

Association of Transforming Growth Factor-Beta Gene Polymorphisms in Recurrent Aphthous Stomatitis

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Abstract- This study has been conducted to evaluate the allele, genotype and haplotype frequencies of the polymorphic gene coding TGF- β in recurrent aphthous stomatitis (RAS). TGF- β gene typing was done by polymerase chain reaction with sequence-specific primers (PCR-SSP) assay. Allele frequencies were estimated by direct gene counting. C allele at codon 25 was significantly increased, while G allele at this position was significantly decreased in patients compared to the controls. A significantly higher frequency of CG genotype at codon 25 was found in control group. CC genotype and TT genotype at codon 10 of the gene was significantly decreased, while CT genotype at the same position was significantly increased in patients, indicating that CT heterozygosity at codon 10 TGF- β is associated with greater risk of RAS. CG and TG haplotypes were significantly decreased while CC and TC haplotypes were significantly increased in patients compared with controls. This study indicates the TGF- β single nucleotide polymorphisms could play a role in RAS pathogenesis. Thereby certain SNPs of TGF- β gene have an association with RAS pathogenesis.

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Introduction

Recurrent aphthous stomatitis (RAS) is the most common oral inflammatory disease, which is an ulcerative painful condition of the oral cavity. It is characterized by recurrent episodes of small painful ulcerations (1). Three of clinical manifestations have been described for RAS: minorRAS (MiRAS), major RAS (MaRAS), Herpetiform Ulcers (HU). MiRAS is accounting for 80% of RAS patients, which usually occurs on the floor of the mouth and the buccal and labial mucosa. The ulcers are round and usually less

than 5 mm in diameter with an erythematous halo and heal within 10-14 days without scarring (2).

The precise etiology of RAS has remained occult, basic studies point to the pivotal role of some extrinsic factors in disease manifestation such as smoking and local trauma, while some intrinsic factors also have been reported in association with the disease including systemic diseases, allergy, psychologic stress, nutritional deficiencies and genetic factors (3). Some forms of immune dysfunction deemed to be critical to initiating an aberrant cytokine cascade leading to RAS ulcers (2).

Transforming growth factor-beta (TGF- β) is a

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regulatory cytokine which modulates immune and inflammatory cell response (4). TGF- β is known as an immunosuppressor cytokine (5), which antagonize the activity of interleukin (IL) 1, interferon gamma (IFN- γ), tumor necrosis factor alpha (TNF- α), and IL6. It plays a pivotal role in fine-tuning the unleash of cytokines and other inflammatory mediators (6,7,8). Lack of TGF- β secretion dysregulates the production of immunosuppressive cytokines including IL-4 and IL-10, leading to an abnormal proinflammatory cytokine cascade (9). Increased systemic production of IL-6 and TNF- α have been detected in RAS patients (10) and also elevated levels of IL-4, IFN- γ and TNF- α in RAS ulcers have been reported (11,12). Decreased level of TGF- β , have been detected in RAS (10).

Evidence support the involvement of genetic polymorphisms in the cytokine secretion. This study conducted to evaluate possible interference of single nucleotide polymorphisms (SNPs) of TGF- β gene in the MiRAS clinical profile. To our best knowledge, this is the first investigation of TGF- β SNPs in individuals with MiRAS.

Materials and Methods

Subjects

Five ml blood was obtained from sixty-four Iranian patients with MinRAS comprised 24 men and 40 women (range of 20-61 years) from Department of Oral Medicine in the school of dentistry of Tehran University of Medical Sciences. All patients were assessed by an oral medicine specialist and diagnosis of RAS was made based on accepted international clinical criteria (13). Twenty-three cases have less than three aphthous episodes per month, 41 cases had 3 or more aphthous episodes per month. One hundred and thirty-eight healthy controls were also enrolled in this study.

Smoking, history of systemic diseases such as Behcet's syndrome, Diabetes, PFAPA syndrome, HIV infection, exposure to radiation and drugs consumption, presenting pregnancy and periodontal diseases considered as exclusion criteria for both experimental and control groups.

The Ethical Committee of Tehran University of Medical Sciences approved this project. Written informed consent was obtained from all subjects included in this study before sampling.

Genotyping

DNA was isolated using the phenol-chloroform method. TGF- β gene typing was done by polymerase

chain reaction with sequence-specific primers (PCR-SSP) assay (PCR-SSP kit, Heidelberg University, Heidelberg, Germany), the method described in detail previously (14). Briefly, amplification was carried out, using a thermal cycler Techne Flexigene apparatus, and the presence or absence of PCR product was visualized by 2% agarose gel electrophoresis. After electrophoresis, the gel was placed on a UV transilluminator, and a picture for interpretation and documentation was taken. The allele, genotype and haplotype frequencies of TGF- β were determined.

Statistics

Data analysis was performed using SPSS statistical software package (version 15.0). Chi-square test was used to compare frequencies of alleles, genotypes, and haplotypes between patients and control groups. The odds ratio (OR) and 95% confidence intervals (CI) were calculated. Comparison of medians of quantitative variables was performed, using Mann-Whitney U-test. P of <0.05 was considered significant. Allele frequencies were estimated by direct gene counting.

Results

Alleles, genotypes and haplotype frequencies

TGF- β allelic, genotype and haplotype frequencies at codon 10 and codon 25 of the gene in RAS patients and healthy controls are tabulated in Table 1.

