

# Association of ABCB1(Rs10276036, C/T) Gene, IL-18, and TNF $\alpha$ as Risk Factors for Nephrotic Syndrome Incidence

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## Abstract

**Background:** The most common cause of Nephrotic Syndrome (NS) in children is idiopathic NS, also called nephrosis. The most prominent clinical signs are hyperlipidemia, severe proteinuria, edema, swelling of body tissues, and an increased risk of infection. The object of this study was to examine the correlation of the ABCB1 gene (rs10276036, C > T), IL-18, and TNF $\alpha$  to the prevalence of NS among Egyptian children having NS.

**Methods:** This study included 100 participants with NS and 100 healthy controls. To analyze the ABCB1 gene (rs10276036 C >T) variant PCR technique was used. IL-18 and TNF levels were estimated using Enzyme-Linked Immunosorbent Assay (ELISA).

**Results:** Increased frequency of CT and TT genotypes of the ABCB1 gene (rs10276036 C / T) in NS patients compared to controls, with p-value = 0.001, OR = 2.270, CI = (1.550-3.327) for CT genotype and p-value = 0.001, OR = 5.070, CI = (2.463-10.438) for TT genotype. The frequencies of ABCB1 (rs10276036 C >T) genotypes were statistically significant in the dominant model (OR 2.560;  $p < 0.001$ ) and in the recessive model OR, 3.231;  $p = 0.001$ ). Significantly high levels of both IL-18 and TNF $\alpha$  were found in NS patients compared to controls.

**Conclusion:** The ABCB1 gene (rs10276036 C/T), IL-18, and TNF $\alpha$  are associated with the prevalence of NS in Egyptian children and might be considered as independent risk factors for its incidence.

**Keywords:** ATP Binding Cassette Transporter, Interleukin-18, Polymerase Chain Reaction, Subfamily B, Tumor Necrosis Factor-alpha.

## Introduction

Nephrotic syndrome (NS) or nephrosis is defined as a glomerular filtration barrier structural and functional impairment (1). It causes an increase in protein loss, resulting in significant proteinuria (2). Nephrotic syndrome (NS) is the second leading cause of chronic kidney disease (CKD) in people under the age of 25 (3). Boys are more susceptible to pediatric NS than girls with a ratio of 2:1. Nephrotic syndrome (NS) affects children of any age, from infancy through adolescence (4). Minimal change disease

(MCD) and focal segmental glomerulosclerosis (FSGS) are both common causes of nephrotic syndrome in children and adults (5). Steroid-sensitive nephrotic syndrome (SSNS) and steroid-resistant nephrotic syndrome (SRNS) are the two forms of NS. Some people with SSNS may eventually acquire SRNS (6).

The ABCB1 gene (multi-drug resistance MDR1) located on chromosome 7q21, consists of 28 exons with sizes ranging from 49 to 209 bp. P-glycoprotein (P-gp), a

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multidrug transporter, is encoded by this gene which functions as a transmembrane efflux pump, and required for drug absorption, tissue targeting, and excretion (7). ABCB1 mRNA expression in lymphocytes has been linked to a poor response to prednisone, cyclophosphamide, and cyclosporine. In children with NS, more than 50 single nucleotide polymorphisms (SNPs) in ABCB1 have been found. Previous studies found that children with SRNS have high ABCB1 expression and P-gp activity. Several studies have investigated the relationship between P-gp polymorphisms and the responsiveness of contradictory glucocorticoids (GCs) (8). The MDR-1 gene product P-glycoprotein is also suggested to be a risk factor for NS and/or steroid resistance (9). Some natural and synthetic GCs have xenobiotic accumulations in the leukocyte membrane cytoplasm. Cytokines promote the role of P-gp in chronic autoimmune disease inflammation (10, 11).

IL-18 is a member of the IL-1 family of cytokines, and is expressed by a wide range of cells, including monocytes, stromal cells, melanocytes, progenitor cells, and glial cells. Tubular epithelial cells are the primary source of IL-18 production in the kidney. IL-18 is involved in inflammation, which is thought to be the root cause of both acute and chronic kidney disease (12). Recently, researchers have investigated the biological and pathological role of IL-18 in different diseases (13).

Tumor necrosis factor-alpha (TNF- $\alpha$ ) is a cytokine that affects a variety of cell types (14). It is produced by activated macrophages, T-lymphocytes, and natural killer cells (15). TNF- $\alpha$  is regarded as the primary regulator of inflammatory responses (16). It plays a role in the development of various inflammatory and autoimmune disorders (17). Tumor necrosis factor receptors (TNFRs) are divided into two types (TNFR1 and TNFR2). TNFR1 is found in all cells. However, TNFR2 is found only in immune cells (18). It was observed that there is an increase in the TNF- $\alpha$  levels in NS

children (19).

