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Original research article

Genetic studies of nephrotic syndrome in Egyptian children

Rehab Elmougy

Mansoura University, Faculty of Science, Chemistry Department, Mansoura, Egypt

Abstract

Introduction: Nephrotic syndrome (NS) might be caused by a kidney disorder or it can be a secondary disease. Untreated or resistant to treatment, NS stimulates glomerular damage that reduces the kidney function. This reduction leads to the end stage of renal failure. Therefore, it is very important to diagnose NS early, with the aim of inhibiting or lessening its associated morbidity and mortality.

Methods: Gene polymorphism analysis for the three genes eNOS 27 bp VNTR, GSTP1 and IL-10(1082 G/A) were checked in 98 children with NS and 101 control subjects.

Results: eNOS 27 bp VNTR genotypes and alleles are significantly different in the group of 98 children with NS compared to the 101 control subjects. The frequencies of ab and bb genotypes are significantly lower in patients than in the control group (ab: 17.2% vs. 22.8%; OR: 0.19; 95% CI: 0.06–0.58; $p = 0.0026$ & bb: 54.7% vs. 70.3%; OR: 0.19; 95% CI: 0.07–0.5; $p = 0.0004$). However, neither GSTP1 nor IL-10(1082 G/A) genotypes showed any significant difference in both groups.

Conclusions: eNOS 27 bp VNTR gene might be considered as an independent risk factor in the early prediction of nephrotic syndrome incidence, which may help prevent/reduce the occurrence of other complications associated with the late diagnosis and treatment of the disease.

Keywords: eNOS 27 bp VNTR; GSTP1 and IL-10(1082 G/A); Nephrotic syndrome

Highlights:

- Gene polymorphism analysis for the three genes eNOS 27 bp VNTR, GSTP1 and IL-10(1082 G/A) were checked in 98 children with NS and 101 control subjects.
- eNOS 27 bp VNTR genotypes and alleles were significantly different in the children group with NS compared to the control subjects' group, while both GSTP1 and IL-10(1082 G/A) genotypes showed no significant difference in both groups.
- eNOS 27 bp VNTR gene might be considered as a tool for the early diagnosis of nephrotic syndrome.

Introduction

Nephrotic syndrome (NS) is a clinical disorder accompanied by proteinuria, which is responsible for various health problems (Vasudevan et al., 2021). These problems include an excessive decrease in albumin levels, hyperlipidemia, and edema (Tapia and Bashir, 2020; Tumlin and Campbell, 2018). These symptoms are caused by increased permeability through the damaged clusters of small blood vessels in the kidney (Hill et al., 2016). The abnormality of glomerular permeability might be associated either primarily with a kidney disease or secondarily with other diseases (e.g., diabetes mellitus and Lupus) or it might be related to the use of some drugs (Raina and Krishnappa, 2019). People of all ages can be affected by this disorder. However, the first sign of NS is swelling around the eyes, feet, and ankles (Dumas et al., 2018).

L-arginine is the source of nitric oxide (NO) by nitric oxide synthase (NOS). In humans, there are three types of NOS. These are endothelial, neuronal, and inducible NOS (Dursun et al., 2013). In this study we selected endothelial NOS 27 bp VNTR gene, which has two alleles. The larger one has five tandem 27-bp repeats (allele b). The smaller has only four repeats (allele a) (Wang et al., 1996). eNOS 27 bp VNTR gene is thought to be the deterioration factor for kidney disease progression and may be involved in the pathogenesis of renal diseases.

Glutathione transferases (GSTs) consist of a multifunctional protein found in all eukaryotic and prokaryotic cells which can detoxify toxic compounds (Gusti et al., 2021; Sharda et al., 2008). The impact of GST polymorphisms (GSTP1) in children suffering from NS has been evaluated.

Cytokines play a vital role as mediators of inflammation and as progressive factors in NS. Various cytokines are considered as prime factors for mediating NS progression. Interleukin-10 (IL-10), produced by helper T (Th2) cells, is immune regulatory cytokine (Krawiec et al., 2021). T cells and monocytes/macrophages are regulated by IL-10 (Jafar et al., 2011).

