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Soluble P-selectin and CD40L levels in subjects with prediabetes, diabetes mellitus, and metabolic syndrome—the Chennai Urban Rural Epidemiology Study☆

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Abstract

The aim of the study was to determine whether the levels of soluble P-selectin (sP-selectin) and soluble CD40L (sCD40L) are elevated in Asian Indian subjects with impaired glucose tolerance (IGT), diabetes, and metabolic syndrome (MS). Study subjects were recruited from the Chennai Urban Rural Epidemiology Study (CURES), an ongoing population-based study on a representative population of Chennai city in southern India, and were grouped as follows: group 1, normal glucose tolerance (NGT) (n = 60); group 2, IGT (n = 60); and group 3, type 2 diabetes mellitus (n = 60). Normal glucose tolerance, IGT, and diabetes were defined using World Health Organization consulting group criteria. The inclusion criteria were nonsmokers; normal resting 12-lead electrocardiogram; absence of angina, myocardial infarction, or history of any known vascular, infectious, or inflammatory diseases; and subjects not on statins or aspirin. Insulin resistance was calculated using the homeostasis assessment model using the formula: fasting insulin (μIU/mL) × fasting glucose (mmol/L)/22.5. Soluble P-selectin and sCD40L were estimated by enzyme-linked immunosorbent assay. Metabolic syndrome was defined using Adult Treatment Panel III guidelines. Subjects with diabetes and IGT were older (diabetes: 53 ± 9 years, $P < .01$; IGT: 51 ± 10 years, $P < .05$) compared with the NGT group (48 ± 10 years). Subjects with diabetes and IGT had higher levels of sP-selectin (diabetes: 162 ± 79 ng/mL, $P < .001$; IGT: 102 ± 37 ng/mL, $P < .001$) compared with the NGT group (55 ± 48 ng/mL). Soluble CD40L levels were also higher in those with diabetes and IGT (diabetes: 3.2 ± 2.0 ng/mL, $P < .001$; IGT: 2.0 ± 1.3 ng/mL, $P < .001$) compared with the NGT group (1.1 ± 0.9 ng/mL). Subjects with MS had significantly higher levels of sP-selectin (with MS, 118 ± 76 ng/mL; without MS, 95 ± 66 ng/mL; $P = .028$) and sCD40L (with MS, 2.4 ± 1.8 ng/mL; without MS, 1.9 ± 1.5 ng/mL; $P = .036$) compared with subjects without MS. Among subjects with NGT and IGT, the mean levels of sP-selectin (tertile I, 65.0 ng/mL; tertile II, 80.0 ng/mL; tertile III, 91.0 ng/mL) and sCD40L levels (tertile I, 1.2 ng/mL; tertile II, 1.7 ng/mL; tertile III, 1.8 ng/mL) increased with increase in tertiles of homeostasis assessment model–insulin resistance, and the difference reached statistical significance in the last tertile compared with the first tertile ($P < .05$). This study demonstrates that increased levels of sP-selectin and sCD40L are seen in Asian Indian subjects with IGT, type 2 diabetes mellitus, MS, and insulin resistance.

1. Introduction

Studies on migrant Asian Indians have consistently shown that they have an increased prevalence of premature coronary artery disease (CAD) compared with the host population [1-5]. More recently, even within the Indian subcontinent, a rising prevalence of CAD has been reported [6]. According to World Bank figures, the overall cardiovascular mortality in Indians is projected to rise by 103% in men and by 90% in women between 1985 and 2015 [6]. Unfortunately, India also has the largest number of diabetic subjects in the world [7]. Diabetic subjects have 2- to 4-fold higher risk of developing CAD compared with nondiabetic subjects [8]. Coronary artery disease is often present even at the time of diagnosis of diabetes mellitus and may indeed be seen even in prediabetic stages like impaired glucose tolerance (IGT) [9]. Glucose intolerance and other metabolic abnormalities may cluster together, resulting in increased risk for CAD [10,11]. Insulin resistance is considered to be one of the underlying causes for both glucose intolerance

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and CAD [12]. It clusters with many other metabolic abnormalities, and this is known as the insulin-resistance syndrome or MS [12]. Earlier studies have shown that changes in blood coagulation homeostasis such as decreased fibrinolysis and hypercoagulation are associated with insulin resistance [13]. In addition, increased platelet activity, particularly soluble P-selectin (sP-selectin) and soluble CD40L (sCD40L) levels, has been reported in diabetic subjects [14,15]. Measurement of these 2 soluble platelet-related factors in the blood have gained a lot of interest as they show a strong association with future cardiovascular events [16,17].

