Specific Polymorphisms of IL-1β Genes (-511, +3953) and Their Probable Effects on IL-1β Production in Idiopatic Hypogonadotropic Hypogonadism

İdiopatik Hipogonadotropik Hipogonadizm Hastalarında IL-1β Genlerindeki Spesifik (-511, +3953) Polimorfizmler ve Bunların IL-1β Üretimindeki Olası Etkileri

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ABSTRACT Objective: In the present study, we aimed to determine a possible association of specific polymorphisms of IL-1β genes with enhanced production of IL-1β in patients with idiopathic hypogonadotropic hypogonadism (IHH). **Material and Methods:** Forty-five male IHH patients who had no familial relationship with each other and 58 ethnically matched healthy male controls were enrolled in the study. Patients were diagnosed with IHH according to failure in spontaneus puberty and decreased serum testosterone concentration below normal range for adults. Serum follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels were low or in normal range in patients with IHH. In this study, we analyzed the gene polymorphisms of IL-1β at positions -511 and +3953 and serum IL-1β levels in patients with IHH and control subjects. For this purpose, we used polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), and enzyme-linked immunosorbent assay (ELISA) methods. **Results:** The frequency of genotype 2/2 of IL-1β-511 gene was significantly higher in patients with IHH compared to healthy control subjects (24.4% vs 8.6%, respectively, p= 0.019). Although there was no significant difference in serum concentrations of IL-1β between total patients with IHH and healthy controls, the levels in the patients with the 1/2 genotype of IL-1β-511 and with the 2/2 genotype of IL-1β-511 were found significantly higher than the levels of patients with the 1/1 genotype of IL-1β-511. **Conclusion:** Our results indicate that IL-1β-511 gene polimorphism may be the cause of increased IL-1β production in patients with IHH.

Key Words: Interleukin-1beta; hypogonadism; cytokines; polymorphism, single nucleotide; hormones

ÖZET Amaç: Bu çalışmada, idiopatik hipogonadotropik hipogonadizm(IHH)'li hastalarda IL-1β genlerindeki spesifik polimorfizmler ile artmış IL-1β üretimi arasındaki muhtemel ilişkiyi otaya koymayı amaçladık. **Gereç ve Yöntemler:** Çalışmaya birbiriyle aile ilişkisi bulunmayan 45 IHH hastası ve etnik olarak bunlar ile uyumlu 58 sağlıklı erkek birey çalışmaya alındı. IHH tanısı spontan pubertenin olmaması ve normal erişkin değerlerinin altında serum testosteron konsantrasyonu bulunması ile konuldu. IHH'li hastalarda serum folikül uyarıcı hormon (FSH) ve luteinizan hormon (LH) düzeyleri düşük veya normal sınırlarda idi. Bu çalışmada IHH'li hastalarda ve normal bireylerde -511 ve +3953 pozisyonlarındaki IL-1β gen poliformizmlerini ve serum IL-1β düzeylerini analiz ettik. Bu amaçla polimeraz zincir reaksiyonu-"restriction fragment length polymorphism" (PCR-RFLP) ve enzyme-linked immunosorbent assay (ELISA) metodlarını kullandık. **Bulgular:** IHH'li hastalarda kontrol grubuna göre IL-1β -511 2/2 genotipinin frekansı anlamlı olarak artmıştır (Sırasıyla %24.4 ve %8.6, p= 0.019). Tüm hastalar ve sağlıklı kontroller arasında serum IL-1β konsantrasyonlarında önemli farklılık olmamasında rağmen, IL-1β-511'in 1/2 ve IL-1β-511'in 2/2 genotiplerini taşıyan hastalardaki düzeyler IL-1β-511'in 1/1 genotipini taşıyan hastalardan anlamlı derecede yüksek bulunmuştur. **Sonuç:** Bulgularımız IHH'lı hastalarda IL-1β-511 gen polimorfizminin, IL-1β üretimindeki artışının nedeni olabileceğini göstermektedir.

