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Original Article

VEGF Gene Polymorphism Among Diabetes Mellitus and Diabetic Retinopathy

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ABSTRACT

Vascular endothelial growth factor (VEGF) is a major angiogenic factor and a prime regulator of endothelial cell proliferation. VEGF gene is located on chromosome 6 (6p21.3). **Objectives:** To determine whether deletion at -2549 position of promoter region of the VEGF gene exert influence on the development of diabetic retinopathy. **Methods:** Diseased and control subjects were selected for blood sampling. The blood samples (n=50) was taken from diabetic retinopathy patients and blood samples(n=50) was taken from type 2 diabetes mellitus patients as control group. After DNA extraction Polymerase Chain Reaction was performed to amplify the VEGF gene and sequencing was commercially done for molecular analysis of VEGF gene. **Results:** The molecular analysis confirmed that deletion at -2549 position of the promoter region of VEGF significantly greater in DR group. 2-11 % deletion was examined at -2549 position of promoter region of VEGF. The DD genotype was responsible for the development of DR. **Conclusions:** This study indicates that DD gene mutation and D allele is an autonomous hazard aspect for the advancement and progression of retinopathy in people with type 2 diabetes, additionally different issues for example diabetic age and family ancestry of diabetes assumes key function in the advancement of retinopathy in diabetic patients.

INTRODUCTION

Retinopathy is a severe disease of the retina of eye. For some cases, it is the primary reason of impaired vision. Among non-diabetic patients, eye disorders related with retinopathy contains visual obstruction such as retinal telangiectasia and microaneurysms in the retina. Chronic disorders linked to retinopathy in patients with no diabetes contains chronic hypertension and general disorders. Retinopathic characteristics namely microaneurysms, dot and blot leakage and spots of cotton wool may be dependable on the identification of DR in certain people above the age of 40 without DM [1]. PDR is the most prevalent vision threatening abrasion mostly in people with type 1 diabetes. But, DME causes much of the blindness suffered by diabetes people since it is the main reason of the blindness in extremely widespread T2D and is typically exists in PDR type 2 diabetes patients [2, 3]. Numerous risk factors have previously been reported for macular edema, for example period of diabetes, impaired glycemic regulation and diabetic nephropathy. Type 2 diabetes symptoms namely increased age, hypertriglyceridemia and high blood pressure can also raise the threat of macular edema [4]. Angiogenesis of retina due to ischemia and too much discharge of extracellular matrix causes the development of fibrovascular tissues in PDR at the vitreoretinal boundary. The development of fibrovascular membrane, which consists of new blood vessles, αsmooth muscle actin (α-SMA)-expressing myofibroblasts and leukocytes frequently causes blindness due to detachment of retina. Hypoxia appears to cause neovascularization by angiogenic factor upregulation in PDR [5, 6]. Prematurity retinopathy (ROP) is an irregular retinal neovascularization of visual condition that infects premature babies. The ailment can assist the retina dispassion and is one of the major reason of everlasting vision loss in preterm infants

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[7]. DR is a multifaceted disorder in addition to prolonged inflammation and oxidative stress induced by leukocytes, some studies report the function of proinflammatory cytokines and angiogenesis stimulating molecules in the pathological process of the disease [8].

METHODS

For this study, a total of 100 people (n=100) were chosen and evenly divided into two groups: control and sick. People with type 2 diabetes (n=50) were termed the control group, whereas those with diabetic retinopathy (DR) (n=50) were considered the sick group. All subjects' blood samples were obtained in labelled falcon tubes containing (200l) 0.5M EDTA solution. All of the falcon tubes were vortexed to mix the blood and EDTA solution, and the tubes were then kept in a freezer at -20oC. To separate DNA from blood tests, an organic standard chloroform and ethanol precipitation method was used, followed by cell disruption, protein destruction via proteinase K, protein deposition using soaking NaCl, and DNA accumulation via isopropanol. Gel electrophoresis confirmed the extracted DNA. By using 8g agarose, 0.8% agarose gel was prepared for this objective. In 100ml of 1X TBE 0.8 g agarose was included during gel preparation, then heated on hot plate till agarose was totally broken down in buffer and a clear solution was found. 4-5µl of EtBr was included in it and mixed it appropriately. Under the gel doc apparatus, gel was visualized. Quantification of DNA was completed in this way.Oligonucleotide primers were used for the amplification of needed gene fragment or polymerase chain reaction (PCR). A particular set of forward and reverse primers were used for VEGF gene Forward: 5′ GCTGAGAGTGGGGCTGACTAGGTA-3′(24bp) and Reverse: 5′ GTTTCTGACCTGGCTATTTCCAGG-3′ (24bp). Starting denaturation for 5 minutes at 95^{0}C, 35 cycles each for 30 seconds at 94^{0}C, annealing at 61.7^{0}C for 1 minute and 20 seconds then extension at 72^{0}C for 1 minute and final extension at 72^{0}C for 10 minutes. PCR products optimization was verified with 2% agarose gel. Each PCR product (3µl) was combined with 2µl of 6X loading dye. The loaded DNA samples were run for 35 minutes at 120V on 2% gel versus 4µl of Marker (1kb DNA ladder). Gel was then shifted to gel doc for the visualization of PCR products. The 18 bp I/D fragment was further confirmed by commercial sequencing to confirm the size of I/D, its sequence and I/D region in the population of Lahore.

