GPx1-rs1050450 Gene Polymorphism Association with End Stage Renal Disease in Type2 Diabetic Patients of Babylon Province/Iraq

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Abstract

Background: Glutathione Peroxidase-1 is an antioxidant enzyme, play an important role in defense against oxidative stress. In Pro198Leu (C/T) polymorphism of Glutathione gene variation which result in lowering enzyme activity increasing microvascular complications of diabetic patients. **Aim:** This study was aimed to estimate the relationship between GPX1 gene variation and the risk of develop and progression of end stage renal disease in type I diabetic nephropathy patients. In this study, it has been examined 140 type I diabetes mellitus patients with nephropathy and 40 healthy control. **Results:** All samples have been genotyped by using polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) techniques. In diabetic nephropathy patients, there were a significant elevation in in the variation (TT) genotype and (T) allele frequencies compared with control group.

Conclusions: Using major Iraqi diabetic patients of Babylon province we studied the transforming effect of GPX1 gene polymorphism on the risk of development diabetic nephropathy, this study was found that the (TT) genotype of GPX1 SNPs increases the risk of development diabetic nephropathy, while (CC) genotype decreases the chance for developing disease. These results will gave the way for better perception of the genetic influencing on the development and progression of diabetic nephropathy.

Keywords: *Diabetes mellitus, Oxidative stress, ESRD, GPx1 gene polymorphism*

Introduction

Diabetes mellitus is a metabolic disorder with abnormal elevation of glucose levels as a result of insulin failure to metabolize elevated carbohydrates, proteins, and lipids leading to sever life threatening diabetic complications. The best known diabetic symptomes include polydipsia, increased urine volume, and excessive hunger, the persistent of disruption and abnormal functions of various body organs such as retinopathy, neuropathy, nephropathy, and atherosclerosis are strongly associated with chronic long lasting hyperglycemia[1,2].

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Oxidative stress is the accumulation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), these molecules play an important roles in the tissue damage and injury by several factors, then the body defense by several mechanisms such as tissuerepair, antioxidant and physical defenses, the association between oxidative stress and type2 diabetes is well revealed as that oxidative stress result from imbalance between oxygen free radicals and the endogenous antioxidant defense, diabetes is accompanied with the augmentation of free radicals formation andddiminished antioxidant ability[3,4]. The balance usually present in cells among free radical formation and their reduction through cellular antioxidants is damaged, these free radicals causese the destruction of cell components such as DNA, lipids, and proteins results in the development of diabetes mellitus and its complications[5,6].

Diabetic patients with renal disease have been a worldwide distributed with continuous increasing in numbers as the age and duration of disease increases, this forms dangerous burden on the presence of 'healthcare resources'. Nearly 30-40% of diabetic populations may develop diabetic nephropathy with high risk for end stage renal disease and several vascular complications. Despite both type1 and type2 diabetes lead to end stage renal disease, the vast majority of patients are those with type2 diabetes[7]. Glutathione peroxidase (GPx1), is the second line of enzymatic antioxidant defense system after superoxide dismutase enzymes, it has six isoforms and the best known isoforms is (GPx1), is the most distributed and the more abundant in body cells especially in vascular endothelial cells. GPx1, act by the conversion of peroxides such as hydrogen peroxide H_2O_2 and 'ROOH into 'water and 'alcohol[8] .Gene that are responsible coding for GPx1 is present on chromosome 3p21.3 with 2 exons (1.42 kb) region, numerous polymorphisms have been established in the GPx1 gene but, the best known one in located at 198 codon (C to T) leading to subsituation of proline (CCC) to leucine (CTC), this amino acid variation lead to modification of active site of enzyme changing its activity[9].

Material and Methods

1.Study subjects

The diagnosis of type2 diabetic patients was determined by the medical experts of Marjan teaching hospital in AL-Hilla City/Babylon province-Iraq. The patients with nephropathy history of 5-6 years with albumin excretion >300mg/day in two out of three urine samples. This study was comprised of end stage renal disease patients who were dependent on hemodialysis for 1-3 years. The questionnaire included diabetic nephropathy family history, age, smoking, hypertension, occupation, and the level of education. The physiological and biochemical parameters associated with diabetic patients include serum fasting blood glucose, HbA_{1c}, BMI, microalbuminurea, and albuminuria.