P-selectin, a member of the selectin family, is expressed on activated platelets. It mediates the rolling of monocytes on activated endothelium and promotes atherosclerotic lesion development. P-selectin–mediated adhesive interaction is important in both hemostatic and inflammatory processes [18]. Although P-selectin is expressed as a functional membrane glycoprotein, a shorter, soluble isoform has been identified. This sP-selectin occurs in humans as the product of the alternative splicing of the exon containing the transmembrane domain [19]. Earlier studies have shown sP-selectin to be associated with atherosclerotic process [20].

CD40L expressed on stimulated platelets enhances the production of adhesion molecules and inflammatory markers. The soluble form of CD40L, which is a cleaved fragment form of surface-expressed CD40L, is considered to be a platelet activation marker [20,21]. Soluble CD40L is pro-inflammatory for endothelial cells and promotes coagulation by inducing expression of tissue factor on monocytes [22] and endothelial cells [23].

Although the studies in the West have reported high levels of sP-selectin [14] and sCD40L [15] both in diabetic subjects and in subjects with CAD compared with healthy subjects [16,17], there are virtually no studies to our knowledge on these markers in subjects with IGT or with MS. Moreover, this is the first report on the levels of sP-selectin and sCD40L in Asian Indian subjects.

2. Research design and methods

The study subjects were recruited from the Chennai Urban Rural Epidemiology Study (CURES), an ongoing epidemiological study conducted on a representative population (aged $\geq$20 years) of Chennai (formerly Madras), the fourth largest city in India. The methodology of the study has been published previously [24]. Briefly, in phase 1 of the urban component of CURES, 26 001 individuals were recruited based on a systematic random sampling technique. Our web site http://www.mvdsc.org (under the link "Publications") shows a map with the location of our center and the sampling frame. Fasting capillary blood glucose was determined using a One Touch Basic glucose meter (LifeScan, Johnson & Johnson, Milpitas, CA) in all subjects. Subjects were classified as "known diabetes" if the subjects stated that they had diabetes and/or if they were on the treatment with antidiabetic drug.

In phase 2 of CURES, all the known diabetic subjects (n = 1529) were invited to the center for detailed studies on vascular complications. In addition, all subjects with fasting blood glucose levels in the diabetic range based on American Diabetes Association fasting criteria [25] underwent oral glucose tolerance test using 75 g oral glucose load (dissolved in 250 mL of water). Those who were confirmed by oral glucose tolerance test to have 2-hour plasma glucose value of 11.1 mmol/L or higher (200 mg/dL) based on World Health Organization consulting group criteria [26] were labeled as "newly detected diabetic subjects"; those with 2-hour postglucose value of 7.8 mmol/L or higher (140 mg/dL) and less than 11.1 mmol/L [26] as having IGT; and those with 2-hour postglucose value of less than 7.8 mmol/L (140 mg/dL) as having normal glucose tolerance (NGT).

Group 1 comprised 60 healthy subjects, group 2 comprised 60 subjects with IGT, and group 3 comprised 60 type 2 diabetic subjects. The inclusion criteria were nonsmokers; normal resting 12-lead electrocardiogram; absence of angina, myocardial infarction, or history of any known vascular, infectious, or inflammatory diseases; and not on statins or aspirin.

2.1. Anthropometric measurements

Anthropometric measurements including weight, height, and waist measurements were obtained using standardized techniques as detailed previously [24]. The body mass index was calculated using the formula: weight (kg)/height (m^2). 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.

2.2. Biochemical parameters

Fasting plasma glucose (glucose oxidase–peroxidase method), serum cholesterol (cholesterol oxidase–peroxidase-amidopyrine method), serum triglycerides (glycerol phosphate oxidase–peroxidase-amidopyrine method), and high-density lipoprotein cholesterol (HDL-C) (direct method–polyethylene glycol–pretreated enzymes) were measured using Hitachi-912 Autoanalyzer (Hitachi, Mannheim, Germany). The intra- and interassay coefficient of variation for the biochemical assays ranged between 3.1% and 7.6%. Low-density lipoprotein cholesterol was calculated using the formula of Friedewald et al [27]. Glycated hemoglobin (HbA_{1c}) was estimated by high-pressure liquid chromatography using the Variant machine (Bio-Rad, Hercules, CA). The intra- and interassay coefficient of variation of HbA_{1c} was less than 10%. Serum insulin concentration was estimated using Dako kits (Dako, Glostrup, Denmark). Insulin resistance was calculated using

the homeostasis assessment model (HOMA-IR) using the formula: fasting insulin (μIU/mL) × fasting glucose (mmol/L)/22.5 [28]. Insulin resistance was diagnosed if subjects have HOMA-IR value of 1.93 or higher (75th percentile of the total study population in the Chennai Urban Population Study) [29].

