Arterial stiffness and proinflammatory cytokines in fibromyalgia syndrome

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ABSTRACT

Objectives. *We assessed arterial stiffness and inflammatory cytokine profiles in fibromyalgia syndrome (FMS) patients and analysed the association between them.*

Methods. *Twenty-seven FMS patients and 29 age-matched premenopausal healthy controls were enrolled in this study. Arterial stiffness was assessed by pulse wave velocity (PWV) and augmentation index (AIx) from pulse waveform analysis. Levels of serum interleukin-1β (IL-1β), IL-6, IL-8, and vascular endothelial growth factor (VEGF) were measured by enzyme-linked immunosorbent assay, and a colorimetric assay was used for measurement of serum nitric oxide (NO) metabolites (nitrate and nitrite, NO_x) level. Statistical analyses included the Mann-Whitney U-test and Spearman's correlation coefficient analysis.*

Results. *Higher AIx and AIx@HR75 (aortic AIx at a heart rate of 75beats/min) were noted in FMS compared to those in the controls after adjustment using covariants (p_{adj}=0.023 and p_{adj}<0.001). However, there were no differences between the three regional PWVs of the two groups at the aorta-femoral, femoral-dorsalis, and aorta-radialis arteries (p_{adj}>0.05 for all). FMS subjects had significantly higher serum IL-8 levels than did the healthy controls (327.9±588.7 vs. 76.4±90.5, p_{adj}=0.041). However, there were no significant differences in serum IL-1β, IL-6, VEGF, or NO_x levels between the FMS patients and the controls (p_{adj}>0.05 of all). Serum IL-8 level did not correlate with PWV and AIx in FMS patients.*

Conclusion. *This study demonstrates higher AIx and IL-8 levels in FMS subjects compared to those of the controls. However, arterial stiffness including AIx in FMS was not determined by the serum IL-8 level.*

Introduction

Fibromyalgia syndrome (FMS) is a relatively common disease characterised by chronic widespread pain and tender points at specific localised anatomical sites; symptoms may also include cognitive dysfunction, sleep disturbance, fatigue, and psychological distress (1). It has been reported that approximately 30% of FMS patients also complain of cold intolerance or Raynaud's phenomenon (2). Disturbances in haemodynamics of microvascular circulation including decreased capillary flow and vasospasm (3-5) as well as abnormalities of morphological changes have been noted in FMS (6-8). Sympathetic hyperactivity induced by various stimuli, a well-established pathogenic feature in FMS patients, may be associated with vasoconstriction. (9). These data may implicate aberrant microvascular circulation in FMS patients.

In addition to microvascular abnormalities in FMS, some data have suggested that macrovascular or cardiovascular problems may be related to the clinical features of FMS. Significantly reduced global longitudinal left ventricular strain in 30 postmenopausal women with FMS was reported (10). Another study demonstrated that higher serum fibronectin level was involved in the non-immunological vascular damage in FMS (11). Although the definite pathogenesis of FMS has not been determined, disturbance of proinflammatory cytokines, including interleukin-8 (IL-8), tumour necrosis factor-α (TNF-α), IL-6, IL-4, and IL-10, may be associated with the pathogenesis of FMS (12, 13-15). Furthermore, these cytokines have been shown to be involved in vascular inflammation and atherosclerosis (16). A recent study demonstrated that subjects with widespread pain have a tendency toward increased mortality risk, especially from cardiovascular diseases (17).

Arterial stiffness is a dynamic vascular property that depends on the functions and structures of larger arteries. The clinical importance of arterial stiffness as a potent predictor for cardiovascular disease has been well established in the elderly and in various clinical entities (such as hypertension, diabetes mellitus) (18). In addition, increased arterial stiffness and development of cardiovascular events have also been established as clinical characteristics in other inflammatory rheumatic diseases, such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) (19, 20).

Our hypothesis in this study is that FMS patients have increased arterial stiffness compared to that of healthy subjects, based on diverse evidence that includes the presentation of microvascular/macrovascular clinical abnormalities and the pathogenic role of inflammatory cytokines in FMS. Therefore, we assessed indicators of arterial stiffness, including PWV of large arteries and augmentation index (AIx) derived from pulse waveform analysis. In addition, we measured the levels of serum inflammatory cytokines related to both vascular inflammation and the pathogenesis of FMS, including serum IL-1β, IL-6, IL-8, VEGF, and nitric oxide (NO) metabolites (nitrate and nitrite, NO_x).

