeği (ÇDSKÖ) ve Çocuklar için yaşam kalitesi ölçeklerinin Ebeveyn ve Çocuk formları (ÇİYKÖ-E ve C) uygulandı.

Bulgular: Büyüme ağrısı olan çocuk ve ergenlerin, kontrol gruplarına göre ÇDSKÖ ve ÇDÖ ölçek puanlari yüksek bulunurken, ÇİYKÖ-E ve C formları puanları istatistiksel olarak anlamlı derecede düşük bulundu ($p<0.01$).

Sonuç: Büyüme ağrısı olan çocukların, kontrol gruplarına göre yaşam kalitesi ölçek puanlarında azalma, kaygı durumlarında yükseklik, depresyona meyil ve yaşam kalitelerinin genel olarak düşük olduğu tespit edildi.

Anahtar kelimeler: Fonksiyonel büyüme ağrısı, çocuk, ergen, yaşam kalitesi

Introduction

In recent years, research has led to a greater understanding of the pathogenesis of acne known as chronic inflammatory disease. The etiology of acne involve polygenic and genetic factors, Propionibacterium acnes (P. Acnes) infection, production of inflammation and enviromental influences (1). Three notable pathophysiological factors influence the development of acne: the increase of sebum production by the sebaceous gland, ductal hypercornification of the pilosebaceous follicle and the presence of P. acnes (1,2).

P. acnes is an anaerobic Gram-positive bacterium which has a proinflammatory activity and takes part in immune reactions modulating the Th1/Th2 cellular response. The main function of this process depends on monocyte derived (Mo-DCs) (3). The mechanism by which *P. acnes* activates monocyte cytokine release is unknown but is thought to involve (PRRs) of the innate immune system. Recently identified Toll-like receptors (TLRs) are one example of pattern recognition receptors (PRRs) (4). TLRs play a central role in determining the Th1/Th2 balance of immune responses and orchestrate the innate immune response by linking pathogen recognition with immune cell activation (5). In most situations, TLR activation promotes the generation of a Th1-dominated immune response and inhibits Th2 cytokine production (6).

In vivo TLR-2 and TLR-4 expression is increased in the epidermis of acne lesions (7). P. acnes induce activation of interleukin (IL)-12 p40 promoter activity via TLR2 and IL-12 and IL-8 protein production by primary human monocytes which is inhibited by anti-TLR2 blocking antibody; and the demonstration of TLR2 expression on the cell surface of macrophages surrounding pilosebaceous follicles taken from acne lesions. This data suggests that P. acnes triggers inflammatory cytokine responses in acne by activation of TLR2 (1, 8). TLR4 has been shown to be a major LPS-recognition receptor (9). LPS, the main component of the cell wall of Gram-negative bacteria, has been shown to elicit an inflammatory response *in vivo* and *in vitro* involving macrophages and monocytes (2, 10). TLR4 is a recognition molecule for Gram-negative pathogens and specifically LPS.

Arg753Gln (refSNP ID: rs5743708) polymorphism located within the signal transduction domain of TLR-2 was found to yield a nonfunctional receptor in in vitro assays (11, 12). Heterozygosity for the Arg753Gln TLR-2 SNP impairs the release of TNF-α and IFN-α (13). Indeed, it has been reported that the TLR2 Arg753Gln polymorphism is associated with susceptibility to tuberculosis (14), diabetes (12) and sepsis (11). TLR2 gene at position 677 (Arg677Trp) was associated with susceptibility to lepromatous leprosy (14). The human TLR4 gene harbours two important nonsynonymous single-nucleotide polymorphisms (SNPs) Asp299Gly (refSNP ID: rs4986790) and Thr399Ile (refSNP ID: rs4986791) reported to reduce LPS responsiveness (15). The biological relevance of these TLR4 SNPs has been widely investigated; individuals carrying the variant alleles are at increased risk of Gram-negative infections (16) and premature birth but are protected from atherosclerosis (17-18). Specific polymorphisms of TLR4 (Asp299Gly and Thr399Ile) are associated with airway hyporesponsiveness to inhaled LPS in normal human volunteers (19). Mice that contain mutations in TLR4 are hyporesponsive to LPS and are more susceptible to Gram-negative bacterial challenge (20).

