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Serum levels of interleukin 6, C-reactive protein, vascular cell adhesion molecule 1, and monocyte chemotactic protein 1 in relation to insulin resistance and glucose intolerance—the Chennai Urban Rural Epidemiology Study (CURES)☆

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Abstract

The aim of this cross-sectional study was to assess the association of insulin resistance (IR) with inflammatory molecules C-reactive protein (CRP), interleukin 6 (IL-6), vascular cell adhesion molecule 1 (VCAM-1), and monocyte chemotactic protein 1 (MCP-1) in urban South Indian subjects. The following groups were selected from the population-based Chennai Urban Rural Epidemiology Study: group 1 composed of 50 healthy subjects with normal glucose tolerance without IR; group 2 consisted of 50 normal glucose-tolerant subjects with IR as defined by homeostasis model assessment of IR (HOMA-IR); group 3 consisted of 50 subjects with impaired glucose tolerance (IGT); and groups 4 and 5 each comprised 50 newly diagnosed and known type 2 diabetic subjects, respectively. The inclusion criteria included nonsmokers; normal resting 12-lead electrocardiogram; and absence of angina, myocardial infarction, or history of any known vascular, infectious, or inflammatory diseases, and not on statins or aspirin. Normal glucose tolerance without IR had the lowest values of CRP, IL-6, and VCAM-1 (CRP, 1.32 mg/L; IL-6, 12.56 pg/mL; VCAM-1, 277 pg/mL) followed by normal glucose tolerance with IR (CRP, 2.25 mg/L; IL-6, 20.97 pg/mL; VCAM-1, 289 pg/mL), impaired glucose tolerance (CRP, 2.37 mg/L; IL-6, 22.11 pg/mL; VCAM-1, 335 pg/mL), newly diagnosed diabetic subjects (CRP, 3.24 mg/L; IL-6, 23.21 pg/mL; VCAM-1, 568 pg/mL), and the highest levels were in the known diabetic subjects (CRP, 4.08 mg/L; IL-6, 29.44 pg/mL; VCAM-1, 577 pg/mL). This trend was statistically significant ($P < .001$). However, monocyte chemotactic protein 1 did not show such a trend and did not differ significantly between groups. In nondiabetic subjects, Pearson correlation analysis revealed that CRP ($r = 0.299$; $P < .001$) and IL-6 ($r = 0.180$, $P = .025$) had a significant correlation with HOMA-IR. Monocyte chemotactic protein 1 did not show any correlation with HOMA-IR. Multiple linear regression analysis revealed CRP to be significantly associated with HOMA-IR ($\beta = .229$; $P < .001$) and this was unaltered by the addition of waist and IL-6 into the model ($\beta = .158$; $P = .028$). In conclusion, this study shows that in Asian Indians, inflammatory markers (CRP, IL-6, and VCAM-1) increase with increasing degrees of glucose intolerance.

1. Introduction

It is well known that insulin resistance (IR) predisposes to type 2 diabetes mellitus and possibly to coronary artery disease (CAD) as well [1]. Low-grade inflammation has been suggested to be the link between IR and the above disease states [2,3]. Earlier studies have shown that the levels of inflammatory markers are elevated in subjects with diabetes and IR, and some of these markers have been shown to predict incident type 2 diabetes mellitus as well as CAD [4-6]. It has been hypothesized that increased levels of interleukin 6 (IL-6) and other cytokines lead to an acute-phase response with increased production of C-reactive protein (CRP), which along with other inflammatory factors increase the production of adhesion molecules [7,8]. Insulin

☆ This is the 33rd paper from CURES.

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resistance also indirectly stimulates endothelial production of vascular cell adhesion molecule 1 (VCAM-1) and monocyte chemotactic protein 1 (MCP-1), which are important triggers of the atherosclerotic process. Recently, MCP-1 and VCAM-1 were also shown to impair adipocyte insulin sensitivity [9,10]. Thus, there appear to be intricate links between cytokines, inflammation, macrovascular disease, and type 2 diabetes mellitus.

