ORIGINAL ARTICLE

Circulating Levels of Markers of Inflammation and Endothelial Activation are Increased in Men with Chronic Spinal Cord Injury

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Background/Purpose: Accelerated atherogenesis is often seen in individuals with chronic spinal cord injury (SCI). However, the mechanisms contributing to this phenomenon remain unclear. This study aimed to evaluate whether SCI *per se* is associated with a low-grade chronic inflammatory state and endothelial activation, both of which are well-documented prerequisites for atherogenesis.

Methods: Serum levels of markers of inflammation (C-reactive protein [CRP], interleukin-6, and soluble CD40 ligand) and endothelial activation (endothelin-1, soluble intercellular adhesion molecule-1, and soluble vascular cell adhesion molecule-1 [sVCAM-1]) were measured in SCI patients with CRP levels < 10 mg/L and with no evidence of active infection. Sixty-two men with traumatic neurologically complete SCI (20 tetraplegics and 42 paraplegics) and 29 age-matched male controls were enrolled.

Results: Compared with able-bodied controls, subjects with SCI had a significantly lower body mass index (BMI) (−7%) and significantly lower serum levels of albumin (−10%), creatinine (−20%), low-density lipoprotein cholesterol (−10%), and high-density lipoprotein (HDL) cholesterol (−25%), and showed a trend toward higher fasting insulin levels. Irrespective of injury level and duration, subjects with SCI had significantly higher serum levels, compared to able-bodied controls, of CRP (mean, 4.0 ± 2.7 mg/L *vs.* 1.4 ± 1.1 mg/L), interleukin-6 (median, 2.5 pg/mL *vs.* 0.4 pg/mL; range, 1.5–3.6 pg/mL *vs.* 0.2–0.5 pg/mL), endothelin-1 (mean, 1.3 ± 0.4 pg/mL *vs.* 0.9 ± 0.3 pg/mL), and sVCAM-1 (mean, 1170 ± 318 ng/mL *vs.* 542 ± 318 ng/mL). The serum levels of all four factors correlated negatively with levels of serum albumin, creatinine and HDL cholesterol, but not with BMI or fasting insulin levels. In multivariate analyses, SCI was the only factor that was independently associated with increased serum levels of CRP, interleukin-6, endothelin-1 and sVCAM-1 after adjustment for confounding factors such as serum albumin and creatinine levels and parameters of dyslipidemia and insulin resistance.

Conclusion: In this study, we have, for the first time, demonstrated that SCI *per se* is associated with a low-grade chronic inflammatory state and endothelial activation, which may partly explain the increased atherogenic risk in patients with long-standing SCI. [*J Formos Med Assoc* 2007;106(11):919–928]

Key Words: adhesion molecule, C-reactive protein, endothelin, interleukin-6, spinal cord injury

As a result of improvements in medical care and increasing survival, atherosclerotic cardiovascular disease is now the leading cause of death in patients with spinal cord injury (SCI).[1,2] Coronary heart disease is more prevalent and occurs earlier in patients with SCI than in the able-bodied population.[3,4] As a consequence of changes in body composition and reduction in physical activity,

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individuals with SCI often show metabolic alterations, such as insulin resistance and dyslipidemia, which may increase the risk of coronary heart disease.[1] However, whether other mechanisms contribute to the accelerated atherogenesis seen in patients with SCI is not clear.

Recently, atherosclerosis has been viewed as a dynamic and progressive disease arising from a combination of endothelial activation and low-grade inflammation.[5–7] Endothelial cells provide the barrier between the circulation and the extravascular space. Endothelial activation and endothelial cell-leukocyte interactions are a necessary prerequisite for initiation of inflammatory processes that predispose to atherogenesis. Moreover, inflammatory mediators appear to play a fundamental role in the initiation, progression, and eventual rupture of atherosclerotic plaques. Measurement of markers of inflammation and endothelial activation may therefore be useful by providing a mechanistic explanation for the increased cardiovascular risk in certain patient populations.

