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Association of Mutations in The NPHS2 Gene and Nephrotic Syndrome in Children and Adults in Middle East

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ABSTRACT

Background: Limited and contradictory pharmacogenetic studies of NPHS2 gene R229Q polymorphism in nephrotic syndrome (NS) children and adults of different ethnicities steered us to investigate the genotype frequency and associated risk of this polymorphism in Middle East NS children and adults.

Objectives: The present work aimed to study the effect of NPHS2 R229Q genetic variations on the susceptibility to idiopathic NS and the treatment response in NS children and adults from Assiut University and major Kuwait Hospitals**.**

Patients and methods: A prospective observational cohort study was conducted which comprised a total of 100 idiopathic NS patients (30 children and 70 adults). Mutation analysis was carried out by Taqman allele discrimination of the NPHS2 gene R229Q polymorphism (rs61747728) using specific primers and probes.

Results: The results indicate the presence of R229Q polymorphism in 9% of our patients. Moreover, R229Q variant in Steroid-resistant nephrotic syndrome (SRNS) adults was observed in a single heterozygous form. A total of 100 patients were genotyped for the variant rs61747728. Ninety-one percent of patients carry the CC genotype (Homozygous), in addition only 9% were carriers of the CT genotype (Heterozygous), whereas no patients were carrying the TT genotype. The minor allele (T) frequency was 0.045, whereas the major allele (C) frequency was 0.955 in our population.

Conclusion: NPHS2 p.R229Q plays an important role in enhancing the susceptibility of minimal change disease (MCD), focal segmental glomerulosclerosis/steroid-resistant nephrotic syndrome (FSGS/SRNS), especially in Middle East population and age of late-onset patients. We recommend to screen for p.R229Q polymorphism in the diagnosis of SRNS among our population.

Keywords: NPHS2 gene, Nephrotic Syndrome, Mutations, Middle East.

INTRODUCTION

Nephrotic syndrome (NS) is characterized by proteinuria, hypoalbuminaemia, oedema, and dyslipidaemia. Clinically, NS has been divided into two categories, based on the response to steroid therapy: steroid-sensitive NS (SSNS) and steroid-resistant NS (SRNS). Approximately 10 % of children and 50 % of adults with idiopathic NS have SRNS, fail to respond to immunosuppressive treatment and progress to end-stage renal disease (ESRD) within seven years. In these cases, renal histology typically shows focal segmental glomerulosclerosis (FSGS) or minimal change disease (MCD) [(1)]. Steroid-resistant nephrotic syndrome (SRNS) is defined as NS resistant to steroid therapy, defined by the absence of complete remission after four weeks of daily prednisone therapy at a dose of 60 mg/m^2 per day [(2)]. Despite representing a smaller proportion of nephrotic syndrome cases, patients with steroid-resistant nephrotic syndrome (SRNS) have proven more difficult to treat, with 36%–50% progressing to end-stage renal disease within 10 years[(3)].

The treatment of SRNS is a challenging task for the nephrologists due to its poor response to immnosupressive drugs. High dose steroids, cyclopsphamide, calcineurin inhibitors (cyclosporine and tacrolimus), mycophenolate mophetil and rituximab have been used with variable success rates in children and adults [(4)]. Adult-onset SRNS is associated with various NPHS2 mutations, mostly with R229Q [(5)]. The NPHS2 gene mapped on chromosome 1q25.2 encodes podocin, which is an important protein expressed by the visceral glomerular epithelial cells (podocytes)[(6)]. Podocin is a 383-amino acid lipid-raft-associated protein localized at the slit diaphragm, where it is required for the structural organization and regulation of the glomerular filtration barrier[(7)].

Podocin mutations are apparently restricted to SRNS[(8)], they represent approximately 40% of familial SRNS patients and have also been found in 10-30% of sporadic SRNS cases in groups from European and Middle Eastern countries[(9)]. The identification of NPHS2 mutations in NS patients is important for therapeutic decisions and genetic counseling, and according to several authors, patients should be tested for mutations in this gene before receiving immunosuppressive therapy[(10)]. Inter-ethnic differences have also been suggested to play a role in the incidence of NPHS2 mutations[(11)].

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The polymorphism R229Q is one of the most commonly reported podocin sequence variations. This polymorphism is more frequent among South Americans, Europeans and European Americans (4–7 %) than among Africans, African Americans, and Asians (0–1.5 %), suggesting that this variant emerged in Europe, although it is not possible to discern its specific geographic origin[12]. In European population, the highest occurrence of p.R229Q polymorphism, 4.5 %, was described in France, 3.2 % in Italian population and 3.1 % in Spanish Population[13].

Our study represents the first cohort of Middle East SRNS patients evaluated for heterozygous for the variant c.686G > A (p.R229Q, rs61747728) and a mutation, including childhood and adult-onset cases.

