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Original Article

VEGF Genotype and Allele Frequency of Diabetes Mellitus and Diabetic Retinopathy in Lahore, Pakistan

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ABSTRACT

Diabetic retinopathy is characterized as basement membrane (BM) thickening, pericyte loss, endothelial cell (EC) dysfunction, microaneurysms, microvascular infarcts and neovascularization in a patient with diabetic retinopathy. **Objectives:** To determine the VEGF genotype and allele frequency of diabetes mellitus and diabetic retinopathy in Lahore, Pakistan. **Methods:** A total of 100 blood samples were taken including diabetes mellitus (50) and diabetic retinopathy patients (50). Diseased and control subjects were selected for blood sampling. Demographic and clinical characteristics was evaluated. The BMI, HbA1c and the blood pressure of both groups were also examined. The VEGF genotype and allele frequency of diabetes mellitus and diabetic retinopathy was done. The statistical analysis was done by chi-square test and SPSS to study significant differences in control and diabetic retinopathy subjects. **Results:** The normal estimation of DM group was 46.18 ± 1.23 years while of DR class was 52.86 ± 1.36 years. The mean value of BMI of DM group was 26.0±0.62 while of DR group was 26.57±0.70. DD genetic mutation was substantially higher in diabetic retinopathy bunch ($p<0.05$) relative to the II gene mutation, the huge contrasts ($p<0.05$) were seen in diabetic group. **Conclusions:** This study concluded that Vascular endothelial growth factor gene was detected in both group of diabetes. In retinopathy people with T2DM the substantial elevated VEGF DD genetic variation was seen relative to retinopathy people without diabetes.

INTRODUCTION

Diabetes mellitus (DM) is a set of metabolic issues categorized by prolonged high blood glucose level made by deficits in insulin function. The significance of insulin as an anabolic hormone causes metabolic anomalies in carbohydrates, lipids and proteins [1].. The standard diabetes grouping suggested by the American Diabetes Association as T1D, T2D and other forms announce in 1997 [2]. Type 1 diabetes is 5%-10% of patients identified with diabetes, and is affected by the damage of β cells of pancreas. Type 1 diabetes constitutes for 80 percent - 90 percent diabetes for teenager and adolescents [3-5]. According to the International Diabetes Federation (2013), the overall frequency of diabetes in individuals (>20-79 years of age) a study released by IDF in 2013 was 8.3 percent, with 14 million greater number of men than women, the bulk aged between 40 and 59, and the figure is expected to increase above 592 million by 2035 with worldwide incidence of 10.1 percent. The maximum incidence of diabetes has been reported in Middle East and North Africa (10.9%), while, maximum adults were identified with diabetes (138.2 millions) in Western Pacific area and there is maximum incidence of diabetes in many states. According to the National Health Survey the frequency of diminished glucose tolerance and diabetes in Pakistan is present amongst the people of age >25 years, is 22.4 percent [6]. Diabetes Mellitus is one of the greatest hazardous sophisticated ailments that affects health and give rise to societal difficulties. It is recognized as a universal epidemic of the twenty first century. The occurrence of this disorder is still growing and is frequently related to critical problems that causes the bigger danger and death in diabetic patients [7]. There are many patients

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who do not experience any problem for several years like DR, diabetic neuropathy and DN. Lifelong consequences of hyperglycemia in the first years after the identification of type 2 diabetes mellitus are believed to rely on epigeneticconditioned metabolic memory [8]. The predictive variability between all people with diabetes designates a possibly heterogenous existence of many diabetic complications that may be correlated with genetic predisposition [9]. Genetic influences assume a significant part in the pathological process of T2DM which defines the clinical course of type 2 diabetes mellitus and associated abnormalities are determined by the environmental conditions [10]. Some microvascular abnormalities correlate unregulated glycemic rates in diabetic subjects [11]. In people of age 20-74 years the primary reason of blindness is DR [12]. From past reports that were done in 1990 to 2010, diabetic retinopathy was considered as the fifth usual reason of unnecessary vision loss and is the source of mild to extreme vision disorders [13].