It is tempting to speculate that the release of proinflammatory cytokines mediated through TLR2 has a harmful effect in acne by promoting inflammation and tissue destruction. Given these data, TLR2 and TLR4 may effect inflammatory

cytokine responses in acne and other inflammatory conditions in which tissue injury is detrimental to the host (4). TLR2 and TLR4 genes may be candidate genes that may have an influence on increased susceptibility to the development and severity of acne disease. We hypothesized that the variation in the TLR2 and TLR4 gene may be associated with decreased or abnormal immune response and predisposition to acne. The present aim was to investigate the possible contribution of the TLR2 and TLR4 gene polymorphism to susceptibility and clinical outcome of a etnically homogeneous Turkish acne patients.

Materials and Methods

Patients

The study population consisted of 100 patients with diagnosed acne who were undergoing acne treatment at the Departent of Dermatology, Firat University Medical Faculty during September 2005–October 2007. The control group was matched in age with the case group. All subjects were living in Elazig Province. The study was approved by the local ethics committee of the Firat University School of Medicine and written informed consent was obtained from all patients and control subjects.

All subjects were examined in our outpatient unit by dermatologists. The clinical grade of acne was assessed based on the Consensus Conference on Acne Classification (21). According to these criteria, mild acne is defined by the presence of comedones, without significant inflammation and a few or no papules, moderate acne by the presence of comedones, with marked inflammatory papules and pustules, and severe acne by the presence of inflammatory nodules, in addition to comedones, papules and pustules.

TLR2 and TLR4 Polymorphisms Genotyping

All patients underwent peripheral blood sampling for genotype analysis. Genomic DNA was isolated from peripheral blood by using Promega Wizard DNA Extraction Kit (Promega, USA). Genotyping was performed using RFLP for TLR4 Asp299Gly and TLR4 Thr399Ile polymorphism (Table 1). PCR reactions were run at 95°C for 4 min followed by 30 cycles with a 95°C 30 s, 62°C 30 s, 72°C 30 s profile. PCR products was digested with the appropriate restriction enzyme and electrophoresed in a 3% agarose gel. RFLP for TLR2 Arg753Gln and Arg677Trp polymorphism was performed according to the protocol provided by Sanchez et al (Table1) (14). PCR were performed under the following conditions: 4 min of initial denaturation at 94ºC, followed by 35 cycles of denaturation for 1 min at 94ºC, annealing for 1 min at 56ºC and extension for 1 min at 72ºC, with variation in the annealing temperature. Final extension was carried out for 10 min at 72ºC. PCR products were subjected to electrophoresis in a 3,5% agarose gel and stained with ethidium bromide. Amplification was carried out in a Eppendorf Thermal Cycler.

Statistical analysis

Data are presented as means with 95% confidence intervals (CI). Mann-Whitney-U-tests were used to compare continuous values. Fisher's exact tests were used to determine genotype distribution and dichotomous values. Student's t-test, and when necessary Mann-Whitney U test were used in the comparison of the means between the groups. After the assessment of normality assumption. A p-value < 0.05 was considered to be statistically significant. For all statistical tests, SPSS version 12.0 was used (SPSS Co. LEAD Techn., IL).

