·Clinical Research·

C-reactive protein and diabetic retinopathy in Chinese patients with type 2 diabetes mellitus

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Abstract

• AIM: To investigate the relationship between C-reactive protein (CRP) and diabetic retinopathy (DR) in a cohort of Chinese patients with type 2 diabetes mellitus (T2DM).

• METHODS: Community-based observational cohort study. There were 1131 participants recruited from November 2009 to September 2011 in Desheng community in urban Beijing. Patients diagnosed T2DM were recruited and underwent a standardized evaluation consisting of a questionnaire, ocular and anthropometric examinations and laboratory investigation. The presence and severity of DR were assessed by seven fields 30° color fundus photographs. Subjects were then classified into groups with no DR, any DR, or vision-threatening DR. CRP was analyzed from serum of study subjects.

• RESULTS: A total of 1007 patients with T2DM were included for analysis, including 408 (40.5%) men and 599 (59.5%) women. The median CRP level was 1.5 mg/L for women and 1.1 mg/L for men (P=0.004, OR 0.37, 95% CI 0.18-0.74). After adjusting for possible covariates, higher levels of CRP were associated with lower prevalence of any DR (P=0.02, OR 0.55, 95% CI 0.35-0.89), but not associated with vision-threatening DR (P=0.62, OR 0.78, 95% CI 0.28-2.14). After stratification by sex, the inverse association between CRP and DR was found to be statistically significant in men (P=0.006, OR 0.35, 95% CI 0.16-0.73), but not in women (P=0.58, OR 0.88, 95% CI 0.29-1.16).

• CONCLUSION: The data drawn from a Chinese population with T2DM suggest that increasing CRP levels may be inversely associated with development of DR.

• KEYWORDS: type 2 diabetes mellitus; C-reactive protein; diabetic retinopathy; inflammation
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INTRODUCTION

Diabetic retinopathy (DR) is the most common microvascular complication of diabetes and the leading cause of legal blindness in working age adults. Elevated blood glucose, longer duration of diabetes, hypertension and hyperlipidemia are well-accepted risk factors for the development and progression of DR based on data from epidemiological studies and clinical trials[1-2]. However, these conventional factors seem to explain only a portion of the risk for DR[3] and other potential risk factors such as chronic subclinical inflammation should be examined and evaluated[4-6].

C-reactive protein (CRP) is the most extensively studied biomarker of systemic inflammation that has been reported to be associated with cardiovascular disease[7], diabetes[8] and micro/macroalbuminuria either in diabetic populations[9] or in the general population [10]. Data on a possible association of CRP with DR, however, are sparse and a limited number of studies show inconsistent results. For example, association of higher CRP level with early and advanced retinopathy was reported in a population-based cohort of 543 subjects with type 1 diabetes mellitus (T1DM) [11] and the Hoorn study including 625 non-diabetic individuals and patients with type 2 diabetes mellitus (T2DM)[12]. In a prospective clinic-based study which included 328 subjects with T2DM, higher CRP level was significantly associated with a higher risk of baseline DR, but not with DR progression over a ten-year

observation [13]. Another prospective study, the diabetes control and complications trial (DCCT) cohort consisted of 1441 subjects with T1DM, showed CRP level may be associated with higher risk of incident clinically significant macular edema (CSME) and macular hard exudate[14]. Other studies, however, showed no associations between CRP and DR [15-18]. Moreover, higher CRP levels were reported to be associated with lower prevalence of any DR in a recent study which included 718 Malay participants with T2DM[19].

In this current study, we aimed to further investigate the possible associations of CRP levels with DR in a large cohort of Chinese patients with T2DM.

SUBJECTS AND METHODS

Patients with T2DM in the Desheng community of urban Beijing were recruited between November 2009 and September 2011 by using posters, pamphlets, and phone calls. Diabetes was defined as either a self-reported history of physician diagnosed T2DM being treated with insulin, oral hypoglycemic agents, and diet only, or by a fasting plasma glucose (FPG) concentration of 7.0 mmol/L (126 mg/dL) or more in at least two previous examinations or a random plasma glucose concentration of ≥11.1 mmol/L (200 mg/dL). The duration of diabetes was defined as the interval between the first diagnosis and the time of enrollment into the present study. Patients with severe media opacity preventing the classification of retinopathy, with shallow anterior chamber or angle-closure glaucoma preventing mydriasis were excluded. The study protocol was approved by the Ethics Committee of the Beijing Tongren Hospital and adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained from all participants before their enrollment.

