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Genetic Predisposition of TNF-α (-308), TGF-β-1 (-508) and IL-35(EBI3) Gene Polymorphisms towards Rheumatic Heart Disease in the Population of Telangana from South India

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ABSTRACT

Background: Rheumatic Heart Disease (RHD) is a complex disease, subject to genetic and environmental factors. Cytokines play an important role in development and pathogenesis of the rheumatic heart disease. TNF-α, TGF-β and IL-35 gene polymorphisms may affect the expression levels of cytokines which may lead to damage to the heart valves.
Objective: This study was intended to explore the association of TNF-α, TGF-β and IL-35 gene polymorphisms with RHD.
Materials and Methods: The present case control study consisted of 145 patients with rheumatic heart disease and 217 control subjects in the same age group. Genotyping was done for the TNF-α (-308), TGF-β-1 (-508) and IL-35(EBI3) gene polymorphisms in both case and control groups.
Results: The results showed Bone differences in the distribution of genotypes in TNF-α, TGF-β and IL-35 genes RHD cases and control groups. However, the statistical analysis of the data showed the differences in the genotypes between TNF alpha (-308 G>A), TGF-β-1 (C-508T) and IL-35 EBI3G/C genes RHD case and control subjects were not found to be statistically significant.
Conclusion: In conclusion our study could not find any significant association between TNF-α (-308), TGF-β-1 (-508) and IL-35(EBI3) gene polymorphisms and RHD.

Keywords: Rheumatic Heart Disease, Cytokines, Tumour Necrosis Factor alpha, Transforming growth factor beta 1, Interleukin, Single Nucleotide Polymorphism.

INTRODUCTION

Rheumatic Fever (RF) is an autoimmune disease arbitrated by humoral and cellular immune responses that follow an untreated pharyngeal Streptococcus pyogenes infection. The gravest complication of RF is rheumatic heart disease (RHD), which occurs in 30 to 45% of RF patients and leads to chronic valvular lesions [1]. RHD is a complex disease, subject to genetic and environmental factors. The autoimmune reactions are the hallmark of the pathogenesis of RHD. The prevalence of RHD was assessed and more than 39 million cases were observed globally by the year 2017 [2]. Molecular mimicry, the sharing of epitopes between antigens of the host and S. pyogenes, has been anticipated to be the causing factor leading to RHD. The cross-reactive antibodies and T cells

play an important role in the cross-recognition between streptococcal antigens and human proteins leading to inflammation and autoimmunity [3]. The polymorphisms in genes such as tumour necrosis factor-alpha (TNFα), interleukin-1 receptor antagonist (IL1RA), tumour growth factor-beta (TGFβ), and cytotoxic T cell lymphocyte antigen 4 (CTLA4) may contribute to the pathogenesis of RF and RHD. Cytokine genes are polymorphic in nature and specific variants of cytokine genes are associated with cytokine levels. It is hypothesized that these polymorphisms may be prognostic of persons disposition to RHD. In the present study we focused on the genetic susceptibility of TNF-α (-308), TGF-β-1 (-508) and IL-35(EBI3) gene polymorphisms in the development of RHD.

MATERIALS & METHODS

Study Subjects

The present case control study consists of 145 patients with Rheumatic Heart Disease patients and 217 control subjects in the same age group. All the patients were recruited from the Department of Cardiology, Mahavir Hospital and Research Centre, Hyderabad and also from other hospitals in twin cities of Telangana State. The control subjects were randomly selected from healthy volunteers who visited the hospital for general health check-up and also hospital staff. This study was approved by Institutional Ethics Committee (IEC) of Bhagwan Mahavir Medical Research Centre. Only Echo cardio graphic ally confirmed RHD patients without congenital heart diseases in the age group of 15 to 60 years were included in the study. Patients with other autoimmune diseases, family history of rheumatic fever, other cardiac diseases and hypertension were excluded from the study.

Genotyping of TNF-α (-308), TGF-β-1 (-508) and IL-35(EBI3) gene polymorphisms

5 ml of peripheral blood samples were collected in EDTA vacationers from RHD patients and control subjects and genomic DNA was isolated and genotyping was done for TNF-α (-308), TGF-β-1 (-508) and IL-35(EBI3) gene polymorphisms in both case and control groups.

Statistical Analysis

The statistical analysis of the data was carried using Epi info software. The significance of the distribution of variants in TNF2 (-308), TGF-B1 (-508), AND il-35 (FBB) genes among the RHD cases and control subjects were assessed using chi-squire test and odds ratios. P value < 0.05 was considered significant.