* **Corresponding author:** Rehab Elmougy, Mansoura University, Faculty of Science, Chemistry Department, Mansoura, Egypt;
e-mail: rehab.elmougy@yahoo.com
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Considering IL-10 as a candidate mediator for controlling the renal inflammation, we chose to analyze IL-10(1082 G/A) in NS patients as well as control subjects.

Materials and methods

Study subjects

Our study included 98 Egyptian children with nephrotic syndrome, of whom 28 were girls and 70 were boys. Their ages ranged from 4 to 12 years old, with mean ± SE = 7.7 ± 3.78 years. They were enrolled in this study at the Urology and Nephrology Center, Mansoura University, Egypt. The healthy group contained 101 subjects who were ethnically matched (with the patients' group) and whose ages ranged from 3 to 16 years old with mean ± SE = 7.3 ± 3.73 years. Moreover, those volunteers had neither renal nor cardiac diseases.

Blood samples

Sterile disposable plastic syringes were used to draw five milliliters of whole blood from the vein puncture of each subject. Of each of these samples, four milliliters were allowed to clot for 10–15 minutes. They were then centrifuged to get the serum used to analyze the biochemical parameters (Albumin, Cholesterol, Triglycerides and Creatinine). The remaining one milliliter of each sample's whole blood was taken in EDTA coated tubes and used to extract the genomic DNA. Finally, the PCR technique was applied on these DNA samples.

Biochemical analyses

Albumin, Creatinine, Cholesterol and Triglycerides were measured using commercially available kits (Spinreact, Co., Spain) with Ref numbers: MX1001020, MD1001111, TK41021 and MX41031, respectively.

Molecular variants genotyping

27bp-VNTR polymorphism of eNOS gene

Genomic DNA from peripheral blood was extracted and purified using Qiagen spin columns (Cat. No. / ID: 51104). Polymerase chain reaction (PCR) was used to detect eNOS gene intron 4, 27 bp VNTR polymorphism (Fig. 1). A detailed description of the method used is shown in Wang et al. (1996) and in Channon et al. (2000). The PCR program for eNOS gene amplification was executed according to that described in Mehmetoğlu et al. (2010).

GSTP1 Ile105val gene polymorphism

Using an amplification refractory mutation system (ARMS), GSTP1 Ile105val polymorphism was analyzed. The primers were designed for tetra-ARMS-PCR using: Forward outer primer 5'-AGGTTACGTAGTTTGCCCAAGGTC-3', Reverse outer primer 5'-CGTTACTTGGCTGGTTGATGTCC-3', Forward inner primer 5'-GAGGACCTCCGCTGCAAATTCG-3' and Reverse inner primer 5'-CATAGTTGGTGTAGATGAGGGAGCT-3'. The PCR product of GSTP1 gene was amplified using the program described thoroughly in Salimi et al. (2015). Three bands appeared at 563 bp for the control product, 360 bp for the Ile variant, and 260 bp for the Val variant (Fig. 2).

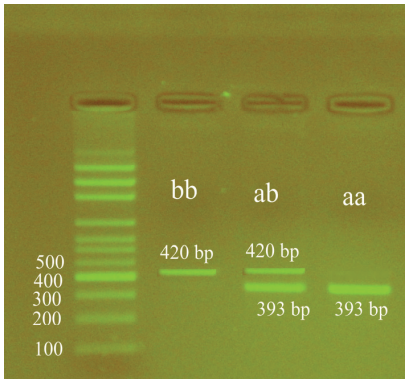

Fig. 1. PCR analysis of eNOS 27 bp polymorphism in agarose electrophoresis [the size of amplified products (420 bp, 393 bp) and different eNOS 27bp genotypes are shown. Bands were visualised using ethidium bromide].