Anahtar Kelimeler: İnterleukin-1beta; hipogonadizm; sitokinler; çok biçimlilik, tek nükleotid; hormonlar

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It is well known that sex hormones have modulatory roles on immune functions and alteration of these hormone levels in circulation is associated with changes in some immunologic parameters.[1,2] The female preponderance to autoimmune diseases is proposed as an evidence for these findings.[3] For example, several autoimmune disorders such as systemic lupus erythematosus (SLE), Sjogren's syndrome, rheumatoid arthritis (RA), Hashimoto's thyroiditis and multiple sclerosis are more common in women than in men.[4,5] While male sex hormones have classically suppressive effects on immune functions, female hormones present a dual action by inhibiting cellular immunity, but enhancing humoral immunity.[6-8] Briefly, clinical and experimental studies showed that there are gender-related differences in immune response of both sexes.[9]

Idiopathic hypogonadotropic hypogonadism (IHH) is a clinically and genetically heterogeneous disorder caused by deficiency of gonadotrophin-releasing hormone (GnRH).[10] Patients with IHH may have small testicular size, low testosterone levels, infetrtility and no history of puberty. In previous studies, some similarities were found between immune status of females and men with IHH.[1,11] According to the findings of these studies, both humoral and cellular immunity measurements are higher in men with hypogonadism than those of healthy men.[11] Additionally, altered immunity may be modulated with gonadotropin therapy in male patients with IHH.[1,2] Although in the literature, there is a lot of accumulated data with regard to effects of sex hormones on immunity, it is not fully understood why autoimmunity is more common in female gender and hypogonadal men. For this reason, recent investigations have focused on explaining this enigma.

Cytokines are the most eleborately studied mediators for clarification of autoimmunity. Cytokine researchs showed that these peptides play a central role in the development of autoimmune diseases. However, it is clear that every cytokine has not equal importance in the pathogenesis of autoimmunity. On the other hand, proinflammatory cytokines such as interleukin (IL)-1b and tumor necrosis factor (TNF)a have a broad spectrum of immunomodulatory activities on immune responses.[12,13] It was demonstrated that these cytokines are involved in the T helper (Th)1/Th2 balance and autoimmunity.[14,15]

Because of gender-dependent variations in the immune response, we were encouraged to investigate immunologic status in hypogonadal men. Thus, we previously decided to perform a study including the effects of testosterone deficiency and gonadotropin therapy upon in vitro production of TNFa and IL-1b in peripheral blood mononuclear cells (PBMCs) obtained from patients with IHH in order to elucidate the modulatory role of androgen in cytokine production. Consequently, we found increased secretion of interleukin-1β (IL-1β) and TNFa in stimulated monocyte cultures in patients with idiopatic hypogonadotropic hypogonadism (IHH), and gonadotropin treatment restored in vitro production of both cytokines.[2] On the other hand, it was shown that IL-1β polymorphisms were reported to correlate with increased IL-1β expression by monocytes in response to stimulants.[16,17] Based on these results, in the present study, we aimed to determine a possible association of specific polymorphisms of IL-1β genes with enhanced production of IL-1β in the patients with IHH.

MATERIAL AND METHODS

Forty-five unrelated male patients with IHH and 58 ethnically matched healthy male controls were enrolled in the study. The patients were diagnosed according to failure of spontaneus puberty before 18 years of age, low serum testosterone concentrations, normal or low gonadotropin levels, absence of a pituitary or hypothalamic mass on computerized tomography (CT) or magnetic resonance imaging (MRI), presence of a gonadotropin response to repetitive doses of GnRH, and a normal karyotype (46, XY). None of the patients had hyposmia, anosmia, or a family history of IHH. All controls had a history of spontaneous puberty, and their physical and biochemical findings were within the normal range. This study was conducted in conformity to the Helsinki Declaration and approved by Ethics

Committee of Gülhane School of Medicine. The samples were obtained from the subjects after they had provided written informed consent.

