Association Of Gpx-198C/T Gene Polymorphism (rs1050450-198C/T) in Sudanese with Diabetic Retinopathy

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Abstract

Background: Diabetic retinopathy is the common micro-vascular complication of diabetes mellitus. It is the main cause of blindness among young adults worldwide. Poor glycemic control in addition to longer diabetic duration is the main risk factors for diabetic retinopathy. Many genes have been postulated as candidates for diabetic retinopathy. Little is known about anti-oxidative enzyme gene polymorphism and its association with diabetic retinopathy, mainly for catalase enzyme and manganese superoxide dismutase and glutathione genes. The study aims to assess the role of glutathone GPX-198C/T (rs1050450) gene polymorphism in diabetic retinopathy Sudanese patients and its relation with GPX level. In addition to determine the association of FBS, HbA1c and lipid in the pathogenesis of diabetic retinopathy.

Methodology: The number of subjects involved were 130 which were classified into (n 60) clinically diagnosed as diabetic retinopathy and (n 70) diabetes mellitus without retinopathy as control group, age ranged from 22 to 80 years old, from Makkah Eye Complex. DNA was extracted and PCR product for GPX, gene segment were digested by AbaI enzymes, moreover gene polymorphisms were determined. Serum GPX, activity and FBS, TG, CHOL and HbA1c level were analyzed using Cobas Int 400 using absorption photometer and immunoassay methods respectively.

Results: The results revealed that, retinopathy is common in female than male by approximately 2 fold =1.9:1. Type II is more common in our population that type 1. The majority of the patients had type II diabetes (128, 98.5%) and only 2(1.5%) patients were type I diabetes mellitus. The activity of GPX, was significantly higher in DNR when compared with DR (p=0.003). Mean HbA1c and FBG concentration were significantly higher among DR than DNR p=0.001 and p=0.001 respectively. In contrast, mean serum CHOL and TG level revealed insignificant differences when compared DR with DNR. The genotyping for GPX-198C/T showed that, the frequency of CC was observed in 33(47%) in control higher than cases 13(22%), theses Associations for CCs, GPX-198C/T SNP rs1050450, decreased risk after correction for multiple testing (OR=0.357 (0.216-0.433), p=0.001). While TT genotype was detected in 25(42%) cases and only 14(20%) in controls, these Associations for TTs, GPX-198C/T SNP rs1050450 increased risk after correction for multiple testing (OR=2.4, 95% CI=1.10-5.90, p=0.004). The frequency of the allele C protective allele was found to be 48% among cases group while allele T-risky allele was higher among cases group 72%, OR=0.357 (0.216-0.433), p=0.001.

Conclusion: The study concludes that there is a significant association between GPX-198C/T (rs1050450) gene polymorphism and the occurrence of diabetic retinopathy in Sudanese population. There is a significant decrease in GPX levels and glycemic control in patients with the mutant allele T.

Keywords: Glutathione peroxidase GPX-198C/T; Gene polymorphism (rs1050450); Diabetic retinopathy, PCR-RFLP

Introduction

No a doubt full diabetic retinopathy is the most common causes of blindness around the world. Which are a multifactorial eye disease and a major cause of the loss of lens transparency in the aging population group 20-60 years moreover has a burden on the economy and community as it’s associated with loss of reproductive. Additionally, WHO added diabetic retinopathy to the priority list of eye disease for catalase enzyme and manganese superoxide dismutase and glutathione genes. The study aims to assess the role of glutathone GPX-198C/T (rs1050450) gene polymorphism in diabetic retinopathy Sudanese patients and its relation with GPX level. In addition to determine the association of FBS, HbA1c and lipid in the pathogenesis of diabetic retinopathy.

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complications as retinopathy [6,7]. ROS reactive oxygen species are most common processes to develop vascular complications.

