RESEARCH ARTICLE

Tumor Necrosis Factor Alpha-863 C/A Single Nucleotide Polymorphisms and Nephrotic Syndrome

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Received: 16th June, 2020; Revised: 13th July, 2020; Accepted: 19th August, 2020; Available Online: 25th September, 2020

ABSTRACT

Introduction: Cytokines act as a mediator of inflammation in childhood nephrotic syndrome. Polymorphisms of cytokines genes may influence susceptibility to nephrotic syndrome (NS), as well as, patients' steroid responses.

Objective: To study the association of tumor necrosis factor-alpha single nucleotide polymorphisms (TNF-α SNP) (-863 C/A) with the development of NS in addition to access to their effects on serum level of TNF and the response to steroid therapy.

Patients and Methods: This study included 60 patients (19 female and 41 male) with nephrotic syndrome; their ages ranged from 2 to 18 years. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to assess the TNF-α gene polymorphism.

Results: According to the digestion pattern of RFLP-PCR products of TNF-α-863, this polymorphism had three genotypes, which were CC, CA, and AA, in both NS patients and controls. Also, the current result observed that -863 SNP do not affect the serum level of TNF-α and steroid responsiveness in patients with nephrotic syndrome.

Conclusion: This polymorphism did not show any significant association with response to steroid therapy and TNF serum level neither at genotype nor at allele level.

Keywords: Nephrotic syndrome, Polymerase chain reaction (PCR), Tumor necrosis factor-alpha (TNF-α).

International Journal of Drug Delivery Technology (2020); DOI: 10.25258/ijddt.10.3.1

How to cite this article: Fadhil ZJ, Abbas Ahmed AH, Ali SH. Tumor necrosis factor alpha-863 C/A single nucleotide polymorphisms and nephrotic syndrome. International Journal of Drug Delivery Technology. 2020;10(3):319-322.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

The NS is an immune-mediated renal disease. It is related to the disorder of T cell and secondary disorder of B-cell that leads to changes in scales of immunoglobulins.[1] This systemic disturbance of function of T cell results in the production of humoral factors or lymphokines that responsible for the rise of permeability of the glomerular basement membrane.[2] Usually, children are more commonly affected, with a decreased quality of life, so they may expose to serious complications related to considerable morbidity and mortality rates.[3]

Many studies mentioned that cytokines play an essential function as mediators of inflammation and are considered as primary candidates for mediating nephrotic syndrome progression.[4] Inflammatory response development and advancement are partly correlated with the pro-inflammatory cytokine TNF-α.[5] TNF has an essential role in the pathogenesis of kidney injury, inflammation, damage of glomerular permeability barrier, and development of albuminuria.[6]

The mechanisms underlying the difference in response to steroid therapy in NS are not well understood, genetic factors may be involved in those mechanisms.[7] Different SNPs in the TNF-α gene promoter have been investigated.[8,9] These SNPs alter circulating TNF-α levels by regulating its production.[6,10]

PATIENTS AND METHODS

The current study comprised of 60 NS patients (41 males and 19 females, with a mean age of 7.23 ± 3.15 years) and 30 healthy controls. They were seeking treatment in the nephrology consultation clinic at Al-Imamain Al-Kadimain Medical City, Central Child Teaching Hospital, Al-Karama Teaching Hospital, and Children Welfare Teaching Hospital, Medical City in Baghdad. DNA was extracted from whole blood samples according to the manufacturer's instructions, using a kit from Promega Company, USA.

The detection of 863 C/A SNP in the promoter of TNF-α-gene was done using PCR-RFLP. The primer sequence for SNP is shown in Table 1. PCR amplification was done using a thermal cycler (Cleaver Scientific Thermal Cycler-TC32/80-UK). PCR was carried out according to the program shown in Table 2. PCR products were exposed to digestion by

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Table 1: Primer sequence of -863 C/A[11]

Polymorphism	*Primer 5'→3'*	*Fragment length*
-863 GlA	F: gGCTCTGAGGAATGGGTTAC R: CCTCTACATGGCCCTGTCTAC	126 bp

Table 2: Thermocycling conditions for amplification of the gene (-863 C/A)

Step	*Temperature and duration*	
Initial denaturation	94ºC for 4 minutes	
Denaturation	94ºC for 45 seconds	35 cycles
Annealing	53ºC for 54 seconds	
Extension	72ºC for 60 seconds	
Final extension	72ºC for 6 minutes	