Subjects and methods

Subjects and data collection

All 27 pre-menopausal patients met the classification criteria for FMS proposed by the American College of Rheumatology in 1990 (1). Twenty-nine age-matched healthy pre-menopausal subjects were consecutively enrolled as a control group. All subjects gave informed consent for participation in this study and for collection of the blood samples used for cytokine analysis. This study was approved by the Institutional Review Border/Ethics Committee at the Catholic University of Daegu.

Information regarding general patient characteristics, including weight, height, body mass index (BMI), symptom duration, disease duration after diagnosis, and duration of education, were assessed at enrolment. Past medical history, such as hypertension, diabetes mellitus, chronic renal disease, thyroid diseases, hypercholesterolemia, and cardiovascular diseases, was collected from all participants. Subjects with a diagnosis of or taking medications for inflammatory rheumatic diseases, including RA, SLE, systemic sclerosis, ankylosing spondylitis, and Raynaud's syndrome, were excluded. Initially, 34 FMS patients and 30 controls were enrolled; seven FMS subjects and one control were eliminated due to the exclusion criteria.

Clinical features related to FMS, including generalised weakness, fatigue, stiffness, parasthesias, febrile sensation, tension-type headache, subjective cognitive dysfunction (worsening concentration and memory disturbance), and dizziness, were obtained from individual interviews and review of medical records. Fatigue is defined as disturbance of patients' function, disproportionateness to exertion, and more than 50% of the time (21). Tension-type headache is considered headache or neck pain lasting for more than 6 months and worsening by stress (22). Functional abilities in patients with FMS were measured using the Korean version of the Fibromyalgia Impact Questionnaire (FIQ), using a scale of 0 to 100 for each patient (23). The Korean version of the Brief Fatigue Inventory (BFI) was used to assess fatigue experienced by FMS patients (24). Pharmacoloical therapeutics persistently used to manage clinical symptoms of FMS for the last 3 months were also reviewed including tricyclic antidepressants (*e.g.* amitriptyline, nortriptyline), selective serotonin reuptake inhibitors (*e.g.* fluxetine), anticonvulsants (*e.g.* gabapentin, pregabalin), non-steroidal anti-inflammatory drugs, tramadol/acetaminophen, and opioids.

Measurement of arterial stiffness

Arterial stiffness was measured using a non-invasive automatic pulse wave velocity analyser (PP-1000, HanByul Meditech Co., Jeonju, Korea) using an applanation tonometry system (27). The pulse wave was assessed in the supine position for approximately 15–20 minutes after 30 minutes of bed rest in a quiet room and a minimum 12-hour abstinence from smoking, alcohol, and coffee consumption. Tonometric sensors were applied on the skin above the left carotid, radial, dorsalis pedis, and femoral arteries. For measurements of brachial and ankle blood pressures, oscillometric cuffs were wrapped around both the left upper forearm and the lower leg. Four electrodes were attached on the four extremities in order to monitor cardiac rhythm, and electrocardiography (ECG) was taken. Phonocardiography (PCG) using heart sounds (including S1 and S2) was recorded using a piezopolymer film contact microphone placed between the left board of the sternum and the fourth intercostal space. Three PWVs, including the aorta-femoral PWV, the femoral-dorsalis PWV and the aorta-radialis PWV, were determined using data derived from ECG, PCG, and arterial pulse waves from the four different arteries. The PWV was measured in m/sec.

AIx was determined by analysis of pulse waves recorded in a PP-1000 pulse wave analyser and was expressed as a percentage value. Aortic AIx at a heart rate of 75 beats/min (AIx@HR75) was also calculated to avoid the influence of changes in heart rate. The interobserver variation coefficient was 88% and the intraobserver coefficient was 92%.

Measurements of IL-1β, IL-6, IL-8, and VEGF levels using enzyme-linked immunosorbent assay (ELISA)

Serum IL-6, IL-8, and VEGF levels were measured using a DuoSet ELISA Development kit (R&D Systems, Minneapolis, MN, USA). Serum IL-1β was also measured using the ELISA method (eBioscience Inc., San Diego, CA, USA) according to the manufacturer's instructions.

