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Original Article

Association of SNP (RS4880) in SOD-2 Gene with Diabetic Nephropathy

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ABSTRACT

Objective: To find an association between SNP (rs4880) in SOD-2 gene with Diabetic Nephropathy.
Study Design: Case Control Study.
Place & Duration of study: Department of Biochemistry and Molecular Biology, Army Medical College Rawalpindi and Armed Forces Institute of Urology, Rawalpindi Pakistan, from Nov 2020 to Mar 2021.
Methodology: A total of 60 peoples were enrolled including 30 healthy individuals and 30 patients of diabetic nephropathy. Both males and females aged 35-85 years were enrolled. Baseline biochemical tests were performed to ascertain diagnosis and exclude comorbidities. DNA was extracted using kit method and sequence of interest was amplified using PCR. Amplified product of genomic DNA of each individual was subjected to restriction fragment length polymorphism (RFLP) analysis. Data was analyzed using SPSS version 22.
Results: The frequency of TT genotype (40%) was higher among type 2 Diabetes Mellitus (T2DM) patients with nephropathy than CT (33.3%) and CC (26.6%) genotypes however the odds ratio for TT with 95% confidence interval (CI) was 2.53(0.69-9.25) with p=0.35 which is statistically non-significant. The association value for genotypes was 1.29 (p-value=0.52) hence not significant.
Conclusion: There was no significant association present between SNP (rs4880) in SOD-2 gene which causes Val16Ala polymorphism in superoxide dismutase 2 gene, with nephropathy in type 2 Diabetes Mellitus (T2DM) patients.

Keywords: Diabetic nephropathy, superoxide dismutase 2 gene, SNP rs4880, Val16Ala polymorphism.

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INTRODUCTION

Diabetic nephropathy (DN) is a major health concern affecting 50% of patients with diabetes mellitus. It is marked by the presence of persistent proteinuria and gradual decline in kidney function leading to glomerulosclerosis.[1] The prevalence of type 2 Diabetes Mellitus (T2DM) in Pakistan is 16.98% and the patients suffering from diabetic nephropathy are 24.4% of these, the major micro-vascular complication of diabetes.[2] DN is a major cause of end stage renal disease and leads to 1.6 million global annual deaths.[3,4]

Superoxide dismutase 2 is a key antioxidant enzyme in the mitochondria, encoded by SOD2 gene, provides antioxidant defense against superoxide radicals by scavenging free reactive oxygen species.[5] In diabetic nephropathy the pro-oxidant levels increase and antioxidant enzyme activity decreases which not only perpetuates glomerular/interstitial injury, but also contributes to endothelial dysfunction.[6] Production of reactive oxygen species in hyperglycemia is the main pathogenesis behind T2DM and its complications as it affects the metabolism of lipids, proteins and causes vascular damage.[7] Super oxide Dismutase (SOD2) converts superoxide radicals to hydrogen peroxide and diatom oxygen.[8] Thus slows down the process of development of diabetes and complications. Gene for this enzyme (SOD2 gene) is located on chromosome number 6 (6q25.3) with 10 exons. Single nucleotide polymorphism Val16Ala registered as SNP rs4880 on exon 2 at 16th amino acid position may be associated with T2DM and its complications as it can affect the transport of superoxide dismutase 2 enzyme inside mitochondria leading to decreased scavenging activity and raised oxidative stress 7.[5,9] The major allele C codes for alanine and minor allele T codes for Valine. When valine to alanine substitution takes place the mobilization of this enzyme from cytosol to mitochondria may get affected. This is likely to increase free radical burden causing oxidative stress.[9]

Lipid profile derangements are common among diabetic patients. Both insulin deficiency and resistance affect the lipid metabolic pathways causing dyslipidemia i.e. hypertriglyceridemia, decreased HDL and raised LDL levels. It is caused by altered role of lipoprotein lipase enzyme causing raised TG levels and low HDL levels, which may cause vascular and renal

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cell damage.[10] Lipid profile parameters were also assessed and analyzed in control and diabetic groups in this study

This study primarily aimed to determine whether SNP (rs4880) Val16Ala polymorphism in SOD2 gene is a factor associated with the development of DN among local Pakistani population of the region. The focus was to determine the genotype distribution and its possible role in disease occurrence and progression.

