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Vascular endothelial growth factor concentrations in synovial fluids of patients with symptomatic internal derangement of the temporomandibular joint

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BACKGROUND: Vascular endothelial growth factor (VEGF) is an inducer of angiogenesis and permeability of small blood vessels. We determined the concentrations of VEGF in synovial fluid of patients with symptomatic internal derangement of the temporomandibular joint (TMJ).

METHODS: Diluted synovial fluid was collected by a pumping procedure from 22 TMJs of patients with internal derangement and 10 control TMJs. VEGF concentration was determined by an enzyme-linked immunosorbent assay.

RESULTS: The VEGF was detected in 14 of the 22 joints (64%) of patients with internal derangement, at a mean concentration of 67 pg/ml, but in only one control joint (12.5 pg/ml) ($P = 0.004$ for the difference in concentration). There was a significant correlation between VEGF concentration and total protein concentration in the synovial fluid ($P = 0.002$).

CONCLUSIONS: The increased concentration of VEGF in patients with symptomatic internal derangement suggests that this growth factor may be involved in the pathogenesis of this condition.

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Keywords: serum; synovial fluid; temporomandibular joint; vascular endothelial growth factor

Introduction

Inflamed synovial tissues associated with internal derangement of the temporomandibular joint (TMJ) are characterized by the presence of many new small blood vessels (1–3). We previously demonstrated that the density of these small blood vessels in synovial tissues with internal derangement was significantly higher than in control synovial tissues, and that the number of small blood vessels correlated significantly with the degree of synovitis diagnosed by arthroscopy (3, 4).

Vascular endothelial growth factor (VEGF) is an inducer of angiogenesis specific to endothelial cells. VEGF has been shown to promote angiogenesis, as well as the increased permeability of small blood vessels, in pathologic conditions (5–7). We have shown distinct immunohistochemical expression pattern of VEGF and VEGF receptor-1 (Flt-1) in synovial tissues with internal derangement, suggesting that the VEGF/VEGF receptor system may contribute to angiogenesis in inflamed synovial tissues (3, 8). This is consistent with findings in other joints. For example, synovial fluids from patients with rheumatoid arthritis (RA) have significantly higher levels of VEGF than do synovial fluids of controls (7, 9). Moreover, the concentration of serum VEGF has been shown to be a useful indicator of the severity of arthritis in patients with RA (10). We hypothesized that synovial fluid and serum VEGF levels are higher in patients with symptomatic internal derangement of the TMJ, and that these levels correlate with severity of disease. There are few reports, however, concerning the detection of VEGF in TM synovial fluid. We therefore assayed VEGF concentrations in synovial fluid and serum in patients with symptomatic internal derangement, and discuss the possible role of VEGF in the pathologic conditions of the TMJ.

Materials and methods

Subjects

We studied 22 joints of 21 patients (19 women and two men) with symptomatic internal derangement of the TMJ, each of whom underwent arthroscopic surgery after appropriate non-surgical treatment failed to resolve the clinical symptoms (Table 1). All of the patients with internal derangement complained of painful hypomobility of the TMJ. The patients ranged in age from 17 to 73 years old (mean: 43.7 years). Their mean

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Table 1 Patient and clinical variables, VEGF concentration, VEGF, and Flt-1 staining

