

Association of Pro198Leu polymorphism in glutathione peroxidase one (GPX1) gene with peripheral neuropathy in type 2 diabetic patients

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Abstract

Objectives: To evaluate the association of Pro198Leu polymorphism in glutathione peroxidase 1 gene in type 2 diabetic patients with neuropathy.

Methods: The comparative cross-sectional study was conducted from February 2 to November 30, 2018, at the Department of Biochemistry and Molecular Biology, Army Medical College, Rawalpindi, Pakistan, in collaboration with the Department of Neurology, Military Hospital, Rawalpindi. Diagnosed type 2 diabetics of either genders aged 40-70 years were divided into two equal groups of neuropathy and non- neuropathy subjects. Deoxyribonucleic acid was subjected to restriction fragment length polymorphism for glutathione peroxidase 1 gene analysis. Hardy Weinberg equation was used to check the genotype frequency equilibrium.

Results: Of the 60 patients, there were 30(50%) each in the two groups. Age, fasting glucose level and diabetes duration were significantly different between the groups ($p < 0.05$). Even though the frequency of TT genotype was higher, no association of the polymorphism and any of the genotypes was found with diabetic neuropathy ($p > 0.05$).

Conclusion: There was no association found between Pro198 Leu polymorphism in glutathione peroxidase 1 and diabetic neuropathy.

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Introduction

Type 2 Diabetes mellitus (T2DM) is a chronic multifactorial, metabolic syndrome characterised by hyperglycaemia with insulin resistance (IR). It is one the most prevalent metabolic disorder associated with high mortality and morbidity.¹ According to the World Health Organisation (WHO), the world diabetic population has increased four times since 1980, reaching 422 million.² Approximately 1.6 million deaths are caused directly by T2DM and its complications.³ According to the latest survey in 2018, the prevalence of T2DM in Pakistani population is 26.3%.⁴

Diabetic peripheral neuropathy (DPN) is the most common morbid diabetic complication, affecting about half of the patients and it is often left undiagnosed,⁵ characterized by pain, loss of sensation and paresthesia. It involves both autonomic and peripheral nerves, resulting in almost 80% non-traumatic leg amputations worldwide.⁶ Prolonged oxidative ravages can intercede to neuron, Schwann cell and peripheral glial cell apoptosis, causing sensory and motor loss.⁷ The prevalence of DPN is documented from 14% to 63%; the variation being

attributable to the criteria used and population from different ethnic origins.⁸ Approximately 30% of the diabetic patients in Pakistan suffer from DPN.⁹

The raised oxidative stress in T2DM is partnered by the constitution of advanced glycation end products (AGEs), provoking dyslipidaemia, inflammation as well as vascular and thrombotic complications.¹⁰ Potential molecular driving forces for the augmentation of oxidative stress (OS) are reactive metabolites, like reactive oxygen species (ROS), free radicals peroxides, lipid peroxides and heavy metals.¹¹

In order to combat this phenomenon, nature has provided the human body with certain defence mechanisms which involve the antioxidant enzymes, glutathione peroxidase (GPX), catalase (CAT), superoxide dismutase (SOD), nitric oxide synthase (NOS) and glutathione S transferase (GST).¹² GPX1 enzyme is a soluble selenoprotein and the master detoxifier as it reduces free radicals, thereby making them less harmful to body tissues.¹³ GPX1 is the most common isoform in human cells. GPX1 Pro198Leu polymorphism (rs1050450) causes amino acid to change from proline to leucine at codon 198, affecting enzyme activity.¹⁴

This enzyme is encoded by the GPX1 gene which is located at chromosome 3p21. It contains two exons where two alternatively spliced transcript variants encode

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two isoforms for this gene. There are three genotypes and two alleles, C and T, and genotypes CC, CT and TT.¹⁵ Expression of GPX is regulated by transcription factor (TF), nuclear factor erythroid2 (Nrf2) and antioxidant responsive element (ARE).¹⁵

The area of genetics in DPN is far less explored compared to other vascular complications of diabetes, such as retinopathy and nephropathy. Up till now about 80 T2DM-susceptible loci in different ethnicities have been documented around the world.⁵

The current study was planned to explore the role of single-nucleotide polymorphism (SNP) in GPX1 gene and its association with DPN among local patients.

