Original Article

Circulating adropin and vascular endothelial growth factor receptor-2 levels in age-related macular degeneration and T2DM patients—A cross-sectional study

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Abstract

Background: Macular drusen formation and angiogenesis are the two chief processes associated with age-related macular degeneration. Adropin and vascular endothelial growth factor receptor-2 (VEGFR-2) may be involved in these pathologies. By altering lipid metabolism, adropin may contribute in the early stages of age-related macular degeneration (AMD). VEGFR-2 may participate in the later form of AMD, by promoting angiogenesis. This study compared the circulatory levels of adropin and VEGFR-2 in AMD and patients without AMD and assessed their association with disease severity, to understand their possible role in AMD. **Objectives:** This study aimed to assess and compare the serum levels of adropin and VEGFR-2 in patients with AMD and type 2 diabetes patients without AMD, and, to investigate the correlation between these two parameters with disease severity. **Methods:** Our study involves two groups of 39 each. Group A (age-related macular degeneration) and Group B (diabetes patients without age-related macular degeneration). Routine parameters fasting blood sugar (FBS), lipid profile, and liver f*unction* tests (LFT) were estimated by using autoanalyzer. Serum adropin and VEGFR-2 were assessed by ELISA. **Results:** Among the basic parameters, systolic blood pressure and fasting blood glucose alone were significantly different across the groups. We did not find significant alterations in adropin and VEGFR-2 levels between the study groups. Our lipid profile parameters (triglycerides and total cholesterol) have significant positive association. VEGFR-2 showed a positive correlation with the severity of AMD. Adropin did not exhibit any correlation with disease severity and with VEGFR-2. **Conclusion:** We could not find any observable alterations of statistical significance, in adropin and VEGFR-2 levels. VEGFR-2's correlation with disease severity could be important. Adropin might have subtler roles in AMD, though not evident from our study, and requires a deeper observation at the molecular level to elucidate its function.

Keywords: Adropin, age-related macular degeneration, angiogenesis, drusen, VEGFR-2

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Introduction

Age-related macular degeneration (AMD) is the leading cause of blindness in elderly people.[1] In the early stage, yellowish-white deposits- drusen, form in the retinal pigment epithelial layer (RPE). In the intermediate stage, they become more confluent. The last

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stage is characterized by choroidal neovascularization (CNV) or retinal angiomatous proliferation (RAP-lesions) in WET AMD. DRY AMD is characterized by geographic atrophy occurring in the outer retina; atrophic lesions expand across the macula, leading to complete loss of the neuroretinal tissue.[2,3]

The accumulation of damage occurs at an age where evolutionary selection is weakening and thus, aging tissue cannot withhold these stochastic damages.[4] The prime factors contributing to AMD include: 1) age-related decline in autophagy; 2) age-dependent endothelial stress; 3) decline in blood perfusion to the retina; 4) damage in the blood retinal barrier (BRB); 5) inflammatory processes, occurring locally in the choroid, the retinal pigment epithelium (RPE), and the neuroretina.[5]

The peptide hormone, adropin, is known for its glucose-lipid homeostasis in various tissues. It is a function of age and the biological clock governing diet consumption.[6] It is a highly conserved polypeptide, encoded by the energy homeostasis associated gene (Enho), which is highly expressed in highly metabolic tissues, such as, liver, brain, skeletal muscle, and endothelium.[7] Adropin participates in regulating lipid accumulation in highly specialized cells, including the retina, and acts through modulating the fatty acid oxidation (FAO) pathway. Retinal cells have a huge dependence on FAO for energy while aerobic glycolysis is utilized mainly for retinal outer segment phospholipid synthesis.[8,9] The mechanism of adropin's role in FAO seems to have conflicting results[10-12] and might be central to understanding FAO's contribution to AMD.

In rat brain tissue, expression of adropin and endothelial nitric oxide synthase (eNOS) declines with age. eNOS attenuates endothelial oxidative damage, and accordingly showed a negative correlation with free radical damage.[7] A similar concomitant decline of adropin and eNOS, in an age-dependent manner, was observed in skeletal muscle feed arteries.[13] This study observed a mechanistic link between adropin and eNOS. Endothelium-dependent arterial vasodilatation declines with age, and could be restored with adropin treatment. Blocking eNOS ablates the positive effects of adropin treatment, suggesting that adropin exerts a vaso-protective role through eNOS.

