Original Research Paper

Volume-8 | Issue-12 | December-2018 | PRINT ISSN No 2249-555X

Cardiology

A STUDY ON THE ASSOCIATION OF TGF β1 (-509) AND TNF- α (-308) GENE POLYMORPHISMS WITH SUSCEPTIBILITY TO RHEUMATIC HEART DISEASE IN SOUTH INDIAN PATIENTS

P. P Reddy	Department of Genetics and Environmental Toxicology, Bhagwan Mahavir Medical Research Centre, Hyderabad
Prashanth. Chiliveri*	Department of Genetics and Environmental Toxicology, Bhagwan Mahavir Medical Research Centre, Hyderabad *Corresponding Author
Vanitha. Baluka	Department of Genetics and Environmental Toxicology, Bhagwan Mahavir Medical Research Centre, Hyderabad
Rupalakshmi Harshavardhan	Kamalamma Hospitals, Chennai
Shivakumar Yerra	Department of Cardiology, Mahavir Hospital and Research Centre, Hyderabad , Telangana

ABSTRACT

BACKGROUND: Rheumatic heart disease (RHD) is one of the major health problems world over and the morbidity and mortality due to this disease is alarming. Cytokines are important mediators of inflammatory and immune responses to RF and RHD.

OBJECTIVE: The aim of the study is to investigate the association of TGFβ1 (-509) and TNFα (-308) gene polymorphisms with susceptibility to RHD in Telangana population and their potential usefulness as biomarkers to identify the individuals with risk for RHD.

METHODS: The study was carried out on 56 RHD and 45 ethnically matched healthy subjects for comparison (controls). Demographic data was collected and 2D echocardiography was carried out for all for confirmation of the disease. 5mL blood sample was drawn from each subject, genomic DNA was isolated from each and subjected to PCR-RFLP to identify the polymorphisms of cytokine genes. The results were analyzed using appropriate statistical tests.

RESULTS: Our results showed that GG genotype of TNFα (-308) (P< 0.05, OR 3.1, CI 1.17-8.4) was significantly associated with increased risk of RHD and G allele was found to be highly significant (P=0.0004, OR 2.81, CI 1.57 to 4.97). AG genotype showed 1.8 folds risk with RHD (P>0.05, 1.8, CI 0.65-5.1) and AG+GG combination showed 1.3 fold risk for RHD (P> 0.05 OR 1.3 CI 0.6-2.8). However, none of the genotypes of TGF-b1 (-509) showed an increase in the RHD patients compared to control subjects thus indicating that gene was not associated with susceptibility for RHD.

CONCLUSION: Our findings suggest that TNFα (-308) gene polymorphism plays an important role in predisposition to RHD in Telangana population. However, our results showed that the TGF β- 1 (-509) was not associated with increased risk for RHD in this population.

KEYWORDS : RHD, Telangana, TNF, TGF, MS, Cytokines.

INTRODUCTION

Despite the tremendous progress made in cardiology, the menace of morbidity and mortality due to Rheumatic Fever and Rheumatic Heart Disease and subsequent consequences remain very high particularly in children and young adults in developing countries like India.The incidence of RF in some developing countries exceeds 50 per 100,000 children. The worldwide prevalence of RHD is at least 15.6 million cases, and this disease is responsible for around 233,000 deaths/year (Carapetis et al., 2005). Rheumatic heart disease (RHD) is still a major health problem in India and the prevalence is 10.8 /1000 in urban population of Andhra Pradesh (Rama et al., 2013). RHD results from Rheumatic Fever in which an autoimmune reactions triggered by an untreated Streptococcus pyogenes throat infection leading to severe valvular damage. Rheumatic Fever (RF) causes an acute generalized inflammatory response and an illness that selectively affects the heart, joints, brain and skin and it is a major health problem in children, adolescents and young adults. Although, it leaves no lasting damage to the brain, joints and skin, it damages the heart valves particularly the mitral and aortic valves and also the tricuspid valve leading to Rheumatic Heart Disease (Debakey et al., 1977). It is the most serious complication with inflammatory condition which develops into progressive calcification and thickening over time as a serious consequence of rheumatic fever (Sarkar et al., 2017).