Production

ROS are generated under physiological and pathological condition, including pathogenesis of diabetes vascular complications. Recently, many studies reported that some diabetic patients develop macrovascular complications while others didn’t develop any complications in spite of sharing the same level of glycemic control, mode of treatment and matching for age. This findings lead to postulation of genetic susceptibility to developing diabetes vascular complications [8,9]. ROS developed in micro vascular cells in retina mostly and the superficial fiber cells, which are highly reactive. The proper regulation of cell functions depend on a certain level of ROS, such as intracellular signal, transcription activation, cell proliferation, inflammation, and apoptosis, but higher amounts of ROS are harmful to macromolecules [10-12]. The main multifunction’s of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX), protection the organisms from oxidative damage [6,8,11]. SOD decomposes superoxide into hydrogen peroxide. CAT catalyzes the decomposition of hydrogen peroxide into water and oxygen, thereby preventing cell damage from high levels of ROS. GPXs are selenoproteins that reduce organic peroxides and hydrogen peroxide through the coupled oxidation of glutathione [10,11].

Genetic variations in the antioxidant genes coding for the SOD, CAT, and GPX enzymes alter ROS resulted from irregulation of their enzymatic activity and alter ROS detoxification [9,11]. ROS with genetic material, polymorphisms in genes coding for antioxidant enzymes have a significant function for inter-individual differences in maintaining the human genome’s integrity. Genetic polymorphisms in SOD, CAT, and GPX have been included in proneness to cancer [8,11]. These potentially significant genetic variants related to oxidative stress have already been studied extensively, including single nucleotide polymorphisms (SNP) GPX–198C/T in the promoter region of the GPX gene (SNP, rs1050450), GPX–198C/T polymorphism gene has significant value as an antioxidant enzymes. Most of these polymorphism result in changes in the levels or the activities of these enzymes, which can lead to reduced protection against oxidative stress. The effect of these variations on the lens has not yet been clarified, so we choose these candidate SNPs to study in our work [9,11,13-18].

The aim of the present study was to evaluate the possible association of GPX–198C/T gene polymorphisms with diabetic retinopathy, in addition to determine the association of FBS and HbA1c and lipid in the pathogenesis of diabetic retinopathy in the Sudanese population.

Materials and Methods

Methodology: In case-control hospital based study (n 130) subject were enrolled, then classified into (n 60) clinically diagnosed as diabetic retinopathy and (n 70) diabetes mellitus without retinopathy as control group, age ranged from 22 to 80 years old, from Makka Eye Complex. DNA was extracted and PCR product for GPX–198C/T gene segment digested by Abal enzyme was used for determination of genotype by (PCR-RFLP).

GPX–198C/T genotypes were determined by using a multiplex PCR-RFLP method (Figure 1). To form undigested fragments of 222 bp by amplifying An SNPS C>T in exon 10 (codon 399) using primers, illustrated in. PCR procedure were started by 35 cycles for the total amplification reaction completed in 1:08 h. 94°C for 5 min, as an initial denaturation temperature 94°C for 30 s, as DNA denaturation temperature 62°C for 30 s, as primers annealing temperature 72°C for 30 s, as Taq polymerase extension temperature 72°C for 10 min, as a final extension step. The 222 bp PCR products were digested with Abal (MBI Fermentas, Burlington, CA) at 37°C for 5 h and analyzed with 2% agarose gels. Abal digestion resulted in one fragment of 222 bp for wild-type (TT), two fragments of 170 and 52 bp for variant homozygous (CC); and three fragments of 222, 170, and 52 bp for heterozygous (CT). (Column L show the DNA ladder 50 bp; columns 1, 5 and 7 GPX heterozygous (CT) genotype; 2-4 and 8 GPX variant homozygous (TT); column 6 GPX wild homozygous (CC).

Figure 1: PCR-RFLP analysis for CAT-262/T polymorphism (222 bp). One fragment of GPX 222bp indicates variant homozygous (TT), two fragments of 170 and 52bp for wild homozygous (CC); and three fragments of 222, 170, and 52bp for heterozygous (CT).
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and most common cause of blindness in people aged 30-60 years. After 15 years almost all patients with type 1 and two thirds of those with type 2 diabetes have a risk of retinopathy. In the retina there is increased oxygen uptake and glucose oxidation relative to any other tissue; consequently, this phenomenon renders retina more vulnerable to oxidative stress, accordingly the present study was carried out to evaluate GPX gene polymorphisms and level in patients with diabetic retinopathy (DR), and to correlate between gene polymorphism and study variables. The frequency showed that, the prevalence of DR in the present study was 60(46.1%), which is similar to previous findings that, in the India 60(42.7%), and was higher than that in the prospective diabetes studies done in Egypt 28(39.84%), KSA 22(32.84%), UK 25(37%) and Melbourne 26(35.7%). A similarity was observed with Pima Native Americans in Arizona 58(41.8%). The high prevalence of DR in our study and the Arizona study might be attributed to the poor glycemic control that increases the risk for diabetic retinopathy. Moreover, a limited period of poor glycemic control can have a prolonged effect on the incidence of diabetic retinopathy ("metabolic memory") as demonstrated by the Epidemiology of Diabetes Interventions and Complications (EDIC) cohort follow-up. The prevalence of DR in our study was also higher than that in another study conducted in Hong Kong 30(28.4%) in which the subjects were recruited from primary health care clinics [18-20].

