ORIGINAL PAPER

Nagoya J. Med. Sci. **63**. 115 ~ 122, 2000

EVALUATION OF VASCULAR INJURY WITH PROINFLAMMATORY CYTOKINES, THROMBOMODULIN AND FIBRONECTIN IN PATIENTS WITH PRIMARY FIBROMYALGIA

SALIH PAY, M.D., MERAL ÇALGÜNERI, M.D., ZAFER ÇALIŞKANER, M.D., AYHAN DINÇ, M.D., ŞULE APRAŞ, M.D., İHSAN ERTENLI, M.D., SEDAT KIRAZ, M.D. and VELI ÇOBANKARA, M.D.

Hacettepe University School of Medicine, Departments of Rheumatology and Haematology, Ankara, Turkey

ABSTRACT

Objective

Cold intolerance, cold induced peripheral vasospasm, Raynaud's phenomenon, livedo reticularis and immunoglobulin deposition in the skin are often encountered clinical and laboratory findings in patients with primary fibromyalgia (FM). These findings are suggestive of vascular injury.

Methods

Eighty patients (4 male, 76 female) with fulfilling primary FM criteria (FM (+) patient group), 60 patients (3 male, 57 female) with chronic musculoskeletal complaints but without FM (FM (–) patient control group) and 40 healthy volunteers (1 male, 39 female) without musculoskeletal complaints (healthy control group) were enrolled in this cross-sectional study. The study was carried out in two steps. In the first step, the clinical findings, routine laboratory tests, autoantibodies and radiological findings were investigated. The second step were consisted of the laboratory investigations of thrombomodulin and fibronectin as the mediators indicating vascular injury and proinflammatory cytokines in FM patients with Raynaud's phenomenon and/or livedo reticularis and in control groups.

Results

There were no differences between study and control groups with regard to laboratory, radiological and immunological (ANA, AntidsDNA, ENA, anticardiolipin IgG and IgM) results. No statistically significant differences were found in the levels of proinflammatory cytokines between FM (+) patient group and control groups ($p > 0.05$). Thrombomodulin was also shown statistically insignificant difference between FM (+) patient group and control groups ($p > 0.05$). However, fibronectin, another mediator of vascular injury, was higher in FM (+) patient group and the differences between FM (+) patients and each control groups were statistically significant ($p < 0.0001$).

Conclusion

Our results were suggestive of the presence of a non-immunological vascular injury in FM patients with Raynaud's phenomenon and/or livedo reticularis

Key Words: Fibromyalgia, proinflammatory cytokines, thrombomodulin, fibronectin.

Correspondence: Salih PAY, M.D., GATA Department of Rheumatology Etlik, Ankara, TR-06018, Turkey
Fax: 00-90-312-323 49 23
E-mail: spay@gata.edu.tr

INTRODUCTION

Fibromyalgia (FM) is a commonly encountered disorder characterised by widespread musculoskeletal pain and easy fatigability along with multiple tender points, which are widely and symmetrically distributed. The patients with FM may also have cold intolerance, cold induced peripheral vasospasm, Raynaud's phenomenon and livedo reticularis which are considered a vascular instability. In addition, the deposition of IgG and IgM which is the characteristic finding of autoimmune disorders have been shown in skin biopsy samples from patients with FM by many researchers. Immunoglobulin deposition in the skin is more common in FM patients with livedo reticularis. Depending on a combination of these clinical and laboratory evidence, the existence of vascular injury in FM can be proposed.

In this study, frequency of Raynaud's phenomenon and livedo reticularis, which are encountered in autoimmune disorders, were investigated in patients with FM and controls. Thrombomodulin and fibronectin that are mediators indicating vascular injury and proinflammatory cytokines were studied in the FM patients who have clinical findings of vascular instability and control groups without these findings.

MATERIAL AND METHODS

Patient selection:

The study was carried out at the Rheumatology division of Hacettepe University School of Medicine. Eighty patients (4 male, 76 female) with fulfilling primary FM criteria (*FM* (+) *patient group*), 60 patients (3 male, 57 female) with chronic musculoskeletal complaints but without FM (*FM* (–) *patient control group*) and 40 healthy volunteers (1 male, 39 female) without musculoskeletal complaints (*healthy control group*) were enrolled in this cross-sectional study. The diagnosis of FM was made according to the 1990 criteria of ACR (1).