Interleukin-10 (1082 G/A) gene polymorphism

The reactions of IL-10-1082-G/A were performed according to the method of Turner et al. (1997). Primers' sequences were: (IL-10 F) 5'-AGCAACACTCCTCGTCGCAAC-3', (IL-10 R1) 5'-CCTATCCCTACTTCCCCC-3', (IL-10 R2) 5'-CCTATCCCTACTTCCCCT-3'. The PCR program for IL-10 gene amplification was executed according to that described in Elsaid et al. (2014). PCR products were resolved by agarose gel electrophoresis (3%) and the specific band appeared at 179 bp (Fig. 3).

Statistical analysis

The statistical discovery software JMP 9 was used to analyze our raw data. Quantitative data were presented as mean ± SE, while qualitative data were presented as frequencies. The Chi-square test with Yates' correction and Fisher exact test were used to compare categorical variables. The genotypic and allelic distributions for the candidate gene polymorphisms were checked for being in Hardy–Weinberg equilibrium.

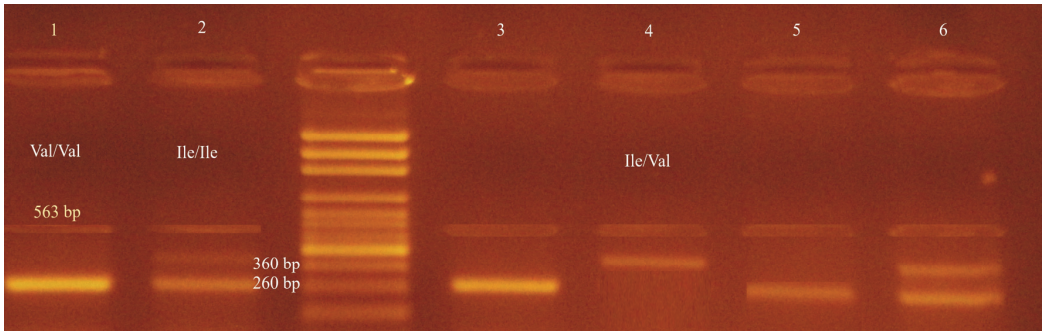

Fig. 2. PCR analysis of GSTP1 polymorphism in agarose electrophoresis [the size of amplified products (563 bp, 360 bp, 260 bp) and different GSTP1 genotypes are shown. Bands were visualised using ethidium bromide].

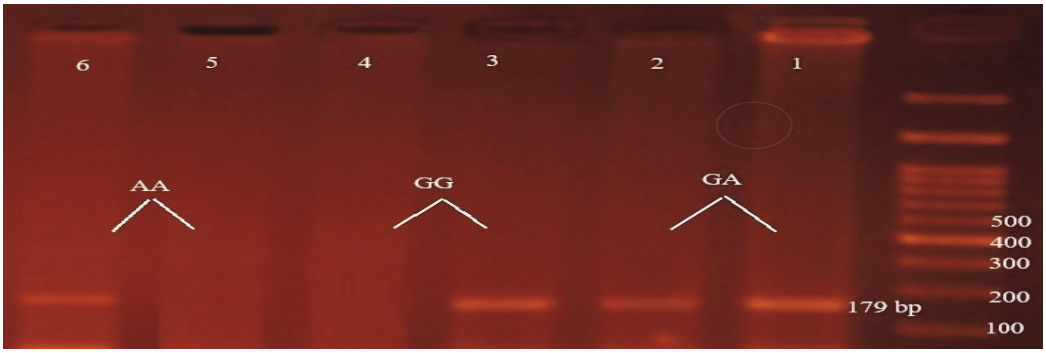

Fig. 3. PCR analysis of IL10-1082 G/A polymorphism in agarose electrophoresis [the size of amplified products (179 bp) and different IL10-1082 G/A genotypes are shown. Bands were visualised using ethidium bromide].

Results

Table 1 shows the demographic variables' distribution among the study of serum levels of albumin, triglycerides, cholesterol, creatinine, and the levels of hemoglobin, white blood cells and platelets in 199 cases, including (98) patients with primary nephrotic syndrome and 101 healthy subjects.