Although several epidemiologic studies point to a causative relationship between inflammation and IR, incident cardiovascular disease, and diabetes, very few studies have looked at these parameters in nondiabetic subjects in relation to IR [11,12]. In addition, there are insufficient data on these biomarkers in Asian Indians, a very high-risk group for diabetes, CAD, and IR [13-16]. Such studies are relevant in this population given the high prevalence of diabetes and CAD in the absence of traditional risk factors such as obesity or excess smoking rates [16,17]. In this study we look at the association of IL-6, CRP, VCAM-1, and MCP-1 with IR in nondiabetic Asian Indians and in subjects with different grades of glucose intolerance.

2. Materials and methods

2.1. Sample selection

The Chennai Urban Rural Epidemiology Study (CURES) is an ongoing epidemiologic study conducted on a representative population (aged ≥20 years) of Chennai (formerly Madras), the fourth largest city in India with a population of about 4.2 million. The methodology of the study has been published elsewhere [18]. Briefly, in phase 1 of the urban component of CURES, 26 001 individuals were recruited based on a systematic random sampling technique. Self-reported diabetic subjects (physician diagnosed) were classified as "known diabetic subjects." In phase 2 of CURES, all known diabetic subjects (n = 1529) were invited to our center for detailed studies on vascular complications. In addition, age- and sex-matched nondiabetic subjects underwent oral glucose tolerance tests (OGTTs) using 75 g of glucose load. Those who were confirmed by OGTT to have fasting plasma glucose of less than 100 mg/dL and 2-hour plasma glucose value of less than 140 mg/dL were categorized as normal glucose tolerance (NGT) [19]. Those who were confirmed by OGTT to have 2-hour plasma glucose value of 200 mg/dL or higher based on World Health Organization consulting group criteria [19] were labeled as "newly detected diabetic subjects," whereas those with 2-hour postglucose value ≥140 mg/dL and <200 mg/dL were diagnosed as impaired glucose tolerance (IGT). For known diabetic subjects, fasting and postprandial plasma glucose after a standard breakfast was measured [20]. All study subjects underwent a 12-lead electrocardiogram (ECG).

Institutional ethical committee approval was obtained for the study, and informed consent was obtained from all study subjects.

2.2. Anthropometric measurements

Anthropometric measurements including weight, height, and waist measurements were obtained using standardized techniques as described earlier [18]. The body mass index was calculated as the weight in kilograms divided by the square of height in meters. Blood pressure was recorded in the sitting position in the right arm to the nearest 2 mm Hg with a mercury sphygmomanometer (Diamond Deluxe BP apparatus, Pune, India). Two readings were taken 5 minutes apart and the mean of the two was taken as the blood pressure.

2.3. Biochemical parameters

Biochemical analyses were done on Hitachi-912 Auto-analyser (Hitachi, Mannheim, Germany) using kits supplied by Roche Diagnostics (Mannheim, Germany). Fasting plasma glucose (GOD-POD method), serum cholesterol (CHOD-PAP method), serum triglycerides (GPO-PAP method), and high-density lipoprotein cholesterol (HDL-C) (direct method-polyethylene glycol–pretreated enzymes) were measured. Low-density lipoprotein cholesterol was calculated using the Friedewald formula [21]. Glycated hemoglobin (HbA_{1c}) was estimated by high-pressure liquid chromatography using the Variant machine (Bio-Rad, Hercules, CA). Serum insulin concentration was estimated using Dako kits (Dako, Glostrup, Denmark).

Insulin resistance was calculated using the homeostasis model assessment of IR (HOMA-IR) [22] using the formula: fasting insulin (μIU/mL) × fasting glucose (mmol/L)/22.5. Subjects whose HOMA-IR values exceeded the 75th percentile of the total population (ie, 1.93) were considered to have IR [23].

The following groups of subjects were selected from CURES:

Group 1 is composed of 50 healthy subjects with NGT without IR. The inclusion criteria for this group included NGT; nonsmokers; normal resting 12-lead ECG; and absence of angina, myocardial infarction, or history of any known vascular, infectious, or inflammatory diseases, and not on statins or aspirin.

Group 2 consisted of 50 normal glucose-tolerant subjects with IR as defined by the HOMA-IR. Group 3 consisted of 50 subjects with IGT. These subjects were also nonsmokers and had no infectious or inflammatory diseases. Diagnosis of IGT was based on the World Health Organization consulting group criteria, that is, 2-hour postglucose value of ≥140 and <200 mg/dL [19]. The other inclusion criteria were similar to that for group 1.

