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Original Research

Superoxide dismutase 1 gene +35A>C (intron3/exon3) polymorphism in diabetic nephropathy patients among Bangladeshi population

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ABSTRACT

Background and Aim: Superoxide dismutase 1 (SOD1) gene +35A>C (intron3/exon3) polymorphism (rs2234694) has been found to be associated with SOD1 enzyme activity modulation. SOD1 is known as free radical scavengers. Along with metabolic processes hyperglycemia also produces free radical particles. Therefore, SOD1 +35A/C polymorphism may interplays in the development of complications of diabetes. The present study has been aimed to investigate the association of this polymorphism in diabetic nephropathy (DN) subjects of Bangladeshi population. **Subjects and Methods:** 150 DN, 109 type2 diabetes mellitus (T2DM) subjects without nephropathy and 144 healthy control subjects were recruited in the study. Genomic DNA was extracted from whole blood using commercial kit. SOD1 gene +35A>C (intron3/exon3) polymorphism was investigated using polymerase chain reaction-restriction fragment length polymorphism method. Data were analyzed using Statistical Package for Social Science for windows version 17. **Results:** The SOD1 A>C genotype frequencies (AA for wild and AC for heterozygous variant [Ht]) were 0.972 and 0.028 for AA and AC in control subjects, 0.963 and 0.037 for T2DM and 0.907 and 0.093 for DN subjects, respectively. These genotype frequency distribution between the groups have shown significant association in c^2-test (χ^2=5.493; P = 0.019). Odds ratio (OR) of the genotypes between controls and DN have shown significant (OR/P = 3.603/0.027; confidence interval=1.157-11.220). Genotypes of Ht in DN are male preponderance. **Conclusions:** (a) +35A>C polymorphism in SOD1-gene possibly involve in the development of nephropathy in Bangladeshi Type 2 diabetic subjects; (b) Male DN subjects of Bangladeshi population are preponderant for +35A>C polymorphism in SOD1-gene.

KEY WORDS: Diabetic nephropathy, superoxide dismutase 1 +35A>C polymorphism, type 2 diabetes mellitus

INTRODUCTION

Diabetes mellitus (DM) is the most prevalent metabolic, non-communicable disorder in the world. Diabetes often remains undiagnosed until its life-threatening complication(s) developed and Diabetic nephropathy (DN) is the most common complication of DM [1]. Oxidative stress has been emerged as an important mechanism for the development of DN [2]. Overproduction of free radicals i.e., oxidative stress can cause oxidative damage to lipids, proteins and DNA, eventually leading to many chronic diseases such as diabetes, myocardial infarction, cardiovascular diseases, atherosclerosis, stroke and other degenerative diseases in humans [3,4]. Moreover, hyperglycemia-induced generation of reactive oxygen species (ROS) at the mitochondrial level is the initial trigger of vicious cycle of oxidative stress in DM [5,6]. There are multiple sources of oxidative stress in diabetes including non-enzymatic, enzymatic and mitochondrial pathways. ROS can activate formation of advance glycation end products [7], polyol pathway [8], hexosamine pathway and PKC [9], involved in the pathogenesis of micro- and macro-vascular complications in T2DM. The most important antioxidant enzyme, superoxide dismutase (SOD), has three isoforms, SOD1 (CuZn-SOD), SOD2 (Mn-SOD) and SOD3 (EC-SOD) where SOD3 expresses only extra-cellularly. SOD1 appears to be expressed at relatively higher levels in all cells, including blood vessels [10]. SOD1 or CuZn-SOD (EC 1.15.1.1) is a copper and zinc-containing homodimer that acts as a scavenger of superoxide through a two-step reaction involving reduction and re-oxidation of the copper ion in its active site where zinc plays a structural role of stabilizing the enzyme thermodynamically [11]. The dismutation of superoxide radical ($\bullet O_2^-$) by SOD was

characterized by McCord and Fridovich [12]. The human SOD1 gene (Entrez Gene ID 6647) is located on chromosome 21q22.11. The coding region of SOD1 gene consists of five exons interrupted by four introns. SOD1 has been found in the cytoplasm, nuclear compartments, and lysosomes of mammalian cells [13-16]. SOD1 gene which is highly polymorphic has ethnic specificity and represents about 50-80% of the total SOD activity [10,17] and is an excellent mechanism against oxidative stress. SOD1catalyze the superoxide radical ($\bullet O_2^-$) into hydrogen peroxide (H_2O_2), has been found to be associated with T2DM and advanced stages of nephropathy in some population [18,19]. In SOD1, the +35A/C polymorphism (rs2234694) is adjacent to the splicing point (exon3/intron3), being related to the SOD1-activity - AA-genotype having the higher SOD1-activity [18].