Measurement of serum NO_x by colourimetric assay

Serum NO_x level was measured by the nitrate/nitrite colourimetric assay (Cayman, Ann Arbor, MI, USA). Assay buffer or water was added to the plate, to which samples and Enzyme Cofactor mixture were added. Nitrate reductase mixture was added to the plate and incubated at room temperature for 1h.

Griess reagent R1 and Griess reagent R2 were added to the plate, followed by incubation for 10min at room temperature. Absorbance was measured at 550nm.

Statistical analysis

Data are described as mean±standard deviation or number (% of cases). Statistical analyses for ordinal to interval variables were verified by normality tests such as the Kolmogorov-Smirnov and Shapiroe-Wilk tests. The Mann-Whitney U-test was applied in the assessment of the differences between sequential variables in the two groups. Analysis of covariace (ANCOVA) was applied to adjust the difference of arterial stiffness and proinflammatory cytokines between two groups using covariants. Correlation analysis was performed using Spearman's correlation coefficient. A p-value<0.05 was considered statistically significant. Statistical analysis was performed using the Statistical Package for the Social Sciences, version 13.0 (SPSS Inc., Chicago, IL, USA).

Results

General characteristics

The study population included 27 premenopausal FMS and 29 age-matched healthy subjects (Table I). The mean disease duration in FMS patients was 2.9 years. There were no differences between FMS subjects and healthy controls in terms of weight, height, or BMI. Scores for the FIQ and BFI were 63.4±16.6 and 6.6±2.0, respectively. Medications for FMS taken by patients were also illustrated.

Comparisons of haemodynamics and parameters of arterial stiffness

In the comparison of blood pressures, ankle DBP differed between FMS and healthy controls (p=0.010), but brachial SBP, brachial DBP, and ankle SBP did not (Table II). The ankle-brachial indices were similar between the two groups. In measurements of aorta haemodynamics, augmentation pressure in FMS patients was significantly higher than that in the controls (p=0.026). The SBP, DBP, mean BP, and pulse pressure at the peripheral artery in FMS patients did not differ from those of the controls. FMS patients had higher AIx and AIx@HR75 measurements than did the controls after adjustment of covariants including ankle DBP, augmentation pressure, and mean peripheral BP (p_{adj}=0.023 and p_{adj}<0.001, Fig. 1A and 1B). However,

Table I. General characteristics of patients with fibromyalgia syndrome and healthy controls.*

	Fibromyalgia syndrome (n=27)	Healthy controls (n=29)	p-value
Age (year)	43.4 ± 6.3	42.7 ± 6.1	NS
Weight (kg)	55.2 ± 7.0	56.1 ± 6.5	NS
Height (cm)	157.8 ± 5.1	158.4 ± 4.0	NS
BMI (kg/m^2)	22.2 ± 2.7	22.3 ± 2.3	NS
Symptom duration (year)	7.1 ± 6.6		
Disease duration after diagnosis (year)	2.9 ± 3.1		
Duration of education (year)	11.0 ± 4.2		
FIQ	63.4 ± 16.6		
BFI	6.6 ± 2.0		
Clinical features, n (%)			
General weakness	22 (81.5)		
Fatigue	23 (85.2)		
Stiffness	24 (88.9)		
Parasthesia	12 (44.4)		
Febrile sensation	17 (63.0)		
Tension-type headache	18 (66.7)		
Subjective cognitive dysfunction	19 (70.4)		
Dizziness	21 (77.8)		
Medications, n (%)			
Tricyclic antidepressant	21 (77.8)		
Selective serotonin reuptake inhibitor	23 (85.2)		
Anti-convulsant	12 (44.4)		
Nonsteroidal antiinflammatory drug	24 (88.9)		
Tramadol/acetaminophen	20 (74.1)		
Opioids	11 (27.0)		

*Data were described as mean ± standard deviation or number of case (%).
BMI: body mass index, FIQ: fibromyalgia impact questionnaire, BFI: brief fatigue inventory.
NS: not significant (p-value>0.05).

Table II. Comparison of parameters for arterial stiffness between fibromyalgia syndrome and healthy controls.

