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Article

Sex Differences in RANTES (CCL5) in Patients With Intermediate Age-Related Macular Degeneration (AMD) and Controls With no AMD

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Purpose: To determine if there are sex differences in levels of regulated upon activation, normal T cell expressed and secreted (RANTES) in patients with intermediate age-related macular degeneration (iAMD) and in controls with no AMD.

Methods: Patients with iAMD and controls defined by multi-modal imaging were recruited into a Colorado AMD registry. Plasma levels of the chemokine RANTES were measured using a multiplex assay. A nonparametric (rank-based) regression model was fit to RANTES with a sex by AMD category interaction.

Results: The plasma level of RANTES was significantly higher in the control group in comparison with the iAMD group. When moderated by sex, RANTES was significantly lower ($P = 0.005$) in males (median, 4525.6 pg/mL; interquartile range, 2589–7861 pg/mL) compared with females (median, 6686 pg/mL; interquartile range, 3485–12488 pg/mL) within the iAMD cohort. No significant difference was found in levels of RANTES between males and females in the control group.

Conclusions: We found that levels of RANTES were moderated by sex in cases with iAMD with lower levels in males compared with females. The findings illustrate the importance of including sex as a biological variable in AMD research. There is a need for further studies of RANTES, stratified by sex, in the advanced phenotypes of AMD.

Translational Relevance: The biomarker RANTES identified in the plasma of patients with iAMD reflects systemic alterations when stratified by sex.

Introduction

The importance of incorporating the critical role of biological sex (being male or female) and gender (including social and cultural factors) into biomedical research has emerged as an area of importance by the National Institutes of Health, the Women's Health Initiative, the Office of Research on Women's Health, and other organizations involved in studies of the health of women over the lifespan.[1–3] It is well-established that there are sex disparities in cardiovascular disease, autoimmune disorders, and several other diseases.[4,5] Moreover, there are sex differences in the presentation of disease and in the response to treatment.[6] In more recent times, studies from the COVID-19 pandemic have demonstrated sex differences in adverse outcomes of COVID-19 infection and

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in the postacute sequelae of the disease.[7,8] As comprehensively reviewed by Miller et al.,[9] presenting data not stratified by sex may obscure important sex-related differences in outcomes. It is, therefore, important to include sex as a biological variable in the study design, analysis, and reporting of study results.[9]

In the area of eye disease, women comprise the majority of individuals who are blind or visually impaired.[10] However, the role of sex is understudied in diseases involving the eye.[11] The research focus of our group is AMD and specifically intermediate AMD (iAMD),[12,13] an early form of AMD.[14] Established risk factors for AMD include age, race, smoking, hypertension, obesity, a family history of AMD, dysregulation of the immune system, and multiple genetic variants.[15–17] It is noteworthy that the relationship of sex with AMD is uncertain.[15,17,18]

Inflammation has been shown to have a prominent role in the development of AMD.[15,19] Indeed, our group has conducted several studies evaluating inflammation in patients with iAMD and found compelling evidence that systemic inflammatory biomarkers distinguished patients with and without iAMD.[12,13] Recently, we described a novel role for the chemokine regulated on activation, normal T cell expressed and secreted (RANTES)/CCL5 in iAMD.[20] In our study comparing RANTES levels in cases with iAMD to controls with no AMD, we found significantly higher levels of RANTES in controls compared with cases with iAMD.[20] The purpose of the epidemiological research described herein was to build on this research and determine if there are sex differences in levels of RANTES. This area of research has not been studied previously in this phenotype of AMD.