Serum follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels were measured by an immunoradiometric assay with reagents from Radim Techland (Angleur, Belgium). Serum free testosterone (FT) was determined by a solid-phase 125I radioimmunoassay (RIA), using reagents from Diagnostic Products (Los Angles, CA). Serum sex hormone binding globulin (SHBG) was measured by RIA with reagents from Radim Techland. The normal ranges in our laboratory are <15 IU/l for FSH, <20 IU/l for LH, 15-45 pg/ml for FT, and 9-55 nmol/l for SHBG. A commercial Human IL-1b ELISA kit (Orgenium Laboratories, Helsinki, Finland) was used to test serum IL-1b levels (Assay Range: 3.9-250 pg/ml, sensitivity: <3 pg/ml).

We used polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) in order to analyse the cytokine gene polymorphism of IL-1β at positions -511 and +3953, in patients with IHH and in control subjects. For this purpose, 100 ng of genomic DNA was extracted from EDTA-anticoagulated peripheral blood by using QIAamp DNA Blood Kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's instruction and it was amplified using 0.5 μM of each primer, 1.0 U of AmpliTaqGold, 200 μM of dNTP, and 1.5 mM MgCl2. Two PCR reactions per polymorphic site were performed on each genomic DNA.

The primers 5′-TGG CAT TGA TCT GGT TCA TC-3′ (forward) and 5′-GTT TAG GAA TCT TCC CAC TT-3′ (reverse) and *Ava*I enzyme were used for screening the region containing a single nucleotide polymorphism (SNP) (C-to-T) at position -511 in the IL-1β promoter (304 bp). Amplified PCR products were visualized by 2% agarose gel electrophoresis with ethidium bromide staining. While the fragments of 190 and 114 bp were produced by *Ava*I in the presence of C allele (allele 1), there was not any digestion product in the presence of T allele (allel 2).

The primers 5'-GTT GTC ATC AGA CTT TGA CC-3' (forward) and 5'-TTC AGT TCA TAT GGA CCA GA-3' (reverse) and *Taq*I enzyme were used for the determination of SNP (C-to-T) in the IL-1β gene at position +3953 (249 bp). *Taq*I enzyme elicited two fragments of the 135 and 114 bp in the presence of C allele (allele 1), however no digestion product was observed in the presence of T allele (allele 2).

Statistical analyses of demographic data and serum parameters of patients and controls were performed using SPSS (SPSS 11.5, SPSS Inc., and Chicago, IL, USA) statistical package. The Kolmogorov-Smirnov test was used to assess whether ages and the levels of FSH, LH, FT, SHBG and IL-1β were normally distributed with a given mean and standart deviation. The differences between two groups were evaluated by Independent samples test or Mann-Whitney U Test, according to the normality of data distribution. Kruskal Wallis tests was used for multiple statistical comparisons. Then, Mann-Whitney U tests was used for post-hoc comparisons in order to determine which groups were significantly different from one another. Comparisons of the allele and genotype frequencies of patients and healthy controls were performed by using Chi-Square test with Yates' correction or Fisher's Exact test, when appropriate. The consistency of the genotype frequencies with the Hardy-Weinberg equilibrium was also determined. The calculations of odds ratios (OR) together with their confidence intervals (CI) were performed using a calculator, which is available at the website "http://www.hutchon.net/ConfidORselect.htm". A P value of <0.05 was considered as indicating a significant difference.

RESULTS

There was no significant difference between the patients with IHH and controls with respect to mean age (mean ± SD age: 22.3 ± 1.8 vs 22.7 ± 1.9 respectively). The mean levels of hormonal parameters are given in Table 1. While the mean levels of FSH, LH, and FT were significantly lower in the patients with IHH than those of controls, the levels of SHBG were significantly higher.

TABLE 1: Hormonal parameters and IL-1β levels of study groups.