Rheumatic Heart Disease may or may not have any symptoms and diagnosed 5 - 10 years or more after the episode of rheumatic fever (Carabello et al., 2005, DeBakey et al., 1977) that occurs in 32-45 % of RF patients (Enas et al., 2010) and mostly depends on genetic and environmental factors (Carapetis et al., 2005). It depends on several host factors that mediate an inflammatory and heart-tissue driven autoimmune response triggered by a protective immune response against S. pyogenes. Several genes contribute to the development of rheumatic heart lesions after S. pyogenes infection but pro and anti-inflammatory cytokines seem to play an essential role in the activation of immunological and inflammatory responses in RF and RHD. The effectiveness of the immune response depends on the production of cytokines, which are important secondary signals following an infection (Guilherme et al., 2013).

TNF- α is a pro-inflammatory cytokine gene that mediates diverse pathological processes, such as shock during infection and inflammation during autoimmunity (Apostolaki et al., 2010). Several studies were carried out the association of TNF–α(-308) and (-238) gene polymorphisms with susceptibility to RHD in different populations and the results are controversial. Some studies have shown that SNPs in the promoter region of TNF-alpha (-308A), (-238) were associated with susceptibility to RHD in populations fromMexico, Turkey, Brazil, Pakistan and Egypt (Rehman et al., 2013, Settin et al., 2007)while some other studies did not providence any evidence for such as association in north indian population (Usha et al., 2016).

Kamal et al., (2010) studied the association of C-509T and T869C of TGF β1 gene polymorphisms in RHD patients and showed T869C TT genotype was found significantly associated with RHD in children and in adults from Egypt with combined valvular disease (CVD) compared to control subjects, whereas chou et al., (2004) showed a significant difference in the distribution of genotypes between patients with RHD and control subjects for both C-509T and T869C polymorphisms of TGF-beta1. Usha et al (2016) studied the association of IL-6, IL-10, TNF- A, IL-1 β, IL-1 VNTR,TGF-β1, CTLA-4 with RHD risk in north Indian population and showed that TGF-β1, IL-1β, IL-1 VNTR gene polymorphisms were significantly associated with RHD susceptibility. Although studies have been carried out on the prevalence of rheumatic fever in school children, studies on role of cytokine gene polymorphisms in causation of RHD were not carried out on south

Indian population especially in Telangana state.Hence, our study was aimed to find the association of TGF β1 (-509) and TNF- α (-308) gene polymorphisms with susceptibility to Rheumatic Heart Disease.

MATERIALS AND METHODS:

Study Subjects

The present study was carried out in 56 RHD patients in the age group of 15 to 60 years attending the cardiology department of Mahavir Hospital and Research Centre in Hyderabad, Telangana State. 45 ethnically matched subjects in the same age group with normal 2D echocardiography and without other autoimmune diseases and without family history of rheumatic fever and heart disease were considered for comparison (control subjects).Demographic data on age, sex, smoking status, alcohol consumption, hygiene, socio economic status, past history of RF, severity of RHD and other associated diseases in patients and control subjects were recorded using a standard questionnaire. 2D echocardiography was done to understand the valvular lesions and the patients were further classified into Mitral Valve Stenosis (MS), Mitral Valve Regurgitation (MR),Tricuspid Stenosis (TS),Tricuspid Regurgitation (TR),Aortic Stenosis (AS) and Aortic Regurgitation (AR) subgroups based on the valvular lesions.The severity in each group of valvular lesion was categorized into mild, moderate and severe as per WHO criteria.

This study was approved by the Institutional Ethical Committee. Written subject's Informed Consent was obtained from both RHD patients and control subjects.

