Gene polymorphisms of TNF-α and IL-10 related to rheumatic heart disease

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ABSTRACT

Background: Rheumatic fever (RF) is inherited as a single recessive gene. Several genes are Likely to predispose an individual to develop rheumatic fever and rheumatic heart disease (RHD). Polymorphisms of TNF-α gene were associated with susceptibility to develop RF.T cells from all rheumatic fever patients produce significant amounts of TNF-α in response to steptococcal peptides with the highest production attained by the chronic rheumatic heart disease patients,and IL-10 expression was characterized in heart tissue of RHD patients by immuno-histochemistry.

Objectives: To test the relation of RHD and gene polymorphisms of pro-inflammatory cytokines TNF-α gene at position -308 and anti–inflammatory IL-10 gene at position -1082.

Subjects and Methods: This study included 20 children with chronic rheumatic heart disease (group A) and 10 healthy children as a control group (Group B). Patients group was classified into patients with single and multiple valvular lesions, both of them were classified according to the severity by Echocardiography into: Group I: mild valvular lesion (n=7) Group II: Moderate lesion (n=4) Group III: severe lesion (n=9) Real time PCR was done for both TNF-α at-308 and IL-10 at position – 1082.

Results: All cases showed significant higher frequency of TNF-α homozygous genotype G/G compared to control group ($p < 0.05$,OR,11). Cases with severe valvular lesions showed increased frequency of homozygous genotype G/G and increased frequency of IL-10 genotype G/A in cases compared to control group ($p \leq 0.05$,OR 13). There was no statistically significant difference in frequency of IL-10 genotypes among cases and control groups regarding to severity of valvular lesions. Composite genotypes (TNF-α G/G,IL-10 G/A) were higher in cases compared to control group ($P \leq 0.01$), while composite genotypes (TNF-α G/A,IL -10 G/G) were higher in control group compared to cases groups.

Conclusions: Susceptibility to RHD is associated with cytokine gene polymorphisms of TNF-α homozygous genotype G/G at-308 and IL-10 G/A at 1082. Composite genotypes (TNF-α G/G,IL-10 G/A) had high risk for RHD, while composite genotypes (TNF-α G/A,IL-10 G/G) may be protective genotypes.

Key Words:

Rheumatic fever, rheumatic, heart disease, TNF-α, IL-10 gene polymorphisms.

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INTRODUCTION

Acute rheumatic fever (ARF) causes an acute generalized inflammatory response and an illness that selectively affects the heart, joints, brain and skin. In spite of the dramatic response nature of acute episode of rheumatic fever (RF), it leaves no lasting damage to the brain, joints and skin[1]. but there is damage in the heart valves particularly the mitral and aortic valves that may persist after an acute episode has resolved[2]. Rheumatic heart disease (RHD) is a residual and progressive valve deformity resulting in stenosis or a combination of stenosis and insufficiency which appears after an episode of acute RF.[3]

Rheumatic fever is inherited as a single recessive gene. Several studies have suggested that genetic susceptibility to RF and RHD is linked to HLA class II alleles[4]. HLA class II genes are located in human chromosome 6 and are often associated with susceptibility to auto- immune disease. There is a strong evidence that an auto-immune disease response to streptococcal antigens mediates the development of RF and RHD in susceptible host. Susceptibility to RF in certain individuals have been associated with immunogenic, genetic and environmental factors.[5]

Cytokines appear to play a critical rule in triggering immunogenic and inflammatory reaction in rheumatic fever. Blood mono-nuclear cell culture from rheumatic children produced more TNF –α than those from controls[6]. Several genes are likely to predispose an individual to develop RF. Polymorphisms of TNF-α gene were associated with susceptibility to rheumatic fever development[7]. Polymorphisms in the promotor region of the TNF-α gene at nucleotide – 308 related to the transcription start site may be important in determining the host TNF –α response[8]. IL-10 is also a cytokine that has well – documented an anti – inflammatory and immune – regulatory activities. IL-10 inhibits the synthesis of pro-inflammatory cytokines and chemokine by monocyte,macrophages, neutrophlis and eosinophils[9]. IL-10 is mainly produced by T helper 2 (TH2) subset of CD 4. However, it is also produced by some activated B cells. Kinetic studies demonstrated that IL-10 is synthesized later than other immuno regulatory cytokines by activated T cells or monocytes. This data may reveal the regulatory role of IL-10 in later phases of the immune response[10]. Also IL-10 is a prominent regulatory cytokine secreted by large numbers of cells in both valve and myocardium tissues[11]. The anti inflammatory cytokine interleukin-10 (IL-10) inhibits monocyte production of pro – inflammatory cytokines including TNF-α. It also reduces MHC expression and attenuates recognition by cytotoxic lymphocytes[12]. Polymorphisms in the promotor region of IL-10 locus exhibit relevance in the pleiotropy of diseases of infectious, auto- immune or immune-responsive nature[13]. Polymorphisms of IL-10 change its production up to tenfold variation between indi-