Study design:

The study was carried out in two steps. In the first step, we investigated the clinical findings, routine laboratory assays and radiological examinations. The second step was consisted of laboratory investigations of proinflammatory cytokines and mediators indicating vascular injury.

Step I:

Detailed medical history including rheumatologic complaints was taken. Morning stiffness, fatigue, photosensitivity, Raynaud's phenomenon, symptoms of spastic colon, eye and mouth dryness, sleep disorders, anxiety or depression symptoms, headache and parestesia were investigated in the rheumatologic anamnesis. Systemic physical and locomotor system examinations were performed.

The duration of morning stiffness was noted as minute. Pain was measured in direct patient interviews using a visual analog scale (VAS) and 0 indicated no pain and 10 indicated most severe pain. Tenderness at specific tender points, dermographism and livedo reticularis were investigated. Body mass index (BMI) was calculated.

Laboratory investigations were performed on each participants including; erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), rheumatoid factor (RF), complete blood count (CBC), urine analysis, routine serum biochemistry (fasting glucose, BUN, creatinine, uric acid, total protein, albumin, calcium, phosphate, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyl transpeptidase (GGT), direct and indirect bilirubin, amylase, creatinine phosphokinase (CPK), lactic dehydrogenase (LDH), total

cholesterol and triglyceride), thyroid function tests (free T3, free T3, TSH), serum autoantibodies (ANA, anti-dsDNA, ENA, anticardiolipin antibody (ACA) IgM and IgG) and complement components (C3 and C4). Chest, lumbosacral, sacroiliac, knee, hands and foot x-rays were also examined.

Following medical history, physical examination and laboratory investigations, the patients whose diagnosed as any rheumatologic or other systemic disease were excluded from the study.

Step II:

In this step, blood samples of 75 study participants were withdrawn for assays of proinflammatory cytokines and vascular injury mediators. The levels of thrombomodulin, fibronectin, IL-1β, TNF-α and IL-6 were measured in 25 patients in FM (+) patient group (23 female, 2 female) with livedo reticularis and/or Raynaud's phenomenon, 25 patients in FM (–) patient control group and 25 volunteers in healthy control group (24 female, 1 male). (Fig. 1).

Laboratory investigations:

The laboratory investigations and radiological examinations of step I were performed at the first visit. CBC and serum biochemistry were tested by automated analyser, CRP and RF by nephelometric method, ESR by Westergren method, ANA by IFA, anti-dsDNA, ENA, ACA IgG and IgM, C3, C4, free T3, free T4 and TSH by ELISA.

In step II, all blood samples were withdrawn at the morning to avoid diurnal variations. Samples centrifuged at room temperature (3000 rpm for 5 minutes) to obtain serum. Collected serum samples stored at –40°C until they were tested.

The measurement of IL-1β, IL-6, TNF-α, fibronectin and thrombomodulin were assessed by solid phase sandwich ELİSA techniques using "IL-1β immunoenzymometric assay kit,