Participants underwent a standardized evaluation consisting of a questionnaire, ocular and anthropometric examinations and laboratory investigation. Basic information (age, sex, income and educational level), information on lifestyle (such as smoking and alcohol intake), medical history (such as medication, the use of insulin and history of systemic diseases) and family history of diseases were elicited from the interview. The level of education was recorded as high school completed or not and family income was self-reported. Smoking status was classified as current smoker, ex-smoker and never smoker. Persons currently smoking more than one cigarette/cigar/pipe a day for at least one year were classified as current smokers. Persons who never smoked were classified as never smokers. Persons currently doing not smoke but with a prior smoking history were classified as ex-smokers.

Anthropometric parameters included body weight and height, waist and hip circumference, systolic blood pressure (SBP) and diastolic blood pressure (DBP) in a resting status for three measurements and 5min apart. Height and weight were measured with subjects in light clothing and not wearing shoes by a trained observer. Body mass index (BMI, kg/m^2) was calculated as the ratio between weight and the square of height of the participant. Waist-to-hip ratio (WHR) was calculated as waist circumference divided by hip circumference. A comprehensive ophthalmological examination included corrected visual acuity, slit-lamp biomicroscopy, and fundus examination. Seven fields 30° color fundus photographs with stereoscopic images of optic disc and macula were taken through dilated pupils for all patients using a digital fundus camera (Zeiss Visucam Pro, Oberkochen, Germany).

One trained ophthalmologist (Yang XF) graded all the images at the University of Wisconsin Fundus Photographic Reading Center, according to the modified Early Treatment Diabetic Retinopathy Study (ETDRS) standard classification. Retinopathy was considered present if any characteristic lesions as defined by the ETDRS severity grading scale, including microaneurysms, hemorrhages, cotton wool spots, intraretinal microvascular abnormalities, hard exudates, venous beading and new vessels [20]. A retinopathy severity score was assigned for each eye according to a scale of ETDRS and the severity of retinopathy for each person was evaluated based on the score of the worse eye. Eyes were graded according to the following criteria: no DR (NDR, levels 10) or any DR (levels 14 and above). Any DR was further divided into mild non-proliferative diabetic retinopathy (NPDR) (levels 14-35), moderate NPDR (levels 43 through 47), severe NPDR (level 53), and proliferative retinopathy (level>60). Macular edema was classified as present or absent and further divided into those with or without CSME. Macular edema was defined as retinal thickening in the macular area according to the ETDRS classification protocol. CSME was considered present when the macular edema was present within 500 μm of the foveal center or focal photocoagulation scars were present. Vision-threatening DR (VTR) was defined as the presence of severe NPDR, proliferative retinopathy, or CSME [20]. Grading reproducibility was assessed by regrading 5% of the eyes by a senior grader at the University of Wisconsin Fundus Photograph Reading Center. Exact agreement on retinopathy severity level and macular edema was tabulated and weighted Kappa was calculated and compared to historical reproducibility from reading center grading.

Overnight fasting blood samples were collected for measurement of FPG, glycosylated hemoglobin A1c (HbA1c), creatinine, uric acid, and lipid profile [levels of total cholesterol, triglycerides, high-density (HDL) and low-density (LDL) lipoprotein cholesterol]. The samples were allowed to stand at room temperature for 30min for coagulation and serum was obtained by centrifugation. CRP concentration was measured through particle-enhanced

immunonephelometry using an automated system (Hitachi analyzer 7080, Japan). The lowest detection limit of the assay was 0.1 mg/L. The intra-assay and inter-assay coefficients of variation for CRP concentration were 5% and 10%, respectively. A first-void, midstream morning spot urine sample was collected, and albuminuria was measured by immunonephelometry (Roche/Cobas C501 analyzer, Ibaraki, Japan). High albuminuria was defined as ≥20 mg/L.