Patient number	*Age/sex (years)*	*Duration of symptom (months)*	*MIO (mm)*	*VAS*	*Synovitis score*	*VEGF concentration in synovial fluid (pg/ml)*	*VEGF concentration in serum (pg/ml)*	*VEGF immunopositive cells (%)*	*Flt-1 immunopossitive cells*	*Total protein concentration in synovial fluid (μg/ml)*	*MVD*
1[a]	36/F	3	35	7	5	32.1	–	7	Presence	2874	33
2[a]	36/F	3	35	7	5	0	–	20	Presence	900	18
3	38/F	7	31	8	5	0	–	17	Presence	462	17
4	23/F	15	36	5	5	0	–	37	Presence	839	36
5	56/F	5	32	3	5	114.6	–	70	Presence	1410	58
6	55/F	5	27	4	4	0	–	3	Absence	1802	7
7	50/F	6	37	6	10	177.9	–	17	Absence	4895	25
8	18/F	11	33	6	6	16.4	–	27	Presence	453	25
9	17/F	4	33	6	5	0	–	17	Presence	389	29
10	73/F	7	29	5	5	70.7	275.5	50	Presence	1746	25
11	52/F	2	36	1	6	0	315.5	0	Absence	704	3
12	40/F	1	30	4	9	45.0	232.5	77	Absence	2355	19
13	39/M	2	33	2	9	72.9	211.0	43	Presence	3968	59
14	52/F	2	32	1	3	160.4	287.0	52	Presence	1805	14
15	56/F	2	38	10	2	150.5	321.5	97	Presence	12 091	8
16	36/F	9	42	8	5	0	201.5	93	Presence	477	56
17	35/F	3	28	4	6	298.5	256.0	74	Presence	5689	29
18	58/F	6	30	6	5	12.0	235.5	53	Presence	1194	75
19	52/F	3	25	5	2	0	219.0	27	Presence	4891	12
20	61/F	2	31	5	6	16.5	83.0	46	Presence	5313	65
21[a]	23/F	10	30	7	6	148.5	–	68	Presence	4123	45
22	48/M	7	35	5	4	149.0	290.5	62	Presence	1857	75
23[a,b]	25/F	2	50	0	0	12.5	135.5	0	Absence	2707	14
24[a,b]	25/F	2	50	0	2	0	–	18	Presence	783	9
25[b]	72/M	11	50	0	0	0	182.0	11	Absence	2280	7
26[a,b]	23/F	–	30	0	1	0	–	–	–	285	–
27[a,b]	36/M	–	48	0	–	0	395.0	–	–	509	–
28[a,b]	36/M	–	48	0	–	0	–	–	–	174	–
29[a,b]	36/F	–	45	0	–	0	234.0	–	–	1431	–
30[a,b]	36/F	–	45	0	–	0	–	–	–	1353	–
31[a,b]	38/F	–	46	0	–	0	305.5	–	–	625	–
32[a,b]	38/F	–	46	0	–	0	–	–	–	420	–

M, male; F, female; MIO, maximum interincisal opening; VAS, visual analogue scale; VEGF immunopositive cells, the percentage of immunopositive cells for vascular endothelial growth factor in the synovial tissues (%); MVD, microvessel density.
[a]Bilateral joints.
[b]Control subjects.

maximum interincisal opening was 32.5 mm, and the mean duration of their symptoms was 5.2 months. Magnetic resonance imaging (MRI) revealed that all of the patients had anterior disk displacement without reduction. Their mean subjective pain level before the operation, rated on a visual analogue scale (VAS) of 0–10, was 5.2.

We assayed 10 joints in six subjects (four women and two men) as controls. Two of the subjects (three joints) underwent arthroscopic eminoplasty (11) because of habitual dislocation without pain, one subject (one joint) had a non-symptomatic joint with internal derangement in the opposite joint (case 26), and three subjects (six joints) were healthy volunteers. The controls ranged in age from 23 to 72 years, with a mean age of 36.5 years. Preoperative MRI revealed that the disk was in normal position in the three controls (four joints), who were not healthy volunteers. MRI was not performed on the three healthy volunteers.

Both the patients and controls in this study also participated in our previous study (12).

All of the participants, except for the healthy volunteers, gave their informed consent to arthroscopy, collection of synovial fluid and serum, and synovial biopsy. The healthy volunteers gave their informed consent to collection of synovial fluid and serum.

Synovial fluid and serum sample preparation

Synovial fluid samples were collected from the superior joint compartment of all the subjects, except the healthy volunteers, just prior to the joint distension during arthroscopic surgery as previously described (12, 13). Synovial fluid samples from the three healthy control subjects were collected in an outpatient manner under local anesthesia. In each case, the surgeons (NS or JS) injected 2.0 ml of normal saline solution into the superior joint space with a 21-gauge needle and aspirated the diluted synovial fluid; this procedure was performed 10 times before drawing off the final sample (13, 14). We excluded samples of smaller than 1.8 ml (90% of the volume of injected saline) or samples that contained an excessive amount of blood. Synovial fluid samples were immediately centrifuged at 1000 ***g*** for 20 min to remove cells and stored at −80°C until assay.

