Molecular Vision 2015; 21:417-427 <http://www.molvis.org/molvis/v21/417>
Received 3 December 2014 | Accepted 8 April 2015 | Published 10 April 2015

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Identification of inflammatory mediators in patients with rhegmatogenous retinal detachment associated with choroidal detachment

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Purpose: To investigate the expression profile of intravitreous cytokines, chemokines, and growth factors in patients with rhegmatogenous retinal detachment associated with choroidal detachment (RRDCD) in comparison with patients with only rhegmatogenous retinal detachment (RRD).

Methods: Twenty RRDCD patients and 30 RRD patients were included in this case-control study. A multiplex bead-based immunoassay was performed to determine the expression of a wide range of 29 inflammatory mediators in undiluted vitreous from the patients. Data were analyzed using the Mann–Whitney U-test for nonparametric values and multivariate logistic regression analysis.

Results: Compared with the patients with RRD, intravitreous inflammatory mediators, including migration inhibitor factor (MIF), interleukin-6 (IL-6), CCL4, CCL11, CCL17, CCL19, CCL22, CXCL9, CXCL8, soluble inter-cellular adhesion molecule 1 (sICAM-1), transforming growth factor β3 (TGF-β3), and platelet-derived growth factor AA (PDGF-AA), were upregulated in patients with RRDCD. After calibrating the factors duration of detachment, preoperative proliferative vitreoretinopathy grade, and presence or absence of macular hole, the PDGF-AA concentrations were not significantly different according to the multivariate logistic regression analysis. MIF and sICAM-1 markers were significantly different between the two groups and represented a forward stepwise logistic regression trend.

Conclusions: This is the first report to use multiplex bead analysis to investigate inflammatory mediators related to RRDCD. We proposed that the upregulated expression of these mediators may be involved in the inflammation process of RRDCD and that regulation of their expression may be potentially therapeutic by altering local inflammation.

Rhegmatogenous retinal detachment associated with choroidal detachment (RRDCD) is an unusual type of rhegmatogenous retinal detachment (RRD) with specific and complicated signs. Patients with RRDCD often suffer from ocular hypotony and secondary uveitis, characteristics that distinguish RRDCD from most cases of primary RRD [1].

RRDCD accounts for 2.0–4.5% of all primary RRD in Western countries but accounts for 1.5–18.1% of cases in China [2-5]. The high prevalence of high myopia in China might be responsible for this significant rate, since high myopia is a risk factor for RRDCD [6,7]. Another possible explanation for this difference is racial differences. For patients with RRDCD, the postoperative prognosis remains unfavorable, and the poor reattachment rate is mainly attributed to the development of proliferative vitreoretinopathy (PVR) after surgery [8,9]. Thus, more attention should be directed toward understanding the pathophysiology of RRDCD. To date, the pathogenesis and pathologic processes underlying this disease remain unclear. It has been widely considered that hypotony secondary to retinal detachment is essential for the initiation of subsequent choroidal detachment and other events [7,10]. However, there is also the view that uveitis secondary to retinal detachment initiates the process. Supporters of this view believe that a severe inflammatory response contributes to the exudation of choroid blood vessels. Consequently, this leakage of fluid causes choroidal detachment and subsequent hypotony, while hypotony in turn enhances this process [1].

Recently, it has been reported that administration of preoperative steroids by local intravitreal injection or oral administration can reduce the ocular inflammatory response and prevent postoperative PVR by reducing protein leakage and inhibiting growth factors [11,12]. However, some patients with RRDCD with comorbid diseases, such as gastric ulcer, should not be administered oral steroids. In addition, local intravitreal injection might bring about ocular adverse effects,

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such as endophthalmitis. Thus, novel therapeutic strategies must be developed to avoid these side effects.

Since steroids can improve prognosis via an anti-inflammatory effect, we assume that inflammatory mediators might be associated with choroidal detachment. Although there is still little evidence regarding the role of inflammatory mediators in RRDCD from vitreous or subretinal fluid, upregulated inflammatory cytokines were found in an animal model of retinal detachment [13]. In addition, the infiltration of inflammatory cells and increase in cytokines were observed in subretinal fluid in vitreoretinal disorders, such as RRD and diabetic retinopathy vitreous (PDR) [14,15]. Several studies have implicated cytokines, chemokines, and growth factors as signaling molecules in the recruitment of inflammatory cells and the aggravation of inflammatory response [16-18]. We investigated whether these inflammatory mediators were associated with choroidal detachment by measuring 29 inflammatory mediators, including cytokines, chemokines, and growth factors, with multiplex bead analysis in undiluted vitreous obtained from patients with RRDCD.

METHODS

Study population: This study adhered to the tenets of the Declaration of Helsinki and the ARVO statement on human subjects. Also, it was met with the approval of the ethics committee of Nanjing Medical University affiliated Wuxi Second Hospital. All patients included in this research signed an informed consent form before surgery.

