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eNOS GENE POLYMORPHISMS AND DIABETIC NEPHROPATHY

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Abstract: *The genes thought to be effective in the pathogenesis of Diabetic nephropathy (DN), one of the important complications of diabetes, have been the focus of attention of researchers. In our study, three eNOS polymorphisms, the 27-bp repeat (4b/a) in Intron 4, the G894T missense mutation in exon 7, and the T786C single nucleotide polymorphism in the promoter region were examined. This study is conducted with the blood specimens of ninety patients with diabetic nephropathy and sixty healthy control groups. eNOS, G/T, and T/C gene polymorphisms were evaluated. The genotypic distribution and demographic/clinical findings obtained in our study were not significant between the two groups ($p>0.05$). But, the genotypical distribution in the patient group was significantly different ($p<0.05$). Our findings are consistent with much literature, and in our population, eNOS is thought to be an important genetic factor contributing to DN pathology. Studies show that NO gene polymorphism seen in diabetes may be a crucial risk factor for DN and atherosclerosis.*

Keywords - *Diabetic nephropathy, diabetes mellitus, endothelial nitric oxide synthase gene, polymorphism*

I. INTRODUCTION

The genes thought to be effective in the pathogenesis of Diabetic nephropathy (DN), one of the important complications of diabetes, have been the focus of attention of researchers [1]. DN is the primary cause of morbidity and mortality in diabetes mellitus patients [2]. The development of diabetic nephropathy depends on multifactorial factors [1-4]. Genes that important in the development of DN are seen in the regulation of hyperlipidemia and hypertension, which affect cardiovascular system diseases [1,5].

Endothelial nitric oxide synthase gene polymorphisms: Endothelial nitric oxide plays a key role in the regulation of blood flow and blood pressure and is kept under control by endothelial nitric oxide synthase (eNOS), an important released from vascular endothelial cells [6,7]. So, the eNOS gene is an important gene for DN and other kidney diseases [6]. It is thought that the contribution of the polymorphism that may occur in this gene to DN is important. However, studies have not clearly reported this relationship [8].

The presence of the eNOS polymorphism may contribute to the reduction in eNOS activity and the NO level is reduced. In addition, studies have reported that it is a crucial factor in the development and pathogenesis of DN [9,10]. Studies show that there are potentially DN-associated three polymorphisms: 27 bp repeats (VNTR) in intron 4, 786-T/C single nucleotide polymorphism (SNP) in the promoter region, and 894-G/T missense mutation in exon 7 [8,11].

There is a great deal of literature that includes genetic polymorphisms and their relationship with DN. In our study, three eNOS polymorphisms, the 27-bp repeat (4b/a) in Intron 4, the G894T (Glu298Asp) missense mutation in exon 7, and the T786C single nucleotide polymorphism in the promoter region were examined.

II. MATERIAL AND METHODS

Ninety diabetic nephropathy patients and sixty healthy controls were included in our study. DNA extraction was performed from the blood samples taken from the patients and the control group into tubes coated with EDTA, using a DNA extraction kit (Biorad, United Kingdom). Then, the purity and quantity determinations of the DNAs were made with a micro-volume spectrophotometer. qPCR Master Mixes (Biorad, United Kingdom) and reverse and forward primers were used for amplification in Table I. The primers contain 20-80% guanine(G)-cytosine (C), especially because of the nucleotides working like G and the absence of a G base at the 5' end of the probe, the C base rather than G in the selected sequence, the melting temperature (Tm) temperature of 65-Care was taken to ensure that it was between 85 ^{o}C.

Statistical Analysis

The statistical Package (SPSS) program (version 20.0, SPSS Inc., Chicago, IL, USA) was used in the analysis of the obtained data. Data are presented as mean values ± standard deviation. Non-parametric tests were used. The statistical significance value was accepted as $p<0.05$ and 95% confidence interval was preferred.

III. RESULTS

In this study, 90 diabetic nephropathy patients and 60 healthy controls were compared in terms of gene polymorphism. Table 2 shows the demographic data of the patient and healthy control groups. There was no statistical difference between the two groups in terms of age and BMI data ($p>0.05$). However, a significant difference was detected in fasting blood glucose, Bun, creatinine, and HbA1c parameters. ($p<0.05$).

eNOS, G/T, and T/C gene polymorphisms were examined in ninety DN with patients and 60 healthy control individuals. The genotype distribution of groups is in Table 3. Genotypical distribution and demographic/clinic findings data were not significant between two groups ($p>0.05$). But, the genotypical distribution of the DN patients was significantly different ($p<0.05$).

IV. DISCUSSION

The DN's etiology is multifactorial, is includes environmental and genetic factors. It is very important to reveal the pathogenesis of DN and to be able to identify patients at risk beforehand. Studies in recent years have aroused serious interest in investigating susceptibility polymorphisms of DN [12,13]. eNOS gene polymorphisms that reduce NO expression are associated with DN, and the possible mechanism between DN risk and eNOS polymorphisms has not been revealed yet. However, variants of the eNOS gene are thought to increase susceptibility to glomerular disease by causing a decrease in NO levels [14,15]. In our study, three gene polymorphisms, which are thought to be effective in the process of diabetic nephropathy, were studied and evaluated together. ENOS 4b/a, T/C single nucleotide polymorphism, and G/T missense mutation.