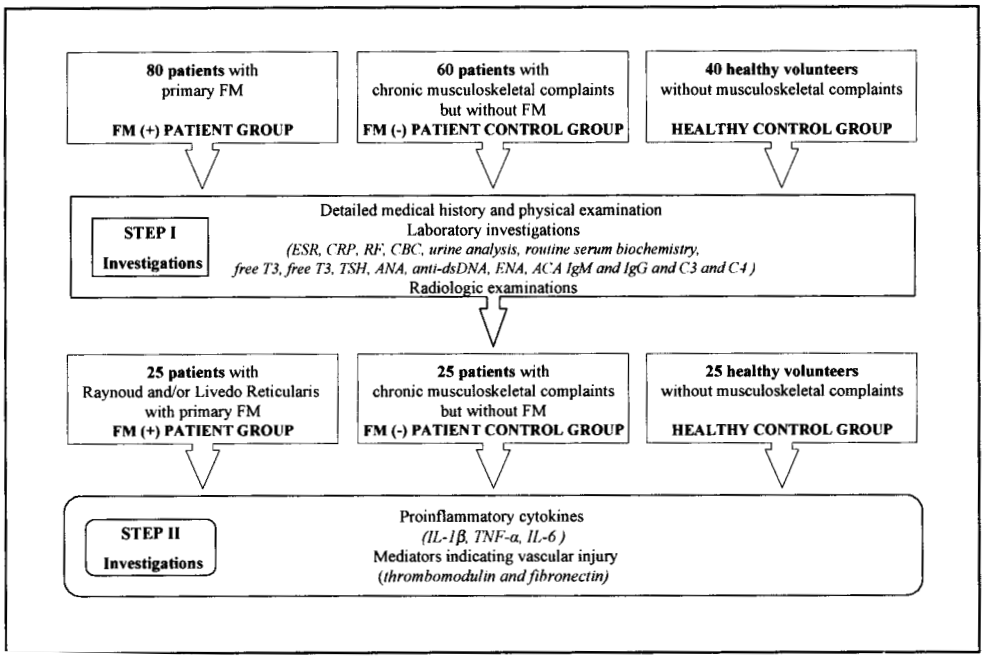

Fig. 1: Study design

Immunotech S.A., France", "Seroscreen human IL-6 ELİSA kit, Serotec, England", "Human TNF-α ELİSA kit, Bender MedSystems, HSTA50, USA", "Asserachrom fibronectin, enzyme immunoassay of fibronectin" and "Asserachrom thrombomodulin Fabrique pour Diagnostica Stago", respectively.

Statistical Analysis:

Data were analysed by using a statistical software package (SPSS for Windows ver. 6.0). The Chi-square test was used to compare the nonparametric values of the groups. The Kolmogorov-Smirnov test confirmed the normal distribution of all study parameters except the cytokines, fibronectin and thrombomodulin. Analysis of variance was used to determine whether there were differences in variables of interest among the groups, than the groups compared to each other. Cytokines, fibronectin and thrombomodulin were compared with Mann-Whitney U test, other parameters with independent sample Student-t test. Correlation between parameters was examined with Spearman correlation analysis. A p value equal or less than 0.05 was considered significant.

RESULTS

Clinical findings:

The mean ages were 40.16 ± 8.81 (range 31 to 48), 40.94 ± 9.85 (range 30 to 50) and 40.75 ± 8.46 (range 32 to 48) years in the FM (+) patient group, FM (–) patient control group and healthy control group, respectively.

The comparison of some clinical findings obtained at step I was presented in table-I.

There were no significant differences between FM (+) patient group and control groups with respect to age, sex and BMD ($p > 0.05$). The mean number of tender point in FM (+) patient group was significantly higher than controls ($p = 0.001$), while no differences between the control groups ($p > 0.660$). Intensity of pain according to VAS in FM (+) patient group was significantly higher than FM (–) patient control group ($p = 0.001$). Since the patients in healthy control group have not complaint of widespread musculoskeletal pain, VAS was not calculated for them.

Statistically significant differences in the livedo reticularis and Raynaud's phenomenon between FM (+) patient group and control groups were found ($p < 0.05$). There were no differences between FM (–) patient and healthy control groups with respect to these parameters ($p > 0.05$).

Table 1: The comparison of clinical findings of study and control groups

Parameter	FM (+) patient group (n=80)	FM (-) patient control group (n=60)	*p* FM (+) vs FM (-)	Healthy control group (n=40)	*P* FM (+) vs Healthy control group	*p* FM (-) vs Healthy control group
Sex (Male / Female)	4 / 80	3 / 60	NS	1 / 39	NS	NS
Patient age	40.16 ± 8.8	40.94 ± 9.8	NS	40.75 ± 8.4	NS	NS
Body Mass Index	25.7 ± 4.16	25.93 ± 3.74	NS	26.14 ± 5.8	NS	NS
Number of Tender Point	13.67 ± 1.94	3.3 ± 1.82	0.001	2.52 ± 1.95	0.001	NS
Skin tenderness	75	50	0.008	45	0.011	NS
VAS	6.86 ± 1.28	3.11 ± 1.05	0.001	-	-	-
Photosensitivity	20.4	3.3	0.03	5	0.011	NS
Livedo reticularis	32.6	7.4	0.04	5	0.035	NS
Raynaud's phenomenon	28.8	7.5	0.009	0	0.007	NS