Inclusion and Exclusion Criteria

The study included echocardiographycally confirmed RHD patients in the age group of 15 to 60 years from Telangana state. Patients with congenital heart diseases. hypertension, other autoimmune disease, family history of RF and other cardiac diseases were excluded.

Blood Collection and DNA Isolation

5mL whole blood samples were collected in ethylene diamine tetra acetic acid (EDTA) vacutainers from RHD patients and control subjects. Genomic DNA was extracted from all the subjects using a standard salting out method(Miller et al., 1988)and quantified the concentration and purity using Biophotometer-D30 (Eppendorf).

Genotyping by PCR - RFLP

TGF β1 (-509)and TNF- α (-308) gene polymorphisms were determined using PCR- RFLP method and a negative control was used to ensure contamination free PCR product. The PCR amplification was carried out in 25 µLof PCR reaction mixture consisting of 100ng DNA template, 10µM of each primer (Forward and Reverse), 0.2 mM of each dNTP, 2.4mM $MgCl_2$, and 1 U Taq DNA polymerase with 1 x Reaction buffer (*Bioline, UK*). 20µL of PCR products of each gene was digested using appropriate enzymes and electrophoresis was done at 15 mA constant for 3-5 hrs in mini vertical gel electrophoresis system (Bangalore Genei, India). For visualization under UV light, gel was soaked in ethidium bromide (0.5 g/mL).

TGF-β1 (-509) and TNF- α (-308) Genotyping

Genotyping of TGF-β1and TNFα −308 genes was carried out using the following specific primer pairs. For TGF-β1, the following primers were used: Forward 5`- CAGACTCTAGAGACTGTCAG-3' Reverse 5`- GTCACCAGAGAAAGAGGAC-3' and for TNFα −308, 5' -ATC TGG AGG AAG CGG TAG TG-'3 (Forward) and 5'-AAT AGG TTT TGA GGG CCA TG-'3(Reverse) were used.

The PCR conditions were 94°C for 3 minutes; 35 cycles of 94°C for 35 seconds, annealing at 58°C for TGF β1 and 55°C for TNF- α for 30 seconds,72°C for 30 seconds, 72°C for 5 minutes for final extension. 20 µL of PCR product was digested with 10 units of Eco81I restriction enzyme (New England Bio labs, Inc., USA) for TGF β1 and N*coI* restriction enzyme (*New England Bio labs, Inc., USA*) for TNF- αat 37°C for overnight and subjected to agarose gel electrophoresis.

STATISTICAL ANALYSIS

The data was expressed as mean (± standard deviation) percentage. Distribution of the TGF-β1and TNF- α genotypes were evaluated and the chi square test or fisher exact probability test was used for analysis of the frequency distribution of subjects in various subgroups. Odds ratio and confidence interval were calculated to estimate the risk of contracting disease in the presence of various risk factors. Statistical significance was accepted as $P < 0.05$ (two-tailed). All analyses were done using GraphPad Prism 5.0 version software

RESULTS

Demographic characteristics of patients with RF and RHD and control subjects were presented in Table-1. Out of 56 (48 RHD and 8 RF) RHD patients, 41 were (73.2%) females and 15 (26.8%) were males in the study subjects as against 28 (62.2 %) females and 17 (37.8%) males in the control group. The mean age of the patients was 39.7 ± 11.3 as against 38.2 ± 9.2 years in control group (Table-1).