METHODS

For this research, total 100 individuals (n=100) were selected and were equally divided in two groups i.e. control group and diseased group. Individuals with type 2 diabetes mellitus (n=50) were considered as control group whereas individuals having diabetic retinopathy (DR) (n=50) were considered as diseased group. All the individuals were selected from District Lahore Punjab, Pakistan. The sampling was done from private sector hospital i.e. Mughal Eye Hospital (Trust), Lahore. Patients were examined by ophthalmologist Doctors. The experimental work was carried out on hi-tech equipment in University of Health Science Lahore, Lahore. Before participation of diseased group in research, each individual was undergone for complete ophthalmological examination i.e. best corrected visual acuity, slit lamp examination and retinal examination. Fluorescein angiography was done for confirming the presence or absence of diabetic retinopathy of diseased group.-Whereas, severity level of retinopathy was determined by diabetic retinopathy severity scale. The following individuals were included for diseased group: Type 2 diabetes patients. Age at onset from above 20 to 75 years. Duration of disease ≥5 years. Diabetic retinopathy patients without macula edema condition. The individuals having ocular trauma, nephropathy or any other blood borne disease were excluded from this research. A questionnaire performa was designed to take general information (age, gender, family history, BMI, smoking, diabetic duration, lifestyle etc.) from individuals of control group and diseased group. Before sampling, all the individuals of control and diseased groups signed a consent form. Blood samples of all the individuals were collected in labeled falcon tubes having (200µl) 0.5M EDTA solution. All the falcon tubes were vortexed to mix the blood and EDTA solution and after vertexing, tubes were stored in freezer at -20ºC. Organic standard chloroform and ethanol precipitation method was used for DNA extraction. 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 through published work which was used in past studies. The analysis of data was done with the help of SPSS software (version 20) to determine the significance and its degree between demographic characteristics of different groups by using Chi-square test.

RESULTS

The present research was intended to discover the relationship of diabetic retinopathy (DR) with VEGF gene and with various clinical and demographical boundaries in the number of inhabitants in Lahore. Within 100 study subjects, 50 subjects having DR considered as unhealthy group and 50 were control having DM. Among the ailing group, fifty-two percent were women and forty-eight percent were men whereas in examination with control, fifty-six percent were women and forty-four percent were men. During sampling, sexual orientation was chosen arbitrarily. Table 1 indicates DR participants average age was 52.85 ± 1.36 whereas in healthy subjects the average age was 46.18 ± 1.23. The normal estimation of DM group was 46.18 ± 1.23 years while of DR class was 52.86 ± 1.36 years. Table 4.1 demonstrates the p estimation of this variable. The information about age was categorized into four classes. Class I (31-40), class II (41-50), class III (51-60), class IV (61-70). The mean value of BMI of DM group was 26.0±0.62 while of DR group was 26.57±0.70. The p value of BMI of two groups was 0.55. A large portion of people were extremely obese. The average length Of DM group was 7.38±0.38 and 11.12±0.75 of DR group (table 1). The average value of systolic blood pressure of DM group was 132.70±1.850 (mmHg) whereas of DR group was 135.32±1.564. The average value of diastolic pulse was 84.84±0.941 for DM group whereas 82.64±0.895was for DR group.

Mean age (years)	46.18±1.23	52.86±1.36	0.000*
Gender (female/male)	49/51	52/48	
Duration (years)	7.38±0.38	11.12±0.75	0.000*
BMI (Kg/m2)	26.0±0.62	26.57±0.708	0.556**
HbA1c level	7.4±0.053	7.6±0.063	0.082***
Diastolic Blood Pressure (mmHg)	84.84±0.941	85.64±0.895	0.539**
Systolic Blood Pressure (mmHg)	132.70±1.850	135.32±1.564	0.282*
Family history (%)	40/50 (80%)	42/50 (84%)	81% (average)

Table 1: Clinical and demographical factors of DM and DR groups
*Significant, **Significant, ***Highly significant

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Vascular endothelial growth factor gene sequencing was financially conducted. In retinopathy people with T2DM the substantial elevated VEGF DD genetic variation was seen relative to retinopathy people without diabetes. Table 2 demonstrates the genetic mutation of vascular endothelial growth factor and rates of alleles in all examination disciplines.

Study Groups	Genotype Frequency			Allele frequency	
	II	ID	DD	I	D
T2DM with DR	12%	36%	52%	0.30	0.70
T2DM without DR	26%	34%	40%	0.43	0.57

Table 2: VEGF genotype and allele frequencies

DD genetic mutation was substantially higher in diabetic retinopathy bunch ($p<0.05$) relative to the II gene mutation as demonstrated in table 3 the huge contrasts ($p<0.05$) were seen in diabetic group. These correlations indicate that huge contrasts were present among gene variation of diabetic retinopathy and diabetes mellitus groups.