viduals[14], A number of polymorphisms of promtor region have been characterized. Variable association between IL-10 polymorphisms and IL-10 production and auto-immune response have been reported.[15]

SUBJECTS AND METHODS

This study included 20 children patients with chronic RHD, 7 males and 13 females and 10 sex and age matched apparently healthy children (7 males and 3 females) were included as a control group. Patients were selected from those attending the out patients cardiology clinic of EL-Zahraa Hospital, AL-Azhar University and Clinics of The General Organization For Teaching National Heart Institute. Their age ranged from 5-15 years (Mean ages were 11.5±2.6years). All patients were under regular follow up in outpatient cardiology clinic. All patients were chosen not during the activity of RHD and free from congenital heart diseases. Children with other immunological diseases were excluded. An informed consent was taken from each patient parent after explaining the purpose of the study.

Our studied patients were divided into two groups: The first group (Group A) compromised twenty patients of chronic RHD while the second group (Group B) compromised ten apparently healthy children who had a negative family history of RHD as a control group.

According to echocardiography, valve lesions divided into single mitral valvular regurgitation (MR) (50%) and multiple valvular lesions as MR and aortic regurge (AR) (50%). Both single and multiple lesions of group A were subdivided according to echocardiography into mild (Group I), moderate (Group II) and severe RHD (Group III).

The studied groups were subjected to the following:

1. ***Full history taking with emphasis on:***

- Detailed history of RF (onset, duration, course of symptoms and possible associated symptoms).
- Past history including recurrent tonsillitis and tonsillectomy.
- Drug therapy (long acting penicillin, salicylates, diuretics, corticosteroids and digoxin).
- Family history of similar conditions.

2. **Clinical examination with special stress on cardiac examination.**

3. ***Laboratory investigations which include:***

- Anti streptolysin-O-titre (ASOT).
- The C-reactive protein (CRP).
- Erythrocyte sedimentation rate (ESR).
- Real time polymerase chain reaction (PCR) for TNF-α and IL-10.

4. **Chest-X-ray and electrocardiogram.**

5. **Echocardiogram.**

Specimen Collection and Methods For Real Time PCR:

Five ml of venous blood samples were collected aseptically from each subject in this study and divided into three tubes:

- In the first tube 2ml were collected in sterile vacutainer tubes containing ethyenediamine tetra acetate (EDTA) and stored at -15 to -25°c

until used in DNA extraction and real-time PCR amplification for processing gene polymorphisms of TNF-α gene at position -308 and IL-10 gene at position -1082.

DNA was extracted promptly using MagNA Pure Compact Nucleic Acid Isolation Kit I (Cat. No. 03730964001) supplied by (Roche, Germany).

Real time PCR amplification was done by Light Cycler-480 High Resolution Melting Master (Cat. No. 04909631001). The kit is easy to use reaction mix (2× conc.) for PCR and high resolution melting using the Light Cycler® 480 Real Time PCR System (Roche, Germany).

- The second tube 0.8ml of blood were collected in sterile vacutainer tubes containing 0.2 Na citrate used to measure ESR by traditional Westergren method read in 1st hour.[16]
- The third tube contained the remaining portion of blood about 2.2 ml. Serum is separated by centrifugation. This serum is used to measure ASOT by using ASO latex agglutation slide kits and CRP by latex agglutation test.[17]

Statistical Analysis

Qualitative data were presented as frequencies and percentages. Chi-square ($\chi 2$) test was used for studying the comparisons between different qualitative variables. Odds ratios (OR) and their 95% confidence intervals (CI) were used to study the association between different variables and valvular lesions. The (OR) is an estimate of relative risk.