Endothelial cells incubated with adropin, also exhibit increased eNOS expression. Adropin may stimulate the activation of eNOS via phosphorylation of its amino acid residues Ser^{1177} and Ser^{437}, mediated through VEGFR-2/PI3K/Akt or VEGFR-2/ERK1/2 pathways.[14]

VEGFR2, a tyrosine kinase receptor, is crucial to VEGF-induced angiogenesis. Trafficking of VEGFR2 in response to membrane binding of VEGF in endothelium activates VEGFR2 to form a coding template for the recruitment of angiogenic downstream signaling proteins.[15-17] Blocking VEGFR2 prevented VEGF-induced Akt phosphorylation and angiogenic tube formation.[18] VEGFR2 may be involved in vascular leakage and choroidal *neovascularization* (CNV) in AMD.[19] But, adropin could protect against endothelial barrier dysfunction during ischemic conditions.[20] The relationship of adropin with VEGFR-2 is, therefore, crucial to understand age-dependent alterations in angiogenic and vaso-protective mechanisms in AMD. Since Lovren *et al.*[14] observed the relation between adropin and VEGFR2 in endothelial cells back in 2010, not much work has progressed in this direction. Hence, we aimed to observe the relationship between adropin and VEGFR-2, and their association with severity of AMD.

Materials and Methods

This is a cross-sectional study conducted by the Department of Biochemistry, JIPMER combined with the Department of Ophthalmology, JIPMER between the period of December 2018 and March 2020. Ethical approval was obtained from the institute ethics committee (JIP/IEC/2018/0347), and informed written consent from was obtained from all the patients participants. All study procedures followed complied with the Helsinki Declaration of 1975, as revised in 2000.

Study protocol

The study consists of two groups, 39 in each. Group A includes age-related macular degeneration (Cases) and Group B includes diabetic patients without age-related macular degeneration (Controls).

Inclusion criteria

Patients with dry or wet type AMD, and T2DM aged above 50–60 years, attending medical consultation in JIPMER hospital, Puducherry, India.

Exclusion criteria

Patients with heart disease, renal/hepatic failure, acute infection, hematologic disorder, systemic autoimmune disease, and other retinal diseases were excluded. Patients on anti-lipidemic drugs were also excluded.

Baseline demographics and clinical data were collected after obtaining informed written consent from each study participants and according to the study criteria patients were recruited. About 5 mL of fasting venous blood sample was drawn from all patients and collected in tubes free of anticoagulant. The serum was separated by centrifugation at 3000 rpm for 10 min at room temperature. The routine biochemical parameters namely, fasting blood glucose and lipid profile were analyzed by the clinical chemistry autoanalyzer (Beckman Coulter AU-680). The remaining serum sample was estimated for Adropin and VEGFR-2 by using a commercially available ELISA kit (Fine test, China). LDL-cholesterol was calculated by using the following Fried Wald's formula.

Statistical analysis

The patient demographic data (age, BMI, and blood pressure), biochemical data (fasting blood glucose, lipid

profile, liver function test) and the outcome variable data (adropin and VEGFR-2) were expressed as mean ± standard deviation and median with interquartile range, as appropriate to the type of distribution. Comparisons of the data were performed using independent t-test or Mann Whitney U test as appropriate. Correlation among study parameters was performed using Spearman's rank correlation test. The relationship of the study parameters with disease severity was assessed using one-way Anova test. *P* value < 0.05 is considered statistically significant. SPSS version 20 was used for all statistical tests.

Results

The comparison of demographic parameters between cases and controls are given in Table 1. Controls have significantly higher levels of systolic blood pressure (SBP) in comparison to cases. We did not observe any statistical significance in the comparison of the other demographic data.

A comparison of biochemical parameters between cases and controls is given in Table 2. We observed significant difference in the levels of only fasting blood glucose, and not in the other biochemical parameters assessed in the patient serum.

Table 1: Demographic data between two groups

Parameters	Cases (*n*=39)	Controls (*n*=39)	*P*
Age (years)	62.00 (52.00-73.00)	62.00 (54.00-71.00)	0.886
Height (cm)	159.00 (155.00-163.00)	160.00 (155.00-161.00)	0.821
Weight (kg)	62.00 (60.00-68.00)	64.00 (60.00-67.00)	0.755
BMI (kg/m^2)	24.65 (22.58-27.68)	25.39 (24.12-26.56)	0.490
SBP (mmHg)	120.00 (110.00-120.00)	130.00 (120.00-130.00)	0.002*
DBP (mmHg)	80.00 (80.00-90.00)	90.00 (80.00-90.00)	0.344

Data were presented as median with interquartile range;*P value<0.05 considered as statistically significant: BMI-body mass index, SBP-systolic blood pressure, DBP-diastolic blood pressure

Comparison of major study parameters of study groups are given in Table 3. We did not find any statistically significant differences in the levels of the two main study parameters. Adropin shows an elevation in cases in comparison to control, but it is not significant.