Serologic levels of inflammatory markers, such as C-reactive protein (CRP) and interleukin-6, have been shown to be significantly elevated in patients with SCI.[8–10] However, the SCI patients included in those studies had either manifest or occult evidence of active infection, as CRP levels were greater than 10 mg/L, the commonly assigned cut-off value for CRP.[9–12] This degree of CRP elevation indicates the presence of acute infection or inflammation,[12] which is different from the chronic low-grade inflammation associated with atherosclerosis.[6] In order to evaluate whether SCI *per se* is associated with a low-grade chronic inflammatory state and endothelial activation, we measured serum levels of markers of inflammation (CRP, interleukin-6, and soluble CD40 ligand) and endothelial activation (endothelin-1, soluble intercellular adhesion molecule-1 [sICAM-1], and soluble vascular cell adhesion molecule-1 [sVCAM-1]) in SCI patients with CRP levels less than 10 mg/L and with no evidence of active infection. We also investigated possible correlations between these serum markers and various factors, such as injury level, duration of injury, body mass index (BMI), serum albumin and creatinine levels, and metabolic parameters of dyslipidemia and insulin resistance.

Methods

Participants

Eighty-nine men with traumatic SCI were recruited from the SCI clinic of National Taiwan University Hospital. Their SCIs were neurologically complete, as defined by the American Spinal Injury Association.[13] The injury levels were from C3 to L1. Those with injury levels at or above C8 were classified as tetraplegics and the others as paraplegics. To avoid the confounding effect of infection, 27 subjects with evidence of active infection or CRP levels ≥ 10 mg/L were excluded. A total of 62 SCI subjects (42 with paraplegia and 20 with tetraplegia) were included in this study. These subjects had been injured at a mean age of 28.0 ± 9.7 years (range, 16.2–59.1 years), and the mean duration of injury was 11.8 ± 7.0 years (range, 1.2–27.7 years). The control group consisted of 29 age-matched healthy men. No participants in either the control or SCI groups had known diabetes or endocrine disorders. Except for a few patients who were taking stool softeners (magnesium oxide) or low-dose antispastic agents (baclofen), none were taking drugs regularly. All participants voluntarily agreed to participate in this study and all gave written informed consent. The study was approved by the research ethics committee of National Taiwan University Hospital.

Laboratory analyses

Blood samples were taken from all participants between 8:00 and 9:00 am after overnight fasting, and serum was prepared and stored frozen at −70°C until assayed. Levels of total cholesterol, total triglycerides, low-density lipoprotein (LDL) cholesterol, and high-density lipoprotein (HDL) cholesterol were assayed by routine laboratory techniques and the methods used in the Lipid Research Clinics, as reported previously.[14] If plasma

triglycerides were ≥ 400 mg/dL, LDL cholesterol was assessed by a direct method.[14] High-sensitivity CRP levels were measured by rate nephelometry (Dade Behring, Newark, DE, USA). Serum levels of insulin, soluble CD40 ligand, endothelin-1, sICAM-1, and sVCAM-1 were determined in duplicate using commercially available ELISA kits (insulin, BIOSOURCE, Camarillo, CA, USA; soluble CD40 ligand, endothelin-1, sICAM-1, and sVCAM-1, R&D Systems, Minneapolis, MN, USA). The marker of insulin resistance, the homeostasis model assessment (HOMA) index, is defined as the fasting plasma insulin level (μU/mL) × fasting glucose level (mmol/L)/22.5. Routine clinical chemical analyses were performed by standard methods subject to strict quality control. The coefficients of variation were < 5% for all types of measurement.[15]

Statistical analyses

Statistical analyses were performed using SPSS version 11.0 (SPSS Inc., Chicago, IL, USA). Data were examined for normality before analysis, and the distributions of levels of interleukin-6, soluble CD40 ligand, fasting glucose, insulin and triglycerides, and the HOMA index were found to be positively skewed. The data were therefore analyzed by nonparametric methods to avoid assumptions about the distribution of the measured variables. Differences in demographic characteristics and various biochemical markers between subjects with SCI and able-bodied controls were compared using the Mann–Whitney *U* test. Differences in the above variables between paraplegics and tetraplegics and between those with injury at T6 and above and those with injury at T7 and below were compared using either the Mann–Whitney *U* test (duration of injury) or one-way analysis of variance (ANOVA) with *post hoc* Bonferroni tests (all other variables). Associations between different variables were assessed using the Spearman rank correlation test. Multivariate regression analysis was used to test the independent association between various serum markers and SCI in the whole study population using a forward stepwise linear regression model with age, BMI, serum levels of albumin, creatinine, glucose and insulin, lipid profiles, and the HOMA index as covariates. The distribution of continuous variables in groups was expressed as mean ± standard deviation or median (interquartile range) as appropriate. Statistical significance was set at $p < 0.05$.