In our department, consecutive 368 cases (including 26 RRDCD and 342 RRD patients) between March 2012 and October 2013 underwent pars plana vitrectomy (PPV). Only twenty RRDCD patients and 30 RRD patients in parallel as control were enrolled in this case-control study. None of the included patients had diabetics, hypertension and diabetic retinopathy, whereas none of them have received anti-VEGF therapy. The age, gender, storage time and axial length were matched between RRDCD patients and RRD case.

All participants underwent a detailed ophthalmic examination on admission, including preoperative logMAR visual acuity, anterior segment evaluation with a slit-lamp microscope, funduscope examination, and B ultrasound by three experienced ophthalmologists. Patients with RRDCD underwent ultrasonic biomicroscopy (UBM) examination, which not only provided objective visual evidence for RRDCD but also determined the quadrants and degrees of choroidal detachment [5]. All selected patients had developed PVR before surgery and had been scored according to the 1983 International Classification [19]. Since most patients were over 45 years old, the lens had a varying degree of opacity. Thus, for these patients, it was appropriate to perform PPV combined with phacoemulsification. Usually, for the patients enrolled in the study, we performed phacoemulsification following PPV to avoid the influence of perfusion on the undiluted vitreous during phacoemulsification. Additionally, all surgical operations were performed by the same ophthalmologist in our department. Patients' clinical parameters and potential risk factors are shown in Table 1.

Sample collection: Under local or general anesthesia, all surgical procedures were performed using the three-port 25-gauge transconjunctival sutureless vitrectomy system (TSV25G; Alcon Constellation; Alcon Laboratories, Fort

Table 1. Clinical characteristics of the study population.

Clinical characteristics	RRDCD n=20	RRD n=30	P value
Gender Male Female	13 7	16 14	0.413
Age (Y) Median (range)	57(32–68)	56(35–74)	0.629
Axial length (mm) Median (range)	24.71(22.59–29.68)	24.66(21.81–29.57)	0.835
Preoperative logMAR Visual Acuity (VA) Median (range)	1.70(1.10–4.00)	1.70(0.50–4.00)	0.876
IOP (mmHg) Median (range)	6.7(5.2–11.1)	13.4(6.1–23.6)	<0.001
Duration of Detachments (Days) Median (range)	15(4–75)	7(2–60)	0.091
Size of RD (quadrants) Median (range)	4(3–4)	4(3–4)	0.876
Macular hole	6/20	8/30	0.797
Preoperative lens status Pseudophakic Phakic	1 19	3 27	0.915
PVR grade Median (range)	3(2–4)	2(2–4)	0.351

P value was calculated by Mann–Whitney U test or Chi-square test.

Table 2. Inflammatory mediators measured by multiplex bead immunoassay.

Biologic Group	Inflammatory mediators
Cytokines	MIF, IFN-γ, GM-CSF, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-10, IL-13, IL-17A,
Chemokine	MCP-1/CCL2, CCL4/MIP-1β, CCL11, CCL17, CCL19, CCL22/MDC, CXCL10, CXCL9, CXCL8, sICAM-1
Growth factors	TGF-β1, TGF-β2, TGF-β3, PDGF-AA, PDGF-AB, VEGF

MIF=macrophage migration inhibitory factor, IFN=interferon, GM-CSF=granulocyte macrophage colony stimulating factor, IL=interleukin, CCL=chemokine (C-C motif) ligand, CXCL=chemokine (C-X-C motif) ligand, ICAM=intercellular adhesion molecule, TGF=transforming growth factor, PDGF=platelet-derived growth factor, VEGF=vascular endothelial growth factors

Worth, TX) with the help of a noncontact wide-angle viewing system (Resight; Carl Zeiss Meditec AG, Jena, Germany). In the inferotemporal, superotemporal, and superonasal quadrants, the 25G trocar together with a microcannula was inserted into the vitreous about 4.0 mm from the corneo-scleral limbus. Then, the microcannula was left, but the trocar was withdrawn. The infusion cannula was placed in the inferotemporal quadrant, and other surgical instruments were placed in the superotemporal or superonasal quadrants. At the beginning of PPV, gas perfusion was set to open. Then, undiluted vitreous samples were collected with 5 ml syringes. Immediately, the samples were transferred into microfuge tubes and placed on ice. Each sample was centrifuged at 1007.1 ×g for 10 min at 4 °C. After centrifugation, approximately 0.5–1.2 ml supernatant was obtained and frozen at −80 °C before analysis. Bicinchoninic acid protein assay (Pierce Biotechnology, Rockford, IL) was conducted to determine the total protein concentration in vitreous. Each assay was performed according to the manufacturer's instructions.