PATIENTS AND METHODS

1. Patient Recruitment:

One hundred patients (30 children and 70 adults) suffering from Nephrotic Syndrome were enrolled in this observational cohort study, in the period from 2017 to 2021. The patients were referred to the nephrology departments in the major hospitals in Kuwait- Mubarak, Amiri, Farwaniya, Adan and Jahra Hospitals. This study also included patients referred to the nephrology unite in medical and pediatric departments in Assiut University Hospitals.

2. Procedures for genetic study:

I - Saliva and blood sampling and DNA extraction:

After obtaining informed consent from parents of children and adults, 3 ml of blood was collected from children and in the event that the patients or their parents refuse then buccal swabs was collected from children, and 5ml of blood was collected from adults. The buccal swabs was processed to extract DNA and stored at -20 till time of genetic analysis. Peripheral blood samples was collected in EDTA anticoagulated tubes and DNA was extracted according to standard methods using QIAGEN DNA blood mini kit (QIAGEN) and stored at -20 till time of analysis.

II- DNA extraction from whole blood (quiagen Kit, Catalog No. 51104):

III- Single nucleotide polymorphism (SNP) Genotyping Technique by Real-Time PCR allelic Discrimination. Real-time Genotyping Assay (Allelic Discrimination).

Ethics approval and consent to participate:

All patients were fully informed about the methods of blood sampling or buccal swabs. A written consent was obtained from adult patients and parents of children. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national review board of Kuwait University-faculty of medicine (IRB approval number MG03 -15) and was approved by the institutional review board of the Faculty of Medicine in Assiut University, Assiut, Egypt (IRB local approval number 17200140). This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Funding:

Funding was by Kuwait University under Grant MG03-15. The money was used to buy reagents for DNA extraction and SNP genotyping and to pay for temporary manpower that helped with the manual laboratory work.

Statistical analysis:

Data was collected and analyzed those using SPSS (Statistical Package for the Social Science, version 20, IBM, and Armonk, New York). Continuous data was expressed in form of mean ± SD or median (range) while nominal data was expressed in form of frequency (percentage).

RESULTS

The mean age of our studied patients was 27.45 ± 16.01 years with range between 6 years and 64 years. The majority (63%) of those patients were males and (37%) were females. The most frequent nationalities in the study were Egyptian (49%), Kuwaiti (19%), Indian (12%), and Saudi Arabian (8%). Twelve percent of the patients had other nationalities in a descending manner. The duration of disease ranged between 2-45 years with mean duration 9.16 ±7 years. Thirty-five percent of patients, disease's duration was 10 years or more. Seven patients had positive family history of NS (table 1).

Table (1): The demographic data of our studied patients with nephrotic syndrome

Variables	**N=100**
Age (years) Range	27.45 ± 16.01 6-64
Gender Male Female	 63 (63%) 37 (37%)
Nationality Egypt Kuwait India Saudi Arabia Others	 49 (49%) 19 (19%) 12 (12%) 8 (8%) 12 (12%)
Duration of disease Range Class < 10 years ≥ 10 years	9.16 ±7 2-45 65 (65%) 35 (35%)
Family history	7 (7%)

Data expressed as frequency (percentage), mean (SD).

Regarding to kidney function of our studied patients**,** 76% of patients had normal kidney function while 20% of patients were classified to CKD and only four patients (4%) had end stage renal stage. All

patients were treated with steroid while 56%, 27%, 13%, 10% and 9% of patients used cyclosporine, MMF, tacrolimus, rituximab, and cyclophosphamide, respectively.

Twenty-six percent of patients used only one immunosuppressant while 48% of patients used two agents. It was noticed that 26% of patients used three or more agents for management. Only 22% of patients were improved on steroid alone while 20%, 7%, 5%, 4% and 3% of patients were improved with addition of cyclosporine, MMF, tacrolimus, rituximab, and cyclophosphamide, respectively.

No improvement was noticed in 39% of patients even with addition of other agent (s) with steroid. Majority (52%) of patients had no comorbidities while 48% of patients had different form of comorbidities. The most frequent comorbidities were chronic kidney disease (20%), hypertension (16%), steroid induced diabetes mellitus (11%) and obesity (4%). Each of pulmonary embolism, steroid induced osteoporosis, avascular necrosis of hip joint and dyslipidaemia was presented in one patient. Eleven patients had other forms of complications that didn't relate to the kidney disease or side effects of therapy (as bronchial asthma, pre-eclampsia, etc) (table 2).