Results

The characteristics of the 62 subjects with SCI and the 29 able-bodied controls are listed in Table 1. Compared to able-bodied controls, subjects with SCI had a significantly lower body weight, BMI, and serum levels of albumin and creatinine. The difference in serum creatinine levels (−20%) between SCI subjects and able-bodied controls was greater than those for body weight (−8%), BMI (−7%), and serum albumin levels (−10%), probably due to a greater reduction in lean muscle mass in SCI subjects or differences in renal function status. As regards the metabolic parameters of dyslipidemia and insulin resistance, subjects with SCI had significantly lower HDL and LDL cholesterol levels, a higher total cholesterol/HDL cholesterol ratio, and a trend toward higher insulin levels than able-bodied controls. Although all participants had CRP levels < 10 mg/L and no evidence of active infection, subjects with SCI had significantly higher serum levels of CRP, interleukin-6, endothelin-1 and sVCAM-1 than able-bodied controls. There were no significant differences between paraplegics and tetraplegics with regard to injury duration, demographic characteristics, parameters of dyslipidemia and insulin resistance, and markers of inflammation and endothelial activation, except that tetraplegic subjects had significantly lower serum albumin and marginally lower sICAM-1 levels than paraplegic subjects. SCI subjects with injury at T6 and above had significantly lower albumin levels than those with injury at T7 and below, but there were no significant differences in any of the other parameters. There were no significant relations between injury duration and markers of inflammation and endothelial activation (data not shown).

Table 1. Characteristics of study participants*

	Control ($n=29$)	SCI ($n=69$)	p	Paraplegic ($n=42$)	Tetraplegic ($n=20$)	p	T6 and above ($n=33$)	T7 and below ($n=29$)	p
Age (yr)	36±11	40±11	0.099	39±12	41±12	0.651	43±12	36±9	0.058
Duration of injury (yr)	–	12±7	–	12±7	10±7	0.240	13±7	11±7	0.293
Weight (kg)	70±10	64±13	0.017	65±13	60±10	0.281	64±12	63±13	0.753
Height (cm)	173±7	171±5	0.163	172±5	170±5	0.755	170±5	172±5	0.281
BMI (kg/m^2)	23.4±2.6	21.7±4.2	0.044	22.2±4.5	20.8±3.6	0.546	22.2±4.0	21.2±4.4	0.986
Albumin (g/dL)	4.8±0.2	4.3±0.3	<0.001	4.4±0.3	4.1±0.3	0.002	4.2±0.3	4.5±0.3	0.001
Creatinine (mg/dL)	1.0±0.1	0.8±0.2	<0.001	0.8±0.2	0.8±0.1	0.434	0.8±0.1	0.8±0.3	0.914
Fasting glucose (mg/dL)	86 (78–92)	84 (77–90)	0.503	84 (77–89)	84 (77–91)	0.723	84 (80–92)	80 (76–88)	0.507
Fasting insulin (μIU/mL)	4.7 (2.5–8.4)	6.7 (4.3–10.9)	0.076	8.0 (4.1–11.5)	5.8 (4.4–9.2)	0.729	7.4 (4.4–12.7)	4.8 (3.9–9.6)	0.370
HOMA index	1.0 (0.5–1.8)	1.3 (0.8–2.3)	0.149	1.6 (0.8–2.3)	1.2 (0.8–1.9)	0.792	1.7 (0.8–2.7)	1.0 (0.8–1.7)	0.406
Total cholesterol (mg/dL)	196±44	184±35	0.142	190±35	171±33	0.214	185±38	184±33	0.910
Triglycerides (mg/dL)	116 (61–157)	111 (81–176)	0.416	121 (93–179)	98 (72–175)	0.352	136 (84–199)	101 (81–135)	0.620
LDL cholesterol (mg/dL)	121±40	109±29	0.048	113±29	100±28	0.414	110±29	108±29	0.854
HDL cholesterol (mg/dL)	48±9	36±6	<0.001	36±6	34±6	0.803	34±7	37±5	0.278
Total/HDL cholesterol	4.2±1.0	5.2±1.0	<0.001	5.3±1.1	5.0±0.8	0.348	5.5±1.1	5.0±0.9	0.161
LDL/HDL cholesterol	2.6±1.0	3.1±0.9	0.051	3.3±0.9	2.9±0.8	0.626	3.3±0.9	2.9±0.8	0.376
CRP (mg/L)	1.4±1.1	4.0±2.7	<0.001	4.0±2.7	3.9±2.9	0.952	4.2±2.9	3.7±2.6	0.516
Interleukin-6 (pg/mL)	0.4 (0.2–0.5)	2.5 (1.5–3.6)	<0.001	2.6 (1.6–3.7)	2.2 (1.3–3.7)	0.614	2.4 (1.6–4.0)	2.6 (1.4–3.5)	0.772
Soluble CD40 ligand (ng/mL)	2.4 (1.6–3.3)	2.4 (1.7–3.6)	0.772	2.4 (1.7–3.5)	2.5 (1.7–3.7)	0.735	2.7 (1.8–3.7)	2.3 (1.5–3.6)	0.603
Endothelin-1 (pg/mL)	0.9±0.3	1.3±0.4	<0.001	1.3±0.4	1.4±0.4	0.577	1.4±0.4	1.2±0.3	0.605
sICAM-1 (ng/mL)	272±81	305±112	0.233	326±112	260±100	0.053	284±101	328±121	0.282
sVCAM-1 (ng/mL)	542±318	1170±318	<0.001	1148±281	1214±388	0.758	1216±353	1117±268	0.668