Quantitative data were presented as minimum, maximum, means and standard deviation (SD) values.

The significance level was set at $P \leq 0.05$ and $P \leq 0.01$ (Highly significant) while P value >0.05 was insignificant. Statistical analysis was performed with SPSS 16.0 (Statistical Package for Scientific Studies) for Windows.

RESULTS

TNF-α Genotypes:

Comparison between group A and group B regarding TNF-α genotype at -308 showed, that homozygous genotype G/G is significantly higher in group A versus group B. On the other hand heterozygous genotype G/A is statistically significant higher in group B than group A. Homozygous genotype A/A showed no significant difference between group A and group B, (Table 1).

Table 1: Comparison between TNF-α-308 genotypes among group A and group B:

TNF-α Genotypes	Grouop (A) Cases (n= 20)		Group (B) Control (n= 10)		P-value
	Frequency	%	Frequency	%	
G/G	11	55	1	10	≤0.05*
G/A	8	40	9	90	≤0.01**
A/A	1	5	0	0	NS●

Odds Ratio results shows a statistically significant strong association between homozygous genotype G/G and RHD patients (Table 2).

Table 2: Association between genotype G/G of TNF-α at -308 and RHD in group A and group B:

TNF-α G/G	Group A Cases (20)		Group B Control (10)		P-value	Odds Ratio	95% CI
	Frequency	%	Frequency	%			
+ve	11	55	1	10	≤0.05*	11.000	1.164-103.944
-ve	9	45	9	90			

Significant increase in TNF-α genotype (G/A) in group B compared to both mild, moderate and severe valvular lesions (Group I and II, III), respectively. No significant difference regarding TNF-α (G/G) and (A/A) genotypes between group (B) and groups I, II and III.

There is significant increase in TNF-α genotype (G/G) in cases with severe valvular lesions (group III) compared to control group while genotype (G/A) showed statistically significant increase in group B than cases with severe valvular lesions (Table 3). Normalized and polymorphism of TNF-α at-308 (Figs 1, 2).

Table 3: Comparison between TNF-α genotypes at -308 among group B and group I, group II and group III:

TNF-α Genotypes	Group B Control (n= 10)		Group I Mild (n= 7)		P-value	Group II Moderate (n= 4)		P-value	Group III Severe (n= 9)		P-value
	Frequency	%	Frequency	%		Frequency	%		Frequency	%	
G/G	1	10	2	28.6	NS	2	50	NS	7	77.8	≤0.05*
G/A	9	90	4	57.1	≤0.05*	2	50	≤0.05*	2	22.2	≤0.05*
A/A	0	0	1	14.3	NS	0	0	NS	0	0	NS●

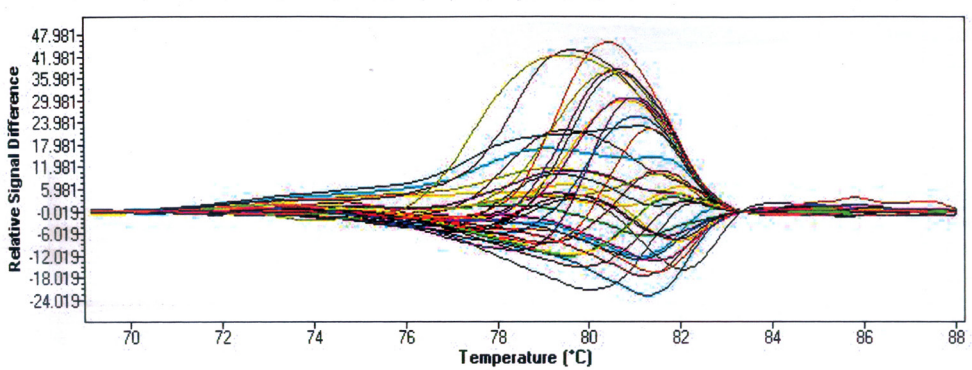

Fig. 1: Normalized and shifted melting curve of TNF-α at position – 308.