Table (2): Baseline data of our studied patients with nephrotic syndrome

Variables	**N=100**
Serum creatinine (umol/L)	
Normal (n= 76)	83.11 ± 12.56
CKD (n= 20)	204 ± 50.76
ESRD (n= 4)	976.50 ± 241.81
Treatment	
Steroid	100 (100%)
Cyclosporine	56 (56%)
MMF	27 (27%)
Tacrolimus	13 (13%)
Rituximab	10 (10%)
Cyclophosphamide	9 (9%)
Number of agents in addition to steroids	
One agent	26 (26%)
Two agents	48 (48%)
≥ three agents	26 (26%)
Improvement with	
Steroids	22 (22%)
Others	
Cyclosporine	20 (20%)
MMF	7 (7%)
Tacrolimus	5 (5%)
Rituximab	4 (4%)
Cyclophosphamide	3 (3%)
No-improvement	39 (39%)
Comorbidities	
CKD	20 (20%)
Hypertension	16 (16%)
Steroid induced DM	11 (11%)
Obesity	4 (4%)
Dyslipidaemia	1 (1%)
Pulmonary embolism	1 (1%)
Steroid induced osteoporosis	1 (%)
Avascular necrosis of hip joint	1 (1%)
Others	11 (11%)
No comorbidities	52 (52%)

Data expressed as frequency (percentage), mean (SD). **CKD**: chronic kidney disease, **ESRD**: end stage renal disease, **MMF**: mycophenolate mofetil. **DM**: diabetes mellitus.

It was noticed that 54% of patients had minimal change disease while 46% of patients had focal segmental glomerulosclerosis. The majority (72%) of enrolled patients had steroid resistance NS while steroid sensitive NS was

noticed in only 14% of patients. Each of steroid dependent, frequent relapse and infrequent relapse NS was presented in 9%, 3% and 2% of patients, respectively (table 3 & figure 1).

Table (3): Histopathological diagnosis and clinical classification of nephrotic syndrome in our studied patients according to treatment with steroids.

Pathological diagnosis	**N= 100**
Minimal change disease	54 (54%)
Focal segmental glomerulosclerosis	46 (46%)
Classification of NS patients according to their response to steroids	
Steroid sensitive	14 (14%)
Steroid dependent	9 (9%)
Frequent relapse	3 (3%)
Infrequent relapse	2 (2%)
Steroid resistance	72 (72%)

Data expressed as frequency (percentage). NS: nephrotic syndrome.

