Original Article

The Impact of Tumor Necrosis Factor-α (−308 G/A) and Transforming Growth Factor Beta 1 (−509C/T) Gene Polymorphism in Egyptian Children with Primary Nephrotic Syndrome

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Abstract

Background: Nephrotic syndrome (NS) is a disease affecting both children and adults. Cytokines act as inflammatory mediators in childhood NS. Excretion of too much protein in patients' urine as well as reduction in albumin levels in their blood are the most common symptoms of NS**. Aim of the Study:** The aim is to assess the potential relationship of tumor necrosis factor (TNF)-α (−308 G/A) and transforming growth factor beta 1 (TGF-β1) (−509C/T) genes with the incidence of NS. **Subjects and Methods:** In this study, 99 healthy children and 98 children with NS have been included. Polymerase chain reaction was used to detect the gene polymorphism of both TNF-α-G308A and TGF-β1 (−509C/T). **Results:** The TNF-α G308A showed a significant different genotype distribution among children with NS compared with the healthy volunteers (GG vs. AA, $P = 0.0009$; [odds ratio [OR] 95% CI = 25.2 [2.45–259.23]); GG vs. GA, $P = 0.001$; (OR *95%* CI = 4.84 [1.74–13.5]); as well as alleles distribution of G vs. A, $P = 0.022$; (OR 95% CI = 1.06 [1.068–2.42]). However, there is a non-significant variation in the frequency of TGF-β1 (-509C/T) genotypes (CC, CT and TT) respectively (11.4%, 78.5% and 10.1) in NS patients, compared with their corresponding levels in healthy control subjects (15.5%, 68% and 16.5). **Conclusion:** TNF-α-G308A polymorphism may help to early predict the incidence of NS in children, while TGF-β1 showed no effect on the disease incidence.

Keywords: Nephrotic syndrome, transforming growth factor-β1, tumor necrosis factor-α

Introduction

Primary nephrotic syndrome (NS) is a renal disease. The patients have edema, proteinuria, and hyperlipidemia. NS patients are either steroid resistant or steroid dependent. NS treatment and its pathogenesis are still not clear.[1] The literature shows that the genetic background might be associated with the disease pathogenesis and its severity.[2]

Tumor necrosis factor (TNF)-α is an inflammatory cytokine and is involved in many inflammatory and immune-mediated responses. The immune cells stimulated by monocytes, macrophages, T- and B-lymphocytes release TNF-α. Stimulating the cytokine cascade to produce immune response is crucial to resist infections and cancers.[3] TNF-α-308 polymorphism is one of the few major polymorphisms identified in the TNF locus. TNF-α-308 polymorphisms are associated with stimulation of TNF-α expression.[4] The susceptibility to inflammation and infectious diseases might be associated with these polymorphisms.[5]

The transforming growth factor-β1 (TGF-β1) is a complex cytokine that performs vital cellular functions. TGF-β1 regulates cell differentiation and the processes of both cell

Access this article online

Quick Response Code:

Website: www.jnsbm.org

DOI: 10.4103/jnsbm.JNSBM_187_20

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Submitted: 01-Oct-2020 **Revised:** 23-Dec-2020
Accepted: 30-Apr-2021 **Published:** 15-Jul-2021

How to cite this article: Elmougy R, Elsaid A, Salah N. The impact of tumor necrosis factor-α (-308 G/A) and transforming growth factor beta 1 (-509C/T) gene polymorphism in Egyptian children with primary nephrotic syndrome. J Nat Sc Biol Med 2021;12:180-4.

migration and progression.[6] The signaling pathway of TGF-β1 regulates response of immunity and mineral maintenance.[7] The literature has shown that TGF-β1 has a crucial role in controlling diseases. The gene is located at 19q13.1–q13.3.[8] It is aimed in this study to assess the potential relationship of TNF-α (−308 G/A) and TGF-β1 (−509C/T) genes with the incidence of NS.