2.Extraction of DNA from blood samples

The blood samples were collected in EDTA tubes and stored at -20ºC. Genomic DNA was extracted from 140 patients blood samples and 80 control blood samples by using the extraction kit (Geneaid/Korea). An Proline/Leucine polymorphism in the (GPx1-rs1050450) gene was determined by PCR-RFLP technique. PCR amplification was performed with a total volume of 20 µl containing 50ng of genomic DNA, 1.25 U of Taq polymerase, 2mM dNTP, 2 mM $MgCL_2$ and 1X PCR buffer in the presence of 0.4 µmol/l of both primers, forward: 5'-TCCAGACCATTGACATCGAG-3' and Reverse: 5'-ACTGGGATCAACAGGACCAG-3'. The PCR amplification was performed in 38 cycles at 96ºC for 5 minutes (initial denaturation), 94ºC for 45 seconds (Denaturation), 59ºC for 30 seconds (annealing), and 72 for 10 minutes (final elongation). The amplification products then digested with the restriction enzymes ApaI (Promega/ USA), the digestion product then being electrophoresis in 3% agarose gel, it will show: 1 fragment of 314 bp for the Pro198 (C) wild type homozygous, 3 fragments of 314, 261, and 53 bp for the Pro198/Leu (CT) heterozygous, and 2 fragment of 261 and 53 bp for the 198Leu (T) mutated homozygous genotype.

3. Statistical analysis

Statistical analysis'were performed by using (SPSS) version (23.0). Chi square'test were used for the distribution of genotypes for deviations from'Hardy-Weinberg' equilibrium. Genotype 'distribution'of GPx1 gene in all 'subjects was analyzed by'Chi-square test.

The results

1. GPx1-rs1050450 genotyping

The results of GPx1-rs1050450 gene genotyping showed that the PCR product had one band about (314bp) for both diabetic patients and control group as shown in figure (1).

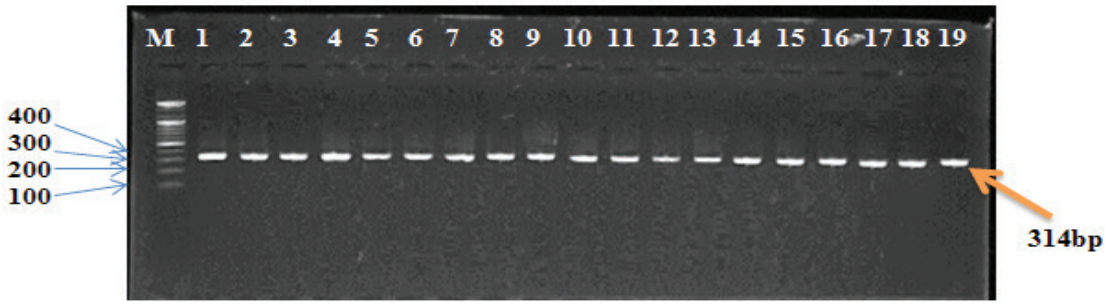

Figure (1): The electrophoresis pattern of PCR product for GPx1-rs1050450 gene. M: DNA marker (100bp), lane 1-10 from diabetic patients, lane 11-19 from control; 1% agarose, 75V, 20mA for 1h.

2. PCR-RFLP of GPx1-rs1050450 gene

The results of PCR-RFLP of GPx1-rs1050450 gene of diabetic patients and control group by using ApaI-restriction enzyme showed that the homozygous CC pattern has one band about (314 bp), the homozygous TT pattern has two bands about (53 bp and 261 bp) and the heterozygous Ff patterns has three bands (53, 261, and 314 bp) as shown in figure (2).