The genotyping for GPX-198C/T showed that, the frequency of genotype CC was significantly lower in cases compared with control, these Associations for SNPs different by type. Among CCs, GPX-198C/T SNP rs1050450, decreased risk after correction for multiple testing (OR=0.310, 95% CI=0.176-0.486, P=0.004), while frequency of the TT genotype were significantly higher in cases than controls. Theses TT's, GPX-198C/T SNP rs1050450, increased risk after correction for multiple testing (OR=2.4, 95% CI=1.10-5.90 P=0.004). The frequency of the allele C protective allele was found to be 48% among cases group while allele T-risky allele was higher among cases group

Discussion

Diabetic retinopathy is the result of metabolic disorder in diabetes

Table 1: SNP Specification and Primer Sequences and PCR-RFLP Products.  

| Gene | Mutation | Forward | Primer | PCR | Restriction enzyme | Allele | PCR-RFLP Products |
|------|----------|---------|--------|-----|---------------------|--------|------------------|
| GPX  | rs1050450-198C>T | 5'-TCCAGACCATGACATCGAG:3' | 40bp | 222bp | AbaI | C/C | 52+170bp |
| Pro200Leu |  | 5'-ACTGGGATCAACAGGACCAG:3' |  |  |  | C/T | 52+170+222bp |
|  |  |  |  |  |  | T/T | 222bp |

Table 2: SNPs Location and Prevalence of Risk Allele among African Population.

| Name of Primer | Primer Sequence |
|---------------|-----------------|
| (GPX) (sense) | 5'- TCCAGACCATGACATCGAG:3' |
| GPX (antisense) | 5'- ACTGGGATCAACAGGACCAG:3' |

Table 3: Glutathione Peroxidase 1 (GPX) Primer.

| Variables | Patients | Controls | p-Value |
|-----------|----------|----------|---------|
| Patients Gender (%) | N=60 | N=70 |  |
| Male | 26(43%) | 30(43%) | 0.075 |
| Female | 34(57%) | 40(57%) | 0.005 |
| | 16.5 ± 7.5 | 16.5 ± 7.5 |  |
| Type of Diabetes (%) |  |
| Type I | 2(4) | 0(0) |  |
| Type II | 58(96) | 70(100) |  |
| Significant difference considered as p-value ≤ 0.05 |

Table 4: Socio-Demographic Comparison between Cases and Control.

| Variables | Patients | Controls | p-Value |
|-----------|----------|----------|---------|
| | N=60 | N=70 |  |
| GPX mIU/ml | 8.2 ± 1.84 | 11.14 ± 2.21 | 0.001 |
| Significant difference considered as p-value ≤ 0.05 |

Table 5: Biochemical Comparison between Cases and Controls.

| Genotype | Patients (%) | Controls (%) | OR (95% CI) | p-Value |
|----------|--------------|--------------|-------------|---------|
| GPX-198C/T | N=60 | N=70 |  |
| CC | 13 (22) | 33 (47) | 0.310 (0.176-0.486) | 0.004 |
| CT | 22 (36) | 23(33) | 0.40 (0.10-0.90) | 0.003 |
| TT | 25 (42) | 14 (20) | 2.4 (1.10-5.90) | 0.004 |
| C allele frequency | 0.48 | 0.89 |  |
| T allele frequency | 0.72 | 0.51 | 0.357 (0.216-0.433) | 0.001 |
| Significant difference considered as p-value ≤ 0.05 |