Methods

This study was conducted on patients with iAMD, whose records and samples were part of an AMD research registry and repository developed by the Division of Ophthalmic Epidemiology, Department of Ophthalmology at the University of Colorado (described in detail elsewhere).[13,21–23] The registry includes patients with AMD receiving care at the retina clinics of the UCHealth Sue Anschutz-Rodgers Eye Center and control postcataract surgery patients with no AMD. This registry conforms with the Declaration of Helsinki and was approved by the Colorado Multiple Institutional Review Board. The informed consent, recruitment, and exclusion and inclusion criteria are described in detail in other registry-related AMD research.[13,21] In brief, each patient is consented for review of the medical history, collection of plasma

and serum for biomarker studies, and disease phenotype classification[14] after an assessment of multimodal imaging (ophthalmic coherence tomography, color fundus photography, and autofluorescence).[13,23] Patients who are between 55 and 99 years of age, have AMD in one or both eyes, and have the capacity to provide consent are eligible for inclusion in the registry. Exclusion criteria are terminal illness, active ocular inflammatory disease, prior treatment with antivascular endothelial growth factor injections, panretinal photocoagulation, and branch and central retinal vein occlusion.

Further exclusion criteria include proliferative diabetic retinopathy, nonproliferative diabetic retinopathy, diabetic macular edema, cystoid macular edema, macula-off retinal detachment, central serous retinopathy, full-thickness macular hole, ocular melanoma, pattern or occult macular dystrophy, macular telangiectasia, corneal transplant, drusen not caused by AMD, current systemic treatment for cancer, or any serious mental health or advanced dementia issues. Control patients are cataract surgery patients enrolled 1 month after cataract surgery who have no evidence of AMD by review of multimodal imaging.[13,21,23] Information on demographic factors, patient comorbidities, and review of retinal imaging is entered into a secure web-based REDCap database.

Image Review

The images on cases and controls were reviewed by two vitreoretinal specialists, focusing on the examination of the anatomic macula, which includes the entire area between the retinal vascular arcades. The images were classified into early, iAMD or advanced AMD using the classification described by the Beckman Initiative for Macular Research Classification Committee.[14] A third vitreoretinal specialist resolved any discrepancies. Based on this classification,[14] iAMD, the focus of this study, was defined as pigmentary abnormalities with at least medium drusen or large drusen (>125 μm) in either eye with no indication of advanced AMD in either eye as evaluated by multimodal imaging.

Collection and Processing Blood Samples

For this study, we collected a plasma sample from each patient. The plasma ethylenediaminetetraacetic acid tube was spun at 3000 rpm in a chilled (4° C) centrifuge for 10 minutes to isolate plasma. The plasma was then pipetted into aliquots and immediately stored in a −80° C freezer. Aliquots of plasma were transferred to the laboratory for measurement of RANTES.

Measurement of Plasma RANTES

RANTES was measured at the Clinical Translational Core laboratory, located at the Children's Hospital Colorado. Multiplex assays were completed using multiplex kits produced by the R&D Systems that use color-coded microparticles coated with analyte-specific antibodies that are analyzed on dual-lase suspension array platforms. We examined 150 microliters of plasma using a magnetic bead-based multiplex method and read on a Luminex FlexMap platform. All samples had an acceptance threshold coefficient of variance of less than 15% and were performed in duplicate.

Statistical Analysis

Patient characteristics were compared between groups using a two-sample *t*-test for continuous variables and a χ^2 test or Fisher's exact test, when indicated, for categorical variables. A nonparametric (rank-based) regression model was fit to RANTES with a sex by AMD category interaction. Least square means were used to evaluate the pairwise comparisons that included iAMD cases versus control, male versus female controls, male versus female cases, male controls versus male cases, and female controls versus female cases. In addition, a sensitivity analysis was performed adjusting for age and family history of AMD as covariates. All analyses were performed using SAS version 9.4 (The SAS Institute, Cary, NC).