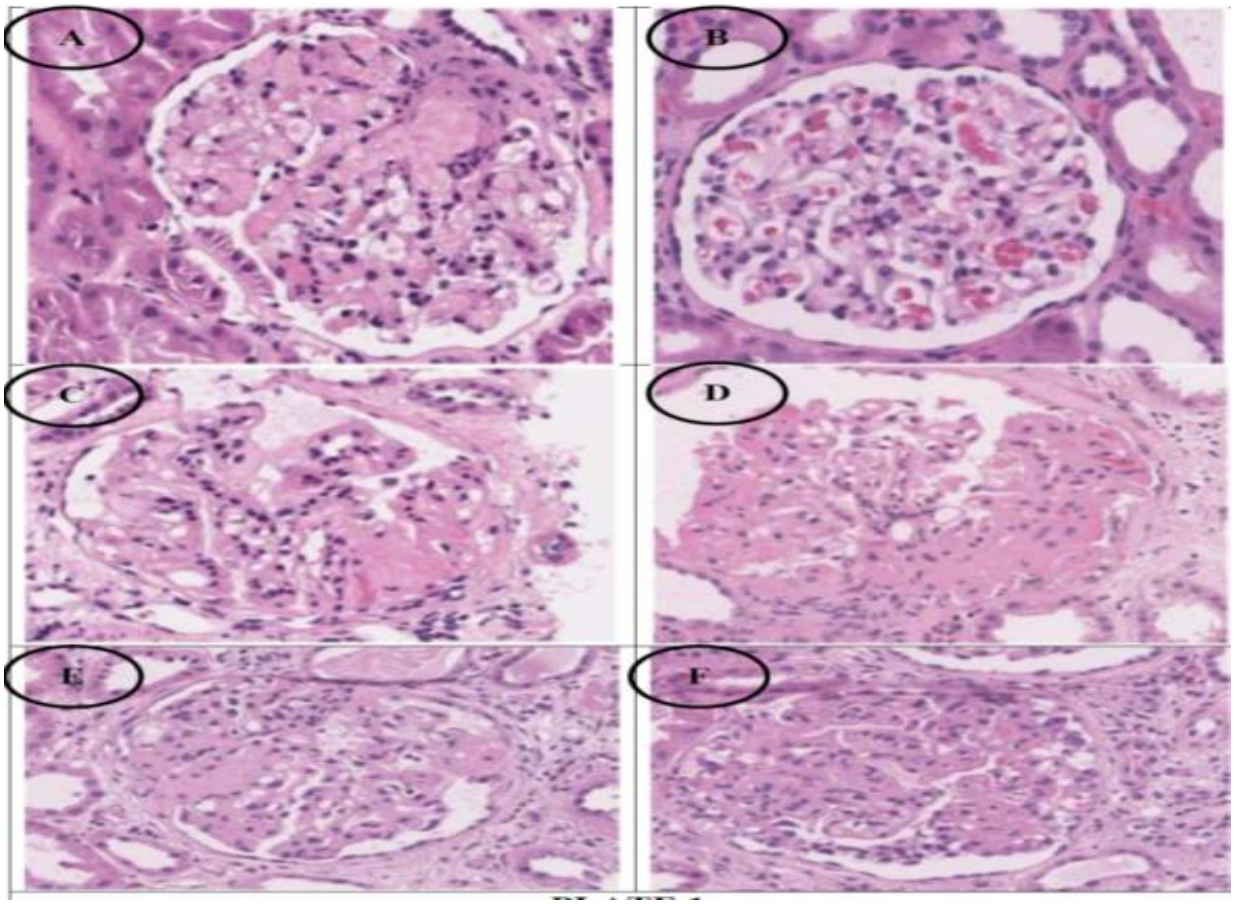

Figure (1): (**A and B**) show that MCD slides with the same comment as follows: H&E stained slides show normal glomeruli by light microscopy, the capillary loops have open Lumina with no basement membrane thickening or any alterations, no mesangial endocapillary or extracapillary proliferation are present. **(C) FSGS 1.**H&E stained slide shows segmental solidification of the glomerular capillary tuft and slight mesangial hypercellularity, focal adhesion of the tuft with Bowman capsule is also present. **(D) FSGS 2**. H&E stained slide shows segmental solidification of the glomerular capillary tuft. **(E)FSGS 3**. H&E stained slide shows segmental solidification of the glomerular capillary tuft and more extensive adhesion between the tufts and Bowman Capsule. **(F)FSGS 4.** H&E stained slide shows segmental sclerosing lesion associated with intra capillary foam cells and epithelial cell hyperplasia.

A total of 100 patients were genotyped for the variant rs61747728. We observed 91% of patients carry the CC genotype (Homozygous), in addition only 9% of patients were carriers of the CT genotype (Heterozygous), whereas no patients were carrying the TT genotype. The minor allele (T) frequency was 0.045 whereas the major allele was

0.955. It was noted that all patients had amplified allele C, while 9% of patients had amplified allele T and 91% of patients had not amplified allele T. Out of the studied patients; 91% and 9% of patients had homogenous and heterogenous NPHS2 gene respectively (table 4 & figure 2).

Table (4): NPHS2 (p.R229Q) variant among studied patients

NPHS2 Genotyping	**N= 100**
Genotype CC (homozygous)	91 (91%)
Genotype CT (Heterozygous)	9 (9%)
Genotype TT (Homozygous)	0

Data expressed as frequency (percentage).

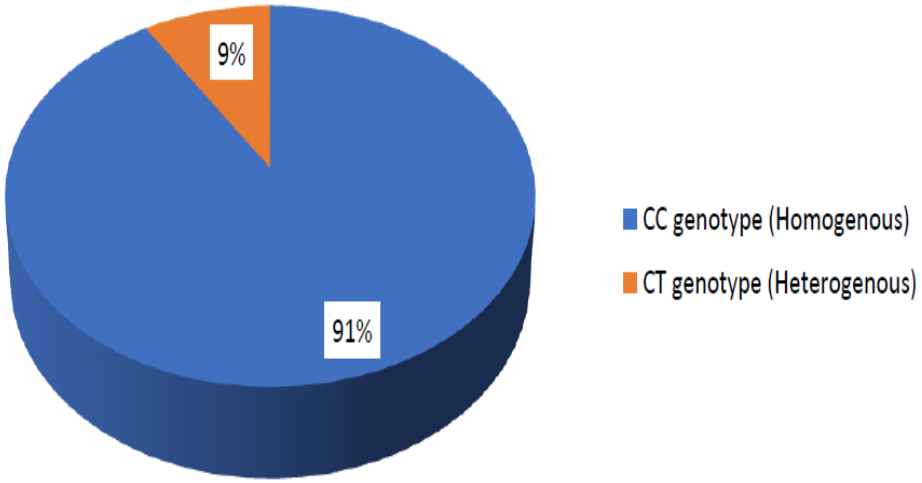

Figure (2): NPHS2 (p.R229Q) variant among enrolled patients

Table (5) illustrates that there was a statistically significant difference between MCD and FSGS patients as regards their mean age with lower mean age in MCD versus FSGS patients (24.20 ± 17.19 vs. 31.26 ± 13.69 (years)). Interestingly, use of MMF was significantly higher among patients with FSGS (37% vs. 18.5%) and majority (56.5%) of patients with FSGS used dual agents for therapy vs. (40.7%) of patients with MCD.

As expected, frequency of improvement with steroid was significantly higher among patients with MCD (35.2% vs. 6.5%) and frequency of no improvement even with addition of other immunosuppressant agents was significantly higher in patients with FSGS (56.5% vs. 24.1%). Majority (70.4%) of patients with MCD had no comorbidities and frequency of no comorbidities was significantly higher among those patients with MCD (70.4% vs. 30.4%).

