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ASSOCIATION OF ENDOTHELIAL NITRIC OXIDE SYNTHASE GENE POLYMORPHISM (G894T) WITH NEPHROPATHY AMONG SUDANESE SICKLE CELL PATIENTS IN KHARTOUM STATE

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ABSTRACT

Background: Sickle cell nephropathy (SCN) is a major cause of morbidity and mortality among patients with sickle cell disease (SCD), arising from chronic hemolysis, oxidative stress, and endothelial dysfunction. Variations in the endothelial nitric oxide synthase (eNOS) gene may influence nitric oxide (NO) bioavailability, potentially affecting renal susceptibility in SCD. **Aim:** This study aimed to investigate the association of eNOS gene polymorphism (G894T) with nephropathy among Sudanese patients with SCD. **Method:** A case-control study was conducted between June 2021 and June 2022 at Soba University Hospital and Jafar Ibn Auf Specialized Hospital, Khartoum. Participants included 65 patients with sickle cell nephropathy, 45 nephropathy patients without SCD, and 45 healthy controls. Samples were used for CBC and DNA extraction. (Hb, PCV, PLTs, WBCs, RBCs count and RBCs indices): Was done by using automated hematology analyzer Genomic DNA was extracted from blood samples and analyzed for G894T polymorphism using polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) techniques. **Result:** The NOS3 (G894T) genotype distributions differed significantly between SCD nephropathy cases and healthy controls(χ^2 = 5.982, p = 0.050). No significant differences were found for G894T or in comparisons between sickle and non-sickle nephropathy groups. Hematological parameters showed no genotype-related variations.

Conclusion: The eNOS3 G894T polymorphism showed a significant difference in genotype distribution between cases and normal controls, though logistic regression analysis did not reveal a statistically significant association in genetic models.

KEYWORDS: SCN,eNOS,NOS3, (G894T).

INTRODUCTION

Sickle cell nephropathy (SCN) is one of the serious complication of sickle cell disease, often underrecognized complication of sickle cell disease (SCD), contributing substantially to long-term morbidity and mortality. It encompasses a wide spectrum of renal abnormalities, ranging from asymptomatic tubular dysfunction and glomerular hyperfiltration to progressive proteinuria, chronic kidney disease, and ultimately end- stage renal disease. The onset of kidney involvement in SCD is often insidious, making early detection and risk stratification critical for improving outcomes.[1]

The pathophysiology of SCN is complex, involving chronic hemolysis, oxidative stress, recurrent vaso-occlusion, and ischemia-reperfusion injury within the renal microvasculature. The renal medulla, characterized by low oxygen tension, hypertonicity, and acidosis, presents an environment that favors hemoglobin S polymerization and red cell sickling. These factors render the kidney, especially the medullary region, highly vulnerable to hypoxic damage and endothelial dysfunction.[2,3] One of the central mediators of endothelial homeostasis is nitric oxide (NO), a potent vasodilator synthesized by endothelial nitric oxide synthase (eNOS). NO plays a protective role by regulating vascular tone, inhibiting platelet aggregation, and reducing inflammation.[4]

In SCD, impaired NO bioavailability is believed to contribute to vascular complications, including nephropathy.

Nephropathy is term used to describe a heterogenous group of patients with either microalbuminuria or varying degrees of proteinuria, with or without maternal hypertension or significant impairment in renal function. It is a broad medical term used to denote disease or damage of the kidney, which can eventually result in kidney failure. The primary and most obvious functions of the kidney are to excrete any waste products and regulate the water and acid-base balance of the body; therefore, loss of kidney function is a potentially fatal condition.[5]

The eNOS gene (NOS3), located on chromosome 7q36, encodes the enzyme responsible for constitutive NO production in endothelial cells. Polymorphisms in the eNOS gene have been shown to affect gene expression and enzymatic activity, potentially influencing NO levels and vascular function. Among the most widely studied variants are T786C, located in the promoter region, which is associated with reduced transcriptional activity, and G894T (Glu298Asp), a missense mutation that may impair enzyme stability and function.[6,7]

While several studies have linked these polymorphisms to cardiovascular and renal diseases in various populations, data on their distribution and association with nephropathy in individuals with SCD, particularly in Sudan, remain limited. Given the high burden of both SCD and renal disease in this region, exploring the genetic determinants of nephropathy is essential for advancing risk prediction and personalized care.[8]

Therefore, this study aimed to investigate the association of endothelial nitric oxide synthase gene polymorphism (G894T) with nephropathy among patients with sickle cell disease in Khartoum State.

