Lack of Association Between TNF-alpha rs1800629 (-308G > A) Polymorphism and Nephrotic Syndrome

Simin Sadeghi-Bojd,[1,2] Mohammad Hashemi,[3†] Mahrokh Firoozi-Jahanigh,[2] Maryam Rezaei,[4] Hosna Sarani,[1] Mohsen Taheri[3,5]

[1]Children and Adolescent Health Research Center, Resistant Tuberculosis Institute, Zahedan University of Medical Sciences, Zahedan, Iran
[2]Department of Pediatrics, School of Medicine, Zahedan University of Medical sciences, Zahedan, Iran
[3]Genetics of Non-communicable Disease Research Center, Zahedan University of Medical sciences, Zahedan, Iran
[4]Department of Clinical Biochemistry, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran
[5]Department of Genetic, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran
[†]Deceased

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Introduction. Idiopathic Nephrotic Syndrome is a multifactorial disease that accompanying with immune system dysfunction. Cytokines as potent immunomodulators have a key role in pathogenesis of the disease. We aimed to evaluate the association between TNFα -308G > A polymorphism with Idiopathic Nephrotic Syndrome and its effect on the response to steroid therapy.
Methods. This case-control study was performed on 168 patients with Nephrotic Syndrome and 153 healthy children. Genotyping of TNF-α rs1800629 (-308G > A) variant was detected by polymerase chain reaction restriction fragment length polymorphism method (PCR-RFLP).
Results. The results revealed that there was no significant difference in allele ($P > .05$, OR = 0.92, 95% CI: 0.59 to 1.43) or genotype ($P > .05$, OR = 1.00; 95% CI: 0.62 to 1.65) frequency of TNF-α rs1800629 (-308G > A) between childhood cases of nephrotic syndrome and healthy controls. Also no association was found between genotype ($P > .05$, OR = 2.28; 95% CI: 1.03 to 5.04), and allele frequency ($P > .05$, OR = 1.93; 95% CI: 0.97 to 3.87) among the SSNS and SRNS groups.
Conclusion. Our results did not support any association between the TNF polymorphism and the risk of nephrotic syndrome in a sample of southeast Iranian population.

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INTRODUCTION

Idiopathic Nephrotic syndrome (INS) is a common glomerular disease in childhood characterized by proteinuria, hyperlipidaemia, hypoalbuminemia and generalized edema.[1,2] Based on the response to steroid therapy, the disease is divided into two sensitive (steroid sensitive nephrotic syndrome - SSNS) and resistant (steroid resistant nephrotic syndrome – SRNS) forms. Approximately 10% of patients do not respond within 4 weeks, which is said to be steroid-resistant and the most common resistant form is focal segmental glomerolosclerosis. The steroid-resistant form has a poor prognosis and may require dialysis and transplantation.[3] Immunologic response and the imbalance between Th1 and Th2 cytokines may play an important role in pathogenesis of INS. Various circulating factors, released from activated T-cells, may affect the pathogenesis and progression of the disease.[4] Th1 obligation is to creation of IL-2, IFN-γ, and tumour necrosis factor-beta (TNF-β) and clarify both macrophage activation and production of complement-fixing and opsonizing antibodies. Tumour necrosis factor alpha (TNF-α) known as a powerful immunomodulator and pro-inflammatory Th1 cytokine that implicated in

many pathological processes.[5] Th2 cells, which can produce IL-4, IL-5, IL-6, IL-10, and IL-13, develop both eosinophil differentiation and activation and mast cell growth, resulting in humoral responses.[3] In previous studies, several cytokines have been measured in the urine and serum of the patients including interleukin IL-2, IFNγ, IL-4, IL8, IL-10, and TNF-α.[6,7,8] Recently, studies of several cytokine gene polymorphisms, including those on the genes encoding IL-1β, interleukin-1 receptor antagonist (IL-1ra), and TNF-α, have demonstrated an association with various inflammatory diseases.[9,10,11] The cytokine selected in the present study was TNF-α-G308A (db SNP ID rs1800629). The reason behind selecting these genes was that the single nucleotide polymorphism of these genes might influence susceptibility or clinical course of the disease. We aimed to compare TNF-α gene polymorphisms between patients with INS and healthy children, and the effects of genetic polymorphisms on steroid response were also studied in INS patients.