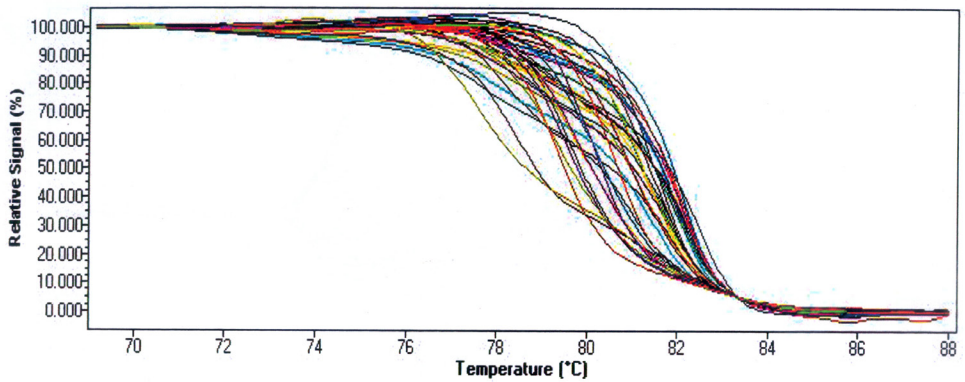

Fig. 2: Difference plots of polymorphism of TNF-α at position – 308.

IL-10 genotypes:

Heterozygous G/A genotype is statistically significantly higher in group A than group B while homozygous G/G and A/A genotypes showed no statistically significant difference between group A and group B (Table 4). This indicates statistical significant strong association between IL-10 heterozygous genotype G/A and RHD (Table 5), normalized and difference plots of IL-10 at position-1082 (Figs 3, 4).

Table 4: Comparison between IL-10 genotypes at -1082 among group A and group B:

IL-10 Genotypes	Group A Cases (n= 20)		Group B Control (n= 10)		P-value
	Frequency	%	Frequency	%	
G/G	1	5	3	30	NS●
G/A	18	90	5	50	≤0.05 *
A/A	1	5	2	20	NS●

Table 5: Association between genotype G/A of IL-10 at -1082 and RHD in group A and control group B:

IL-10 G/A	Group A Cases		Group B Control		P-value	Odds Ratio	95% CI
	Frequency	%	Frequency	%			
+ve	18	90	5	50	≤0.05*	13.500	1.955-93.246
-ve	2	10	5	50			

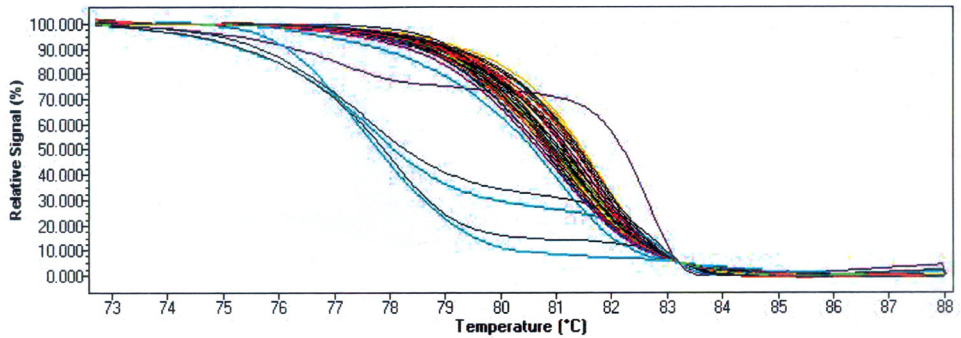

Fig. 3: Normalized and shifted melting curve of IL-10 at position – 1082.

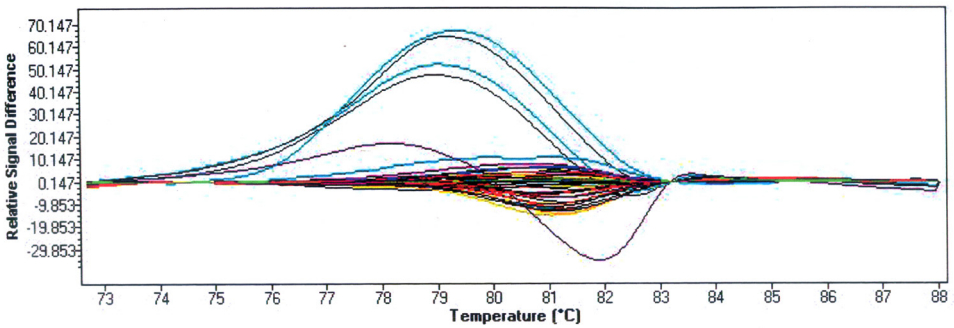

Fig. 4: Difference plots of polymorphism of IL-10 at position – 1082.