Statistical analysis was performed using the R statistical analysis package (http://www.r-project.org/). Given CRP is an inflammatory marker and may increase sharply by infectious disease, CRP levels of more than 10 mg/L were initially excluded from the analysis as they might indicate the presence of acute inflammatory disease [10,21-22]. The data with CRP levels >10.0 mg/L were then included in the supplementary analyses to examine the potential effect of data exclusion. Mean and standard deviation (SD) were calculated for continuous variables with normal distribution. For variables presenting a non-normal distribution the median with 25th percentile and 75th percentile were used. For categorical analysis, CRP level was grouped into categories as quartiles based on its distribution among total samples. For continuous analysis, CRP value was log-transformed because of its skewed distribution. Differences in clinical characteristics of participants with or without DR were assessed using the two-sample t-test for continuous variable and Chi-square test for categorical variables. Selected characteristics of the study population stratified by CRP quartiles were analyzed with ANOVA or Chi-squared tests as appropriate. To test the dependent relationships between CRP and severity of DR, a multivariate logistic regression analysis was carried out with DR as the dependant variable initially adjusted by established DR risk factors, including duration of diabetes, HbA1c, SBP, high albuminuria, use of insulin, and BMI (model 1). Variables with P value ≤0.2 in the univariate analysis shown in Table 1 were further controlled in regression model, including age of diabetic onset, current smoking, salary and education status, DBP, uric acid and creatinine (model 2). An additional model controlled for the use of oral hypoglycemic medications (either metformin and/or rosiglitazone), antihypertensive medication (such as beta blockers, angiotensin-converting enzyme inhibitors and angiotensin receptor blockers), use of lipid-lowering medications and aspirin (model 3). Regression analyses using CRP as continuous covariates and categorical covariates were repeated separately. Subjects in the upper quartile of CRP distributions were defined as the high category group with the lowest quartile used as the reference category. Test for trend was performed using linear test across the CRP quartiles in corresponding multiple logistic regression models. Results were expressed as P-value, odds ratio (OR) and 95% confidence intervals (CI). Statistical significance was set at $P<0.05$.

RESULTS

This study included 1131 subjects with T2DM. Those with missing data of CRP (n=72) and fundus photos (n=15) were excluded in the analysis. A further 37 participants who had CRP concentrations of >10.0 mg/L were excluded from statistical analyses because they were assumed to have infections or other diseases. A total of 1007 individuals with a mean age of 64.96±8.17y were available for the current analysis, including 408 (40.5%) men and 599 (59.5%) women. The mean age was 64.47±7.49y for women and 65.71±9.03y for men. The median CRP level was 1.5 mg/L for women and 1.1 mg/L for men (P=0.004, OR 0.37, 95% CI 0.18-0.74). Exact agreement on retinopathy level and presence of macular edema were 86% and 61.9% respectively. Weighted Kappa was 0.82. These statistics are in agreement with published reproducibility from the reading center[23].

Table 1 shows characteristics of the study population. Any DR was diagnosed in 358 (35.6%) subjects, among them 61 (6.1%) subjects were diagnosed as VTR, as assessed by fundus photography. Among the 408 men and 599 women, 165 (40.4%) men and 193 (32.2%) women had DR (P=0.008). The age of diabetic onset in any DR group (52.58±9.81y) tended to be younger compared with NDR group (57.52±8.63y) (P<0.001). Compared with the NDR group, any DR group had a longer duration of diabetes (P <0.001), lower education (P =0.001) and salary levels (P =0.04). Patients in the any DR group were more likely to be smoker (P=0.01) and to use insulin (P <0.001). Subjects with DR had significantly higher levels of FPG (8.59±2.83 *vs* 7.50±2.10 mmol/L) and HbA1c (7.40±1.64 *vs* 6.67±1.23 mmol/L) than those without DR. Moreover, compared to NDR group, the any DR group had higher levels of creatinine (P =0.007) and SBP (P <0.001), as well as DBP (P =0.03) and high albuminuria (P <0.001). There were no differences in BMI, WHR, and lipid profile between the any DR and NDR groups.