Table 7: Distribution of Genotype Frequencies of GPX-198C/T Polymorphisms among Cases and Controls.
A large number of studies on GSTM1-0 and/or GSTT1-0 null genotypes reported an increased risk for development and progression of rheumatoid arthritis and asthma. However, one study reported an association of GSTM1 gene with protection from development of type 1 diabetes in a group of 14 to 20-year-old children. We conclude that GPX1 is significant to evaluate the combinatorial association of gene variants in DR in T2DM. Another study from India, reported a significant association of GSTM1 null with T2DM with no significant association with GSTT1 null genotype. We hypothesized that genetic variability of GPX enzymes regulating oxidative stress could be involved in development of microangiopathic complications in people with diabetes. From these results demonstrated that the development and progression of DR affect by the multiple risk factors. In this study, and showed in other studies. Moreover other study from India was reported that GPX1 C allele indicated a 1.362 times higher risk of T2DM. Additionally other study from China was reported that there was no statistically significant association between the GPX1-198C/T gene polymorphisms and the risk of DR [18,20,21,30]. Serum anti-oxidant activity (GPxs) was significantly lower among DR (P=0.001), compared with DNR individuals. This may be revered to the poor glycomic control of DR patients. Poor glycomic control may be the key factor enhancing AGE formation, which may be associated with lower GPX activity in DR [18,22]. Interestingly, one study in Hungarian patients had reported that an increased frequency of diabetes with catalase deficiency compared with both healthy relatives and the background population [24]. Free radical formation in diabetes mellitus and increase over time may play a role in the development of diabetic retinopathy, which is an important complication of the disease [26]. Oxidative stress can influence the expression of multiple genes, including signalling molecules; over expression of these genes may cause mitochondrial dysfunction and peroxidization of the lipid and protein structure, which induce a variety of cellular dysfunctions leading to retinopathy [23]. Elivated oxidized lipids, DNA and protein in diabetics, represent a diminished capacity to decrease toxic reactive oxygen [26]. A relation was found between Reducing Glutathione Concentration and Diabetic Complications (USA and UK) [18,25]. Reduction in Glutathione Levels Occurs in Patients with Primary Open-Angle Glaucoma (USA) [18,27]. And the level of glycomic control as measured by HbA1c, fasting blood glucose is a marker for both development and progression of DR. There was a significant increased main level of HbA1c, fasting blood glucose concentration in the DR patients when compared with DNR (p=0.001, p=0.001), contradicted with patient. This was in accordance with findings obtained from another study [18,29]. Also of interest, it has been reported that the serum fasting plasma glucose levels correlated well with the progression of DR [18,30]. Finally, poor glycomic control increases the risk for diabetic retinopathy. Moreover, a limited period of poor glycomic control can have a prolonged effect on the incidence of diabetic retinopathy (“metabolic memory”) as demonstrated by the Epidemiology of Diabetes Interventions and Complications (EDIC) cohort follow-up [23]. The association between serum lipid levels and DR has been investigated in many studies, several studies have shown that the serum cholesterol level was associated with increased risk and severity of retinal hard exudates [18,26]. Treatment of hard exudates by atorvastatin has a significant associated with reduction in the severity and decrease the risk of the lipid in clinically significant macular edema [18,23]. The present study observed that, there was significant association between hyperlipidemia and DR [18]. The current study provide evidence that the lipid profile concentration in the DR patients when compared with DNR was insignificant (p=0.463, p=0.335, and p=0.327) this agree with result obtained by Zhang et al. [29]. Recently recommend that glycomic and lipid emic control be widely promoted and used as routinely for diagnoses [18,27]. This agrees with result obtained from Wisconsin Epidemiologic Study. The results should be compared with those of the Wisconsin Epidemiologic Study of diabetic retinopathy and its relation to various risk factors [18,23]. The Wisconsin study reported that the glycated hemoglobin is associated with increased risk of incidence of PDR. Also, the result of present study show that there was significant different in main concentration of serum GPX antioxidant enzymes of DR in compared with DNR with (p=0.001). There is a decrease in GPX activity in diabetic patients with retinopathy in comparison to without retinopathy control with significant difference between diabetic patients with retinopathy than without retinopathy.

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