Groups 4 and 5 each comprised 50 newly diagnosed and known type 2 diabetic subjects, respectively. The other inclusion criteria were similar to that for group 1.

C-reactive protein (BioCheck, Foster City, CA; intra- and interassay coefficient of variation, 4.0% and 7.8%, respectively), IL-6 (R&D System, Minneapolis, MN; intra- and interassay coefficient of variation, 3.2% and 6.0%, respectively), VCAM-1 (BioSourse International, Camarillo, CA;

Table 1
Clinical and biochemical characteristics of the study groups

Variables	Normal glucose-tolerant subjects without IR (n = 50)	Normal glucose-tolerant subjects with IR (n = 50)	Impaired glucose tolerance (n = 50)	Newly diagnosed type 2 diabetic subjects (n = 50)	Known type 2 diabetic subjects (n = 50)
Age (y)	45 ± 11	45 ± 13	47 ± 10	49 ± 10	54 ± 11‡
Sex (M/F)	17/33	18/32	19/31	22/28	24/26
Body mass index (kg/m^2)	22.5 ± 4.4	24.6 ± 3.6*	26.3 ± 3.8†	25.4 ± 4.1**	25.0 ± 4.7**
Waist circumference (cm)	83.8 ± 11.9	87.2 ± 9.1	93.2 ± 9.2††	91.2 ± 10.1†	92 ± 10.7†
Systolic BP (mm Hg)	122 ± 20	121 ± 17	128 ± 19	132 ± 20†	129 ± 15
Diastolic BP (mm Hg)	77 ± 12	77 ± 11	79 ± 14	81 ± 10*	77 ± 10
Fasting plasma glucose (mg/dL)	87 ± 6	88 ± 8	100 ± 15††	153 ± 60‡	169 ± 69‡
Total cholesterol (mg/dL)	181 ± 41	204 ± 47*	195 ± 35	211 ± 42*	207 ± 46*
Triglycerides (mg/dL)[a]	96	126**	129**	160***	146***
HbA_{1c} (%)	5.7 ± 0.5	5.9 ± 0.5	6.4 ± 0.8††	8.2 ± 2.1‡	9.0 ± 2.1‡
HOMA-IR[a]	1.1	2.7***	2.8***	2.7***	2.5***

BP indicates blood pressure.
[a] Data expressed as a geometric mean.
* $P < .05$ compared to NGT without IR.
** $P < .01$ compared to NGT without IR.
*** $P < .001$ compared with NGT without IR.
† $P < .05$ compared to NGT without IR and NGT with IR.
†† $P < .01$ compared to NGT without IR and NGT with IR.
‡ $P < .01$ compared to NGT without IR, NGT with IR, and IGT.

intra- and interassay coefficient of variation, 3.0% and 5.3%, respectively), and MCP-1 (R&D System; intra- and interassay coefficient of variation, 4.0% and 7.6%, respectively) were measured by enzyme-linked immunosorbent assay.

2.4. Statistical analysis

One-way analysis of variance or Student *t* test, as appropriate, was used to compare groups for continuous variables. χ^2 Test or Fisher exact test, as appropriate, was used to compare proportions. Triglycerides, HOMA-IR, CRP, VCAM-1, MCP-1, and IL-6 values were log transformed to obtain a normal distribution. To examine the association of various cardiovascular risk factors with inflammatory markers and adhesion molecules, we performed Pearson correlation analysis.

To determine the association of the study parameters with diabetes, we categorized the study subjects into diabetic and nondiabetic subjects by combining the groups together, that is, groups 1, 2, and 3 were taken together as the nondiabetic group and groups 4 and 5 as the diabetic groups. Multiple logistic regression analysis was done to determine the association of inflammatory markers and adhesion molecules with diabetes.

The regression analysis was restricted to nondiabetic subjects, to determine the association of IR with CRP, IL-6, VCAM-1, and MCP-1 in nondiabetic subjects, as it is well known that diabetic subjects have altered insulin sensitivity. Factor analysis was done to determine the relation of inflammatory markers with the components of metabolic syndrome by extracting the initial components by principal component analysis, followed by rotation of principal components by varimax method. Factors with loading of ± 0.3 or more were used to define a significant relationship.