Table (5): Demographic and baseline data of our studied patients based on their histopathological diagnosis

Variables	MCD (n= 56)	FSGS (n= 46)	*P* value
Age (years)	24.20 ± 17.19	31.26 ± 13.69	**0.02**
Range	6-64	6-54	
Treatment			
Steroid	54 (100%)	46 (100%)	---
Cyclosporine	27 (50%)	29 (63%)	0.13
MMF	10 (18.5%)	17 (37%)	**0.03**
Tacrolimus	7 (13%)	6 (13%)	0.61
Rituximab	3 (5.6%)	7 (15.2%)	0.10
Cyclophosphamide	7 (13%)	2 (4.3%)	0.12
Number of agents in addition to steroids			**0.02**
One agent	20 (37%)	6 (13%)	
Two agents	22 (40.7%)	26 (56.5%)	
≥ three agents	12 (22.2%)	14 (30.4%)	
Improvement with Steroids	19 (35.2%)	3 (6.5%)	**0.01**
Others			
Cyclosporine	13 (24.1%)	7 (15.2%)	0.11
MMF	3 (5.6%)	4 (8.7%)	0.22
Tacrolimus	2 (3.7%)	3 (6.5%)	0.10
Rituximab	2 (3.7%)	2 (4.3%)	0.09
Cyclophosphamide	2 (3.7%)	1 (2.2%)	0.19
No improvement	13 (24.1)	26 (56.5%)	**0.01**
Comorbidities			
Chronic kidney disease	2 (3.7%)	16 (34.8%)	< **0.001**
Hypertension	2 (3.7%)	14 (30.4%)	< **0.001**
Steroid induced diabetes mellitus	8 (14.8%)	3 (6.5%)	0.15
Obesity	1 (1.9%)	3 (6.5%)	0.25
Dyslipidaemia	0	1 (2.2%)	0.46
Pulmonary embolism	1 (1.9%)	0	0.54
Osteoporosis	1 (1.9%)	0	0.54
AVN	0	1 (2.2%)	0.46
Others	5 (9.3%)	6 (13%)	0.38
No comorbidities	38 (70.4%)	14 (30.4%)	< **0.001**

Data expressed as frequency (percentage), mean (SD), MMF: mycophenolate mofetil, AVN: avascular necrosis of hip joint, MCD: minimal change disease, FSGS: focal segmental glomerulosclerosis. P value was significant if < 0.05.

Table (6) illustrates that there were no significant differences between those patients with genotype CT (heterogenous) and genotype CC (homogenous) as regarding demographic data with exception of patients with homozygous gene had significantly lower mean age in comparison to those with heterozygous gene (26.12 ± 15.66 vs. 40.88 ± 13.61 (years)).

Majority of patients with homogenous gene was from Egypt (51.6%) while minority (33.3%) of patients with heterogenous gene came from India. Where there were no significant differences between those patients with genotype CT (heterogenous) and genotype CC (homogenous) except in the frequency of DM was significantly higher among patients with heterogenous gene (44.4% vs. 7.7%) in comparison to those with homogenous gene.

Table (6): Demographic and baseline data of our studied patients based on NPHS2 (p.R229Q) variant

Variables	Genotype CC (Homozygous; n= 91)	Genotype CT (Heterozygous; n= 9)	*P* value
Age (years)	26.12 ± 15.66	40.88 ± 31.61	**< 0.001**
Range	6-57	19-64	
Nationality			
Egypt	47 (51.6%)	2 (22.2%)	**0.04**
Kuwait	18 (19.8%)	1 (11.1%)	
India	9 (9.9%)	3 (33.3%)	
Saudi Arabia	7 (7.7%)	1 (11.1%)	
Others	3 (6.5%)	2 (22.2%)	
Comorbidities			
CKD	17 (18.7%)	1 (11.1%)	0.49
Hypertension	13 (14.3%)	3 (33.3%)	0.15
Steroid induced DM	7 (7.7%)	4 (44.4%)	**< 0.001**
Obesity	4 (4.4%)	0	0.86
Dyslipidaemia	1 (1.1%)	0	0.91
AVN	0	1 (11.1%)	0.09
Osteoporosis	0	1 (11.1%)	0.09
Pulmonary embolism	1 (1.1%)	0	0.91
Others	9 (9.9%)	2 (22.2%)	0.25
No comorbidities	49 (53.8%)	3 (33.3%)	0.20

Data expressed as frequency (percentage), mean (SD). CKD: chronic kidney disease; AVN: Avascular necrosis of hip joint; DM: diabetes mellitus. P value was significant if < 0.05.

Table (7) illustrates that there was statistically significant difference between number of CT genotype and CC genotype steroid resistant patients.