Blood samples were obtained from a vein using a separate tube (about 5 ml) just prior to collection of synovial fluids from 12 patients with internal derangement and five control subjects (JS). After clotting, blood was centrifuged at 1000 rpm for 30 min, and serum was removed and stored at −80°C until assay.

Measurement of VEGF concentrations

The concentrations of VEGF in synovial fluid and serum were determined using an enzyme-linked immunosorbent assay (ELISA) kit (Analyza; Techne Corporation, Minneapolis, MN, USA; product number 8059), according to the manufacturer's instructions. This assay recognizes recombinant and natural human VEGF (VEGF165 and VEGF121) and has no significant cross-reactivity or interference, except with the VEGF165/PlGF heterodimer. The minimum detectable level of VEGF in this assay is <9 pg/ml. The measurement was performed by two of the authors (KK and JS).

Measurement of total protein concentrations in the synovial fluid

The total protein concentration was determined using a protein assay kit (Bio-Rad, Hercules, CA, USA), according to the manufacturer's instructions (KK and JS).

MRI

A preoperative MRI was administered to all subjects, except for the three healthy volunteers, using a 1.5 Tesla MRI scanner equipped with a 3-in surface coil (Siemens, Erlangen, Germany). The mean time between the MRI and operation was 30 days (range: 0–90). In spin-echo sequence, proton and T2-weighted images were taken of sections 3 mm thick with the mouth open and closed. The MRI settings have been described (13, 15). High signals on the T2-weighted images were classified into four grades of joint effusion, where nothing was classified as '−' or 'none', a distinct line was classified as '+' or 'mild', a thick line was classified as '++' or 'moderate', and a band was classified as '+++' or 'severe' (16). Two authors (JS and NS) independently graded each images unaware of the patient's clinical condition. In the internal derangement group, seven joints (32%) showed no sings of joint effusion, whereas 15 joints (68%) did (+, five joints; ++, seven joints; +++, three joints). In contrast, none of the four joints in the control group showed joint effusion.

Diagnostic arthroscopy and synovial biopsy

Conventional diagnostic arthroscopy in the whole area of the upper joint compartment was performed by a standard technique (17). The degree of synovitis was estimated using the scores described by Murakami et al. (18) (Table 2). The mean (SD) synovitis scores were 5.4 (2.0) in the internal derangement group and 0.8 (1.0) in the control group ($n = 4$).

Two or three synovial tissue specimens about 2 mm in diameter were obtained arthroscopically from the region of the posterior disk attachment using the triangular technique (19). Biopsy specimens were obtained from 22 joints in the internal derangement group and three joints

Table 2 Scoring with intensity of synovitis

Score	*Findings*
0	Normal pale, almost translucent, synovial lining with a fine network of anastomozing small blood vessels
1	Increased vascularity and capillary hyperemia (mild)
2	Increased vascularity and capillary hyperemia (moderate)
3	Increased vascularity and capillary hyperemia (severe)
4	Capillary dilatation and increasing network (mild to moderate)
5	Capillary dilatation and increasing network (severe)
6	Contact bleeding occurs on probe palpation (mild to moderate)
7	Contact bleeding occurs on probe palpation (severe)
8	Microbleeding and effusion
9	Granulative change, effusion and debris (mild to moderate)
10	Granulative change, effusion and debris (severe)

in the control group with dislocation joints. Diagnostic arthroscopy and synovial biopsies were carried out by two of the authors (NS and JS).

Immunohistochemical staining

Tissue specimens were fixed in 4% paraformaldehyde for 8 h and embedded in paraffin. Immunohistochemical staining was performed using an avidin-biotin technique (Vector Laboratories, Burlingame, CA, USA) (20). Details of the immunohistochemical technique and the evaluation of immunopositive cells have been described (3, 8). The primary antibody, mouse anti-VEGF monoclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA; sc-7269), at a concentration of 1 μg/ml was applied for 1 h at room temperature (8, 15). Color was developed with 3-amino-9-ethyl carbazole, followed by counter-staining with hematoxylin. For each section, the number of immunopositive cells was counted in two regions, each containing 200–500 cells, by two authors (JS and NS) blinded as to the origin of the samples.