Subjects and Methods

Patients and controls

Ninety-eight patients with primary NS (70 boys and 28 girls) aging 4–12 years (mean 7.7 ± 0.375 years) were recruited from Mansoura University Children's Hospital, Mansoura University, Egypt. Patients with heavy proteinuria (>3.5 g 24-h urea) and Hypoalbuminemia (<2.5 g/dL) were included in this study. Ninety-nine healthy participants (53 boys and 46 girls) aging between 4 and 12 years (mean 7.3 ± 0.373) were included in the study. None of the controls had any signs or symptoms suggesting NS. Based on medical history and primary NS questionnaire, all controls were free from any other diseases.

Blood samples

Five ml of whole blood were collected from the subjects under investigation (patients and controls) by vein puncture using sterile disposable plastic syringes. The blood sample was divided into two parts. One part allowed to clot for 10–15 min then it was centrifuged. The levels of albumin, cholesterol, triglycerides and creatinine were measured in serum. The second part of blood sample was taken in ethylenediaminetetraacetic acid coated tubes for extraction of DNA. The PCR technique on these DNA samples was applied.

Determination of genotypes

DNA was isolated and purified from whole blood using QIAamp DNA Mini Kit (Cat No./ID: 51104), TNF-α (−308 G/A) genotypes were classified as GG, GA, and AA. TGF-β1 (−509C/T genotypes were CC, CT, and TT and these polymorphisms were determined by polymerase chain reaction (PCR). The reactions of TNF-α (−308 G/A) were performed according to the method of Perrey *et al*.[9] The primer sequences used for ARMs PCR TNF-α genotyping are:

- -TNFα-308 A 5-ATAGGTTTTGAGGGGCATGA-3
- -TNFα-308 G 5-ATAGGTTTTGAGGGGCATGG-3
- -Genetic antisense 5-TCTCGGTTTCTTCTCCATC-3.

The total volume of PCR reaction mixture was 20 µl. The reaction contained 4 µl of antisense primer (10 pmol/µl), 10 µl of Green master mix (Promega), mixed with 3 µl of DNA in PCR tube. This mixture was added to 3 µl of specific primer (10 pmol/µl) G or A primer in separate tubes. Cycling conditions included an initial denaturation of 95°C for 1 min. It was followed by 95°C for 15 s, 65°C for 50 s and 72°C for 40 s (10 cycles). Finally, 95°C for 50 s, 59°C for 50 s, and 72°C for 50 s (20 cycles). The products of PCR were electrophoresed on 2% agarose gel. It has been visualized after staining with ethidium bromide under UV illumination. The mutant AA and wild GG genotypes appeared at 184 bp[9] [Figure 1].

The PCR reaction of TGF-β1 (−509C/T) was performed according to the two complementary reactions.

The region in TGF-β1 promoter, which was targeted for amplification, is 340 bp [Figure 2]. The sequences of primers used in the study are

- 5í AAGGGGCAACAGGACACCTGGG 3í,
- 5í AAGGGGCAACAGGACACCTGGA 3í
- 5í CTACGGCGTGGAGTGCTGAG 3í.

The PCR reaction had 40 ng of DNA sample. The reaction volume was 30 µl. The reaction contained 0.16 µM of each primer. The reaction also contained 30 µM of each dNTP, 10 mM Tris-HCl (pH 9.0. Finally, 0.3 U of Taq DNA polymerase was added (Bangalore Genei, Bangalore). Amplification was taken place for 35 cycles. Each cycle consisted of denaturation at 94°C for 30 s. Annealing at 61°C for 20 s. Finally, extension at 72°C for 20 s.[10]

Statistical analysis

Statistical analysis was performed with the statistical discovery software JMP 9. Quantitative data were presented as mean and standard deviation (for normally distributed data), Qualitative data were presented as frequencies. The Chi-square test with Yates' correction and Fisher exact test were used to compare categorical variables. The odds ratio (OR) with 95% confidence interval (CI) was calculated to study the association between single nucleotide polymorphisms (SNPs) and NS disease. Significance was accepted at $P < 0.05$.

Results

Table 1 shows the biochemical parameters of primary NS patients and healthy volunteers such as albumin, triglycerides, cholesterol, creatinine, and the levels of hemoglobin, white blood cells and platelets in 197 cases, including (98) patients with primary NS and 99 healthy controls.