	Patients with IHH*	Healthy Controls*		
	n = 45	n = 58	F	p**
FSH (mUI/ml)	0.47 ± 0.17	3.81 ± 1.03	80.229	<0.001
LH (mUI/ml)	0.89 ± 0.62	3.94 ± 0.98	8.278	<0.001
FT (pg/ml)	0.86 ± 0.45	31.32 ± 9.62	28.063	<0.001
SHBG (nmol/l)	45.40 ± 8.06	28.77 ± 3.73	25.777	<0.001
IL-1β (pg/ml)	12.93 ± 5.44	11.50 ± 5.30	0.001	0.170

FSH; follicle-stimulating hormone, LH; luteinizing hormone, FT; free testosterone, SHBG; sex hormone binding globulin, IL-1β; interleukin-1β. *Values are as arithmetic mean ± standart deviation notation. **Independent samples test was used for comparison of hormonal parameters and IL-1β levels between the patient and the control groups.

The distribution of the IL-1β -511 and +3953 alleles and genotype frequencies in patients with IHH and controls are summarized in Table 2. There were no statistically significant differences between the patients with IHH and healthy controls with regard to the frequencies of allele 1 and allele 2 of the IL-1β -511 gene. However, the frequency of genotype 2/2 of the IL-1β -511 gene was significantly higher in the patients with IHH compared with healthy control subjects (24.4% vs. 8.6%, respectively, p= 0.019). No significant difference was observed between the patients with IHH or controls with regard to both allele and genotype frequencies of IL-1β +3953 (p> 0.05).

There was no significant difference in serum concentrations of IL-1b between total patients with IHH and healthy controls (Table 1). Statistical comparisons of serum IL-1b levels were also performed in the subgroups of patient and healthy controls subdivided according to genotypes of IL-1b-511 and IL-1b + 3953. The serum levels of IL-1b in the patient subgroup with the 1/2 genotype of IL-1b-511 were not signicantly different from those of the levels of healthy control subgroup with same genotype (Table 3). When the patient subgroups with the different genotypes of IL-1b-511 were compared to one another, the serum levels of IL-1b in the patients with the 1/2 genotype and with the 2/2 genotype were significantly higher than the levels of patients with the 1/1 genotype. IL-1b levels of the patients with the 2/2 genotype of IL-1b-511 were also higher than the levels of patients with the 1/2 genotype of IL-1b-511. No significant difference in the levels of IL-1b was found among the subgroups of patients with the different genotypes of IL-1b + 3953 and among healthy controls with the different genotypes of IL-1b + 3953. Similarly, there was no significant difference between the subgroups of patient and healthy controls who had the same genotypes of IL-1b + 3953.

DISCUSSION

Our previous study had demonstrated an increased secretion pattern of IL-1b in the stimulated monocyte cultures prepared from peripheral blood of the patients with IHH.[2] Therefore, in the present study, we investigated the possible effect of IL-1β polymorphisms on increased IL-1β production in the patients with IHH. We have not encountered

TABLE 2: The distribution of the IL-1β -511 and +3953 alleles and genotype frequencies in patients with IHH and controls.

	Alleles (%)		Genotype (%)		
	1	2	1/1	1/2	2/2
IL-1β (-511)					
Patients with IHH (n= 45)	42 (46.7)	48 (53.3)	8 (17.8)	26 (57.8)	11 (24.4)*
Controls (n= 58)	58 (50.0)	58 (50.0)	5 (8.6)	48 (82.8)	5 (8.6)
IL-1β (+3953)					
Patients with IHH (n= 45)	54 (60.0)	36 (40.0)	10 (22.2)	34 (75.6)	1 (2.2)
Controls (n=58)	79 (68.1)	37 (31.9)	21 (36.2)	37 (63.8)	0 (0.0)

Chi-square analysis was used for comparisons of allele and genotype frequencies of the patients and controls. *P= 0.019 (OR= 3.42) between the patients with IHH and controls.

TABLE 3: The levels of IL-1β in the subgroups of patients and healthy controls according to the IL-1β -511 and +3953 genotypes.

