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Original Article

Searching for Biomarkers in Proliferative Diabetic Retinopathy: Amphiregulin and Progranulin

Proliferatif Diyabetik Retinopatide Yeni Biyomarker Arayışı: Amphiregulin ve Progranulin

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ABSTRACT

Objective: Diabetic retinopathy is a common diabetic microvascular problem. Its diagnosis and classification are based on visible changes in clinical fundus examination. However, the discovery of possible vitreous biomarkers in patients with proliferative and nonproliferative diabetic retinopathy may guide both the differentiation and degree of retinopathy. Biomarkers that will be accepted can be also a treatment target. Amphiregulin (AREG) promotes proliferative and regenerative activity and repairs most cell types by binding and activating epidermal growth factor receptors. Progranulin (PGRN) has complex functions in many physiological and pathological processes. Thus, this study aimed to report vitreous AREG and PGRN levels in patients with diabetes and proliferative retinopathy and compare the results with those without diabetes.

Methods: Thirty-three eyes of 33 patients with proliferative diabetic retinopathy and 31 eyes of 31 patients without diabetes were included in this study. Vitreous humor samples were collected from all patients at the time of pars plana vitrectomy surgery immediately before the surgical procedure. Vitreous AREG and PGRN values were determined by the ELISA method.

Results: The mean AREG and PGRN values were similar in the groups (p=0.427, p=0.459, respectively).

Conclusions: The results demonstrated that vitreous AREG and PGRN levels have no significant relationship with proliferative diabetic retinopathy.

Keywords: Amphiregulin, progranulin, vitreous, diabetes mellitus, proliferative diabetic retinopathy

ÖZ

Amaç: Diyabetik retinopati, diabetes mellitusun yaygın bir mikrovasküler komplikasyonudur. Hastalığın teşhisi ve sınıflandırılması, klinik fundus muayenesindeki gözle görülür değişikliklere dayanmaktadır. Ancak proliferatif diyabetik ve diyabetik olmayan retinopatili hastaların olası vitreus biyobelirteçlerin keşfedilmesi hem retinopatinin ayrımı hem de derecesi hakkında yol gösterici olabilir. Ayrıca kabul görecek biyomarkerler birer tedavi hedefi de olabilir. Amfiregülinin (AREG), epidermal büyüme faktörü reseptörlerini bağlayarak ve aktive ederek çoğu hücre tipinin çoğalmasını, yenilenmesini ve onarımını uyardığı bilinmektedir. Progranülin (PGRN), birçok fizyolojik ve patolojik süreçte karmaşık fonksiyonlara sahiptir. Bu çalışmada, proliferatif diyabetik retinopatili hastalarda vitreus AREG ve PGRN düzeylerinin raporlanması ve sonuçların diyabetik olmayan kişilerle karşılaştırılması amaçlandı.

Yöntemler: Bu çalışmaya 33 proliferatif diyabetik retinopatili hastanın 33 gözü ve 31 diyabetik olmayan bireyin 31 gözü dahil edildi. Pars plana vitrektomi ameliyatı sırasında cerrahi işlemden hemen önce tüm hastalardan vitreus örnekleri toplandı. Vitreus AREG ve PGRN seviyeleri ELISA yöntemi ile ölçüldü.

Bulgular: Ortalama AREG ve PGRN seviyeleri gruplarda benzerdi (sırasıyla p=0,427, p=0,459).

Sonuçlar: Vitreus AREG ve PGRN düzeylerinin proliferatif diyabetik retinopati ile anlamlı bir ilişkisinin olmadığı gösterildi.

Anahtar kelimeler: Amfiregülin, progranülin, vitreus, diabetes mellitus, proliferatif diyabetik retinopati

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INTRODUCTION

Diabetic retinopathy (DR) is a common diabetic microvascular problem. Its diagnosis and classification are based on visible changes in clinical fundus examination. If needed, treatment decision is also based on clinical examination. An inflammatory process begins long before the visible anatomical changes in which trophic factors, complements, cytokines, and many mediators are important in DR development. Inflammatory changes increase endothelial dysfunction, and the DR process starts[1,2]. The inflammatory process appears to be the main contributor to microvascular damage, which finally results with ocular complications of diabetes mellitus (DM). The activation of growth and trophic factors, cytokines, and mediators may cause inflammatory cells to move into the retinal microvasculature, contributing to DR development[3].