Table -1. Demographic Characteristics of RF and RHD Patients and Control Subjects

Parameters	RF and RHD patients (n=56)	Control Subjects (n=45)
Age (Mean ± SD)	39.7 ± 11.3	38.2 ± 9.2
Sex		
Males	15 (26.8)	17 (37.8)
Females	41 (73.2)	28 (62.2)
Smoking Status		
Smokers	11 (19.6)	8 (17.8)
Non Smokers	45 (80.4)	37 (82.2)
Socio Economics Status		
Poor	26 (46.4)	11 (24.4)
Middle	30 (53.6)	25 (55.6)
Rich	0	9 (20)
Religion		
Hindu	28 (50)	22 (48.9)
Muslim	28 (50)	15 (33.3)
Others	0	8 (17.8)
Population		
Rural	12 (21.4)	15 (33.3)
Urban Population	44 (78.6)	30 (66.7)
Alcohol Consumption		
Yes	3 (5.4)	8 (17.8)
No	53 (94.6)	37 (82.2)

The values given in parentheses are percentages

Diagnosis of RF was done by ASO Titre followed by 2D Echo in controls whereas the severity of RHD was assessed by 2D Echocardiography in RHD. Based on the 2D ECHO reports of the cases, the severity and types of valvular lesions of Rheumatic Heart Disease were determinedand the results are shown Table –2.The analysis of valvular lesions showed that a high percentage of Mitral Valve Stenosis (44%) followed by Tricuspid Regurgitation (35.39%), Mitral valve Regurgitation (31.35 %) and Aortic Regurgitation (25.42%).

Mitral Valve Stenosis (MS)		Total
Mild	14 (7.6)	52(44.06)
Moderate	9 (4.9)	
Severe	29 (15.8)	
Mitral Valve Regurgitation (MR)		37 (31.35)
Mild	26 (14.1)	
Moderate	5 (2.7)	
Severe	6 (3.3)	
Tricuspid Stenosis (TS)		
Mild	1 (0.5)	5 (4.23)
Moderate	0	
Severe	4 (2.2)	
Tricuspid Regurgitation (TR)		
Mild	33 (17.9)	42 (35.59)
Moderate	6 (3.3)	
Severe	3 (1.6)	
Aortic Stenosis (AS)		
Mild	9 (4.9)	18 (0.15)
Moderate	8 (4.3)	
Severe	1 (0.5)	
Aortic Regurgitation (AR)		
Mild	21 (11.4)	30 (25.42)
Moderate	9 (4.9)	
Severe	0	
Total Valvular lesions (n=56)		118

The values given in parentheses are percentages

Table – 3: Genotypes of TNF-αand TGF-β1 in patients and control subjects

Name of the Gene	Genotype	Patients (%) n=56	Controls (%) n=45	P Value	Odd Ratios (95% CI)
TNF-α (-308)	AA	22(39.3)	8 (17.8)	**Reference**	
	AG	24(42.9)	16 (35.6)	0.31	1.8 (0.65 – 5.1)
	GG	10 (17.9)	21 (46.7)	**0.03***	**3.1 (1.17 – 8.4)**
	AG + GG	34 (60.7)	37(82.2)	0.55	1.3 (0.6 to 2.8)
	A Alleles	68 (60.7)	32 (35.6)	**Reference**	
	G Alleles	44 (39.2)	58 (64.4)	**0.0004**	2.81 (1.57 to 4.97)
TGF-β- 1 (-509)	CC	22 (39.3)	18 (40)	Reference	
	CT	25 (44.6)	22 (48.9)	1.0	1.0 (0.46 – 2.50)
	TT	9 (16)	5 (11.1)	0.5	0.6 (0.68 – 1.18)
	CT + TT	34(60.7)	27(60)	0.1	1.9 (0.76 -4.89)
	C Alleles	69 (61.6)	58 (64.4)	**Reference**	
	T Alleles	43 (38.3)	32 (35.6)	0.7	0.8 (0.49 to 1.57)

* P < 0.05 (significant); P < 0.001 (Highly Significant)

The genotypic distribution for TGF-β- 1 (-509) and TNF –α (-308)in patients with RHD & RF and in controls is shown in Table -3. The frequencies of AA, AG, GG and AG + GG genotypes of TNF –α in patients were 39.3, 42.9, 17.9 and 60.7 %, as against 17.8, 35.6,46.7 and 82.2 % in control subjects respectively. GG genotype of TNF–α. showed a significant association (P< 0.05, OR 3.1, CI 1.17-8.4) with susceptibility for RHD.G allele was found to be highly significant in the RHD patients (P=0.0004, OR 2.81, CI (1.57-4.97) compared to control subjects.AG genotype showed 1.8 folds risk for RHD (P>0.05, 1.8, CI 0.65-5.1) and AG+GG combination showed 1.3 fold risk for RHD (P> 0.05 OR 1.3 CI 0.6-2.8).

