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Research Article

ASSOCIATION OF HBA1C, SERUM VASCULAR ENDOTHELIAL GROWTH FACTOR, INSULIN RESISTANCE IN PREDIABETES, DIABETES, AND DIABETIC RETINOPATHY PATIENTS

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ABSTRACT

Objective: In the micro- and macrovascular pathophysiology of diabetes mellitus, vascular endothelial growth factor (VEGF) is crucial. It has become recognized as a critical element contributing to the emergence of diabetes-related retinopathy and as a critical prognostic marker for the illness.

The aim of this study is to assess the level of glycated hemoglobin (HbA1c), serum VEGF, insulin resistance (IR) in prediabetes, type-2 diabetic patients without microvascular complications, and diabetic retinopathy patients

Methods: Values for serum VEGF, HbA1c, and IR were evaluated. Spearman coefficient correlation was used to perform the correlation.

Results: Our results demonstrate that fasting insulin levels were almost completely correlated with homeostasis model assessment index of IR (HOMA-IR) (r=0.99) and highly correlated with homeostasis model assessment of β-cell function (r= −0.84). We also looked at if there was any relationship between blood VEGF levels and biochemical results (HbA1c, total cholesterol, triglycerides, low-density lipoprotein). According to a study, IR and serum levels of VEGF were considerably greater in diabetic patients than in controls.

Conclusion: Both prediabetes and type 2 diabetes mellitus (T2DM) patients had elevated serum VEGF levels, and these individuals also had favorable relationships with their HOMA-IR scores. In comparison to controls, prediabetes and T2DM patients may have higher VEGF levels due to increased IR.

Keywords: Glycated hemoglobin, Serum vascular endothelial growth factor, Insulin resistance.

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INTRODUCTION

Chronic hyperglycemia, a feature of type 2 diabetes mellitus (DM), eventually causes damage to several organs, including the kidneys, the eyes, and peripheral nerves if left untreated. Diabetes-related retinopathy, glaucoma, cataracts, abnormalities of the cornea, neuropathies, and neovascularization of the iris are among the ophthalmic consequences. The most prevalent and feared ophthalmic side effect of DM and diabetic retinopathy (DR) is now the main factor in vision loss in working-age individuals. Over 60% of DM type 2 patients will acquire DR after 20 years from the time of illness onset, and signs of DR are evident in one-third of patients at the time of diagnosis [1,2].

When plasma glucose levels fall between those of normoglycemia and diabetes, it is said to be prediabetes. About 86 million persons in the US, or one in three, were assessed by the Centers for Disease Control to have prediabetes in 2012. The yearly progression rate to diabetes is 5–10% [3], with the risk of progression being increased in older people, those with significant insulin resistance (IR), poor insulin production, and other diabetes risk factors [4].

The best methods for avoiding the development of diabetes and its accompanying problems are lifestyle and pharmaceutical therapies. After altering one's lifestyle, it was shown that β-cell function was preserved and that IR and diabetic consequences such as retinopathy, cardiovascular disease (CVD), and mortality from all causes were decreased. The U.S. Diabetes Prevention Program, the Finnish Diabetes Prevention Study, and the DA QING Diabetes Study in China all demonstrated that dietary modifications, weight loss, and increased physical activity all lowered the chance of developing diabetes [5,6].

A common result of chronically uncontrolled DM and DR causes substantial vision loss and blindness in people. Around 150 million people are already impacted by DR globally, and the World Health Organization predicts that figure will quadruple by the year 2025. The most used biomarker for detecting diabetes and prediabetes is glycated hemoglobin (HbA1c). When glucose binds to the amino-terminal group of the β subunit of hemoglobin, HbA1c is created.

HbA1c measures chronic glycemia rather than a single point in time's glucose levels. At present, the American Diabetes Association criteria for diabetes are HbA1c ≥6.5% (48 mmoL/moL) and 5.7–6.4% (39–46 mmoL/moL) for prediabetes. Increased morbidity and mortality are linked to higher HbA1c values. Higher HbA1c levels were linked to more CVD, cancer, and all-cause death in the Norfolk prospective research. Long-term prospective studies have shown that diabetic complications are directly correlated with the mean HbA1c, with a level below 6.5% (48 mmoL/moL) being associated with retinopathy. These studies include the diabetes control and complications trial, the UK prospective diabetes study group, and the epidemiology of diabetes interventions and complications study. Furthermore, retinopathy and HbA1c showed a stronger correlation than retinopathy and fasting plasma glucose (FPG). As a result, HbA1c rather than FPG may be a better indicator of microvascular problems. The signaling protein known as vascular endothelial growth factor (VEGF) is a member of a subfamily of growth factors that has a role in angiogenesis. Retinal pigment epithelium cells, pericytes, astrocytes, Muller cells, glial cells, and endothelial cells all release VEGF, which functions as a biomolecule. Blood retinal barrier collapse, capillary non-perfusion, and endothelial cell damage result from VEGF's induction of retinal intercellular adhesion molecule-1 expression and retinal leukocyte adherence. The

