Association between Superoxide Dismutase 2 p.(Ala16Val) and Superoxide Dismutase 3 p.(Arg213Gly) Genetic Variants and Risk of Peripheral Neuropathy in Children and Adolescents with Type 1 Diabetes

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Abstract

Diabetic neuropathy (DN) is one of the microvascular complications of diabetes. Marked increase in oxygen free radicals (OFR) results in oxidative stress that leads to development of DN. Antioxidant enzymes play a major role in protection against progression of DN, by reducing OFR. This study aimed to investigate the association between superoxide dismutase 2 *SOD2*:p.(Ala16Val) and superoxide dismutase 3 *SOD3*:p.(Arg213Gly) genetic variants and the risk of neuropathy in Type 1 Diabetes children and adolescent. The study included 80 children with type 1 diabetes divided into 2 groups, group 1 of 40 patients with clinical DN and group 2 of 40 patients without DN. HbA1c levels were measured and genetic variants of *SOD2*:p.(Ala16Val) and *SOD3*:p.(Arg213Gly) were assessed by Taqman Real time Polymerase Chain Reaction (PCR) for both groups. The frequency of Ala/Ala genotype (OR=0.28 with 95% CI of 0.11-0.71) and Ala allele (OR=0.33 with 95% CI of 0.17-0.65) of *SOD2*:p.(Ala16Val) were significantly lower in group 1 (27.5%, 50% respectively) than group 2 (57.5%, 75% respectively) (p=0.007, p=0.001 respectively). In contrast the frequency of Val/Val genotype (OR=4.68 with 95% CI of 1.19-18.3) and Val allele (OR=3 with 95% CI of 1.54-5.86) were significantly higher in group 1 (27.5%, 50% respectively) than group 2 (7.5%, 25% respectively) (p=0.019 and p=0.001 respectively). Regarding *SOD3*:p.(Arg213Gly) gene variants the frequency of Arg/Arg genotype and Arg allele were higher in group 1 (100%,100% respectively) than group 2 (90%,95% respectively) but with statistical insignificance (P=0.06, P=0.058 respectively), however the frequency of Arg/Gly genotype and Gly allele were higher in group 2 (10%, 5% respectively) than group 1 (0%, 0% respectively) but also with no statistical significance (P=0.058 and P=0.06 respectively). There is a possible association between *SOD2*:p.(Ala16Val), but not with *SOD3*:p.(Arg213Gly) genetic variants and the occurrence of DN in patients with type 1 diabetes mellitus.

Keywords: *Type 1 diabetes- neuropathy- SOD2 gene- SOD3 gene- Real Time PCR.*

Introduction

Diabetes Mellitus (DM) is a collection of metabolic abnormalities characterized by hyperglycemia which results from defects in insulin secretion, insulin action or both.[1] Diabetes Mellitus Type 1 is caused by destruction of the insulin-producing beta cells in the pancreas[2] and can lead to microvascular complications as DN, affecting somatic and/or autonomic nervous system.[3] About 20% of patients with diabetes will develop clinically significant neuropathy within 10 years of diabetes onset[4] the molecular mechanisms involved in development of diabetic peripheral neuropathy (DPN) is complex process that includes over activation of polyol pathway and protein kinase C in presence of