In the present study, we have investigated the distribution of SOD1 +35A>C (rs2234694) gene polymorphism in diabetic and DN subjects of Bangladeshi population.

MATERIALS AND METHODS

Subjects

A total number of 403 unrelated subjects (109 type 2 diabetes, 150 DN and 144 healthy controls without family history of diabetes) of Bangladeshi population were recruited in this study. Male-female distribution in the DM group was 64 and 45, DN group 100 and 50 respectively and in the control 71 and 73 respectively. DM and DN patients (age range 30-60 years) were consecutively recruited from the outpatient department, BIRDEM Hospital, a referral center for diabetes in Bangladesh and the central Institute of Diabetic Association of Bangladesh (DAB). Healthy control subjects were recruited through personal communication from the friend circle of the patients and were confirmed as non-diabetic through Oral Glucose Tolerance Test. The theme of the study was explained to the subjects and written consent was taken from all the volunteers. The study was approved by the Ethical Review Committee of DAB.

Methodology

Anthropometric measurements were taken using standard methods. Fasting and postprandial serum Glucose were measured using glucose-oxidase method. Genomic DNA was extracted from peripheral blood leucocytes obtained from 200 μl of EDTA anticoagulated blood samples using FavorPrep™ DNA Extraction Kit (FAVORGEN®, Taiwan). DNA yield for each sample was checked by agarose gel (1%) electrophoresis.

SOD1 Gene Polymorphic Marker Analyses

SOD1 gene polymorphic marker was analyzed by polymerase chain reaction (PCR) followed by restriction fragment length polymorphism method using the primers specific for SOD1 gene amplification [18] and the restriction enzyme Hha I shown in Table 1.

Table 1: Sequences of primers for PCR and recognition site of Hha I

Polymorphism	SOD1+35 A>C (refSNP ID: rs2234694)
Sequence of used primers (location)	Forward primer: 5′-CTATCCAGAAAACACGGTGGGCC-3′ (Exon 3) Reverse primer: 5′-TCTATATTCAATCAAATGCTACAAAAC-3′ (Intron 3)
Annealing temperature	55°C
Restriction endonuclease	Hha I
Recognition site	5′…G C G↓C…3′ 3′… C↑G C G…5′
Restriction fragments	C allele 71 bp and 207 bp A allele 278 bp

PCR: Polymerase chain reaction

PCR was carried out in 10 μl reaction volume. Product size for the above-mentioned primer set is 278 bp. Three μl of PCR product has been checked for amplification in 2% agarose gel. The optimum size of the product was ascertained comparing it with 100 bp DNA ladder. Restriction enzyme digestion was performed using standard digestion protocol. Genotypes of SOD1 were determined after digestion with Hha I restriction enzyme for the +35A>C (intron3/exon3) polymorphism [Figure 1].

Statistical Analyses

Statistical analyses were performed using Statistical Package for Social Science (SPSS Inc. USA) software for Windows version 17. Data were expressed as mean ± standard deviation, number (percentage) as appropriate. Difference between two groups was determined by unpaired Student's *t*-test and Chi-square test where applicable.

RESULTS

Anthropometric and Biochemical Characteristics of the Total Study Subjects

Age (years), body mass index (BMI) (kg/m^2), systolic blood pressure (SBP) (mm-hg) and diastolic BP (DBP) (mm-hg) were significantly higher in DN subjects compared to control subjects ($P = 0.001$) [Table 2]. Diabetic subjects did not show significant differences of these variables.