IL-10 genotypes showed no significant difference between cases subgroups compared to control group (Table 6). Also there is high significant association between composite genotypes (TNF-α G/G, IL-10 G/A) and RDH (Table 8) and (Fig 5).

Table 6: Comparison between IL-10 genotypes at -1082 among group B and group I, group II and group III:

IL-10 Genotypes	Group B Control (n= 10)		Group I Mild (n= 7)		P-value	Group II Moderate (n= 4)		P-value	Group III Severe (n= 9)		P-value
	Frequency	%	Frequency	%		Frequency	%		Frequency	%	
G/G	3	30	1	14.3	NS	0	0	NS	0	0	NS●
G/A	5	50	6	85.7	NS	3	75	NS	9	100	NS●
A/A	2	20	0	0	NS	1	25	NS	0	0	NS●

Table 7: The odds ratios for association between composite genotypes (TNF-α G/G, IL-10 G/A) and chronic RHD in group A and group B:

TNF-α G/G, IL-10 G/A	Group A Cases (n= 20)		Group B Control (n= 10)		P-value	Odds Ratio	95% CI
	Frequency	%	Frequency	%			
+ve	10	50	0	0	≤0.01**	2.000	1.290-3.100
-ve	10	50	10	100			

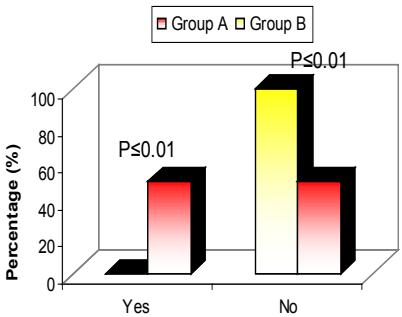

Fig. 5: Association between composite genotypes (TNF- α G/G,IL-10 G/A) and chronic RHD in group A and group B.

Also there is statistically significant association between composite genotypes (TNF-α G/A, IL-10 G/G) in group B (Control) than group A (Patients), so this composite genotype is considered as a protective genotype against RHD, (Tables 7 and 9) and (Fig. 6) Normalized and difference spots of IL-10 polymorphisms at-1082 (Figs. 3, 4).

Table 8: Comparison between composite genotypes TNF-at -308 and IL-10 at -1082 in group A and group B:

Composite Genotypes	Group A Cases (n= 20)		Group B Control (n= 10)		P-value
	Frequency	%	Frequency	%	
TNF-α G/G, IL-10 G/G	1	5	1	10	NS●
TNF-α G/G, IL-10 G/A	10	50	0	0	≤0.01**
TNF-α A/A, IL-10 G/A	1	5	0	0	NS●
TNF-α G/A, IL-10 G/G	0	0	2	20	≤0.05*
TNF-α G/A, IL-10 A/A	1	5	2	20	NS●
TNF-α G/A, IL-10 G/A	7	35	5	50	NS●

Table 9: The odds ratio for association between composite genotypes (TNF αG/A, IL-10 G/G) and chronic RHD in group A and group B:

TNF αG/A, IL-10 G/G	Group A Cases (n= 20)		Group B Control (n= 10)		P-value	Odds Ratio	95% CI
	Frequency	%	Frequency	%			
Yes	0	0	2	20	≤0.05*	3.500	1.949-6.287
No	20	100	8	80			

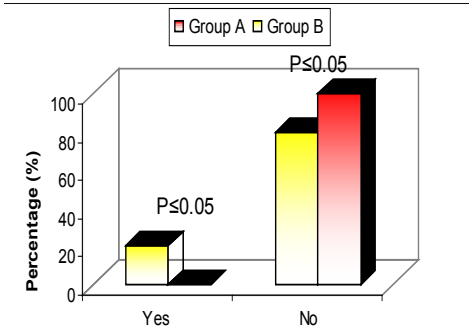

Fig. 6: Association between composite genotypes (TNF-G/A, IL-10 G/G) and chronic RHD in group A and group B.