MATERIALS AND METHODS

This case-control study was conducted on patients suffering from nephrotic syndrome in the paediatric department of Ali-ebneh Abitaleb Hospital, Zahedan, Iran. The same paediatric nephrologist examined all participants. All patients were diagnosed according to the International Study of Kidney Disease in Children (ISKDC) criteria and were < 5 years old.[12] Patients with familial, infantile, congenital, and secondary NS were excluded from the study. Thus patients were categorized according to their clinical response to glucocorticoids into the following groups: steroid resistant patients (SR, no achievement of remission despite treatment with prednisolone, 60 mg/m^2/d for 6 to 8 weeks); and steroid sensitive group that consisted of frequent relapsers and infrequent relapsers. The steroid sensitive group was compared with the steroid resistant group in terms of genotype and alleles of TNF-α.

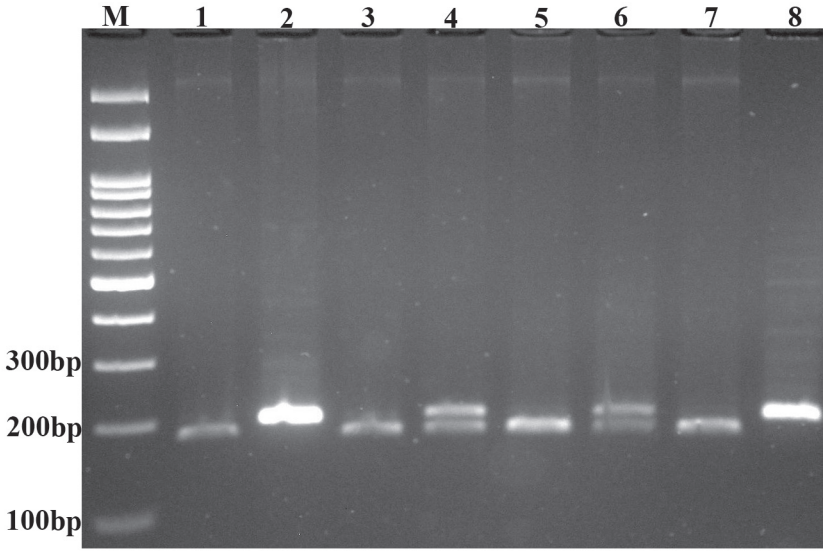
Demographic characteristics of cases and controls are summarized in Table 1. The local Ethical Committee of Zahedan University of Medical Sciences approved the study protocol (IR.ZAUMS.REC.1392.6014), and written informed consent was obtained from each subject s parents. Routine laboratory investigation included BUN and creatinine level, total protein, albumin and 24 hours urine collection in terms of protein and creatinine. One ml of venous blood was drawn from each subject, and genomic DNA was extracted from peripheral blood as described previously.[3,4] Samples were stored at −80°C until final analysis. Evaluation of TNF-α rs1800629 (−308 G > A) polymorphism was done by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Forward and reverse primers were 5′-GGAGGCAATAGGTTTTGAGGGCCAT-3′ and 5′-CTGCACCTTCTGTCTCGGTTTCT-3′, respectively. 0.20 mL reaction solution included 1 µl genomic DNA (approximately 100 ng/mL), 1 µL forward and reverse primers and 10 µL 2X Prime Taq Premix (Genet Bio, Korea) and 7 µL ddH2O.

Table 1. Demoghraphic and Biochemical Parameter in Two Groups

Parameter	Case (n = 168)	Control (n = 153)	*P*
Sex (M/F)	96 / 72	74 / 79	> .05
Age, y	5.77 ± 3.01	6.03 ± 2.3	> .05
Total Protein, g/dL	4.63 ± 0.79	-	-
Serum Albumin, mg/dL	2.42 ± 0.53	-	-
Triglyceride, mg/dL	296.92 ± 138.6	-	-
Cholesterol, mg/dL	382.48 ± 130.91	-	-

The PCR axle described as 5 min at 95°C followed by 30 cycles by duration of 30s at 95°C, 30s at 66°C, and 30s at 72°C with a final step of 72°C for 10 min to allow for complete extension of all PCR fragments. Ten microliters of PCR product were later digested (NcoI restriction enzyme) and analysed by electrophoresis on a 3% agarose gel containing 0.5µg/mL ethidium bromide and visualized by transillumination with UV light and a photograph was then taken .The G allele was digested and produced 180-bp and 24-bp, while the A allele was undigested and produced 204-bp fragment (Figure 1).