SOD 1 Gene +35 A>C Genotype of the Total Study Subjects

The Hardy-Weinberg equilibrium analysis among the control, DM and DN subjects has not shown any statistical significance [Table 3]. The genetic polymorphisms in SOD1 gene (+35 A>C) has been investigated, and the genotypes are shown in Figure 1. The SOD1 A>C genotype frequencies (wild AA and heterozygous variant [Ht] AC) in control were 0.972 and 0.028 for AA and AC; 0.963 and.037 for DM and 0.907 and 0.093 for DN group respectively. These genotype frequency distribution between the groups have shown statistical significant association

Table 2: Anthropometric and biochemical characteristics of the total study subjects

Variables	Control (n=144)	T2DM (n=109)	DN (n=150)	t/P values	
				Count versus T2DM	Count versus DN
Age (years)	42.0±10.5	44.5±9.15	56.2±10.1	−1.901/0.058	−11.707/0.001
BMI (kg/m²)	24.9±4.34	25.3±3.56	22.4±4.10	−0.609/0.543	5.255/0.001
BFM (%)	28.9±7.88	28.8±7.58	27.2±7.38	0.121/0.903	1.983/0.048
SBP (mmHg)	114±13	116±14	150±16	−1.135/0.257	−21.070/0.001
DBP (mmHg)	76±9	77±10	89±9	−1.127/0.261	−12.118/0.001

Results are expressed as mean±standard deviation, statistical comparison between groups was performed using unpaired Student's *t*-test, $P<0.05$ was considered statistically significant level. BMI: Body mass index, BFM: Body fat mass, SBP: Systolic blood pressure; DBP: Diastolic blood pressure, DN: Diabetic nephropathy, T2DM: Type 2 diabetes mellitus

Table 3: Hardy Weinberg equilibrium of the control, T2DM and DN subjects

Groups	Total	Obs AA, (n)	Obs AC, (n)	Obs CC, (n)	P	q	χ^2/P
Control	144	140	4	0	0.9861	0.0138	0.02856/0.86578
T2DM	109	105	4	0	0.9816	0.0183	0.03808/0.84528
DN	150	136	14	0	0.9533	0.0466	0.35943/0.54882

DN: Diabetic nephropathy, T2DM: Type 2 diabetes mellitus

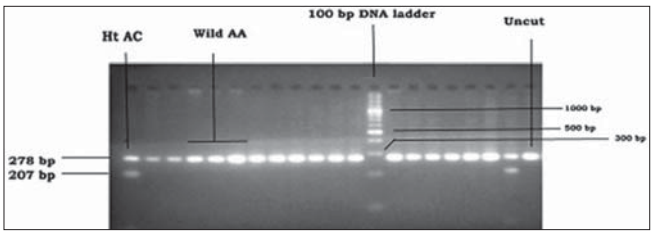

Figure 1: Superoxide dismutase 1 gene +35A>C candidate marker analysis by Hha I restriction enzyme digestion

(χ^2=5.493; P = 0.019). Also allele frequency distribution among the groups have shown statistical significant association (χ^2=2.358; P = 0.019) [Table 4].

Clinical Characteristics According to SOD 1 Gene A>C Genotype

Age, BMI, body fat mass and BP of the study subjects were analyzed according to SOD1 +35 A>C genotype (homozygous wild [AA] and Ht [AC]) and no differences have been found among DM, DN and control subjects [Table 5].

SOD 1 Gene A>C Genotype According to age, Gender and BP

Again the genotype frequencies were analyzed according to age group, gender and BP in control, DM and DN subjects. On the basis of age (years), the study population was divided into four groups as: <35 years, 35-45 years, 46-55 years and >55 years. The genotype frequency distribution among these groups did not shown any statistical significance association.

Frequency distribution of genotypes in male and females have showed significant association in DN subjects (χ^2 = 7.72, P = 0.005) but not in DM subjects. Control subjects have also shown significance association (χ^2 = 4.23, P = 0.040).

On the basis of SBP and DBP the study subjects of each group has been divided into two categories as normal and high. The genotype frequency distributions of SBP and DBP in the control group showed statistical significance but not in DM and DN groups [Table 6].