Multiplex bead immunoassay: For measurement with the multiplex bead immunoassay, undiluted vitreous from all patients was simultaneously analyzed for 29 cytokines, chemokines, and growth factors with Luminex 200 (Luminex Corporation, Austin, TX). Luminex xMAP technology is characterized by high throughput detection and high sensitivity, which allows for detection hundreds of molecules per sample and simultaneous measurement of 96 samples. Table 2 lists 29 inflammatory mediators, and magnetic bead kits were purchased from EMD Millipore (Millipore, Billerica, MA). To ensure experimental reliability, quality controls were provided in the kits. For the vitreous samples, we used the serum matrix offered in the kits as the diluent. Concentrations of inflammatory mediators at or below minimum detectable levels were considered immeasurable and assigned a value of zero. The assay sensitivity was as follows: cytokines, migration inhibitor factor (MIF) 7.67pg/ml, interferon (IFN)-γ) 1.90 pg/ml, GM-CSF 2.50 pg/ml, IL-1β1.86 pg/ml IL-2 1.55 pg/ml, IL-4 2.39 pg/ml, IL-5 1.27 pg/ml, IL-6 1.51 pg/ml, IL-7 1.71 pg/ml, IL-10 1.19 pg/ml, IL-13 1.85 pg/ml, IL-17A 1.37 pg/ml, IL-23 43.62 pg/ml; chemokines, CCL2 1.92 pg/ml, CCL4 1.59 pg/ml, CCL11 2.17 pg/ml, CCL17 0.66 pg/ml, CCL19 5.16 pg/ml, CCL22 3.0 pg/ml, CXCL10 2.2 pg/ml, CXCL9 46.08 pg/ml, CXCL8 1.88 pg/ml, sICAM-1 39.92 pg/ml; growth factors, TGF-β1 10.81 pg/ml, transforming growth factor (TGF)-β2 0 4.61 pg/ml, TGF-β3 2.64 pg/ml, platelet-derived growth factor (PDGF)-AA 1.25 pg/ml, PDGF-BB 1.84 pg/ml, and vascular endothelial growth factor (VEGF) 54.74 pg/ml. Each assay was conducted in accordance with the instructions.

Statistical analysis: All analyses were performed with SPSS 17.0 statistical software (Chicago, IL), and diagrams were drawn in GraphPad Prism 5.0 (La Jolla, CA). For most demographic variables, the non-parametric Mann–Whitney U test was used to compare groups. The chi-square test was performed for clinical variables, such as gender. Multivariate logistic regression was performed to identify inflammatory mediators associated with RRDCD after correction for possible confounders, including the duration of detachment, macular hole, and preoperative PVR grade. Forward stepwise logistic regression analysis was conducted to analyze data as well. The correlation between each mediator was determined with Spearman's non-parametric test. A value of $p<0.05$ was considered significantly different.

RESULTS

Demographics and clinical variables: For statistical analysis, PVR grade A was recorded as 1, B as 2, C as 3, and D as 4. In total, 50 vitreous samples from 50 eyes were analyzed. Clinical variables, such as age (p=0.629), gender (p=0.413), and axial length (p=0.835), were matched between the two groups. Seven women and 13 men with a median age of 57 years comprised the RRDCD group, and 14 women and 16 men with a median age of 56 years constituted the control group. The median axial length of two groups was 24.71 and 24.66 mm, respectively. Preoperative visual acuity was

similar between the two groups (p=0.876). Intraocular pressure (IOP) was significantly different between the RRDCD and RRD groups (p<0.001), with a median IOP of 6.7 mmHg and 13.4 mmHg for the RRDCD and RRD cases, respectively. Regarding other demographics, including duration of retinal detachment (p=0.091), size of retinal detachment in quadrants (p=0.876), macular hole (p=0.797), preoperative lens status (p=0.915), and PVR grade (p=0.351), there was no statistically significant difference between the two groups. In the RRDCD group, six out of 20 patients had macular hole, and they were related to high myopia.