About 30 years ago, amphiregulin (AREG) was first identified as a member of the epidermal growth factor (EGF) family[4]. Members of the EGF family are known to stimulate cell proliferation, differentiation, and regeneration. Originally referred to as an epithelial cell-associated factor, recently, AREG was reported to express in many activated immune cells such as monocytes, brain tissue, central nervous system, ovary, testis, placenta, pancreas, heart, kidney, spleen, bone marrow, lung, colon, breast, and blood[5-7]. Many studies have reported that AREG participates in various physiological processes[8]. Currently, AREG is thought to promote the proliferation, regeneration, and repair of most cell types by binding and activating EGF receptors[9].

Progranulin (PGRN), also known as pro-epithelium, is a multifunctional protein expressed in various cell types, including immune and epithelial cells, adipocytes, neurons, reproductive organs, gastrointestinal tract, and retina[5,10-13]. PGRN has complex functions in many physiological and pathological processes[5,14]. After it was first identified in the early 90s, many studies have investigated its growth factor-like trophic properties and role in wound repair and tissue remodeling[15-17]. PGRN was found related to the pathogenesis of diabetic microangiopathies such as retinopathy and its severity because of its roles in glycometabolism, insulin resistance, and inflammatory response[18,19]. Increased serum PGRN levels have also been reported in patients with type 2 DM (T2DM) compared with individuals without DM[20-22].

To our knowledge, no data have been published regarding vitreous concentrations of AREG and PGRN in patients with DM. Thus, this study aimed to measure the intravitreal levels of AREG and PGRN in patients with proliferative DR and compare them with healthy individuals.

MATERIALS and METHODS

The study adhered to the Declaration of Helsinki's principles and was approved by the Kahramanmaras Sutcu Imam University Clinical Research Ethics Committee (decision no: 03, date: 10.10.2018). All participants signed an informed consent form. This study included 33 eyes of 33 patients with proliferative DR and 31 eyes of 31 patients without DM. The case group consisted of patients with proliferative DR who had undergone pars plana vitrectomy (PPV) surgery for tractional retinal detachment. The control group included patients without DM who had undergone PPV surgery for other reasons such as rhegmatogenous retinal detachment, macular hole, and epiretinal membrane. The exclusion criteria were as follows: history of previous PPV surgery, intravitreal hemorrhage, active inflammation, retinal laser photocoagulation in the past 3 months, intravitreal injection of corticosteroids in the past 6 months, and intravitreal injections of anti-VEGF agents in the past 3 months.

Material Collection and Handling

All vitreoretinal surgery procedures and collection of vitreous materials were performed by the same operator (M.G.). Special care was taken to avoid bleeding, which may cause contamination of the samples. In this study, 1 mL of vitreous humor was collected, transported to the laboratory on ice, and divided into two Eppendorf tubes of 0.5 mL each. Vitreous samples were stored at -80 °C following the collection.

Measurement of AREG and PGRN

Vitreous humor samples, previously frozen at -80 °C, were incubated at room temperature for 2 h before assay. After defrosting, the samples were added to microplate wells in a short time for quantitative measurement. All reagents were brought to room temperature before use. The tests were conducted at room temperature. AREG and PGRN levels were determined with the ELISA kit, according to the manufacturer's guidelines (Quantikine R&D Systems, MN, USA) with catalog nos. DAR00 and DPGRN0, respectively. The program of the Biotek_ ELx808 (Winooski, Vermont, USA) device was used to plot all concentration and absorption graph curves of the data obtained from AREG and PGRN measurements. All measurements were performed twice. The lowest detectable value of human AREG level was 3.56 pg/mL,

and the standard curve range was 15.6-1000 pg/mL. The intra and inter-assay coefficients were 8.7% and 9.3%, respectively. The lowest detectable value of the human PGRN level was 0.54 ng/mL, and the standard curve range was 1.6-100 ng/mL. The intra and inter-assay coefficients were 7.7% and 8.9%, respectively.