Patients and Methods

The comparative cross-sectional study was conducted from February 2 to November 30, 2018, at the Department of Biochemistry and Molecular Biology, Army Medical College (AMC), Rawalpindi, Pakistan, in collaboration with the Department of Neurology, Military Hospital (MH), Rawalpindi. Approval from Ethical Review Committee (ERC) of AMC was taken prior to the commencement of study. The sample size was calculated using WHO calculator by taking 30% population proportion,⁹ 95% confidence interval (CI) and 11% absolute precision. Patients were selected using non-probability purposive sampling. Those included were T2DM patients of either gender aged 40-70 years diagnosed by a neuro-physician on the basis of clinical examination, nerve conduction study (NCS), and electromyography (EMG). Non-diabetic neuropathic patients were excluded. After getting informed consent from the subjects, they were divided into two equal groups, with Group I having T2DM patients without neuropathy, and Group II having T2DM patients with neuropathy.

Demographic and clinical details were recorded on a structured proforma. Fasting blood samples of 5ml were drawn and stored at 40C, which were later analysed for fasting blood glucose (FBG), glycosylated haemoglobin (HbA1C) and genotypes.

Deoxyribonucleic acid (DNA) was extracted from the whole blood by using Thermo scientific DNA purification kit. Extracted DNA samples were analysed for quality and quantity via 1.5% agarose gel electrophoresis (AGE) and stored at -200C. The following primers against the GPX1

gene fragment were designed using National Center for Biotechnology Information (NCBI) primer blast.¹⁶

Forward primer: 5'_TTGACATCGAGCCTGACATC_3'

Reverse primer: 5'_CAGGTGTTCTCCCTCGTAG__3'

A 161 base pair (bp) fragment of GPX1 gene containing the polymorphic segment was amplified by conventional polymerase chain reaction (PCR). The amplification was confirmed by 1.5% AGE. Genotyping was performed using the analysis of fragment length polymorphism (RFLP) among restriction fragments of amplified products, with hereditary angioedema-III (HEA III) restriction digestion enzyme (Thermo scientific). The polymorphism was visualised by separating the digested fragments via 2.5% AGE on the basis of their size. The respective genotypes were identified according to the digested fragment length (Figure-1).

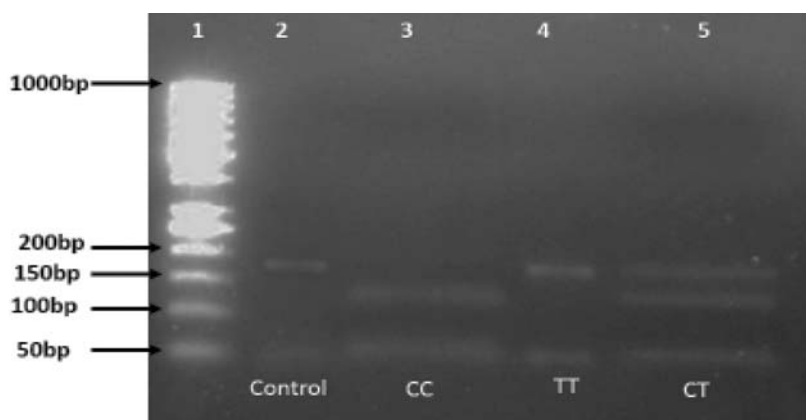


Figure-1: Restriction fragments of glutathione peroxidase 1 (GPX1) gene amplified products. Lane 1 deoxyribonucleic acid (DNA) molecular weight marker (50bp); lane 2 control; lane 3 Pro/Pro wild type genotype; lanes 4 Leu/Leu mutated homozygous genotype; lane 5 Pro/Leu mutated heterozygous genotype.

Data was analysed using SPSS 22. Descriptive statistics were calculated for quantitative variables and compared using independent t test. Categorical variables were presented as frequencies and percentages. Hardy-Weinberg equilibrium (HWE) principle was assessed by using chie-square with one degree freedom to find the association of polymorphic frequencies in the groups. $P \leq 0.05$ was considered statistically significant.

Results

Of the 60 T2DM patients, there were 30 (50%) each in the two groups. In terms of baseline characteristics, age, FBG and T2DM duration were significantly different between the groups (Table).

Table: Clinical profile of participating subjects.

Variables	Diabetics without Diabetic Neuropathy (DPN) (Group I) (N=30) Mean $\pm$ SD	Diabetics with Diabetic Neuropathy (DPN) (Group II) (N=30) Mean $\pm$ SD	Independent t-test P-values
Age (years)	50.17 $\pm$ 10.22	58.57 $\pm$ 8.93	0.002*
Fasting Blood Glucose (FBG) mg/dl	180.45 $\pm$ 49.74	219.74 $\pm$ 82.7	0.030*
Glycosylated Haemoglobin (HbA1C) %	8.39 % $\pm$ 1.88%	9.0% $\pm$ 2.53%	0.300
Body Mass Index (BMI)	26.9643 $\pm$ 3.85896	26.0267 $\pm$ 4.04596	0.362
Duration of diabetes (years)	6.08 $\pm$ 6.028	10.82 $\pm$ 8.683	0.017*

*p value $\leq$ 0.05 was deemed as significant.