RESULTS

The present research was intended to discover the relationship of diabetic retinopathy (DR) with VEGF gene polymorphism. Within 100 study subjects, 50 subjects having DR considered as unhealthy group and 50 were control having DM. The PCR products were sent for commercially sequencing. Following are the sequences of diseased and control samples. These sequences then further blast on NCBI to check the alignments. Following are the alignments of diseased and control subjects. These sequences showed 2-11% mutation (deletion) at -2549 position of the promoter region of the VEGF gene in diabetic retinopathy subjects (Figure 1-Figure 04).

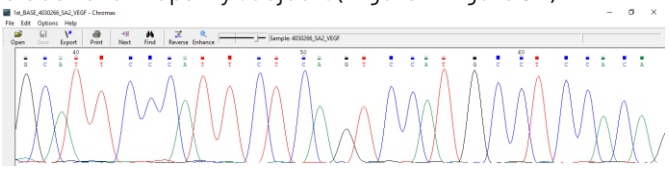

Figure 1: DNA Sequencing chromatogram demonstrating deletion (mutation) at -2549 promoter region of VEGF gene in diseased group (DR)

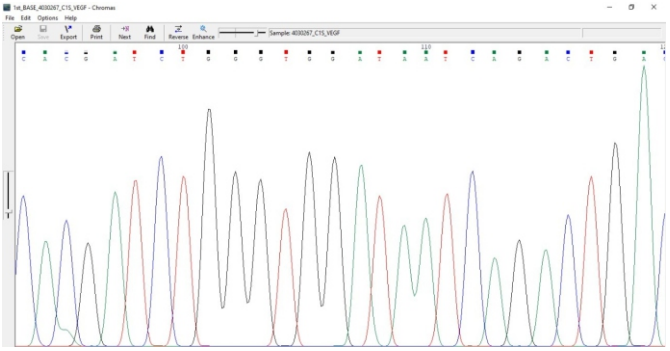

Figure 2: DNA Sequencing chromatogram demonstrating Insertion (mutation) at -2549 promoter region of VEGF gene in Control group (DM)

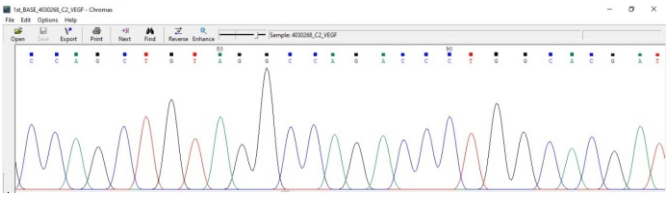

Figure 3: DNA Sequencing chromatogram demonstrating Insertion (mutation) at -2549 promoter region of VEGF gene in Control group (DM)

Vascular endothelial growth factor gene VEGF I/D mutation are seen in figure 4. The band for DD gene mutation is seen at 211bp in diabetic retinopathy subjects. Huge contrasts were seen in diabetic retinopathy and diabetic people.

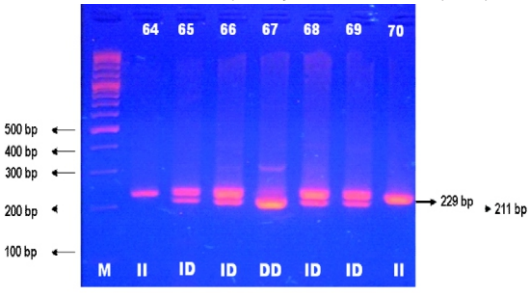

Figure 4: PCR amplification products of VEGF I/D at -2549 position of promoter region