Table 2 summarizes the selected demographic, clinical and biochemical parameters of the study participants stratified by quartiles of CRP level. Significant difference in CRP level was observed between men and women (P =0.002). In unadjusted analyses of quartiles, increasing CRP levels were associated with higher levels of BMI, WHR, total cholesterol, blood glucose, HbA1c, SBP, DBP, and lower level of HDL or younger age (P <0.01).

Table 3 shows the relationship between CRP and any DR or VTR. Regression analyses were performed separately for any DR and VTR to examine their independent contribution. The multivariate models showed that elevated CRP levels were strongly associated with a lower risk of any DR. After adjustment for cofactors including age, sex, duration of

Table 1 Clinical characteristics of the 1007 participants with type 2 diabetes *n* (%)

Sample characteristics	VTR	Any DR[1]	NDR	Any DR and NDR	
	n=61	*n*=358	*n*=649	*P*	OR (95%CI)
Age (a)	63.56±8.23	64.60±8.23	65.17±8.13	0.29	0.99 (0.97-1.00)
Sex (male)	30 (49.2)	165 (46.1)	243 (37.4)	0.008	0.70 (0.54-0.91)
Age of diabetic onset (a)	47.28±9.59	52.58±9.81	57.52±8.63	<0.001	0.94 (0.93-0.96)
Duration of diabetes (a)	16.28±8.25	12.02±7.49	7.65±5.95	<0.001	1.10 (1.08-1.13)
Education lower than high school	27 (44.3)	177 (49.4)	251 (38.7)	0.001	0.65 (0.50-0.84)
Salary lower than 2 000 CNY per month	29 (47.5)	144 (40.2)	219 (33.7)	0.04	0.76 (0.58-0.99)
Alcohol consumption	15 (24.6)	72 (20.1)	111 (17.1)	0.26	1.21 (0.86-1.69)
Smoking status					
Current smoking	13 (21.3)	63 (17.6)	77 (11.9)	0.01	1.6 (1.11-2.29)
Ex-smoking	16 (26.2)	74 (25)	124 (21.6)	0.25	1.26 (0.89-1.78)
Never smoking	32 (52.5)	217 (61.3)	447 (69.0)	0.01	0.7 (0.52-0.92)
BMI (kg/m^2)	25.71±3.42	25.54±3.67	25.41±3.68	0.59	1.01 (0.97-1.04)
WHR	0.93±0.06	0.92±0.07	0.92±0.06	0.94	0.93 (0.13-6.90)
SBP (mm Hg)	140.6±15.66	138.4±16.80	134.1±16.77	<0.001	1.02 (1.01-1.02)
DBP (mm Hg)	79.17±9.51	79.38±9.90	78.04±9.44	0.03	1.01 (1.00-1.03)
Use of insulin	50 (82.0)	147 (41.1)	93 (14.3)	<0.001	4.14 (3.06-5.61)
Use of aspirin	31 (50.8)	179 (50.0)	301 (46.4)	0.40	0.89 (0.69-1.16)
High albuminuria	33 (54.1)	88 (26.2)	103 (15.8)	<0.001	1.89 (1.35-2.65)
HbA1c (mmol/mol)	7.71±1.88	7.40±1.64	6.67±1.23	<0.001	1.43 (1.30-1.57)
FPG (mmol/L)	9.68±3.55	8.59±2.83	7.50±2.10	<0.001	1.20 (1.14-1.27)
Creatinine (mg/dL)	87.82±86.03	72.35±41.47	67.03±16.46	0.007	1.01 (1.00-1.02)
Uric acid (μmol/L)	287.9±68.61	281.6±76.96	291±78.78	0.07	0.99 (0.99-1.00)
Total cholesterol (mmol/L)	5.17±1.11	5.15±1.12	5.09±0.99	0.41	1.05 (0.93-1.19)
Triglycerides (mmol/L)	1.84±1.48	1.69±1.44	1.76±1.54	0.47	0.97 (0.88-1.06)
HDL (mmol/L)	1.19±0.30	1.24±0.30	1.24±0.29	0.91	1.03 (0.66-1.59)
LDL (mmol/L)	2.99±0.89	3.10±0.90	3.06±0.84	0.51	1.05 (0.91-1.22)