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hyperglycemia, increase of free radical and oxidative stress are incriminated in the pathogenesis of DPN[5], also genetic factors such as single nucleotide polymorphism (SNP) in the superoxide dismutase and catalase gene may be a risk factor for DN.[6] Superoxide dismutases are the primary antioxidant defense system, catalyzing dismutation of superoxids into O2 and H2O2. In mammals there are cytosolic (SOD1), mitochondrial (SOD2) and extracellular (SOD3) isoforms that are all products of distinct genes.[7,8] Mitochondrial SOD2 gene is located on the chromosome 6q25.3, it contains five exons, coding for 223 amino acids which undergo post translational modifications via the mitochondrial translocases to facilitate the import process in the mitochondria.[9] The rs 4880 of *SOD2* p. (Ala-9Val) or p.(Ala16Val) is the most studied variant in exon 2 of *SOD2* gene[10] the replacement of alanine to valine affects the α-helix configuration in the mitochondrial targeting sequence (MTS) affecting the influx to mitochondria causing enzyme degradation and decrease enzyme activity.[11] Extracellular superoxide dismutase 3 gene lies on chromosome 4p15.2, it contains 3 exons. The entire 720bp coding region lies within exon 3.[12] The rs 1799895 (Arg213Gly) is present in exon3 of *SOD3* gene at codon 213.SOD3 with Gly-213 inhibits ionic interactions between heparin and SOD3 enzyme so it binds less tightly to the plasma membrane, and its serum concentration is about nine times or more that of the isoezyme with Arg-213.[13] This study aimed to investigate the association between *SOD2*:p.(Ala16Val) and *SOD3*:p.(Arg213Gly) genetic variants and the risk of peripheral neuropathy in Type 1 Diabetes children and adolescents with more advanced method than the one used by ElMasry et al. (2005), which was Real time PCR using Taqman probes. Moreover the present study targeted a younger age group with neuropathy with short duration of diabetes compared to that done by El Masry et al. (2005).[14]

Method

This case control study was conducted on 80 patients with T1DM, diagnosed according to American Diabetes Association (ADA) criteria 2018[1]that were recruited from outpatient clinic of the Diabetes, Endocrine and Metabolism Pediatric Unit (DEMPU), at Children Hospital, Cairo University in the period from January 2018 to December 2018.

We divided 80 patients into two groups, group 1 consisted of 40 Type 1 diabetes patients with clinical DN, diagnosed on basis of symptomatic symmetrical distal neuropathy (reduced or absent ankle reflexes, vibration sense at the medial malleolus and/or reduced sense of position with one or more typical symptoms, such as burning sensation, cramps, parasthesia or numbness) using scores for neuropathy symptoms and neuropathy examination regardless of age, or degree of glycemic control, with diabetes duration of 5 years or less.

Group 2 included 40 patients (age and sex matched to group 1 with diabetes duration of more than 5 years regardless of degree of glycemic control without any symptom of neuropathy as a control group. Patients with type 2 diabetes or those with any other cause of neuropathy were excluded from the study. All participants in this study were informed and consents were taken from their parents. All subjects were subjected to thorough history taking including: age of onset of symptoms and duration of diabetes. Full clinical assessment was done.

Four milliliters of blood were collected from each participant and divided into 2 tubes: one EDTA tube for measurement of Glycated Hemoglobin (HbA1c) and another EDTA tube for DNA isolation and PCR procedure. Glycated Hemoglobin (HbA1c) Assay: Withdrawn samples were assayed immediately after collection on Dimension clinical chemistry system using FDA approved kits supplied by Siemens healthneers*®.

*Dimension (Siemens): Siemens Healthcare Diagnostics Inc. 511 Benedict Ave/Tarrytown, NY 10591 Phone: +1 914 631-8000).

The HbA1c measurement was based on a turbidimetric inhibition immunoassay (TINIA) principle which is NGSP certified as traceable on the DCCT.[15] Determination of *SOD2*:p.(Ala16Val) (rs4880) and *SOD3*:p.(Arg213Gly) (rs1799895) gene variants by Taqman Real Time Polymerase Chain Reaction included two steps: Extraction of genomic DNA from peripheral blood leucocytes of EDTA anticoagulated blood using QIAamp DNA blood Mini kit (Qiagen) by spin columns,[16] then, amplification of extracted DNA and analysis of gene variants by real time PCR technique using TaqMan single nucleotide polymorphism (SNP) genotyping assay (Applied Biosystems) performed on Step One™ Real Time PCR System (Applied Biosystems). The reaction volume was 20µl/well, and the thermal cycler was adjusted for initial step for activation of AmpliTaq Gold DNA polymerase enzyme, by adjusting temperature at 95°C for 10 minutes followed by 40 PCR cycles; each cycle of PCR consisted of 3 steps: denaturation step at 92°C for 15 seconds, annealing step reaction mixture was cooled to 60°C for 30 seconds, extension reaction,

done at 60°C for 30 seconds. After PCR amplification, an endpoint plate read was performed using Step One™ Real Time PCR System (Applied Biosystems). The Sequence Detection System (SDS). Software used the fluorescence measurements made during the plate read to plot fluorescence (Rn) values based on the signals from each well. The plotted fluorescence signals indicated which alleles were in each sample and alleles were converted to genotypes.