Concentration of total protein and inflammatory mediators in vitreous: The total protein concentration in vitreous was a median of 9,163 µg/ml and 2,605 µg/ml in patients with RRDCD and RRD, respectively, suggesting the breakdown of the blood–retinal barrier (BRB) [20] and exudation of blood vessels in the RRDCD condition, indirectly (Figure 1). To investigate the inflammatory mediators associated with disease, we used a multiplex bead immunoassay to measure the concentration of 29 cytokines, chemokines, and growth factors in undiluted vitreous. The expression of these inflammatory mediators is summarized in Table 3. Of the 29 inflammatory mediators analyzed, eight cytokines (IL-1β, IL-2, IL-4, IL-5, IL-10, IL-13, IL-17A, and IL-23) and PDGF-AB were immeasurable, since the concentrations of these mediators were below the minimum detectable levels in the majority of the vitreous. CCL2 levels exceeded the maximum detection range in approximately 17% of the patients with RRD and were assigned the greatest value. Among the other 19 inflammatory mediators, 12 inflammatory mediators displayed significantly higher concentrations in the RRDCD cases than in the RRD cases (Figure 2). These mediators were as follows: cytokines MIF (p<0.001) and IL-6 (p<0.001); chemokines CCL4 (p=0.011), CCL11 (p=0.007), CCL17 (p=0.007), CCL19 (p<0.001), CCL22 (p=0.003), CXCL9 (p<0.001), CXCL8 (p=0.001), and sICAM-1 (p<0.001); and growth factors TGF-β3 (p=0.002) and PDGF-AA (p=0.048). IFN-γ, GM-CSF, IL-7, CCL2, CXCL10, TGF-β1, TGF-β2 and VEGF were not significantly different between the two groups. Previous studies demonstrated that the majority of these mediators had a close relationship with postoperative PVR, but none had previously been linked to RRDCD [21-23].

Multivariate logistic regression between inflammatory mediators: We used multivariate logistic regression analysis to correct the influence of possible confounders. The PDGF-AA concentrations were significantly upregulated in the RRDCD group, according to the Mann–Whitney U test, but significance was lost after correction (p=0.072). Similar results were found for other mediators. Of all the inflammatory mediators detected, MIF and sICAM-1 were the only statistically significant variables according to the forward stepwise logistic regression analysis.

Correction between inflammatory mediators: Since 11 inflammatory mediators were statistically significant,

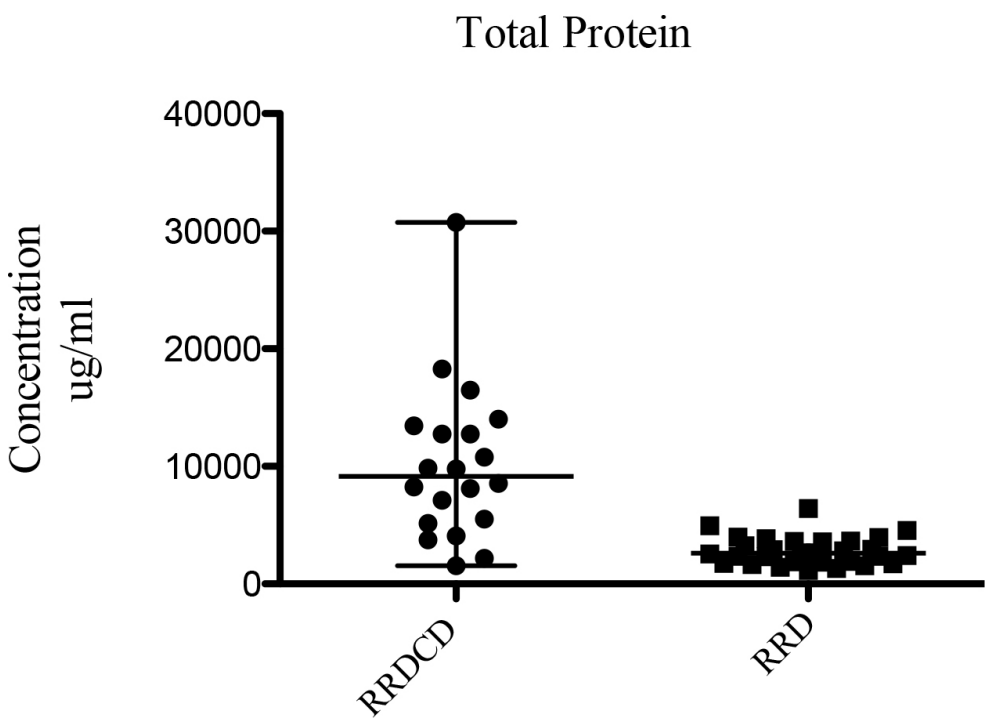

Figure 1. Scatter plots of total protein in the vitreous of patients with RRDCD and RRD. The bicinchoninic acid protein assay was used to compare the total protein concentrations in the vitreous between the rhegmatogenous retinal detachment associated with choroidal detachment (RRDCD) and rhegmatogenous retinal detachment (RRD) groups. The vertical bars represent the median and the range.

Table 3. Concentrations of inflammatory mediators.