DISCUSSION

DN is a micro-vascular complication which results from long term uncontrolled blood glucose and it is the leading cause of end-stage renal disease (ESRD) throughout the world. Although this disease progressively imposes an increased burden on the health care system, its pathological basis still remains poorly understood. In addition to the environmental factors, genetic susceptibility has been postulated in its development [20]. About 10-21% of Type 2 DM (T2DM) patients are found to present with renal functional abnormalities at the time of diagnosis [21]. However renal function deteriorates progressively with the duration of diabetes in both Type 1 and Type 2 varieties. About 50% of T1DM patients are found to have ESRD (80% of overt nephropathy) after 10-15 years of diagnosis [21]. In case of T2DM 20% (from 20 to 40% of overt nephropathy) of patient developed ESRD after 20 years of onset of the disease [21]. Although uncontrolled diabetes attributed to the most likely risk factor, but it is known that a portion of T1DM and T2DM with more than 25 years are likely to develop DN irrespective of hyperglycemia [22]. This suggests a possible genetic susceptibility, if not anything else, contribute its pathogenesis. Possible likely genetic susceptibility largely implicated in the both grounds of familial clustering of nephropathy case within the families [23]. Genome-wide linkage scans identified several chromosomal regions likely to contain DN susceptibility genes, and association analyses have evaluated positional candidate genes under linkage peaks. One of the most promising candidate genes susceptibility to DN is SOD1 gene. The SODs are the most important line of antioxidant enzyme defense systems against ROS and particularly superoxide anion radicals [24]. SODs are metalloenzymes that catalyze superoxide radical $\bullet O_2^-$ into H_2O_2. SOD1 is a key enzyme in DN because its renal level is decreased in this disease [25]. Involvement of low levels of SOD1 in DN, the existence of some polymorphisms which diminish SOD1-activity and the evidence of genetic susceptibility for diabetic kidney disease [26-29], render the study of SOD1-gene functional mutations as risk factors for DN. As oxidative stress is a common pathogenic factor for the dysfunction of beta and endothelial cells, polymorphisms

Table 4: SOD1 gene+35 A>C genotype and allele frequency of the total study subjects

Variables	Control (n=144)	T2DM (n=109)	DN (n=150)	Count versus DM	Count Versus DN
Genotype					
Wild AA, % (n)	0.972 (140)	0.963 (105)	0.907 (136)	χ^2/P=0.161/0.688	χ^2/P=5.493/0.019
Ht AC, % (n)	0.028 (4)	0.037 (4)	0.093 (14)	OR/P=1.333/0.689	OR/P=3.603/0.027
				95% CI=0.326-5.455	95% CI=1.157-11.220
Allele frequency					
A	0.986	0.982	0.954	0.478/0.633	2.358/0.019
C	0.014	0.018	0.047		

Data are presented as frequency (number of subjects), Chi-squared (χ^2) test (Fisher's Exact) was performed to calculate statistical association, $P<0.05$ was considered statistically significant level, SOD 1: Superoxide dismutase 1; DN, Diabetic nephropathy, Ht, Heterozygous variant

Table 5: Anthropometric and Biochemical characteristics of the total study subjects according to SOD1 gene+35A/C genotype

Variables	Controls			T2DM			DN		
	Wild AA	Ht AC	P value	Wild AA	Ht AC	P value	Wild AA	Ht AC	P value
Age (years)	42±10	51±7	0.076	45±9	43±11	0.660	56±10	59±14	0.218
BMI (kg/m^2)	24.9±4.4	24.5±2.8	0.847	25.2±3.6	27.2±3.1	0.267	22.3±4.3	22.9±1.2	0.617
BFM (%)	29±8	25±4	0.318	29±8	32±7	0.451	27±8	25±6	0.235
SBP (mmHg)	114±13	122±19	0.195	116±14	115±6	0.884	150±16	152±17	0.640
DBP (mmHg)	76±9	84±12	0.075	77±11	75±6	0.643	89±9	87±6	0.405

Results are expressed as mean±standard deviation, statistical comparison between groups was performed using unpaired Student's t-test, $P<0.05$ was considered statistically significant level. BMI: Body mass index; BFM: Body fat mass; SBP, Systolic Blood Pressure; DBP: Diastolic blood pressure

Table 6: SOD1+35A>C genotype frequencies (number) of the study subjects on the basis of BP