Study subjects	Chi-square value	Degree of freedom	p-value	95% Confidence Interval
DM vs. DR	9.490	2	<0.05(0.009)	0.009-0.013

Table 3: Comparison of (I/D) VEGF genotypes using Chi-square

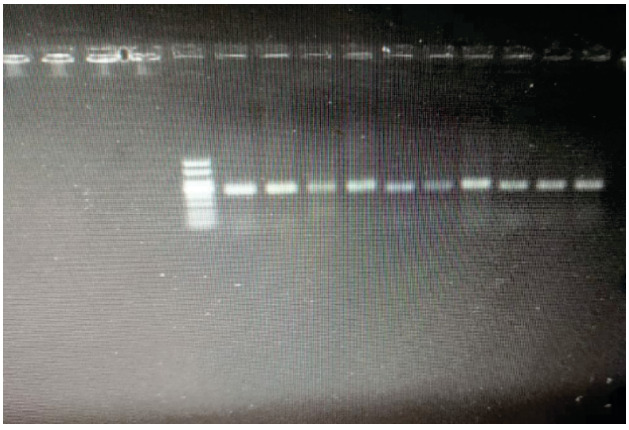

Figure 1: 2 % agarose gel showing the optimization of PCR product

DISCUSSION

Diabetes Mellitus (DM) is not only a disorder that involves ailment yet in addition results in financial issues that likewise influences the estimation of life, therefore it is one of the greatest disturbing illness of general public. It is additionally perceived as highly predominant disorder of twenty first epoch worldwide [7]. Whereas diabetic retinopathy (DR) because of microvascular complexity of uncontrolled and life-long diabetes mellitus which results in visual deficiency in people. The common frequency of diabetic retinopathy was 34.6 percent whereas occurrence of extreme phases of this aliment was 10.2 percent [14]. The joined frequency was 28.78 percent in diabetic patients of Pakistan with contrast from 10 percent to 90 percent [15]. The expanded ischemical states of the tissue and hyperporouness in retina define DR. VEGF is a glycoprotein because of its attributes of formation of new blood vessels formation appeared to be a conceivable applicant quality of diabetic retinopathy. Vascular endothelial growth factor guideline for the formation of new blood vessels is enacted when variables lead towards low oxygen that causes hypoxia are attached to the segment of hypoxia response and starts the VEGF release that consequently starts formation of new blood vessels [16]. The mechanism of formation of new blood vessels formation is strongly regulated by non-developmental and encouraging stimuli [17]. A latest analysis showed that vascular endothelial growth factor grafting is changed from non developmental to a pro-developmental state in the eyes of patients with diabetes and 460 allele may be a crucial element in the control of isoforms of VEGF [18]. In this investigation, we likewise surveyed demographical and clinical factors such as, sex, age, HbA1c percentage, BMI, financial status as a hazardous aspect for expectation of diabetic retinopathy. In this present investigation, women are more predominant when contrasted with men. Yang *et al.* (2011) saw similar consequences of sex in people of China. Sexual orientation as such does not make a difference in diabetic retinopathy [19]. However, different components for example HbA1c percentage, body mass index and period of diabetes with retinopathy with diabetes and associated with the commencement and progression of diabetic retinopathy. Body mass index and hemoglobin percentage were non-critical in this investigation. However, a few investigations indicated contradictory effects about relationship of body mass index with diabetic retinopathy [20]. Diabetes mellitus span is significant indicator of diabetic retinopathy. There will be multiplied the frequency of diabetic retinopathy as the people experiencing ten to nineteen years of diabetes yet with over twenty years of diabetes have right around multiple times more risks of diabetic retinopathy [21]. Smoking additionally watched hazard predictor for some infections. In this investigation, the level of smoking for diabetic retinopathy was two percent, whereas seven percent was for diabetes bunch. It is determined that smoking does not impact diabetic retinopathy positively. Some past investigations clarified that smoking did not establish a fundamental aspect for diabetic retinopathy. Solid family ancestry was additionally go about as danger aspect in the progression of numerous ailments. Particularly the disorder like DM indicates the significance of family ancestory. Particularly more than half considered groups had acquired this issue from fatherly or maternal foundation.

CONCLUSIONS

This study concluded that Vascular endothelial growth

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factor gene was detected in both group of diabetes. In retinopathy people with T2DM the substantial elevated VEGF DD genetic variation was seen relative to retinopathy people without diabetes

Conflicts of Interest

The authors declare no conflict of interest

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