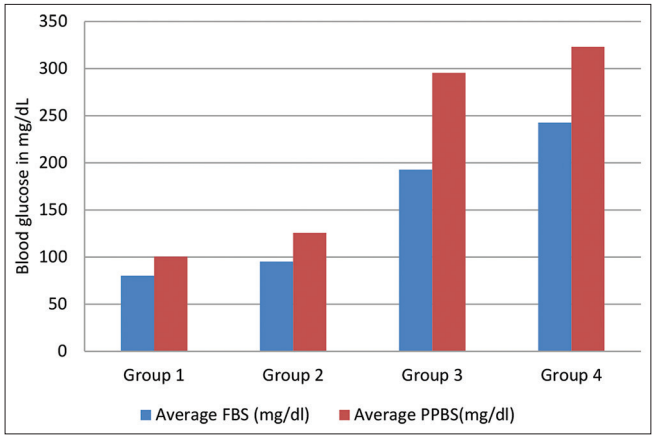

Fig. 1: Average fasting blood sugar and post-lunch blood sugar when compared in all 4 groups

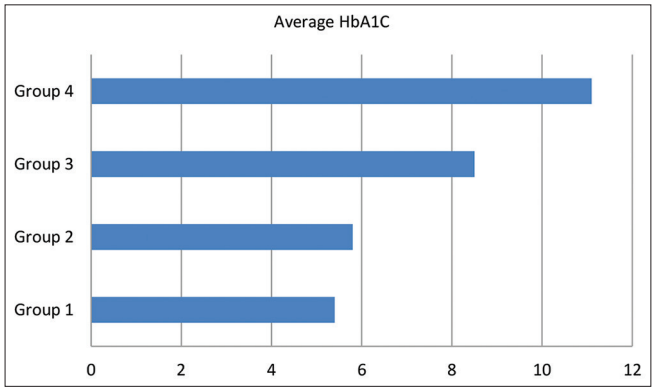

Fig. 2: Average glycated hemoglobin when compared in all 4 groups

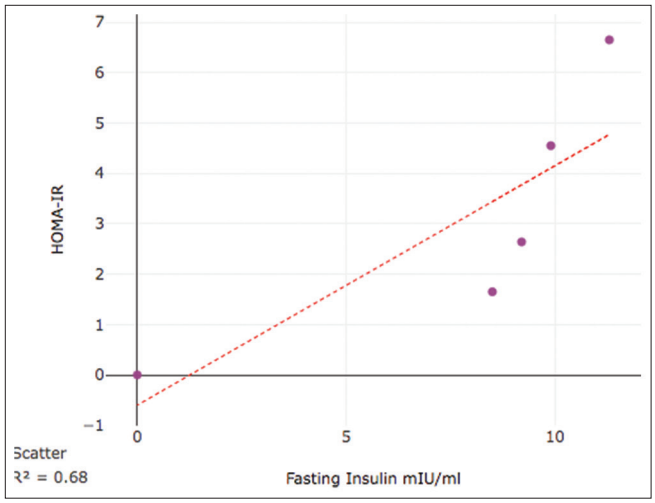

Fig. 3: Fasting insulin levels were almost completely correlated with homeostasis model assessment index of insulin resistance

main factor controlling angiogenesis and proliferation in DR is the balance between VEGF and angiogenic inhibitors [3,7].