METHODOLOGY

The case control study was carried out at Department of Biochemistry and Molecular Biology, Army Medical College, National University of Medical Sciences, Rawalpindi Pakistan, in collaboration with Armed Forces Institute of Urology (AFIU), Rawalpindi Pakistan, from November 2020 to March 2021. The ethical approval was taken from the institutional Ethical Review Committee, Army Medical College (ERC/ID/78, Dated 12-11-2020). Subjects who were part of research had signed a written informed consent form. Sample size was calculated by WHO calculator using 95% confidence interval, 80% power of study with anticipated minor allele frequency (MAF) of 0.09 in control group and 0.55 in patients with type 2 diabetes mellitus.[7] Calculated sample size was 20 individuals in each group. In order to eliminate the chances of dropout we enrolled 30 persons in each group.

Inclusion Criteria: Both males and females between the ages of 35-85 years were included. Group-I included 30(16 male and 14 female) healthy control individuals while Group-II included 30 patients (18 male and 12 female) of T2DM patients with nephropathy, properly diagnosed by a nephrologist. Known diabetics were enrolled in patient group if they had ≥7 mmol/l of fasting blood glucose levels or ≥6.5% HbA1C levels and estimated glomerular filtration rate (eGFR) <60 mL/min/1.73m^2 or raised urinary albumin to creatinine ratio i.e. > 3mg/mmol.[11]

Exclusion Criteria: The patients with type I diabetes, non-diabetics with kidney disease, females with gestational diabetes, patients with chronic thyroid and other endocrine disorders were excluded.

Non-probability purposive sampling technique was used. Biochemical parameters estimation was carried out using commercially available laboratory kits implying photometric methods and ELISA for HBA1c. DNA extraction was done from the collected venous blood sample by Thermo scientific (Walton, MA, USA) DNA extraction kit (Gene JET) and the purified DNA was stored at -20°C. Quality of extracted DNA was analyzed on 1% agarose gel electrophoresis with 1kb DNA ladder (Fermentas-Gene ruler). The gene sequence was downloaded from human genome data base (ensemble) and primers were designed using the online primer designing tool Primer 3 plus available at (https://www.bioinformatics.nl›cgi-bin›primer3plus). The sequences of forward and reverse primers used are as under:

Forward primer: 5'_CTGACCGGGCTGTGCTTTCT_3'

Reverse primer: 5'_CAACGCCTCCTGGTACTTCT_3'

The PCR program was optimized for 25µL reaction mixture at 59.9°C annealing temperature for 40 seconds. The constituents of PCR mixture were 2.5µl of 1x Taq Buffer, 1.7µl of $MgCl_2$, 0.5µl of dNTPs, 0.8µl of Forward and Reverse primer each, 0.5units of Taq DNA polymerase, 1µl of sample DNA and NF water to amplify the desired bands of target sequence in exon 2. PCR reaction was run using following protocol: 94°C for 4 minutes followed by 35 cycles of 94°C for 30 seconds, primer annealing at 59.9°C for 40 seconds, 72°C for 35 seconds with a final extension at 72°C for 12 minutes and the amplified product was analyzed using 2% agarose gel electrophoresis. The genotype SNP (rs4880) in SOD2 gene was determined by restriction fragments length polymorphism (RFLP) technique using restriction endonuclease enzyme BsaWI (New England Bio labs). 0.5ul (10000units/ml) of the restriction enzyme was added to 15ul amplified DNA product along with 3ul restriction buffer. The reaction mixture was incubated at 60°C in water bath for 60 minutes. The respective genotypes were identified and documented according to the digested fragment lengths. RFLP reaction was confirmed through the analysis of digested fragments length on 3% agarose gel, against the molecular weight marker. CC genotype had 225bp undigested single fragment, CT genotype had three fragments sized 225bp, 175bp and 50bp, and TT genotype was supposed to have 2 fragments of 175bp and 50bp length as shown in Figure-1.

In this figure, left most lane is molecular marker, then control group lane. The next two lanes from the left, labelled CC show Ala/Ala wild type homozygous genotype. The next two lanes labelled CT show Ala/Val mutant heterozygous genotype. While last lane from the left labelled TT shows Val/Val mutant homozygous genotype.

Gene Calculator (Gene Calc.) software was employed to evaluate the obtained genotypic and allelic frequencies between two groups. The genotype frequ-

ency distribution was compared using chi-square test. Data analysis was done on Statistical Package for Social Sciences (SPSS) version 22. The mean and standard deviation of biochemical parameters were calculated and significance of difference was determined by independent t-test. Statistically the *p*-value ≤0.05 considered as significant.