2.3. Soluble P-selectin and soluble CD40L

Soluble P-selectin and sCD40L concentrations were measured by enzyme-linked immunosorbent assay (ELISA) (Biosource, Camarillo, CA). In brief, microtiter plates precoated with monoclonal antibodies were incubated with serum samples. Bound sP-selectin and sCD40 ligand were revealed by incubation with a secondary antibody coupled to horseradish peroxidase. Plates were then washed, and antibody binding was determined by colorimetry with substrate. Absorbance was read at 450 nm. The intra- and interassay coefficient of variation ranged between 3.8% and 7.4%.

All biochemical analyses were done at Madras Diabetes Research Foundation, Chennai.

2.4. Definition

2.4.1. Metabolic syndrome

Metabolic syndrome was diagnosed based on modified Adult Treatment Panel III guidelines [30] if any 3 of the following abnormalities were present: abdominal obesity (defined as waist circumference ≥90 cm for males and ≥80 cm for females as per modified Asia Pacific World Health Organization guidelines [31]), high blood pressure (subjects who were on antihypertensive medication or had systolic blood pressure ≥130 mm Hg or diastolic blood pressure ≥85 mm Hg), elevated fasting glucose (fasting plasma glucose ≥6.1 mmol/L), hypertriglyceridemia (serum triglycerides ≥1.7 mmol/L), and low HDL levels (males, HDL-C <1.04 mmol/L; females, HDL-C <1.3 mmol/L).

Table 1
Clinical and biochemical characteristics of the study subjects

	Group 1 (subjects with NGT; n = 60)	Group 2 (subjects with IGT; n = 60)	Group 3 (diabetic subjects; n = 60)
Age (y)	48 ± 10	51 ± 10*	53 ± 9**
Males, n (%)	22 (36.7)	24 (40.0)	26 (43.3)
Body mass index (kg/m^2)	23.8 ± 4	24.8 ± 3.7	24.6 ± 4.0
Systolic blood pressure (mm Hg)	123 ± 14	125 ± 17	131 ± 21*
Diastolic blood pressure (mm Hg)	76 ± 9	77 ± 11	77 ± 11
Fasting plasma glucose (mmol/L)	4.8 ± 0.3	5.2 ± 0.7	9.7 ± 4.5***,††
HOMA-IR[a]	1.8 ± 1.1	3.1 ± 1.6***	4.4 ± 2.6***,†
HbA_{1c} (%)	5.8 ± 0.42	6.1 ± 0.57***	9.1 ± 2.3***,††
Serum cholesterol (mmol/L)	4.9 ± 0.8	4.9 ± 0.9	5.4 ± 1.1**,†
Serum triglycerides (mmol/L)	1.3 ± 0.6	1.6 ± 0.7**	1.9 ± 1.0**

[a] Eight diabetic subjects on insulin therapy were excluded for calculation of insulin resistance using the HOMA-IR formula.

* $P < .05$ compared with subjects with normal glucose tolerance.

** $P < .01$ compared with subjects with normal glucose tolerance.

*** $P < .001$ compared with subjects with normal glucose tolerance.

† $P < .01$ compared with subjects with impaired glucose tolerance.

†† $P < .001$ compared with subjects with impaired glucose tolerance.

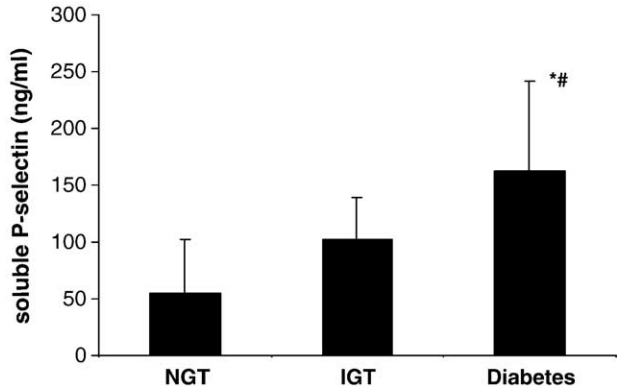

Fig. 1. Distribution of sP-selectin levels in the study groups. *$P < .001$ compared with subjects with NGT. #$P < .001$ compared with subjects with NGT and IGT.