## Materials and Methods

### Study Population

This study included 200 children, with 100 diagnosed with nephrotic syndrome at Mansoura University's Urology and Nephrology Centre in Egypt, and 100 healthy volunteers visiting the hospital for routine check-ups. The two groups were matched in age and sex. Ethical approval for the study was granted by the ethical committee of Mansoura University (Code Number: MDP.20.12.51; Date: 12/01/2021). Nephrotic syndrome was diagnosed based on plasma albumin levels  $<2.1$  g/dL, proteinuria  $\geq 40$  mg/m<sup>2</sup> per hour, and the presence of peripheral edema. Additionally, total cholesterol levels  $>350$  mg/dL were indicative of nephrotic syndrome (Bagga and Mantan 2005). Cases with complications other than nephrotic syndrome were excluded from the study. Informed written consent was acquired from the legal guardians of all investigation participants with the declaration of data privacy. This study was performed by the "Declaration of Helsinki."

### Sample collection and examination

Five ml of peripheral blood was collected from each participant. The blood sample was divided into two parts: two ml were deposited in EDTA-coated tubes for the analysis of both hematological markers and PCR, while the remaining part was drawn into ordinary vacutainer tubes for biochemical analysis.

### Biochemical, hematological parameter estimation

A fully automated biochemical analyzer was utilized to measure creatinine, albumin, total cholesterol, triglycerides, Alanine Aminotransferase (ALT/GPT), and Aspartate Aminotransferase (AST/GOT) in both control and patient groups. Additionally, a complete blood count was performed for each participant using a cell analyzer.

**Extraction of whole genomic DNA and Genotyping of ABCBI gene (rs10276036 C>T) variants**

DNA was extracted from the whole blood samples using a GeneJET DNA extraction kit.

The ABCBI (NCBI accession number:

5243) gene was genotyped using the T-ARMS-PCR technique, as described by Faraji et al. (20). The ABCBI primers were used in this study and PCR condition are shown in Table 1.

**Table 1.** PCR primers and PCR condition for ABCBI.

Primer name	Sequences (5'-3')		
ABCBI1R	GAGCCCAGGAGGTAGAGGTT		
ABCBI1WF	CCATCAGGCTACTGAGATAGTGTC		
ABCBI1MF	CCATCAGGCTACTGAGATAGTGTC		
PCR program			
Steps	Temperature (°C)	Time	Cycles
Initial denaturation	95	5 min.	1
Denaturation	94	35 (s)	35
Annealing	59	35 (s)	35
Extension, Final extension	72,72	50 (s), 5 min	35, 1

The PCR reaction of the ABCBI gene (C >T) was carried out by utilizing the technique described in detail in (20). The amplicons size was 260 bp.

**Estimation of IL-18 and TNF- $\alpha$**

IL-18 levels were assessed with the Human IL-18 ELISA Kit (Catalog No: EH0011, China) (21), while TNF- $\alpha$  levels were assessed using the TNF- $\alpha$  ELISA Kit (Catalog No: ELH-TNF- $\alpha$ , China).

**Statistical Analysis**

The collected data were revised, coded, and tabulated using the Statistical Package for Social Science (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.). Qualitative data were presented as frequencies. The selected study groups were assessed for Hardy-Weinberg equilibrium (HWE) to ensure suitability for genetic analysis of this SNP. Normality of data distribution was assessed using the Kolmogorov-Smirnov test. Mean ( $\pm$  SD) was reported for normally

distributed numerical data, while median and range were reported for non-normally distributed numerical data. Non-numerical data were presented as frequency and percentage. The ROC Curve (receiver operating characteristic) was utilized to evaluate the sensitivity and specificity of quantitative diagnostic measures categorizing cases into two groups. Logistic regression analysis was employed to predict risk factors when dependent variables were categorical, utilizing generalized linear models (22).

Sample size was determined using the Quanto calculator software program (version 1.2.4) for Sample Size or Power for Association Studies (23).

**Results**

Our study comprised two groups: the first group consisted of one hundred children with nephrotic syndrome, and the second group included one hundred healthy children. Age and gender were matched between the two groups ( $p = 0.660$  and  $p = 0.74$ , respectively (Table 2).

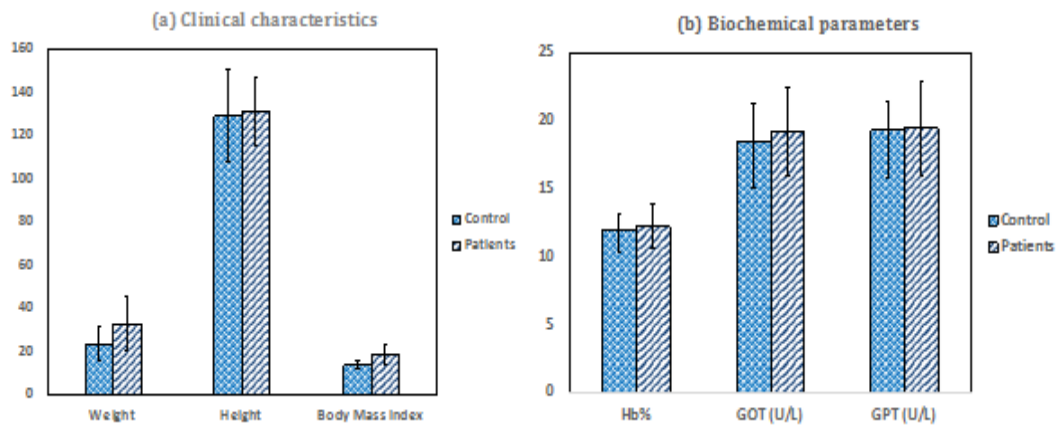
**Table 2.** Basic clinical characteristics of NS patients and controls.