**Data are presented as mean±standard deviation or median (interquartile range). SCI=spinal cord injury; BMI=body mass index; HOMA=homeostasis model assessment; LDL=low-density lipoprotein; HDL=high-density lipoprotein; CRP=C-reactive protein; sICAM-1=soluble intercellular adhesion molecule-1; sVCAM-1=soluble vascular cell adhesion molecule-1.*

The associations between markers of inflammation and endothelial activation in all study participants are shown in Table 2. CRP levels were significantly correlated with all other parameters, most notably interleukin-6. Soluble CD40 ligand levels were only correlated with CRP levels. Endothelin-1 levels were significantly correlated with levels of CRP, interleukin-6 and sVCAM-1, but not sICAM-1, whereas sICAM-1 levels were correlated with levels of CRP and interleukin-6, but not with other markers of endothelial activation (endothelin-1 and sVCAM-1).

The associations between the above markers and age, BMI, serum albumin and creatinine levels, and parameters of dyslipidemia and insulin resistance in all study participants are shown in Table 3. Higher CRP levels were significantly associated with lower levels of albumin and creatinine, higher fasting glucose and insulin levels, and lower HDL cholesterol levels, but not with BMI. Interleukin-6 had a similar correlation pattern to CRP. Both endothelin-1 and sVCAM-1 levels were correlated positively with age and negatively with serum levels of albumin, creatinine and HDL cholesterol, but not with BMI or fasting glucose and insulin levels. Higher sICAM-1 levels were significantly associated with higher BMI and higher fasting insulin and triglyceride levels.

To determine whether there were independent associations between SCI and increased serum levels of CRP, interleukin-6, endothelin-1 and sVCAM-1, we performed stepwise multiple linear regression analyses including each single serum marker as the dependent variable and age, the

Table 2. Spearman rank correlation coefficients between levels of markers of inflammation and endothelial activation in all study participants ($n = 91$)

	Interleukin-6	Soluble CD40 ligand	Endothelin-1	sICAM-1	sVCAM-1
CRP	0.668*	0.239†	0.216†	0.398*	0.373*
Interleukin-6	—	0.101	0.458*	0.324‡	0.585*
Soluble CD40 ligand	—	—	0.092	0.171	–0.025
Endothelin-1	—	—	—	0.065	0.332‡
sICAM-1	—	—	—	—	0.205

**p < 0.001; †p < 0.05; ‡p < 0.01. sICAM-1 = soluble intercellular adhesion molecule-1; sVCAM-1 = soluble vascular cell adhesion molecule-1; CRP = C-reactive protein.*

Table 3. Spearman rank correlation coefficients between levels of markers of inflammation and endothelial activation and biochemical and anthropometric variables in all study participants ($n = 91$)