VTR: Vision-threatening diabetic retinopathy; WHR: Waist and hip ratio; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; HbA1c: Glycosylated hemoglobin A1c; FPG: Fasting plasma glucose; BMI: Body mass index; HDL: High-density lipoprotein; LDL: Low-density lipoprotein. The data are expressed as numbers (%) for categorical variable, mean±standard deviation (SD) for continuous variable. Differences between diabetic retinopathy (DR) and diabetic without retinopathy (NDR) groups were compared using *t*-test or Chi-squared test. $P<0.05$ considered significant. Odds ratios (OR) with 95% confidence intervals (CI) were obtained by logistic regression. [1]Any DR group included the 61 subjects with VTR.

diabetes, HbA1c, SBP, high albuminuria, use of insulin and BMI (model 1), subjects with highest quartile of CRP were found to be inversely associated with the risk of any DR (P=0.022, OR 0.61, 95% CI 0.39-0.93). In further analysis including age of diabetic onset, current smoking, salary and education status, DBP, uric acid and creatinine (model 2), a significant inverse association was found for developing any DR among subjects with the highest quartile of CRP compared with the lowest quartile (P=0.02, OR 0.59, 95% CI 0.38-0.92). Associations between CRP and any DR did not significantly change when further adjustment was undertaken (model 3) that included the use of oral hypoglycemic medications, antihypertensive, lipid lowering drug and use of aspirin (P=0.02, OR 0.55, 95% CI 0.35-0.89). Similar results were obtained by using log transformed CRP levels for the analysis (P=0.004, OR 0.76, 95% CI 0.63-0.92 in model 1; P=0.003, OR 0.75, 95% CI 0.62-0.91 in model 2; P=0.005, OR 0.75, 95% CI 0.61-0.92 in model 3). Although a similar trend was noted for VTR, the difference between VTR and NDR groups was not statistically significant with each model tested.

A significant influence of gender on the association of log transformed CRP with any DR were found (P=0.003). Table 4 shows the relationship between CRP and any DR under stratification by gender. After adjusting for covariates (model 1), subjects with highest quartile of CRP in men's group were significantly less likely to have any DR (P=0.004, OR 0.37, 95% CI 0.19-0.73). Among men in the highest CRP quartile an OR of 0.37 for developing any DR (95% CI 0.18-0.73) were found compared with the lowest quartile including confounding factors in model 2. Further analysis that included oral glucose lowering medications in model 3 did not lead to significant changes in the observed OR (OR 0.35, 95% CI 0.16-0.73). After log transformation of CRP, the same significant associations were found in men after adjustment of possible covariates. In women, however,

Table 2 Characteristics of the type 2 diabetic participants, by quartiles of C-reactive protein *n* (%)

Sample characteristics	CRP				*P* for trend
	1st quartile (*n*=298)	2nd quartile (*n*=221)	3rd quartile (*n*=237)	4th quartile (*n*=251)	
Sex					
M	132 (44.3)	104 (47.1)	88 (37.1)	84 (33.5)	0.002
F	166 (55.7)	117 (52.9)	149 (62.9)	167 (66.5)	
Age (a)	65.79±7.72	64.91±8.47	64.63±8.22	64.37±8.32	0.04
Duration of diabetes (a)	9.25±6.95	10.25±7.26	8.91±6.61	8.52±6.57	0.09
BMI (kg/m^2)	24.17±3.36	25.20±3.64	25.86±3.35	26.84±3.83	<0.001
WHR	0.91±0.07	0.93±0.07	0.93±0.06	0.93±0.06	<0.001
SBP (mm Hg)	133.6±15.73	135.20±17.48	135.7±16.36	138.5±17.88	0.001
DBP (mm Hg)	77.36±9.20	77.24±9.33	79.09±8.76	80.46±10.77	<0.001
Use of insulin	63 (21.1)	57 (25.8)	59 (24.9)	61 (24.3)	0.38
Use of aspirin	138 (46.3)	105 (47.5)	119 (50.2)	118 (47.0)	0.71
High albuminuria	36 (12.1)	41 (18.6)	51 (21.5)	63 (25.1)	<0.001
HbA1c (mmol/mol)	6.57±1.10	6.97±1.53	6.91±1.44	7.34±1.57	<0.001
Blood glucose (mmol/L)	7.16±1.81	8.01±2.69	7.98±2.50	8.54±2.60	<0.001
Creatinine (μmol/L)	70.65±40.84	70.11±22.00	68.10±18.29	66.60±21.87	0.07
Uric acid (μmol/L)	281±78.62	290.50±80.83	288.48±75.49	292±77.94	0.12
Total cholesterol (mmol/L)	4.99±0.99	5.14±1.04	5.08±1.00	5.25±1.13	0.01
Triglycerides (mmol/L)	1.43±0.84	1.71±1.37	1.84±1.52	2.01±2.04	<0.001
HDL cholesterol (mmol/L)	1.34±0.33	1.23±0.27	1.18±0.25	1.17±0.27	<0.001
LDL cholesterol (mmol/L)	2.97±0.84	3.10±0.88	3.07±0.83	3.16±0.89	0.02