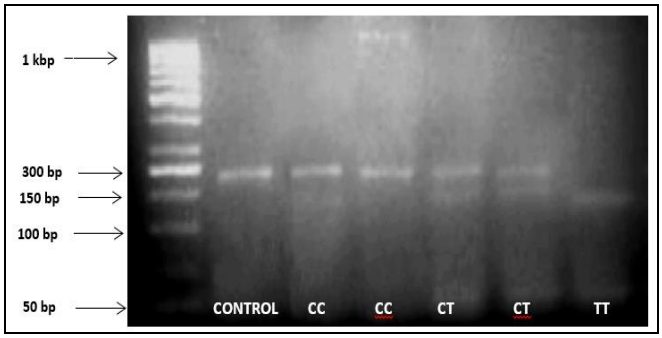

Figure-1: Gel Electrophoresis Restriction fragments of SOD2 gene amplified products

RESULTS

The genotypic distribution was similar among the patient and control group. The gender was not associated with any specific genotype. In Group-I the frequency of CC and CT genotype were 12(40%) and 11(36.7%) respectively, whereas TT genotype was 7 (23.3%) in count. Among cases, the frequency of CC, CT and TT genotype was found as 8(26.6%), 10(33.3%), and 12(40%) respectively. as mentioned in Table-I. There was no significant association found between SNP rs(rs4880) in SOD2 gene and development of diabetic nephropathy in our cohort.

Table I: Association analysis of genotypes between two groups (n=60)

Genotypes	Group-I	Group-II	OR (95% CI)	Chi Square X2	*p*-value
CC	12(40%)	8(26.6%)	-	1.29	0.52
CT	11(36.7%)	10(33.3%)	1.36(0.39-4.69)		
TT	7(23.3%)	12(40%)	2.53(0.69-9.25)		

The allelic count for both the groups was obtained and assessed for the association of gene variant among study population as shown in Table-II. There was no association present for any genotype and allele among cases in our study population.

Additionally, TC, HDL-C, LDL-C and TG showed significant difference (p <0.05) between the groups. Group II has deranged lipid profile as compared to group-I.

Table II: Allelic frequency and association analysis between two groups (n=60)

Alleles	Group I	Group II	OR (95% CI)	Chi- Square X2=2.13 *p*-value
C	35(58.3%)	26(43.3%)	1.83(0.89-3.78)	0.144
T	25(41.6%)	34(56.6%)		

Table III: Comparison of biochemical parameters between the groups (n=60)

Variables (mmol/L)	Group-I Mean±SD (n=30)	Group-II Mean±SD (n=30)	*p*-value
TC	2.57±0.73	3.60±0.74	<0.01
HDL-C	1.17±0.16	0.79±0.09	<0.001
LDL-C	2.47±0.31	3.72±0.34	<0.01
TG	1.46±0.29	2.14±0.67	<0.01

DISCUSSION

In this study there was no association found between the SNP rs4880 and TT genotype with risk of developing T2DM associated nephropathy in our small cohort of Pakistani population. However, the study provides molecular cognizance regarding susceptibility of nephropathy and its association with this polymorphism because many other studies have found a positive relationship between TT genotype and development of diabetic nephropathy. A study carried out in 2016, postulated that 85.7% of the TT homozygotes were diabetic while the percentage of patients was very in those having CC genotype. They concluded that T allele has 78% more frequency of developing diabetic complications than C allele, which has 22% frequency of developing diabetic retinopathy and nephropathy. This study is partially consistent with our study as TT genotype is frequent among cases but this association was not significant.[12] According to Da Cruz Jung *et al.* TT genotype was linked to the development of inflammatory state by enhancing the amount of interleukin levels in body, which further boost the oxidative stress in body.[13] The SNP rs4880 polymorphism is associated with both T1DM and T2DM with a genotype TT that is homozygous Val/Val and the CT genotype that is Val/Ala is more common among healthy individuals. They reported that Ala allele has a protective effect among Chinese population in the development of T2DM associated nephropathy while T allele is a risk allele common among cases.[9] Another research undertaken in 2016 stated that the patients having T2DM associated microvascular complications i.e. diabetic neuropathy and nephropathy have high fasting blood glucose levels and low HDL-C levels along with that they have high mean serum urea levels which leads to progressive

renal and peripheral neuronal disease.[14] T2DM is known to be a secondary cause of dyslipidemia which exacerbate the risk of development of atherosclerosis which leads to progressive decline of renal functions.[15]