Correlation of study parameters within cases are given in Table 4. In Table 4a, we observe a positive correlation between the two lipid profile parameters, total cholesterol and triglycerides, assessed in the patients with AMD.

We attempted to identify any association between the levels of adropin and VEGFR-2. In Table 4b, we show our results that there was no statistically significant relationship, although they were bordering on the possibility of a positive correlation.

Correlation between adropin and VEGFR-2 with disease severity is given in Table 5. We found a significant correlation between the severity of AMD with the levels of VEGFR-2 but not with respect to adropin.

Discussion

Adropin is involved in both energy metabolism, and in aging-related systemic changes. Hence, we aimed to evaluate its association with AMD. Being known for the neovascularization events, we compared AMD with a similar disease involving angiogenesis, i.e., type 2 diabetes mellitus.

Our study population exhibited uniformity of age, gender, and BMI. There was a significant increase in the systolic blood pressure in controls. Among the biochemical parameters, blood glucose level was elevated with statistical significance in the T2DM group. Adropin and VEGFR2 had no significant

Table 2: Biochemical parameters of study participants

Parameters	Cases (*n*=39)	Controls (*n*=39)	*P*
FBS (mg/dL)	141.00 (95.00-224.00)	193.00 (151.00-254.00)	0.017*
Total cholesterol (mg/dL)	175.00 (145.00-220.00)	165.00 (137.00-203.00)	0.353
HDL (mg/dL)	42.00 (38.00-49.00)	41.00 (36.00-50.00)	0.774
LDL (mg/dL)	114.00-(93.00-151.00)	103.00 (83.00-133.00)	0.459
TG (mg/dL)	155.00 (87.00-179.00)	145.00 (110.00-225.00)	0.136
Albumin (g/dL)	4.200 (4.00-4.50)	4.10 (3.90-4.30)	0.163
Total bilirubin (mg/dL)	0.580 (0.420-1.010)	0.500 (0.370-0.690)	0.130
Direct bilirubin (mg/dL)	0.100 (0.070-1.010)	0.090 (0.060-0.130)	0.357
AST (IU/L)	23.00 (19.00-0.160)	21.00 (18.00-28.00)	0.289
ALT (IU/L)	20.00 (14.00-28.00)	19.00 (13.00-29.00)	0.734
ALP (IU/L)	93.00 (77.00-110.00)	101.00 (85.00-123.00)	0.165

Data were expressed as median with interquartile range: *P value <0.05 considered as statistically significant: FBS-fasting blood sugar, HDL-C-high density lipoprotein, LDL-C-low density lipoprotein, TG-triglycerides, AST-aspartate transaminase, ALT-alanine transaminase, ALP-alkaline phosphatase

Table 3: Assayed special parameters among the two groups

parameter	Cases (*n*=39)	Controls (*n*=39)	*P*
Adropin (Pg/mL)	886.10 (765.92-943.77)	812.34 (703.99-912.24)	0.05
VEGFR-2 (Pg/mL)	1301.12 (608.29-2867.33)	1839.668 (1207.77-2777.60)	0.133

Data were expressed in terms of median with interquartile range. P value <0.05- statistically significant: VEGFR-2-vascular endothelial growth factor receptor-2

Table 4: Correlation among study parameters within cases

(4a). total cholesterol Vs HDL-C, LDL-C and TG

Cases (*n*=39)	HDL-C (mg/dL)		LDL-C (mg/dL)		TG (mg/dL)	
	r	*P*	*r*	*P*	*r*	
Total cholesterol (mg/dL)	1.00	0.385	0.951	0.666	0.522	0.001*

Data were expressed by using Spearman's rank correlation test:**P* value<0.05 - considered as statistically significant: HDL-C- High density lipoprotein cholesterol, LDL-C- Low density lipoprotein cholesterol, TG- Triglyceride