Genotypes	Patients with IHH*	n	Healthy Controls*	n
IL-1β (-511)				
1/1	8.12 ± 4.48[a]	8	8.60 ± 3.84	5
1/2	12.57 ± 4.37[b]	26	11.25 ± 5.07[d]	48
2/2	17.27 ± 5.40[c]	11	16.80 ± 6.05	5
IL-1β (+3953)				
1/1	14.10 ± 6.27	10	12.57 ± 6.04	21
1/2	12.73 ± 5.25	34	10.89 ± 4.84	37
2/2	8.00	1	-	0

Kruskal-Wallis test was used for multiple comparisons of IL-1β levels of patient subgroups. Then, Mann-Whitney U tests were used as Post-hoc to Kruskal-Wallis. Independent samples test was used for comparison of patient and health control subgroups. P values were 0.003, 0.005, 0.012, 0.264 for [a] vs. [b], [a] vs. [c], [b] vs. [c], and [b] vs. [d], respectively.

any research on the relation between IL-1β polymorphisms and IHH in the literature. According to our results, while the genotype 2/2 of the IL-1β -511 gene associates with IHH in male patients, allelic frequencies of the IL-1β-511 gene in men with IHH are not different from those of healthy controls. Concordantly, increased serum levels of IL-1β were found in the patients with the 1/2 genotype of IL-1b-511 and with the 2/2 genotype of IL-1b-511 compared to the patients with the 1/1 genotype of IL-1b-511. However, there is no association between the IL-1β +3954 gene polymorphism and IHH in male patients. In addition, serum levels of IL-1b are not associated with the IL-1β+3954 gene polymorphism in the patients and healthy controls.

As mentioned before, it is well known that gender difference influences the development of autoimmune diseases due to different hormonal status. Similarly, we previously reported that both humoral and cellular immunity are enhanced in male hypogonadism and testosterone deficiency is the primary responsible factor for increased autoantibody production in Klinefelter's syndrome.[11] On the other hand, it has been proposed that IL-1β polymorphism is associated with inflammation and susceptibility to autoimmune disease such as RA, SLE and SS.[18,19] You et al.'s study is one of the leading investigations attempted to use sex stratification to examine gender specific cytokine polymorphisms in an autoimmune disorder, RA.[18] The authors suggested that the association of IL-1β-511 and IL-1β+3954 polymorphisms with susceptibility to RA appears to be significantly affected by gender.[18] In their study, the frequencies of the IL-1β+3954 allele and genotype in female patients with RA were found significantly higher when compared to the healthy controls; but in males, only the frequency of the IL-1β + 3954 allele was found statistically different when compared to the controls. However, in You et al.'s study, the frequencies of the IL-1β-511 allele and genotype in the patients with RA were not found different from that of healthy control subjects. In contrast with the study done by You et al., in Camargo et al.'s study, neither the infuence of IL-1β-511 nor +3954 polimorphism on susceptibility to RA was observed, and allele +3953T and the haplotype -511C +3953T were found to be protective for SLE.[19] The relationship between IL-1β-511 polymorphism (CT genotype and T allele) and SLE was also observed in African Americans but not in Whites from the Southeastern US.[20] A study showing an association of IL-1β-511 polymorphism with increased IL-1β production in inflammatory bowel disease was carried out by Nemetz et al.[21] In their paper, IL-1β-511T (allel 2) carriers were reported to be higher producers of IL-1β than IL-1β-511C (allel 1) carriers. A similar finding was observed in patients with systemic sclerosis (SSc) whore are carriers of at least one copy of the IL1β-511T allele since they have an increased risk of SSc.[22] When the results of last two studies were compared with ours, it may be proposed that enhanced production of IL-1β in men with IHH may be due to increased frequency of genotype 2/2 of the IL-1β-511 when compared to healthy controls.