There is a highly significant increase in the serum level of triglycerides (TG) and cholesterol in patients with primary nephrotic syndrome ($P < 0.0001$) when compared to healthy volunteers. However, a highly significant decrease can be observed in the level of serum albumin in patients with primary nephrotic syndrome ($P < 0.0001$) when compared to healthy volunteers. Table 1 shows also that there are significant increases in the levels of hemoglobin and platelets in the group of patients versus the control group, while there are no significant differences in the levels of serum creatinine and white blood cells (WBC's) between both groups. The table also shows that there are no significant differences between the two groups regarding age and residency.

In the final analysis of the genotype eNOS27bp, only 64 children suffering from primary nephrotic syndrome and 101 control subjects were included (Table 2). The table shows the genotypic and allelic frequencies of the eNOS27bp polymorphism in both the control and patient groups. It has been checked that both genotype and allele frequencies are in Hardy–Weinberg equilibrium. The table also shows that there is a significant difference in the genotypic and allelic frequencies between the control and patient groups, and especially that the frequencies of ab and bb genotypes are significantly lower in patients than in the control group (ab: 17.2% vs. 22.8%; OR: 0.19; 95% CI: 0.06–0.58; $p = 0.0026$ & bb: 54.7% vs. 70.3%; OR: 0.19; 95% CI: 0.07–0.5; $p = 0.0004$).

Similarly, for the gene GSTP1, only 70 children suffering from nephrotic syndrome and 99 healthy ones were included in its genotypic and allelic final analysis, the results of which are shown in Table 3. The analysis showed no significant difference in the genotypic or the allelic frequencies of GSTP1 in both groups. Also, the genotypic and allelic analysis of the gene IL-10-1082G/A showed no significant difference between both healthy and nephrotic syndrome children's groups (Table 4).

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

Parameter	Control (n = 101) Mean ± SE or N (%)	Patients (n = 98) Mean ± SE or N (%)	p-value OR (CI)
Age (years)	7.3 ± 3.73	7.7 ± 3.78	0.43
Residency:			
Rural	67 (66.34)	62 (63.27)	0.65
Urban	34 (33.66)	36 (36.73)	1.14 (0.64–2.05)
Serum Albumin g/dl	4.49 ± 0.081	2.02 ± 0.081	<0.0001
Serum Creatinine mg/dl	0.48 ± 0.012	0.46 ± 0.012	0.14
Albumin/Creatinine ratio × 10^3	9.88 ± 0.245	4.68 ± 0.246	<0.0001
Serum Triglycerides mg/dl	76. 9 ± 5.9	185. 87 ± 5.93	<0.0001
Serum Cholesterol mg/dl	156.88 ± 9.23	448.88 ± 9.27	<0.0001
Hemoglobin g/dl	11.38 ± 0.15	11.86 ± 0.151	0.025
WBC's × 10^9/l	10.08 ± 0.313	10.45 ± 0.315	0.41
Platelets × 10^9/l	344.4 ± 11.99	396.9 ± 12.05	0.023

OR = Odds Ratio; CI = confidence interval; SE = standard error.

Table 2. ENOS 27 bp gene distributions in both primary nephrotic syndrome cases and healthy volunteers

Polymorphism	Controls *n* = 101	Patients *n* = 64	*p*-value	OR (95% CI)
Genotypes: *n* (%)				
aa	7 (6.9)	18 (28.1)	Ref.	
ab	23 (22.8)	11 (17.2)	0.0026	0.19 (0.06–0.58)
bb	71 (70.3)	35 (54.7)	0.0004	0.19 (0.07–0.5)
Alleles: *n* (%)				
a	37 (18.32)	47 (36.72)	Ref.	
b	165 (81.68)	81 (63.28)	0.0002	0.39 (0.23–0.64)

OR = Odds Ratio; CI = confidence interval.