Data obtained from the study was coded and entered using the software SPSS (Statistical package for social science) version 17(Chicago,IL,USA). Parametric data was summarized using mean and standard deviation. Frequency and percentages were used for qualitative variables. Comparison between groups was done using Chi square and Fischer exact test for qualitative variable. Student's t test was used to compare two groups. The odds ratio (OR) and their 95% confidence intervals (CI) were calculated to estimate the strength of the association between each of genotypes and alleles and patients and controls. P-value is considered significant if <0.05.[17]

Results

The baseline clinical, demographic features and genetic variant analysis of SOD2 and SOD3 genes of both studied groups are showed in (Table 1).

There was no statistically significant difference as regards the age of participants, or their HbA1c levels (P=0.12, and P=0.92 respectively), but there was a statistically significant difference regarding diabetes duration among the studied groups (P=<0.0001).

The frequency of Ala/Ala genotype (OR=0.28 with 95% CI of 0.11-0.71) and Ala allele (OR=0.33 with 95% CI of 0.17-0.65) of *SOD2*:p.(Ala16Val) were significantly lower in group 1 than group 2 (P=0.007 and P=0.001 respectively). In contrast the frequency of Val/Val genotype (OR=4.68 with 95% CI of 1.19-18.3) and Val allele (OR=3 with 95% CI of 1.54-5.86) were significantly higher in group 1 than group 2 (P=0.019 and P=0.001 respectively).

Regarding *SOD3*:p.(Arg213Gly) genetic variants the frequency of Arg/Arg genotype and Arg allele were higher in group 1 than group 2 but with statistical insignificance (P=0.058 and P=0.06 respectively). However the frequency of Arg/Gly genotype and Gly allele were higher in group 2 than group 1, but also with no statistical significance (P=0.058 and P=0.06 respectively (Table 1).

Table 1: Baseline, demographic, biochemical data and univariate analysis of factors with SOD

Variables	Group 1 (n= 40)	Group 2 (n= 40)	P- value
Age (Years) mean ± SD	13.45(±2.64)	12.67(±1.75)	0.12
Sex (male/female)	27/13	22/18	0.25
Diabetes duration (Years) mean ± SD	3.75 (±1.07)	7.75 (±1.03)	<0.0001
HbA1c (%) mean ± SD	8.86 (±0.97)	8.84 (±0.91)	0.92
SOD2Ala/Ala (%)	11 (27.5)	23 (57.5)	0.007
SOD2Ala/Val (%)	18 (45)	14 (35)	0.363
SOD2 Val/Val (%)	11 (27.5)	3(7.5)	0.019
SOD2Ala allele (%) n= 80	40 (50)	60 (75)	0.001
SOD2Val allele (%) n= 80	40 (50)	20 (25)	0.001
SOD3Arg/Arg (%)	40 (100)	36 (90)	0.058
SOD3Arg/Gly (%)	0 (0)	4 (10)	0.058
SOD3Arg allele (%) n= 80	80 (100)	76 (95)	0.06
SOD3Gly allele (%) n= 80	0 (0)	4 (5)	0.06

P-value is considered significant if <0.05

Discussion

The current study threw the light on different genotypes of Superoxide dismutase 2 and 3 in type 1 diabetes children and aimed at finding an association between the different genotypes and the risk of diabetic neuropathy.

