ISSN- O: 2458 - 868X, ISSN–P: 2458 – 8687
National Library of Medicine - ID: 101731606

International Journal of Medical Science and Innovative Research (IJMSIR)
IJMSIR : A Medical Publication Hub
Available Online at: www.ijmsir.com
Volume – 7, Issue – 5, October – 2022 , Page No. : 43 – 48

Correlation between serums vascular endothelial growth factor with severity of diabetic retinopathy

[1]Vishwanath H S, Assistant professor, Department of Ophthalmology, JJMMC, Davangere, Karnataka, India
[2]Madhavi gupta S V, Professor, Department of Ophthalmology, JJMMC, Davangere, Karnataka, India
[3]Deepak T Swamy, Senior resident, Department of Ophthalmology, Siddartha Medical College, Tumkur, Karnataka, India
[4]Bhavya S.O, Department of Paediatrics, SSIMS and RC, Davangere, Karnataka, India
Corresponding Author: Bhavya S.O, Department of Paediatrics, SSIMS and RC, Davangere, Karnataka, India

Citation this Article: Vishwanath H S, Madhavi Gupta S V, Deepak T Swamy, Bhavya S O, "Correlation between Serum Vascular Endothelial Growth Factor with Severity of Diabetic Retinopathy", IJMSIR- October - 2022, Vol – 7, Issue - 5, P. No. 43 – 48.

Type of Publication: Original Research Article

Conflicts of Interest: Nil

Abstract

Background: Diabetic retinopathy, being a major complication of diabetes mellitus (DM) has significant ocular morbidity affecting the working population. Diabetic retinopathy is the leading cause of blindness in the worldwide amongst 25-74 year age group. Vascular endothelial growth factor leading to neovascularisation, fibrovascular proliferation and bleeding causing blindness, if not treated in early stage

Objectives

- To estimate serum VEGF level in no diabetic retinopathy, NPDR and PDR.
- To correlate serum VEGF with severity of diabetic retinopathy.

Methodology: This is a descriptive hospital based cross sectional study. Study population includes 90 Type 2 diabetic patients grouped into no DR, NPDR and PDR after complete ophthalmic examination. FBS, PPBS, HbA1c were estimated and Serum VEGF is estimated by the ELISA technique.

Results: There was significant mean difference found between the severities of diabetic retinopathy with mean duration of diabetes, mean FBS, mean HbA1C, serum VEGF. Serum VEGF had a decreased trend with severity of diabetic retinopathy (123.4 pg/ml - No DR, 100 pg/ml - NPDR, 88.5 pg/ml - PDR).

Conclusion: FBS, PPBS, HbA1c correlated with increase in severity of diabetic retinopathy and can be used as long-term predictor of severity of diabetic retinopathy.

Although Serum VGEF levels are elevated in the entire diabetic population irrespective of whether they had diabetic retinopathy or not there was no significant relationship between the serum VEGF levels and the severity of DR

Abbreviation: DM- diabetic mellitus, DR - Diabetic retinopathy PDR - proliferative diabetic retinopathy, NPDR – Non proliferative diabetic retinopathy, VEGF- Vascular Endothelial Growth Factor

Introduction

Diabetic retinopathy, being a major complication of diabetes mellitus (DM) has significant ocular morbidity affecting the working population. Almost 40% of diabetics would develop diabetic retinopathy, which is one of the potential complications of diabetic mellitus.[1] Diabetic retinopathy is the leading cause of blindness in the worldwide amongst 25-74 year age group.[1]

Micro vascular changes, inflammation and neurodegenerative changes are some of the important early changes in diabetic retinopathy, which progresses to release of growth factors like Vascular endothelial growth factor leading to neovascularisation, fibrovascular proliferation and bleeding causing blindness, if not treated in early stage.[2,3]

VEGF is an angiogenic mitogen secreted from various types of cells and plays an important role in angiogenesis under physiological and pathological conditions.[4,5] This vascular endothelial growth factor is found physiologically in serum and tears but increased expression occurs during oxidative stress, ischemia explaining its role in diabetic retinopathy.[6]