METHOD

This study was a case-control study conducted from June 2021 to June March 2022 at two major Sudanese healthcare institutions: Soba University Hospital and Jafar Ibn Auf Specialized Hospital for Children, both located in Khartoum, Sudan. The study involved 65 sickle cell nephropathy cases 36 (55.4%) male and 29 (44.6%) were female, 45 non-sickler nephropathy patients 28 (62.2%) male and 17 (37.8%) were female, and 45 healthy controls patients 23 (51.1%), 22 (48.9%) male and female respectively. Exclusion criteria patients with sickle cell nephropathy who had concurrent systemic illnesses and patient who declined genetic testing or sample provision. Venous blood (3 ml) was collected from each participant using EDTA tubes. Samples were used for CBC and DNA extraction. (Hb, PCV, PLTs, WBCs, RBCs count and RBCs indices): Was done by using automated hematology analyzer DIRUI(DIRUI BCC-3600). Genotyping of the G894T polymorphisms in the NOS gene was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis, as described by Omneya -Moguib et al[9] and Genomic DNA was extracted from whole blood samples using the G-DEX™ IIb DNA extraction kit, following the manufacturer’s protocol. Statistical assessment was carried out with a statistical package for social sciences (SPSS).

Ethical considerations

Ethical approval was obtained from the Ethical Committee of the Faculty of Medical Laboratory Sciences, Omdurman Islamic University, and the Khartoum State Ministry of Health. Written informed consent was obtained from all participants or their guardians. Permissions were granted by the administrative authorities of both hospitals. All collected data were handled with strict confidentiality and used solely for research purposes.

RESULT

In this study the distribution of the NOS3 (984G/T) polymorphism among the study groups is presented in Table 1. In the case group, 57 patients (87.7%) exhibited the homozygous G/G genotype, while 5 (7.7%) were heterozygous (G/T), and 3 (4.6%) were homozygous for the T/T variant. In contrast, all individuals in the normal control group (100%) demonstrated the G/G genotype. The abnormal control group had 43 individuals (95.6%) with the G/G genotype and 2 individuals (4.4%) with the G/T genotype; none exhibited the T/T genotype.

Chi-square analysis revealed (Table 2), a statistically significant difference in the distribution of the NOS3 (984G/T) genotype between cases and normal controls ($X^2 = 5.973$, $p = 0.050$).

Comparison of red blood cell indices based on NOS3 (G894T) genotypes is shown in Table 4. No significant differences were observed in RBC count, hemoglobin levels, packed cell volume, MCV, MCH, or MCHC across the G/G, G/T, and T/T genotypes (all $p > 0.05$).Similarly, Table 5 illustrates the comparison of total white blood cell counts (TWBCs) and platelet counts among the different NOS3 (G894T) genotypes. The mean TWBC count ranged from 10.6 ± 3.3 in the G/T genotype to 16.2 ± 9.6 in the T/T genotype, while platelet counts ranged from 172.0 ± 24.4 (T/T) to 220.7 ± 86.8 (G/G). However, these differences were not statistically significant (TWBCs $p = 0.426$; Platelets $p = 0.555$).

For the dominant genetic model analysis (Table 3) of NOS3 (984G/T) between cases and abnormal controls, the association was not statistically significant (OR = 3.018, 95% CI: 0.610 – 14.935, $p = 0.176$).

Notably, not all polymorphisms demonstrated sufficient genotype variation across all study groups to permit full application of all genetic models. In some comparisons, certain genotypes (such as C/C or T/T) were either absent or extremely rare, limiting the feasibility of codominant and recessive analyses. These limitations were considered in the model selection

process, and only statistically valid comparisons based on available genotype distributions were included in the final analysis.

Table 1: Distribution of NOS3 (G894T) among study population.

