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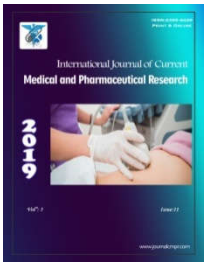

GENETIC PREDISPOSITION ANALYSIS BY GLUTATHIONE PEROXIDASE IN PATIENTS ON DIABETIC PERIPHERAL NEUROPATHY [DPN] MEDICATION

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ABSTRACT

To evaluate response of drugs used in the treatment of Neuropathy in patients with different genetic variants of Glutathione peroxidase enzyme. The details of Antihypertensive, Oral Hypoglycemic and Lipid lowering agents used by the patients was acquired in order to correlate with severity of Neuropathy using Toronto Clinical Score System. An Observational study was performed to ascertain prevalence of Peripheral Neuropathy in Type 2 Diabetes mellitus patients visiting department of Neurology and General Medicine in Princess Esra Hospital using patients of Diabetes mellitus without Peripheral Neuropathy as controls. The Allele specific analysis of odds ratio revealed that T allele carrying subjects may have nearly 2-fold increased risk of developing Peripheral Neuropathy. It is fascinating to know that individuals with CC genotype are headed to be associated with reduced risk of Neuropathy. We found that no specific pattern of a particular Neuropathy drug being used could be shown as accompanying with specific Glutathione Peroxidase genotype (CC, CT, TT).

INTRODUCTION

[1] Diabetic Peripheral Neuropathy [DPN] isone of the major micro vascular complication of Diabetes Mellitus advances with decreasing nerve functionality and Nerve blood perfusion which may result in malnourished nerve and leads to permanent nerve damage. Diabetes mellitus is becoming pandemic in last century distressing with the exponential rise of other afflictions and has become one of the dominating cause of death worldwide consequently DPN sinister 70% of diabetic population.[7]

Glutathione peroxidase (EC 1.11.1.9.) is a selenoenzyme, which is part of the biological antioxidant defense mechanism, plays an important role in preventing free radical initiated peroxidative damage by catalyzing the reduction of hydrogen peroxide and a wide range of lipid hydroperoxides[2,3,5] Since its first identification in 1957 five isoforms have been typified. Several environmental factors, like feed composition, trace element status and vitamin intake, are known to affect the activity of this enzyme, but there are some publications suggesting significant role of genetics, as well[8].There are some preliminary population level studies on genetic regulation of the enzyme[6,9]Diagnosis of DPN[10]Screening for DPN is done by using Monofilament, tuning fork and Toronto clinical scoring system for neuropathy.

Methodology It is a Prospective Observational (non-interventional study) which was carried out. At Owaisi Research Centre For Cellular and Molecular Medicine & In patient &outpatient departments of Princess Esra Hospital for the period of six months. The study design includes: Review of medication charts every day in selected patients, Documentation of findings and Polymerase chain reaction (PCR). The Patients with Age 35 and above suffering from Wound, Ulcers, Coronary Artery Disease, Hypertension, Dyslipidemia with Past Medical History of Diabetes Mellitus with duration of 5 years are involved in inclusion criteria. The geriatric population with Type 1 diabetes including pregnant women those with thyroid disorder along with pediatric population were excluded from the study. The study data was obtained from Patient data collection form, Treatment chart/ case sheet and PCR obtained results. DNA extraction was carried out by salting out method for genotypic determination

➢PCR TECHNIQUE[4]: GPxgenotype was obtained by PCR using 2 primers the sequence of primer pair used is as follows:

- 5'-TGTGCCCCTACGGTACA-3'
- 5'-CCAAATGACAATGACACAGG-3'

The amplicon was then digested with ApaI restriction enzyme and separated on a 2% agarose gel.

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Statistical analysis: Data entry was done using MS Excel and it was statistically analyzed using Statistical package for social sciences (SPSS version 16) for MS Windows. Descriptive statistical analysis was carried out to explore the distribution of several categorical and quantitative variables. Percentage distribution of GPX genotype polymorphism should be determined and the differences in genotype and allele frequencies between the specified groups should be compared using the Pearson's chi-square, Fischer's Exact Test as appropriate. The risk estimates for alleles and genotype contrasts should be obtained by computing odds ratio (OR).