Parametric values were given as mean ± SD, nonparametric values given as (%)
NS: statistically insignificant

Table 2: The comparison of IL-1β, IL-6, TNF-α, fibronectin and thrombomodulin levels in study and control groups

Parameter	FM (+) patient group (n=25)	FM (-) patient control group (n=25)	*p* FM (+) vs FM (-)	Healthy control group (n=25)	*P* FM (+) vs Healthy control group	*p* FM (-) vs Healthy control group
IL-1β (pg/ml)	30.7 (13.1-88.5)	33.2 (16.4-92.7)	NS	29.8 (15.6-87.3)	NS	NS
IL-6 (pg/ml)	9.6 (3.7-25.2)	8.8 (2.8-23.4)	NS	7.9 (2.6-24.1)	NS	NS
TNF-α (pg/ml)	8.5 (5.1-18.7)	9.2 (3.7-19.6)	NS	8.1 (4.9-21.4)	NS	NS
Thrombomodulin (ng/ml)	30.84 (18.5-69.5)	33.2 (17.4-53.8)	NS	36.8 (30.7-44.1)	NS	NS
Fibronectin (ng/ml)	18.7 (13.5-35.5)	6.17 (2.7-25.3)	0.0001	5.67 (1.1-28.9)	0.0001	NS

Values were given as median and range
NS: statistically insignificant

Laboratory findings:

The comparison of laboratory investigations which were performed in the step I, were statistically insignificant between the FM (+) patient, FM (–) patient control and healthy control groups ($p > 0.05$).

The results of step II investigations were shown in Table-2.

No statistically significant differences were found in the levels of proinflammatory cytokines, IL-1β, IL-6, and TNF-α, between FM (+) patient group and control groups ($p > 0.05$). Thrombomodulin was also shown statistically insignificant difference between FM (+) patient, FM (–) patient control and healthy control groups ($p > 0.05$). However, fibronectin was higher in FM (+) patient group and the differences between FM (+) patient group and each control groups were statistically significant ($p < 0.0001$).

DISCUSSION

Fibromyalgia is a chronic medical condition characterised by widespread body pain and multiple tender points. It is often accompanied by many other problems such as cold intolerance. Increased sensitivity to cold has been known for many years.[2,3] Cold-induced vasospasm has also been demonstrated in nearly 40% of the patients with primary FM.[4] Raynaud's phenomenon is another feature, which is observed in patients with primary FM. Dinerman et al. described Raynaud's phenomenon in 30% of FM patients with only medical history. In other study, it has been determined in 39% of FM patients with sensitive finger plethysmography by Ingram *et al.*[5,6] In our study, Raynaud's phenomenon were investigated based on medical history and determined in 28.8% of patients in FM (+) patient group. This frequency was statistically significant when compared with control groups. Our results were concordant with previously reported studies mentioned above.

Another cutaneous clinical manifestation of FM is livedo reticularis. Caro *et al.* reported that 64% of FM patients (40% mild, 24% moderate) have livedo reticularis.[7] We also observed this manifestation in our FM patients. Although, livedo reticularis was less frequent in our patients than Caro *et al.*'s found, the difference between FM (+) patient group and control groups with respect to the presence of livedo reticularis was statistically significant.

The observation of fibromyalgia-like symptoms with parenteral recombinant IL-2 therapy, leads to further studies on the role of the cytokines in FM. Immune mediated increase in cap-

illary permeability is expected to be responsible for the most of the side effects which is observed during IL-2 therapy. The close resemblance of the clinical features of the patients receiving IL-2 therapy to the patients with FM, suggest that both of these clinical pictures may be due to the same pathophysiological factor. Wallace *et al.* determined the elevated levels of IL-2 and interferon-α in 8 of 18 patients with FM.[8] However, they could not found statistically significant differences between patients and controls when compare all cytokines that they were studied (IL-1β, IL-2, IL-2R, interferon-α. interferon-γ and TNF-α). We studied proinflammatory cytokines, (IL-1β, TNF-α and IL-6) in FM patients with clinical evidence of vasculitis, because elevated levels of these cytokines were detected in some vasculitic syndromes. However, levels of these cytokines were in normal ranges, as in Wallace's study.