Variables	Control			T2DM			DN		
	AA (n)	AC (n)	χ^2/P	AA (n)	AC (n)	χ^2/P	AA (n)	AC (n)	χ^2/P
Age group									
<35 years	1.0 (39)	0	6.863/0.076	0.947 (18)	0.053 (1)	0.932/0.818	1.0 (1)	0	1.266/0.737
35-45 years	0.982 (54)	0.018 (1)		1.0 (19)	0		0.909 (20)	0.091 (2)	
46-55 years	0.970 (32)	0.030 (1)		0.959 (47)	0.041 (2)		0940 (47)	0.060 (3)	
>55 years	0.875 (14)	0.125 (2)		0.955 (21)	0.045 (1)		0.883 (68)	0.117 (9)	
Gender									
Male	0.944 (67)	0.056 (4)	4.23/0.040	0.969 (62)	0.031 (2)	0.130/0.718	0.86 (86)	0.14 (14)	7.72/0.005
Female	1.0 (73)	0		0.956 (43)	0.044 (2)		1.0 (50)	0	
SBP									
Normal	0.979 (137)	0.021 (3)	7.52/0.006	0.956 (86)	0.044 (4)	0.375/0.543	0.923 (48)	0.077 (4)	0.253/0.615
High	0.75 (3)	0.25 (1)		1.0 (8)	0		0.898 (88)	0.102 (10)	
DBP									
Normal	0.979 (138)	0.021 (3)	10.59/0.001	0.951 (77)	0.049 (4)	0.875/0.350	0.899 (107)	0.101 (12)	0.39/0.566
High	0.667 (2)	0.333 (1)		1.0 (17)	0 (0)		0.933 (28)	0.067 (2)	

Data presented as frequency. Chi-square test was performed to calculate statistical association. A two-tailed $P<0.05$ was considered statistically significant. SOD1: Superoxide dismutase, AA, Wild type, AC, Heterozygous variant (Ht), Normal value of systolic blood pressure 120 mmHg and diastolic blood pressure 80 mmHg, DBP: Diastolic blood pressure, SBP: Systolic blood pressure, BP: Blood pressure

of SOD1 gene and oxidative stress become subject of intense scrutiny for their association with DN.

BMI of DN subjects in the present study was significantly lower compared to control subjects (P = 0.001). This may be explained that long time uncontrolled diabetes develops DN and at the same time decreases body weight. DN has a strong correlation with BP [30,31]. Consistent with this, our study have also documented that both SBP and DBP of DN subjects were significantly higher compared to control subjects (P = 0.001 and P = 0.001 respectively). A previous study has been reported hemodynamic factors that contribute to the development of DN includes increased systemic and intra-glomerular pressure, as well as activation of vasoactive hormone pathways including the renin-angiotensin system and endothelin [32].

Test for Hardy-Weinberg equilibrium of the study subjects did not show any significant deviation which justifies the lack of selection bias of this study subjects. Genotype frequencies of SOD1 +35A>C variant were 0.972 and 0.028 for homozygous wild type (AA) and heterozygous (Ht) variant (AC) respectively in the control group. In the T2DM group the frequencies were 0.963 and 0.037, and in the DN group, 0.907 and 0.093 respectively. The genotype frequency distribution between the groups showed statistical significant association (χ^2=5.493; P = 0.019; odds ratio = 3.60, P = 0.027). The allele frequency distribution between the groups also show statistical significant association (χ^2=2.358; P = 0.019) which may indicates that the mutations in +35SOD1 gene may have links in the development of nephropathy in diabetic population of Bangladesh. A study done in Romania on DN

population [33] have supported our study where it has been reported that +35A>C (intron3/exon3) polymorphism in SOD1-gene confers a significant risk ($P = 0.008$) for diabetes nephropathy.