The studies on the association of TGF-β- 1 with RHD showed that CC, CT, TT and CT + TT genotype frequencies were 39.3, 44.6, 16 and 60.7 % in patients while the frequencies for the same genotypes were 40, 48.9, 11.1 and 60% in the control subjects respectively. None of the genotypes showed an increase in the RHD patients compared to control subjects thus indicating that TGF-β- 1 gene was not associated with susceptibility for RHD.

DISCUSSION:

Rheumatic heart disease is the inflammatory disease of the heart valves caused by a combination of immune, genetic and environmental factors. Cytokines are the mediators of inflammation and play an important role in the pathogenesis of Rheumatic hearts disease. In the present study we have investigated the role of polymorphisms of TNF – β1 and TNF-αcytokine genes and their usefulness as biomarkers in RHD patients from a south Indian (Telangana) population.

TNF – β1 and TNF-α are very important cytokine genes that are proved to influence the clinical outcome of the Rheumatic heart disease. In the present study we have screened 56 RHD patients and 45 ethnically matched controls for TGF –β1 (-509) and Tumor necrosis factor TNF-α, (-308) and observed only GG genotype of TNF-α gene was significantly associated with RHD.

TNF-α, gene is located on chromosome 6p21.33 and TGF –β1 gene is located on chromosome19q13.2 and both genes are shown to be associated with susceptibility in causation of various autoimmune disorders (Guilherme et al., 2011). Earlier studies were carried out on the association of TNF-α and TGF β, IL-4, IL 1, VNTR, MDR gene polymorphisms with susceptibility to RHD in different populations. Our results are in agreement with that of Rehman et al (2013) who showed significant association of TNF-α(-308) with susceptibility to RHD in Pakistan patients. The present results are also in accordance with that of Amal et al (2010) who reported that TNF-α -238 and -308 polymorphisms were associated with susceptibility to RHD in Egyptian children. However, Hernandez, et al (2003) showed that -238 A allele was found to cause protection. Sallakci et al (2005) and Hernandez- Pachecco et al (2003) also showed strong association of TNF-α -308 A allele with RHD in patients from Turkey and Mexico respectively.

Ramaswamy et al (2007) showed borderline association of TNF-α (-308) and (-238) with rheumatic fever whereas Settin et al., (2007) tested the cytokine gene polymorphisms with susceptibility to RHD in children from Egypt and observed significantly higher frequencies of homozygous genotypes of TNF-α in RHD and concluded SNPs of promoter region of TNF -α (-308) were associated with RHD. However, Usha et al (2016) did not find the association of TNF –α (-308) with RHD susceptibility in North Indian population.

The results showed wide variation on the association of TNF-α variants with RHD in different studies. The discrepancy in the outcome / variation might be due to the ethnicity of the populations studied and sample size. Genetic and environmental factors of the region/country also influenced the susceptibility to the disease. Very few studies were carried on the role of TNF-α in Indian population and it is the first report from South India.