All of these different results may be due to genetic differences in the studied population, besides the type of autoimmune disease and inadequate sample size. Additionally, the effects of sex difference and hormonal factors on modulation of immune response gene expression make this phenomenon more complex and the relationships among all these factors become more obscure. It was reported that the influence of gender on the incidence, progression and pathogenesis of many diseases are attributed, at least partially, to the influence of sex hormones on some cytokine gene expression.[23,24] Accordingly, the modifier effect of sex steroids upon humoral and cellular immune responses is the best evidence supporting these data.[25,26] The studies performed in the light of such information have shown that androgens and estrogens may stimulate the production of inflammatory cytokines in the synovial fluid of the patients with RA by influencing gene expression.[8,27] The accumulated data from those studies have allowed better understanding of the effects of sex and hormonal status on immune response.

Although there are limited number of studies related to gender-specific associations between cytokine gene polymorphisms and immunity,[23,28] this is the first study on IL-1β polymorphism in men

with IHH. According to our findings, IL-1β-511 gene polimorphism may be the cause of increased IL-1β production in the patients with IHH. However, the IL-1β gene polymorphism might not be the only factor of the IL-1β production in IHH. The other gene polymorphisms and genetic defects should be considered in the studies investigating the cytokine gene polymorphisms in IHH. It is probable that more detailed clinical and laboratory studies in the future will elucidate the relationship between the cytokine gene polymorphisms and IHH, and the consequences of this relation.