Table 7: Classification of nephrotic syndrome based on NPHS2 (p.R229Q) variant

Classification of NS according to treatment with steroids	CCgenotype (Homozygous; n= 91)	CTgenotype (Heterozygous; n= 9)	*P* value
Classification			**0.01**
Steroid sensitive	14 (15.4%)	0	
Steroid dependent	9 (9.9%)	0	
Frequent relapse	3 (3.3%)	0	
Infrequent relapse	2 (2.2%)	0	
Steroid resistance	63 (69.2%)	9 (100%)	

Data expressed as frequency (percentage). P value was significant if < 0.05

Table (8) illustrates that there was no statistically significant difference between MCD and FSGS patients as regarding NPHS2 (p.R229Q) variant.

Table (8): Histopathological diagnosis of our studied patients based on NPHS2 (p.R229Q) variant

Histopathological diagnosis	CC genotype (Homozygous; n= 91)	CT genotype (Heterozygous; n= 9)	*P* value
Pathological diagnosis			0.33
MCD	48 (57.7%)	6 (66.7%)	
FSGS	43 (47.3%)	3 (33.3%)	

Data expressed as frequency (percentage). MCD: minimal change disease; FSGS: focal sclerosis glomerulosclerosis. P value was significant if < 0.05.

Table (9) illustrates that there were insignificant differences between **SRNS** and **Non-SRNS** groups except in, use of cyclosporine was significantly higher among patients with SRNS than Non-SRNS (69% vs. 24.1%), while use of MMF was also, significantly higher among patients with SRNS than Non-SRNS (35.2% vs. 6.9%). The majority (54.9%) of patients with SRNS used dual agents of immunosuppression while majority (58.6%) of patients with Non-SRNS used only one agent of immunosuppression with significant statistically differences between both groups, improvement with steroids was significantly higher among Non-SRNS patients than SRNS (75.8% vs 0%), while no improvement at all even with addition of other agent (s) than steroids in 54.9% of patients with SRNS with highly significant statistically differences between both groups.

Table (9): Demographic and baseline data of our studied patients based on nephrotic syndrome according to response to steroid therapy

Variables	SRNS (n= 72)	Non-SRNS (n= 28)	*P* value
Age (years)	26.47 ± 15.59	29.82 ± 17.01	0.35
Range	6-64	6-57	
Gender			0.06
Male	41 (57.7%)	22 (75.9%)	
Female	30 (42.3%)	7 (24.1%)	
Nationality			0.43
Egypt	37 (52.1%)	12 (41.4%)	
Kuwait	12 (16.9%)	7 (24.1%)	
India	7 (9.9%)	5 (17.2%)	
Saudi Arabia	4 (5.6%)	3 (10.3%)	
Others	11 (15.5%)	2 (6.9%)	
Onset of disease			0.54
Before time of biopsy	67 (94.4%)	28 (96.6%)	
At time of biopsy	4 (5.6%)	1 (3.4%)	
Duration of disease	8.41 ± 2.04	11 ± 2.77	0.43
Range	2-24	2-45	
Class			0.14
< 10 years	49 (69%)	16 (55.2%)	
≥ 10 years	22 (31%)	13 (44.8%)	
Family history	4 (5.6%)	3 (10.3%)	0.32
Treatment			
Steroid	72 (100%)	28 (100%)	---
Cyclosporine	49 (69%)	7 (24.1%)	< **0.001**
MMF	25 (35.2%)	2 (6.9%)	< **0.001**
Tacrolimus	12 (16.9%)	1 (3.4%)	0.06
Rituximab	8 (11.3%)	2 (6.9%)	0.40
Cyclophosphamide	5 (7%)	4 (13.8%)	0.24
Number of agents in addition to steroids			< **0.001**
One agent	9 (12.7%)	17 (58.6%)	
Two agents	39 (54.9%)	9 (31%)	
≥ three agents	23 (32.4%)	3 (10.3%)	
Improvement with			
Steroids	0	22 (75.8%)	< **0.001**
Others			
Cyclosporine	14 (19.7%)	6 (20.7%)	0.10
MMF	6 (8.5%)	1 (3.4%)	0.32
Tacrolimus	5 (7%)	0	0.10
Rituximab	4 (5.6%)	0	0.09
Cyclophosphamide	3 (4.2%)	0	0.18
No improvement	39 (54.9%)	0	< **0.001**
Comorbidities			
Chronic kidney disease	16 (22.5%)	2 (6.9%)	0.05
Hypertension	14 (19.7%)	2 (6.9%)	0.09
Steroid induced diabetes mellitus	6 (8.5%)	5 (17.2%)	0.17
Obesity	3 (4.2%)	1 (3.4%)	0.67
Dyslipidaemia	1 (1.4)	0	0.71
Pulmonary embolism	0	1 (3.4%)	0.29
Osteoporosis	1 (1.4%)	0	0.71
AVN	1 (1.4%)	0	0.71
Others	7 (9.9%)	4 (13.8%)	0.39
No comorbidities	35 (49.3%)	17 (58.6%)	0.26

Data expressed as frequency (percentage), mean (SD). **SRNS**: steroid resistance nephrotic syndrome; **Non-SRNS**: non-steroid resistance nephrotic syndrome. **MMF**: mycophenolate mofetil; **AVN**: avascular necrosis of hip joint; *P* value was significant if < 0.05.