The possible mechanism for this polymorphism over the action of SOD1-activity may include the production of large quantities of NO which lead to SOD1-activation in mesangial cells in order to compensate an endothelial dysfunction [34]. This fact is also confirmed by the increase of SOD1-levels in patients with ESRD where the endothelial dysfunction is marked [35]. In addition, the circulating levels of SOD1 in adolescents with Type 1 diabetes seems to be protective against endothelial dysfunction, the low SOD1-levels being a susceptibility marker for diabetic vascular complications [36]. Tubular cell and podocyte apoptosis is an early event in DN, and the simultaneous release of SOD1 and cytochrome C regulates the mitochondrial apoptosis [37]. Hypertension and the renin–angiotensin system are key factors in DN, closely related to SOD1-levels [38]. The fibrosis mediated by transforming growth factor-beta (TGF-β) is the corner-stone of glomerulosclerosis in DN, and SOD1 is a strong antifibrotic agent, lowering the TGF-β1-expression [39]. The insulin resistance is increased in patients with nephropathy [40,41] in inversely proportion to glomerular filtration rate [42] and seems to be the most important predictor for the development of DN [43-45]. It is hard to guess the mechanism through which the decreased SOD1-expression and activity leads to the development of nephropathy, but our results suggest that this polymorphism (+35 A/C) with functional role in antioxidant defense is associated with DN in Bangladeshi Type 2 diabetic subjects.

Clinical studies have shown a decrease in SOD activity in aged, African-Americans with hypertension and in T2DM subjects compared to control subjects [46,47] but we failed to found any association of SOD1 polymorphism with age of the studied subjects. It has been found that increased systolic BP in diabetic subjects may have associations with increased polymorphism in SOD1 gene [10]. However, this study has not found any association of this polymorphism with SBP or DBP i9n DM or DN subjects. Although SBP and DBP in control subjects have shown an association with this polymorphism, it may be incidental (1 out of 4).

DN has been reported to more common in males (22.55%) when compared to females (6.25%) [48]. Consistent with this, the frequency distribution of +35A>C polymorphism in SOD1 gene between the male and female groups in the DN subjects (14% of male) have shown statistical significant association (χ^2=7.72 and $P = 0.005$). In control subjects, 5.6% have also shown this polymorphism.

From the viewpoint of above discussion it may be concluded: (i) +35A>C polymorphism in SOD1-gene may involve in the development of nephropathy in Bangladeshi Type 2 diabetic subjects and (ii) Male subjects of Bangladeshi DN subjects are preponderance for +35A>C polymorphism in SOD1-gene.