Shoukry et al. (2012) a study of 400 diabetic patients found an association between diabetic nephropathy and three eNOS polymorphisms (894G > T, 27-bp-VNTR, and -786T > C) [16]. Similarly, Ma et al. (2014) reported a significant relationship between diabetic nephropathy and eNOS-4b/a polymorphism in their meta-analysis study [17]. However, some studies did not show a significant relationship, [7,18], found no association between DN and NOS polymorphism in patients with type 1 diabetes. El-Din and Hamdy (2011) reported that the risk of end-stage renal disease in patients with type 2 diabetes is associated with the TT genotype of eNOS, therefore it may be an important marker in identifying high-risk diabetic patients [19]

V. FIGURES AND TABLES

TABLE 1. PRIMER SEQUENCES

Primers	Sequences
EcNOS Polymorphism 4b/a	AGGCCCTATGGTAGTGCCTTT-F TCTCTTAGTGCTGTGGTCAC-R
G/T Polymorphism eNOS G894T	AAGGCAGGAGACAGTGGATGGA-F CCCAGTCAATCCCTTTGGTGCTCA-R
T/C Polymorphism eNOS T786C	GTGTACCCCACCTGCATTCT-F CCCAGCAAGGATGTAGTGAC-R

TABLE 2. Characteristics of the patient and healthy control groups.

Parameters	Control (n=60)	DN (n=90)	p-value
Age (year)	56±6	62±8	p>0.05
Male/Female	26/34	38/52	p<0.05
Body mass index (kg/m^2)	25.6±4.2	28.6±4.8	p<0.05
Fasting Blood Sugar (mg/dl)	92.6±8.2	198.4± 42.8	p>0.05
Bun (mg/dl)	7.6±2.8	44.3±15.6	p>0.05
Creatinine (mg/dl)	0.5±0.2	5.2±2.6	p>0.05
Potassium (mmol/L)	4.0±0.5	5.2±1.4	p<0.05
Sodium (mmol/L)	142±18	143±15	p<0.05
HbA1c (mg/dl)	4.1±1.2	8.2±1.6	p>0.05

Table 3. Gene polymorphisms among study groups

Genotypes	Control (n=60)	DN (n=90)	p-value
4b/a	12.25±0.16	39.67±2.36	
G/T	8.61±0.32	29.21±1.70	p<0.05
T/C	8.14±0.18	28.24±2.97	

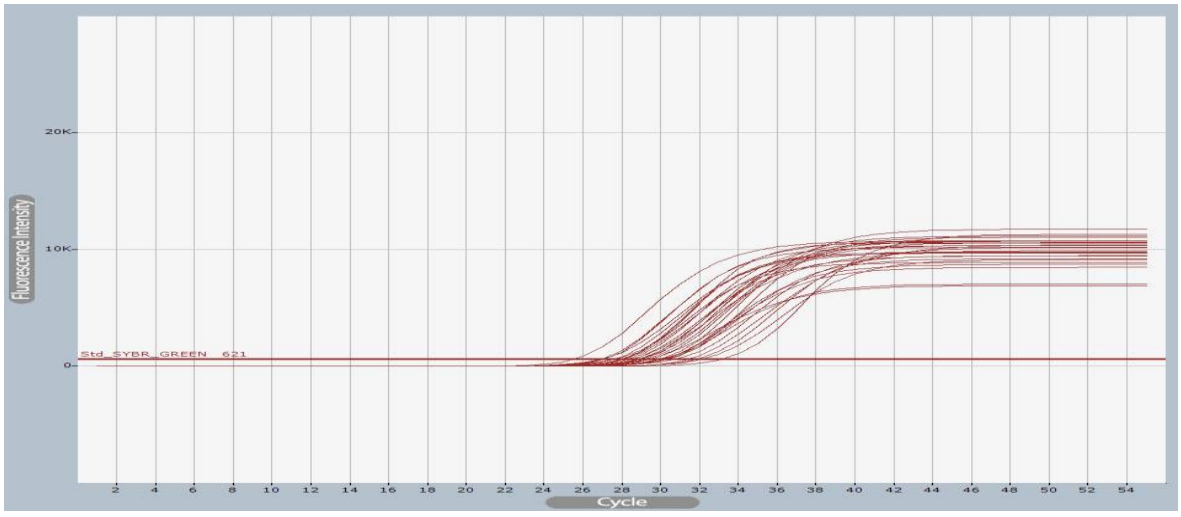

Figure 1. Sample amplification curve obtained as a result of the study

VI. CONCLUSION

Our findings are consistent with much literature, and in our population, eNOS is thought to be an important genetic factor contributing to DN pathology. Studies show that eNOS polymorphism seen in patients with diabetes may be a crucial risk factor for DN and atherosclerosis.