Genotypic analysis of *SOD2*:p.(Ala16Val) gene variant revealed that the frequency of homozygous Val/ Val genotype and Val allele of *SOD2* was significantly increased in DN patients (27.5%, 50%) than in those without neuropathy (7.5%,25%) (P=0.019).

Val/Val genotype was found to be a significant risk factor for developing peripheral neuropathy in a diabetes patient, (OR=4.68 with 95% CI of (1.9-18.3), P= 0.019). So this genetic variation tend to be the main risk factor for diabetic neuropathy regardless the glycemic control which was comparable between our two groups and regardless the duration of diabetes which was intended to be more than 5 years in patients without neuropathy and 5 years or less in patients with neuropathy to identify the impact of genetic variation on the development of diabetic neuropathy.

It was also found that the frequency of Ala/Ala genotype was significantly lower in patients with DN (27.5%) compared to those without neuropathy (57.5%) (OR=0.28 with 95%CI of (1.9-18.3), P = 0.007). Hence this genotype was associated with lower risk of DN.

In agreement with these results, Strokov et al. (2003)[6] and Zotova et al. (2003)[18]showed that *SOD2*:p. (Ala16Val) gene variant in a Russian population was associated with a high risk of the development of neuropathy in type 1 diabetic patients they stated that Val/Val genotype was more common in diabetic neuropathy patients (18.6%,20.4% respectively) than in diabetic patients without neuropathy (5.4%,5.6 respectively) (P=0.009, P=0.02 respectively).Regarding Ala/Ala genotypes, it was found that, it was significantly more common in diabetic patients without neuropathy (P=0.03,P=0.03 respectively).

These observations are also consistent with the findings of El Masry et al. (2005),[14] who demonstrated that Val/Val genotype of *SOD2* (rs 4880) was a significant risk factor in diabetes patients with neuropathy but not nephropathy and the Ala/Ala genotype was more common in diabetes patients without neuropathy or other microangiopathic complications than those having neuropathy.

On the contrary, in 2015 a study on the Polish population which was done by, Wegner et al. (2015)[19]found that, Val/Val genotype of *SOD2* (rs4880) did not increase the risk of T1DM or chronic diabetes complications in Polish T1DM patients (P= 0.761, OR= 0.407). This discrepancy may relate to different genetic background in sample selection.

Regarding rs1799895 of *SOD3*:p.(Arg213Gly) gene variants, our current study revealed that the frequency of Arg/Arg genotype and Arg allele were higher in group 1 (100%,100%respectively) than group 2 (90%,95% respectively) but with statistical insignificance (P=0.058, P=0.06 respectively), however the frequency of Arg/Gly genotype and Gly allele were higher in group 2(10%,5% respectively) than group 1(0%,0%respectively) but also with no statistical significance (P=0.058 and P=0.06 respectively). In harmony with our results, Zhai and his coworkers on 2017[20] declared that no significant association was found between *SOD3*:p.(Arg213Gly) genetic variants and development of type 2 diabetes mellitus and its complications in Chinese population (P=0.66).On other hand, Zotova et al. (2003)[18]reported that the *SOD3*:p.(Arg213Gly) genetic variant was significantly associated with the development of diabetic polyneuropathy in T1DM patients in a Russian population. This was attributed to significantly higher frequency of homozygous wild Arg/Arg genotype in diabetic neuropathy patients (27.9%) than the diabetic patients without neuropathy (9.7%) (P=0.002). It is recommended that the results obtained by this study would be confirmed on larger studies on wider scale including other microvascular complications as nephropathy and retinopathy and its relation to glycemic variability.

Conclusion

This study suggested the presence of a possible association between the *SOD2*:p.(Ala16Val) (rs4880), but not *SOD3*:p.(Arg213Gly) (rs1799895) gene variants and the susceptibility of diabetic polyneuropathy in children and adolescents with type 1 diabetes mellitus. This susceptibility is independent of glycemic control or diabetes duration.

Conflict of Interest: None

Ethical Clearance: Ethical approval was granted by Clinical and Chemical Pathology Department, Faculty of Medicine, Cairo University, Egypt.

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