REFERENCES

1. Yesilova Z, Ozata M, Kocar IH, Turan M, Pekel A, Sengul A, et al. The effects of gonadotropin treatment on the immunological features of male patients with idiopathic hypogonadotropic hypogonadism. J Clin Endocrinol Metab 2000;85(1):66-70.
2. Musabak U, Bolu E, Ozata M, Oktenli C, Sengul A, Inal A, et al. Gonadotropin treatment restores in vitro interleukin-1beta and tumour necrosis factor-alpha production by stimulated peripheral blood mononuclear cells from patients with idiopathic hypogonadotropic hypogonadism. Clin Exp Immunol 2003;132(2):265-70.
3. Whitacre CC, Reingold SC, O'Looney PA. A gender gap in autoimmunity. Science 1999;283(5406):1277-8.
4. Ansar Ahmed S, Penhale WJ, Talal N. Sex hormones, immune responses, and autoimmune diseases. Mechanisms of sex hormone action. Am J Pathol 1985;121(3):531-51.
5. Grossman CJ. Interactions between the gonadal steroids and the immune system. Science 1985;227(4684):257-61.
6. Fimmel S, Zouboulis CC. Influence of physiological androgen levels on wound healing and immune status in men. Aging Male 2005;8(3-4):166-74.
7. Ansar Ahmed S, Dauphinée MJ, Montoya AI, Talal N. Estrogen induces normal murine CD5+ B cells to produce autoantibodies. J Immunol 1989;142(8):2647-53.
8. Cutolo M, Villaggio B, Seriolo B, Montagna P, Capellino S, Straub RH, et al. Synovial fluid estrogens in rheumatoid arthritis. Autoimmun Rev 2004;3(3):193-8.
9. Sperry JL, Minei JP. Gender dimorphism following injury: making the connection from bench to bedside. J Leukoc Biol 2008;83(3):499-506.
10. Pedersen-White JR, Chorich LP, Bick DP, Sherins RJ, Layman LC. The prevalence of intragenic deletions in patients with idiopathic hypogonadotropic hypogonadism and Kallmann syndrome. Mol Hum Reprod 2008;14(6):367-70.
11. Oktenli C, Yesilova Z, Kocar IH, Musabak U, Ozata M, Inal A, et al. Study of autoimmunity in Klinefelter's syndrome and idiopathic hypogonadotropic hypogonadism. J Clin Immunol 2002;22(3):137-43.
12. Nakae S, Komiyama Y, Yokoyama H, Nambu A, Umeda M, Iwase M, et al. IL-1 is required for allergen-specific Th2 cell activation and the development of airway hypersensitivity response. Int Immunol 2003;15(4):483-90.
13. Iwamoto S, Iwai S, Tsujiyama K, Kurahashi C, Takeshita K, Naoe M, et al. TNF-alpha drives human CD14+ monocytes to differentiate into CD70+ dendritic cells evoking Th1 and Th17 responses. J Immunol 2007;179(3):1449-57.
14. Chen Z, O'Shea JJ. Th17 cells: a new fate for differentiating helper T cells. Immunol Res 2008;41(2):87-102.
15. Elenkov IJ, Iezzoni DG, Daly A, Harris AG, Chrousos GP. Cytokine dysregulation, inflammation and well-being. Neuroimmunomodulation 2005;12(5):255-69.
16. Buchs N, di Giovine FS, Silvestri T, Vannier E, Duff GW, Miossec P. IL-1B and IL-1Ra gene polymorphisms and disease severity in rheumatoid arthritis: interaction with their plasma levels. Genes Immun 2001;2(4):222-8.
17. Mark LL, Haffajee AD, Socransky SS, Kent RL Jr, Guerrero D, Kornman K, et al. Effect of the interleukin-1 genotype on monocyte IL-1beta expression in subjects with adult periodontitis. J Periodontal Res 2000;35(3):172-7.
18. You CG, Yin YS, Xie XD, Ju J, Wang ZP, Chen YR. Sex influences on the penetrance of IL-1beta and IL-1RN genotypes for rheumatoid arthritis in the Chinese population. J Int Med Res 2007;35(3):323-8.
19. Camargo JF, Correa PA, Castiblanco J, Anaya JM. Interleukin-1beta polymorphisms in Colombian patients with autoimmune rheumatic diseases. Genes Immun 2004;5(8):609-14.
20. Parks CG, Cooper GS, Dooley MA, Treadwell EL, St Clair EW, Gilkeson GS, et al. Systemic lupus erythematosus and genetic variation in the interleukin 1 gene cluster: a population based study in the southeastern United States. Ann Rheum Dis 2004;63(1):91-4.
21. Nemetz A, Nosti-Escanilla MP, Molnár T, Köpe A, Kovács A, Fehér J, et al. IL1B gene polymorphisms influence the course and severity of inflammatory bowel disease. Immunogenetics 1999;49(6):527-31.
22. Mattuzzi S, Barbi S, Carletto A, Ravagnani V, Moore PS, Bambara LM, et al. Association of polymorphisms in the IL1B and IL2 genes with susceptibility and severity of systemic sclerosis. J Rheumatol 2007;34(5):997-1004.
23. Baynam G, Zhang G, Khoo SK, Sly P, Holt P, Goldblatt J, et al. Gender-specific effects of cytokine gene polymorphisms on childhood vaccine responses. Vaccine 2008;26(29-30):3574-9.
24. Bottema RW, Reijmerink NE, Koppelman GH, Kerkhof M, Postma DS. Phenotype definition, age, and gender in the genetics of asthma and atopy. Immunol Allergy Clin North Am 2005;25(4):621-39.
25. Polan ML, Daniele A, Kuo A. Gonadal steroids modulate human monocyte interleukin-1 (IL-1) activity. Fertil Steril 1988;49(6):964-8.
26. Homo-Delarche F, Fitzpatrick F, Christeff N, Nunez EA, Bach JF, Dardenne M. Sex steroids, glucocorticoids, stress and autoimmunity. J Steroid Biochem Mol Biol 1991;40(4-6):619-37.
27. Cutolo M, Seriolo B, Villaggio B, Pizzorni C, Craviotto C, Sulli A. Androgens and estrogens modulate the immune and inflammatory responses in rheumatoid arthritis. Ann N Y Acad Sci 2002;966:131-42.
28. Lio D, Scola L, Crivello A, Colonna-Romano G, Candore G, Bonafè M, et al. Gender-specific association between -1082 IL-10 promoter polymorphism and longevity. Genes Immun 2002;3(1):30-3.