All analyses were done using Windows-based SPSS statistical package (version 10.0, Chicago, IL), and *P* values of less than .05 were taken as the level of significance.

Table 2
Levels of inflammatory molecules in the study groups

Variables	Normal glucose-tolerant subjects without IR (n = 50)	Normal glucose-tolerant subjects with IR (n = 50)	Impaired glucose tolerance (n = 50)	Newly diagnosed type 2 diabetic subjects (n = 50)	Known type 2 diabetic subjects (n = 50)	*P* for linear trend
CRP (mg/L)	1.32 (0.11-7.54)	2.25** (0.11-7.24)	2.37** (0.36-8.14)	3.24*** (0.46-8.38)	4.08†† (0.99-8.27)	<.001
IL-6 (pg/mL)	12.56 (1.86-77.60)	20.97* (2.05-88.80)	22.11* (2.60-80.00)	23.21* (3.40-91.8)	29.44** (3.40-90.20)	<.001
VCAM-1 (pg/mL)	277 (90-836)	289 (51-1798)	335 (65-1850)	568† (134-2064)	577†† (103-2014)	<.001
MCP-1 (pg/mL)	7.10 (0.40-11.90)	8.40 (0.50- 44.50)	6.90 (1.10-28.8)	8.3 (2.0-60.2)	9.07 (1.50-61.60)	.695

Data expressed as geometric mean (range).
* $P < .05$ compared to NGT without IR.
** $P < .01$ compared to NGT without IR.
*** $P < .001$ compared to NGT without IR.
† $P < .05$ compared to NGT without IR, NGT with IR, and IGT.
†† $P < .01$ compared to NGT without IR, NGT with IR, and IGT.

Table 3
Pearson's correlation analysis in nondiabetic subjects

Variables	CRP		IL-6		MCP-1		VCAM-1	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Total study subjects								
Age	0.026	.684	0.055	.379	0.092	.138	0.010	.879
Body mass index	0.174	.006	0.034	.581	0.050	.419	0.038	.554
Waist circumference	0.241	<.001	0.110	.049	0.048	.439	0.138	.032
Fasting Plasma glucose	0.321	<.001	0.127	.041	0.072	.247	0.163	.010
HbA_{1c}	0.271	<.001	0.088	.160	0.103	.960	0.177	.005
Triglycerides	0.126	.045	0.015	.812	0.088	.155	0.035	.588
HOMA-IR	0.295	<.001	0.080	.200	0.114	.066	0.069	.280
CRP	–	–	0.485	<.001	0.026	.678	0.206	.001
IL-6			–	–	0.036	.564	0.479	<.001
MCP-1	–	–	–	–	–	–	0.097	.128
Nondiabetic subjects								
Age	0.043	.592	0.030	.711	0.103	.202	0.152	.063
Body mass index	0.283	<.001	0.127	.115	0.032	.693	0.009	.916
Waist circumference	0.309	<.001	0.122	.133	0.117	.151	0.060	.465
Fasting Plasma glucose	0.166	.040	0.072	.375	0.052	.517	0.044	.592
HbA_{1c}	0.033	.687	0.114	.157	0.114	.157	0.070	.391
Triglycerides	0.071	.386	0.045	.582	0.119	.180	0.131	.110
HOMA-IR	0.299	<.001	0.180	.025	0.040	.625	0.061	.460
CRP	–	–	0.537	<.001	0.049	.546	0.086	.301
IL-6			–	–	0.078	.337	0.378	<.001
MCP-1	–	–	–	–	–	–	0.022	.790

3. Results

The clinical and biochemical characteristics of the study group are shown in Table 1. Nondiabetic subjects with IR had higher body mass index ($P < .05$) and triglyceride ($P < .01$) and serum cholesterol ($P < .05$) levels compared with those without IR. Subjects with IGT had higher body mass index ($P < .05$), waist circumference ($P < .05$), triglycerides ($P < .01$), and increased HOMA-IR ($P < .001$) compared with nondiabetic subjects without IR. Both newly diagnosed and known diabetic subjects had higher body mass index ($P < .01$), waist circumference ($P < .05$), total cholesterol ($P < .05$), and triglycerides ($P < .001$), whereas the difference in systolic blood pressure ($P < .05$) and diastolic blood pressure ($P < .05$) reached significance only in subjects with newly diagnosed diabetes compared with nondiabetic subjects without IR.