Assessing IR and beta-cell activity using the homeostasis model, previous cross-sectional investigations on Japanese, Mexican-American, and non-Hispanic revealed that both high homeostasis model assessment index of IR (HOMA-IR) and low homeostasis model assessment of β-cell function (HOMA-β) were linked to higher prevalences of impaired

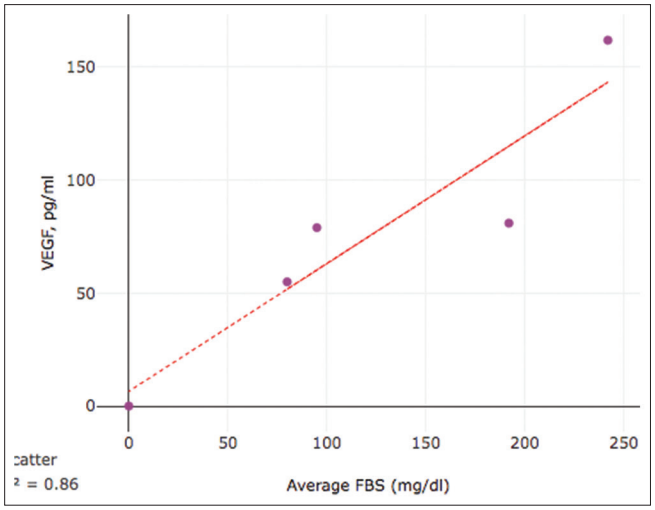

Fig. 4: Correlation between serum vascular endothelial growth factor concentrations and biochemical findings

Table 1: Comparison of the demographic details in present study groups

Parameters	Group 1	Group 2	Group 3	Group 4
Number of participants	50	50	50	50
Sex (M/F)	24/26	23/27	26/24	26/24
Average age (years)	44	46	51	52
Average duration of diabetes mellitus (years)	-	-	3 years or more	3 years or more

Age and gender-matched subjects are selected for study

Table 2: Categories in persons with and without diagnosed diabetes mellitus

Parameters	Group 1	Group 2	Group 3	Group 4
Average FBS (mg/dL)	80.21	95.11	192.78	242.43
Average PPBS (mg/dL)	100.34	125.62	295.45	323.1
Average HbA1C	5.4	5.8	8.5	11.1

Average FBS, PLBS, and HbA1C are significant when compared in all 3 groups, i.e., group, 2, 3, 4 when compared to controls, i.e., group-1. FBS: Fasting blood sugar, HbA1c: Glycated hemoglobin, PPBS: Postprandial blood sugar

glucose tolerance (IGT) and type 2 diabetes. The ability of HOMA-IR, HOMA-β, or both to predict future risk of type 2 diabetes and/or IGT in a variety of groups has been demonstrated in a number of prospective studies [8,9]. It is uncertain, nevertheless, if there is an ethnic variation in the relationship between HOMA indices and risk of type 2 diabetes. In addition, little research has been done on the relative significance of HOMA-IR and HOMA-β in relation to the risk of type 2 diabetes.

METHODS

A case–control study with four groups and a total of 200 individuals were conducted in a hospital setting. Group 1 (control group, n=50) was made up of 50 non-diabetic individuals who were randomly chosen based on their age and sex. They were free of any condition that would have an impact on the study's variables (no clinical history or investigational findings implicating any organ system). They were chosen among the medical and paramedical personnel, patient visitors, and those coming to the hospital for physical therapy.

Group-2 (n=50), prediabetes patients. HbA1C value between 5.7 and 6.4%, fasting blood sugar (FBS) - 110–126 mg/dL, and post-lunch blood sugar (PLBS) - 140–199 mg/dL.

Table 3: General parameters in patients in present study

Parameters	Group 1	Group 2	Group 3	Group 4
Average blood urea (mg/dL)	20.22	21.74	25.61	29.33
Average serum creatinine (mg/dL)	1.0	1.0	0.8	0.9
Average serum total protein (g/dL)	6.0	6.2	6.3	7.6
Average serum albumin (g/dL)	3.5	3.4	3.6	3.6
Average serum total cholesterol (mg/dL)	139.12	141.22	183.67	172.91
Average serum low-density lipoprotein cholesterol (mg/dL)	75.45	76.56	102.27	100.18
Average serum triglycerides (mg/dL)	95.19	96.23	149.23	159.15
Average urinary protein/creatinine ratio	0.1	0.1	0.5	1.1

When compared to controls and Group 2, 3, and 4, the average blood urea, total protein, total cholesterol, LDL, and triglycerides are significant.