Moreover, another primary antibody, rabbit anti-Flt-1 polyclonal antibody (dilution rate 1:200; Santa Cruz Biotechnology; sc-9029) were applied for overnight at 4°C in order to investigate the expression and localization of VEGF receptor-1 (Flt-1) (3).

Microvessel density of the tissues was determined by staining endothelial cells with a monoclonal antibody to CD34 (1:50, Nichirei, Tokyo, Japan; NU-4A1), according to Weidner et al.'s method with minor modifications (21, 22). Microvessel density was evaluated as the total number of blood vessels in two areas of maximal vascularization under a light microscope (×20 objective and ×10 ocular, 0.74 mm^2 per field).

Statistical analysis

The Mann–Whitney test was used to check for intergroup differences in VEGF concentration in synovial fluids and serum. The Mann–Whitney test was also used to compare the levels of total protein in the synovial fluid between the internal derangement and control groups. Spearman's correlation coefficient was used to assess the correlation between the concentration of VEGF in synovial fluids and the clinical symptoms, MRI findings, arthroscopic findings, and immunohistochemical results. STAT VIEW J-5.0 statistical software (Abacus Concepts, Berkeley, CA, USA) was used. *P*-values of < 0.05 were considered statistically significant.

Results

VEGF concentrations

We detected VEGF in synovial fluids from 14 of the 22 joints (64%) in the internal derangement group (Table 1). The mean ± SD (median) concentration of VEGF in the 22 joints was 66.6 ± 82.5 (24.3) pg/ml and ranged from 0 to 298.5 pg/ml. In the control group, VEGF was detected in only one of 10 joint (12.5 pg/ml). Thus, the concentration of VEGF in the synovial fluid was significantly higher in the internal derangement group than in the control group ($P = 0.004$) (Fig. 1).

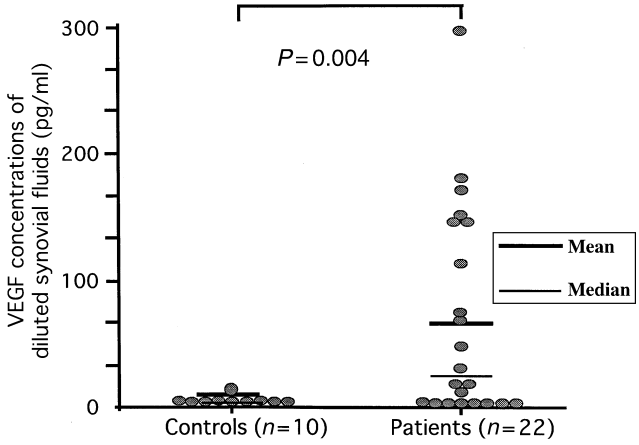

Figure 1 Vascular endothelial growth factor (VEGF) concentrations in the diluted synovial fluids of patients with internal derangement and controls. We observed significant higher VEGF concentration in the internal derangement group than in the control group ($P = 0.004$ by Mann–Whitney test).

The VEGF was detected in all of the 12 serum samples of the internal derangement group and five samples of the control group. The mean ± SD (median) serum VEGF concentration in the internal derangement group was 244.0 ± 64.6 (245.8) pg/ml and ranged from 83 to 321.5 pg/ml. In the control group, the mean ± SD (median) serum VEGF concentration was 250.3 ± 102.5 (234) pg/ml and ranged from 135.5 to 395 pg/ml. There was no significant difference between the two groups ($P = 0.92$) (Fig. 2).

Total protein concentrations

Bio-Rad protein analysis detected total protein levels in all of synovial fluids from both the internal derangement and the control subjects. The results are shown in Table 1. The concentration of total protein in the synovial fluid was significantly higher in the internal derangement group than in the control group ($P = 0.035$).