	Fibromyalgia syndrome (n=27)	Healthy controls (n=29)	p-value
Blood pressure			
Brachial SBP (mmHg)	122.9 ± 16.5	120.5 ± 17.2	NS
Brachial DBP (mmHg)	78.4 ± 12.4	76.3 ± 11.8	NS
Ankle SBP (mmHg)	138.6 ± 17.6	136.4 ± 15.4	NS
Ankle DBP (mmHg)	76.2 ± 11.8	70.5 ± 8.8	0.010
Ankle brachial index	1.1 ± 0.1	1.1 ± 0.1	NS
Heart rate (beats/min)	68.4 ± 11.7	61.2 ± 10.3	NS
Aorta haemodynamics			
SBP (mmHg)	108.8 ± 25.5	106.1 ± 17.4	NS
DBP (mmHg)	77.6 ± 12.6	72.9 ± 12.2	NS
Mean BP (mmHg)	93.3 ± 13.9	87.6 ± 13.8	NS
Pulse pressure (mmHg)	34.8 ± 7.2	33.4 ± 6.7	NS
Augmentation pressure (mmHg)	9.5 ± 4.1	7.2 ± 3.4	0.026
Peripheral artery hemodynamics			
SBP (mmHg)	120.9 ± 16.3	116.6 ± 16.2	NS
DBP (mmHg)	76.4 ± 12.3	72.2 ± 12.1	NS
Mean BP (mmHg)	92.9 ± 14.0	87.0 ± 13.9	0.047
Pulse pressure (mmHg)	44.4 ± 9.1	44.4 ± 6.3	NS

SBP: systolic blood pressure, DBP: diastolic blood pressure, mean BP: mean blood pressure.
NS: not significant (p value>0.05).