There is a highly significant increase in the level of serum TG and cholesterol in patients with primary NS ($P < 0.0001$) in comparison with healthy volunteers. However, a highly significant decrease has been observed in the level of serum albumin in patients with primary NS ($P < 0.0001$) when compared to healthy volunteers.

The genotype analysis of TNF-α (−308 G/A) gene showed a higher frequency of GG genotype in NS patients amounting to

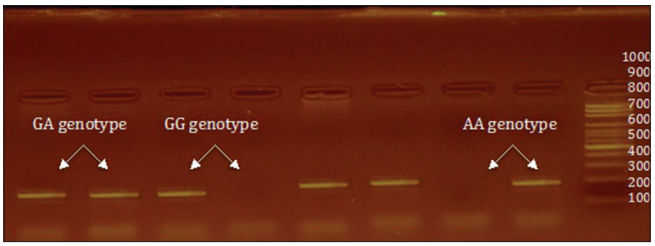

Figure 1: Agarose gel shows polymerase chain reaction product of tumor necrosis factor-α (−308 G/A) polymorphism in agarose gel electrophoresis (the size of amplified products [184 bp] and different tumor necrosis factor-α [−308 G/A] genotypes are shown)

22.3% versus 5.3% in healthy controls. However, the frequency of AA genotype was significantly lower in NS cases with a relative frequency of 1.1% in comparison with 6.4% in healthy volunteers [Table 2]. The frequency of A allele in NS patients was significantly lower (38.83%) in comparison with the level of the control group (50.53%).

Table 3 shows a nonsignificantly variation in the frequency TGF-β1 (−509C/T) genotypes (CC, CT and TT), respectively, (11.4%, 78.5%, and 10.1 in NS patients, compared with their corresponding levels in healthy controls (15.5%, 68%, and 16.5). The frequency of both alleles (C and T) has no significant difference in NS patients in comparison with healthy subjects.

Discussion

NS is a kidney disease. It is characterized by (proteinuria) and (hypoalbuminemia). Among the NS symptoms are hyperlipidemia and peripheral edema.[11,12] Patients suffered from edema and fatigue. They had neither heart failure nor chronic liver disease. The etiology of NS in adults is complex. It ranges from primary glomerular nephrtitis to secondary forms.[13] The Study of the genetic role in the progression or response to treatments in NS is considered to be important clinical approaches in pediatric nephrology.

Our study aims to assess TNF-α (−308 G/A) as a candidate gene with the risk of incidence of NS. Our results show that the AA genotypes and A allele are significantly decreased in NS patients in comparison with healthy controls. This result conflicts the results of Youssef *et al*.[3] and Madani *et al*.[14] in Egypt, Jafar *et al*. in India[15] and Fadhil *et al*. in Iraq,[16] who registered a significant higher AA genotypes and A allele. Kim *et al*.[17] and Tieranu *et al*.[18] stated that TNF-α (−308 G/A) is not related to the risk of NS. However,

Figure 2: Agarose gel shows polymerase chain reaction product of transforming growth factor-β1 (−509C/T) polymorphism in agarose gel electrophoresis (the size of amplified products [340 bp] and different transforming growth factor-β1 [−509C/T] genotypes are shown)

Table 1: Biochemical parameters of primary nephrotic syndrome patients and healthy volunteers

Parameter	Mean±SE		*P*, OR (CI)
	Control (*n*=99), *n* (%)	Patients (*n*=98), *n* (%)	
Age	7.3±0.373	7.7±0.375	0.43
Gender			
Female	46 (46.46)	28 (28.57)	0.009, 2.16 (1.2-3.9)
Male	53 (53.54)	70 (71.43)	
Residency			
Rural	66 (66.67)	62 (63.27)	0.61, 1.16 (0.65-2.09)
Urban	33 (33.33)	36 (36.73)	
Serum albumin	4.49±0.081	2.02±0.081	<0.0001
Serum triglycerides	76.9±5.9	185.87±5.93	<0.0001
Serum cholesterol	156.88±9.23	448.88±9.27	<0.0001
Serum creatinine	0.48±0.012	0.46±0.012	0.14
Hemoglobin	11.38±0.15	11.86±0.151	0.025
WBC's	10.08±0.313	10.45±0.315	0.41
Platelets	344.4±11.99	396.9±12.05	0.023