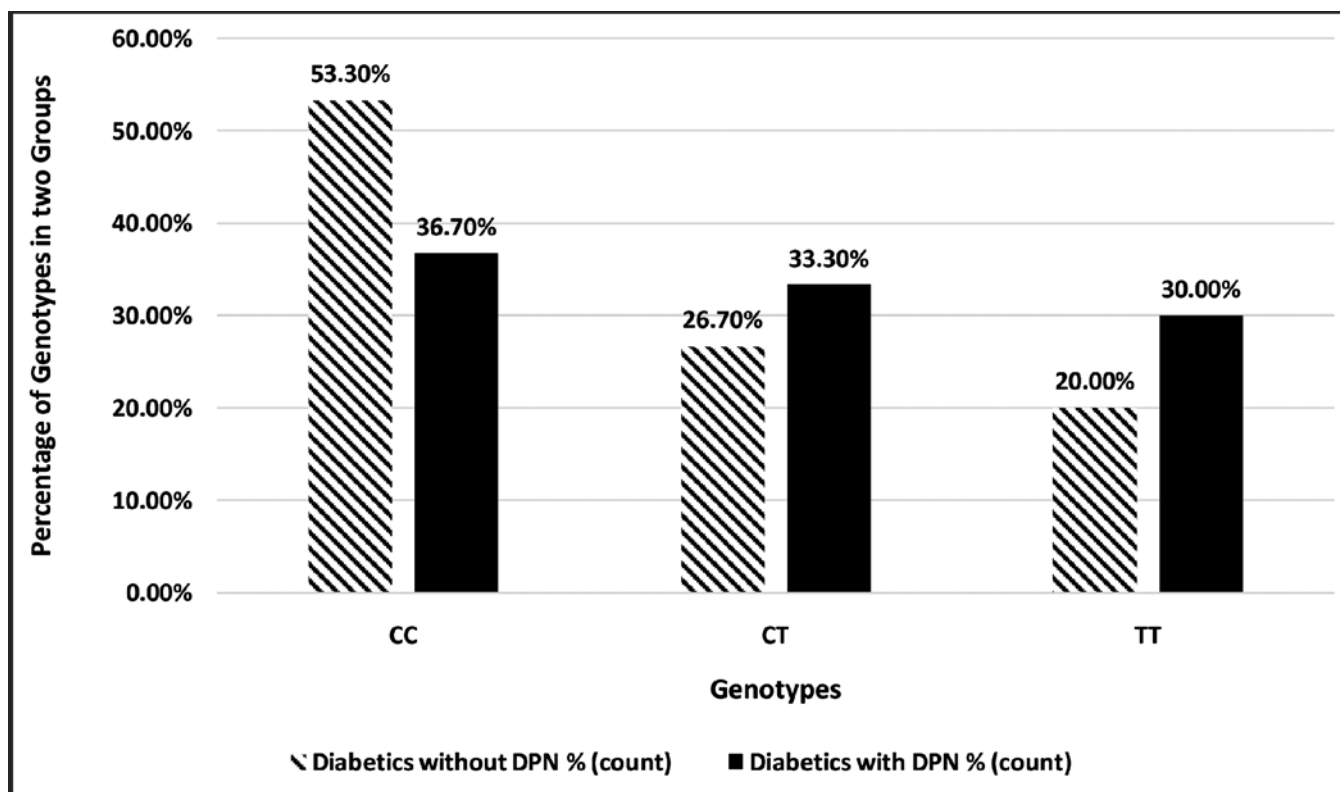


Figure-2: Comparison of genotype frequencies between the two groups. The Pearson chi square association value was $p=0.417$ ($p>0.05$; non-significant), hence, no association.

HWE was 8.4 ($p=0.03$), depicting significant difference in the existence of allelic frequencies. Despite the frequency of TT genotype being higher, there was no association of the polymorphism and any of the genotype with DPN (Figure-2).