Angiogenesis depends on both pro and anti-angiogenic factors. Some of the pro angiogenic factors include vascular endothelial growth factor (being the most important), insulin likegrowth factor 1 (ILGF-1), beta fibroblast growth factor (b-FGF), platelets derived growth factor (PDGF), pro inflammatory cytokines, angiopoietins. Some of the anti-angiogenic factors include pigment epithelial derived factor (PEDF), Transforming growth factor beta (TGF-B), Tissue stimulating plasminogen (TSP) and somatostatin.[3]

Without treatment it is estimated that 50% of proliferative diabetic retinopathy will lead to blindness within 5 years.[7] To preserve vision and proliferative changes of diabetic retinopathy; early detection of Diabetic retinopathy through screening programmes and appropriate referral for therapy is important at timely interval.[8] Serum and vitreous level of VEGF can be estimated by Enzyme Linked Immuno Sorbent Assay, this can be used as a biomarker for severity of diabetic retinopathy.[9]

In the present study, we aimed to estimate the serum levels of VEGF in grades of diabetic retinopathy and also to determine the correlation between serum VEGF and the grading of DR.

Material and Methods

This is a hospital based cross sectional study conducted on a total of 90 diabetes patients who presented to department of Ophthalmology of a medical college and hospital from December 2019 to April 2021. The study subjects were included after their consent. The study was conducted after obtaining approval from the institutional ethical committee.

Inclusion Criteria

Patients in the age group of 35-70 years who were diagnosed to have Type 2 Diabetic Mellitus with Diabetic retinopathy (non-proliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR) and Type2 Diabetic Mellitus without Diabetic retinopathy.

The Diagnosis of Diabetes is based on the criteria of American Diabetes Association (FBS>/=126mg/dl or oral glucose tolerance test >/= 200mg/dl) or who were known case of diabetes under the treatment of physician.

Exclusion Criteria

Type1 diabetic Mellitus, Diabetic Nephropathy**,** Pregnancy**,** Ischemic Heart Diseases**,** Glaucoma, History of past ocular surgery or trauma**,** Active eye infection or inflammation**,** Anti VEGF treatment, Prior photocoagulation patients, Systemic illnesses such as known case of or clinically diagnosed case of

Rheumatoid arthritis, systemic lupus erythematous, Ocular Ischemic syndromes, Retinopathy of blood disorders, Retinal Vascular disorders.

Sample size: It is calculated by using the mean difference formula. The values are taken from the study done by Ahuja S et al., [10]

$$n = \frac{2Z1^2S^2}{d^2}$$

M1	Mean VEGF values in mild NPDR	189.48
M2	Mean VEGF values in Severe NPDR	434.34
S1	Standard deviation of M1	35.37
S2	Standard deviation of M2	66.67
S	Pooled SD	53.3663
AH	Alternate hypothesis	1
1-α	Level of confidence	0.95
1-β	Power of test	0.8
Z1	Z value associated with alpha	1.64485
Z2	Z value associated with beta	0.84162
d	Absolute precision	23
n	Minimum sample size	30

Thus, 30 study subjects were included in each group.

Considering inclusion and exclusion criteria, patients under inclusion criteria were enrolled in the study. Written informed consent was taken from them. A detailed history with demographic characteristics including age, sex, age at onset of diseases (diabetic mellitus/ Diabetic retinopathy) and duration of illness and history of medications used, family history of illness were obtained. Through ocular examination was performed in all patients. Cases were divided into 3 subgroups as T2DM with NO DR, T2DM with NPDR, T2DM with PDR based on ETDRS classification.

Procedure

After satisfying inclusion criteria, informed consent was taken from subjects and a 5 ml of blood was collected from median cubital vein under aseptic precautions with 2 ml syringe and centrifugated at 3000 rpm for 10 minute, separated serum was collected by disposable pipettes and transferred into a plain vacutainer which is stored at -35 to -40^{o} C.

Semi-Automated ELISA reader is used to determine optical density at 450 nm of the microplate within 30 minutes of preparation and the results obtained in IU/L (international unit per litre) is converted to picogram per millilitre.