Table 3. GSTP1 gene distributions in both primary nephrotic syndrome cases and healthy volunteers

Polymorphism	Controls *n* = 99	Patients *n* = 70	*p*-value	OR (95% CI)
Genotypes: *n* (%)				
GG	19 (19.19)	9 (12.86)	Ref.	
GA	64 (64.65)	61 (87.14)	0.1	0.5 (0.21–1.18)
AA	16 (16.16)	0 (0.00)	–	–
Alleles: *n* (%)				
G	102 (51.52)	79 (56.43)	Ref.	
A	96 (48.48)	61 (43.57)	0.37	1.2 (0.8–1.9)

OR = Odds Ratio; CI = confidence interval.

Table 4. IL10 gene distributions in both primary nephrotic syndrome cases and healthy volunteers

Polymorphism	Controls *n* = 98	Patients *n* = 58	*p*-value	OR (95% CI)
Genotypes: *n* (%)				
GG	5 (5.1)	5 (8.6)	Ref.	
GA	85 (86.7)	52 (89.7)	0.45	1.63 (0.45–5.9)
AA	8 (8.2)	1 (1.7)	0.06	8 (0.7–90)
Alleles: *n* (%)				
G	95 (48.47)	62 (53.45)	Ref.	
A	101 (51.53)	54 (46.55)	0.4	1.2 (0.77–1.93)

OR = Odds Ratio; CI = confidence interval.

Discussion

Patients suffering from nephrotic syndrome have edema and fatigue. These patients have no evidence of heart failure or liver disease. The diagnosis of NS is based on typical clinical features. Heavy proteinuria and hypoalbuminemia are tested. NO has a vital role in kidney disease progression (Kodner, 2016). In our study, it is examined whether eNOS27bp gene is implicated by the incidence of NS in Egyptian patients.

In our study, which included 64 children diagnosed with NS, we found that eNOS 27 bp gene is significantly different from that of the 101 control subjects. This finding matches the results reported by Ahluwalia et al. (2008) who reported a significant association of aa genotype (27VNTR) with a high risk of nephropathy in patients with type 2 diabetes. Our result also agrees with that obtained by Buraczynska et al. (2004), who conducted their study on patients with end stage renal disease (ESRD). Consequently, an association between eNOS gene polymorphism and the increased risk of chronic kidney failure may exist. On the contrary, in a study on diabetic patients with and without nephropathy, Shoukry et al. (2012) reported no significant difference in the frequency of either eNOS (27 VNTR) genotype or its allele. Also, Dursun et al. (2013) showed that the eNOS (27 VNTR) gene polymorphisms were not associated with the development of nephrotic syndrome.

In our study, neither the genotypes nor the alleles of GSTP1 were significantly different in NS patients compared to the control subjects. This finding agrees with other studies (Gsur et al., 2001; Murata et al., 1998; Steinhoff et al., 2000) as it shows there is no significant difference between the NS and control groups. However, this result differed from that found by Agrawal et al. (2007) who conducted their study on Indian patients. The authors registered an association between GSTT1, GSTM1, GSTP1 genes and the risk of developing ESRD.

Also, Sharda et al. (2008) conducted their study on children with INS treated with cyclophosphamide. They indicated that an association exists between a combination of GSTP1 Ile105 polymorphism and the null genotypes of GSTT and GSTM in these patients.

Both IL-10 (1082 G/A) genotypes and alleles have no significant differences in the NS group compared to the control one. This result confirms the findings published by Dudnyk et al. (2019) who reported that IL-10 may not be considered an independent risk factor for the incidence of NS. However, the result disagrees with that published by Naing et al. (2018) who conducted their study on an Asian population. The authors showed that for patients with type 2 diabetes, both IL-10 (1082A/G) and IL-10 (819G/A) polymorphisms significantly affect the risk of developing diabetic nephropathy. Also, the results reported by Polina et al. (2017) support the hypothesis that the IL-10 (1082A>G) gene might be associated with Diabetic Kidney Disease in white Brazilians with type 2 diabetes.

Conclusions

eNOS 27 bp VNTR gene might be considered as an independent risk factor in the early prediction of nephrotic syndrome incidence, which may help prevent/reduce the occurrence of other complications associated with the late diagnosis and treatment of the disease.

Conflict of interests

The author has no conflict of interests to declare.

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