The abnormal cutaneous deposition of immunoreactants, such as IgG, IgM and albumin, in patients with FM have been reported by many researcher up to day.[5,9-11] These reports are remarkable for the finding of an extravascular accumulation of plasma proteins which are normally confined to the vascular space. Caro summarised these interesting findings as: 1) the pattern of immunofluorescent staining, particularly that found in the dermoepidermal junction, is rarely, seen in normal individuals; 2) the conditions in which these immunofluorescent findings are described typically demonstrate an immunologic component; 3) one of the fundamental common denominators in such cutaneous immunoreactant deposition is the concept of "enhanced vascular permeability". That is, there has been a fundamental change in microvascular permeability to explain the blood vessel and dermoepidermal junction abnormalities seen in these types of immunofluorescent investigations. This enhanced vascular permeability can not be explained by any other mechanism but endothelial cell injury in patients with FM.[12]

When the patients with Fibromyalgia divided into groups with respect to the skin immunofluorescent findings, manifestations that commonly seen in autoimmune disorders such as hair loss, oral ulcer and photosensitivity, are more frequent in patients with positive for skin immunofluorescent than those patients with negative for skin immunofluorescent.[9,13] However, opposite to the results, no evidence for the immunological disturbance in FM has been obtained. Serum autoantibodies and cytokine profiles had been found as similar within FM patients and controls.[14,15] The deposition of immunoglobulin in dermis probably depends on non-immunological mechanisms. Ig deposition without C3 and the absence of circulating immune complexes in FM supports this opinion.[15,16]

Some findings such as reactive hyperemia observed in patients with FM, thought to be due to the neurological inflammation. Neurogenic inflammation results from peripheral release of neuropeptides, such as calcitonin gene-related peptide, substance P and neurokinin A, from primary afferent nerve fibers. These neuropeptides increase skin blood flow, vascular permeability, and tissue accumulation of polymorphonuclear leukocytes. Calcitonin gene-related peptide acting via CGRP1 receptors is the principal transmitter of neurogenic dilatation of arterioles whereas substance P and neurokinin A acting via NK1 receptors mediate the increase in venular permeability.[17,18] The plasma extravasation is also increased in FM patients. This finding may be explaining the protein deposition in dermo-epidermal junction in FM.[19]

Trombomodulin and fibronectin are endothelial cell derived products and elevated levels of these mediators reflect the vascular injury. Thrombomodulin has been demonstrated as a marker of vascular injury in collagen vascular diseases, in several studies.[20,21] Ohdama et al. reported that the level of thrombomodulin was correlated with vascular injury and disease activity in patients with Wegener's granulomatosis.[22] It has been also suggested that thrombomodulin might be a marker for endothelial cell injury in Behcet's disease.[23]

In this respect, fibronectin has similar features as thrombomodulin. Elevated levels of fibronectin have been reported after experimental vascular injury and inflammation,[24] and in

active phase of vasculitis.[25] In a recently reported study, elevation in fibronectin level in rheumatoid vasculitis has been shown.[26]

Another disorders that fibronectin investigated are pre-eclampsia and eclampsia which are resulted from endothelial dysfunction. In both clinical picture elevated fibronectin levels had been detected and correlation between fibronectin level and the severity of clinical findings observed.[27,28]

In the present study, we studied pro-inflammatory cytokines (IL-1β, TNF-α and IL-6), fibronectin and thrombomodulin in FM patients with clinical findings of vascular instability. Trombomodulin levels were similar in FM (+) patient group and controls, but fibronectin levels were significantly higher in FM (+) patient group then controls. We could not found any study to compare our results. However we tried to explain the question of "why only fibronectin elevated?", a study reported by Shaarawy *et al.* was aroused our interest.[29] They studied thrombomodulin, fibronectin and plasminogen activator inhibitor-1, to determine biological markers of endothelial injury in patients with pre-eclampsia and eclampsia. They divided the patients into three subgroups as mild pre-eclampsia, severe pre-eclampsia and eclampsia, and compared them with normotensive pregnants. They found elevated levels of all study parameters in patients with severe pre-eclampsia and eclampsia. However, the most significant elevation observed in fibronectin level. In mild pre-eclampsia the levels of plasminogen activator inhibitor-1 and fibronectin also found elevated, while thrombomodulin within normal ranges. They suggested that, fibronectin is a more sensitive biological marker for endothelial injury in patients with pre-eclampsia and eclampsia.[29]