The levels of inflammatory and adhesion molecules in the study subjects are presented in Table 2. Levels of CRP, IL-6, and VCAM-1 progressively increased with increasing

Table 4
Association of biomarkers with diabetes

Total study population—multiple logistic regression analysis (dependent variable: diabetes)	Odds ratio	95% Confidence intervals	*P*
CRP			
Unadjusted	17.18	6.077-48.586	<.001
Adjusted for age, waist circumference, blood pressure, lipids	15.92	5.225-48.549	<.001
Adjusted for age, waist circumference, blood pressure, lipids, IL-6, VCAM-1, and MCP-1	30.21	6.795-134.308	<.001
IL-6			
Unadjusted	2.31	1.199-4.439	.012
Adjusted for age, waist circumference, blood pressure, lipids	2.13	1.048-4.382	.024
Adjusted for age, waist circumference, blood pressure, lipids, CRP, VCAM-1, and MCP-1	0.39	0.117-1.332	.134
VCAM-1			
Unadjusted	8.77	4.011-19.188	<.001
Adjusted for age, waist circumference, blood pressure, lipids	12.46	4.987-31.139	<.001
Adjusted for age, waist circumference, blood pressure, lipids, CRP, IL-6, and MCP-1	13.40	4.446-40.371	<.001
MCP-1			
Unadjusted	1.77	0.845-3.705	.130
Adjusted for age, waist circumference, blood pressure, lipids	1.82	0.807-4.108	.149
Adjusted for age, waist circumference, blood pressure, lipids, CRP, IL-6, and VCAM-1	1.249	0.434-3.589	.680

degrees of glucose intolerance. Normal glucose-tolerant subjects without IR had the lowest values of CRP, IL-6, and VCAM-1 followed by normal glucose-tolerant subjects with IR, IGT, newly diagnosed diabetes, and the highest levels were in the known diabetic subjects; this trend was statistically significant ($P < .001$). However, MCP-1 did not show such a trend, and there was no significant difference between the groups.

When compared with nondiabetic subjects without IR, difference in CRP levels and IL-6 levels reached statistical significance in nondiabetic subjects with IR ($P < .05$), IGT ($P < .05$), newly diagnosed type 2 diabetes mellitus ($P < .05$), and known diabetic subjects ($P < .01$). However, the difference in VCAM-1 levels reached statistical significance only in subjects with diabetes (both newly diagnosed [$P < .001$] and known diabetic subjects [$P < .001$]) compared with other groups.

Table 3 shows the Pearson correlation analysis for the markers studied. In the total study population, CRP ($r = 0.241$, $P < .001$), and IL-6 ($r = 0.110$, $P = .049$) and VCAM-1 ($r = 0.138$, $P = .032$) showed a correlation with waist circumference. C-reactive protein and IL-6 showed a significant correlation with fasting plasma glucose (CRP: $r = 0.321$, $P < .001$; IL-6: $r = 0.127$, $P = .041$). C-reactive protein also showed an association with body mass index ($r = 0.174$, $P = .006$), waist circumference ($r = 0.241$, $P < .001$), triglycerides ($r = 0.271$, $P < .001$), HbA_{1c} ($r = 0.271$, $P < .001$), HOMA-IR ($r = 0.295$, $P < .001$), IL-6 ($r = 0.485$, $P < .001$), and VCAM-1 ($r = 0.206$, $P = .001$). Vascular cell adhesion molecule 1 showed an association only with fasting plasma glucose ($r = 0.138$, $P = .032$) and IL-6 ($r = 0.479$, $P < .001$). Monocyte chemotactic protein 1 failed to show any correlation with the study parameters.

3.1. Association of CRP, IL-6, VCAM-1, and MCP-1 with diabetes

For this analysis we categorized the study subjects into diabetic (groups 4 and 5) and nondiabetic subjects (groups 1-3).