Results

We screened in the TLR2 and TLR4 genes polymorphism, and examined possible associations of these polymorphisms with acne patients in Turkish population. Of the 100 study patients, 58 were female and 42 male. Their mean±SD age was 21.9±4.46 yrs. All of the patients and controls were ethnically of

Turkish origin and living in Elazig city and its provinces. 30% of patients have a family history for acne disease . 43% of patients are associated with good response to the treatment. We observed a very low frequency of the Arg753Gln polymorphisms in our study population. The TLR2 Arg753Gln polymorphism A allele occurred in 11 (11%) of the 100 acne patients, whereas homozygous carriers of the A/A allele polymorphism not detected in the acne patients. The TLR2 Arg753Gln A allele occurred in four (4%) subjects in the control group, whereas homozygous carriers of A/A genotype were not detected in controls. There was no difference in TLR2 Arg753Gln polymorphism genotype and allelic distribution. between patients with acne and healty controls ($p>0.05$). The genotype and alleles frequency of the TLR4 polymorphisms were not statistically significantly in patients with acne than controls ($p>0.05$). Genotype frequencies of the patient and control groups are presented in Table 2. No significant association was observed between personality traits, inheritance, severity and etc, and TLR2 and TLR4 genotypes ($p>0.05$). Neither did we find a difference between female patient groups and the control group when the mild-,moderate- and severe-acne subgroups were considered separately. Treatment response was not statistically difference in patients having the Arg677Trp and Arg753Gln genotypes in TLR 2 gene or Asp299Gly and Thr399Ile genotypes in TLR4 gene.

Discussion

The microbiology of acne and its immunological implications are the main aim of the present research in the elucidation of the pathogenesis of the inflammatory acne lesions. In the present study we examined the effects of the TLR2 and TLR4 polymorphisms on clinical features in acne patients. It is first study the association between TLR2, TLR4 gene polymorphisms and acne in Turkish population. A significant difference was not found between with acne patients and healthy controls in both TLR2 and TLR4 polymorphisms genotype and allelic distribution.

The results of the reports in the literature of the association of TLR2 and TLR4 polymorphisms and acne are controversial. Tian et al (22). have studied in 93 acne vulgaris patients and 90 healthy subjects from the Chinese Han ethnic group to examine the association between acne vulgaris and the polymorphisms in the TNFR2 M196R as well as TLR2 Arg753Gln gene. They showed a significant difference in the frequency of TLR2 Arg753Gln genetic polymorphisms between the severe-acne subgroup and control group ($p < 0.05$).and stated that the 753Gln allele of TLR2 Arg753Gln are risk factors for acne vulgaris in Chinese Han patients (22). Koreck et al (10). have investigated TLR2 and TLR4 genotype distrubution in 63 acne vulgaris patients and 38 healthy subjects from the caucasians and reported that TLR2 and TLR4 polymorphisms were not significantly associated with the susceptibility to acne vulgaris (10). Our results were consistent with the report of Koreck et al that no association was found between TLR2,TLR4 genotypes and acne in Turkish population. One possible explanation is that such a difference may reflect the genetic differentietion between Causasians and the Han etchnic group.

Acne therapy may have its effect via TLR. Liu et al (23). demonstrated that the treatment of primary human monocytes with all-trans retinoic acid led to the down-regulation of TLR2 and its coreceptor CD14, but not TLR1 or TLR4 (23). Zinc salts could have a beneficial effect on mild and moderate inflammatory acne lesions. The increase of TLR2 surface expression in skin upon membrane fraction (FM) of P. acnes challenge was decreased as compared to that in control samples. However, this

inhibition does not modify IL-8 secretion by keratinocytes. Jarrousse et al (24). stated that the inhibition of TLR2 surface expression by keratinocytes could be one of the anti-inflammatory mechanisms of zinc salts in acne (24). Toll-like Receptor 2 (TLR2) expressed on mononuclear inflammatory cells and possibly on keratinocytes and sebocytes is thought to be of vital importance in mediating P. acnes-induced inflammatory response in acne vulgaris. Fathy et al (25). suggest that TLR2 expression on PBM is an important event in acne pathogenesis and targeting this molecule might be a useful therapeutic goal in the future (25). Kuuliala et al (26) reported that the polymorphic *TLR4* +896G allele may impair treatment response to single DMARD treatment in recent onset rheumatoid arthritis (26). Not only may current acne treatments at least partially have their therapeutic effect via TLR (23), but we not detected any association TLR2 and TLR4 genotypes and the response of different therapies.