Discussion

To the best of our knowledge, the current study is the first to have conducted genetic analysis of GPX1 gene among DPN patients in Pakistan. It provides insight into GPX1 as a potential molecular risk factor for the onset and prognosis of DPN, which may well be a step towards

personalised medicine. The main goal of the study was to find association of Pro198Leu polymorphism in the GPX1 (rs1050450, 198C > T) gene with DPN risk. Although there was increased frequency of T allele and TT genotype in the DPN patients compared to diabetics without DPN, but no association was observed between the studied SNP and DPN as well as TT genotype. Our data coincided with the Hardy-Weinberg principle and genotypic frequency. There was difference in allelic frequency of alleles C and T, showing that our diabetic population exhibited greater chance of genotype CC and CT compared to TT, but the association of this polymorphism with risk of neuropathy

was not found (Figure-2). However, the study provided new clinically relevant information regarding genetic determinants of susceptibility to DPN.

T2DM duration and FBG levels of the two groups exhibited significant difference. Although HbA1C and body mass index (BMI) showed a difference in the values of the two groups but it was not statistically significant. As T2DM duration was more in DPN patients, they were usually taking hypoglycaemic drugs, which leads to lower glycaemic levels; explaining the non-significant difference (Table). As perceived from previous researches, the pre-diabetics are more prone to developing DPN compared to the diagnosed diabetics.^{7,17,18}

No significant association between GPX1 Pro198Leu SNP and DPN, myocardial infarction (MI) and stroke was observed by Malazy et al. in their research on polymorphism of antioxidant genes in relation to T2DM management.¹⁹ These findings are similar to the results of the current study. In a research on Kurdish population to see the effect of polymorphism in three major antioxidant genes, including manganese superoxide dismutase (MnSOD), CAT and Pro198 Leu polymorphism in GPX1 (rs1050450) in relation to polycystic ovarian syndrome (PCOS), no association was found between the risk of PCOS and Pro198Leu (rs1050450) polymorphism, which is in line with the results of the current study as IR and raised OS are encountered in both cases.¹⁴

Kasznicki et al. studied Pro197Leu polymorphism in GPX1 gene in T2DM Polish patients with diabetic symmetrical polyneuropathy (DSPN) and reported no association,^{18,19} which is in line with our study. On the other hand, a study in Polish population showed significant association of Pro198Leu polymorphism with DPN patients compared to diabetics without DPN. It also observed significant increase in T allele frequency associated with DPN.¹⁵ These findings are in contrast to our results, but the other study found no association of Pro198Leu polymorphism in GPX1 gene when subgroups of patients with cardiovascular disease (CVD) and those without CVD were compared.

Hishida et al. studied gene polymorphism in different antioxidant genes including SOD2, CAT, GPX1, thioredoxin reductase (TXNRD), selenoprotein P (SEPP1), 15-kDa selenoprotein (SEP15) and selenoprotein S, (SELS) in reference to kidney disease at stage 2 to 5 demonstrated no significant associations between the GPX1 polymorphisms with chronic kidney disease (CKD) risk.²⁰ Yosra et al. conducted a research on Tunisian men suffering from chronic heart disease (CHD) and healthy controls, and showed a lack of association of Pro198Leu

GPX1 polymorphism with CHD risk and severity.²¹

In contrast, Politi et al. examined the association between this gene variant and DPN among diabetic Caucasian subjects and observed that the polymorphism was present more in patients with DPN than in those without DPN.²² The results of this study suggest that the T allele of Pro198Leu (rs1050450) is a risk factor for DPN.²²

Two studies elaborated the fact that South Asians have more conserved small nerve fibre function compared to the Europeans, and the amendable risk factors for CHD were the main contributors to these ethnic differences. They suggested that improved autonomic neurogenic control of cutaneous blood flow in Asians may contribute to their protection against foot ulcers.^{23,24} Another possible reason for the less severe neuropathic effects in South Asian population could be the lower frequency of TT genotype, which is held responsible for DPN by many of the researchers around the world as already mentioned.^{15,22} Our results also favour this concept as no association was found between DPN and TT genotype in the study population.

The association between Pro198Leu polymorphism in GPX1 gene with DPN in different population of a particular ethnicity may show a different effect completely. Hence, it is important to study the polymorphic variants of significant genes in Pakistani population so that the risk genotype/haplotype can be identified. This will help in the identification of susceptible individuals, making them aware of the risks, related complications and preventive measures to delay or avert the disease onset.

Due to resource constraints, the sample size was small which is a limitation of the current study.

Conclusion

There was no association between Pro198Leu polymorphism in GPX1 and DPN. Moreover, no association was found between TT genotype and DPN.

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Conflict of Interest: None.

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