2.4.2. Statistical analysis

Statistical analysis was carried out using Windows-based SPSS package (version 10.0; Chicago, IL). Values are expressed as mean ± SD. Mann-Whitney U test or Kruskal-Wallis H test, as appropriate, was used for comparisons between groups for the parameters.

χ^2 Test or Fisher exact test, as appropriate, was used to compare proportions. Correlation between variables was tested using Spearman rank correlation analysis. Diabetic subjects on insulin therapy (n = 8) were excluded for analysis of HOMA-IR. P values of less than .05 were taken as significant.

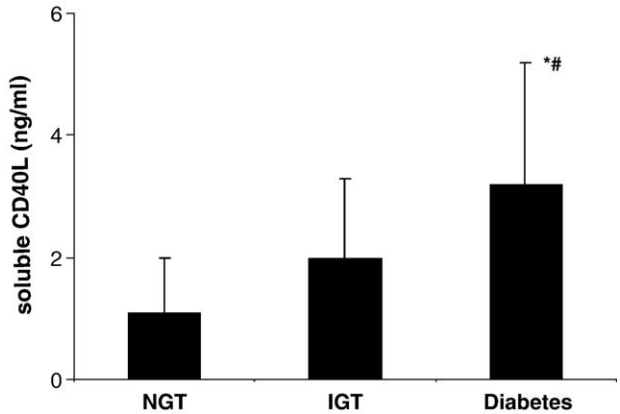

Fig. 2. Distribution of sCD40L levels in the study groups. *$P < .001$ compared with subjects with NGT. #$P < .001$ compared with subjects with NGT and IGT.

3. Results

The clinical and biochemical profile of the study subjects is shown in Table 1. Subjects with diabetes and IGT were older (diabetes, $P < .01$; IGT, $P < .05$) and had significantly higher serum triglyceride levels ($P < .01$) compared with healthy subjects. Diabetic subjects had significantly higher serum cholesterol levels compared with healthy subjects and subjects with IGT ($P < .01$). Among the diabetic subjects, 14 were newly diagnosed and 46 were known diabetic subjects, and of the latter, 35 (76.1%) were on oral hypoglycemic drugs, 8 (17.4%) were on oral drugs plus insulin, and 3 (6.5%) were on diet alone.

Soluble P-selectin values were higher in subjects with IGT ($P < .001$) compared with subjects with NGT and highest in subjects with diabetes ($P < .001$) (Fig. 1). Soluble CD40L levels were highest in subjects with diabetes ($P < .001$) followed by subjects with IGT ($P < .001$) and NGT (Fig. 2).

Levels of sP-selectin and sCD40L were computed for subjects with and without MS. Subjects with MS had significantly higher levels of sP-selectin (with MS, 118 ± 76 ng/mL; without MS, 95 ± 66 ng/mL; $P = .028$) and sCD40L (with MS, 2.4 ± 1.8 ng/mL; without MS, 1.9 ± 1.5 ng/mL; $P = .036$) compared with subjects without MS. When categorized based on diabetes status, diabetic subjects with MS had higher levels of sP-selectin (157 ± 79 ng/mL) and sCD40L (3.3 ± 2.0 ng/mL) compared with nondiabetic subjects with MS (sP-selectin, 79 ± 49 ng/mL; sCD40L, 1.5 ± 1.1 ng/mL) and without MS (sP-selectin, 78 ± 48 ng/mL; sCD40L, 1.5 ± 1.1 ng/mL) ($P < .001$).

For the determination of the association of sP-selectin and sCD40L with HOMA-IR in nondiabetic subjects, mean values of sP-selectin and sCD40L were computed in relation to tertiles of HOMA-IR in the study population after excluding diabetic subjects.

Table 2
Levels of sP-selectin and CD40L in relation to HOMA-IR in subjects with NGT and IGT

HOMA-IR	sP-selectin (ng/mL)	sCD40L (ng/mL)
(A) Subjects with NGT and IGT		
First tertile (n = 39)	65 ± 50, reference	1.2 ± 0.93, reference
Second tertile (n = 40)	80 ± 43, .085	1.7 ± 1.6, .416
Third tertile (n = 41)	91 ± 50, .029	1.8 ± 0.92, .002
(B) Subjects with NGT		
HOMA-IR <1.93 (n = 41)	54 ± 48, reference	1.0 ± 0.96, reference
HOMA-IR ≥1.93 (n = 19)	57 ± 48, .812	1.1 ± 0.79, .556

Values are expressed as mean ± SD, *P* value. Subjects with NGT and IGT were combined together for the first analysis (A). The study subjects were categorized into tertiles based on the calculated HOMA-IR values. Mean values of sP-selectin and sCD40L were computed for the tertiles of HOMA-IR. The second analysis (B) was done in subjects with NGT. Mean values of sP-selectin and sCD40L were calculated in NGT subjects with and without insulin resistance. Insulin resistance was determined by using a HOMA-IR cutoff of 1.93, which is the 75th percentile of the total population in the Chennai Urban Population Study [29].