DISCUSSION

Rheumatic fever is an auto reactive disease secondary to group A streptococcal (GAS) infection involving the heart, joints, skins and brain. Acute rheumatic fever (ARF) is an infective illness that has an impact on the heart and gives rise to chronic rheumatic heart disease[2]. Rheumatic fever and rheumatic heart disease continue to be an important cause of cardiovascular morbidity and mortality in developing world and account of all pediatric cardio admission. Carditis is the only manifestation of RF that leads to permanent disability. Recurrences' of RF are likely to cause further damage of the heart with higher morbidity and mortality.[18]

The production of interlukine (IL) -10, TNF-α and IL-2 in the valvular lesions of ARF patients was correlated with Aschoff nodule progression. IL-1 and TNF-α were secreted by monocytes/macrophages in stages 1 and 2, IL-2 was secreted by T lymphocytes in stage 3. These results suggest a major role for inflammatory cytokines in mediating heart lesions in RF.[11]

WHO[2] reported that the immune response is genetically controlled with high responsiveness to streptococcal cell wall antigen which is being expressed through a single recessive gene and with low responsiveness through a single dominant gene. Immuno- histochemical analysis of surgical heart tissue fragments from patients with RHD showed predominance of cells positive for TNF-α and IL-10 in lesion sites in both myocardium and valves[11]. The mononuclear cells from rheumatic heart lesions predominantly secrete INF-α and IL-10 in both myocardium and valves.[19]

Hafez, et al.[20] found the relation between the degree of the concentration of INF-γ and the severity of carditis which indicates variability in genetic susceptibility and hence the degree of response. Tumor necrosis factor α is a potent immune-modulator and pro-inflammatory cytokine that has been implicated in the pathogenesis of a large number of human diseases. The location of its gene within MHC and biological activities has raised the possibility that polymor-

phisms within this locus may contribute to the pathogenesis of wide large autoimmune and infectious diseases.[21]

Our study demonstrated that there is highly significant frequency of homozygous genotype TNF-α G/G at position-308 in cases ($p < 0.05$, odd ratio= 2 and 95% confidence interval ;CI) than control group. So there is significant strong association between homozygous TNF-α -308 genotype G/G and RHD.

On the other hand Settein, et al.[22] studied polymorphisms of TNF-α gene at -308 and found that TNF-α G/A was higher in cases compared to control group. The differences can be attributed to regional differences as well as difference in sample size.

Hernandes Pacheco, et al.[23] reported that RHD is associated with TNF-α polymorphisms at position -308 in the Mexican population, he found that increased frequencies of TNF-α G/A at-308 and found that TNF-α G/A at-308 and decreased frequencies G/G genotype in cases compared to control group. The difference can be attributed to different races. In this work our results showed no significant difference in frequency of homozygous genotypes of TNF-α A/A and G/G of mild and moderate valvular lesions compared to control group. However in severe cases of RHD the frequency of homozygous genotype G/G was significantly higher in patients than in control group while the frequency of heterozygous genotype G/A is significantly higher in control group compared to cases.

On the other hand Settein, et al.[22] found that patients with moderate severity showed high frequency of homozygous genotypes TNF-α A/A and G/G,while patients with severe lesions showed significant high frequency of homozygous genotype A/A.

IL-10 is an important component of anti inflammatory cytokines network suppressing gene expression and synthesis of pro-inflammatory cytokines[24]. In our study using real time PCR detected significant increase in heterozygous genotypes G/A of IL-10 at position -1082 in cases ($p \leq 0.05$, odds ratio, 13.5, CI, 95%) than in control. So there was a statistically strong association between heterozygous G/A and RHD while homozygous G/G and A/A genotypes of IL-10 showed no statisticaly significant difference.

Settein, et al.[22] reported that higher frequency of homozygous A/A and G/G of IL10 at position -1082 with RHD. On the other hand there were studies of IL10 at -1082 with other diseases. Germano, et al.[25] reported that IL10 gene polymorphisms at –1082 and expression are important in determining susceptibility to Cardiomyopathy and Nemec, et al.[26] reported that an association between the -1082 G/A polymorphisms in IL10 gene promoter and the production of serum levels of rheumatoid factors in patients with rheumatoid arthritis. Our results showed no statisticaly significant differences in comparison between IL10 genotype frequency at -1082 with severity of RHD in cases groups compared to control. On the other hand Settin, et al.[22] showed that homozygous genotype A/A was related to a more severe form of RHD.