Inflammatory mediators	RRDCD n=20	RRD n=30	Mann–Whitney U test	Multivariate	Forward stepwise
MIF Median (range)	2023.5(861.86–5008)	942.63(273.39–3224)	p<0.001	p=0.002	p=0.009
IFN-γ Median (range)	0.3(0–2.67)	0.15(0–5.21)	NS	NS	NS
GM-CSF Median (range)	6.87(0–25.11)	8.18(4.09–15.85)	NS	NS	NS
IL-1β Median (range)	ND	ND	-	-	-
IL-2 Median (range)	ND	ND	-	-	-
IL-4 Median (range)	ND	ND	-	-	-
IL-5 Median (range)	ND	ND	-	-	-
IL-6 Median (range)	232.2(38.7–7393)	32.18(6.05–135.24)	p<0.001	p=0.008	NS
IL-7 Median (range)	9.26(2.81–31.45)	6.71(4.23–20.04)	NS	NS	NS
IL-10 Median (range)	ND	ND	-	-	-
IL-13 Median (range)	ND	ND	-	-	-
IL-17A Median (range)	ND	ND	-	-	-
IL-23 Median (range)	ND	ND	-	-	-
CCL2/MCP-1 Median (range)	5145(1856->12191)	6389(1906->12191)	NS	NS	NS
CCL4 /MIP-1β Median (range)	33.05(7.69–76.46)	23.88(0–60.57)	p=0.011	p=0.017	NS
CCL11 Median (range)	15.88(3.67–38.73)	12.15(0–26.62)	p=0.007	p=0.025	NS
CCL17 Median (range)	1.48(0.85–4.19)	1.06(0–1.89)	p=0.007	p=0.015	NS
CCL19 Median (range)	546.86(4.2–1364.0)	172.23(0–1256)	p<0.001	p=0.003	NS
CCL22/MDC Median (range)	76.31(0–287.51)	14.48(0–113.88)	p=0.003	p=0.003	NS
CXCL10/ IP10 Median (range)	1327.5(291.03–11868)	1018.63(411.08–5082)	NS	NS	NS
CXCL9 Median (range)	2108.5(645.59–7633)	655.64(0–3613)	p<0.001	p=0.004	NS
CXCL8 Median (range)	42.92(18.87–168.19)	26.13(7.25–90.78)	p=0.001	p=0.005	NS
sICAM-1 Median (range)	29,606.5(5322–85287)	6928(1924–19756)	p<0.001	p=0.005	p=0.009
TGF-β1 Median (range)	42.66(0–182.29)	25.58(0–75.18)	NS	NS	NS
TGF-β2 Median (range)	9693(0–15259)	8953.5(5783–13061)	NS	NS	NS
TGF-β3 Median (range)	49.30(32.17–98.49)	37.13(18.98–61.18)	p=0.002	p=0.003	NS
PDGF-AA Median (range)	247.55(73.3–978.86)	193.22(63.26–396.18)	p=0.048	NS	NS
PDGF-AB Median (range)	ND	ND	-	-	-
VEGF Median (range)	307.58(159.53–1093)	307.58(0–468.13)	NS	NS	NS

ND: Not detected; NS: not significant.