The mean levels of both sP-selectin (tertile I, 65.0 ng/mL; tertile II, 80.0 ng/mL; tertile III, 91.0 ng/mL) and sCD40L (tertile I, 1.2 ng/mL; tertile II, 1.7 ng/mL; tertile III, 1.8 ng/mL) increased with increasing tertiles of HOMA-IR and reached statistical significance in the last tertile (Table 2). Finally, NGT subjects were categorized as those with and without insulin resistance (HOMA-IR cutoff, 1.93; 75th percentile of the total population in the Chennai Urban Population Study) [29]. Subjects with insulin resistance had higher levels of sP-selectin and sCD40L, but the differences did not reach statistical significance, probably because of the small numbers (Table 2).

4. Discussion

The prevalence of CAD is 2 to 3 times higher in diabetic subjects and nearly 1.5 times higher among IGT subjects compared with those with NGT [9]. This indicates that the atherosclerotic risk factors start operating even at the stage of prediabetes. In this regard, this study is of interest as it reports increased levels of both sP-selectin and sCD40L, not only in diabetic subjects but also in subjects with IGT.

Diabetic subjects had higher levels of both sP-selectin and sCD40L, and this is similar to that reported by Lim et al [32]. Harding et al [33] showed that platelet- monocyte aggregates and CD40L levels were significantly elevated in type 1 diabetic subjects. Yngen et al [34] showed that acute hyperglycemia induced by oral glucose tolerance test led to elevated levels of sP-selectin in males with type 2 diabetes mellitus. Thus, among the several reasons for excess CAD in subjects with diabetes, increased platelet activation could be one.

Earlier studies have demonstrated that elevated levels of both sP-selectin and sCD40L predict an increased risk for future cardiovascular risk even among healthy subjects [16,17]. We therefore felt it was of interest to look at these markers in IGT subjects as earlier studies have shown that the relation between blood glucose and CAD is a continuous one [35]. In the present study, IGT subjects, who are considered to be at an increased risk for cardiovascular disease [36], had higher levels of both sP-selectin and sCD40L. In the Chennai Urban Population Study, we reported that 14.7% of subjects with IGT had CAD compared with 9.0% in subjects with NGT, indicating the higher risk for CAD is also seen among Asian Indians with IGT [5]. Various studies have documented that inflammatory markers and other atherosclerotic risk factors are altered in subjects with IGT [37,38]. However, to our knowledge, there have been no studies that have looked at these 2 platelet activation markers in subjects with IGT.

We also observed that subjects with MS had higher levels of sP-selectin and sCD40L compared with subjects without MS. In addition, diabetic subjects with MS had the highest values of sP-selectin and sCD40L, indicating that this group of subjects are at higher risk for CAD as demonstrated in the ARIC study [39].

Another interesting observation in this study is that both sP-selectin and sCD40L showed an association with insulin resistance as assessed by HOMA-IR in subjects without overt diabetes. Earlier studies have included hypercoagulation and decreased fibrinolysis as components of MS [13]. This study suggests that platelet activation may also be a part of the metabolic syndrome, and this needs to be confirmed by future studies.

There are several limitations to this study. Being a cross-sectional study, it cannot explain a cause-and-effect relationship of sP-selectin and sCD40L with glucose intolerance. Secondly, the sample size is small, and, hence, the results should be interpreted with caution. Finally, sP-selectin and sCD40L had a high degree of variability, and, hence, we used nonparametric analysis to compare groups. The strengths of the study are that it is population-based, and these are the first results on the relationships of sP-selectin and sCD40L with IGT and MS and the first data on these markers in a high-risk Asian Indian population.

In conclusion, this study demonstrates increased levels of sP-selectin and sCD40L in subjects with IGT, diabetes, and MS. Further prospective studies are required to establish the value of sP-selectin and sCD40L in subjects with IGT. Furthermore, as drugs like statins and aspirin can alter platelet activation, it is also necessary to establish the significance of lowering sP-selectin and sCD40L levels with respect to the reduction of macrovascular events.

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