RESULTS

Out of all diabetic population 76 subjects were found to be hypertensive among them 75% are positive for DPN and 25%were negative for DPN. In DPN positive patients the ratio of male: female is found to be 23:34 whereas, in DPN negative patients is found to be 6:13 respectively.The different categories of antihypertensives used by the patients are as follows:

1. Calcium channel blockers (CCBs)
2. Beta blockers (β-Blockers)
3. Adrenergic blockers (ARBs)
4. Combination of various classes which include

- ARBs+CCBs
- ARBs+βBs
- Combination of CCBs

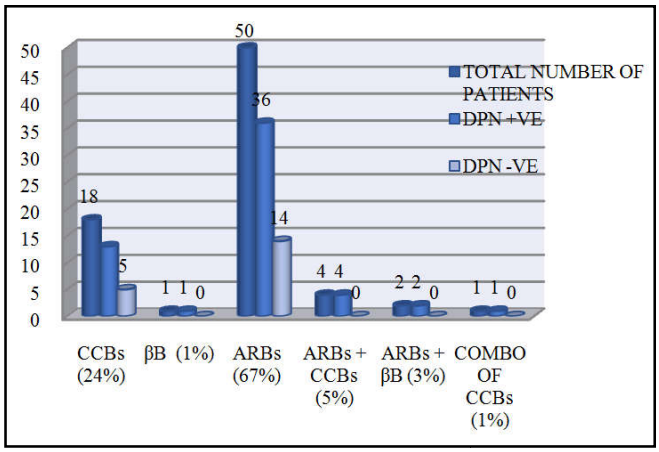

Figure No 1 Frequency of various drugs prescribed

Among the DPN positive patients' females are found in higher number taking LLDs compared to males. In DPN negative subjects only 3 males were found taking LLDs

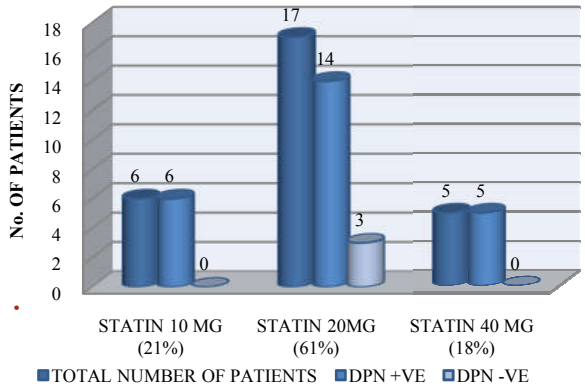

Figure No 2 Frequencies of DM positive patients taking statins in three different doses which include 10mg, 20mg and 40 mg respectively.

The PCR fragments corresponded to three genotypes The GPx-1 homozygous TT allele showed a 222-bp PCR product which is resistant to enzyme digestion, whereas CC and CT allele is digested and gave 170 and 52 bp bands. Patients with TT genotype have reduced activity of glutathione peroxidase and hence appeared to be at a higher risk of diabetic peripheral neuropathy

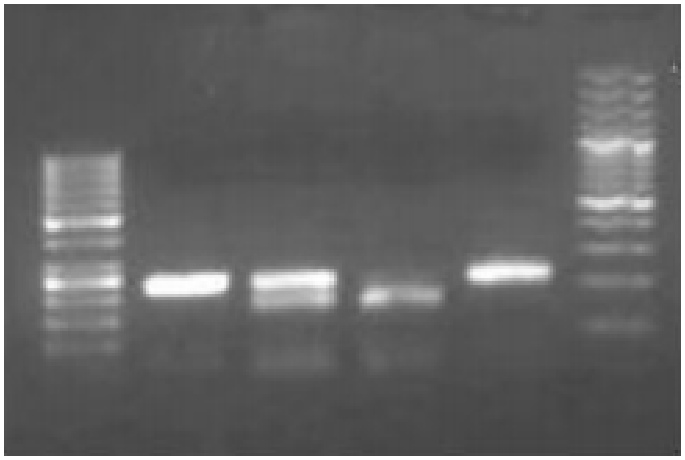

Fig 9 PCR restriction fragment length polymorphism analys is of GPx-1 C198T polymorphism