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REFERENCES

1. Vithian K, Hurel S. Microvascular complications: Pathophysiology and management. Clin Med 2010;10:505-9.
2. Araki E, Nishikawa T. Oxidative stress: A cause and therapeutic target of diabetic complications. J Diabetes Investig 2010;1:90-6.
3. Fridovich I. Fundamental aspects of reactive oxygen species, or what's the matter with oxygen? Ann N Y Acad Sci 1999;893:13-8.
4. Fang YZ, Yang S, Wu G. Free radicals, antioxidants, and nutrition. Nutrition 2002;18:872-9.
5. Nishikawa T, Edelstein D, Du XL, Yamagishi S, Matsumura T, Kaneda Y, *et al.* Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. Nature 2000;404:787-90.
6. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature 2001;414:813-20.
7. Tan AL, Forbes JM, Cooper ME. AGE, RAGE, and ROS in diabetic nephropathy. Semin Nephrol 2007;27:130-43.
8. Hammes HP. Pathophysiological mechanisms of diabetic angiopathy. J Diabetes Complications 2003;17:16-9.
9. Nishikawa T, Araki E. Impact of mitochondrial ROS production in the pathogenesis of diabetes mellitus and its complications. Antioxid Redox Signal 2007;9:343-53.
10. Faraci FM, Didion SP. Vascular protection: Superoxide dismutase isoforms in the vessel wall. Arterioscler Thromb Vasc Biol 2004;24:1367-73.
11. Fridovich I. Superoxide dismutases. Annu Rev Biochem 1975;44:147-59.
12. McCord JM, Fridovich I. Superoxide dismutase: The first twenty years (1968-1988). Free Radic Biol Med 1988;5:363-9.
13. Chang LY, Slot JW, Geuze HJ, Crapo JD. Molecular immunocytochemistry of the CuZn superoxide dismutase in rat hepatocytes. J Cell Biol 1988;107:2169-79.
14. Keller GA, Warner TG, Steimer KS, Hallewell RA. Cu,Zn superoxide dismutase is a peroxisomal enzyme in human fibroblasts and hepatoma cells. Proc Natl Acad Sci U S A 1991;88:7381-5.
15. Crapo JD, Oury T, Rabouille C, Slot JW, Chang LY. Copper,zinc superoxide dismutase is primarily a cytosolic protein in human cells. Proc Natl Acad Sci U S A 1992;89:10405-9.
16. Liou W, Chang LY, Geuze HJ, Strous GJ, Crapo JD, Slot JW. Distribution of CuZn superoxide dismutase in rat liver. Free Radic Biol Med 1993;14:201-7.
17. Fukai T, Galis ZS, Meng XP, Parthasarathy S, Harrison DG. Vascular expression of extracellular superoxide dismutase in atherosclerosis. J Clin Invest 1998;101:2101-11.
18. Flekac M, Skrha J, Hilgertova J, Lacinova Z, Jarolimkova M. Gene polymorphisms of superoxide dismutases and catalase in diabetes mellitus. BMC Med Genet 2008;9:30.
19. Ghattas MH, Abo-Elmatty DM. Association of polymorphic markers of the catalase and superoxide dismutase genes with type 2 diabetes mellitus. DNA Cell Biol 2012;31:1598-603.
20. Arya A, Aggarwal S, Yadav HN. Pathogenesis of diabetic nephropathy. Int J Pharm Sci 2010;2:249.
21. American Diabetes Association (ADA). Position statement: Diabetic nephropathy. Diabetes Care 1999;22:66-9.
22. Nordwall M, Bojestig M, Arnqvist HJ, Ludvigsson J, Linköping Diabetes Complications Study. Declining incidence of severe retinopathy and persisting decrease of nephropathy in an unselected population of Type 1 diabetes-the Linköping diabetes complications study. Diabetologia 2004;47:1266-72.
23. Freedman BI, Bostrom M, Daeihagh P, Bowden DW. Genetic factors in diabetic nephropathy. Clin J Am Soc Nephrol 2007;2:1306-16.
24. Zelko IN, Mariani TJ, Folz RJ. Superoxide dismutase multigene family: A comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2), and EC-SOD (SOD3) gene structures, evolution, and expression. Free Radic Biol Med 2002;33:337-49.
25. Fujita H, Fujishima H, Chida S, Takahashi K, Qi Z, Kanetsuna Y, *et al.*