Statistical Analysis

SPSS Statistics version 17 was used to analyze data. The Shapiro-Wilk test was used to determine normality. Normally distributed data were compared using independent samples t-test. Nonnormally distributed data were compared using the Mann-Whitney U test. The Mann-Whitney U test was also used to compare AREG and PGRN levels. Independent samples t-test was performed to compare the age of the patients between the two groups. The chi-square test was performed to compare the sex of the patients between the groups. Statistical significance was defined as a p-value of <0.05. Minimum and maximum values of age and AREG and PGRN levels are shown in parenthesis.

RESULTS

The DM group consisted of 16 (48.5%) female and 17 (51.5%) male patients. The control group consisted of 14 (45.2%) female and 17 (54.8%) male patients. The sex distribution in both groups was comparable (p=0.808). The mean age values were 61.45±9.18 (43-79) years in the DM group and 61.54±8.54 (48-82) years in the control group. The average age of the groups was comparable (p=0.966). The mean AREG levels were 23.55±5.81 (13.71-32.88) pg/mL in the DM group and 26.76±9.20 (16.01-52.93) pg/mL in the control group. The mean AREG levels were comparable in both groups (p=0.427). The mean PGRN level was 13.78±3.87 (6.80-19.83) ng/mL in the DM group and 15.85±5.93 (8.66-32.36) ng/mL in the control group. The mean PGRN levels were comparable in both groups (p=0.459). The demographic characteristics of the patients and results are shown in Table 1. Comparison of AREGamphiregulin and progranulin levels between the diabetic and control groups was demonstrated in Figure 1. As regards vitreal AREG and PGRN levels, no significant difference was found between the groups.

DISCUSSION

The DR process starts with inflammatory changes, which will later cause endothelial dysfunction and microvascular damage[1,2]. Many inflammatory molecules, cytokines, and factors have been studied in human vitreous, and their roles in DR were investigated[3]. AREG and PGRN have previously been studied in the blood samples of patients with DM; however, no study has

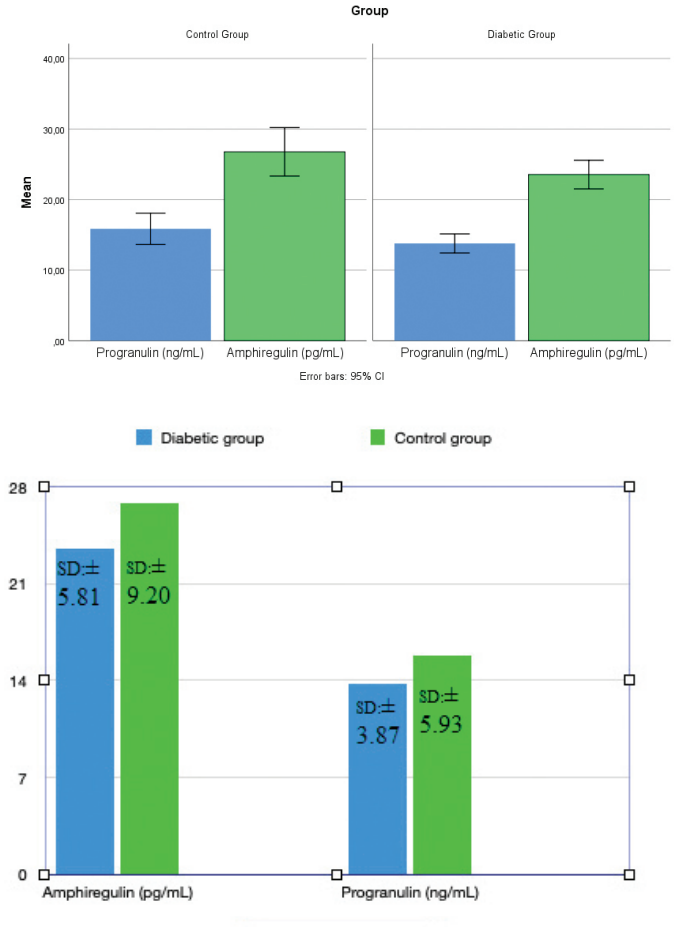

Figure 1. Comparison of amphiregulin and progranulin levels between the diabetic and control groups.