Table No 1 ODD'S RATIO FOR TT vs CT+CC

Odd's Ratio	TT	CT + CC
Patients with DPN	15	35
Patients without DPN	9	41

The ODDs ratio was calculated and it was found to be 1.9

DISCUSSION

The Risk factors for DPN are: Age, Oxidative stress, Duration of disease, Genetic deficiency of GPx and Hypertension.. Among all the diabetic patients 75%of population were prescribed drugs used in the treatment of neuropathy 76%of patients were found to be hypertensive and they were on antihypertensive therapy, 28%of population were taking lipid lowering agents and oral hypoglycemic agents were taken by all the diabetic population. A large number of DPN positive patients were found under the class interval of 51-60 and 61-70 followed by the class interval of 41-50. Patients with TT genotype have reduced activity of glutathione peroxidase and hence appeared to be at a higher risk of Diabetic peripheral Neuropathy. In the present study frequency of TT genotype among the DPN patients is 30% compared to only 18% in T2 DM without DPN. Risk analysis revealed more than 2-fold increased risk of developing DPN among individual with TT genotype. CC genotype is associated with highest activity while CT heterozygous show intermediate activity of the enzyme GPx. The frequency of CC genotype was 18(36%) in patient population while it was22 (44%) in controls. The frequency of heterozygote CT genotype were 17(34%) in patients and 19(36%) in controls with regards to homozygous TT genotype frequencies were much higher in patients compared to controls (15 (30%): and 9(18%).The GPx-1 homozygous TT allele showed a 222-bp PCR product which is resistant to enzyme digestion, whereas CC and CT allele is digested and gave 170 and 52 bp bands.

CONCLUSION

Deficiency of Glutathione peroxidase is one of the leading cause for the development of neuropathyGPx TT genotype is associated with about 2-fold increase in risk of peripheral neuropathy in diabetes patients. The allele specific analysis of odds ratio revealed that T allele carrying subjects may have nearly 2-fold increased risk of developing Peripheral Neuropathy. It is interesting to know that individuals with CC genotype are found to be associated with reduced risk of Neuropathy We found that no specific pattern of a particular Neuropathy drug being used could be shown as associated with specific Glutathione Peroxidase genotype (CC, CT, TT).

References

1. O, Ugoya A, Clinically diagnosed diabetic Neuropathy: Frequeny, types, and Severity, International Journal of national medical association (2006) 98 p. 1763-1775.
2. Hiragi O, Vilela A, Htegima A, Superoxide dismutase, catalase, glutathione peroxidase and glutathione S-transferases M1 and T1Gene polymorphisms in 3 Brazilian population, Genetics and molecular biology (2011) 34: p 11-18.
3. Briggs O, Brown H, Superoxide dismutase and glutathione peroxidase levels in patients with long standing type 2 diabetes in port harcourt, rivers state, Nigeria, *International Journal of science and research* (IJSR) (2016) 5: p 417-424.
4. Haren G, Olscn A, Associations between gpx1 pro 198 leu polymorphism, erythrocyte Gpx activity, alcohol consumption and breast cancer risk in a prospective cohort study. Carcinogenesis (2018) 27: p. 820-825.
5. Albadawy NMN, Ismail AM, Association of Gpx-198 C/T gene polymorphism (rs 1050450-198C/T) in Sudanese with diabetic retinopathy. *Journal of blood and lymph* (2017) 7: p 265-270.
6. IDF Diabetes Atlas, International Diabetes Federation, 6th edition, 2013.
7. U. Schmid, H. Stopper, A. Heidlanf, and N. Schupp, Benfoatamine exhibits direct antioxidative capacity & prevents induction of DNA damage in vitro, Diabetes Metabolism Research and Reviews (2008) 24: p.371-377.
8. Edwares FJ, Casellini MC, Role of Peroxynitrite in the development of Diabetic Peripheral Neuropathy, Diabetes care (2015) 38: p 101-103.
9. Kish JS, Morito HLC, Hornykiewicz O, Brain Glutathione Peroxidase in Neurodegenerative Disorders, Neurochemical Pathology (2006) 4: p 23-24.
10. Oyenihi BA, Ayeleso OA, Antioxidant strategies in the management of Diabetic Neuropathy, BioMed Research international (2015) p.1

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