Table 3: Socio-demographic and clinical characteristics of steroid-sensitive and steroid-resistant patients with nephrotic syndrome

Variables	*Steroid sensitive patients (30)*	*Steroid resistant patients (30)*	*p value*
Age, years (mean ± SD)	6.96 ± 2.87	7.49 ± 3.44	0.517
Sex, No (%)			
Male	20 (66.67%)	21 (70%)	0.781
Female	10 (33.33%)	9 (30%)	
Family history, No (%)			
No family history	25 (83.33%)	23 (76.67%)	0.314
Nephrotic syndrome	4 (13.33%)	2 (6.67%)	
Asthma	1 (3.33%)	4 (13.33%)	
Dermatitis	0 (0%)	1 (3.33%)	
Blood urea, mg/dL (mean ± SD)	24.93 ± 11.69	24.7 ± 10.05	0.934
Serum creatinine, mmol/L (mean ± SD)	52.67 ± 32.04	58.53 ± 36.89	0.514
Serum albumin, g/dL (mean ± SD)	1.64 ± 0.62	2.29 ± 0.96	0.003
Total cholesterol, mg/dL (mean ± SD)	166 ± 83.65	128.87 ± 82.42	0.089

Table 4: Frequency of different genotypes and alleles of TNF-α-863 polymorphism in nephrotic syndrome patients and controls

TNF-α-863	*Patients (60)*	*Controls (30)*	*p value*	*OR (95% CI)*
Genotypes				
CC	32 (53.33%)	19 (63.33%)	0.661	1 reference
CA	21 (35%)	8 (26.67%)	0.381	1.559 (0.578–4.206)
AA	7 (11.67%)	3 (10%)	0.663	1.385 (0.32–6.006)
HWE	0.236	0.163		
Alleles				
C	85 (70.83%)	46 (76.67%)	0.408	1 reference
A	35 (29.17%)	14 (23.33%)		1.353 (0.661–2.769)

restriction enzymes (Biolab, England). Then, the fragments were electrophoresed in a 2.5% agarose gel (Promega, USA) using 50 bp marker (Promega, US), stained with ethidium bromide, and visualized under a UV transilluminator.

Data Analysis

Statistical analysis was conducted using Statistical Package for Social Science (SPSS) software version 20. A $p < 0.05$ was accepted as the level of significance.

RESULTS

Both patients and controls are compatible regarding age and sex. Mean age in patients and controls were 7.23 ± 3.15 and 8.08 ± 3.64 years, respectively ($p = 0.252$). There were 41 males and 19 females among patients *vs.* 22 males and 8 females among controls ($p = 0.624$).

Table 3 shows the socio-demographic and clinical characteristics of steroid-sensitive and steroid-resistant patients. All the variables did not differ significantly between the two groups except serum album, which was higher in steroid resistance patients (2.29 ± 0.96 g/dL) than steroid-sensitive patients (1.64 ± 0.62 g/dL) with a highly significant difference ($p = 0.003$).

The distribution of different genotypes of the SNP was in accordance with the Hardy Weinberg equilibrium (HWE). According to the digestion pattern of PCR products of TNF-α-863 (Figure 1), this polymorphism had three genotypes, which were CC, CA, and AA, in both NS patients and controls.

Table 4 shows the frequency of different genotypes and alleles of this polymorphism in patients and controls. No significant differences between the two groups neither in genotype nor in allele frequencies.

Table 5: Frequency of different genotypes and alleles of a TNF-α-863 polymorphism in steroid-sensitive and steroid-resistant patients

TNF-α-863	*Steroid sensitive patients (30)*	*Steroid resistant patients (30)*	*p value*	*OR (95% CI)*
Genotypes				
CC	18 (60%)	14 (46.67%)	0.405	1 reference
CA	8 (26.67%)	13 (43.33%)	0.199	2.089 (0.679–6.429)
AA	4 (13.3%)	3 (10%)	0.966	0.964 (0.185–5.03)
Alleles				
C	44 (73.33%)	41 (68.33%)	0.547	1 reference
A	16 (26.67%)	19 (31.66%)		1.274 (0.579-2.807)

Table 6: Impact of TNF-α-863 on serum levels of TNF-α measured by pg/mL (median, range)