Table 4: Correlation between VEGF and other variables

Parameters	Group 1	Group 2	Group 3	Group 4
Average FBS (mg/dL)	80.21	95.11	192.78	242.43
Fasting insulin mIU/mL	8.5	9.2	9.9	11.3
HOMA-IR	1.65	2.64	4.55	6.65
HOMA-β	71.38	79.43	43.45	35.24
VEGF, pg/mL	55.20	79.82	81.55	162.13

VEGF: Vascular endothelial growth factor, HOMA-β: Homeostasis model assessment of β-cell function

Patients in Group 3 (type 2 DM patients without any microvascular complications, n=50) were those who had had diabetes for at least 3 years were taking oral anti-diabetic medications and lifestyle changes and had no clinical or laboratory evidence of any microvascular complications related to DM. Patients in Group 4 (type 2 DR patients including all stages, n=50) are those who have had diabetes for 3 years or longer, are taking insulin, oral anti-diabetic medications, lifestyle changes, or a combination of all three, and have been diagnosed with DR (diagnosed by direct ophthalmoscopy after mydriasis) (Figure 1 and Table 1).

Exclusion criteria

Patients with type 1 DM, pregnancy or possible pregnancy, and other conditions that might significantly alter the serum protein profile, such as hepatic diseases, hematological malignancies, chronic infections and inflammations such as tuberculosis, sarcoidosis, rheumatological conditions, infectious mononucleosis, and acquired immunodeficiency syndrome, were excluded from the study (Figure 2 and Table 2).

Each participant gave written informed permission and received written information about the study. The study was approved by the ethics committee of ESIC Medical College and Hospital.

Data collection and laboratory tests

Fasting serum insulin (FINS) was measured using a chemiluminescence immunoassay using an access 2 immunoassay system (reference interval: 1.9–23 mIU/mL; Beckman Coulter). All individuals completed clinical examinations of age, sex, height, and weight (Table 3). Enzyme-linked immunosorbent assay was used to measure the levels of serum VEGF in line with the manufacturer's (KRISHGEN BioSystems) instructions (Figures 3 and 4 and Table 4).

HPLC was used to measure HbA1c.

The HOMA-IR and HOMA-β were calculated using the following equation:

$$\text{HOMA} - \text{IR} = \frac{(\text{fasting insulin} \times \text{FPG})}{22.5}$$

$$\text{HOMA} - \beta = 20 \times \frac{\text{fasting insulin}}{(\text{FPG} - 3.5)}$$

Statistics

Data were analyzed using MedCalc version 12.4. Continuous data (e.g., age, fasting blood glucose [FBG], HbA1C, total cholesterol [TC], low-density lipoprotein [LDL-C], triglycerides [TGs]) are expressed as mean±standard deviation. Because some data (i.e., TG, FINS, HOMA-IR, HOMA-β, and serum VEGF level) were not normally distributed, the values are expressed as median (interquartile range).

Before statistical analysis, non-normally distributed variables were log-transformed. The analysis of variance with *post hoc* least significant difference test was used to compare the groups. Multiple linear regression analysis and Pearson's or Spearman's rank correlation studies were used to explore relationships between the serum VEGF level and other parameters. Two-tailed $p<0.05$ was considered statistically significant.

RESULTS

Our findings show that fasting insulin levels were strongly connected with HOMA-IR and virtually fully correlated with HOMA-β. Fasting glucose had a substantial correlation with HOMA-IR and a weak correlation with HOMA-β. In addition, there was a correlation between the two HOMA indices.

We also looked at if there was any relationship between blood VEGF levels and biochemical results (HbA1c, TC, TGs, and LDL).

Spearman correlation analyses revealed significant positive correlation with HbA1c ($p=0.0002$) and a significant negative correlation with age ($p=0.0109$).

Our findings show that DM type 2 patients with DR had considerably greater serum VEGF levels than those without DR. Thus, despite the difference in VEGF levels between studies, which may represent innate demographic sensitivity, considering VEGF levels as a useful tool for risk assessment in DM type 2 patients regarding microangiopathic consequences is an interesting notion.

Traditional diabetes risk variables were more prevalent among diabetes-case individuals than among controls at baseline. According to the study, those with diabetes had substantially greater baseline levels of fasting insulin, glucose, and HOMA-IR, as well as lower HOMA-β than their matched control patients (all $p<0.0001$). Increasing fasting glucose, insulin, and HOMA-IR readings were strongly linked to an increased risk of diabetes once matching variables were taken into account.

DISCUSSION

An important regulator of angiogenesis in both healthy and pathological situations, VEGF is a growth factor that may cause angiogenesis in vascular endothelial cells. Under physiological circumstances, VEGF plays a critical role in maintaining healthy endothelial function; nevertheless, inappropriately high VEGF concentrations can lead to pathological angiogenesis.