Results

The differences in demographic characteristics and select comorbidities between controls ($n = 100$) and iAMD ($n = 211$) patients are shown in Table 1. There were 63 female control subjects and 129 female subjects in the iAMD cohort. The iAMD group had a higher mean age and higher rates of family history of AMD

Table 1. Differences in Clinical Characteristics in iAMD Cases and in Controls With no AMD

	Control ($n = 100$)	iAMD ($n = 211$)	P Value
Sex, female	63 (63%)	129 (61%)	0.75
Family history of AMD			<0.01
None	79 (79%)	109 (52%)	
Yes	16 (16%)	73 (35%)	
Uncertain	5 (5%)	29 (14%)	
Age, mean (SD)	74.6 (4.4)	76.6 (7.2)	0.01
Body mass index, mean (SD)	27.1 (5.4)	26.9 (5.3)	0.80
	$n = 98$	$n = 202$	
BMI categories	$n = 98$	$n = 202$	0.34[†]
Underweight	2 (2%)	4 (2%)	
Normal weight	42 (43%)	73 (36%)	
Overweight	28 (29%)	79 (39%)	
Obese	26 (27%)	46 (23%)	
Smoking			0.52[†]
Never	51 (51%)	97 (46%)	
Current	1 (1%)	6 (3%)	
Former	48 (48%)	108 (51%)	
History of			
Treated hypertension	55 (55%)	113 (54%)	0.81
Kidney disease	12 (12%)	26 (12%)	0.94
Peripheral vascular disease	20 (20%)	34 (16%)	0.40
Cardiac disease	32 (32%)	74 (35%)	0.59
Medications			
NSAIDs	15 (15%)	25 (12%)	0.44
Aspirin	42 (42%)	95 (45%)	0.62

NSAIDs, nonsteroidal anti-inflammatory drugs.

P values obtained from χ^2 for categorical variables and *t*-test for continuous variables unless noted otherwise.

[†]*P* values obtained from Fisher's exact test.

Table 2. Differences in Clinical Characteristics Across Sex Groups

	Males				Females			
	Total ($n = 119$)	Control ($n = 37$)	iAMD ($n = 82$)	P Value	Total ($n = 192$)	Control ($n = 63$)	iAMD ($n = 129$)	P Value	P Value[*]
Family History of AMD				0.01[†]				<0.01[†]	0.62
None	76 (64%)	31 (84%)	45 (55%)		112 (58%)	48 (76%)	64 (50%)		
Yes	31 (26%)	4 (11%)	27 (33%)		58 (30%)	12 (19%)	46 (36%)		
Uncertain	12 (10%)	2 (5%)	10 (12%)		22 (11%)	3 (5%)	19 (15%)		
Age, mean (SD)	76.8 (6.5)	75.6 (4.9)	77.4 (7.1)	0.19	75.4 (6.4)	74.0 (3.9)	76.1 (7.2)	0.03	0.06
Body mass index, mean (SD)	27.1 (4.6)	27.2 (5.0)	27.1 (4.4)	0.92	26.8 (5.8)	27.0 (5.6)	26.7 (5.8)	0.81	0.61
	$n = 116$				$n = 184$				
BMI categories		$n = 116$		0.83		$n = 184$		0.31[†]	0.23[†]
Underweight	0	0	0		6 (3%)	2 (3%)	4 (3%)		
Normal weight	43 (37%)	15 (41%)	28 (35%)		72 (39%)	27 (44%)	45 (37%)		
Overweight	45 (39%)	13 (35%)	32 (41%)		62 (34%)	15 (25%)	47 (38%)		
Obese	28 (24%)	9 (24%)	19 (24%)		44 (24%)	17 (28%)	27 (22%)		
Smoking				0.80[†]				0.47[†]	0.57[†]
Never	52 (44%)	18 (49%)	34 (41%)		96 (50%)	33 (52%)	63 (49%)		
Current	3 (3%)	1 (3%)	2 (2%)		4 (2%)	0	4 (3%)		
Former	64 (54%)	18 (49%)	46 (56%)		92 (48%)	30 (48%)	62 (48%)		
History of									
Treated hypertension	61 (51%)	25 (68%)	36 (44%)	0.02	107 (56%)	30 (48%)	77 (60%)	0.11	0.44
Kidney disease	21 (18%)	10 (27%)	11 (13%)	0.07	17 (9%)	2 (3%)	15 (12%)	0.06[†]	0.02
Peripheral vascular disease	23 (19%)	9 (24%)	14 (17%)	0.35	31 (16%)	11 (17%)	20 (16%)	0.73	0.47
Cardiac disease	47 (39%)	16 (43%)	31 (38%)	0.57	59 (31%)	16 (25%)	43 (33%)	0.26	0.11
Medications									
NSAIDs	15 (13%)	2 (5%)	13 (16%)	0.14	25 (13%)	13 (21%)	12 (9%)	0.04	0.92
Aspirin	55 (46%)	18 (49%)	37 (45%)	0.72	82 (43%)	24 (38%)	58 (45%)	0.37	0.54