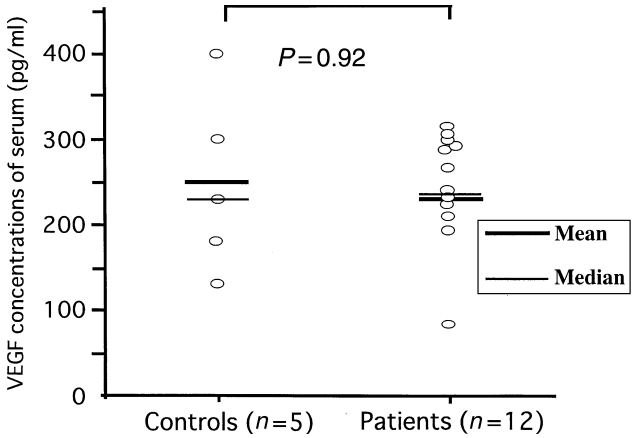

Figure 2 Vascular endothelial growth factor (VEGF) concentration in sera of patients with internal derangement and controls. There was no significant difference between the two groups ($P = 0.92$ by Mann–Whitney test).

Immunohistochemical findings

Of the 22 synovial tissue specimens from the internal derangement group, 21 (95%) were reactive with antibody to VEGF (Table 1). Staining was observed in the cytoplasm of the cells lining the synovium, in the cytoplasm of the fibroblasts beneath, and in the cytoplasm of the endothelial cells of the blood vessels (Fig. 3a). All the negative control sections showed only background staining (data not shown). The mean (SD) percentage of immunopositive cells was 44 (29)% and ranged from 0 to 97%. Specimens from two of the three control subjects also showed staining, with 11 and 18% of the cells in these two samples being immunopositive (Fig. 3c).

Specimens from 18 of the 22 (82%) internal derangement subjects and from one of the three control subjects showed staining for antibody to Flt-1. Flt-1 was present in the endothelial cells of the blood vessels under the synovial lining and in some lining cells (Fig. 3b, d).

Staining with antibody to CD34 was positive for endothelial cells in all tissue samples from the internal derangement and control group (data not shown). The average ± SD microvessel density was 33 ± 18 in samples from the internal derangement group and 10 ± 4 in the control tissues samples.

Correlations with clinical findings

We observed significant correlations in the internal derange group between the concentrations of VEGF and total protein in synovial fluids ($P = 0.002$, $r = 0.52$) (Fig. 4), and between VEGF concentration in synovial fluid and the percentage of VEGF immunopositive cells ($P = 0.02$, $r = 0.48$) (Fig. 5). In this group, however, there were no significant correlations between the concentrations of VEGF in the synovial fluids and patient age ($P = 0.43$), VAS of pain ($P = 0.40$), maximum interincisal opening ($P = 0.96$), duration of symptoms ($P = 0.40$), and degree of synovitis ($P = 0.39$). There were also no significant correlations between the concentration of VEGF in synovial fluid and joint effusion determined by MRI ($P = 0.08$) and the microvessel density assessed by CD34 staining ($P = 0.34$).

In the internal derangement group, there were no significant correlations between serum VEGF concentrations and patient age ($P = 0.54$), VAS of pain ($P = 0.71$), maximum interincisal opening ($P = 0.44$), duration of symptoms ($P = 0.84$), degree of synovitis ($P = 0.18$), total protein in synovial fluid ($P = 0.96$), percentage of cells immunopositive for VEGF ($P = 0.99$), and microvessel density ($P = 0.13$).