According to a number of studies, people with peripheral vascular disease and coronary artery disease had greater blood levels of VEGF than healthy individuals. Acute myocardial infarction patients also had blood VEGF levels that were considerably greater than those of

healthy individuals; however, these levels were lowered with heparin administration or percutaneous coronary intervention. Serum VEGF levels and 10-year CVD risk both showed strong correlations with cerebrovascular accident risk scores [10].

In addition, serum VEGF levels were greater in the prediabetic group, according to our study, which indicated that serum VEGF levels in the T2DM group were considerably higher than those of controls. According to recent research from Finland, patients with prediabetes and diabetic subjects had considerably greater blood VEGF levels than did those with normoglycemia. Therefore, we assume that both IGT and diabetes patients have high cardiovascular risks.

In our investigation, the HOMA-IR value was favorably correlated with the serum VEGF level. According to multiple linear regression analyses, the HOMA-IR score was the sole reliable indicator of rising blood VEGF levels. IR has a strong link to endothelial dysfunction and CVD. Environmental/ambient oxidative stress influences changes in the production of VEGF by proinflammatory cells that have been activated. Proliferative DR is thought to neovascularize in part due to VEGF.

Clinicians currently provide biologic anti-VEGF injections intravitreally to lessen VEGF signaling in the retina. Circulating VEGF in diabetic patients may develop as a key marker for estimating the severity of diabetic renal impairment. Diabetes may cause VEGF release and neovascularization due to insufficient insulin absorption and action. In addition, VEGF overexpression can impair insulin sensitivity.

According to the findings of our study, serum levels of VEGF were significantly higher in the T2DM and IGT groups than in the control group. Serum levels of VEGF were also higher in the IGT group than in the T2DM group, although there was no statistically significant difference between the two groups. We hypothesize that in T2DM and IGT individuals, the rise in blood VEGF levels during the early stages of diabetes may have advantageous compensating effects [11,12].

Our findings show that DM type 2 patients with DR had considerably greater serum VEGF levels than those without DR. Thus, despite the difference in VEGF levels between studies, which may represent innate demographic sensitivity, considering VEGF levels as a useful tool for risk assessment in DM type 2 patients regarding microangiopathic consequences is an interesting notion.

We confirm that the risk of developing diabetes was consistently correlated with both HOMA-IR and HOMA-β derived from baseline levels of fasting insulin and glucose.

A few prospective studies have also assessed the effectiveness of HOMA-IR and HOMA- in predicting the likelihood of developing type 2 diabetes and/or IGT in the future [13,14].

Increased HOMA-IR and reduced HOMA-β have been demonstrated to strongly predict type 2 diabetes in 1,449 Mexicans over the course of a 3.5-year follow-up, in 644 Chinese over the course of a 4.5-year follow-up, and in 81 healthy first-degree relatives of African-American patients with type 2 diabetes over the course of a 6-year follow-up [15,16].

Similar results were obtained from our extensive prospective data, which further demonstrated the independent and additive relationships between HOMA-IR and HOMA-β and diabetes risk. This information highlights the significance of evaluating both IR and beta-cell activity in connection to diabetes risk.

CONCLUSION

Through this study, we can detect prediabetes early and stop its progression to diabetes as well as its consequences. Both IGT and T2DM patients had elevated serum VEGF levels, and these patients also had a favorable correlation between their HOMA-IR readings. Compared to controls, IGT and T2DM patients had higher VEGF levels, which may be partially attributed to greater IR.

The basal levels of HOMA indices, particularly HOMA-IR, were independently and consistently linked with diabetes risk in patients with prediabetes, diabetes, and DR, according to our prospective data. These potential relationships proved to be strong across many ethnic groupings.

According to our research, the HOMA indices may be used to measure IR and cell function and identify those who are at high risk for developing diabetes and who could benefit from interventions to avoid it.

Serum VEGF level is a quick, reliable laboratory test for detecting the development of DR in eyes without any signs of DR. We come to the conclusion that blood VEGF levels are a suitable biomarker for DR early identification and therapy in this ethnic group since they are substantially linked with the existence of DR. In addition, a statistical correlation between serum VEGF concentrations and various biochemical results (HbA1c, high-density lipoprotein) and demographic results (age) was discovered. It is urged that more research should be done to examine these connections in various civilizations and with other diabetes problems.