Parameter	Control group (n: 100)	Nephrotic Syndrome group (n: 100)	P-value
Age (y), mean $\pm$ SD	9.791 $\pm$ 0.411	9.550 $\pm$ 0.381	0.66
Sex (boys / girls), N (%) / N (%)	76(76%) / 24 (24%)	76 (76%) / 24 (24%)	0.7400
SDNS number (%)		24.0 (24%)	
SRNS number (%)		76.0(76%)	
<b>Biopsy of the kidney n (%)</b>			
MCD	25 (25)		
FSGS	16 (16)		
MPGN	7 (7)		
No -biopsy	52 (52)		

NS: Nephrotic Syndrome, SDNS: Steroid dependent nephrotic syndrome, SRNS: Steroid resistant nephrotic syndrome, MCD: Minimal change disease, FSGS: Focal segmental glomerulosclerosis, MPGN: Membranoproliferative glomerulonephritis (MPGN). The statistics were presented as mean  $\pm$  standard deviation (SD), statistically significant if  $p < 0.05$ . () number (%)

The results indicate a significant increase in the weight and body mass index of the nephrotic syndrome (NS) patients compared to the healthy volunteers. However, there was no significant

difference observed in either the hemoglobin (Hb%), GOT, or GPT levels between the two study groups (Fig. 1).



**Fig. 1.** Some clinical characteristics and biochemical parameters for both healthy volunteer and nephrotic syndrome groups. A: weight, height, and body mass. B: hemoglobin (Hb%), GOT, and GPT comparisons between NS patients and healthy controls.

The results revealed significantly higher total leucocyte blood counts (TLC) levels in the nephrotic syndrome (NS) group compared to the normal group ( $p < 0.001$ ). However, there were no significant differences observed in either creatinine levels or platelet counts between the two groups. Albumin, cholesterol, triglycerides,

sodium ( $\text{Na}^+$ ) levels, and potassium ( $\text{K}^+$ ) levels were also measured. Additionally, the study showed significant increases in cholesterol and triglyceride levels in the NS group ( $p < 0.001$ ), while albumin levels were significantly lower in NS patients ( $p < 0.001$ ) compared to the controls (Table 3).

**Table 3.** Biochemical data of NS cases and healthy volunteers.

Parameter (unit)	Control group (n: 100), M (R)	Nephrotic Syndrome group (n: 100)	P-value
TLCs ( $\times 10^9/L$ )	7.001 (4.011- 9.651)	14.091 (5.51 - 28.21)	$p < 0.001^*$
Platelet ( $\times 10^9/L$ )	335.51 (206.01 – 480.0)	318.51 (191.01 – 645.01)	$p = 0.521$
Creatinine (mg/dl)	0.581 (0.421 - 0.751)	0.601 (0.301 - 7.801)	$p = 0.704$
Albumin (g/dl)	4.221 (3.901 - 5.101)	2.101 (1.201 - 5.201)	$p < 0.001^*$
Cholesterol (mg/dl)	154.01 (120.01 – 187.01)	574.51 (100.0 – 917.01)	$p < 0.001^*$
Triglycerides (mg/dl)	85.01 (50.01 - 135.01)	319.01 (68.01 - 710.01)	$p < 0.001^*$
Sodium Level (mmol/L)	139.561 (135.21 – 149.01)	138.91 (130.80 – 152.01)	$p = 0.0912$
Potassium Level (mmol/L)	4.11 (3.61 - 4.91)	4.081 (2.471 – 5.01)	$p = 0.4811$

The statistics were presented as mean  $\pm$  standard deviation (SD), M = median, R = Range, statistically significant if  $p < 0.05$ .

**ABCBI gene analysis**

The fit of goodness between both observed and expected genotype frequencies of the ABCBI

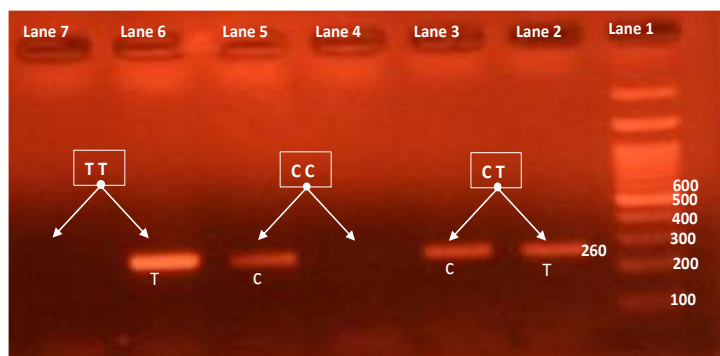
gene (rs10276036, C to T) genotype for NS and control groups was in alignment with the Hardy-Weinberg equilibrium (HWE) (Table 4).