NOS3(984G/T)	Case	Normal Control	Abnormal Control
G/G	57 (87.7%)	45 (100.0%)	43 (95.6%)
G/T	5 (7.7%)	0 (0.0%)	2 (4.4%)
T/T	3 (4.6%)	0 (0.0%)	0 (0.0%)
Total	**65 (100.0%)**	**45 (100.0%)**	**45 (100.0%)**

Table 2: Distribution of NOS3 (G894T) polymorphism between Sickler patients and normal controls.

Genotype/Allele	Sickler Cases (n = 65)	Normal Controls (n = 45)	Chi-square(X^2)	P-value
NOS3 (G894T)				
G/G	57 (87.7%)	45 (100.0%)	5.973	**0.050***
G/T	5 (7.7%)	0 (0.0%)		
T/T	3 (4.6%)	0 (0.0%)		
T allele carriers (G/T + T/T)	8 (12.3%)	0 (0.0%)	-	-

Table 3: Distribution of NOS3 (G894T) polymorphism between Sickler patients and abnormal controls.

Genotype/Allele	Sickler Cases (n = 65)	Abnormal Controls(n = 45)	Chi-square(X^2)	P-value
NOS3 (G894T)				
G/G	57 (87.7%)	43 (95.6%)	2.699	0.259
G/T	5 (7.7%)	2 (4.4%)		
T/T	3 (4.6%)	0 (0.0%)		
T allele carriers (G/T + T/T)	8 (12.3%)	2 (4.4%)	3.600	0.058

Table 4: Comparison of RBCs, Hb and red cells indices according ofNOS3 (G894T) polymorphisms.

NOS3 (G894T)	RBCs ($\times 10^{12}$/L)	Hb (g/dL)	PCV (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)
G/G	2.5 ± 0.6	6.7 ± 1.6	22.1 ± 4.3	81.6 ± 7.2	26.8 ± 2.3	32.3 ± 3.0
G/T	2.6 ± 0.9	6.7 ± 1.4	22.2 ± 5.1	77.1 ± 8.1	27.1 ± 2.5	32.1 ± 2.2
T/T	2.4 ± 0.7	6.6 ± 2.2	22.2 ± 6.1	81.5 ± 1.3	26.2 ± 2.5	33.9 ± 4.2
P-value	0.912	0.985	0.997	0.391	0.864	0.643

Table 5: Comparison of Total White Blood Cell Count (TWBCs) and Platelet Count according to NOS3 (G894T) polymorphisms.

NOS3 (G894T) Genotype	TWBCs ($\times 10^9$/L)	Platelet Count ($\times 10^9$/L)
G/G	11.7 ± 6.1	220.7 ± 86.8
G/T	10.6 ± 3.3	201.0 ± 22.4
T/T	16.2 ± 9.6	172.0 ± 24.4
P-value	0.426	0.555

Table 6: Genetic Model Analysis of NOS3 (984G/T) polymorphisms between cases and control groups.

Polymorphism	Genetic Model (Cases vs.)	Comparison	P-value	Odds Ratio (OR)	95%
NOS3 (984G/T)	Dominant (vs. Abnormal Control)	G/T + T/T vs. G/G	0.176	3.018	0.61

DISCUSSION

This study investigated the distribution and association of NOS3 (G894T) polymorphism among patients with sickle cell nephropathy in comparison to normal healthy controls and nephropathy patients without sickle cell disease (SCD). The NOS3 (G894T) polymorphism showed a significant difference in genotype distribution between cases and normal controls, though logistic regression analysis did not reveal a statistically significant association in genetic models.

In contrast, the genotype distribution and allele frequencies in the sickle cell nephropathy cases were not significantly different from those in the nephropathy patients without SCD (abnormal controls), suggesting a shared or overlapping genetic risk background for nephropathy in these two groups.

The endothelial nitric oxide synthase enzyme (eNOS), encoded by the NOS3 gene, plays a vital role in vascular function by producing nitric oxide (NO), a potent vasodilator and regulator of endothelial homeostasis. Variants such as the G894T and T786C polymorphisms have been shown to reduce eNOS expression or activity, leading to decreased NO bioavailability, which is implicated in endothelial dysfunction and the pathogenesis of renal disease.[10]

Our findings align with the review by Medina et al.[10], which highlighted that NOS3 polymorphisms, including G894T, T786C, and intron 4 VNTR variants, accelerate kidney function decline through endothelial dysfunction and oxidative stress pathways. Importantly,

Medina et al. emphasized that the association of these variants with chronic kidney disease (CKD) exhibits ethnic and population variability, consistent with the variable genotype distributions observed in our Sudanese cohort. Similar observations were reported by Tanus-Santos et al.[11], who described significant differences in NOS3 polymorphism frequencies across ethnicities, underscoring the importance of population-specific genetic investigations.