RESULT

The distribution of genotypes among the study subject's statistical analysis of the data is represented in Table 1. Results of polymorphism in the promoter region of TNF alpha (308G>A) showed high frequency of heterozygous variants AG (53.10%) followed by homozygous major variant AA (24.83%) and homozygous minor variant GG (22.07) in the RHD patients. Similar trend was observed in the control subjects. TGF-β-1 (C-508T) gene polymorphism results showed high frequency of heterozygous variant TC (54.48%) followed by homozygous major variant TT (34.41%) and homozygous minor variant CC (13.10%) in the RHD patients. Same pattern was observed in the control subjects. The results of IL-35 EBI3G/C polymorphism showed high frequency of homozygous major variants GG (59.31%) followed by heterozygous variants GC (31.03%) and homozygous minor CC (9.66%) variants in the RHD patients. Exact pattern was observed in the control subjects. The statistical analysis of the results using x2, odds ratios and p value into consideration, did not show any significant association between TNF alpha (-308 G>A), TGF-β-1 (C-508T) and IL-35 EBI3G/C gene polymorphisms and RHD.

Table 1. Genotypic and allelic distribution of TNF-α (-308), TGF-β-1 (-508) and IL-35(EBI3) gene polymorphisms among RHD patients and controls subjects.

Polymorphism	Genotype	RHD Patients N=145 (%)	Healthy Controls N=217(%)	χ2	OR (95%CI)	P value
TNF-α (-308)	AA	36 (24.83)	75 (34.56)	1	Reference	
	AG	77 (53.10)	102 (47.01)	2.8	1.57 (0.96-2.58)	0.09
	GG	32 (22.07)	40 (18.43)	2.21	1.67 (0.90-3.07)	0.13
	AG+GG	109 (75.17)	142 (65.44)	3.43	1.60 (1.00-2.56)	0.06
	A allele	149 (51.38)	252 (58.06)	1	Reference	
	G allele	141 (48.62)	182 (41.94)	2.88	1.31(0.97-1.77)	0.08
TGF-β-1 (-508)	TT	47 (32.41)	82 (37.79)	1	Reference	
	TC	79 (54.48)	106(48.84)	0.99	1.30(0.81-2.06)	0.31
	CC	19 (13.10)	29 (13.36)	0.04	1.14 (0.57-2.25)	0.83
	CC+TC	98(67.59)	135 (62.21)	0.87	1.26 (0.81-1.97)	0.35
	T allele	173 (59.66)	270 (62.21)	1	Reference	
	C allele	117 (40.34)	164 (37.79)	0.37	1.11(0.82-1.50)	0.53
IL-35(EBI3)	GG	86 (59.31)	97 (44.70)	1	Reference	
	GC	45 (31.03)	90 (41.47)	5.43	0.56 (0.35-0.89)	0.019
	CC	14 (9.66)	30 (13.82)	2.72	0.52 (0.26-1.05)	0.09
	GC+CC	59 (40.69)	120 (55.30)	6.84	0.55 (0.36-0.84)	0.008
	G	217 (74.83)	284 (65.44)	1	Reference	
	C	73 (25.17)	150 (34.56)	6.75	0.63(0.45-0.88)	0.009

DISCUSSION

Defence against pathogens depends on the complex interactions between the genetically controlled innate and adaptive immune responses. The innate immune response provides the first line of defence against S. pyogenes infections in RF, through complement cascade activation. Throughout the innate response, the adaptive response is initiated through antigen processing and presentation to T cells and by cytokine secretion [4]. Gene polymorphisms that code for molecules involved in increasing the effectors innate and adaptive immune response contribute to RF and RHD susceptibility.

TNF-α is the most studied immune related gene in RHD. TNF-alpha is a proinflammatory cytokine mainly produced by monocytes, macrophages, T and B lymphocytes and maps within the MHC class III region on chromosome 6. Previous studies have revealed that the elevated serum levels of TNF-alpha are associated with various inflammatory and autoimmune diseases [5]. At position -308 of its promoter G/A polymorphism has functional implications as the A allele results in higher levels of transcription compared to the G allele [6]. Higher serum levels of TNF-α have been detected in RF cases, occurring as a result of a SNP (-308G>A) in the promoter site of the TNF-α gene [7]. Candidate gene studies have reported association between tumour necrosis factor alpha (TNF-α) [8], transforming growth factor beta 1 (TGF-β1) genes [9] and RHD. Rajendranath et al (2007) reported border line association of TNFA-308G/A polymorphism with rheumatic fever [10]. Amal et al (2010) reported association of TNF alpha -308G/A polymorphism with RHD susceptibility and increased production of TNF alpha, and also related with valve damage and more severe outcome of RHD [11]. According to Settin et al (2007) predisposition to RHD is influenced by composite polymorphisms of TNF-α−308 A/A and IL-10–1082 A/A among cases [12]. Rehman et al (2013) showed that the TNF-alpha -308 AA, GA genotypes and A allele were associated with susceptibility to RHD while the GG genotype conferred resistance [13]. A meta-analysis by Ruo et al (2014) demonstrated that the TNF-a 308G>A polymorphism is associated with RHD susceptibility, and it contributes to the increased risk of RHD [14]. In contrary earlier meta-analysis, some authors did find any association between the TNF-α -308G>A polymorphism and RHD [15-18]. A recent meta-analysis by Babu et al (2020) also could not find any evidence for the association between the TNF-α -