NSAIDs, nonsteroidal anti-inflammatory drugs.

P values obtained from χ^2 for categorical variables and t-test for continuous variables unless noted otherwise.

[*]*P* value for comparison between males and females.

[†]*P* values obtained from Fisher's exact test.

compared with the control group ($P = 0.01$ and $P < 0.01$, respectively). In Table 2, we show differences in demographic characteristic and select comorbidities between the control and iAMD groups stratified by sex. Both the male and female cohorts with iAMD had higher rates of family history in comparison with the male and female controls ($P = 0.01$ and $P < 0.01$, respectively). We found no significant differences between any of the risk factors with sex, except for kidney disease.

In Figure, we present the analyte levels for controls and iAMD cases comparing male versus female groups. As previously reported,[20] we found significantly higher level of RANTES in controls compared with iAMD cases: median, 10,678 pg/mL (interquartile range, 5656–18,851 pg/mL) versus median, 5405 (interquartile range, 3129–11,066 pg/mL; $P < 0.001$). RANTES levels did not differ between males and females in the control group (Fig.). In contrast, we found significantly lower levels of RANTES in male iAMD cases: median, 4525.6 (interquartile range, 2589–7861) compared with female iAMD cases: median, 6686 (interquartile range, 3485–12,488; $P = 0.005$). It is noteworthy that male and female controls demonstrated significantly higher levels of RANTES compared with male and female cases ($P < 0.001$). The sensitivity analysis which included

age and family history of AMD as covariates yielded similar results.

Discussion

In this study, we describe levels of RANTES in patients with iAMD compared with controls with no AMD stratified by sex in a cohort of patients who were part of a Colorado AMD Registry. In the overall cohort, the levels of RANTES were significantly higher in controls compared with iAMD cases.[20] Significantly elevated levels of RANTES were also found in male and female controls in comparison with male and female iAMD cases, respectively. Males with iAMD had significantly lower levels of RANTES compared with females with iAMD. It is striking that, among the groups examined, the lowest level of this biomarker was in the male cases (Fig.). There was no significant difference in levels of RANTES between males and female controls.

It has been recognized that sex plays a vital role in many diseases, including cardiovascular disease, cancer, type 2 diabetes, chronic kidney disease, chronic liver disease, depression, and autoimmune disease.[4,5] In the area of eye-related research, some studies have