At the end of our study, it is worth mentioning that out of 100 enrolled patients, 49 patients (49%) were of Egyptian nationality. 28 (57%) were children with age group from 6 to 16 years, and 21 (43%) were adults. Majority (63%) of these patients were males. As regarding pathological diagnosis there were 26 (53%) MCD and 23 (47%) FSGS. As regarding type of NS there were 4 (8%), 4 (8%), 1 (2%), 40 (82%) patients has SSNS, SDNS, frequent relapse NS and SRNS respectively. We found single heterozygous mutation in R229Q variant in two adults (9.5%) out of 21 adults enrolled in our study which is going with other international studies. From these two adults, one patient has MCD and the other has FSGS. We did not find mutation of p.R229Q in Egyptian children enrolled in our study.

DISCUSSION

Interestingly, our results showed that (9%) of patients were heterozygotes for the common NPHS2 (R229Q) variant among children and adults. Fortunately, none of our patients with the SNP had a family history of NS which might suggest that the SNP is predominantly associated with sporadic cases in our population and not familial cases. Moreover, none were children and none were on dialysis. Eventually, all 9 patients with NPHS2 (R229Q) variant had SRNS.

Our results were in agreement with the findings of **Boyer *et al.*[14], Mohamed Ahmed and Mohamed Farhan [15] and Tsukaguchi *et al.*[16]**. Our study results found that no polymorphism in R229Q variant in children which is compatible with **Otukesh and colleagues [8] and Hashemi M *et al.*[17].**

Moreover, our study results were interestingly in keeping with the national study done in the Pediatric Nephrology Clinic, Cairo University Children's Hospital and Genetics Department, National Research Centre (2017). They did not discover mutation of p.R229Q in exon 5 among Egyptian children[18].

On the contrary of our results, **Ruf *et al.* [9], Weber *et al.* [19] and McKenzie *et al.* [20]** reported a relatively similar frequency of the p.R229Q (c.686G>A) variant in patients with SRNS and normal control subjects, hence suggesting that this variant is not a risk factor for SRNS in the heterozygous state. Notably, **Basiratnia *et al.* [21]** investigated the prevalence of NPHS2 mutation in SRNS and SSNS patients in south-west of Iran. They did not find any mutation at rs61747728 (R229Q) position.

Moreover, **Shatha *et al.* [22], Phelan *et al.* [23], Behnam *et al.* [24]** were in contrast to our findings. They concluded that polymorphism R229Q of NPHS2 gene is prevalent in Iraqi children with SRNS and SSNS. In addition, **Phelan *et al.* [23]** found that R229Q polymorphism was associated with early onset childhood SRNS. Moreover, **Behnam *et al.* [24]** revealed that both NPHS1 and NPHS2 were prevalent in Iranian children with SRNS. No mutation of p.R229Q was reported in Iranian adolescent with SRNS.

The most frequent nationalities in our study were Egyptian, which is about half of enrolled patient. So our study results can give us a good idea about single heterozygous mutation in R229Q variant in Egyptian adult patients rather than in middle east populations.

In the current study, regarding treatment with corticosteroids, all patients were treated with steroids while more than half of the patients used cyclosporine and few patients used cyclophosphamide. In fact most of our patients used two immunosuppressive medications.

In the present study we noticed that nearly equal percentage of patients improved on steroids alone and or with addition of cyclosporine while the majority showed no improvement even with addition of other immunosuppressant drugs with steroids. The PodoNet registry concluded that, in immune-mediated forms of SRNS, calcineurin inhibition was demonstrated to be the most efficacious second-line therapy following the diagnosis of steroid resistance. Children resistant to calcineurin inhibitors are usually also resistant to other immunosuppressive agents. Moreover, initial responsiveness to calcineurin inhibitors is uniquely predictive of long-term preservation of kidney function [25]. The highest rates of complete (30%) or partial (19%) remission were achieved with CNI based protocols. The variation of the response rates may be related to the selection and composition of the individual study cohorts and the chosen set of response criteria [26]. Indeed, the efficacy of CNI-based therapies in the PodoNet cohort was by far superior to steroid pulses, cyclophosphamide, and MMF monotherapy, all of which did not show any therapeutic effect in around 85% of patients as first line therapy and were completely non-efficacious as second- or third-line therapies in CNI resistant patients. These findings confirm previous studies and provide strong evidence against the use of these therapeutics in SRNS [27]. Notably, B-cell depleting therapy with Rituximab induced complete remission in 44%, and partial remission in 15% of patients [28].