**Table 4.** Assessment of Hardy Weinberg equilibrium (HWE) for studied SNPs.

Genotype	Nephrotic syndrome cases n = 100		Control cases n = 100	
	Observed	Expected	Observed	Expected
CC	24	28.09	59	60.84
CT	58	49.82	38	34.32
TT	18	22.09	3	4.84
P	0.101		0.284	

Using Tri ARMS-PCR, the ABCBI gene (rs10276036) was studied and electrophoresed

giving CC, CT, and TT genotypes, (Fig. 2).



**Fig. 2.** The PCR products after electrophoresis on a 3% agarose gel. Lane (1) represents the DNA marker (100 bp), lanes (2, 3) show a CT genotype. Lanes (4, 5) show CC genotype. Lanes (6, 7) are TT genotypes.

The frequency of the minor T-allele was significantly higher in NS patients than in the control group. However, the frequency of the common allele (C-allele) was lower in NS

cases than in the control group. The most prevalent genotype CC was significantly lower in the NS patients than in the control group (Table 5).

**Table 5.** ABCB1(rs10276036, C>T) genotypic and allelic frequencies in NS and normal groups.

ABCB1	NS		Control		P value	OR (95 % CI)			
	N = 100		n = 100						
	N	%	N.	%					
Genotypes	CC	24	24	59	59	Reference			
	CT	58	58	38	38	<0.001*	2.270	1.550	3.327
	TT	18	18	3	3	<0.001*	5.070	2.463	10.438
Dominant model	CC	24	24	59	59	Reference			
	CT+TT	76	76	41	41	<0.001*	2.560	1.771	3.701
Recessive model	CC+CT	82	82	97	97	Reference			
	TT	18	18	3	3	0.001*	3.231	1.623	6.431
Alleles	C	106	53	156	78	Reference			
	T	94	47	44	22	<0.001*	2.008	1.547	2.605

C, cysteine; T, Thymine; OR odds ratio; CI, confidence interval; OR>1 is considered risky; OR<1 is considered protective.

**The polymorphism of ABCB1 (rs10276036 C>T) in NS patients**

A significant association of ABCB1 (rs10276036 C>T) polymorphism with the steroid resistance utilizing multiple genetic association models including co-dominant,

dominant, recessive, and allelic models had been revealed (Table 6). The TT genotype is significantly increased in SRNS cases in comparison with SDNS cases. In the dominant model, CT+TT genotypes were significantly increased in SRNS cases than in SDNS cases.

**Table 6.** ABCB1 among SDNS and SRNS patients.

ABCB1	SDNS		SRNS		P value	OR (95 % CI)			
	N = 26		n = 74						
	N.	%	N.	%					
Genotypes	CC	18	69.2	6	8.1	Reference			
	CT	7	26.9	51	68.9	<0.001*	6.335	3.188	12.587
	TT	1	3.8	17	23.0	0.001*	9.657	3.247	28.720
Dominant model	CC	18	69.2	6	8.1	Reference			
	CT+TT	8	30.8	68	91.9	<0.001*	6.866	3.535	13.335
Recessive model	CC+CT	25	96.2	57	77.0	Reference			
	TT	1	3.8	17	23.0	0.031*	2.953	1.102	7.913
Alleles	C	43	82.7	63	42.6	Reference			
	T	9	17.3	85	57.4	<0.001*	3.094	1.972	4.853

C, cysteine; T, Thymine; OR odds ratio; CI, confidence interval; OR>1 is considered risky; OR<1 is considered protective.

**TNF-α and IL-18 levels**

The level of TNF-α was estimated in 54 patients with NS and 29 healthy individuals, while the serum level of IL-18 was estimated

in 57 patients with NS and 32 healthy volunteers (Table 7). The levels of both TNF-α and IL-18 were significantly increased in NS patients than in controls.

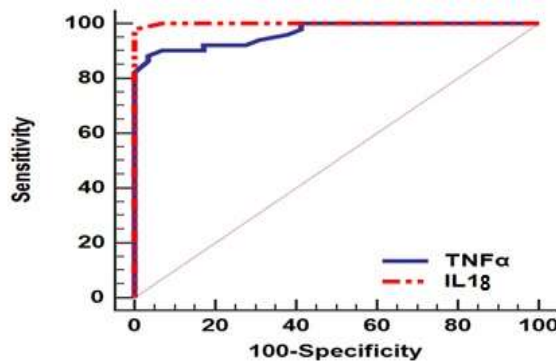
**Table 7.** The levels of TNF- $\alpha$  and IL-18 in patients with NS and control groups.

	NS	Control	p-value
<b>TNF-<math>\alpha</math></b>	<b>N = 54</b>	<b>N = 29</b>	
Mean $\pm$ SE.	0.32 $\pm$ 0.013	0.21 $\pm$ 0.005	U=53.5
Median	0.29	0.21	P< 0.001*
Range	0.22-0.61	0.14-0.25	
<b>IL-18</b>	<b>N = 57</b>	<b>N = 32</b>	
Mean $\pm$ SE	0.42 $\pm$ 0.022	0.26 $\pm$ 0.005	U=1
Median	0.36	0.26	P< 0.001*
Range	0.30-0.93	0.18-0.30	

The results were described as mean  $\pm$  S.E and median (range.). \* = Statistically significant ( $p < 0.050$ ), TNF- $\alpha$  = Tumor necrosis factor-alpha. IL-18= Interleukin-18, NS= Nephrotic syndrome.