We could not find any study indicated that fibronectin is more sensitive vascular injury marker than thrombomodulin. Since Shaarawy *et al.*'s study is best established the correlation between clinical findings and levels of these markers, we compared our results with this study, although FM and eclampsia are different disorders, however. Depending on Shaarawy et al.'s results, it may be concluded that, only fibronectin was elevated in our patients, because neurogenic inflammation is minimal in FM.

The results of the presented study and other studies reported up to day, suggestive of the non-immunological vascular injury and endothelial dysfunction in patients with FM, particularly in patients with clinical evidence of vascular injury. Depending on the current data, it may be suggested that, the pathophysiological mechanism of this vascular injury might be related to the neurological inflammation.

Further detailed studies are necessary to establish the pathophysiology and clinical importance of endothelial dysfunction and vascular injury in FM. These studies will be able to an important step to expose the pathogenesis of FM.

REFERENCES

1) WOLFE F, SMYTHE HA, YUNUS MB, *et al.* American College of Rheumatology 1990 criteria for classification of fibromyalgia. Report of multicenter criteria committee. *Arthritis Rheum* 1990; 33: 160–172
2) YUNUS MB, MASI AT, CALABRO JJ, MILLER KA, FEIGENBAUM SL: Primary fibromyalgia (fibrositis): clinical study of 50 patients with matched normal controls. *Semin Arthritis Rheum.* 1981; 11: 151–71.
3) BENGTSSON A, HENRIKSSON K-G, JORFELDT L, *et al.* Primary fibromyalgia. *Scand J Rheumatol* 1986; 15: 340–344
4) LAPOSSY E, GASSER P, HRYCAJ P, DUBLER B, SAMBORSKI W, MULLER W. Cold-induced vasospasm in patients with fibromyalgia and chronic low back pain in comparison to healthy subjects. *Clin Rheumatol* 1994; 13: 442–445
5) DINERMAN H, GOLDENBERG DL, FELSON DT. Prospective evaluation of 118 patients with fibromyalgia syndrome: prevalence of Raynaud's Phenomenon, sicca symptoms, low complement, and Ig