Hyperglycemia and hyperlipidemia generates Reactive Oxygen Metabolites,[16] which are detrimental for DNA and promotes the instigation of micro and macro vascular complications.[17] Predominant molecular mechanisms which produce excess reactive metabolites are increased synthesis of advanced-glycation-products,[18] enhanced production of glucose through polyol-pathway, hyperglycemia induced by elevated HMP-shunt and excessive formation of mitochondrial superoxide hydroxyl radicals.[19] This causes mesangial expansion and thickness of mesangial basement (GBM) leading to glomerulosclerosis.[20] The risk associated to its initiation and progression is polygenetic which varies among different ethnicities.[21]

Moreover, a study conducted in 2019 reported that different polymorphisms are linked to development of nephropathy in Chinese, Malaysians and Indians and four oxidative stress related genes including SOD2 (MnSOD-rs4880) are reported in this regard.[5] Another analysis done by Banerjee *et al.* reported analogous results to our study that individuals with TT genotype at SNP rs4880 in SOD2 develop diabetic complications,[22] though our findings were not significant. A similar study performed on SOD2 in Han Chinese population showed diabetic patients who had CC genotype of SNP rs4880 of SOD2 gene were prone to renal failure and had higher morbidity rate but the difference among distribution of genotypes was insignificant 6 which is a similar result to that of our study. This genotypic distribution is in contrary to common findings in other studies.

A recent study on Sudanese population to identify link of this polymorphism with diabetic microvascular complication i.e. retinopathy. Their genotypic analysis corresponds to our results because 20% cases showed CC genotype while this frequency was 50% in controls. It concluded CC as a protective allele and presence of T allele is associated with decreased SOD2 activity and raised HbA1C level along with fasting blood glucose.[23]

Hence, various ethnicities display varying patterns of association of SNP rs4880 in SOD2 gene with microvascular complications of T2DM including nephropathy. It is the need of the hour to identify other genetic polymorphisms related to this gene and its association with nephropathy for timely risk identification and preclude further life threatening complications in Pakistani population.

CONCLUSION

There was no significant association of SNP rs4880 of SOD2 gene with diabetic nephropathy found in our study population. Risk association of minor allele in one population may not necessarily be associated with the pathogenicity in another population due to difference in genetic makeup. In our study population minor allele (VV genotype) was found in abundance among diseased group as compare to healthy group but this association was not statistically significant.

Conflict of Interest: None.

Author's Contribution

Following authors have made substantial contributions to the manuscript as under:

SU & JY: Study design, drafting the manuscript, data interpretation, critical review, approval of the final version to be published.

AR& AM: Data acquisition, data analysis, approval of the final version to be published.

SZHS: Critical review, concept, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investi-gated and resolved.

REFERENCES

1. Selby NM, Taal MW. An updated overview of diabetic nephropathy: Diagnosis, prognosis, treatment goals and latest guidelines. Diabetes Obes Metab2020; 22: 3-15. https://doi.org .10.1111/dom.14007
2. Aamir AH, Ul-Haq Z, Mahar SA. Diabetes prevalence survey of Pakistan (DPS-PAK): pre-valence of type 2 diabetes mellitus and prediabetes using HbA1c: a population-based survey from Pakistan. BMJ Open 2019; 9(2): e025300.
3. Selby NM, Taal MWJD, Obesity, Metabolism. An updated overview of diabetic nephropathy: Diagnosis, prognosis, treatment goals and latest guidelines. Obes Metab 2020; 22: 3-15. https:// doi.org. 10.1111/dom.14007
4. Nazarian-Samani Z, Sewell RD, Lorigooini Z, Rafieian-Kopaei MJCdr. Medicinal plants with multiple effects on diabetes mellitus and its complications: A Systematic review. Curr Diab Rep 2018; 18(10): 72. https://doi.org.10.1007/s11892-018-1042-0.
5. Yahya MJ, Ismail PB, Nordin NB, Akim ABM, Binti Md Yusuf WS, Adam NLB, et al. CNDP1, NOS3, and MnSOD Polymorphisms as Risk Factors for Diabetic Nephropathy among Type 2 Diabetic Patients in Malaysia. J Nutr Metab 2019; 2019: 8736215. https://doi.org.10.1155/2019/8736215.
6. Chao C-T, Chen Y-C, Chiang C-K, Huang J-W, Fang C-C, Chang C-C, et al. Interplay between superoxide dismutase, glutathione peroxidase, and peroxisome proliferator activated receptor gamma polymorphisms on the risk of end-stage renal disease among Han Chinese patients. Oxid Med Cell Longev 2016; 2016. 8516748. https://doi.org.10.1155/2016/8516748.
7. Muriki Naresh Kumar AK, Pallavi Anand. Manganese Superoxide Dismutase (SOD2) Gene Val16Ala polymorphism in type 2 diabetic patients. Al Ameen J Med Sci 2020; 13(4): 242-247.