The sensitivity and specificity of the elevation of both IL-6 and TNF- $\alpha$  at the diagnosis of nephrotic syndrome were determined by the receiver operating characteristic (ROC) curve which is a plotting of sensitivity versus (1-specificity = false positive results). The accuracy was measured by the area under the

ROC curve (AUC). Results of ROC curves of TNF- $\alpha$  and IL-18 showed high AUC values (0.966 and 0.999 respectively) which reflects a perfect accuracy of the test. The appropriate cut-off value at which the sensitivity and specificity have the highest values was  $> 0.25$  for TNF- $\alpha$ , and  $> 0.296$  for IL-18 (Fig. 3).



**Fig. 3.** ROC of TNF- $\alpha$  and IL-18 for diagnosis of NS.

When the levels of both TNF- $\alpha$  and IL-18 were compared between the steroid dependent patient group and the steroid resistant patient

group, no significant differences were noticed (Table 8).

**Table 8.** TNF- $\alpha$  and IL-18 levels among SRNS and SDNS cases.

	SDNS	SRNS	P
<b>TNF-<math>\alpha</math></b>	<b>N = 26</b>	<b>N = 74</b>	
Mean $\pm$ SE.	0.34 $\pm$ 0.036	0.32 $\pm$ 0.014	U=167.5
Median	0.30	0.28	P=0.242
Range	0.26-0.61	0.22-0.61	
<b>IL-18</b>	<b>N = 26</b>	<b>N = 74</b>	
Mean $\pm$ SE	0.43 $\pm$ 0.048	0.41 $\pm$ 0.026	U=0.283
Median	0.40	0.36	P=0.954
Range	0.30-0.87	0.30-0.93	

Data were described by mean  $\pm$  SE and median (range), U= Mann-Whitney U test, TNF- $\alpha$ = Tumor necrosis factor-alpha, IL-18= Interleukin-18, (SDNS)= steroid-dependent nephrotic syndrome, (SRNS)= steroid-dependent nephrotic syndrome.

## Discussion

Nephrotic syndrome in children and adolescents encompasses a spectrum of renal abnormalities, with minimal change disease (MCD) and focal segmental glomerulosclerosis (FSGS) being the most prevalent causes, leading to glomerular sclerosis. Membranoproliferative glomerulonephritis (MPGN) represents another significant etiology of nephrotic syndrome. In our study, MCD accounted for 24% of nephrotic syndrome cases, FSGS for 14%, and MPGN for 5%, with the remaining 57% lacking kidney biopsy data. This distribution highlights MCD as the predominant histopathologic variant in our cohort, while MPGN was the least common. These findings are consistent with previous studies by Mubarak *et al.* (24) and with that of Arbus *et al.* (25) which also noted a decline in MPGN prevalence in regions such as France, Italy, and Canada. Conversely, Ozkaya *et al.* (26) reported MPGN as the most frequent subtype among Turkish patients, underscoring regional variability in nephrotic syndrome etiology.

Our study found a higher incidence of nephrotic syndrome (NS) in boys compared to girls, consistent with findings reported by Arif *et al.* (27) and Siddiq *et al.* (28), who similarly observed greater susceptibility to NS among males. Additionally, our study revealed a significant increase in the frequency of the TT genotype in NS patients compared to controls. These results align with studies by Moussa *et al.* (29) and Youssef *et al.* (30) which highlighted higher TT genotype and T allele frequencies in NS patients compared to controls. Farhat *et al.* (31) also noted a higher prevalence of the TT genotype in Asian populations compared to European populations, further supporting our findings.

Furthermore, our study corroborates the findings of Wasilewska *et al.* (32), and Aziz and Islam (33), regarding the significant association of ABCB1 gene polymorphisms with NS, particularly in Asian and African populations. However, our results contrast

with those presented by Han *et al.* (9), who reported a higher frequency of the CC genotype in European populations compared to Asian populations. Additionally, Cizmarikova *et al.* (34) found a non-significant difference in the distribution of ABCB1 genotypes between NS patients and controls in the Slovak population.

Our findings indicate significantly higher frequencies of the CT and TT genotypes in the steroid-resistant nephrotic syndrome (SRNS) subgroup compared to the steroid-dependent nephrotic syndrome (SDNS) subgroup. These results are consistent with the findings of Ganesan *et al.* (35) who similarly reported increased frequencies of CC, CT, and T alleles associated with SRNS in children compared to SSNS and control groups. Choi *et al.* (36) have suggested that the CC genotype and C allele in MDR1 may serve as predictors for better initial steroid responsiveness, contrasting with our findings.

In contrast, our results differ from those reported by Jafar *et al.* (10), who found no significant difference in mutant genotype frequencies between the two subgroups.