CRP: C-reactive protein; HbA1c: Glycosylated hemoglobin A1c; FPG: Fasting plasma glucose; BMI: Body mass index; WHR: Waist-to-hip ratio; HDL: High-density lipoprotein; LDL: Low-density lipoprotein. SBP: Systolic blood pressure; DBP: Diastolic blood pressure. Data presented were means (SD) or numbers (%), as appropriate. *P* for trend was performed using linear test across the CRP quartiles based on Chi-square test or ANOVA.

Table 3 Independent predictors of any DR or vision-threatening diabetic retinopathy in all type 2 diabetic participants by multiple logistic regression and linear regression analyses

Quartile of CRP	At risk (*n*)	Any DR				Vision threatening retinopathy			
		n (%)	Model 1	Model 2	Model 3	*n* (%)	Model 1	Model 2	Model 3
1st quartile (0-0.70)	297	105 (35.2)				16 (5.4)			
2nd quartile (0.71-1.30)	221	84 (37.8)	0.88 (0.59-1.32)	0.84 (0.55-1.27)	0.75 (0.48-1.16)	20 (9.0)	1.21 (0.53-2.77)	1.30 (0.54-3.14)	1.25 (0.49-3.16)
3rd quartile (1.31-2.40)	239	74 (30.8)	0.60 (0.39-0.90)	0.60 (0.39-0.92)	0.63 (0.40-0.99)	13 (5.4)	0.72 (0.29-1.78)	0.88 (0.34-2.24)	0.95 (0.36-2.55)
4th quartile (2.41-10)	250	79 (31.5)	0.61 (0.39-0.93)	0.59 (0.38-0.92)	0.55 (0.35-0.89)	12 (4.8)	0.54 (0.21-1.43)	0.69 (0.25-1.88)	0.78 (0.28-2.14)
P for trend			0.006	0.007	0.009		0.096	0.19	0.11
LogCRP			0.76 (0.63-0.92)	0.75 (0.62-0.91)	0.75 (0.61-0.92)		0.70 (0.46-1.06)	0.75 (0.49-1.15)	0.80 (0.52-1.24)
P			0.004	0.003	0.005		0.09	0.18	0.32

Model 1: Adjusted for age and sex, plus established risk factors of DR, including duration of diabetes, HbA1c, SBP, high albuminuria , use of insulin, BMI; Model 2: Model 1 plus potential confounding variables with $P \leqslant 0.20$ shown in Table 1; Model 3: Model 2 plus additional variables, adding the use of oral hypoglycemic medications (such as metformin and rosiglitazone) and antihypertensive medication (such as beta blockers, angiotensin-converting enzyme inhibitors and angiotensin receptor blockers), use of lipid-lowering medications and aspirin. HbA1c: Glycosylated hemoglobin A1c; LogCRP: Log transformed CRP value; OR: Odds ratio; CI: Confidence interval. Data presented as OR with 95% CI.

association between CRP and any DR was not statistically significant in any models analyzed.