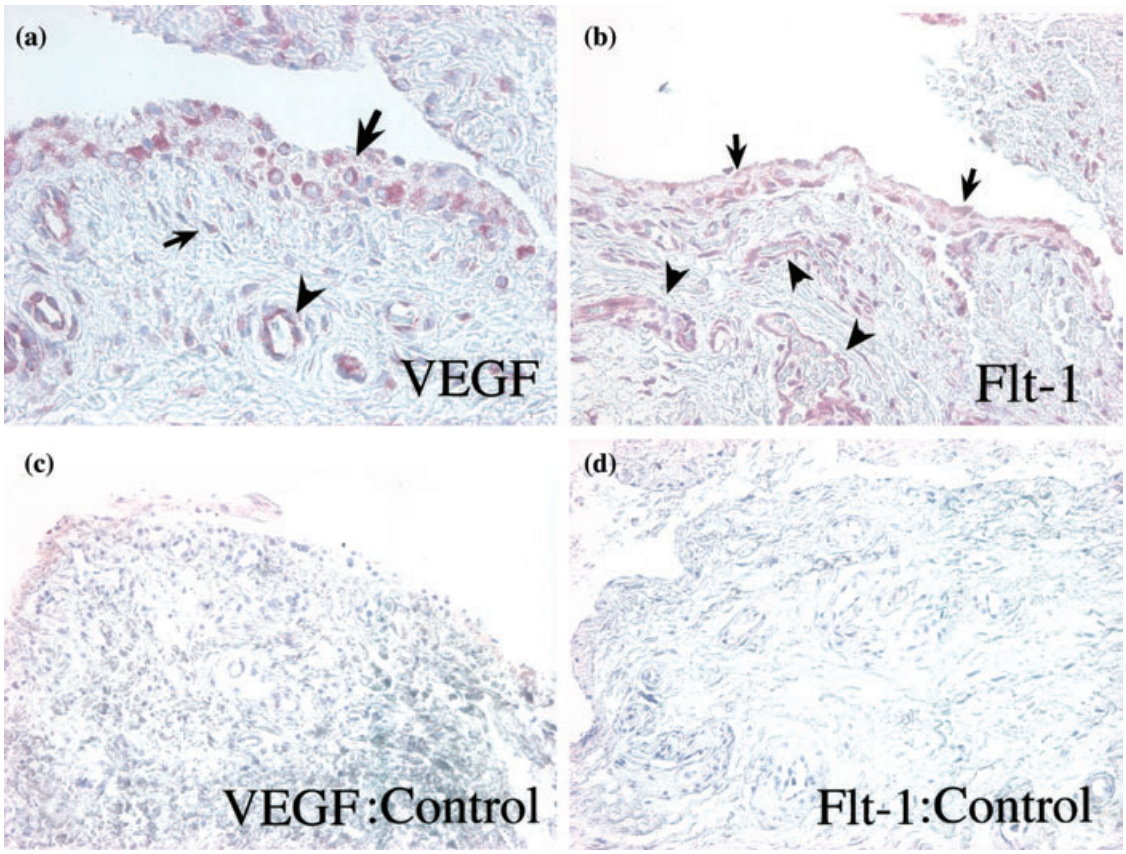

Figure 3 (a) Vascular endothelial growth factor (VEGF)-stained sections obtained from patient number 17. Immunopositivity was observed in cell lining the synovium (big arrows), in endothelial cells of the blood vessels (arrow heads), and in fibroblasts (small arrows) (×150). (b) VEGF receptor-1 (Flt-1)-stained sections obtained from the same patient (number 17). Immunoreactivities are observed in the endothelial cells of the blood vessels (arrow head) and cell lining the synovium (arrow) (×150). (c) VEGF-stained section from control patient number 23 with habitual dislocation. No immunoreactivity for VEGF was observed in the synovial tissue (×120). (d) Flt-1-stained section from control patient number 23 with habitual dislocation. No immunoreactivity for Flt-1 was observed in the synovial tissue (×120).

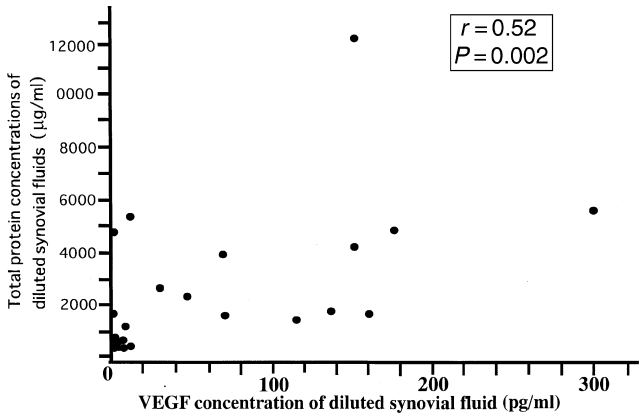

Figure 4 Correlation between the concentrations of vascular endothelial growth factor (VEGF) and total protein in diluted synovial fluids. Spearman's correlation coefficient showed a significant correlation ($P = 0.002$, $r = 0.52$).