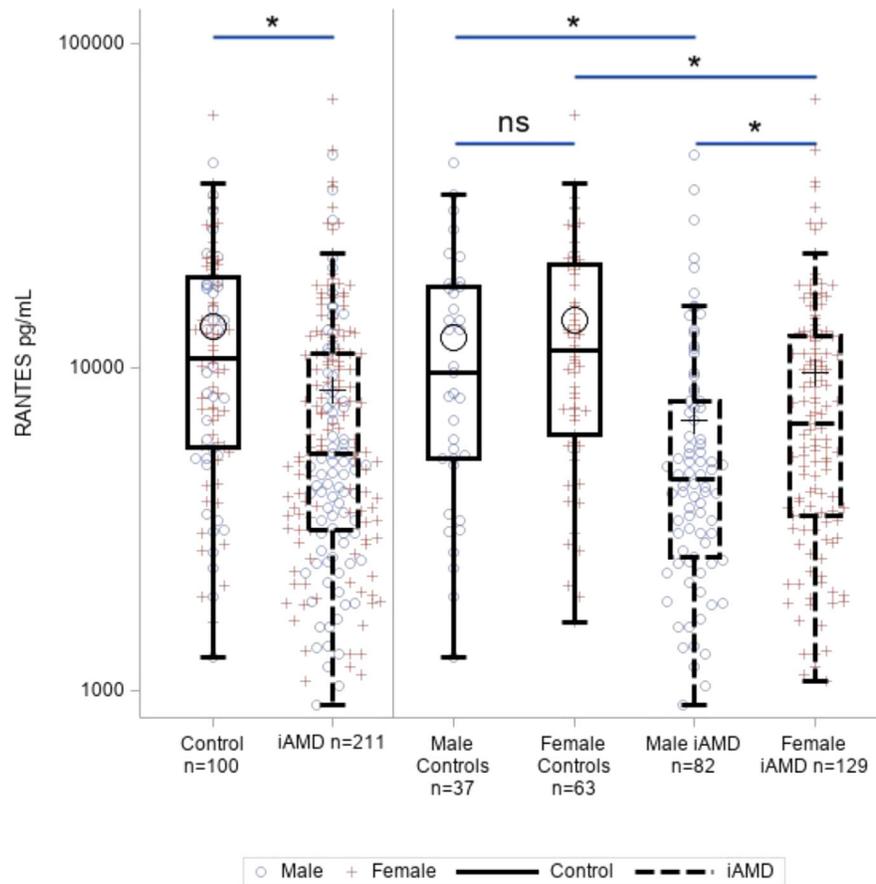translational vision science & technology

Figure. Boxplot showing the differences between groups in patients with iAMD and controls stratified by sex. The box extends to the 25th and 75th percentiles, the line represents the median and the large black circles/plus correspond to the mean values. Individual colored circles/plus points illustrate the raw values. Male patients are represented as blue circles and female patients are represented in red pluses. Statistically significant difference between groups. ns represents no statistical difference between groups.

demonstrated that sex has an impact on the prevalence and presentation of different ocular diseases.[11] Some authors have suggested a sex difference in systemic and genetic factors in early forms of AMD.[24] Other investigators have shown an association between females and the presence of extensive small drusen, one or more intermediate drusen or pigment abnormalities associated with AMD,[17] and higher rates of progression to advanced stages of AMD in females.[25] However, to the best of our knowledge, there are no studies that have specifically studied sex differences in iAMD. As shown in Figure, a key finding of the study was that levels of RANTES were significantly lower in male compared with female cases. The cause of the significant difference in RANTES between males and females at this juncture of our research is unclear. The human X chromosome is unique and replication of its genetic material in females requires systemic compensation of the gene dosage to silence one copy of the X chromosome.[26] Vladan et al.[27] suggest that epige-

netic alteration on the inactivation centers of the X chromosome is not only correlated with aging but could be a unique property that affects women with AMD more than men. Our study's novel finding underscores the importance of moderation by sex in iAMD research.[9]

RANTES, a chemokine, is secreted by activated T cells, smooth muscle cells, endothelial cells, macrophages, and platelets. RANTES is chemotactic for T cells, eosinophils, and basophils; can activate natural killer cells; and has been implicated in moderating the immune responses and is associated with acute and chronic phases of inflammation.[28–30] This chemokine binds to several receptor proteins, including CCR3 and CCR5.[29] Several investigators have demonstrated that RANTES is significantly elevated in patients with cardiovascular disease,[31–36] as well as in autoimmune[37,38] and age-related neurodegenerative diseases such as Parkinson's disease.[39] Regarding sex differences, one study showed that the plasma levels

of RANTES was significantly higher in male patients with stable angina and acute myocardial infarction, in comparison with females,[40] suggesting that there are sex differences in levels of RANTES in nonocular pathologies.