In supplementary analyses, we repeated the analyses by including those subjects with CRP > 10.0 mg/L. Compared with quartile 1 of CRP, the ORs of any DR were 0.17 (0.48-1.14) in quartile 2, 0.66 (0.42-1.03) in quartile 3, and 0.50 (0.30-0.82) in quartile 4 (*P* for trend=0.004) in model 3. The relationship between CRP and any DR under stratification by gender also revealed a significant association in men's group but not in women's group. Compared with quartile 1 of CRP, the ORs of any DR in men's group were 0.48 (0.24-0.94) in quartile 2, 0.49 (0.24-1.01) in quartile 3, and 0.35 (0.16-0.75) in quartile 4 (*P* for trend=0.007) in model 3.

DISCUSSION

The findings of this study suggest a possible inverse association of the CRP levels with the development of any DR after adjustment for several established DR risk factors

Table 4 Independent predictors of any DR in men or women by multiple logistic regression or linear regression analyses

Quartile of CRP	At risk (*n*)	Any DR in men's group				Quartile of CRP	At risk (*n*)	Any DR in women's group			
		n (%)	Model 1	Model 2	Model 3			*n* (%)	Model 1	Model 2	Model 3
1st quartile (0-0.60)	107	54 (50.5)				1st quartile (0-0.70)	166	49 (29.5)			
2nd quartile (0.70-1.10)	102	41 (40.2)	0.54 (0.29-1.00)	0.54 (0.29-1.02)	0.43 (0.21-0.87)	2nd quartile (0.80-1.50)	133	48 (36.1)	0.89 (0.52-1.54)	0.89 (0.50-1.57)	0.79 (0.42-1.46)
3rd quartile (1.10-2.20)	101	39 (38.6)	0.54 (0.29-1.02)	0.51 (0.26-0.97)	0.56 (0.28-1.15)	3rd quartile (1.60-2.70)	151	48 (31.8)	0.74 (0.41-1.34)	0.74 (0.40-1.36)	0.77 (0.40-1.48)
4th quartile (2.20-10)	98	31 (31.6)	0.37 (0.19-0.73)	0.37 (0.18-0.73)	0.35 (0.16-0.73)	4th quartile (2.80-10)	149	48 (32.2)	0.66 (0.36-1.21)	0.63 (0.33-1.19)	0.58 (0.29-1.16)
P for trend			0.002	0.003	0.01	*P* for trend			0.16	0.11	0.12
LogCRP			0.62 (0.46-0.84)	0.61 (0.45-0.84)	0.64 (0.46-0.88)	LogCRP			0.89 (0.7-1.15)	0.88 (0.68-1.15)	0.88 (0.66-1.16)
P			0.002	0.002	0.006	*P*			0.35	0.35	0.36

Model 1: Adjusted for age, plus established risk factors of DR, including duration of diabetes, HbA1c, SBP, high albuminuria, use of insulin, BMI; Model 2: Model 1 plus potential confounding variables with $P \leqslant 0.20$ shown in Table 1; Model 3: Model 2 plus additional variables, adding the use of oral hypoglycemic medications (such as metformin and rosiglitazone) and antihypertensive medication (such as beta blockers, angiotensin-converting enzyme inhibitors and angiotensin receptor blockers), use of lipid-lowering medications and aspirin. HbA1c: Glycosylated hemoglobin A1c; LogCRP: Log transformed CRP value; OR: Odds ratio; CI: Confidence interval. Data presented as OR with 95% CI.

and potential confounding factors in the Chinese patients with T2DM. This inverse association seems to be more prominent in men's group but not statistically significant in women's group.