TGF- β codon 25 C allele was significantly (OR=11.36, CI=6.20-20.98, $P=0.0000$) increased among patients while cytokine G allele at the same position was significantly decreased (OR= 0.09, CI=0.05-0.16, $P=0.000$) in RAS patients compared with control group. This was reflected in the significantly increased number of CG genotype (OR=206.41, CI=43.22-1348.59, $P=0.000$) at codon 25 of the gene in RAS patients while GG genotype at this position was significantly decreased (OR=0.01, CI=0.00-0.03, $P=0.0000$) in patients with RAS in comparison with controls.

No significant differences between alleles were found for TGF- β (C/T) at codon10, while CC genotype (OR=0.000, CI=0.00-0.51, $P=0.0043$) and TT genotype (OR=0.000, CI=0.00-0.35, $P=0.0005$) at this position were significantly decreased in RAS patients, CT genotype at the same position was significantly increased ($P<0.0001$) in patients compared with controls.

Comparison of TGF- β haplotypes between patients and controls indicated significant differences among two groups. CG haplotype (OR=0.51, CI=0.35-0.76,

$P=0.0007$) and TG haplotype (OR=0.31, CI=0.21-0.46, $P=0.0000$) were significantly decreased in patients, while CC haplotype (OR=3.96, CI=2.25-7.00,

$P=0.0000$), and TC haplotype ($P<0.0001$) were significantly increased in RAS patients.

Table 1. Comparison of alleles, genotypes and haplotypes frequencies of TGF-β between patients with RAS and the control group

Position	Alleles/ Genotypes/ Haplotypes	RAS (n=60), n(%)	Controls (n=138), n(%)	P	Odds Ratio (95% Confidence Interval)
Codon 10	C	60(50)	131(47.5)	0.722	1.11(0.70-1.74)
	T	60(50)	145(52.5)	0.722	0.90(0.58-1.42)
	CC	0(0)	20(14.5)	0.004	0.00(0.00-0.51)
	CT	60(100)	91(65.9)	<0.001	-
	TT	0(0)	27(19.6)	<0.001	0.00(0.00-0.35)
Codon 25	C	58(48.3)	21(7.6)	<0.001	11.36(6.20-20.98)
	G	62(51.7)	255(92.4)	<0.001	0.09(0.05-0.16)
	CC	0(0)	2(1.5)	0.484	0.00(0.00-9.49)
	CG	58(96.7)	17(12.3)	<0.001	206.41(43.22-
	GG	2(3.3)	119(86.2)	<0.001	1348.59)0.01(0.00-0.03)
Codon 10, Codon 25	CG	60(25.4)	110(39.9)	<0.001	0.51(0.35-0.76)
	TG	60(25.4)	145(52.5)	<0.001	0.31(0.21-0.46)
	CC	58(24.6)	21(7.6)	<0.001	3.96(2.25-7.00)
	TC	58(24.6)	0(0)	<0.001	-

Discussion

Although the association of several cytokines gene polymorphisms with RAS have been investigated (15-17) to our best knowledge, the association of TGF-β gene polymorphism with RAS has not been investigated yet. Lewkowicz *et al.*, have been reported the decreased level of TGF-β in RAS (10). TGF-β is a homodimeric, 25-kDa peptide, which is essential for immunologic self-tolerance as a suppressive cytokine (18). Also, it is a key cytokine involved in modulating immune and inflammatory cell response (4) and plays a critical role in regulating the secretion of immunosuppressive cytokines (9).

Considering that aberrant cytokine cascade is the culprit in damage of the oral mucosa leading to the formation of RAS ulcers (11) together with giving the multifaceted role of TGF-β in modulating various cytokines release, we investigated the genetic polymorphisms of TGF-β cytokine in MiRAS patients, while significant differences were found between patients and the control groups. A significant association with the codon 10 TGF-β TT and CC and CT polymorphisms were detected in our patients, where no significant differences were found for T and C alleles at this position. All the patients were heterozygote at this codon and CC, and TT genotype was not detected in patients. This indicates that CT heterozygosity at the codon 10 TGF-β is associated with greater risk of RAS.

In our investigation, the codon25 C allele occurred

with higher frequencies in patients, and G allele at this codon was significantly decreased in RAS patients. We found that GG genotype at codon 25 TGF-β gene was significantly decreased while CG genotype at the same position was significantly overrepresented in patients with RAS. The codon 25 CG genotype was the most common genotype in patients (96.7%), while the codon 25 GG genotype was the most prevalent genotype in controls (86.2%), and the majority of controls carry G allele (92.4%) at this position. Indicating that the G to C exchange at codon 25 of the TGF-β gene is associated with decreased TGF-β secretion .

All TGF-β haplotypes at the codon 10 and codon 25 were associated with RAS. CG and TG haplotypes were underrepresented in the patients group, while CC and TC haplotypes were overrepresented in the patients group. It is notable that TC haplotype was not found in healthy controls. It has been suggested that TG is the high production haplotype and TC is the low production haplotype of the TGF-β (19). Thereby the significantly lower frequency of the high production haplotype (TG) and the significantly higher frequency of the low production haplotype (TC) of TGF-β are responsible for the low production of TGF-β in patients with RAS (10). It has been hypothesized that the immune response and the cytokine profile in RAS indicate a Th1 type immune response (20). This has been confirmed by finding the overexpression of IFN-γ, TNF-α, and IL-2 and low expression of IL-5, IL-4, and TGF-β (10). Indeed decreased levels of TGF-β as an immunosuppressor cytokine leads to elevated levels of IL-1, IFN-γ, TNF-α

and IL-6, which have been detected in RAS patients (11,12,21). The results of our investigation indicate that greater risk of RAS occurs in individuals with CT genotype at the codon 10 and with CG genotype at the codon 25 of TGF- β gene, which leads to low production of TGF- β . Indeed more studies in other ethnic groups and larger populations are needed to confirm these findings.

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