Reduction of renal superoxide dismutase in progressive diabetic nephropathy. J Am Soc Nephrol 2009;20:1303-13.
26. Borch-Johnsen K, Nørgaard K, Hommel E, Mathiesen ER, Jensen JS, Deckert T, *et al.* Is diabetic nephropathy an inherited complication? Kidney Int 1992;41:719-22.
27. Karter AJ, Ferrara A, Liu JY, Moffet HH, Ackerson LM, Selby JV. Ethnic disparities in diabetic complications in an insured population. JAMA 2002;287:2519-27.
28. Seaquist ER, Goetz FC, Rich S, Barbosa J. Familial clustering of diabetic kidney disease. Evidence for genetic susceptibility to diabetic nephropathy. N Engl J Med 1989;320:1161-5.
29. Quinn M, Angelico MC, Warram JH, Krolewski AS. Familial factors determine the development of diabetic nephropathy in patients with IDDM. Diabetologia 1996;39:940-5.
30. Tzeng TF, Hsiao PJ, Hsieh MC, Shin SJ. Association of nephropathy and retinopathy, blood pressure, age in newly diagnosed type 2 diabetes mellitus. Kaohsiung J Med Sci 2001;17:294-301.
31. Hypertension in Diabetes Study (HDS): I. Prevalence of hypertension in newly presenting type 2 diabetic patients and the association with risk factors for cardiovascular and diabetic complications. J Hypertens 1993;11:309-17.
32. Hargrove GM, Dufresne J, Whiteside C, Muruve DA, Wong NC. Diabetes mellitus increases endothelin-1 gene transcription in rat kidney. Kidney Int 2000;58:1534-45.
33. Panduru NM, Cimponeriu D, Cruce M, Ion DA, Mota E, Mota M, *et al.* Association of +35A/C (intron3/exon3) polymorphism in SOD1-gene with diabetic nephropathy in type 1 diabetes. Rom J Morphol Embryol 2010;51:37-41.
34. Frank S, Zacharowski K, Wray GM, Thiemermann C, Pfeilschifter J. Identification of copper/zinc superoxide dismutase as a novel nitric oxide-regulated gene in rat glomerular mesangial cells and kidneys of endotoxemic rats. FASEB J 1999;13:869-82.
35. Pawlak K, Pawlak D, Mysliwiec M. Cu/Zn superoxide dismutase plasma levels as a new useful clinical biomarker of oxidative stress in patients with end-stage renal disease. Clin Biochem 2005;38:700-5.
36. Suys B, de Beeck LO, Rooman R, Kransfeld S, Heuten H, Goovaerts I, *et al.* Impact of oxidative stress on the endothelial dysfunction of children and adolescents with type 1 diabetes mellitus: Protection by superoxide dismutase? Pediatr Res 2007;62:456-61.
37. Li Q, Sato EF, Zhu X, Inoue M. A simultaneous release of SOD1 with cytochrome c regulates mitochondria-dependent apoptosis. Mol Cell Biochem 2009;322:151-9.
38. Tang Z, Shou I, Wang LN, Fukui M, Tomino Y. Effects of antihypertensive drugs or glycemic control on antioxidant enzyme activities in spontaneously hypertensive rats with diabetes. Nephron 1997;76:323-30.
39. Vozenin-Brotons MC, Sivan V, Gault N, Renard C, Geffrotin C, Delanian S, *et al.* Antifibrotic action of Cu/Zn SOD is mediated by TGF-beta1 repression and phenotypic reversion of myofibroblasts. Free Radic Biol Med 2001;30:30-42.
40. Yip J, Mattock MB, Morocutti A, Sethi M, Trevisan R, Viberti G. Insulin resistance in insulin-dependent diabetic patients with microalbuminuria. Lancet 1993;342:883-7.
41. Trevisan R, Bruttomesso D, Vedovato M, Brocco S, Pianta A, Mazzon C, *et al.* Enhanced responsiveness of blood pressure to sodium intake and to angiotensin II is associated with insulin resistance in IDDM patients with microalbuminuria. Diabetes 1998;47:1347-53.
42. Thorn LM, Forsblom C, Fagerudd J, Thomas MC, Pettersson-Fernholm K, Saraheimo M, *et al*. Metabolic syndrome in type 1 diabetes: Association with diabetic nephropathy and glycemic control (the FinnDiane study). Diabetes Care 2005;28:2019-24.
43. Kilpatrick ES, Rigby AS, Atkin SL. Insulin resistance, the metabolic syndrome, and complication risk in type 1 diabetes: "double diabetes" in the Diabetes Control and Complications Trial. Diabetes Care 2007;30:707-12.
44. Orchard TJ, Chang YF, Ferrell RE, Petro N, Ellis DE. Nephropathy in type 1 diabetes: A manifestation of insulin resistance and multiple genetic susceptibilities: Further evidence from the Pittsburgh epidemiology of diabetes complication study. Kidney Int 2002;62:963-70.
45. Pambianco G, Costacou T, Orchard TJ. The prediction of major outcomes of type 1 diabetes: A 12-year prospective evaluation of three separate definitions of the metabolic syndrome and their components and estimated glucose disposal rate: The Pittsburgh epidemiology of diabetes complications study experience. Diabetes Care 2007;30:1248-54.
46. Yamashita K, Takahiro K, Kamezaki F, Adachi T, Tasaki H. Decreased plasma extracellular superoxide dismutase level in patients with vasospastic angina. Atherosclerosis 2007;191:147-52.
47. Liao M, Liu Z, Bao J, Zhao Z, Hu J, Feng X, *et al.* A proteomic study of the aortic media in human thoracic aortic dissection: Implication for oxidative stress. J Thorac Cardiovasc Surg 2008;136:65-72.
48. Agarwal N, Sengar NS, Jain PK, Khare R. Nephropathy in newly diagnosed type 2 diabetics with special stress on the role of hypertension. J Assoc Physicians India 2011;59:145-7.

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