Although direct studies on NOS3 polymorphisms and sickle cell nephropathy are limited, the pathophysiology of SCD nephropathy shares mechanisms with other nephropathies, particularly endothelial dysfunction. Naik and Derebail[12] reviewed the spectrum of sickle hemoglobin-related nephropathy and highlighted impaired NO signaling, where eNOS/NOS3 plays a central role, as a key contributor to glomerular injury and progressive CKD in SCD patients.

Nishank et al.[13] reported similar findings in Indian SCD patients, where the G894T polymorphism was significantly associated with SCD complications. Conversely, Thakur et al. (2014) in Malian SCD patients and Navarro et al.[8,14] in African-American populations reported no significant differences in G894T allele and genotype distributions between patients and controls, reflecting the ethnic and geographical variability in genetic susceptibility, which is consistent with our observations.

Furthermore, Padhi et al.[15] reported a significant association between the NOS3 intron 4 a/b polymorphism and end-stage renal disease (ESRD) in autosomal dominant polycystic kidney disease, reinforcing the broader involvement of NOS3 genetic diversity in nephropathies. The lack of significant association of G894T polymorphism in our SCD cohort echoes findings from Padhi et al.'s meta-analysis, suggesting variant-specific effects that may vary by population and disease context.

Notably, the absence of significant differences in hematological indices (RBCs, Hb, PCV, TWBCs, and platelets) across NOS3 genotypes in our cohort indicates that these polymorphisms likely exert their effect via endothelial and vascular pathways rather than through direct hematological alterations.

Regarding the abnormal control group (patients with nephropathy but without SCD), the lack of significant genotype distribution differences compared to SCD nephropathy cases suggests that NOS3 polymorphisms may represent a general genetic risk factor for nephropathy,

irrespective of underlying etiology. This observation supports the notion that endothelial dysfunction is a converging pathogenic mechanism across various nephropathies.

Studies investigating NOS3 polymorphisms in nephropathy have primarily focused on diabetic populations. Armenis et al.[16] demonstrated a significant association between NOS3 polymorphisms and diabetic nephropathy in Greek patients, while Dellamea et al.[17] reported similar findings in a systematic review and meta-analysis. Although these studies pertain to diabetic nephropathy, they further highlight the universal impact of NOS3 genetic variability on renal disease susceptibility across different clinical settings. The lack of available studies specifically exploring NOS3 polymorphisms in sickle cell nephropathy underscores the novelty of our findings and the need for further research.

In alignment with our genetic findings, Chenou et al.[18] conducted a study on Brazilian sickle cell anemia patients, assessing eNOS polymorphisms (T786C, G894T, and VNTR intron 4) alongside markers of hemolysis, inflammation, and endothelial dysfunction. The study found the allelic/genotypic frequencies did not statistically differ between patient and control groups. Their findings are in agreement with Thakur et al.[8] and Navarro et al.[14], who found no association, but contrary to Nishank et al.[13] Additionally, Chenou et al.[18] highlighted the complex interplay between eNOS polymorphisms and endothelial dysfunction markers, suggesting that these genetic variants may contribute to phenotypic variability and disease progression in SCD through endothelial pathways.

Strengths and Limitations

A major strength of this study is its well-characterized case-control design, incorporating both healthy and nephropathy controls, enabling a more precise assessment of genotype-disease associations. The inclusion of Sudanese Sicklers also provides valuable insights from a region with limited genetic epidemiological data.

However, limitations exist. The sample size, while sufficient to detect associations in dominant genetic models, may lack the statistical power to capture subtle effects of less frequent genotypes.

CONCLUSION

The NOS3 G894T polymorphism showed a significant difference in genotype distribution between cases and normal controls, though logistic regression analysis did not reveal a

statistically significant association in genetic models.

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