The TLR4 receptor is the major transmembrane signaling receptor for LPS. Variant alleles of the TLR4 receptor affecting the immune response to LPS could lead to an increased susceptibility to P. acnes. The variant TLR4 receptor genotype would decrease the acute innate immune response to these pathogens, increasing the susceptibility to microbial invasion. It is also important to recognize that the potential functional relevance of these TLR4 SNPs has come under question. The initial description of the TLR4 SNPs demonstrated that individuals heterozygous for these polymorphisms have reduced airway responsiveness to inhaled LPS *in vivo*, and primary airway epithelial cells derived from heterozygous donors have a diminished LPS response *in vitro* (15). However, a number of recent studies have failed to reproduce these initial findings. Peripheral blood cells derived from individuals heterozygous and homozygous for the Asp299Gly and Thr399Ile TLR4 variants have been shown to respond to LPS in a fashion indistinguishable from wild-type controls (27-29). Hence, our inability to demonstrate an association between TLR4 polymorphisms and the risk of acne may reflect the fact that the Asp299Gly and Thr399Ile TLR4 SNPs do not modify the capacity of cells to respond to TLR4 agonists.

Asp299Gly and Thr399Ile polymorphisms frequently cosegregate, and the allelic frequency of these polymorphisms has been estimated at 3–11% of the general population (17,30,31). In our study, co-segregation was observed in 3% of controls and 4% of the susceptible individuals.

A number of previous studies have explored the occurrence of TLR2 polymorphisms in various diseased and healthy populations. The findings of these studies reveal marked variability depending on the ethnicity of the population scanned. While the Arg753Gln SNP has been reported exclusively in the Caucasian population, the Arg677Trp polymorphism has been observed in Asian and African populations (9,11, 20, 32) (Table 3). We found that the allelic frequency of the TLR2 Arg753Gln polymorphism gene (A allele) was 4% heterozygous in healthy Turkish controls. In separate studies, the Arg753Gln allele was observed among 10.34% (9), 12.3% (31), 4,7% (11) of healthy Turkish subjects, while the Arg677Trp was not observed. The low presence of this polymorphism in Caucasian population has been described, although a recent report found a higher frequency amongst whites, around 9% (20). The absence of the TLR2 Arg677Trp in the present study, which concurs with most of the Turkish studies (9, 11, 31) and is in contrast to studies in Caucasian or African populations, could mirror the ethnic relatedness of the populations being compared. The possible explanations for the divergent frequencies

of TLR2 genotypes may be found in populational, geographical, environmental and genetic heterogeneities, beyond methodological detection problems. Further data are needed to determine whether the polymorphic TLR2 gene shows an ethnically varying distribution.

We suggest that there is no evidence for an association of TLR2 and TLR4 genotypes with the clinical parameters of the disease. Because cases and controls were matched in age and gender, the difference between normal control and patients was not likely caused by these factors. Absence of an association between this specific polymorphism of TLR2 and TLR4 and acne does not eliminate the importance of TLR2 and its ligands in the aetiopathogenesis of this disease. Other reported or unidentified polymorphisms of TLR2 and TLR4 and many other factors affecting the function of TLR2 and TLR4 could be important in the occurrence and progression of this disease. In addition, the study designed the population-based and genetic polymorphisms often vary between ethnic groups, these results may not be generalizable to the general population. Althought, the number of the acne cases in our study compared with other studies was large, but the sample size was not enough to detect a small effect from very low penetrance genes or SNPs.