Statistical Analysis

SPSS version 20. Was used to perform the statistical analysis. Data was entered in the excel spread sheet. Descriptive statistics of the explanatory and outcome variables were calculated by mean, standard deviation for quantitative variables, frequency and proportions for qualitative variables. Chi square was applied to test the statistical association between qualitative variables. ANOVA was applied to test the mean difference of quantitative variables with respect to groups. The level of significance was set at 5%.

Results

In the present study, a total of 90 diabetic cases were included. The characteristics of the study subjects along with diabetic status are briefed in table 1. Majority of the study subjects were males (54.4%) and belonged to the age group of 51-60 years (37.8%). Duration of diabetis mellitus, mean FBS, mean PPBS and HbA1c showed significant mean difference on ANOVA test ($p < 0.001$)

Table 1: Characteristics of the study subjects.

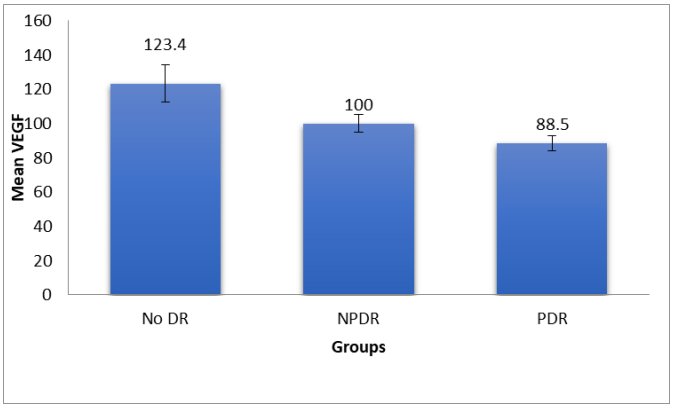
Variables	No DR	NPDR	PDR	p value
Male	14 (46.7 %)	17 (56.7%)	18 (60%)	0.63$
51 - 60 years	12 (40%)	11(36.7%)	11(36.7 %)	0.56$
Duration of DM (years)	3.6 ± 2.2	11 ± 5.5	15.3 ± 5.9	0.001 *
Mean FBS (mg/ dl)	132.4 ± 10	191.7 ± 37.8	204 ± 50.6	0.001 *
Mean PPBS (mg/ dl)	179.4 ± 16	237.7 ± 66.7	248 ± 58.3	0.001 *
HbA1c (%)	6.7 ± 0.44	8.24 ± 1.34	9.97 ± 0.78	0.001 *

$Chi square test

*ANOVA

VEGF levels in three groups is described in fig.1 There is significant mean difference (p – 0.001) found between the VEGF levels among the three groups where mean VEGF was high in the study subjects with no diabetic retinopathy (123.4 ± 10.7) , followed by NPDR (100 ± 5.1) and PDR (88.5 ± 4.3)

Fig.1. VEGF levels in study groups



Visual acuity: Visual acuity of right eye and left eye are grouped as 6/6-6/9, 6/12-6/18, 6/24-6/36, less than 6/60.

In Right eye, 32.2% patients had less than or equal to 6/60 visual acuity, 25.6% had 6/24-6/36, 23.3% had 6/6-6/9,18.9% from 6/12-6/18. In which 70% of PDR had visual acuity <6/60, 40% NPDR had visual acuity range from 6/24 to 6/36 and 50% of No DR had visual acuity from 6/6 to 6/9.

In Left Eye, 34.4% had visual acuity equal to or less than 6/60, 27.8% from 6/6-6/9, 26.7% from 6/24-6/36, 11.1% 6/12-6/18.70% PDR had visual acuity <6/60, 40% NPDR from 6/24-6/36 and 60% of NO DR from 6/6 to 6/9.

Discussion

This study includes diabetic patients in the age group of between 39 to 72 years and the mean age in each group is 56.7years, 57.3years and 56.03years with NO DR, NPDR and PDR respectively. The gender distribution of the population was (54.4%) males and (45.6%) females. The male to female ratio was: 1.19:1.