In the present study, no significant differences in variants of TGF β1 (-509) were found between RHD and control subjects indicating no genetic (TGF β1) predisposition to RHD. However Ushaet al(2016) reported that TGF-β1 (-869) plays an important role in RHD susceptibility in north Indian population. Bhatt el al (2018) carried out a meta-analysis of 23 published case control studies and showed that TGF β1 (-869T/C), -TGF β1 -509, TNF–α and IL-1 β -509 were significantly associated with increased risk of RHD.Some others have also shown strong association of IL-6 (-174) (Rehman et al., 2013) and IL-6(-174), IL-10 (-1082), IL-1Ra (VNTR) (Settin et el., 2007) with risk to RHD. From the present study and from earlier reports in different ethnic groups, it can be inferred that TNF-α allele variants are one of the predisposition risk factors for RHD.

CONCLUSION

In conclusion, our results suggest that TNF-α (-308)polymorphism may contribute to the pathogenesis of RHD. These polymorphisms may be considered as useful markers to detect the individuals susceptible to RHD in Telangana Population and aggressive prophylactic treatment might help to prevent the mobility associated with RHD.

ACKNOWLEDGEMENTS:

The authors are highly grateful to patients for their whole hearted support and to Shri.Motilal Jain, Chairman, Shri.Mahendra Ranka, Vice Chairman, Shri.Sushil Kapadia, Managing Trustee andShri.Ashok Kothari, Trustee Treasurer, Bhagwan Mahavir Memorial Trust, Hyderabad for their encouragement and for providing infrastructure facilities to carry out the work. Thanks are also due to Mrs.Jyothi and Ms.Zareena, Staff of the Cardiology Department, Mahavir Hospital and Research Centre, Hyderabad for their help and cooperation.

REFERENCES:

1. A. Settin, H. Abdel-Hady, R. El-Baz, I. Saber, Gene polymorphisms of TNF-alpha(-308), IL-10(-1082), IL-6(-174), and IL-1Ra(VNTR) related to susceptibility and severity of rheumatic heart disease, Pediatr. Cardiol. 28 (5) (2007) 363–371.
2. Arkwright P. D. et al. Atopic dermatitis is associated with a low-producer transforming growth factor beta(1) cytokine genotype. J Allergy Clin Immunol 108, 281–4 (2001). [PubMed]
3. Carapetis JR, Steer AC, Mulholland EK, Weber M. The global burden of group A streptococcal disease. Lancet Infect Dis. 2005;5:685–94. [PubMed]
4. Chou HT, Chen CH, Tsai CH, Tsai FJ ; Association between transforming growth factor-beta1 gene C-509T and T869C polymorphisms and rheumatic heart disease. Am Heart J. 2004 Jul; 148(1):181-6. [PubMed].
5. Cuenca J, Pe´rez C, Aguirre A, Schiattino I, Aguillo´n JC. Genetic polymorphism at position 2308 in the promoter region of the tumor necrosis factor (TNF): Implications of its allelic distribution on susceptibility or resistance to diseases in the Chilean population. Biol Res 2001;34:237–41.
6. Elahi MM, Asotra K, Matata BM, Mastana SS. Tumor necrosis factor alpha -308 gene locus promoter polymorphism: an analysis of association with health and disease. Biochim Biophys Acta 2009;1792:163–72.
7. Fabris M, Di Poi E, D'Elia A, Damante G, Sinigaglia L, Ferraccioli G. Tumor necrosis factor-alpha gene polymorphism in severe and mild–moderate rheumatoid arthritis. J Rheumatol 2002;29:29–33
8. Hernández-Pacheco G, Flores-Domínguez C, Rodríguez-Pérez JM, PérezHernández N, Fragoso JM, Saul A, et al. Tumor necrosis factor alpha promoter polymorphisms in Mexican patients with rheumatic heart disease. J. Autoimmun. 2003;21:59–63.
9. Hajeer AH, Hutchinson IV. TNF-alpha gene polymorphism: Clinical and biological implications. Microsc Res Tech. 2000;50:216–28. [PubMed]
10. L. Guilherme,R. Ramasawmy, J. Kalil, Rheumatic Fever and Rheumatic Heart Disease: Genetics and Pathogenesis.2007.scandinavian journal of immunology.0.1111/j.1365-3083.2007.01974.x
11. L. Guilherme, P. Cury, L.M. Demarchi, V. Coelho, L. Abel, A.P. Lopez, S.E. Oshiro, S. Aliotti, E. Cunha-Neto, P.M. Pomerantzeff, A.C. Tanaka, J. Kalil, Rheumatic heart disease: proinflammatory cytokines play a role in the progression and maintenance of valvular lesions, Am. J. Pathol. 165 (5) (2004) 1583–1591.
12. Lacki J, Moser R, Korczowska I, Mackiewicz S, Muller W. TNF-alpha gene polymorphism does not affect the clinical and radiological outcome of rheumatoid arthritis. Rheumatol Int 2000;19:137–40. Kaijzel EL, van Krugten MV, Brinkman BM et al. Functional analysis of a human tumor necrosis factor alpha (TNF-alpha) promoter polymorphism related to joint damage in rheumatoid arthritis. Mol Med 1998;