Nevertheless, the etiology of acne is multifactorial, and a few genes are related to the condition. Beyond genetic factors, the environmental factors also deserve investigation to understand acne etiopathogenesis. Thus, it is not likely that a single gene mutation would be the only cause of acne development. Evolutionary pressures would be expected to eliminate the mutated gene over time. It seems more likely that a combination of mutations rather than a single mutation contributes to the risk of acne development. Human cytochrome P450 1A1 gene, steroid 21-hyroxylase gene, epithelial mucin gene, CYP17 and androgen receptor gene have proposed several genetic markers of risk for acne development (35-39). The current challenge is to identify the combination of markers that are clinically useful in identifying and explaining the development of acne. It is hoped that identification of a panel of risk factors will eventually provide insight for future diagnosis and treatment of recurrent pregnancy loss. Further study on interactions between such genes and environmental factors would shed light on the possible genetic mechanisms of acne.

In summary, from our results, we did not observe any statistical association between moderate and mild acne and the polymorphisms. Since acne has the charasteristics of a multifactorial disease, the pentration of this phenotypes is more readily influenced by other factors than the severe acne phenotype, such as enviromental and other genetic elements (Li he). A case-control study was used to analyze for the first time the influence of TLR2 and TLR4 gene polymorphism on the predisposition and clinical characteristics of acne disease in Turkish population but provided no evidence for association with either disease in the population under study.

Table1 : Primers, PCR conditions and restriction enzymes for the restriction fragment length polymorphism

Gene 'olymorphism	Primer	Annea ling	Restriction enyzme PCR products
TLR2 Arg677Trp	5'-CCTTCAAGTTGTGTCTTCATAAC -3'. 5'-GGCCACTCCAGGTAGGTCTT-3'	56°C	HpaII Arg/Arg: 89bp Arg/Trp: 289bp+266bp+23bp Trp/Trp: 266bp+23bp
TLR2 Arg753Gln	5'-CCTTCAAGTTGTGTCTTCATAAC -3'. 5'-GGCCACTCCAGGTAGGTCTT-3'	56°C	PstI Arg/Arg: 89bp Arg/Gly:289bp+246bp+43bp Gly/Gly: 246bp+43bp
TLR4 Asp299Gly	5'-ATTAGCATACTTAGACTACTACCTCCATG -3' 5'-GATCAACTTCTGAAAAAGCATTCCC AC-3'.	62°C	NcoI Asp/Asp:249 bp Asp/Gly:249 bp+196bp+23bp Gly/Gly: 196bç+23bp
TLR4 Thr399Ile	5'-GGTTGCTGTTCTCAAAGTGATTTTGGGAGAA-3' 5'-CCTGAAGACTGGAGAGTGAGTTAAATGCT -3'	62°C	HinfI Thr/Thr: 406 bp Thr/Ile: 406bp+377bp+29 bp Ile/Ile: 377bp+29 bp

Table 2: TLR2 and TLR4 genotype distribution in acne (n=100) and controls (n=100) subjects.

Gene	Patients (n=100)	Controls (n=100)	X^2	p
TLR2 Arg753Gln				
Arg/Arg	89	96	3,5	0.06
Arg/Gln	11	4		
Gln/Gln	0	0		
TLR2 Arg677Trp				
Arg/Arg	100	100		
Arg/Trp	ND	ND		
Trp/Trp	ND	ND		
TLR4 Asp 299 Gly				
Asp/Asp	93	94	1	0.6
Asp/Gly	6	6		
Gly/Gly	1	0		
TLR4 Thr399Ile				
Thr/Thr	92	92	1	0.5
Thr/Ile	7	8		
Ile/Ile	1	0		

ND: Not detected.

Table3: Incidence of TLR-2 Polymorphisms Arg753Gln and Arg677Trp among the populations studied.

Author	Population	Polymorphism rate (%)	
		Arg753Gln	Arg677Trp
Ogus et al (11).	Turkish	4.7%	ND
Berdeli et al (31).	Turkish	12.3%0	ND
Schorder et al (32).	German	9.4%0	ND
Yoon et al (33).	Korea	ND0	ND
Ryu et al (34).	Korea	ND0	ND
Lorenz et al (9).	France	3%	ND

ND: not detected.

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