308G>A and TGF-β1 509C>T polymorphisms with RHD [19]. Even in the present study no significant association was observed between TNF-α 308G>A polymorphism and RHD.

TGF-β 1 is a multifunctional cytokine, which controls the proliferation and differentiation
of many cell types [20] and also involved in the production and degradation of the extracellular matrix. TGFβ acts as an important modulator of the immune response and may have both pro- and anti-inflammatory effects. TGFβ exists in three is forms TGFβ1, TGFβ2, and TGFβ3 with a wide variety of biological functions which include wound healing, fibrosis, immune suppression, and angiogenesis. Chemotactic properties of TGFβ can stimulate cells to produce cytokines such as interleukin (IL) 1, IL6, and tumour necrosis factor a (TNFα) at sites of inflammation [21]. Previous studies reported that the transforming growth factor-β1 exists in calcified aortic valve cusps and promotes aorticvalve interstitial cell calcification through apoptosis, and may be responsible for the increased myocardial collagen expression and myocardial fibrosis in human aortic valve disease [22,23]. TGF-β1 is secreted by many cells such as T lymphocytes, monocytes, endothelial cells, fibroblasts, etc. While in normal condition it is mainly secreted by platelets and released in the α-granules [24]. TGF- β 1 increases the synthesis and secretion of collagen and other matrix proteins and activates gene transcription [25]. Moreover, it decreases the synthesis of proteolytic enzymes collagenase, which degrades the matrix proteins, and increases the synthesis of protease inhibitors like plasminogen activator inhibitor, that block the activity of the proteolytic enzymes [26]. Thus, TGF-β1 may play an important role in fibrotic conditions.

The TGF-β1 gene is located on chromosome 19q13.The human TGF-β1 gene contains 7 exons, which gives rise to a precursor protein of 390 amino acids, that is proteolytically processed to create the mature protein of 112 amino acids [27]. A C-509T polymorphism on the promoter region of the TGF-β1 gene has been associated with high TGFβ -1 levels in population of Caucasian patients [28]. TGFβ1 -509T polymorphism was found to be a risk factor for the development of valvular RHD lesions in Taiwanese population [29]. However, meta-analysis by Babu et al (2020) could not find evidence of an association between TGF-β1 509C>T polymorphisms with RHD [19]. In accord to the meta-analysis, no significant association was found in the present study between C-509T polymorphism on the promoter region of the TGF-β1 gene and RHD.

Interleukin- (IL-) 35 is a heterodimeric cytokine composed of the Epstein-Barr virus-induced 3 (EBI3) and p35 subunits and belongs to IL-6/IL-12 cytokine family, which includes IL-12, IL-23, IL-27, and IL-35 molecules [30]. EBI3 gene encodes the βsubunit (EBI3) of IL-35 and located on chromosome 19q13.3 and consists of 5 exons.IL-35 is nominally expressed inhuman tissues, mostly induced in inflammatory conditions and mainly secreted fromCD4+ Foxp3+ Treg cells [31]. Tregcells play a foremost role in autoimmune control and protect from inflammation, IL-35 may participate in the inflammatory process of RHD development. According to Rosalinda et al (2017) the IL-12A and EBI3 polymorphisms were associated with decreased risk of developing premature coronary artery disease in Mexican population [32]. Li et al (2021) carried out a study on the interaction between interleukin-35 gene polymorphisms and risk factors on susceptibility to coronary heart disease in the Chinese Han population and could not find significant association, however increased risk was observed in smokers [33]. In the present study no significant association was observed between IL-35(EBI3) gene polymorphism and RHD.

CONCLUSION

In conclusion, our study could not find any significant association between TNF-α (-308), TGF-β-1 (-508) and IL-35(EBI3) gene polymorphisms and RHD.

Declaration by Authors

Ethical Approval: Approved

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Conflict of Interest: The authors declare no conflict of interest.

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