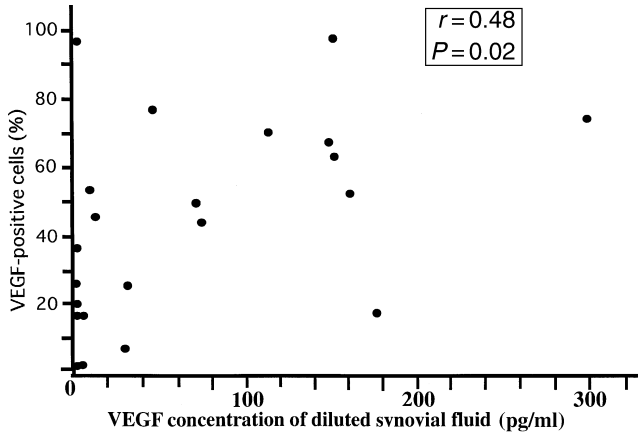

Figure 5 Correlation between vascular endothelial growth factor (VEGF) concentration in diluted synovial fluid and percentage of VEGF-positive cells in synovial tissues. Spearman's correlation coefficient showed a significant correlation ($P = 0.02$, $r = 0.48$).

Discussion

In this study, we detected VEGF in synovial fluids of 14 of 22 (64%) joints from patients with symptomatic internal derangement of the TMJ. Moreover, we demonstrated that the concentration of VEGF in synovial fluid was significantly higher in the internal derangement group than in the control group. Using immunohistochemical assays, we previously showed that the expression of VEGF in synovial tissues from patients with symptomatic internal derangement was up-regulated (3, 8), and was correlated significantly with the degree of synovitis seen by arthroscopy, the extent of joint effusion seen on MRI, and microvessel density (3, 8, 15). These findings suggest that the expression of VEGF in synovial tissues is involved in the pathogenesis of symptomatic internal derangement of the TMJ. VEGF is well-known to be a multifunctional cytokine that acts as an endothelial cell mitogen and induces microvascular permeability (23–26). Synovial fluids from the joints of patients with RA have extremely high levels of VEGF (27), and serum VEGF concentration has been regarded as a useful indicator of the severity of arthritis and the effectiveness of treatments in these patients (10, 27). In large joint such as knee with RA, binding of VEGF is thought to render the endothelial cells of small blood vessels hyperpermeable to plasma protein. This cause leakage into the joint cavity producing a protein-rich effusion (9). The findings in RA suggested that expression of VEGF in synovial fluids and serum may also be related to clinical parameters of symptomatic internal derangement of the TMJ.

We found a significant correlation between VEGF and total protein concentrations in synovial fluid. Inflammatory changes of synovial tissues, including capillary hyperemia and synovial hyperplasia, are thought to lead to an increase in permeability, resulting in the exudation of various proteins into the joint space (28). The ability of VEGF to increase vascular permeability is greater than that of histamine or bradykinin (29, 30). Yeo et al. (29) reported that VEGF renders the microvasculature hyperpermeable to circulating macromolecules with a potency of some 50 000 times greater than histamine. These findings, together with those reported here, suggest that VEGF in synovial fluid acts on the endothelial cells in the synovial tissues to increase their permeability, resulting in the production of protein-rich synovial fluid.