Logistic regression analysis was used to determine the association of CRP, IL-6, MCP-1, and VCAM-1 with diabetes (Table 4). C-reactive protein (odds ratio [OR], 17.18; $P < .001$), IL-6 (OR, 2.3; $P < .012$), and VCAM-1 (OR, 8.7; $P < .001$) showed an association with diabetes, and this association persisted even after adjusting for age, waist circumference, blood pressure, and lipids (CRP: OR, 15.9, $P < .001$; VCAM-1: OR, 12.5, $P < .001$; IL-6, OR, 2.3, $P = .024$). Adding other biomarkers (MCP-1, IL-6, and VCAM-1 for CRP and likewise others) into the model did not alter the association of CRP (OR, 30.2; $P < .001$) and VCAM-1 (OR, 13.4; $P < .001$) with diabetes, but abolished the association of IL-6 with diabetes (OR, 0.69; $P = .417$). Monocyte chemotactic protein 1 failed to show any association with diabetes.

Table 5
Association of biomarkers with IR

Nondiabetic subjects—multivariate linear regression analysis (dependent variable: IR)		
	β	P
CRP		
Unadjusted	.229	<.001
Adjusted for waist circumference and IL-6	.158	.028
IL-6		
Unadjusted	.134	.022
Adjusted for waist circumference and CRP	.031	.640

Table 6
Factor loading pattern of inflammatory markers after varimax rotation of principle components in nondiabetic subjects

Phenotype	Factor loadings		
	Factor 1	Factor 2	Factor 3
Systolic blood pressure	0.920	0.047	0.017
Diastolic blood pressure	0.910	0.083	0.071
Waist circumference	0.188	0.389	0.465
Log-transformed triglycerides	0.288	−0.038	0.706
HOMA-IR	0.017	0.527	0.430
Log-transformed CRP	0.029	0.842	0.084
Log-transformed IL-6	0.068	0.840	−0.168
% of variance	65.4		

3.2. Association of CRP, IL-6, VCAM-1, and MCP-1 with IR

To determine the relation of HOMA-IR with the study markers in nondiabetic subjects, we restricted the rest of the analysis to nondiabetic subjects for which groups 1, 2, and 3 were combined together.

Pearson correlation analysis revealed that only CRP ($r = 0.299$; $P < .001$) and IL-6 ($r = 0.180$, $P = .025$) had a significant correlation with HOMA-IR (Table 3). Multiple linear regression analysis was done to determine the association of CRP and IL-6 with HOMA-IR. The variables, which showed significant correlation with these parameters, were incorporated into the regression model as independent variables to assess their effect on the association of HOMA-IR with CRP and IL-6 (Table 5).

C-reactive protein showed a significant association with HOMA-IR ($\beta = .229$; $P < .001$); this was unaltered by the addition of waist and IL-6 into the model ($\beta = .158$; $P = .028$). However, the association of IL-6 with HOMA-IR abolished when CRP was introduced into the model (Table 5).

3.3. Factor loading pattern of CRP and IL-6 after varimax rotation of principal components

In the nondiabetic subjects, 3 factors explained 65.4% of the variance (Table 6). Factor 1, which represents the blood pressure factor, had positive loadings of systolic and diastolic blood pressure. Factor 2, which represented the inflammatory, IR factor, was positively loaded with CRP, IL-6, and HOMA -IR. Factor 3, which represented the obesity, IR factor, was positively loaded with waist circumference, triglycerides, and HOMA-IR and negatively loaded with HDL-C.

4. Discussion

The main findings from the study are the following: (i) levels of CRP, IL-6, and VCAM-1 increased with increase in grades of glucose intolerance; (ii) CRP and IL-6 showed a significant correlation with IR among nondiabetic subjects; (iii) CRP and IL-6 clustered with waist and HOMA-IR and shared a correlation with triglycerides and HDL-C.

Earlier studies have assessed the relation of inflammatory markers with glucose intolerance and diabetes [2,5,6]. This study shows that in Asian Indians, inflammatory markers such as CRP, IL-6, and VCAM - 1 show an increasing trend with values, higher in nondiabetic subjects with IR, higher still in those with IGT, and highest in diabetic subjects. An earlier study has shown that subjects with IGT had higher IL-6 levels compared with normal glucose-tolerant subjects [12], and several other studies have shown CRP to be increased in the IGT phase [24,25]. However, in contrast, a Korean study demonstrated no increase in these markers in those with IGT [26]. This could indicate ethnic differences in the association of inflammatory markers with glucose intolerance and hence the significance of this study in Asian Indians who have higher risk of diabetes [27] and CAD [28].