	CRP	Interleukin-6	Soluble CD40 ligand	Endothelin-1	sICAM-1	sVCAM-1
Age	0.067	0.048	0.024	0.308*	0.079	0.281*
BMI	0.097	–0.065	0.211†	–0.145	0.237†	–0.127
Albumin	–0.366‡	–0.529‡	–0.028	–0.418‡	0.044	–0.562‡
Creatinine	–0.439‡	–0.450‡	–0.096	–0.235†	–0.097	–0.549‡
Fasting glucose	0.212†	–0.011	0.044	0.036	–0.078	–0.105
Fasting insulin	0.290*	0.212†	0.121	–0.012	0.268†	0.182
HOMA index	0.315*	0.193	0.147	–0.004	0.253†	0.154
Total cholesterol	–0.131	–0.131	0.192	0.019	0.063	–0.145
Triglycerides	0.203	0.143	0.310*	0.201	0.286*	0.064
LDL cholesterol	–0.090	–0.166	0.211†	–0.075	0.077	–0.136
HDL cholesterol	–0.519‡	–0.574‡	–0.113	–0.326*	–0.125	–0.491‡

**p < 0.01; †p < 0.05; ‡p < 0.001. CRP = C-reactive protein; sICAM-1 = soluble intercellular adhesion molecule-1; sVCAM-1 = soluble vascular cell adhesion molecule-1; BMI = body mass index; HOMA = homeostasis model assessment; LDL = low-density lipoprotein; HDL = high-density lipoprotein.*

Table 4. Multivariate forward stepwise linear regression analysis of variables significantly related to levels of CRP, interleukin-6, endothelin-1, or sVCAM-1 in all study participants ($n = 91$)

	Standardized β coefficient	*p*
Dependent variable: CRP (model $r = 0.549$, $p < 0.001$)		
HDL cholesterol	−0.290	0.006
Creatinine	−0.182	0.021
Spinal cord injury	0.199	0.025
Dependent variable: interleukin-6 (model $r = 0.552$, $p < 0.001$)		
Spinal cord injury	0.552	< 0.001
Dependent variable: endothelin-1 (model $r = 0.635$, $p < 0.001$)		
Spinal cord injury	0.586	< 0.001
Age	0.340	< 0.001
Creatinine	−0.289	0.003
Dependent variable: sVCAM-1 (model $r = 0.699$, $p < 0.001$)		
Spinal cord injury	0.600	< 0.001
Creatinine	−0.178	0.004

CRP = C-reactive protein; HDL = high-density lipoprotein; sVCAM-1 = soluble vascular cell adhesion molecule-1.

presence or absence of SCI, BMI, serum albumin and creatinine levels, and parameters of dyslipidemia and insulin resistance as covariates (Table 4). Multivariate analyses showed that SCI was the only factor that was independently associated with increased serum levels of CRP, interleukin-6, endothelin-1 and sVCAM-1. Serum creatinine levels were independently and negatively associated with levels of CRP, endothelin-1 and sVCAM-1, whereas HDL cholesterol levels were negatively correlated with CRP.

Discussion

In this study, we have, for the first time, shown that in men with chronic SCI (duration of injury > 1 year) and with no clinical or serologic evidence of active infection, serum levels of CRP, interleukin-6, endothelin-1 and sVCAM-1 are significantly increased, irrespective of injury duration and injury levels, compared to able-bodied controls. Although SCI subjects usually have a reduced lean muscle mass (as manifested by decreased creatinine levels) and features of the insulin-resistant metabolic syndrome (lower HDL cholesterol levels and hyperinsulinemia),[16] the positive associations between SCI and serum levels of CRP, interleukin-6, endothelin-1 and sVCAM-1 remained statistically significant after adjustment for all these confounding factors. These findings indicate that SCI *per se* is associated with a low-grade chronic inflammatory state and endothelial activation, which may partly explain the increased atherogenic risk in patients with long-standing SCI.

The inflammatory etiology of atherosclerosis has prompted a search for biomarkers of inflammation that predict risk for coronary heart disease and its sequelae. The marker of inflammation that has received the most attention recently as a potential marker of atherosclerotic risk is CRP. CRP, an acute-phase protein and a member of the pentraxin family, is produced by hepatocytes and possibly smooth muscle cells and monocytes/macrophages in response to inflammatory cytokines, including interleukin-6.[5] CRP levels increase several hundred-fold in response to acute injury, infection or other inflammatory stimuli. Traditionally, CRP has been used to monitor infectious and rheumatologic diseases, with a commonly assigned cut-off value of < 10 mg/L.[12] As a consequence of recurrent infections from pressure ulcers and urinary tract infections, which are frequently

encountered in patients with long-term SCI, several previous studies have shown that serologic CRP levels are markedly elevated (> 10 mg/L) in both symptomatic and asymptomatic patients with SCI compared with able-bodied individuals.[9–11] Although asymptomatic SCI patients were also included in these studies, the markedly elevated CRP levels indicate that they probably had occult infections. Likewise, recently published recommendations regarding the application of CRP to clinical practice suggest that, if CRP level is ≥ 10 mg/L, the test should be repeated and the patient examined for sources of infection or inflammation.[12]