Figure (2): The electrophoresis pattern of PCR-RFLP for PCR product (314bp) with restriction enzyme ApaI, 3% agarose, 75V, 20mA for 2h. (7µl in each well). Lane M: DNA marker100bp, Lane: 3,4,7 ,8,10,11 showing homozygote type (CC) genotype, Lane:2,9 heterozygote mutant type (CT) genotype, Lane:1,showing homozygote mutant type (TT).

In this study, the results showed that the frequency of mutant homozygote pattern TT were significantly higher (P≤0.0001) in renal failure diabetic patients with (55%) than control group (15%), also the mutant allele frequency T was the most common in diabetic patients group (0.68 %) compared with only (0.25%) in control group. This means that the mutant homozygote pattern were significantly associated with renal failure in type 2 diabetic patients.

Table (1): GPx1-rs1050450 genotype and allele frequency among diabetic pateints with end stage renal disease and control groups.

Genotype Pattern	Genotype Frequency (%)				
	Diabetic Patients with ESRD No=140(%)	Control No= 80(%)	P-Value	Odds Ratio	95% CI
CC	28 (20%)	52 (65%)	Reference		
CT	35 (25%)	16 (20%)	0.39	1.3333	0.6835-2.6009
TT	77 (55%)	12 (15%)	0.0001**	6.9259	3.4455-13.9221
Allele Frequency%	C (0.32)	C (0.75)			
	T (0.68)	T (0.25)			

****signification at P value ≤0.001**

Discussion

Diabetic nephropathy is a common microvascular complications of diabetes. Candidate gene polymorphisms analysis in diabetic nephropathy could be important in determining patients have ability for the development and progression of this disease. Oxygen free radicals is associated in the evolution of diabetes and its resulting problems (Vincent *et al*., 2004). Many studies have documented that the glutathione C/T variation increases the occurrence of free radicals-related diseases (Cao *et al*., 2014).

Glutathione, a selenoenzyme which induces the liberation numerous of peroxides into O_2 and H_2O, it serve as a cellular barrier against damage by oxygen free radicals and it produced mainly by all tissues in the body. Previous studies were focused on the role of GPX1-rs1050450 which result in the substitution of Proline into Leucine (C/T), this mutation was studied by many studies to determine the risk of lung and bladder cancer[10]. In addition to the risk of cardiovascular disease. Some studies found that there is no association between GPX1 gene polymorphism and cardiovascular disease or chronic kidney failure in type II diabetic patients[11], but in this study it has been found a significant influence between GPX1 gene polymorphism and the risk for developing diabetic nephropathy in Iraqi population/ Babylon province. From results of this study it was determined that diabetes mellitus and TT genotype may act together to raise the risk for development diabetic nephropathy (Table 1). On the other hand, the patients were at lower risk for developing diabetic nephropathy if they have CC genotype of GPX1 gene polymorphism.

In some studies were performed on type I diabetic patients, these studies explained that the GPX1 gene polymorphism did not show any significant correlation with oxidative stress. Mohammedi *et al*. they studied three out from four GPX1 SNPs (rs-1987628, rs-8179164, rs-9819758, and rs-3448) and there analysis showed no any association with diabetic nephropathy or with oxidative stress in type I diabetic patients[12].

The process of the detected effect of glutathione gene polymorphism on predisposition of diabetic nephropathy is obscure. Various studies established the relation of Pro198Leu variation on glutathione activity[13]. The variation of antioxidant enzyme capacity perhaps result in elevation of oxidative destruction connected with the T allele. Earlier studies have been showed the relation between GPX1 activity and low density lipoprotein (LDL) oxidation, and it is very important in the distribution of microvascular injury of kidney disease[14,15].

The conclusion of our findings proposed that glutathione Pro198Leu genotypes are remarkably associated with the risk of nephropathy development in type II diabetic patients. Further studies is required for this type of mutations and it is association with other diabetic complications. The influence on detected genetic capability is little but it is important in the case of presence of other genetic factors. This study introduced novel clinical related information for genetic factors that plays a role in diabetic nephropathy susceptibility.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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