CI: Confidence interval, SD: Standard deviation

Table 1. Demographic characteristics of the patients and results.

Patient demographics	Diabetic group	Control group	p-value
Mean age	61.45±9.18	61.54±8.54	0.966
Sex	17 men	17 men	0.808
	16 women	14 women	
Mean amphiregulin levels	23.55±5.81	26.76±9.20	0.427
Mean progranulin levels	13.78±3.87	15.85±5.93	0.459

investigated the vitreous levels of these proteins in the literature[9,20-22]. This prospective study analyzed patients with PPV whose vitreous levels of AREG and PGRN were measured and compared between those with proliferative DR and those without DM. In this study, we hypothesized that vitreous AREG and PGRN levels of patients with proliferative DR would be higher than those without DM, and these molecules may be used as potential biomarkers or predictors of DR.

AREG is member of the EGF family[4]. Similar to other members of the EGF family, it plays a role in the stimulation of cell proliferation, differentiation, and regeneration by binding and activating EGF receptors[8,9]. Evidence suggests that AREG is generated in many cell types[5-7]. Harmed epithelial cells produce IL-25, IL-33, and some other cytokines that cause AREG release. AREG starts recovery from injury by binding EGF receptors and stimulating proliferation and regeneration[23,24]. Stressful conditions, such as ischemia, hypoxia, and inflammation, result in AREG expression in the brain to provide protection against neuronal cell death[25]. The retina is an extension of the central nervous system, both physically and developmentally. The central nervous system includes retinal ganglion cells and the axons that make up the optic nerve. Surface molecules and cytokines in the retina and response to injury are similar to those in the brain and spinal cord[26]. Therefore, DM-induced retinal inflammation, ischemia, and hypoxia would cause a similar response by the retina, such as upregulating AREG expression. However, we have not found a significant difference in vitreous AREG levels of patients with DM compared with those without DM.

PGRN is a multifunctional protein that acts in various physiological and pathological processes[5,14]. It is expressed in various cell types such as the retina[13]. PGRN is closely associated with DR pathogenesis and its severity by taking roles in glycometabolism, insulin resistance, and inflammatory response[18,19]. Recent studies have found that PGRN promotes IL-6 expression, which is linked to obesity, dyslipidemia, glucose metabolism, and insulin resistance[20,22,27]. T2DM is characterized by resistant response to insulin, leading to hyperglycemia[28]. In many studies, a positive correlation was reported between PGRN and hemoglobin A1c and fasting glucose[20,22,29]. Several studies have reported that serum PGRN levels are significantly increased in patients with DM[20,30,31]. Finch et al.[30] reported that the increase in serum PGRN levels was 1.4 fold in patients with DM compared to healthy controls. Xu et al.[19] also showed that PGRN levels and other inflammatory mediators such as IL-6 and TNF-α are significantly increased in the serum of patients with T2DM and micro-angiopathic complications. As mentioned in previous studies, the relationship between serum PGRN levels and diabetic microangiopathy caught our attention because no study has investigated vitreous PGRN levels in patients with DM. Thus, we hypothesized that vitreous PGRN levels would be higher in patients with DM than in those without DM. However, we have not found a significant difference in vitreous PGRN levels of patients with DM compared with healthy subjects.

The strengths of this study are the strong hypothesis and the fact that this was the first study investigating vitreous AREG and PGRN levels in patients with DM and healthy individuals. However, this study has some limitations. Simultaneous measurement of serum AREG and PGRN levels would make the results stronger. We have not assessed the presence or absence of a posterior hyaloid detachment, which was reported to affect cytokine levels[32].