Data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using SPSS software by independent sample t-test and paired sample t-test. Allele frequency was calculated as the number of occurrence of the test allele in the population divided by the total number of alleles. Statistical analyses for the genotypic and allelic frequencies were performed using SPSS 15. *P* values < .05 were considered statistically significant. Odds ratios (OR) and 95% confidence intervals (CI) were calculated.



Photograph of the PCR Product of the TNF-alpha Polymorphism. M: DNA marker; Lane 1, 3, 5, and 7: rs1800629 GG; Lanes 2 and 8: rs1800629 AA; Lanes 4,6: rs1800629 GA.

RESULTS

168 children with NS (Male = 96, Female = 72) who were admitted to the nephrology clinic from 2011 to 2014; and 153 (Male = 74, Female = 79) healthy children were included as controls in this study. The mean age at diagnosis of NS group and healthy children group was 5.77 ± 3.01 years and 6.03 ± 2.3, respectively. Sex and age distribution of participants in two case and control groups shows no significant differences ($P > .05$, $P > .05$; respectively). Table 1 shows the demographic and biochemical profiles of both INS and control groups.

Among the case subjects, 121 had the GG genotype (72.0%), 47 had the GA genotype (28.0%) and 47 had combined GA + AA genotype (28.0%). Among control group, the GG genotype was also the most frequent; 109 had the GG genotype (71.2%), 42 had the GA genotype (27.5%), 44 had combined GA + AA genotype (28.8%) and 2 had the AA genotype (1.3%).

Frequencies for G and A alleles were 289 (86.0%) and 47 (14.0%) respectively in children with nephrotic syndrome, and the frequencies for G and A alleles were 260 (85.0%) and 46 (15.0%) respectively in control group. Our results showed there was no difference in the control group and childhood NS group neither at the genotypic level ($P > .05$, OR = 1.00, 95% CI: 0.62 to 1.65), nor at the allelic level ($P > .05$, OR = 0.92, 95% CI: 0.59 to 1.43) (Table 2).

A total of 80.6% of the patients were steroid sensitive, and 19.4% were steroid resistant. Thus, we evaluated the effects of TNF-α gene polymorphisms on the response to steroid, and no association was found in genotype ($P > .05$, OR = 2.28, 95% CI: 1.03 to 5.04) and at the allelic level ($P > .05$, OR = 1.93, 95% CI: 0.97 to 3.87) between SSNS and SRNS groups (Table 3).

Table 2. Genotype and Allele Frequency of TNF-alpha rs1800629 Gene in Patients with Nephrotic Syndrome and Control Subjects

TNF-alpha rs1800629	Case n (%)	Control n (%)	OR (95% CI)	*P*
Genotypes				
GG	121 (72.0)	109 (71.2)	1.00	-
GA	47 (28.0)	42 (27.5)	1.00 (0.62 to 1.65)	> .05
AA	0 (0.0)	2 (1.3)	-	-
GA + AA	47 (28.0)	44 (28.8)	0.96 (0.60 to 1.56)	> .05
Allele				
G	289 (86.0)	260 (85.0)	1.00	-
A	47 (14.0)	46 (15.0)	0.92 (0.59 to 1.43)	> .05

Table 3. Genotype and Allele Frequency of TNF-alpha rs1800629 Gene Among Patients with Nephrotic Syndrome

TNF-alpha rs1800629	Steroid Resistance n (%)	Steroid Sensitive n (%)	OR (95% CI)	*P*
Genotype				
GG	19 (57.6)	102 (75.6)	1.00	-
GA	14 (42.4)	33 (24.4)	2.28 (1.03 to 5.04)	> .05
AA	0 (0.0)	0 (0.0)	-	-
Allele				
G	52 (87.1)	237 (84.4)	1.00	-
A	14 (22.9)	33 (15.6)	1.93 (0.97-3.87)	> .05