Inflammation is recognized as a pivotal factor in the pathogenesis of nephrotic syndrome (NS). Investigating certain cytokines as potential biomarkers could offer NS patients more effective therapeutic strategies and help mitigate glucocorticoid toxicity. Our study found significantly elevated levels of IL-18 in the NS group compared to the control group. This aligns with findings from Saleem *et al.* (11) suggested that inflammatory factors, such as interleukin-18, play vital roles in the pathological who proposed that inflammatory mediators, such as interleukin-18, play crucial roles in the pathological processes underlying nephrotic syndrome.

Additionally, VanderBrink *et al.* (37) demonstrated that inflammatory factors, including IL-18 and TNF- $\alpha$ , are closely associated with the progression of nephrotic syndrome and contribute to kidney cell

damage. These insights underscore the potential of cytokines like IL-18 as important biomarkers in NS pathogenesis and their relevance in developing targeted therapeutic interventions.

These findings are corroborated by Kho et al. (38), who suggested that elevated IL-18 levels post-therapy may contribute to the development of steroid-resistant nephrotic syndrome (SRNS). Our results are consistent with Ahmadian et al. (16), who reported an association between high IL-18 levels and nephrotic syndrome (NS). Schachter (39) similarly observed significantly higher serum IL-18 concentrations in children with NS compared to controls. Additionally, Moharrerpour et al. (40) documented increased serum IL-18 levels in children with steroid-sensitive nephrotic syndrome (SSNS). Moreover, Attalla and Ahmed (41) found significantly elevated IL-18 levels in the acute kidney injury group compared to controls, further highlighting the role of IL-18 as a potential biomarker in kidney-related disorders. These studies collectively underscore the importance of IL-18 in the pathophysiology of NS and its potential implications for therapeutic strategies and monitoring disease progression.

TNF- $\alpha$  is widely recognized for its significant association with various inflammatory diseases, including glomerulonephritis. Our study reveals markedly elevated levels of TNF- $\alpha$  in patients with nephrotic syndrome (NS) compared to controls. However, we did not observe any significant difference in TNF- $\alpha$  levels between steroid-resistant nephrotic syndrome (SRNS) and steroid-sensitive nephrotic syndrome (SSNS) subgroups. This finding is consistent with the results reported by Al-Assadi et al. (42). Additionally, our findings align with those of Suranyi et al. (43), who observed elevated TNF- $\alpha$  levels in both plasma and urine of NS patients compared to healthy controls. Liang et al. (44) further suggested that TNF- $\alpha$  might interfere with glucocorticoid activity and directly impact glucocorticoid

receptor function, potentially influencing treatment responses in NS patients. These studies collectively underscore the role of TNF- $\alpha$  in the pathophysiology of nephrotic syndrome and its implications for therapeutic strategies targeting inflammatory pathways.

Various inflammatory mediators can interfere with glucocorticoid binding to target cells, potentially impacting their therapeutic efficacy. The expression of P-glycoprotein (P-gp), encoded by the ABCB1 gene, plays a crucial role in modulating the response of nephrotic syndrome (NS) patients to pharmacological treatments. P-gp is involved in the transport of many drugs, including steroids, across cell membranes, influencing their bioavailability and therapeutic effects. Inflammatory processes can alter ABCB1 gene expression, thereby affecting drug responses in NS.

The ABCB1 gene, along with TNF- $\alpha$  and IL-18, are emerging as independent risk factors associated with the prevalence of NS and serve as potential biomarkers for early prediction of disease onset. These biomarkers not only aid in early diagnosis but also have the potential to mitigate complications associated with advanced stages of NS.

Understanding the interplay between inflammatory responses, ABCB1 gene expression, and cytokine levels such as TNF- $\alpha$  and IL-18 is crucial for developing targeted therapies and improving outcomes for NS patients, particularly in managing treatment resistance and reducing disease progression to end-stage complications.

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### **Conflict of interest**

The authors declare that they have no conflict of interests.