In an earlier study, based on regression analysis, we had shown that the relation between diabetes and body fat was abolished by the introduction of CRP into the model, indicating that this relation was possibly mediated through CRP [4]. In this study we show that the association of CRP with diabetes is independent of IL-6 and other risk factors. This supports the findings of a nested case-control study by Hu et al [29] that IL-6 had a weaker predictive value for diabetes compared with CRP.

The chemokine MCP-1 recruits and activates monocytes from the circulation to inflammatory site, and this activation is stimulated by VCAM-1 [30]. Monocyte chemotactic protein 1 has also been shown to induce adipocyte differentiation, increase IR, and indirectly increase the risk for atherogenic changes [9,30]. However, in our study, MCP-1 did not show any significant association with IR or diabetes. The reason for this could probably be the small sample size. Alternatively, this might represent an ethnic difference in the association of MCP-1 with CAD and diabetes.

An interesting observation in this study is that both IL-6 and CRP showed a strong association with IR even in nondiabetic subjects. Both these markers also showed a good association with waist circumference, a marker for abdominal obesity. Abdominal obesity is considered to play a major role in inducing inflammatory responses, which results in IR in various tissues. Cytokines produced by the adipose tissue could induce an endocrine effect resulting in IR in liver, muscle, and vascular tissues. In turn, these inflammatory markers also induce vascular changes leading to macrovascular diseases. A recent review on metabolic actions of insulin suggests insulin to be an anti-inflammatory hormone; however, it also explores the role of various pro-inflammatory molecules that induces changes at insulin receptor levels resulting in IR [31]. Further studies have shown circulating levels of IL-6, and other inflammatory markers impair insulin sensitivity and glucose regulation [2,32]. Cellular studies support the evidence for a cross-link between IL-6 and insulin intracellular pathways, leading to development of IR [31]. More evidence for the hypothesis that IR is a consequence of inflammatory mediators in obesity is available from a study on peripheral mononuclear cells, which indicated that the mononuclear cells in obese subjects are in proinflammatory state [33].

The association of inflammatory markers with obesity is evident from the clustering of these markers with IR and waist circumference. Factor analysis revealed that the inflammatory markers cluster with IR and waist circumference and shared a correlation with triglycerides and HDL-C. However, blood pressure clustered into a separate factor as reported in other studies [34]. Similar results have been reported from UK, but on subjects with myocardial infarction [35].

Atherosclerosis, which is consequent to IR and diabetes, is now believed to be an inflammatory disease [1,36]. However, very few studies have looked at the relation between these markers with early changes in biomarkers like adhesion molecules in subjects without heart disease, particularly in Asian Indians, who are shown to have very high rates of CAD [28]. In this study we report that both IL-6 and CRP are associated with VCAM-1 and exert a synergistic effect on its levels. However, the association of IL-6 was stronger with VCAM-1 than with CRP.

Interleukin 6 induces inflammatory changes by triggering the production of both CRP and VCAM-1. Although the role of CRP is still not very clear, VCAM-1 enhances adhesion of monocytes and advances progression of the atherosclerotic process. The Edinburgh Artery Study supports these findings as it was shown that IL-6 was an independent predictor of peripheral atherosclerosis in contrast to other markers such as CRP and ICAM-1 [37]. However, some in vitro studies have shown that CRP-induces VCAM-1 messenger RNA expression through nuclear factor κB activation in vascular cells [38]. In this study, IL-6 was found to influence the association of CRP with VCAM-1. This suggested that IL-6 probably induces more aggressive modification of VCAM-1 compared with CRP in vivo.

There are several limitations of this study. Firstly, the sample size is small, which is due to the strict exclusion criteria. However, the subjects were recruited from a population-based study and were carefully selected and, moreover, this study appears to be adequately powered. Secondly, this is a cross-sectional study and, hence, we are unable to comment about the causative roles of the various biomarkers studied.

In conclusion, this study shows that in Asian Indians, a very high-risk group for diabetes and CAD, inflammatory markers increase with increasing degrees of glucose intolerance.

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