It is well recognized that CRP levels measured to assess atherosclerotic risk caused by chronic low-grade inflammation are much lower than those measured in acute inflammation.[12] CRP measurements improve the accuracy in coronary risk assessment over and above the use of conventional risk factors.[17,18] The cut-off points for CRP levels recommended for coronary risk assessment are < 1.0 mg/L (low risk), 1.0–3.0 mg/L (average risk), and > 3.0 mg/L (high risk). The present study is the first to deliberately exclude the confounding effect of infection on CRP levels in patients with long-term SCI by including only asymptomatic patients with CRP levels < 10 mg/L. We clearly demonstrated that the mean CRP levels in patients with long-term SCI were in the high-risk category. The observed concomitant elevation of circulating interleukin-6 levels is consistent with the fact that interleukin-6 is the primary cytokine mediator of CRP production.[6] Despite increases in the levels of both CRP and interleukin-6 indicating that patients with long-term SCI are associated with chronic low-grade inflammation, it is noteworthy that serum levels of soluble CD40 ligand did not differ between SCI patients and able-bodied individuals. Soluble CD40 ligand is a proinflammatory and prothrombotic molecule, which plays a role in the pathogenesis of atherosclerosis and acute coronary syndromes.[19,20] Recent studies have shown that circulating soluble CD40 ligand is largely derived from activated platelets.[19] The lack of a difference in soluble CD40 ligand levels between SCI patients and able-bodied individuals might therefore suggest that SCI is not associated with clinically significant platelet activation.

The etiology of the increases in CRP and interleukin-6 levels in SCI patients free of coexisting infections appears to be multifactorial. First, in non-disabled people, higher circulating levels of CRP and interleukin-6 are significantly associated with components of the insulin-resistant metabolic syndrome, which is characterized by low levels of HDL cholesterol, elevated levels of fasting glucose, insulin and triglycerides, and abdominal obesity.[15,21] As a consequence of the reduction in physical activity and a loss of muscle mass due to atrophy by paralysis below the level of injury, patients with long-term SCI often have greater adiposity for a given BMI and, therefore, are more insulin-resistant than able-bodied individuals.[1,22] In this study, we showed that SCI patients had significantly lower HDL cholesterol and creatinine levels than able-bodied controls. Moreover, CRP and interleukin-6 levels correlated negatively with HDL cholesterol and creatinine levels in the study participants. This finding suggests that both body composition changes and the metabolic syndrome contribute to the increased CRP and interleukin-6 levels seen in SCI patients. Second, several recent studies have shown that sympathetic overactivity is associated with elevated CRP levels and subclinical low-grade inflammation.[23,24] In patients with long-term SCI, somatosensory and visceral stimuli below the level of the lesion can give rise to exaggerated sympathetic activity and marked norepinephrine spillover.[1] This may partly explain the independent association between elevated CRP levels and SCI after excluding the possible confounding effects of various metabolic parameters. However, despite sympathetic overactivity being primarily observed in SCI patients with an injury level above T7,[25] we found no differences in circulating CRP and interleukin-6 levels between SCI patients with an injury level above or below T7. Third, although SCI patients with CRP levels ≥ 10 mg/L were excluded in this study, it is still possible that very subtle

infections and bladder management techniques might influence CRP and interleukin-6 levels in these long-term SCI patients.