8. SOD2 superoxide dismutase 2 [Homo sapiens (human)] 2016 [cited December 2019]. [Internet] Available from: https://www .ncbi.nlm.nih.gov/gene/6648.
9. Pourvali K, Abbasi M, Mottaghi A. Role of superoxide dismutase 2 gene Ala16Val polymorphism and total antioxidant capacity in diabetes and its complications. Avicenna J Med Biotechnol 2016; 8(2): 48-56.
10. Kawanami D, Matoba K. Dyslipidemia in diab-etic nephropathy. Renal Replacement Therapy 2016; 2(1): 16. https://rrtjournal .biomedcentral.com/articles/10.1186/s41100-016-0028-0
11. Jiang W, Wang J, Shen X, Lu W, Wang Y, Li W, et al. Establishment and validation of a risk prediction model for early diabetic kidney disease based on a systematic review and meta-analysis of 20 cohorts. Diabetes care 2020; 43(4): 925-933. https://doi.org.10.2337/dc19-1897.
12. Zahreddine AM, Moustafa ME. Susceptibility of patients with manganese superoxide dismutase Ala16val genetic polymorphism to type 2 diabetes mellitus and its complications in a sample of lebanese population. J Genet Genome Res 2016; 3(1): 3-6.
13. da Cruz Jung IE, da Cruz IBM, Barbisan F, Trott A, Houenou LJ, Osmarin Turra B, et al. Superoxide imbalance triggered by Val16Ala-SOD2 polymorphism increases the risk of depression and self-reported psychological stress in free-living elderly people. Mol Genet Genom Med 2020; 8(2): e1080.
14. MohaPatra D, DaMoDar KS. Glycaemia status, lipid profile and renal parameters in progressive diabetic neuropathy. J Clin Diagn Res JCDR 2016; 10(9): CC14.
15. Pinnelli VBKJIJoRiMS. Role of glycated hemoglobin (HBA1c) as a dual marker to predict glycemic status and dyslipidemia in type II diabetes mellitus. Int J Res Med Sci 2016; 4(10): 4524. https:// doi.org/10.18203/2320-6012.ijrms20163322
16. Neelofar K, Ahmad JJD, Research MSC, Reviews. A comparative analysis of fructosamine with other risk factors for kidney dysfunction in diabetic patients with or without chronic kidney disease. Clini Res Rev 2019; 13(1): 240-244. https://doi: 10. 1016/j.dsx.2018.08.007.
17. Spallone VJDmr, reviews. Might genetics play a role in understanding and treating diabetic polyneuropathy? Diab/ Metab Res Rev 2017; 33(4): e2882.
18. Vougioukalou S, Boaz A, Gager M, Locock L. The contribution of ethnography to the evaluation of quality improvement in hospital settings: reflections on observing co-design in intensive care units and lung cancer pathways in the UK. Anthropol Med 2019; 26(1): 18-32.
19. Abbasi M, Daneshpour MS, Hedayati M, Mottaghi A, Pourvali K, Azizi F, et al. The relationship between MnSOD Val16Ala gene polymorphism and the level of serum total antioxidant capacity with the risk of chronic kidney disease in type 2 diabetic patients: a nested case-control study in the Tehran lipid glucose study. Nutr metabol 2018; 15(1): 25.
20. Dieter C, Assmann TS, Lemos NE, Massignam ET, Souza BMd, Bauer AC, et al. -866G/A and Ins/Del polymorphisms in the UCP2 gene and diabetic kidney disease: case-control study and meta-analysis. Genet Molecul biol 2020; 43(2): 12-15.
21. Umanath K, Lewis JBJAJoKD. Update on diabetic nephropathy: core curriculum 2018. Ame J Kidney Dis 2018; 71(6): 884-895. https://doi: 10.1053/j.ajkd.2017.10.026
22. Banerjee M, Vats P, Kushwah AS, Srivastava N. Interaction of antioxidant gene variants and susceptibility to type 2 diabetes mellitus. Br J Biomed Sci 2019; 76(4): 166-171. https://doi: 10.1080/09674845.2019.1595869.
23. Naser M. Naser SEB, Mayada Abdo, Amar M. Ismail, Sawsan Altoum and Khalid H, et al. Association of MnSOD-47C/TGENE polymorphism (Rs 4880-47C/T) in sudanese patient with diabetic retinopathy. Asian J Med Health 2019; 15(1): 1-9. https://doi.org.10.9734/ajmah/2019/v15i130111

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