REFERENCES

1. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2014;37:S81-90.
2. Atlas D, International Diabetes Federation. IDF Diabetes Atlas. 7th ed. Brussels, Belgium: International Diabetes Federation; 2015.
3. American Diabetes Association. Standards of medical care in diabetes--2014. Diabetes Care 2014;37 Suppl 1:S14-80. doi: 10.2337/dc14-S014, PMID 24357209
4. Bansal N. Prediabetes diagnosis and treatment: A review. World J Diabetes 2015;6:296-303. doi: 10.4239/wjd.v6.i2.296, PMID 25789110
5. Gong Q, Gregg EW, Wang J, An Y, Zhang P, Yang W, *et al*. Long-term effects of a randomized trial of a 6-year lifestyle intervention in impaired glucose tolerance on diabetes-related microvascular complications: The China Da Qing Diabetes Prevention Outcome Study. Diabetologia 2011;54:300-7. doi: 10.1007/s00125-010-1948-9, PMID 21046360
6. Li G, Zhang P, Wang J, An Y, Gong Q, Gregg EW, *et al*. Cardiovascular mortality, all-cause mortality, and diabetes incidence after lifestyle intervention for people with impaired glucose tolerance in the Da Qing Diabetes Prevention Study: A 23-year follow-up study. Lancet Diabetes Endocrinol 2014;2:474-80. doi: 10.1016/S2213-8587(14)70057-9, PMID 24731674
7. Gupta N, Mansoor S, Sharma A, Sapkal A, Sheth J, Falatoonzadeh P, *et al*. Diabetic retinopathy and VEGF. Open Ophthalmol J 2013;7:4-10. doi: 10.2174/1874364101307010004, PMID 23459241
8. Han L, Zhang L, Xing W, Zhuo R, Lin X, Hao Y, *et al*. The associations between VEGF gene polymorphisms and diabetic retinopathy susceptibility: A meta-analysis of 11 case-control studies. J Diabetes Res 2014;2014:805801. doi: 10.1155/2014/805801, PMID 24868559
9. Behl T, Kotwani A. Exploring the various aspects of the pathological role of vascular endothelial growth factor (VEGF) in diabetic retinopathy. Pharmacol Res 2015;99:137-48. doi: 10.1016/j.phrs.2015.05.013, PMID 26054568
10. Laakkonen JP, Lähteenvuo J, Jauhiainen S, Heikura T, Ylä-Herttuala S. Beyond endothelial cells: Vascular endothelial growth factors in heart, vascular anomalies and placenta. Vasc Pharmacol 2019;112:91-101. doi: 10.1016/j.vph.2018.10.005, PMID 30342234
11. Saukkonen T, Mutt SJ, Jokelainen J, Saukkonen AM, Raza GS, Karhu T, *et al*. Adipokines and inflammatory markers in elderly subjects with high risk of Type 2 diabetes and cardiovascular disease. Sci Rep 2018;8:12816. doi: 10.1038/s41598-018-31144-8, PMID 30143687
12. Earle KA, Zitouni K, Nourooz-Zadeh J. Lipopolysaccharide-induced VEGF production and ambient oxidative stress in Type 2 diabetes. J Clin Endocrinol Metab 2019;104:1-6. doi: 10.1210/jc.2018-00836, PMID 30032215
13. Simó R, Sundstrom JM, Antonetti DA. Ocular anti-VEGF therapy for diabetic retinopathy: The role of VEGF in the pathogenesis of diabetic

retinopathy. Diabetes Care 2014;37:893-9. doi: 10.2337/dc13-2002, PMID 24652720

14. Carroll RM, Yu Y, VanderBeek BL. Predictive factors for patients receiving intravitreal anti-vascular endothelial growth factor for the treatment of diabetic macular edema. Eur J Ophthalmol 2020;30:72-80.
15. Felmeden DC, Spencer CG, Belgore FM, Blann AD, Beevers DG, Lip GY. Endothelial damage and angiogenesis in hypertensive patients: Relationship to cardiovascular risk factors and risk factor management. Am J Hypertens 2003;16:11-20. doi: 10.1016/s0895-7061(02)03149-7, PMID 12517677
16. Elias I, Franckhauser S, Bosch F. New insights into adipose tissue VEGF-A actions in the control of obesity and insulin resistance. Adipocyte 2013;2:109-12. doi: 10.4161/adip.22880, PMID 23805408