The differences between our results and the other study may be related to sam-

ple size, regional variations and also our work was done using real time PCR which is more accurate than PCR and this may account for conflicting results.

An auto regulatory loop appears to exist in which TNF-α Stimulates IL-10 production which in turn reduces TNF-α synthesis. Stimulation of human blood samples with bacterial lipo-polysaccharide showed large inter individual variations of IL10 and TNF-α production suggesting a genetic component.[27]

In present study our results showed that composite genotypes of (TNF-α G/G, IL-10 G/A) were statistically significant high in cases compared to control groups) (p value ≤0.01, Odds Ratio= 2, 95% CI), so there was association between composite genotype (TNF-α G/G, IL-10 G/A) and the RHD while composite genotype (TNF-α G/A, IL-10 G/G) showed statistically significant higher in control group than cases (p value ≤0.05, Odds Ratio= 3.5, 95% CI). So there was association between composite genotype (TNF-α G/A, IL-10 G/G) and control group.

Settin, et al.[22] found that composite genotype of cases compared to control showed significantly high frequency in genotype TNF-α at-308 A/A with IL10 at-1082 A/A. Also composite heterozygous genotypes of TNF –α at-308 G/A and IL10 at- 1082 G/A was significantly among control.

We suggest the discrepancy between our results and others could be attributed to different geographic distribution regional variations sample size and methodology.

CONCLUSION

Susceptibility to RHD is associated with certain cytokine gene polymorphisms that is considered a risk genotypes as homozygous genotype TNF-α at -308G/G and heterozygous genotype IL-10 at -1082 G/A, So they considered as risk genotypes.

- Cases with severe RHD was associated with homozygous TNF-α at -308 genotype G/G while heterozygous genotype G/A had low risk for RHD and may be considered as protective genotype.

- Severity of RHD has no significant association with gene polymorphisms of IL-10 at -1082.

- Composite genotypes (TNF-α G/G, IL-10 G/A) had high risk for RHD while composite genotypes (TNF-α G/A, IL-10 G/G) had low risk for RHD and may be regarded as protective genotypes.

REFERENCES

1. Carapetis JR, Brown A, Wilson NJ and Edwards KN. An Australian guideline for rheumatic fever and rheumatic heart disease: An abridged outline. Med.J.Aust. 2007 4;186(11):581-6.

2. World Health Organization. Rheumatic fever and rheumatic heart disease. 1994. 923. World Health Organization, Geneva.

3. Saleh HK. Pattern of rheumatic heart disease in Southern Yemen. Saudi Med.J. 2007; 28(1):108-13.

4. Stanevicha V, Eglite J, Sochnevs A, Gardovska D, Zavadska D and Shantere R. HLA class II associations with rheumatic heart disease among clinically homogeneous patients in children in Latvia. Arthritis Res.Ther. 2003;5(6):R340-6.

5. Ayoub EM, Nelson B, Shulman ST, Barrett DJ, Campbell JD, Armstrong G, et al. Group A streptococcal antibodies in subjects with or without rheumatic fever in areas with high or low incidences of rheumatic fever. Clin.Diagn. Lab.Immunol. 2003;10(5):886-90.

6. 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(11):1436-42.

7. Guilherme L, Ramasawmy R and Kalil J. Rheumatic fever and rheumatic heart disease: Genetics and pathogenesis. Scand.J.Immunol. 2007; 66(2-3):199-207.

8. Kroeger KM, Carville KS and Abraham LJ. The -308 tumor necrosis factor-alpha promoter polymorphism effects transcription. Mol.Immunol. 1997; 34(5):391-9.

9. Stämpfli MR, Cwiartka M, Gajewska BU, Alvarez D, Ritz SA, Inman MD, et al. Interleukin-10 gene transfer to the airway regulates allergic mucosal sensitization in mice. Am.J.Respir.Cell Mol.Biol. 1999;21(5):586-96.