Regarding the role of RANTES in other nonocular pathologies, Lipkova et al.[41] showed that lower RANTES in patients with acute coronary syndrome correlated with severity of myocardial infarction and progression. Another study showed that, in male patients who were referred for coronary angiography, lower baseline RANTES was independently associated with an increased risk of cardiac death at the 2-year follow-up.[42] Badacz et al.[43] also showed that lower RANTES is associated with the degree of carotid artery stenosis and an increased risk of a major adverse coronary or carotid ischemic event. The authors suggest that RANTES migration to the interior of the arterial wall and plaque leading to decreasing levels in the peripheral blood might be responsible for this phenomenon.[42,43] Another proposed mechanism is that lower RANTES causes an upregulation of the CCR5 receptor, a crucial RANTES receptor associated with atherosclerosis, which is known to mediate transmigration of leukocyte on inflamed endothelium.[42] Thus, changes in the plasma concentrations of RANTES may have a role in regulating inflammatory processes in the pathologies of aging.[20]

There is a limited amount of literature linking RANTES with ocular diseases. RANTES has been found to be elevated in patients with severe diabetic retinopathy,[44] dysfunctional tear syndrome with meibomian gland disease,[45] and has a role in dry eye disease.[46] In regard to AMD, RANTES has been shown to be crucial in the recruitment of infiltrating immune cells found in donor retina and choroid of patients with AMD.[28] Nagineni et al. showed that cytokines associated with chronic inflammation induce RANTES release in human RPE and choroidal fibroblast cultures.[47] In a recent study from Nielsen et al, individuals with geographic atrophy had higher plasma levels of RANTES compared with healthy controls, which contrasts with the results of our study, where we found lower levels in our iAMD cases. These findings suggest that RANTES may be a marker of modified T-cell or macrophage migration in patients with iAMD.[20] RANTES may play a role in both the development and advancement of iAMD. Changes in the plasma levels of RANTES as AMD progresses could be a longitudinal study goal.[20]

There are a few etiologies that could be studied to explain this finding, including the finding that RANTES increases expression of several immune modulators including tumor necrosis factor alpha and IL-6 in dendritic cells.[48] There is also a negative relationship between the RANTES receptor and $CD8^+$ cells with geographic atrophy progression.[28] Moreover, the chemokine receptor protein CCR3 is actively expressed on choroidal blood vessels and increased production of human RPE cells of RANTES in response to cytokine in patients with AMD, suggest a potential mechanism for the interaction of local and plasma RANTES with macrophage migration and choroidal blood vessels.[20,47] Because RANTES levels are not consistently increased or decreased in aging diseases, the interaction of this chemokine and its receptor with ocular cells requires further study to speculate these mechanisms.[20]

We suggest that the behavior of this chemokine may be different depending on the type of disease studied, at what point in the natural history of the disease the biomarker is measured, the severity of the disease, and the sex composition of the cohort studied and, hence, may represent a dynamic plasma marker of disease evolution. The sex differences found in our study further emphasize the possible role of the cytokine in evaluating the stage of AMD. There is undoubtedly a need for more research on this biomarker specifically in the different phenotypes of AMD. RANTES may emerge as an excellent marker in disease evolution.

One limitation of our study is the relatively small sample size. Another limitation is the single time point measurement of these biomarkers. In addition, case patients for this study were recruited from a retina service and may not represent the spectrum of all patients with iAMD seen in the general aging population. Another limitation of the study is that we did not examine sex-related risk factors, such as the hormonal status of the cases, which has been reported by several authors to have a role in the immune system and on aging.[49] Recruitment into our registry is ongoing. It is our intention to include addition risk factors and systemic biomarkers and hormone levels in research on this cohort.

In conclusion, we demonstrated that patients with iAMD had significantly lower plasma RANTES compared with controls, and further differences were found when stratified by sex. Moving forward, we intend to build on this research in a larger cohort and examine levels of RANTES moderated by sex in longitudinal blood samples. Moreover, we are also interested to learn if RANTES is related to progression to the advanced stages of AMD. Understanding of the role of inflammatory markers in male and female patients could guide future intervention and treatment strategies of this visually threatening eye disease.

Acknowledgments

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