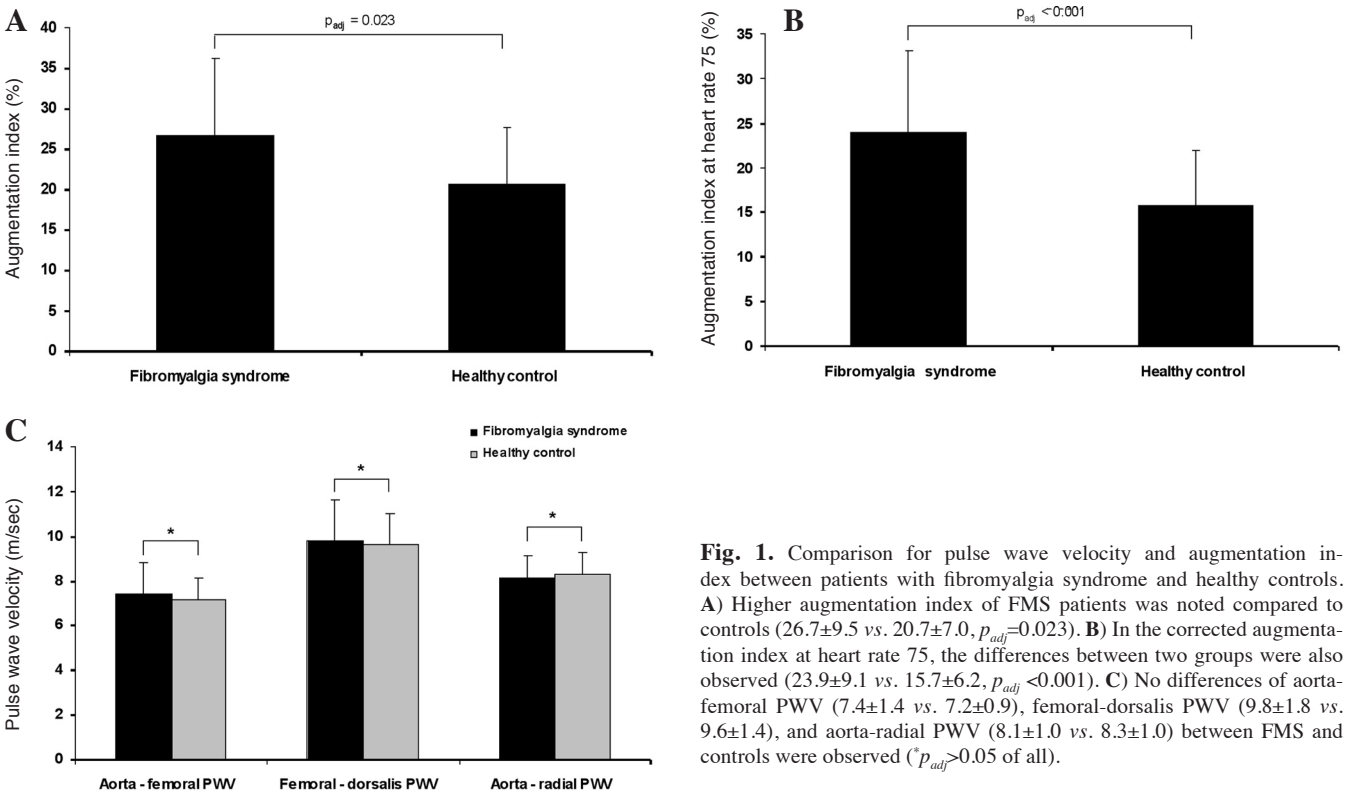

Fig. 1. Comparison for pulse wave velocity and augmentation index between patients with fibromyalgia syndrome and healthy controls. **A**) Higher augmentation index of FMS patients was noted compared to controls (26.7±9.5 *vs*. 20.7±7.0, p_{adj}=0.023). **B**) In the corrected augmentation index at heart rate 75, the differences between two groups were also observed (23.9±9.1 *vs*. 15.7±6.2, p_{adj} <0.001). **C**) No differences of aorta-femoral PWV (7.4±1.4 *vs*. 7.2±0.9), femoral-dorsalis PWV (9.8±1.8 *vs*. 9.6±1.4), and aorta-radial PWV (8.1±1.0 *vs*. 8.3±1.0) between FMS and controls were observed ($^{*}p_{adj}$>0.05 of all).

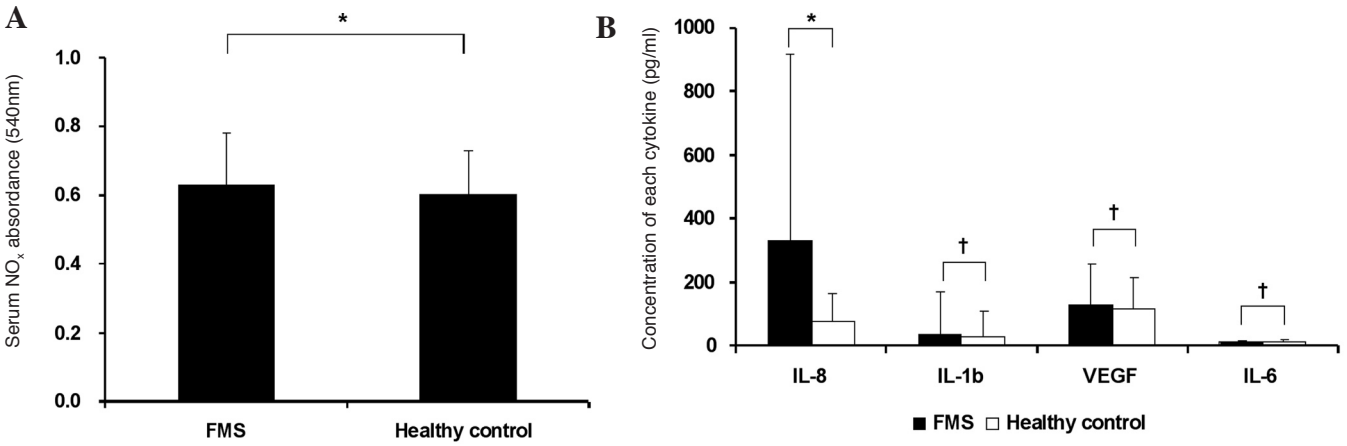

Fig. 2. Comparison for serum nitric oxide metabolites (NO_x) and inflammatory cytokine levels between patients with fibromyalgia syndrome and healthy controls. **A**) Serum NO_x was similar between two groups ($^{*}p_{adj}$>0.05). **B**) Fibromyagia patients showed higher serum IL-8 levels, compared to those of healthy controls ($^{*}p_{adj}$=0.041 for IL-8, and $^{\dagger}p_{adj}$>0.05 for IL-1b, VEGF, and IL-6). NO_x: nitric oxide metabolites (nitrate and nitrite).

the three regional PWVs (aorta-femoral, femoral-dorsalis, and aorta-radialis arteries) did not differ between the two groups (p_{adj}>0.05 for all, Fig. 1C).