Duration of Diabetes mellitus

The mean duration of diabetes mellitus was 10 years. Mean duration of diabetes mellitus in non-proliferative diabetic retinopathy was 11years.Mean duration in proliferative retinopathy was 15.3 years.Mean duration of NO diabetic retinopathy was 3.6years. There was high statistical significance of duration of diabetes with severity of diabetic retinopathy in our study (p<0.001). This was similar to another study which showed longer the duration of diabetes higher the prevalence of diabetic retinopathy.11

According to Muhammad Khizar Niazi et al., duration of diabetes is an independent risk factor for progression of diabetic retinopathy12 which is in similar to our study. The results by Pragatigarg et al., were also in line with our study13

Fasting blood glucose levels

The mean FBS in No DR, NPDR and PDR was 132.4mg/dl, 191.7mg/dl and 204mg/dl respectively which was more than the normal and increasing with severity of Diabetic retinopathy in our study. This was in par with study by Yilling j chenget al.,12 where it was shown that FBS is a discriminator of Diabetic retinopathy

severity. As per many studies estimation of long term control of FBS will impact on the outcome of long-term complications of diabetes14.

Haemoglobin A1C levels

The mean HbA1c levels are 6.70%, 8.24% and 9.97% in No DR, NPDR and PDR respectively. It indicates state of blood glucose control for past 3 month duration. Our study showed increase in HbA1c with severity of diabetic retinopathy was similar to other studies 15. The overall mean HbA1c level in our study was 8.3% which was close to other study by Claus zehetner et al. which showed mean value of 7.5%.16 Another study by Yilling j cheng et al., showed that HbA1c as a strong discriminator of diabetic retinopathy.12 According to Marcus lind et al., the risk of proliferative diabetic retinopathy increases with HbA1c >8.3%17, which was close to our study in which mean HbA1c in NPDR and PDR groups was >8.24%. According to Wenjunzou et al., HbA1c more than and equal to 7% has increased risk of development of diabetic retinopathy.[18]

Serum VEGF levels

In our study Mean Serum VEGF for overall population was 103.9 pg/ml. Mean Serum VEGF level in NO DR, NPDR and PDR was 123.4pg/ml, 100pg/ml and 88.5pg/ml respectively which implies statistically significant ($p<0.001$) with diabetic retinopathy.

According to Claus Zehetner et al., value of mean Serum VEGF level was 34.5 pg/ml with range between 15-217 pg/ml, similarly our study estimated serum VEGF levels falls within this range.16 In a study Jebinath Brayan et al., value of mean serum VEGF level was found to be 577.01 ± 291.13 pg/ml, with mean No DR, NPDR, PDR being in the range of 620 to 496pg/ml which were higher compared to our study, but levels of VEGF decreased with severity of diabetic retinopathy which was similar to our study.[14]

The lowest value of Serum VEGF was in PDR group which was 80pg/ml, the highest value of Serum VEGF was in NO DR group which was 144 pg/ml which was different from another study in which severe NPDR showed highest value.[16]

In study by Sukriti Ahuja et al., serum VEGF in NO DR, NPDR and PDR showed incremental trend from 138.96pg/ml to 457pg/ml respectively which was contradictory to our study.[10]

Limitations

Study should be done in large number of patients to use serum VEGF as a biological biomarker in diabetics. Other biological fluids like Tears, Vitreous and Aqueous samples should have been considered for estimation of VEGF as it gives more significant values.

Conclusion

Duration of diabetes, FBS, PPBS, HbA1c correlated with increase in severity of diabetic retinopathy. HbA1c serves as a good indicator for severity of diabetic retinopathy and can be used as long-term predictor of severity of diabetic retinopathy.

Although Serum VEGF levels are elevated in the entire diabetic population irrespective of whether they had diabetic retinopathy or not.. There was no significant relationship between the serum VEGF levels and the severity of DR.