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DISCUSSION

As per the past examinations, vascular endothelial growth factor has solid relationship with diabetic retinopathy. Vascular endothelial growth factor has double function in functioning as just in pathology of retina. Regularly it is discharged in least quantity from cells of retina yet, when high blood sugar level problems because of diabetes then oxidative pressure and irritation in endothelial cells results in vascular breakdown and deterioration of neuroglia [9]. In the present study the potential association of the 18bp fragment (I/D) polymorphism of promoter region at -2549 site has been identified in the Lahore population. The result of my study was that DD genotype was seen in diabetic patients with retinopathy relative to diabetic patients without retinopathy. The band for DD genotype is analyzed at 211bp and the band for II genotype was seen at 229bp. This 18bp section I/D mutation of vascular endothelial growth factor gene was additionally defined through sequence examination of heterozygous section for I/D mutation. Though, in this study the progressive relationship of the DD gene mutation or D allele was observed as autonomous probability aspect for the development of retinopathy in diabetic people as this research included two groups such as Control (DM) and diseased (DR). Vascular endothelial growth factor (VEGF) is chosen in this investigation because of its significant impacts on retina of eye after the development of diabetic retinopathy. It is seen that extent of vascular endothelial growth factor additionally increments during the cycle of arrangement of fresh blood vessels from remaining new blood vessels in eye in diabetic retinopathy [10]. Hietala *et al.* (2008) reported a hereditary association of proliferative retinopathy in type 1 diabetes patients [11]. This finding indicates the pathology of PDR in type 1 diabetes is a genetic aspect. Approximately all people with type 1 diabetes have been shown to have the extent of retinopathy after living with diabetes for 20 years [12]. The mutation in this gene analyzed in various nations and in Pakistan. Some of analysis are contrasted with distinctive mutation of this gene and their outcomes indicated that vascular endothelial growth factor gene has solid relationship with the advancement of diabetic retinopathy. Various possible polymorphisms have been identified in the VEGF gene. The polymorphism of _2578C/A for molecular testing in the promoter area of VEGF quality was reported, since this mutation was observed in Egyptian people in various groups. VEGF polymorphism has been reported to affect the vulnerability to proliferative diabetic retinopathy. In this study, the results revealed a greater range of the genetic mutantion (C/A and A/A) in both of non-proliferative diabetic retinopathy and the PDR relative to control C/C gene mutation [13]. Buraczynska *et al.* (2007) reported the possible interaction of 18 base pair section Insertion/Deletion mutation of regulatory area at -2549 site in Caucasian population [14]. The specific gene expression shows a central part in the pathogenesis of diabetic retinopathy problems. The higher incidence of DD genetic variation of polymorphism of vascular endothelial growth factor Insertion/Deletion in retinopathy people in Egyptian population relative to the DM people without retinopathy and in control did not display major variations in their genotype expression. They noticed the higher incidence of DD gene mutation in people with DR over 2 ways and greater chance of multiple and half crease with D factor in people with DR relative to normal group [15]. VEGF has three fundamental areas for example promoter area, 3′ untranslated region and 5′ untranslated region. Vascular endothelial growth factor gene is profoundly multifactorial and different mutations are observed on every one of these areas and known as significant gene in the progression of diabetic retinopathy and furthermore induces formation of new blood vessels in different disorders such as joint inflammation, DN and cancer. In this investigation, significant spotlight was on regulatory area. In past reports, six VEGF polymorphisms were detected in a screening report: G(-1877)A, T(-1498)C, G(-1190)A, G(-1154)A in the regulatory area and C(-634)G, C(7)T in the 5′ untranslated region. The other five polymorphisms and C (936) T and G(1612)A mutations in the 3′ untranslated region were identified to be normal in the people of Japan. Past reports have also revealed that the production of VEGF is affected by mutation in both the regulatory region and 3′ untranslated regions of the VEGF [16]. It was reported that the alleles possessing the VEGF -152A (rs13207351) and -116A (rs1570360) were strongly linked with PDR. Three promoters linked with PDR, the single nucleotide polymorphisms 160, 152 and 116. Proliferative stage of diabetic retinopathy is strongly correlated with the _160CC genotype. The genotype _152AA is closely related to PDR (OR_3.5). The _116 SNP displayed the strong correlation in the analysis, with PDR group demonstrating a substantially greater incidence of the AA genotype [17]. The existence of D factor in the regulatory area of vascular endothelial growth factor gene at -2549 position contribute to increased gene expression. Past reports have also revealed that VEGF output is correlated with mutation in the promoter region along with 3′ untranslated region of VEGF gene [18]. The impact of 3 mutations of VEGF on the progression of DME has been investigated in Japanese population. Their findings indicate that polymorphism of VEGF at C -634 G is a contributing hazard predictor for both ME and DR with T2DM [19]. In the regulatory area of VEGF on 405, presence of G factor contributes to increased gene

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expression in Caucasian population. The SNP rs699947 exhibited full association of Insertion/Deletion mutation on -2549 site from interpretation binding facility, that is located 29bp downstream from rs699947 [20]. In alternative investigation performed in north Indian people of Amritsar the Insertion/ Deletion mutation of vascular endothelial growth factor gene has been recognized in irregular bosom malignant growth patients and strikingly they have discovered the critical relationship of II genotype and I allele in bosom disease patients when contrasted with control bunch [21].

CONCLUSION

This study indicates that DD gene mutation and D allele is an autonomous hazard aspect for the advancement and progression of retinopathy in people with type 2 diabetes, additionally different issues for example diabetic age and family ancestry of diabetes assumes key function in the advancement of retinopathy in diabetic patients. In DR subjects 2-11% deletion was observed. Conversely there was huge relationship of II gene mutation or I allele in type 2 diabetic patients.

Conflicts of Interest

The authors declare no conflict of interest

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