Comparison of serum IL-1β, IL-6, IL-8, VEGF, and nitric oxide levels

Serum NO_x absorbance in FMS patients was similar to that observed in healthy controls (0.63±0.2 *vs*. 0.60±0.1, p_{adj}>0.05) (Fig. 2). FMS subjects had significantly higher serum IL-8 level compared to that of the healthy controls (327.9±588.7 *vs*. 76.4±90.5, p_{adj}=0.041). However, no differences in serum IL-1β, IL-6, or VEGF levels were observed between FMS subjects and the controls (p_{adj}>0.05 for all).

We compared the serum inflammatory cytokine levels between patients with and without the diverse clinical features assessed in this study, including general weakness, fatigue, stiffness, paresthesias, febrile sensation, tension-type headache, subjective cognitive dysfunction, and dizziness (Table III). Significant differences were noted in the following cytokine-symptom pairs: serum IL-6 levels in patients with fatigue, VEGF in patients with stiffness, IL-8 in patients with febrile sensation, and $\underline{NO_x}$ in patients with subjective cognitive dysfunction (p=0.011, p=0.047, p=0.025, and p=0.034, respectively). However, no differences were noted in cytokine levels in those with general weakness, paresthesias, tension-type headaches, or dizziness (data not shown).

Table III. Comparison of inflammatory cytokines between presence and absence of clinical features.

	Fatigue		Stiffness		Febrile sensation		Subjective cognitive dysfunction	
	Absence (n=4)	Presence (n=23)	Absence (n=3)	Presence (n=24)	Absence (n=10)	Presence (n=17)	Absence (n=8)	Presence (n=19)
IL-1β	6.6 ± 2.4	41.4 ± 146.0	6.6 ± 2.6	40.0 ± 143.0	8.9 ± 5.5	52.3 ± 169.8	11.4 ± 11.4	46.7 ± 160.8
VEGF	173.5 ± 162.1	117.8 ± 126.3	307.9 ± 283.7	103.3 ± 85.5††	119.9 ± 116.9	129.7 ± 140.7	133.1 ± 123.7	123.1 ± 136.0
IL-6	4.7 ± 1.8	10.5 ± 5.9†	7.4 ± 2.3	9.9 ± 6.1	7.3 ± 3.8	11.0 ± 6.4	10.2 ± 8.6	9.4 ± 4.5
NO_x	0.7 ± 0.2	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.2	0.6 ± 0.2	0.6 ± 0.1	0.7 ± 0.1	0.6 ± 0.2‡
IL-8	82.0 ± 115.2	370.6 ± 628.3	126.3 ± 118.3	353.3 ± 620.0	62.1 ± 36.4	484.2 ± 701.6*	324.5 ± 677.8	329.3 ± 567.3

IL-1β: interleukin-1β; IL-6: interleukin-6; IL-8: interleukin-8; VEGF: vascular endothelial growth factor; NO_x: nitric oxide metabolites (nitrate and nitrite); †*p*: 0.011 of serum IL-6 levels between absence and presence of fatigue; ††*p*: 0.047 of serum VEGF levels between absence and presence of stiffness; **p*: 0.025 of serum IL-8 levels between absence and presence of febrile sensation; ‡*p*: 0.034 of serum NO_x levels between absence and presence of subjective cognitive dysfunction.

Correlation of serum cytokines and parameters of arterial stiffness

Serum NO_x level was positively correlated with brachial SBP (*p*=0.041), while a negative relationship was noted between IL-1β and ankle SBP (*p*=0.026) (Table IV). IL-1β level was negatively associated with ankle SBP (*r*=-0.427, *p*=0.026). Age was closely related with pulse pressure, augmentation pressure, AIx, and AIx@HR75 (*p*=0.021, *p*=0.002, *p*=0.009, and *p*=0.040). FMS patients with higher FIQ and BFI (activity markers for FMS) showed a tendency toward increased heart rate (*r*=0.452, *p*=0.020 and *r*=0.482, *p*=0.011, respectively). BFI in FMS patients was negatively associated with pulse pressure at aorta (*r*=-0.445, *p*=0.020). Patients with longer symptom duration had higher brachial DBP and mean BP (*p*=0.034 and *p*=0.043, respectively).