As regarding NPHS2 (p.R229Q) rs61747728 variation among studied patients, we found that a total of 100 patients were genotyped for the variant rs61747728. We observed 91% of patients to carry the CC genotype (Homozygous), in addition only 9 (9%) were carriers of the CT genotype (Heterozygous), whereas no patients were carrying the TT genotype. The minor allele (T) frequency was 0.045 whereas the major allele (C) frequency was 0.955 in our population. It was noted that all patients had amplified allele C, while 9 (9%) and 91 (91%) patients had amplified and not amplified allele T respectively. Out of the studied patients; 9/100 adult patients (9 %) were heterozygotes

for the common SNP R229Q (c.686G>A; rs61747728) variant.

On the other hand, our results showed that only 35% patients with MCD were improved on steroid alone while 24% patients were improved with addition of cyclosporine. No improvement was noticed in 24% patients with MCD even with addition other agent (s) with steroid. As reported by **Prasad *et al.*** [(29)], Calcineurin inhibitors (CNIs) are the preferred drugs for treatment of childhood steroid-resistant nephrotic syndrome (SRNS) who are also resistant to cyclophosphamide (CYC). The long-term outcome of renal function was significantly better in patients who were treated with tacrolimus (TAC) as compared to cyclosporine (CSA).

Also, our study determined that only 5.6% of patients with MCD had positive family history of nephrotic syndrome and none of them was on dialysis and majority of patients with MCD had no comorbidities. The most frequent comorbidities, in patients with MCD, were steroid induced diabetes mellitus (14.8%), chronic kidney disease (3.7%) and hypertension (3.7%).

In the present study, patients with FSGS were treated with steroids while in majority of patients cyclosporine and MMF were added. As regarding the improvement; it was noticed that only few patients with FSGS were improved on steroid alone and also few patients were improved with addition of cyclosporine, MM, tacrolimus, rituximab, and cyclophosphamide. In majority of FSGS patients no improvement was noticed even with addition of other agent (s) with steroid. Fourteen (30.4%) of patients with FSGS had no comorbidities while the most frequent comorbidities, in patients with FSGS, were CKD (34.8%), hypertension (30.4%), steroid induced diabetes mellitus (6.5%) and obesity (6.5%). Both avascular necrosis of hip joint and dyslipidaemia were detected in one patient.

Our results investigated the genotype phenotype relationship between R229Q polymorphism and mean age. We reported that patients without R229Q polymorphism had significantly lower mean age in comparison to those with R229Q polymorphism. Also, results investigated the genotype phenotype relationship between R229Q polymorphism and frequency of DM. We reported that Frequency of DM was significantly high among patients with R229Q polymorphism, and this finding could be attributed to small number of patients with R229Q polymorphism.

It was noticed that patients without R229Q polymorphism; 48 (57.7%) and 43 (47.3%) patients had MCD and FSGS, respectively while 6 (66.7%) and 3 (33.3%) patients with R229Q polymorphism had MCD and FSGS, respectively. The most remarkable results to emerge from baseline data of enrolled patients based on pathological diagnosis in the present study is that patients with MCD had significantly lower mean age in comparison to those with FSGS. Also In results showed that majority of patients with FSGS used dual agents for therapy than those with MCD. Moreover, frequency of no improvement even with addition of other immunosuppressant agents was significantly higher in patients with FSGS than those in patients with MCD.

Furthermore, patients with FSGS had significantly higher CKD and hypertension. The majority of patients with SRNS used dual agents of immunosuppression while majority of patients with Non SRNS used only one agent of immunosuppression with significant differences between both groups. And logically we found that improvement with steroid occurred in most patients with non SRNS while in more than half of patients with SRNS no improvement at all was detected even with addition of other agent (s) than steroids.

LIMITATIONS

We want to draw the reader attention to some limitations of this study. Our enrolled patients from multiple nationalities and this may not give us a precise results of frequency of R229Q polymorphism in these cohort of patients. Also some of ethnic groups enrolled in our study had small sample size so our results cannot be validated except if there is large sample size from these races. Moreover, we included few patients (7%) had family history of NS. This means that our results as regarding familial R229Q polymorphism cannot be validated.

CONCLUSION

Our study drew its strength from having a head start to investigate the association between the R229Q variant and idiopathic NS syndrome in children and adults in the Arab world as this field hasn't been studied extensively. In fact the general molecular mutational status of FSGS/MCD hasn't been investigated at a large scale except for small cohorts in a few Arab countries. Based on our results, we could conclude that the initial screening for the R229Q SNP amongst patients with NS was mandatory. As R229Q variant is considered the most commonly found NPHS2 mutation in the general population, and ultimately we were working on the whole exome sequencing to screen for all known mutations in NS and possibly discover new novel mutations that might be present in our part of the world with racial differences.

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