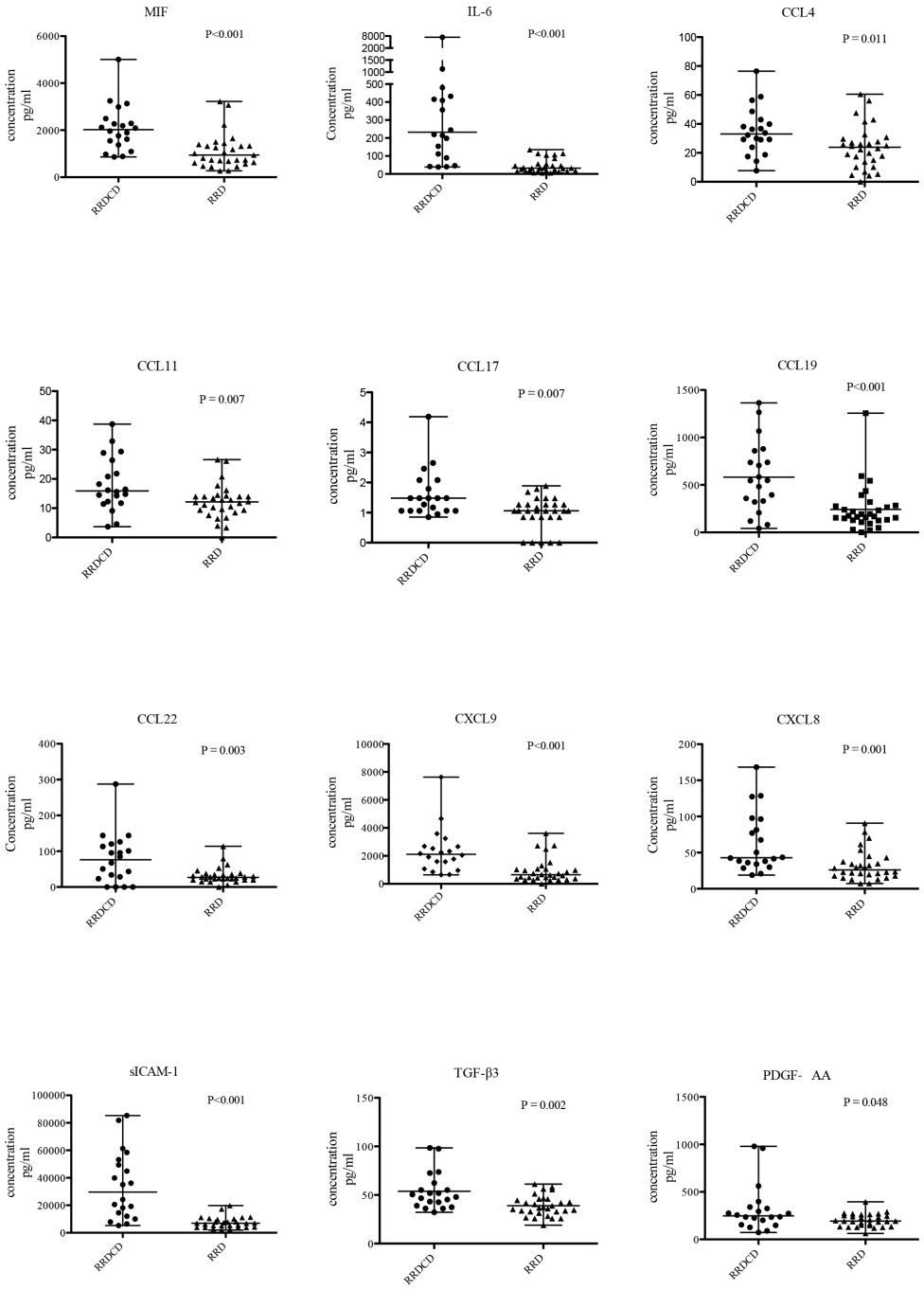

Figure 2. Scatter plots of significantly elevated mediator levels in the vitreous of the patients with RRDCD. The multiplex beads immunoassay identified 12 inflammatory mediators as significantly elevated in vitreous of patients with rhegmatogenous retinal detachment associated with choroidal detachment (RRDCD; the Mann–Whitney U test). The following mediators increased: MIF, IL-6, CCL4, CCL11, CCL17, CCL19, CCL22, CXCL9, CXCL8, sICAM-1, TGFβ3, and PDGF-AA. After correction with multivariate logistic analysis, PDGF-AA was no longer significant. The vertical bars represent the median and the range.

Spearman's non-parametric test was performed to analyze the correlation between the mediators in each group. For instance, the correlation between CCL22 and CCL17 was investigated in the RRDCD cases. The p value was 0.004, and R value was 0.613, suggesting that CCL22 and CCL17 were significantly correlated in the patients with RRDCD. IL-6/CXCL8 (p=0.008, R=0.571), IL-6/TGF-β3 (p=0.029, R=0.489), CCL4/CXCL8 (p=0.006, R=0.590), CCL11/CCL19 (p=0.009, R=0.567), CCL17/CCL22 (p=0.004, R=0.613), CCL17/sICAM-1 (p=0.025, R=0.499), CCL19/CCL22 (p=0.019, R=0.520), CCL19/CXCL8 (p=0.028, R=0.490), CCL19/sICAM-1 (p=0.035, R=0.474), and CCL22/sICAM-1 (p=0.007, R=0.580) showed positive correlations in patients with RRDCD, while significant correlation was established in the RRD cases for IL-6/CCL4 (p=0.041, R=0.375), CCL4/CCL11 (p=0.005, R=0.498), CCL17/CCL19 (p=0.002, R=0.539), CCL17/CCL22 (p=0.022, R=0.417), CCL17/CXCL9 (p=0.012, R=0.451), CCL19/CCL22 (p=0.047, R=0.365), CCL19/CXCL9 (p=0.005, R=0.503), CCL19/sICAM-1 (p=0.001, R=0.563), and CCL22/CXCL9 (p=0.020, R=0.421).

DISCUSSION

In this study, concentrations of MIF, IL-6, CCL4, CCL11, CCL17, CCL19, CCL22, CXCL9, CXCL8, sICAM-1, TGF-β3, and PDGF-AA were significantly elevated in patients with RRDCD compared with the controls. In addition, the total protein concentrations were statistically higher in the RRDCD cases than controls. After correction for detachment duration, preoperative PVR, and macular hole, MIF, IL-6, CCL4, CCL11, CCL17, CCL19, CCL22, CXCL9, CXCL8, sICAM-1, and TGF-β3 remained statistically significant. Of all inflammatory mediators investigated, MIF and sICAM-1 were independent predictors for RRDCD according to the stepwise forward logistic regression analysis.