## References

1. Yousefinejad A, Siassi F, Javanbakht MH, Mohammadi H, Ghaedi E, Zarei M, Djalali E, Djalali M. Effect of Genistein and L-carnitine and Their Combination on Lipid Profile and Inflammatory Cytokines in Experimental Nephrotic Syndrome. *Rep Biochem Mol Biol*. 2018;7(1):1-8.
2. Gordillo R, Spitzer A. The nephrotic syndrome. *Pediatr Rev*. 2009 Mar;30(3):94-104; quiz 105. doi: 10.1542/pir.30-3-94. Erratum in: *Pediatr Rev*. 2009;30(10):408.
3. Shabaka A, Tato Ribera A, Fernández-Juárez G. Focal Segmental Glomerulosclerosis: State-of-the-Art and Clinical Perspective. *Nephron*. 2020;144(9):413-427.
4. Schijvens AM, Ter Heine R, de Wildt SN, Schreuder MF. Pharmacology and pharmacogenetics of prednisone and prednisolone in patients with nephrotic syndrome. *Pediatr Nephrol*. 2019;34(3):389-403.
5. Candelier JJ, Lorenzo HK. Idiopathic nephrotic syndrome and serum permeability factors: a molecular jigsaw puzzle. *Cell Tissue Res*. 2020;379(2):231-243.
6. Johnstone DB, Zhang J, George B, Léon C, Gachet C, Wong H, et al. Podocyte-specific deletion of Myh9 encoding nonmuscle myosin heavy chain 2A predisposes mice to glomerulopathy. *Mol Cell Biol*. 2011;31(10):2162-70.
7. Schwab M, Eichelbaum M, Fromm MF. Genetic polymorphisms of the human MDR1 drug transporter. *Annu Rev Pharmacol Toxicol*. 2003;43:285-307.
8. Xu P, Jiang ZP, Zhang BK, Tu JY, Li HD. Impact of MDR1 haplotypes derived from C1236T, G2677T/A and C3435T on the pharmacokinetics of single-dose oral digoxin in healthy Chinese volunteers. *Pharmacology*. 2008;82(3):221-7.
9. Han SS, Xu YQ, Lu Y, Gu XC, Wang Y. A PRISMA-compliant meta-analysis of MDR1 polymorphisms and idiopathic nephrotic syndrome: Susceptibility and steroid responsiveness. *Medicine (Baltimore)*. 2017;96(24):e7191.
10. Jafar T, Prasad N, Agarwal V, Mahdi A, Gupta A, Sharma RK, et al. MDR-1 gene polymorphisms in steroid-responsive versus steroid-resistant nephrotic syndrome in children. *Nephrol Dial Transplant*. 2011;26(12):3968-74.
11. Saleem MA, Kobayashi Y. Cell biology and genetics of minimal change disease. *F1000Res*. 2016;5:F1000 Faculty Rev-412.
12. Narayanan KB, Park HH. Purification and analysis of the interactions of caspase-1 and ASC for assembly of the inflammasome. *Appl Biochem Biotechnol*. 2015;175(6):2883-94.
13. Jorgensen I, Miao EA. Pyroptotic cell death defends against intracellular pathogens. *Immunol Rev*. 2015;265(1):130-42.
14. Agnihotri SK, Kumar B, Jain A, Anjali A, Negi MPS, Sachan R, et al. Clinical Significance of Circulating Serum Levels of sCD95 and TNF- $\alpha$  in Cytoprotection of Cervical Cancer. *Rep Biochem Mol Biol*. 2022;10(4):711-721.
15. Aldhalmi AK, Al-Athari AJH, Makki Al-Hindy HA. Association of Tumor Necrosis Factor- $\alpha$  and Myeloperoxidase enzyme with Severe Asthma: A comparative study. *Rep Biochem Mol Biol*. 2022;11(2):238-245.
16. Ahmadian E, Rahbar Saadat Y, Dalir Abdolahinia E, Bastami M, Shoja MM, Zununi Vahed S, Ardalan M. The Role of Cytokines in Nephrotic Syndrome. *Mediators Inflamm*. 2022;2022:6499668.
17. Jang DI, Lee AH, Shin HY, Song HR, Park JH, Kang TB, et al. The Role of Tumor Necrosis Factor Alpha (TNF- $\alpha$ ) in Autoimmune Disease and Current TNF- $\alpha$  Inhibitors in Therapeutics. *Int J Mol Sci*. 2021;22(5):2719.
18. Fischer R, Maier O. Interrelation of oxidative stress and inflammation in neurodegenerative disease: role of TNF. *Oxid Med Cell Longev*. 2015;2015:610813.
19. 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(3):164-168.