An unexpected result of this study was our finding of a lack of correlation between VEGF concentration in synovial fluid and microvessel density in the synovial tissues. VEGF is a direct angiogenic agent, which promotes angiogenesis during physiologic and pathologic conditions (31). We previously detected a significant correlation between VEGF expression in synovial tissue and microvessel density (3). This discrepancy may be due to our method of sampling synovial fluid and/or the small number of samples. Although it is almost impossible to aspirate enough synovial fluid directly from the TMJ (32), the ratio between the true synovial fluid concentration and the saline aspirate concentration has been reported to differ among samples obtained by the dilution method (33), which we used here. Although we excluded all samples in which over 10% of the volume of saline was lost in order to decrease sample bias, other sample bias may have been introduced. Another unexpected result was the absence of a significant correlation between the concentration of VEGF in the synovial fluid and the degree of synovitis evaluated by arthroscopy. In previous studies, elevated total protein levels in the synovial fluid correlated with synovitis (13, 28). In the present study, the concentration of total protein in the synovial fluid was significantly higher in the internal derangement group than in the control group. Moreover, a significant correlation was observed between the concentrations of VEGF and total protein in synovial fluids. One possible explanation for this unexpected result is that VEGF may not be a major component of the total protein (13) and other proinflammatory cytokines such as interleukin (IL)-6, IL-8, or IL-1 may contribute directly to synovitis (13).

Maeno et al. (27) reported that VEGF concentrations in synovial fluids from patients with RA were higher than those in serum. Moreover, they demonstrated that VEGF concentrations in synovial fluid seemed to correlate with those in serum. They reported that synovial fluid/serum ratio of VEGF concentration was 2:1–4:1, but we did not observe significant correlation between the two or between VEGF concentration in serum and clinical parameters. Moreover, there was no significant difference in serum VEGF concentrations between the internal derangement and control groups, suggesting that serum VEGF concentration is not useful indicator of the severity of internal derangement of the TMJ. This result is consistent with findings that internal derangement of the TMJ is a local disease rather than a systemic disease. An additional problem with this study may arise from the degree of dilution of synovial fluid obtained by our pumping procedure. Aghabeigi et al. (28, 34) reported that the mean synovial fluid volume of the superior joint compartment in the normal human TMJ was 37 μl (range: 0.9–185). We injected 2.0 ml normal saline into the superior joint compartment and aspirated at least 1.8 ml of diluted solution. The original synovial fluid would therefore be diluted by a factor of 10–2000. The accurate mean VEGF concentration in undiluted synovial fluid may thus be >670 pg/ml, which is higher than the 220 pg/ml observed in serum. These calculations suggest that the most of the VEGF present in synovial fluid is derived from local tissues such as the synovial membrane. In both our previous study (3, 8) and in this report, we have shown that VEGF is expressed in cells lining the synovium, as well as in endothelial cells, and fibroblasts in synovial tissues. During internal derangement, synovial tissues may release VEGF into joint cavity. Our finding, of a significant correlation between VEGF concentrations in synovial fluid and the percentage of cells immunopositive for VEGF in synovial tissues, indicates that synovial tissue is the source of VEGF in synovial fluid.

The major limitations of the present study are the small number of samples and the disparity among the control patients. These problems are difficult to overcome, as it is very difficult to obtain fresh samples of synovial tissues and synovial fluids from healthy individuals (12). We thought, however, that these patients were suitable controls because the patients with dislocation were basically non-symptomatic except for an occasional difficulty in closing their mouths and their MRI revealed a normal disk position. None of the control subjects felt pain. There was no significant difference of age of the patients between the internal derangement and the control groups ($P = 0.35$ by Mann–Whitney test). The conditions of the joints of the control subjects are obviously different from those of the joints with symptomatic internal derangement, which are painful and have limited movement of the condyle.

Although a certain concentration of VEGF may be necessary to maintain the homeostasis of the blood vessels under normal conditions, the up-regulation of VEGF in synovial fluid of the symptomatic internal derangement patients may contribute to the pathology of this condition. The biologic activities of VEGF are mediated by binding to specific cell surface tyrosine kinase receptors, such as Flt-1 (VEGF receptor-1) and KDR (VEGF receptor-2) (35, 36). Pufe et al. (37) demonstrated that the Flt-1-positive endothelial cells are closely adjacent to the VEGF-positive cells in the synovial tissues from patients with RA. In both our previous study (3) and in this report, we have shown that Flt-1 is expressed in the endothelial cells beneath the lining cells in the synovial tissues. Thus, VEGF in the synovial fluids may bind to its cell surface receptors on endothelial cells of synovial tissues, thus increasing the permeability of small blood vessels and, worsening pathologic conditions in symptomatic internal derangement of the TMJ.

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