Interestingly, positive correlations between IL-6, CXCL8, CCL4, CCL11, CCL17, CCL19, CCL22, sICAM-1, and TGF-β3 were found in the RRDCD cases. For instance, CCL22 correlated with CCL17 significantly and positively in the RRDCD cases. Importantly, chemokines could lead to enhancement of inflammation, and CCL17 and CCL22 were recognized as Th2 chemokines [23,24]. A statistical correlation was found between CCL22 and CCL17 in patients with idiopathic pulmonary fibrosis [24]. The positive correlation between mediators in RRDCD cases suggested a possibly complex network of regulation. However, whether CCL22 could contribute to the increase in CCL17 remains unclear. The real relationship between CCL22 and CCL17 requires further investigation.

Upregulated expression of inflammatory mediators in vitreous or subretinal fluid from patients with vitreoretinal disorders might aggravate the inflammation response and induce the proliferation and differentiation of RPE cells [21-23,25]. To better understand vitreoretinal disorders, such as RRDCD, analyzing the mediators in vitreous seemed appropriate and necessary. In addition, such information could identify potential therapeutic targets, especially for patients unsuitable for steroid administration. We detected these potentially related inflammatory mediators with multiplex bead immunoassay in the vitreous samples, because the subretinal fluid is mainly produced from vitreous in RRD cases whereas protein leakage is the main source of the subretinal fluid in the RRDCD cases.

Since MIF and sICAM-1 were significantly different according to the stepwise forward logistic regression analysis and represented independent predictors for RRDCD, we focused on them first. Recently, MIF, known as a proinflammatory cytokine, was reported to participate in the inflammation process and joint destruction of arthritis [26]. Choi et al. [27] confirmed that siRNA against MIF could suppress the airway inflammation induced by particulate matter. The expression and secretion of MIF can be obtained in ocular tissues, such as the cornea, trabecular meshwork, lens, and retina [28-30]. More importantly, previous findings indicated that the role of MIF in intraocular inflammation was essential [28,31]. A significant increase in MIF was found in the serum, aqueous, and vitreous in patients with uveitis compared with the controls [28,31]. In addition, Taguchi et al. [31] demonstrated that MIF levels in the vitreous of patients with uveitis reflected the intensity of ocular inflammation. Thus, we maintain that MIF might be associated with the inflammation process of choroidal detachment. We also found that the total concentrations of sICAM-1 in the vitreous in the two groups were significantly different. Despite the high expression of sICAM-1 in normal human serum, the sICAM-1 levels in the vitreous of patients with vitreous hemorrhage were very low [32]. This observation suggested that plasma leakage was not the source of sICAM-1. Furthermore, gene transcription and protein translation of sICAM-1 were markedly upregulated in human RPE cells and retinal endothelial cells after modulation by inflammatory mediators, such as IFN-γ [33,34]. Thus, sICAM-1 is likely derived from resident retinal cells. It was previously reported that sICAM-1 is elevated in the vitreous of some patients with uveitis [32,35]. In addition, Limb et al. [36] reported that increased sICAM-1 levels in vitreous contributed to the amplification of inflammation and formation of neovascularization in patients with PDR. To our knowledge, the multifunction adhesion molecule sICAM-1 participates in the processes of cell signal transduction and

cell activation. We hypothesized that increased expression of sICAM-1 was involved in the inflammation process of RRDCD by mediation the migration of lymphocyte, accumulation of the extracellular matrix, and the elicitation of an inflammatory reaction [35,37]. Nevertheless, the mechanism of sICAM-1 related to choroidal detachment remains to be clarified. Identification of inflammatory mediators in the vitreous could provide new insight into the process of diseases and design a strategy for potential targeted treatment. Injection of intravitreal triamcinolone acetonide was an effective method for inhibiting inflammatory reaction [38-40]. In addition, an increasing number of investigators have been concerned with the intravitreal pharmacological agents for reducing the occurrence of PVR [41,42]. Perhaps, the specificity of the inhibition of MIF and sICAM-1 through the injection of intravitreal agents during the PPV process could improve patients' prognosis.

To the best of our knowledge, this is the first report describing TGF-β3 in patients with RRDCD and RRD. TGF-β3 is usually secreted by cells of mesenchymal origin [43]. Lutty et al. [44] found TGF-β3 expression in isolated individual cells of the choroid and the retina, such as microglia, indicating that local resident cells of mesenchymal origin can produce TGF-β3. In addition, protein leakage might be another source of TGF-β3 due to the breakdown of the BRB. There is a link between abnormal expression of TGF-β isoforms and inflammatory disease [45]. In mouse lungs with allergic airway inflammation, an apparent increase in TGF-β3 mRNA was observed [46]. Li et al. [46] also pointed out that the non-receptor tyrosine kinase Lyn might provide clues for the development of treatment targets for severe chronic asthma and attributed the effect of Lyn on the mitigation of airway remodeling to directly downregulation of TGF-β3 expression in house dust mite models. However, TGF-β3 is a multifunctional inflammatory mediator, and thus is essential for the promotion of wound healing, reduction of scar formation, and modulation of the inflammatory environment [47]. Whether the high expression of TGF-β3 played a protective or destructive role in RRDCD was not determined in this study, and further investigation is needed.