To our knowledge, we demonstrated for the first time that vitreous AREG and PGRN levels have no significant relationship with proliferative DR. Further prospective research with larger populations is necessary to better understand the molecular aspect of proliferative DR.

CONCLUSION

The inflammatory process appears to be the major mechanism that contributes to microvascular damage resulting from ocular complications of DM. In various studies, blood serum AREG and PGRN levels are elevated in patients with DM. We found no significant difference in vitreous AREG and PGRN levels between patients with and without DM.

Ethics

Ethics Committee Approval: The study adhered to the Declaration of Helsinki's principles and was approved by the Kahramanmaras Sutcu Imam University Clinical Research Ethics Committee (decision no: 03, date: 10.10.2018).

Informed Consent: All participants signed an informed consent form.

Peer-review: Externally and internally peer-reviewed.

Author Contributions

Surgical and Medical Practices: B.B., S.U., Concept: B.B., M.G., Design: H.C., B.M.S., Data Collection and/or Processing: B.B., Analysis and/or Interpretation: S.U., G.K., Literature Search: M.G., Writing: S.U., B.M.S.

Conflict of Interest: The authors have no conflict of interest to declare.

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REFERENCES

1. Antonetti DA, Klein R, Gardner TW. Diabetic retinopathy. N Engl J Med. 2012;366:1227-39.
2. Bromberg-White JL, Glazer L, Downer R, Furge K, Boguslawski E, Duesbery NS. Identification of VEGF-independent cytokines in proliferative diabetic retinopathy vitreous. Invest Ophthalmol Vis Sci. 2013;54:6472-80.
3. Kaul K, Hodgkinson A, Tarr JM, Kohner EM, Chibber R. Is inflammation a common retinal-renal-nervepathogenic link in diabetes? Curr Diabetes Rev. 2010;6:294-303.
4. Shoyab M, Plowman GD, McDonald VL, Bradley JG, Todaro GJ. Structure and function of human amphiregulin: a member of the epidermal growth factor family. Science. 1989;243:1074-6.
5. He Z, Bateman A. Progranulin (granulin-epithelin precursor, PC-cell-derived growth factor, acrogranin) mediates tissue repair and tumorigenesis. J Mol Med (Berl). 2003;81:600-12.
6. Mograbi B, Rochet N, Imbert V, et al. Human monocytes express amphiregulin and heregulin growth factors upon activation. Eur Cytokine Netw. 1997;8:73-81.
7. Plowman GD, Green JM, McDonald VL, et al. The amphiregulin gene encodes a novel epidermal growth factor-related protein with tumor-inhibitory activity. Mol Cell Biol. 1990;10:1969-81.
8. Schneider MR, Wolf E. The epidermal growth factor receptor ligands at a glance. J Cell Physiol. 2009;218:460-6.
9. Avraham R, Yarden Y. Feedback regulation of EGFR signaling decision making by early and delayed loops. Nat Rev Mol Cell Biol. 2011;12:104-17.
10. Nguyen AD, Nguyen TA, Martens LH, Mitic LL, Farese RV Jr. Progranulin: at the interface of neurodegenerative and metabolic diseases. Trends Endocrinol Metab. 2013;24:597-606.
11. Tolkatchev D, Malik S, Vinogradova A, et al. Structure dissection of human progranulin identifies well-folded granulin/epithelium modules with unique functional activities. Protein Sci. 2008;17:711-24.
12. Ong CH, Bateman A. Progranulin (granulin-epithelin precursor, PC-cell derived growth factor, acrogranin) in proliferation and tumorigenesis. Histol Histopathol. 2003;18:1275-88.
13. Tsuruma K, Yamauchi M, Sugitani S, et al. Progranulin, a major secreted protein of Mouse adipose-derived stem cells, inhibits light-induced retinal degeneration. Stem Cells Transl Med. 2014;3:42-53.