In addition to subclinical inflammation, we have, for the first time, showed that chronic SCI is associated with serologic evidence of endothelial activation, as manifested by significant increases in endothelin-1 and sVCAM-1 levels and a marginal increase in sICAM-1 levels. Endothelin-1 is a potent vasoconstrictor peptide secreted by endothelial cells in response to insulin, catecholamines, and other agonists, and has been shown to play a seminal role in the atherogenic process by enhancing mitogenesis and inducing extracellular matrix formation.[26,27] It is of interest that the serum endothelin-1 levels in our long-term SCI patients were similar to those in patients with insulin-resistant metabolic syndrome, which is known to be associated with increased endothelin-1 levels.[28,29] Likewise, serum endothelin-1 levels were shown to correlate negatively with serum HDL cholesterol levels in the study population, suggesting that the increased endothelin-1 levels were partly attributable to the metabolic syndrome.

Leukocyte adhesion to the endothelial surface, resulting from the expression of cell surface adhesion molecules, is a key factor in the atherogenic process. Two molecules of the transmembrane immunoglobulin superfamily, ICAM-1 and VCAM-1, are prototypic cell adhesion molecules and serve as endothelial ligands for the integrins expressed on both leukocytes and platelets and mediate tight attachment to the endothelium.[5] Soluble forms of both adhesion molecules have been detected in human plasma and serum and their levels have been found to be elevated in various inflammatory disorders.[5,30] In this study, we demonstrated that patients with chronic SCI had significantly elevated sVCAM-1 levels and mildly elevated sICAM-1 levels. This discordance in the elevation of circulating sVCAM-1 and sICAM-1 levels in SCI patients is intriguing. There are several plausible explanations for this finding, other than it being a chance finding. First, a recent study demonstrated that circulating sVCAM-1 levels, but not sICAM-1 levels, are elevated differentially in various stages of coronary atherosclerosis.[30] This finding suggests that sVCAM-1 is more sensitive in reflecting the activity and stability of atherosclerotic plaques. Because the sample size in the present study was limited, it is possible that only differences in the more sensitive markers were identified. Second, it is of interest that sVCAM-1 and sICAM-1 levels correlated with different components of the metabolic syndrome in this study population: sVCAM-1 levels correlated significantly with HDL cholesterol levels, whereas sICAM-1 levels correlated with insulin and triglyceride levels. A similar finding was found in able-bodied individuals of South Asian origin.[31] Although low HDL cholesterol and high triglyceride and insulin levels are all components of the metabolic syndrome,[1] only low HDL cholesterol was found in our SCI patients. Thus, the contribution of the metabolic syndrome to levels of markers of endothelial activation in chronic SCI was more evident from the change in sVCAM-1 levels than that in sICAM-1 levels. Third, it has been shown that endothelin-1, by activating nuclear factor κB, can increase the expression of cardiac VCAM-1, but not that of ICAM-1, in hypertensive rats.[32] Consistent with this observation, we found that sVCAM-1 levels correlated significantly with endothelin-1 levels, which might also contribute to the more significant increase in sVCAM-1 levels than in sICAM-1 levels in SCI patients.

There were several limitations in this study. First, the serum markers were measured at a single time point for each patient, so we cannot exclude variability in the levels of these markers with time. However, a previous study showed that in about 90% of cases, two independent CRP measurements taken 3 months apart were within one quartile of each other.[12] Second, we used BMI as a surrogate marker for adiposity. However, BMI does not distinguish the anatomic site of fat mass, and subjects with paraplegia and tetraplegia have different body compositions compared to each other or to able-bodied individuals. Given that the amount of fat mass has been shown to correlate with levels of markers of inflammation and

endothelial activation,[33] we may therefore have underestimated the influence of body composition on these markers. To reduce the effect of this limitation, we also included serum creatinine levels, a surrogate marker of muscle mass,[16] in addition to BMI, in the multivariate analysis model to control for the confounding effect of body composition. Third, the case number in the present study was relatively small. Hence, findings from the present study need to be verified in further larger-scale studies.

In conclusion, we have demonstrated that individuals with SCI have serologic evidence of subclinical inflammation and endothelial activation and features of the metabolic syndrome and changes in body composition. Given that accelerated atherogenesis is often seen in individuals with chronic SCI, our findings suggest that subclinical inflammation and endothelial activation may serve as possible mechanisms linking chronic SCI and atherogenic risk. Further studies are needed to explore the relationships between levels of serum markers of inflammation (CRP and interleukin-6) and endothelial activation (endothelin-1 and sVCAM-1) and parameters of subclinical atherosclerosis (carotid intima-media thickness, brachial artery vasoreactivity, and coronary calcification) or the development of cardiovascular events in SCI subjects and to validate the clinical utility of these markers.

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