IL-6 is a recognized marker of acute inflammatory reaction, and exhibits a wide range of biologic activity. IL-6 not only attracts chemokines but also recruits leukocytes to local inflammatory sites [48].

In addition to recruiting leukocytes, chemokines are critical for angiogenesis regulation, abnormal proliferation, and enhancement of inflammation [23,49]. In general, subfamilies of chemokines are different in their chemotactic effect. CC chemokines are likely to act on monocytes and lymphocytes whereas CXC chemokines tend to attract neutrophils and lymphocytes to inflammatory sites [50]. CCL11 is universally recognized by its specific attraction of eosinophils and its role in allergic diseases [51], and recent studies suggested an association between CCL11 and ocular diseases. For example, CCL11 was markedly upregulated in RPE cells in a mouse model of choroidal neovascularization (CNV) [38], and CCL11 expression was statistically elevated in the RRDCD cases in the present study.

Overexpression of CXCL9 dysregulates immune function and amplifies local inflammatory reaction by interacting with the specific receptor CXCR3. Downregulating CXCL9 could prevent damage to the retina due to excessive inflammatory stimulation [52]. Moreover, Chen et al. [53] found that CXCR3 and its ligands were associated with T-lymphocyte-mediated inflammation. CXCL8 could participate in an inflammatory process via the recruitment of neutrophils and T lymphocytes. CCL17, known as a thymus and activation regulated chemokine, specifically evokes chemotaxis effects and recruits T cells by binding the high-affinity receptor CCR4 [54]. Similarly, the involvement of CCL22, CCL4, and CCL19 is essential to balance the immunomodulation, infiltration of T cells, and regulation of T cell homing [55,56]. Regardless of T-lymphocyte-mediated diseases, overexpression of chemokines together with recruited lymphocytes could aggravate local inflammation and imbalance localized immunomodulation.

Within those inflammatory mediators, except TGF-β3, others have been proven to be associated with the development of postoperative PVR in individuals with RRD [21-23]. Increases in these factors suggested higher morbidity of postoperative PVR in RRDCD cases, and these mediators provided potential targeted therapeutic opportunities. In addition to inhibition of inflammatory reaction, regulation of these mediators could reduce the occurrence of postoperative PVR, and thus improve patients' prognosis. However, the positive or negative aspects of TGF-β3 in postoperative PVR remain unclear.

The small number of samples was the main limitation of the current study. Perhaps because of the small number, some mediators, such as TGF-β1, tended to increase and decrease, but no statistical significance was observed between the two groups. Because of the small number, PDGF-AA was no longer significant after correction for possible confounding factors. Moreover, our observation indicates only that there may be a correlation between elevated inflammatory mediators and RRDCD. Whether these statistical mediators are the cause or the result of RRDCD requires further investigation. Elevated mediators could be probable triggers for

amplification of inflammation and enhance the inflammatory process of choroidal detachment. Another possible explanation is that the elevation of inflammatory mediators is the result of the severe inflammatory response to RRDCD. To our knowledge, however, our report is the first to investigate the link between inflammatory mediators in vitreous and RRDCD. Understanding the molecular mechanism of inflammatory factors associated with RRDCD is necessary, and we will continue to investigate the molecular mechanism of mediators at the cellular level in the future.

In conclusion, our results indicated that several inflammatory mediators were significantly increased in RRDCD cases relative to the control group. Moreover, those elevated mediators in the vitreous might be associated with the inflammatory process of RRDCD. According to the forward stepwise logistic regression analysis, only MIF and sICAM-1 were significant variables and might be independent predictors for RRDCD. Positively significant correlations between certain mediators were observed in patients with RRDCD, suggesting a complex interaction between mediators and the modulation mechanism. Importantly, we believe that these mediators may be novel and efficacious therapeutic targets for treating RRDCD.

ACKNOWLEDGMENTS

Supported by Six Talents Summit Project of Jiangsu Province (2011-WSK-011) and the Wuxi Hospital administrative center Foundation (YGZZ1106). None of the authors have any conflicting interests to disclose.

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Articles are provided courtesy of Emory University and the Zhongshan Ophthalmic Center, Sun Yat-sen University, P.R. China. The print version of this article was created on 10 April 2015. This reflects all typographical corrections and errata to the article through that date. Details of any changes may be found in the online version of the article.