References

1. Porta M, Bandello F. Diabetic retinopathy. Diabetologia. 2002 Dec;45(12):1617-34.
2. Wang W, Lo AC. Diabetic retinopathy: pathophysiology and treatments. International journal of molecular sciences. 2018 Jun;19(6):1816.
3. Simo R, Carrasco E, Garcia-Ramirez M, Hernandez C. Angiogenic and antiangiogenic factors in proliferative diabetic retinopathy. Current diabetes reviews. 2006 Feb 1;2(1):71-98.

4. Awata T, Inoue K, Kurihara S, Ohkubo T, Watanabe M, Inukai K, Inoue I, Katayama S. A common polymorphism in the 5′-untranslated region of the VEGF gene is associated with diabetic retinopathy in type 2 diabetes. Diabetes. 2002 May 1;51(5):1635-9.
5. Frank RN. Vascular endothelial growth factor--its role in retinal vascular proliferation. N Engl J Med 1994 Dec 1;331(22):1519-20.
6. Boulton M, Foreman D, Williams G, McLeod D. VEGF localisation in diabetic retinopathy. British Journal of Ophthalmology. 1998 May 1;82(5):561-8.
7. Yang QR, Zwijsen A, Slegers H, Berghe DV. Purification and Characterization of VEGFNPF Secreted by Human Retinal Pigment Epithelial Cells. Endothelium. 1994 Jan 1;2(1):73-85.
8. Ding J, Wong TY. Current epidemiology of diabetic retinopathy and diabetic macular edema. Current diabetes reports. 2012 Aug;12(4):346-54.
9. Muhiddin HS, Kamaruddin MI, Andi Muhammad Ichsan B. Vitreous and serum concentrations of vascular endothelial growth factor and platelet-derived growth factor in proliferative diabetic retinopathy. Clinical Ophthalmology (Auckland, NZ). 2020;14:1547.
10. Ahuja S, Saxena S, Akduman L, Meyer CH, Kruzliak P, Khanna VK. Serum vascular endothelial growth factor is a biomolecular biomarker of severity of diabetic retinopathy. International journal of retina and vitreous. 2019 Dec;5(1):1-6.
11. Jenchitr W, Samaiporn S, Lertmeemongkolchai P, Chongwiriyanurak T, Anujaree P, Chayaboon D, Pohikamjorn A. Prevalence of diabetic retinopathy in relation to duration of diabetes mellitus in community hospitals of Lampang. J Med Assoc Thai. 2004 Nov 1;87(11):1321-6.
12. Niazi MK, Akram A, Naz MA, Awan S. Duration of diabetes as a significant factor for retinopathy. Pakistan Journal of Ophthalmology. 2010 Dec 31;26(4).
13. Garg P, Misra S, Yadav S, Singh L. Correlative study of diabetic retinopathy with HbA1c and microalbuminuria. International Journal of Ophthalmic Research. 2018 Aug 12;4(2):282-6.
14. Brayan J, Sundar D, Singh SB, Singh NB, Thomas M, Chandrakanth P. Relationship between levels of vascular endothelial growth factor in blood and severity of diabetic retinopathy. TNOA Journal of Ophthalmic Science and Research. 2021 Jan 1;59(1):5.
15. Manaviat MR, Afkhami M, Shoja MR. Retinopathy and microalbuminuria in type II diabetic patients. BMC ophthalmology. 2004 Dec;4(1):1-4.
16. Zehetner C, Kirchmair R, Kralinger M, Kieselbach G. Correlation of vascular endothelial growth factor plasma levels and glycemic control in patients with diabetic retinopathy. Acta ophthalmologica. 2013 Sep;91(6):e470-3.
17. Cheng YJ, Gregg EW, Geiss LS, Imperatore G, Williams DE, Zhang X, Albright AL, Cowie CC, Klein R, Saaddine JB. Association of A1C and fasting plasma glucose levels with diabetic retinopathy prevalence in the US population: implications for diabetes diagnostic thresholds. Diabetes care. 2009 Nov 1;32(11):2027-32.
18. Zou W, Ni L, Lu Q, Zou C, Zhao M, Xu X, Chen H, Zheng Z. Diabetes onset at 31–45 years of age is associated with an increased risk of diabetic retinopathy in type 2 diabetes. Scientific reports. 2016 Nov 29;6(1):1-5