DISCUSSION

In the present study, the possible association between TNF-alpha polymorphism and sporadic nephrotic syndrome was investigated. Children with INS were genotyped for the TNF-α gene polymorphisms. Findings did not demonstrate correlation between TNF-α-G308 A gene polymorphism and sporadic nephrotic syndrome. A polymorphism at -308 position in the TNF-α promoter gene sequence has been associated with elevated transcription and production of TNF-a.[13,14] Concentration and function of cytokines can alter due to various changes in the gene sequence. Bakr A. *et al.*[15] demonstrated patients with active primary NS (PNS) had higher TNF-α production level compared with controls and normalization of TNF-α production in duration of remission. In addition, there was a positive correlation between TNF-α production and the degree of proteinuria (r = 0.34, $P < .05$), mesangial hypercellularity (r = 0.42, $P < .05$), and glomerulosclerosis (r = 0.46, $P < .05$). This may clear the role of TNF-α in the pathogenesis of proteinuria as well as the pathological changes that occur in INS. Consequently, the level of TNF-α could be helpful to predict the pathological type of INS and the response to steroid therapy.[15] Zachwieja *et al.*[16] investigated intracellular synthesis of TNF-α in children with NS relapse and confirmed the involving role of TNF-α in abnormal cellular response in NS. Tain *et al.* studied blood soluble TNF-α receptor (sTNFR) and cell surface receptor (cTNFR) in children with nephrotic syndrome and found a higher plasma sTNFR level with lower cTNFR expression reflects NS activity, whereas a higher initial granulocyte cTNFR expression tends to predict steroid resistance. Therefore, the expressed TNFR may play a pathophysiological role in childhood NS.[17] Previous studies, have been revealed that the imbalance of TNF-β/IL-13 levels can contribute to illness development in childhood NS.[17,18,19] Jafar *et al.* evaluated the role of TNF-α cytokine polymorphism in children diagnosed with INS and strong association was confirmed with TNF-α-G308A.[13] In a study by Lama *et al,* uprising levels of TNF-α were found in SS and SRNS patients versus controls.[3] In the present study, there was no association between TNF-α gene polymorphism in patients diagnosed with NS. This can be due to ethical differences between these studies. Various alterations in cytokine production during INS have been described by many studies that had different results. In the Kim study the association between IL-1β, IL-1ra, and TNF-α gene polymorphisms and childhood nephrotic syndrome (NS) was evaluated. The allele frequency of G allele and A allele were 92.1 and 7.9%, respectively, in the patient group. These findings showed no significant differences between controls and patients.[7] In the Shimoyama H study, there was no significant difference in TNF-α concentration of urine and plasma in both acute and chronic phases.[20] Their result for association between TNF polymorphism and childhood nephrotic syndrome was the same as our data.

The steroid response in treatment of INS patients may affected by some gene polymorphism as genetic risk factors. Tripathi G *et al.* revealed that non-responsiveness of steroid treatment in INS children may cause by the AA genotype of TNF-α-G308A cytokine gene polymorphisms.[18] In our study, there was no subject with AA genotype of TNF-α polymorphism in both SSNS and SRNS groups. Madani *et al.* investigated the TNF-α-G308A polymorphism and corticosteroid therapy response in children with INS. They found the AA genotype and the A alleles in TNF-α-G308A were significantly associated with INS group compared with the control. The A allele distribution was higher significantly in SSNS compared with SRNS.[21] On

the other hand, Jafar *et al.* indicated the A allele was higher in SRNS group than in SSNS.[13] We found no significant differences between SSNS and SRNS patients.

The result of present study may be a point for further studies. There were some limitations in our study; first of all sample size was limited, second, we examined only one variant of TNF-α gene. Finally, we did not determine the serum levels of TNF.

CONCLUSION

The current study on Iranian children with INS revealed that there is no association between TNF-α-G308A genotype and alleles in the INS patients, control group, SSNS and SRNS groups; however, the role of other genetic and environmental factors cannot be ruled out. Further studies are required to confirm these results and the findings of other cytokines to elucidate the role of TNF-α gene polymorphism in development of INS and response to steroid therapy.

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CONFLICT OF INTEREST

All authors declare no conflict of interest.

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Correspondence to:
Mohsen Taheri, PhD
Professor of Genetic, Department of Genetic, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran
Tel: 0098 541 3337 2116
E-mail: Mohsen.taheri.gene@gmail.com, taheri@zaums.ac.ir

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