14. Liu CJ. Progranulin: a promising therapeutic target for rheumatoid arthritis. FEBS Lett. 2011;585:3675-80.
15. Daniel R, Daniels E, He Z, Bateman A. Progranulin (acrogranin/PC cell-derived growth factor/granulin-epithelin precursor) is expressed in the placenta, epidermis, microvasculature, and brain during murine development. Dev Dyn. 2003;227:593-9.
16. Bateman A, Bennet HP. The granulin gene family: from cancer to dementia. Bioessays. 2009;31:1245-54.
17. Zhou J, Gao G, Crabb JW, Serrero G. Purification of an autocrine growth factor homologous with mouse epithelium precursor from a highly tumorigenic cell line. J Biol Chem. 1993;268:10863-9.
18. Tang W, Lu Y, Tian QY, et al. The growth factor progranulin binds to TNF receptors and is therapeutic against inflammatory arthritis in mice. Science. 2011;332:478-84.
19. Xu L, Zhou B, Li H, et al. Serum levels of progranulin are closely associated with microvascular complications in type 2 diabetes. Dis Markers. 2015;2015:357279.
20. Qu H, Deng H, Hu Z. Plasma progranulin concentrations are increased in patients with type 2 diabetes and obesity and correlated with insulin resistance. Mediators Inflamm. 2013;2013:360190.
21. Tönjes A, Fasshauer M, Kratzsch J, Stumvoll M, Blüher M. Adipokine pattern in subjects with impaired fasting glucose and impaired glucose tolerance in comparison to normal glucose tolerance and diabetes. PLoS One. 2010;5:e13911.
22. Youn BS, Bang SI, Klöting N, et al. Serum progranulin concentrations may be associated with macrophage infiltration into omental adipose tissue. Diabetes. 2009;58:627-36.
23. Li L, Yi Z, Seno M, Kojima I. Activin A and betacellulin: effect on the regeneration of pancreatic beta-cells in neonatal streptozotocin-treated rats. Diabetes. 2004;53:608-15.
24. Mashima H, Ohnishi H, Wakabayashi K, et al. Betacellulin and activin A coordinately convert amylase-secreting pancreatic AR42J cells into insulin-secreting cells. J Clin Invest. 1996;97:1647-54.
25. Ogata T, Dunbar AJ, Yamamoto Y, Tanaka Y, Seno M, Kojima I. Betacellulin-delta4, a novel differentiation factor for pancreatic beta-cells, ameliorates glucose intolerance in streptozotocin-treated rats. Endocrinology. 2005;146:4673-81.
26. London A, Benhar I, Schwartz M. The retina as a window to the brain-from eye research to CNS disorders. Nat Rev Neurol. 2013;9:44-53.
27. Matsubara T, Mita A, Minami K, et al. PGRN is a key adipokine mediating high fat diet-induced insulin resistance and obesity through IL-6 in adipose tissue. Cell Metab. 2012;15:38-50.
28. American Diabetes Association. Classification and diagnosis of diabetes. Diabetes Care. 2015;38:8-16.
29. Li H, Zhou B, Xu L, et al. Circulating PGRN is significantly associated with systemic insulin sensitivity and autophagic activity in metabolic syndrome. Endocrinology. 2014;155:3493-507.
30. Finch N, Baker M, Crook R, et al. Plasma progranulin levels predict progranulin mutation status in frontotemporal dementia patients and asymptomatic family members. Brain. 2009;132:583-91.
31. Faerch K, Vaag A, Witte DR, Jørgensen T, Pedersen O, Borch-Johnsen K. Predictors of future fasting and 2-h post-OGTT plasma glucose levels in middle-aged men and women-the Inter99 study. Diabet Med. 2009;26:377-83.
32. Takahashi H, Nomura Y, Tan X, Fujino Y, Kawashima H, Yanagi Y. Effects of posterior vitreous detachment on aqueous humor levels of VEGF and inflammatory cytokines. Br J Ophthalmol. 2015;99:1065-9.