(4b). Adropin Vs VEGFR-2

Cases (*n*=39)	VEGFR-2 (pg/mL)	
	r	*P*
Adropin	0.305	0.05

Data were expressed by using Spearman's rank correlation test: **P* value<0.05 - considered as statistically significant

Table 5: Correlation between adropin and VEGFR-2 with disease severity

Outcome variables	*F*	*P*
Adropin (pg/mL)	2.085	0.132
VEGFR-2 (pg/mL)	3.339	**0.041***

Correlation data were shown by using one way anova; **P* value<0.05 - considered as statistically significant; VEGFR-2 shows a significant positive correlation with disease severity were as adropin does exist a significant correlation with disease severity

difference between the cases and controls. We expected an increase in adropin levels among the cases that related to the pathogenesis of drusen formation in dry AMD.[10] Previous work had identified that adropin can exert its vaso-protective function by increasing VEGFR2 expression. However, concordant with Ornek *et al.*, we observed no significant change in the serum levels of adropin and VEGFR2 in AMD.[21] The absence of a difference in VEGFR-2 could be due to the nature of the study groups, where, both T2DM, as well as AMD are known to have upregulated angiogenic activities in the system.

An interesting trend has been observed in our study, and it was the apparent blood glucose-lowering trend of adropin among the cases. Adropin is at the threshold of significance ($P = 0.05$), exhibiting the expected increase among the cases, though not significantly. The apparently higher adropin could be assumed to have a role in this regard though this observation has to be thoroughly evaluated.[22]

Lipid profile parameters exhibited elevation, indicating the prevalence of dyslipidemia in both study groups. The abnormal lipid profile could be postulated as a function of adropin.[10] The mechanism by which adropin modulates lipid metabolism, however, needs to be determined due to conflict between a theory of reduction in fatty acid uptake and theory of active beta-oxidation.[10,11] The effect of adropin in altering the lipid profile did not reflect in our study, as adropin and lipid profile parameters were not associated. Among the cases, there was a strong statistical correlation observed positively, among total cholesterol, HDL-C, LDL-C, and TG. Abnormal lipid metabolism has long had a suspected role in AMD. Effect of adropin in retinal lipid metabolism could, therefore, shed light upon lipid-mediated AMD pathogenesis.

Analysis of outcome variable association with disease severity shows that there is a positive association of VEGFR2 levels with disease severity, as previously observed in the literature.[15,16,18] However, adropin does not exhibit a significant association. Adropin was also not significantly in association with VEGFR2. In a serum analysis such as ours, finer aspects of association may be hidden due to unavoidable confounders in a systemic level. The effect may be revealed better at a molecular level, in a larger cohort, as literature has strong evidence in metabolic diseases to support this association. One assumption we make, however, is that there may be multitudes of other molecules interacting with adropin and VEGFR-2, and as such may differentially alter the expected functional associations. E.g. Possible differential interactions like VEGF-VEGFR2, Adropin-VEGFR2, Adropin-VEGF interactions. As a step further, studies comparing adropin, VEGF, and VEGFR2 levels may need to be performed in AMD, in a larger cohort, to understand their alterations in circulation.

Skeletal muscle (in T2DM) and retina (in AMD), are much similar in their energy consumption, where age-dependent decline in adropin and its downstream effector, VEGFR-2; can potentially result in metabolic derangements. The roles of adropin and VEGFR-2 might be quite similar across T2DM and AMD, and would be beneficial if studied in larger cohorts. Our study has thus observed the data comparing the effects of chronic derangements arising in different metabolically active cells, affected in two different etiologies, i.e., age and metabolism. From this study, we understand that, metabolic derangements in the retina can be approached as in T2DM in patients to appreciate age-induced changes in retinal energy metabolism.

Conclusion

The current study observed the comparison of adropin and VEGFR-2 levels, across an aging-related and a nonageing-related disease, i.e., AMD and T2DM. We did not find a significant difference in adropin and VEGFR-2 between the study groups. But adropin did exhibit an increasing trend in the AMD group. We found the prevalence of dyslipidemia in both groups. Only VEGFR-2 was associated with the severity of disease and not adropin. Our study hints at the possibility of similar

metabolic alterations occurring in both diseases. A differential observation in the VEGFR2 association, backed by literature suggests age-dependent modulations that might govern disease progression in AMD. Further studies at a molecular level should be performed to validate the usefulness of this hormone in AMD.

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Conflicts of interest

There are no conflicts of interest.

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