10. Spencer SD, Di Marco F, Hooley J, Pitts Meek S, Bauer M, Ryan AM, et al. The orphan receptor CRF2-4 is an essential subunit of the interleukin 10 receptor. J.Exp.Med. 1998 16;187(4):571-8.

11. Guilherme L, Cury P, Demarchi LMF, Coelho V, Abel L, Lopez AP, et al. Rheumatic heart disease: Proinflammatory cytokines play a role in the progression and maintenance of valvular lesions. Am.J.Pathol. 2004;165(5):1583-91.

12. Asseman C and Powrie F. Interleukin 10 is a growth factor for a population of regulatory T cells. Gut 1998; 42(2):157-8.

13. Kurreeman FAS, Schonkeren JJM, Heijmans BT, Toes REM and Huizinga TWJ. Transcription of the IL10 gene reveals allele-specific regulation at the mRNA level. Hum.Mol.Genet. 2004;13(16):1755-62.

14. Ates Ö, Musellim B, Ongen G and Topal-SarIkaya A. Interleukin-10 and tumor necrosis factor-α gene polymorphisms in tuberculosis. J.Clin.Immunol. 2008;28(3):232-6.

15. D'Alfonso S, Rampi M, Rolando V, Giordano M and Momigliano Richiardi P. New polymorphisms in the IL-10 promoter region. Genes Immun. 2000; 1(3):231-3.

16. Cheesbrough M. District laboratory practice in tropical countries. 2nd ed. Cambridge University Press; 2006.

17. Chernecky CC, Berger BJ. Laboratory tests and diagnostic procedures. 5th ed. Saunders; 2007.

18. Sadiq M, Islam K, Abid R, Latif F, Rehman AU, Waheed A, et al. Prevalence of rheumatic heart disease in school children of urban Lahore. Heart 2009;95(5):353-7.

19. Guilherme L and Kalil J. Rheumatic fever: From innate to acquired immune response. Ann.N.Y.Acad.Sci. 2007;1107:426-33.

20. Hafezh H, El-Mursi Z, El-Shennawi FA, Hawwas SA, Sheaishaa AA, El-Marssafawi HM, et al. Cytokine gene expression in rheumatic fever. Egypt.J.Immunol. 2001;8(1):61-76.

21. Elahi MM, Asotra K, Matata BM and Mastana SS. Tumor necrosis factor alpha - 308 gene locus promoter polymorphism: An analysis of association with health and disease. Biochim.Biophys.Acta Mol.Basis Dis. 2009;1792(3):163-72.

22. Settin A, Abdel-Hady H, El-Baz R and Saber I. Gene polymorphisms of TNF-α-308, IL-10-1082, IL-6-174 and IL-1RaVNTR related to susceptibility and severity of rheumatic heart disease. Pediatr.Cardiol. 2007;28(5):363-71.

23. Hernandez Pacheco G, Flores Dominguez C, Rodriguez Perez JM, Perez Hernandez N, Fragoso JM, Saul A, et al. Tumor necrosis factor-alpha promoter polymorphisms in Mexican patients with rheumatic heart disease. J.Autoimmun. 2003; 21(1):59-63.

24. Reuss E, Fimmers R, Kruger A, Becker C, Rittner C and Höhler T. Differential regulation of interleukin-10 production by genetic and environmental factors - a twin study. 2002 Genes and Immun. 3 (7), pp. 407-413.

25. Costa GC, Rocha MODC, Moreira PR, Menezes CAS, Silva MR, Gollob KJ, et al. Functional IL-10 gene polymorphism is associated with chagas disease cardiomyopathy. J.Infec.Dis. 2009;199(3):451-4.

26. Nemec P, Pavkova-Goldbergova M, Gatterova J, Fojtik Z, Vasku A and Soucek M. Association of the -1082 G/A promoter polymorphism of interleukin-10 gene with the autoantibodies production in patients with rheumatoid arthritis. Clin.Rheumatol. 2009;28(8):899-905.

27. Koch W, Kastrati A, Bottiger C, Mehilli J, von Beckerath N and Schomig A. Interleukin-10 and tumor necrosis factor gene polymorphisms and risk of coronary artery disease and myocardial infarction. Atherosclerosis 2001; 159(1):137-44.