SE: Standard error, OR: Odds ratio, CI: Confidence interval, WBC: White blood cell

Table 2: Tumor necrosis factor-alpha gene distributions in both primary nephrotic syndrome cases and healthy volunteers

Polymorphism	Controls (*n*=94), *n* (%)	Patients (*n*=94), *n* (%)	*P*	OR (95% CI)
TNF-α (−308 G/A)				
Genotypes				
GG	5 (5.3)	21 (22.3)	Reference	
GA	83 (88.3)	72 (76.6)	0.001	4.84 (1.74-13.5)
AA	6 (6.4)	1 (1.1)	0.0009	25.2 (2.45-259.23)
Alleles				
G	93 (49.47)	115 (61.17)	Reference	
A	95 (50.53)	73 (38.83)	0.022	1.06 (1.068-2.42)

OR: Odds ratio, CI: Confidence interval, TNF-α: Tumor necrosis factor alpha

Table 3: Transforming growth factor beta 1 (−509C/T) gene distributions in both primary nephrotic syndrome cases and healthy volunteers

Polymorphism	Controls (*n*=97), *n* (%)	Patients (*n*=79), *n* (%)	*P*	OR (95% CI)
TGF-β1 (−509C/T)				
Genotypes				
CC	15 (15.5)	9 (11.4)	Reference	
CT	66 (68.0)	62 (78.5)	0.3	1.56 (0.64-3.84)
TT	16 (16.5)	8 (10.1)	0.76	0.83 (0.25-2.72)
Alleles				
C	96 (49.48)	80 (50.63)	Reference	
T	98 (50.52)	78 (49.37)	0.83	0.955 (0.63-1.45)

OR: Odds ratio, CI: Confidence interval, TNF-β1: Tumor necrosis factor beta 1, TGF-β1: Transforming growth factor beta 1

our finding is similar to that of Ayelign *et al*.[19] who found that the GG genotypes are more frequent in Type 2 Diabetes Mellitus (T2DM) patients than in healthy controls in Ethiopia and that of El Gendy *et al*.[20] who found that GG genotypes are more frequent in children with community-acquired pneumonia than in healthy children. The cause of this similarity might be due to the NS patients suffering from hyperlipidemia. Hyperlipidemia may be a common factor between NS and T2DM.

The analysis of the TGF-β1 (−509C/T) gene frequency revealed that it is non-significantly different in both NS and control groups. This finding agrees with the results reported by Li *et al*.[2] who found no statistical differences in the genotype or allele frequencies of TGF-β1 (−509C/T) gene between primary NS cases and normals. However, our results are not in agreement with the results reported by Mao *et al*.[21] who stated that T allele at the − 509 T/C polymorphism may be associated with chronic kidney disease risk in Asian population. This variation in − 509 T/C distributions may be due to the variety in race and population size.