Discussion

Several features of FMS, including microvascular morphological abnormalities, haemodynamic disturbances, and Raynaud's phenomenon, have been identified by many studies, although the precise mechanisms of these manifestations have been not fully understood (2-8). In addition, there also exists evidence of an association between those with widespread pain or FMS and cardiovascular vascular damage or risk (10, 11, 17). Until now, there are rare investigations enough to understand functional or morphological changes in large vessels in FMS. The major concern in this study is whether there is an association between FMS presenting with chronic widespread pain and functional changes in large arteries. Therefore, we investigated PWV and AIx using pulse waveform analysis, frequently used determinants of arterial stiffness. The main finding of this study is that FMS patients showed increased arterial stiffness, noted by increased AIx, but not PWV, compared to those of the controls.

Cold intolerance, Raynaud's phenomenon, and lower blood flow rate are considered objective findings that are related to microcirculation disturbances in FMS (2-5). In addition, Lindh *et al.* observed significantly lower capillary dimensions and numbers in FMS subjects using muscle histological analysis (8), suggesting aberrant microvascular circulation in FMS patients. However, the mechanism for this microcirculation disturbance has not been identified. Positive Nielson tests and elevated platelet α2-adrenergic receptors were revealed (3). It suggested that up-regulation of α2-adrenergic receptors may explain the enhanced vasospasms seen in FMS. Data from previous studies indicate that microcirculatory vessels should be considered a potential target organ related to the clinical manifestations of FMS.

A definite pathogenic mechanism for FMS has not been determined; however, potential candidates have been suggested, including inflammatory cytokine disturbances, genetic predisposition, negative environmental influences, and psychological factors. A number of studies have shown that inflammatory cytokines, including IL-1, IL-6, IL-8,

Table IV. Correlation analysis between clinical parameters and variables for arterial stiffness.

	Clinical parameters					
Variables for arterial stiffness	NO_x	IL-1β	Age	Symptom duration	FIQ	BFI
Brachial SBP	0.396*					
Brachial DBP				0.416*		
Ankle SBP		–0.427*				
Mean BP at aorta				0.401*		
Pulse pressure at aorta			0.443*			–0.445*
Augmentation pressure			0.575†			
Alx			0.491†			
Alx@HR75			0.398*			
Heart rate					0.452*	0.482*

Data were described as correlation coefficient and p values in each cell (**p*<0.05 and †*p*<0.01).
SBP: systolic blood pressure; DBP: diastolic blood pressure; IL-1β: interleukin-1β; NO_x: nitric oxide metabolites (nitrate and nitrite); BFI: brief fatigue inventory; PWV: pulse wave velocity; Alx: augmentation index; Alx@HR75: adjusted augmentation index at heart rate 75 per minute; FIQ: fibromyalgia impact questionnaire.

TNF-α, IL-4, and IL-10, contribute to the pathogenesis of FMS (12-15). However, results have not always been consistent and are sometimes contradictory. In this study, the serum IL-1β, IL-6, IL-8, VEGF, and $\underline{NO_x}$ were analysed. Our data shows that serum IL-8 level in FMS were significantly higher than those in the controls, while no differences in serum IL-1β, IL-6, VEGF, or $\underline{NO_x}$ levels were noted. This is consistent with previous studies, suggesting that IL-8 may be important in the pathogenesis of FMS.