REFERENCES

Journal Papers:

[1] E Avci, G Alp Avci, ACE I/D Polymorphisms in patient with Diabetic Nephropathy and Non-Diabetic Nephropathy. *International Journal of Applied Science and Research*, 2021, 116-119.
[2] CC Chen , CH Tseng, SH Cheng, Continuity of care, medication adherence, and health care outcomes among patients with newly diagnosed type 2 diabetes: a longitudinal analysis. *Med Care*, 2013 ;51(3):231-7
[3] Z Rahimi, A Hasanvand ,V Fellehgary, Interaction of MTHFR 1298C with ACE D allele augments the risk of diabetic nephropathy in Western Iran, *DNA Cell Biol* 2012;31:553-559.
[4] S Li, J Wang, B Zhang ,X Li, Y Liu, Diabetes Mellitus and Cause-Specific Mortality: A Population-Based Study. *Diabetes Metab J*. 2019;43(3):319-341
[5] N Samsu, Diabetic Nephropathy: Challenges in Pathogenesis, Diagnosis, and Treatment. *BioMed Research International, 2021*, DOI:10.1155/2021/1497449
[6] B Degen ,S SchmidtC, E Ritz, A polymorphism in the gene for the endothelial nitric oxide synthase and diabetic nephropathy. *Nephrology, dialysis, transplantation*, 2001; 16(1):185.
[7] JD Rippin, A Patel, ND Belyaev, GV Gill, AH Barnett, SC Bain, Nitric oxide synthase gene polymorphisms and diabetic nephropathy. *Diabetologia*, 2003; 46(3):426-28.
[8] BS Dellamea , LC Pinto , CB Leitao, KG Santos, LH Canani, Endothelial nitric oxide synthase gene polymorphisms and risk of diabetic nephropathy: a systematic review and meta-analysis. *BMC Medical Genetics*, 2014; 15:9.
[9] TS Ahluwalia, M Ahuja, TS Rai, HS Kohli, K Sud , A Bansali ,M Khullar, Endothelial nitric oxide synthase gene haplotypes and diabetic nephropathy among *Asian Indians*. *Mol Cell Biochem*. 2008;314(1-2):9-17.
[10] A Mollsten, M Lajer, A Jorsal, L Tarnow, The endothelial nitric oxide synthase gene and risk of diabetic nephropathy and development of cardiovascular disease in type 1 diabetes. *Mol. Genet. Metab*,2009, 97, 80–84.
[11] M Bellini, MN Figueira, MF Piccoli et al., Association of endothelial nitric oxide synthase gene intron 4 polymorphism with end stage renal disease. *Nephrology* 2007, 12, 289–29
[12] ME Hellemons, J Kerschbaum, SJ Bakker, H Neuwirt, B Mayer, G Mayer, D de Zeeuw, HJ Lambers Heerspink, M Rudnicki, Validity of biomarkers predicting onset or progression of nephropathy in patients with Type 2 diabetes: a systematic review. *Diabet. Med* , 2012: 29, 567–577
[13] MC Thomas, PH Groop, K Tryggvason, Towards understanding the inherited susceptibility for nephropathy in diabetes. *Curr. Opin. Nephrol. Hypertens*, 2012, 21, 195–202.
[14] E Zintzaras, AA Papathanasio,I Stefanidis, Endothelial nitric oxide synthase gene polymorphisms and diabetic nephropathy: A HuGE review and meta-analysis. *Genetics in Medicine*, 2009; 11(10):695-706
[15] SMA El Dayem, AA Battah, AEM El Bohy, S Ahmed , M Hamed , SNA El Fattah , Nitric Oxide Gene Polymorphism is a Risk Factor for Diabetic Nephropathy and Atherosclerosis in Type 1 Diabetic Patients. *Open Access Maced J Med Sci*. https://doi.org/10.3889/oamjms.2019.831
[16] A Shoukry, SM Shalaby, S Abdelazim, M Abdelazim,A Ramadan, MI Ismail, et al. Endothelial nitric oxide synthase gene polymorphisms and the risk of diabetic nephropathy in type 2 diabetes mellitus. *Genetic Testing and Molecular Biomarkers*. 2012; 16(6):574-79.
[17] ZJ Ma , R Chen ,HZ Ren, X Guo, JG Chen, LM Chen, Endothelial nitric oxide synthase (eNOS) 4b/a polymorphism and the risk of diabetic nephropathy in type 2 diabetes mellitus: A metaanalysis. *Meta Gene*, 2014; 2:50-62.
[18] A Momeni, MH Chaleshtor, S Saadatmand, S Sheiri, Correlation of Endothelial Nitric Oxide Synthase Gene Polymorphism (GG, TT and GT Genotype) with Proteinuria and Retinopathy in Type 2 Diabetic Patients. *Journal of Clinical and Diagnostic Research*. 2016; 10(2):32-35.
[19] SS El-Din Bessa, SM Hamdy, Impact of nitric oxide synthase Glu298Asp polymorphism on the development of end-stage renal disease in type 2 diabetic Egyptian patients. *Renal Failure*. 2011; 33(9):878-84.