4:724–33.
13. MarcotorchinoJ, RomierB, GourantonE, RiolletC, Gleize B, Malezet-Desmoulins C, Landrier JF. Lycopene attenuates LPS-induced TNF-alpha secretion in macrophages and inflammatory markers in adipocytes exposed to macrophage-conditioned media. Mol Nutr Food Res 56, 725–32 (2012). [PubMed]
14. M.R. Stampfli, M. Cwiartka, B.U. Gajewska, D. Alvarez, S.A. Ritz, M.D. Inman, Z. Xing, M. Jordana, Interleukin-10 gene transfer to the airway regulates allergic mucosal sensitization in mice, Am. J. Respir. Cell Mol. Biol. 21 (5) (1999) 586–596.
15. Miller LC, Gray ED, Mansour M, Abdin ZH, Kamel R, Zaher S, et al. Cytokines and immunoglobulin in rheumatic heart disease: Production by blood and tonsillar mononuclear cells. J Rheumatol. 1989;16:1436–42. [PubMed]
16. Ramasawmy R, Faé KC, Spina G, Victora GD, Tanaka AC, Palácios SA, et al. Association of polymorphisms within the promoter region of the tumor necrosis factor-alpha with clinical outcomes of rheumatic fever. Mol Immunol 2007;44:1873–8
17. Ramasawmy R, Faé KC, Spina G, Victora GD, Tanaka AC, Palácios SA, et al. Association of polymorphisms within the promoter region of the tumor necrosis factor-alpha with clinical outcomes of rheumatic fever. Mol Immunol. 2007;44:1873–8.[PubMed]
18. Rehman S, Akhtar N, Saba N, Munir S, Ahmed W, Mohyuddin A, Khanum A ; A study on the association of TNF-α(-308), IL-6(-174), IL-10(-1082) and IL-1Ra(VNTR) gene polymorphisms with rheumatic heart disease in Pakistani patients. Cytokine. 2013 Feb;61(2):527-31. doi: 10.1016/j.cyto.2012.10.020. Epub 2012 Nov 17.
19. Sallakci N, Akcurin G, Köksoy S, Kardelen F, Uguz A, Coskun M, et al. TNF-alpha G-308A polymorphism is associated with rheumatic fever and correlates with increased TNF-alpha production. J Autoimmun 2005;25:150–4.
20. Subendu Sarkar , Mukul Rastogi , Priyanka Chaudhary , Rajesh Kumar , Priya Arora , Vivek Sagar , Inderpal Singh Sahni , Sunil Shethi , Khemraj Thaku , Sourav Ailawadhi , Devinder Toor & Anuradha Chakraborti" Association of rheumatic fever & rheumatic heart disease with plausible early & late-stage disease markers"(2017).
21. Wilson A. G., Symons J. A., McDowell T. L., McDevitt H. O. & Duff G. W. Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. Proc Natl Acad Sci U S A 94, 3195–9 (1997). [PMC free article] [PubMed]