This study aimed to investigate the relationship between clinical features and inflammatory cytokines in FMS patients. We identified significant associations between IL-6 and fatigue, VEGF and stiffness, IL-8 and febrile sensation, and $\underline{NO_x}$ and subjective cognitive dysfunction. The findings for IL-8 and IL-6 are in part in agreement with data published by Wallace *et al*. (12). Interestingly, serum VEGF levels in FMS patients with stiffness and fatigue were significantly lower than those in the controls, although statistical significance was not noted for fatigue, and the number of patients was very small. Inherited α1-antitrypsin deficiency (AATD) has been considered a novel genetic candidate involved in the pathogenesis of FMS. Plasma VEGF levels in AATD subjects with FMS are lower than those in subjects without FMS (25). Lower VEGF expression may be a characteristic feature of FMS and may be associated with the pathogenesis of FMS. Nitric oxide production from L-arginine is determined by nitric oxide synthases, and it is known that NO plays a role as both a reactive oxygen species and a neurotransmitter in the central and peripheral nerve systems. Li *et al*. demonstrated that nociceptive activation by the NMDA receptor was maintained through the production of NO (26). However, a depressive effect of NO on spinal nociceptive neurons has also been noted (27). Our finding that serum $\underline{NO_x}$ levels in FMS were lower than those in controls is more compatible with the latter finding.

Data from a number of studies have suggested that vascular inflammation or atherosclerosis results from a disturbance of balance between proinflammatory and antiinflammatory cytokines (16). We compared arterial stiffness (using PWV and AIx) between FMS and controls and investigated whether the cytokines IL-1β, IL-6, IL-8, VEGF, and $\underline{NO_x}$ influence the indicators for arterial stiffness assessed in this study. Our results show that AIx and AIx@HR75 but not PWV significantly increased in FMS, compared to controls. PWV is sensitive to blood pressure, vessel diameter, and vessel thickness, whereas AIx is mostly influenced by heart rate, gender, age, height, and vasoactive drugs and also dependent on high frequent signals from peripheral reflection wave (28). Activation of sympathetic nerve system generally induces elevation of mean arterial pressure and arteriolar vasoconstriction, which cause enlargement of reflection wave from peripheral artery, and then finally increased AIx. Carotid and radial AIx well reflected activation of sympathetic nerve system after post-exercise muscle ischemia (29). Considering similar blood pressure, age, gender, and heart rate between two groups in our study, the significant difference of AIx might in part be associated with sympathetic activation of FMS. We suggest that AIx, which well reflect vascular remodeling or tone by sympathetic activity, may be a more practical parameter for arterial stiffness rather than PWV in clinical practice of FMS.

Autonomic dysfunction especially sympathetic hyperactivity is considered a major characteristic of FMS (9). It has reported close relationship of sympathetic nerve system activation with IL-6 and of sympathetic pain with IL-8 (30). Wallace *et al*. also demonstrated that IL-8 promotes sympathetic pain in FMS patients (12). However, the role of proinflammatory cytokines released from FMS patients has not been determined, especially with sympathetic activity of FMS. One hypothesis suggested that IL-6 and IL-8 stimulated by substance P and/or cathecholamine might lead to proliferation of two pain sense fibers, afferent C fibres and B fibres associated with FMS pain, and then evoke the development of sympathetic pain (12). Based on these evidences, our hypothesis is that proinflammatory cytokines released from FMS may be closed associated with arterial stiffness. However, our study found that inflammatory cytokines, IL-1β, IL-6, and IL-8, are not associated with PWV and AIx. Further study for relationship among cytokine, arterial stiffness, and sympathetic activity should be needed.

There are some limitations to this study. First, the sample size in this study was small. There may be insufficient data, especially for association analysis between clinical features and cytokines. Therefore, a larger study population is needed to confirm these relationships. Second, this study was cross-sectional in nature, and changes in cytokine levels and parameters of arterial stiffness could not be observed in relation to clinical responses to medications. Third, we focused on the association between cytokine profiles and indicators for arterial stiffness in this study; the results failed to identify a close relationship between them. Sympathetic activity is generally related to vascular tone. FMS patients with vasospasm demonstrate enhanced expression of platelet α2-adrenegic receptors (3), and it has been established that sympathetic hyperactivity is involved in the pathogenesis of FMS (9). Assessment of the autonomic nervous system is therefore necessary to identify the mechanism of increased arterial stiffness in FMS.

In conclusion, we found that serum IL-8 levels and AIx were significantly higher in FMS patients compared to those of the controls. These findings indicate that IL-8 may be a crucial cytokine in the pathogenesis of FMS. However, this study did not show a close association between AIx and serum IL-8. Increased arterial stiffness in FMS may, therefore, be independent of inflammatory cytokine level. A prospective study assessing arterial stiffness and sequential serum cytokine analysis in a larger FMS population is needed.

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