CRP, the classical acute phase plasma protein, is an exquisitely sensitive, non-specific marker of inflammation and tissue damage. CRP may contribute to development of the atherosclerotic lesion and the subsequent acute cardiovascular events *via* its role in a large number of biological pathways by interacting with vascular cells like endothelial cell, smooth muscle cell and monocytes, as well as modulating expression of some factors such as nitric oxide[24]. As an inflammatory biomarker that is easily measured, the association between CRP and systemic disease has been investigated broadly and a large amount of published data show that higher CRP concentration consistently correlates significantly with increased risk of atherosclerosis [25], cardiovascular disease [7], and diabetes [8]. The present study showed that elevated serum CRP levels are associated with traditional cardiovascular and diabetic risk factors, such as BMI, elevated serum glucose and triglyceride levels and LDL cholesterol level, while negatively associated with HDL cholesterol. This is consistent with data from previous reports. Despite the consistency of this association, recently there is a major debate on whether that CRP is merely a risk marker or a potential causative role in cardiovascular pathogenesis [26-29]. For example, recent genetic findings offer opportunities for testing the causal relevance of CRP using the principles of Mendelian randomization. CRP levels confer a moderate risk of artherosclerosis [25] and diabetes[30], no causal relationship has been shown in genetic studies of CRP variants and either artherosclerosis disease or diabetes using Mendelian randomization approach and the multivariable regression analysis approach [28-29]. It implied that previously demonstrated associations between CRP and diseases may be better explained by CRP marking the presence of them, or other risk factors rather than having a direct causal role itself.

Studies examining the possible association of CRP with DR in diabetic patients have shown conflicting results, with studies demonstrating higher CRP levels associated with higher risk of DR [11-14,19] and others showing no association[15-18]. The present study in Chinese population showed that higher levels of CRP have an inverse association with the development of any DR. This unexpected finding is consistent with one latest report from the Singapore Malay Eye Study [19], in which such an inverse relationship between CRP and DR was also reported. In contrast, we failed to find an association of CRP with the subgroup of VTR although a similar trend was noted. It may be due to a small number of subjects in VTR subgroup and the trend might be significant with a larger sample. In support of the Singapore Malay Eye Study[19] and the present study, recently increasing evidences have shown that increased CRP levels may have a possible anti-inflammatory [31] or anti-atherosclerotic [32-33] effect, and CRP is believed to have dual effects on the inflammatory process [34]. Possible mechanisms for this protective effect of CRP have been hypothesized, such as down-regulating the neutrophil surface expression of L-selectin thus preventing migration of neutrophils into inflammatory sites [35], modulating the expression of genes that contribute to both pro- and anti-inflammatory responses in human monocytes[31], and mediating the complement activation thus promoting the removal of debris from tissues [32]. Based on the previous investigation of CRP, it has been hypothesized that elevated CRP levels may be beneficial in the pre-proliferative stages of DR by relieving ischemia, up-regulating the expression of vascular endothelial growth factor A and thus increasing retinal perfusion [19]. Collectively, the role of CRP in the inflammatory process is rather complicated as this protein may initiate and/or modulate multiple responses of the host and in this current study it is suggested that CRP may play a protective role in the DR pathogenesis.

Consistent with previous population-based studies [36-37], the present study showed higher prevalence of any DR in men

(40.4%) than that in women (32.2%). Moreover, the data showed that women had relatively higher levels of CRP than men, which is consistent with the data from the previous epidemiological studies [38]. By analyzing the association of CRP with any DR separately in men and women, the data showed that the elevated levels of CRP were statistically significantly associated with a relatively lower risk of DR in men but this association was not statistically significant in women. Gender differences of endogenous sex hormone levels and the potential confounding effects of lifestyles might explain the higher levels of CRP in women [38]. Given the possible protective effect of elevated CRP levels against DR, it seems reasonable to hypothesis that the lower prevalence of DR in women may be to some extent related to the relatively higher mean concentration of CRP. It should be noted, however, that the true function of systemic CRP in the pathogenic process of DR is not fully understood and further investigation should be warranted.

The strength of the present study is that the participants with T2DM were recruited from a relatively homogenous population of a well-defined geographical area. To the best of our knowledge, this is a study with the largest sample size to date that has studied the association of CRP with the development of DR in the Chinese patients with T2DM. Additionally, standardized protocols were used to obtain photographs and to assess the grading of DR. However, limitations of the study should be noted. Being a cross-sectional survey, the findings only support an association between CRP and DR but do not prove a causal relationship. Moreover, the study participants were not randomly selected so that selection bias could potentially be introduced.

In summary, the present study showed that T2DM patients with higher levels of CRP were less likely to have DR, with significant association found in men but not in women. Further clinical studies to confirm this observation would be warranted. Investigations in appropriate animal models may also be needed to distinguish the role of CRP as either a marker or a factor that directly causes a biological effect.

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