deposition at the dermal-epidermal junction. *J Rheumatol* 1986; 13: 368–373

6) INGRAM S, NELSON D, PORTER J, *et al.* An association of cold-induced vasospasm and fibrositis. *Arthritis Rheum* 1987; 31: 4S:13
7) CARO XJ. Immunofluorescent detection of IgG at the dermal-epidermal junction in patients with apparent primary fibrositis syndrome. *Arthritis Rheum* 1984; 27: 1174–1179
8) WALLACE DJ, BOWMAN RL, WORMSLEY SB, PETER JB. Cytokines and immune regulation in patients with fibrositis. (letter) *Arthritis Rheum.* 1989; 32. 1334–1335
9) CARO XJ, AHMED R. Immunofluorescent (IF) detection of IgG at the dermal-epidermal junction in patients with apparent primary fibrositis syndrome (abstract). *Arthritis Rheum.* 1983; 26: s44
10) CARO XJ. Immunofluorescent studies of skin in primary fibrositis syndrome *Am J Med* 1986; 81: 43-50
11) BURDA CD. Immunoglobulin-G deposits at the dermal-epidermal junction in secondary (traumatic) fibromyalgia syndrome (letter). *Clin Exp Rheumaol* 1984; 2. 195–202
12) CARO XJ. Is there an immunologic component to the fibrositis syndrome. *Rheum Dis Clin N Am* 1989; 15: 169–186
13) CARO XJ. Immunofluorescence and fibromyalgia (letter). *Arthritis Rheum.* 1985; 28: 836-841
14) BENGTSSON A, ERNERUDH J, VRETHEM M, SKOGH T. Absence of autoantibodies in primary fibromyalgia. *J Rheumatol.* 1990; 17: 1682–1683
15) WALLACE DJ, PETER JB, BOWMAN RL, WORMSLEY SB, SILVERMAN S. Fibromyalgia, cytokines, fatigue syndrome, and immune regulation. In: FRICTION JR, AWAD E, (ed). *Advances in pain Research and therapy*, New York, Raven Press 1990; 17: 277–287
16) ENESTRÖM S, BENGTSSON A, LINDSTÖRM F, JOHAN K. Attachment of IgG to dermal extracellular matrix in patients with fibromyalgia. *Clin Exp Rheumatol* 1990; 8: 127–135
17) BALUK P. Neurogenic inflammation in skin and airways. *J Invest Dermatol Symp Proc* 1997; 2: 76–81
18) KILO S, HARDING-ROSE C, HARGREAVES KM, FLORES CM. Peripheral CGRP release as a marker for neurogenic inflammation: a model system for the study of neuropeptide secretion in rat paw skin. *Pain* 1997; 73: 201–207
19) LITTLEJOHN GO, WEINSTEIN C, HELME RD. Increased neurogenic inflammation in fibrositis syndrome. *J Rheumatol.* 1987; 14: 1022–1025
20) TAKAYA M, ICHIKAWA Y, KOBAYASHI N, KAWADA T, SHIMIZU H, UCHIYAMA M, *et al.* Serum thrombomodulin and anticardiolipin antibodies in patients with systemic lupus erythematosus. *Clin Exper Rheumatol* 1991; 9: 495–499
21) BOEHME MWJ, NAWROTH PP, KLING E, LIN J, AMIRAL J, RIEDESEL J, *et al.* Serum thrombomodulin: A novel marker of disease activity in systemic lupus erythematosus. *Arthritis Rheumatol* 1994; 4: p.572–577
22) OHDAMA S, MATSUBARA O, AOKI N. Plasma thrombomodulin in Wegener's granulomatosis as an indicator of vascular injuries. *Chest.* 1994; 106: 666–671
23) HAZNEDAROĞLU İC, ÖZDEMIR O, ÖZCEBE O, DÜNDAR SV, KIRAZLI Ş. Circulating thrombomodulin as a clue of endothelial damage in Behçet's disease (letter). *Thromb Haemost* 1996; 75: 974-975
24) PETERS JH, GINSBERG MH, BOHI BP, SKLAR LA, COCHRANE CG. Intravascular release of intact cellular fibronectin during oxidant-induced injury of the in vitro perfused rabbit lung. *J Clin Invest.* 1986; 78: 1596–1603
25) PETERS JH, MAUNDER RJ, WOOLF AD, GINSBERG MH. Elevated plasma levels of ED1+ fibronectin in patients with vascular injury. *J Lab Clin Med* 1989; 113: 586–597
26) VOSKUYL AE, EMEIS JJ, HAZES JMW, VAN HOGEZAND RA, BIEMOND I, BREEDVELD FC. Levels of circulating cellular fibronectin are increased in patients with rheumatoid vasculitis. *Clin Experimental Rheumatol* 1998; 16: 429–434
27) DENG L, BREMME K, HANSSON LO, BLOMBACK M. Plasma levels of von Willebrand factor and fibronectin as markers of persisting endothelial damage in preeclampsia. *Obstet Gynecol.* 1994; 84: 94194–5
28) PAARLBERG KM, DE JONG CL, VAN GEIJN HP, VAN KAMP GJ, HEINEN AG, DEKKER GA. Total plasma fibronectin as a marker of pregnancy-induced hypertensive disorders: a longitudinal study. *Obstet Gynecol.* 1998; 91: 383–388
29) SHAARAWY M, DIDY HE.Thrombomodulin, plasminogen activator inhibitor type 1 (PAI-1) and fibronectin as biomarkers of endothelial damage in preeclampsia and eclampsia. *Int J Gynaecol Obstet.* 1996; 55: 135–139