Conclusion

In this study, we found GG genotype of TNF-α (−308G/A) polymorphism is more frequent in Egyptian children with NS. Individuals with GG genotype could develop primary NS faster than individuals with AA/AG genotypes. G allele in TNF-α (−308G/A) might be considered as an independent risk factor in Egyptian children with NS.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Shahid S, Abid A, Mehdi SQ, Firasat S, Lanewala A, Naqvi SA, *et al*. Association of the ACE-II genotype with the risk of nephrotic syndrome in Pakistani children. Gene 2012;493:165-8.
2. Li Y, Liu FY, Peng YM, Li J, Sun L, Chen X, *et al*. The relationship between the TGF-beta1 gene -509C/T polymorphism and tubulointerstitial damage resulting from primary nephrotic syndrome. Ren Fail 2010;32:420-7.
3. Youssef DM, El-Shal AS, Hussein S, Salah K, Ahmed AE. Tumor necrosis factor alpha gene polymorphisms and haplotypes in Egyptian children with nephrotic syndrome. Cytokine 2018;102:76-82.
4. Al-Kholy W, Elsaid A, Sleem A, Fathy H, Elshazli R, Settin A. TNF-α-308 G & A and IFN-γ+874 A & T gene polymorphisms in Egyptian patients with lupus erythematosus. Meta Gene 2016;9:137-41.
5. Siddall EC, Radhakrishnan J. The pathophysiology of edema formation in the nephrotic syndrome. Kidney Int 2012;82:635-42.
6. Chen G, Hu C, Lai P, Song Y, Xiu M, Zhang H, *et al*. Association between TGF-β1 rs1982073/rs1800469 polymorphism and lung cancer susceptibility: An updated meta-analysis involving 7698 cases and controls. Medicine (Baltimore) 2019;98:e18028.
7. Gordon KJ, Blobe GC. Role of transforming growth factor-beta superfamily signaling pathways in human disease. Biochim Biophys Acta 2008;1782:197-228.
8. Guo P, Liu S, Sun X, Xu L. Association of TGF-ß1 polymorphisms and chronic hepatitis C infection: A meta-analysis. BMC Infect Dis 2019;19:758.
9. Perrey C, Turner SJ, Pravica V, Howell WM, Hutchinson IV. ARMS-PCR methodologies to determine IL-10, TNF-alpha, TNF-beta and TGF-beta 1 gene polymorphisms. Transpl Immunol 1999;7:127-8.
10. Kumar A, Gupta V, Changotra H, Sarin BC, Sehajpal PK. Tumor necrosis factor–Alpha and transforming growth factor–Beta1 polymorphisms in bronchial asthma. Indian J Med Sci 2008;62:323-30.
11. Bierzynska A, Saleem MA. Deriving and understanding the risk of post-transplant recurrence of nephrotic syndrome in the light of current molecular and genetic advances. Pediatric Nephrology. 2018 Nov;33(11):2027-35-40.
12. Cadnapaphornchai MA, Tkachenko O, Shchekochikhin D, Schrier RW. The nephrotic syndrome: Pathogenesis and treatment of edema formation and secondary complications. Pediatr Nephrol 2014;29:1159-67.
13. Kodner C. Diagnosis and management of nephrotic syndrome in adults. Am Fam Physician 2016;93:479-85.
14. Madani HA, Bazaraa HM, Rady H. Association of cytokine genes polymorphisms and the response to corticosteroid therapy in children with idiopathic nephrotic syndrome: A pilot study in Egypt. Int Res J Med Med Sci 2014;4:84-90.
15. Jafar T, Agrawal S, Mahdi AA, Sharma RK, Awasthi S, Agarwal GG. Cytokine gene polymorphism in idiopathic nephrotic syndrome children. Indian J Clin Biochem 2011;26:296-302.
16. Fadhil ZJ, Abbas AA, Ali SH. Tumor necrosis factor alpha gene polymorphism in Nephrotic syndrome. J Pharm Sci Res 2019;11:418-22.
17. Kim SD, Park JM, Kim IS, Choi KD, Lee BC, Lee SH, *et al*. Association of IL-1beta, IL-1ra, and TNF-alpha gene polymorphisms in childhood nephrotic syndrome. Pediatr Nephrol 2004;19:295-9.
18. Tieranu I, Dutescu MI, Bara C, Tieranu CG, Balgradean M, Popa OM. Preliminary study regarding the association between tumor necrosis factor alpha gene polymorphisms and childhood idiopathic nephrotic syndrome in romanian pediatric patients. Maedica (Bucur)

2017;12:164-8.
19. Ayelign B, Genetu M, Wondmagegn T, Adane G, Negash M, Berhane N. TNF-α (−308) Gene Polymorphism and Type 2 diabetes mellitus in ethiopian diabetes patients, diabetes, metabolic syndrome and obesity: Targets Ther 2019; 12: 2453-9.
20. El Gendy FM, El-Mekkawy MS, El-Naidany SS, El-torgoman ST. The role of tumor necrosis factor alpha − 308 G>A promoter polymorphism in pediatric community acquired pneumonia. Egypt Pediatr Assoc Gaz 2020;68;5.
21. Mao S, Yan B, Zhang J. Association of transforming growth factor-β1 polymorphisms with the risk of chronic kidney diseases. Ren Fail 2015;37:304-11.