20. Faraji A, Dehghan Manshadi HR, Mobaraki M, Zare M, Houshmand M. Association of ABCB1 and SLC22A16 Gene Polymorphisms with Incidence of Doxorubicin-Induced Febrile Neutropenia: A Survey of Iranian Breast Cancer Patients. *PLoS One*. 2016;11(12):e0168519.
21. Dong M, Zhao M, Cui M, Sun J, Meng X, Sun W, et al. Interleukin-18 binding protein attenuates renal injury of adriamycin-induced mouse nephropathy. *Int J Clin Exp Pathol*. 2019;12(8):3005-3012.
22. Kwok PY. Approaches to allele frequency determination. *Pharmacogenomics*. 2000;1(2):231-5.
23. Gauderman WJ, Morrison JM. QUANTO 1.1: A computer program for power and sample size calculations for genetic-epidemiology studies. 2006. <http://hydra.usc.edu/gxe>.
24. Mubarak M, Kazi JI, Lanewala A, Hashmi S, Akhter F. Pathology of idiopathic nephrotic syndrome in children: are the adolescents different from young children? *Nephrol Dial Transplant*. 2012;27(2):722-6.
25. Arbus G, Getu H, Baumal R, Mraz V, Jabs D, Eddy A. Long term follow up, including renal transplantation, of children with membranoproliferative glomerulonephritis. In: Murakami K, Kitagawa T, Yabuta K, Sakai T (eds). *Recent Advances in Pediatric Nephrology*. Excerpta Medica, Amsterdam, 1987; 241–246.
26. Ozkaya N, Cakar N, Ekim M, Kara N, Akkök N, Yalçinkaya F. Primary nephrotic syndrome during childhood in Turkey. *Pediatr Int*. 2004;46(4):436-8.
27. Arif MK, Arif M, Amjad N. A histopathological outlook on nephrotic syndrome: A pediatric perspective. *Indian J Nephrol*. 2016;26(3):188-91.
28. Siddique AB, Hanif M, Ahmed F, Hossain N, Uddin M. Correlation between Serum Prednisolone and Serum Albumin level in Childhood with Nephrotic Syndrome: A study in tertiary care hospital in Bangladesh. *IOSR J Dent Med Sci*. 2018;17(9):62–66.
29. Moussa A, Mabrouk S, Hamdouni H, Ajmi M, Tfifha M, Omezzine A, et al. MDR-1 and CYP3A5 Polymorphisms in Pediatric Idiopathic Nephrotic Syndrome: Impact on Susceptibility and Response to Steroids (Preliminary Results). *Clin Lab*. 2017;63(7):1233-1242.
30. Youssef DM, Attia TA, El-Shal AS, Abduelometry FA. Multi-drug resistance-1 gene polymorphisms in nephrotic syndrome: impact on susceptibility and response to steroids. *Gene*. 2013;530(2):201-7.
31. Farhat K, Waheed A, Hussain A, Iqbal J, Mansoor Q, Ismail M. Polymorphisms of the ABCB1 Gene in the Pakistani Population. *J Coll Physicians Surg Pak*. 2015;25(7):482-5.
32. Wasilewska AM, Zoch-Zwierz WM, Pietruczuk M. Expression of P-glycoprotein in lymphocytes of children with nephrotic syndrome treated with glucocorticoids. *Eur J Pediatr*. 2006;165(12):839-44.
33. Aziz MA, Islam MS. The role of ABCB1 gene polymorphisms in steroid-resistant nephrotic syndrome: Evidence from a meta-analysis of steroid-receiving patients. *J Gene Med*. 2022;24(7):e3436.
34. Cizmarikova M, Podracka L, Klimcakova L, Habalova V, Boor A, Mojzis J, Mirossay L. MDR1 polymorphisms and idiopathic nephrotic syndrome in Slovak children: preliminary results. *Med Sci Monit*. 2015;21:59-68.
35. Ganesan A, Mini Jacob S, Arvind Selvin Kumar R, Padmaraj R, Anandan B, Sambantham S, et al. Identification of Functional Single Nucleotide Polymorphisms of Multidrug Resistance Gene-1 Among Nephrotic Syndrome Children in South India. *Asian J Pharm Clin Res*. 2017;10(2):418-422.
36. Choi HJ, Cho HY, Ro H, Lee SH, Han KH, Lee H, et al. Polymorphisms of the MDR1 and MIF genes in children with nephrotic syndrome. *Pediatr Nephrol*. 2011;26(11):1981-8.
37. VanderBrink BA, Asanuma H, Hile K, Zhang H, Rink RC, Meldrum KK. Interleukin-18 stimulates a positive feedback loop during renal obstruction via interleukin-18 receptor. *J Urol*. 2011;186(4):1502-8.
38. Kho MC, Park JH, Han BH, Tan R, Yoon JJ, Kim HY, et al. *Plantago asiatica* L. Ameliorates Puromycin Aminonucleoside-Induced Nephrotic Syndrome by Suppressing Inflammation and Apoptosis. *Nutrients*. 2017;9(4):386.
39. Schachter AD. The pediatric nephrotic syndrome spectrum: clinical homogeneity and

molecular heterogeneity. *Pediatr Transplant.* 2004;8(4):344-8.

40. Moharrerpour SS, Nickavar A, Bojd SS, Makhtomi S, Ghorbani H. Serum interleukin-18 in children with steroid sensitive nephrotic syndrome. *J Renal Inj Prev.* 2019;8(4): 289-291.

41. Attalla HA, Ahmed AM. Prediction of Acute Kidney Injury in Critically-Ill Pediatric Patients Admitted to PICU: The Role of Serum Cystatin C and Serum Interleukin-18. *Egyptian J Hosp Med.* 2020;80(2):943-950.

42. Al-Assadi AB, Abdulmohammed N, Ali SH. Serum Tumor Necrosis Factor-Alpha (TNF- $\alpha$ ) Levels in Children with Nephrotic Syndrome and Its Correlation with Biochemical Parameters. *Int J Curr Microbiol App Sci.* 2018;7(9):3464-3470.

43. Suranyi MG, Guasch A, Hall BM, Myers BD. Elevated levels of tumor necrosis factor-alpha in the nephrotic syndrome in humans. *Am J Kidney Dis.* 1993;21(3):251-9.

Liang Y, Chen Y, Chen Y, Gong Y. Role of the glucocorticoid receptor in the recurrence of primary nephrotic syndrome. *Exp Ther Med.* 2015;10(4):1556-1562.

44. Liang Y, Chen Y, Chen Y, Gong Y. Role of the glucocorticoid receptor in the recurrence of primary nephrotic syndrome. *Exp Ther Med.* 2015;10(4):1556-1562.