Status	*CC*	*CA*	*AA*
Controls (30)	3 (1–28)[a]	4.5 (0–47)[a]	2 (1–24)[a]
NS patients (60)	9.5 (0–71)[a]	7.5 (1–11)[a]	11 (0–99)[a]
Steroid sensitive patients (30)	8 (0–73)[a]	12.5 (0–99)[a]	9 (0–111)[a]
Steroid resistant patients (30)	14.5 (0–71)[a]	14 (1–86)[a]	16 (2–111)[a]

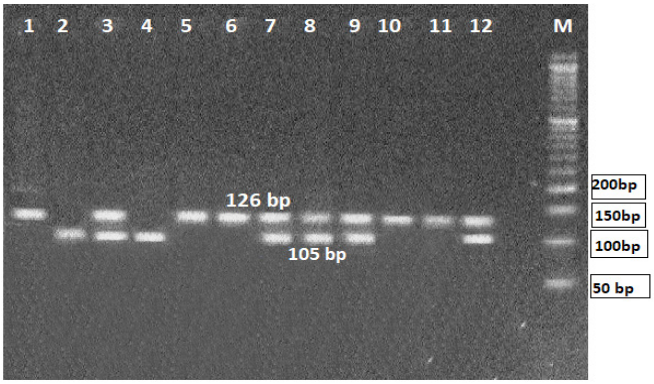

Figure 1: Gel electrophoresis for TNF-α-863 PCR products visualized under UV light after staining with ethidium bromide; M: 50–1,000 bp ladder; lanes 1, 5, 6, 10, and 11: wild-type homozygous genotype (CC); lanes 2 and 4: mutant homozygous genotype (AA); lanes 3, 7, 8, and 9: heterozygous genotype (CA)

Also, this polymorphism showed no association with the steroid effect, both at genotype and allele level (Table 5).

Comparison between overall patients and controls revealed a higher level of serum TNF-α in patients (median = 15.5, range = 0–111 pg/mL) than control (median = 3 pg/mL, range 0–47), with highly significant difference (p = 0.001). Besides, there were no significant differences in serum levels of TNF-α between the three genotypes of this polymorphism in all subject statuses (Table 6).

DISCUSSION

The demographic and biochemical profiles of both Steroid-sensitive nephrotic syndrome (SSNS) and Steroid-resistant nephrotic syndrome (SRNS) patients are shown in Table 3, in which there is no significant difference between both groups at all the parameters, except serum albumin. Hypo-albuminemia was a special characteristic of NS, so for hypoalbuminemia to develop, there must be either an increase in the catabolism of albumin or an insufficient increase in synthetic rate.[12] Several cytokine gene polymorphisms, like TNFα, have been associated with different inflammatory diseases as glomerulonephritis and multiple sclerosis.[13] SNPs in the promoter region of this cytokine gene can influence the activity of the gene promoter and its output quantity.[14] So the investigation of the genetic role in the pathophysiology of nephrotic syndrome or disease improvement, and the rate of response to the drug has an important clinical impact in pediatric nephrology. The prediction of early markers in nephrotic syndrome is important to determine the rate of response to glucocorticoid therapy and would allow optimization of the glucocorticoid dose and duration of the therapy.[10,15]

TNF promoter SNPs are likely to be in linkage with other genes in the human leukocyte antigen locus, which may, in turn, impact resistance, susceptibility, and severity to disease, independent of their effect on TNF gene expression.[11] However, this study observed no significant association between a TNF-α-863 polymorphism (regardless of genotype and an allele), and NS. Similar to the current finding, Youssef *et al.* showed an insignificant correlation of TNF-α -863 with NS.[10] On the contrary, a previous study reported no association between this polymorphism and some inflammatory disease, like ulcerative colitis and Chron's disease among Iranian people,[16] as well as, the current study observed that the level of serum TNF-α among nephrotic syndrome cases was higher than the control group. Also, in children with steroid-sensitive NS, TNF-α level was lower than steroid-resistant patients. This finding suggests that disease activity, at least in some NS children, may be associated with TNF-α serum levels. This assumption is supported by the lack of a reduction in serum TNF-α levels in patients with SRNS. In addition, the current result observed that -863 SNP have no effect on the serum level of TNF-α.

AUTHOR'S CONTRIBUTION

Zainab J. Fadhil: Preparation, performing, doing the tests of the research, and writing of the thesis; Dr. Ahmed Abdul-Hassan Abbas: Project design, reviewing the thesis, and interpretation of the results done under his supervision; Dr. Shathaa H. Ali: Help in the collection of samples.

CONCLUSION

This polymorphism did not show any significant association with response to steroid therapy and TNF serum level neither at genotype nor at allele level.

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