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RESEARCH ARTICLE

Plasma and synovial fluid connective tissue growth factor levels are correlated with disease severity in patients with knee osteoarthritis

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Abstract

Background: Connective tissue growth factor (CTGF) has been implicated in development of osteoarthritis (OA).

Objective: To determine the correlation between plasma and synovial fluid CTGF levels and the severity in knee osteoarthritis patients.

Methods: A total of 100 subjects were recruited into this study (75 OA patients and 25 controls). CTGF concentrations in plasma and synovial fluid were analyzed by enzyme-linked immunosorbent assay.

Results: Plasma and synovial fluid CTGF concentrations were correlated with radiographic severity. There was a positive correlation between plasma and synovial fluid CTGF levels.

Conclusion: CTGF could be useful for monitoring the severity and progression of OA.

Keywords: Connective tissue growth factor, knee osteoarthritis, plasma, severity, synovial fluid

Introduction

Osteoarthritis (OA) is the most common degenerative joint disorder that ultimately results in the progressive destruction of articular cartilage with joint-space narrowing, bony enlargement at joint margins, subchondral sclerosis, and synovitis (Kraus, 1997). The clinical features of OA include chronic pain, joint stiffness, limited range of motion, swelling, crepitation, and disability. Currently, a conventional modality to examine the affected joint is radiological investigation which reflects disease severity by grading the joint degeneration. Although radiographs are commonly utilized to assess the OA severity, it would be advantageous to have a biochemical marker that could accurately reflect disease severity and progression of OA. Several environmental, mechanical, and biochemical factors have been recognized as playing a crucial role in OA development; however, the etiology and pathophysiology of OA remain poorly understood.

Connective tissue growth factor (CTGF or CCN2) is a 38 kDa heparin-binding glycoprotein belonging to the connective tissue growth factor/cysteine-rich 61/nephroblastoma overexpressed (CCN) family, which is a group of secreted multifunctional polypeptides that contain high levels of cysteine (Takigawa et al., 2003). It has

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diversely been known as FISP12, Hcs24, ecogenin, IGM2, IGFBP8, IGFBP-rP2, and CCN2 (Kubota and Takigawa, 2007). Importantly, CTGF participates in crucial process such as differentiation, development, wound healing, angiogenesis, and chondrogenesis (Cicha and Goppelt-Struebe, 2009; Ivkovic et al., 2003). In recent years, the expression of CTGF in osteoarthritic articular cartilage has been explored and implicated in development of OA (Kumar et al., 2001). Previous studies have also demonstrated upregulated CTGF expression in chondrocytes in human OA cartilage at protein and mRNA levels, which suggests that human chondrocytes can synthesize CTGF (Omoto et al., 2004). These findings prompted us to speculate that CTGF may be responsible for the pathogenesis of OA.

Although plasma and/or synovial fluid levels of several cytokines and growth factors have been studied in patients with knee OA, to our knowledge, there have been no published data regarding the association of circulating and synovial fluid levels of CTGF in various clinical stages of primary knee OA (Saetan et al., 2011; Honsawek et al., 2009; Huang et al., 2011; Anitua et al., 2009; Ku et al., 2009). Hence, we have hypothesized that CTGF in plasma and synovial fluid could be correlated with the severity of clinical outcomes in knee OA patients. To prove this hypothesis, we have analyzed the plasma and synovial fluid levels of CTGF in knee OA patients and healthy controls. The objective of the present study was to evaluate both plasma and synovial fluid levels of CTGF in patients with primary knee OA, and determine the possible relationships between plasma and synovial fluid CTGF with the radiographic severity of knee osteoarthritis.

Materials and methods

Study subjects

This study was approved by the Institutional Review Board on Human Research of the Faculty of Medicine, Chulalongkorn University and was conducted in compliance with the guidelines of the Declaration of Helsinki. Written informed consent was obtained from the patients and healthy volunteers prior to their participation in this study.

Seventy-five patients aged 53 to 83 years with primary knee osteoarthritis (60 females and 15 males; mean age 68.9 ± 8.1 years) based on the criteria of the American College of Rheumatology were enrolled in the study. Body mass index (BMI) was calculated as weight in kilograms divided by height squared in meters (kg/m^2). The OA severity was determined using weight-bearing anteroposterior radiographs of the affected knee. Knee radiographs were taken when each participant was standing on both legs with fully extended knee and the X-ray beam was centered at the level of the joint. Assessment of radiographic severity was performed by two graders who were blinded to the results according to the Kellgren and Lawrence (KL) grading system (Kellgren and Lawrence, 1957). OA patients were defined as having radiographic knee OA of KL grade ≥ 2 in at least 1 knee. Controls were defined as having neither radiographic hip OA nor knee OA, as indicated by KL grades of 0 for both hips and both knees. The grading scale used for analysis was the one found higher upon comparison between both knees. We also recruited 25 gender and age matched subjects (20 females and 5 males; mean age 67.8 ± 7.5 years) with normal knee radiographs as controls. None of the participants had underlying diseases such as diabetes, histories of corticosteroid medication, other forms of arthritis, cancer, or other chronic inflammatory diseases.

Laboratory analysis

Synovial fluid was aspirated from the affected knee using sterile knee puncture, centrifuged to remove cells and joint debris, and stored at –80°C until the day of measurement. No synovial fluid was extracted from the controls due to ethical concerns. Venous blood samples collected from all participants were centrifuged and stored at –80°C until utilized. Double-blind quantitative measurement of plasma and synovial fluid CTGF was performed with Human CTGF ELISA Development Kit (Peprotech, Rocky Hill, NJ, USA), a quantitative sandwich enzyme immunoassay using a purified rabbit antibody against human CTGF precoated onto an enzyme-linked immunosorbent assay (ELISA) plate. Following four washes in phosphate buffer saline (PBS) containing 0.05% Tween-20 (Sigma), the plate was blocked with 300 μL/well of 1% bovine serum albumin in PBS, for one hour at room temperature. Plasma and synovial fluid samples were applied to the plate following blocking, alongside a standard curve, from 4,000 pg/mL down in doubling dilutions, constructed from a stock recombinant human CTGF. Samples and standards were incubated (100 μL/well) at room temperature for 2 h following which the plate was washed a further three times with wash buffer. Detection of bound CTGF was performed using 100 μL/well of biotinylated detection antibody at a concentration of 0.5 μg/mL for 2 h at room temperature. After further four washes the plate was incubated with a 1:2,000 dilution of avidin-horseradish peroxidase conjugate for 30 min at room temperature. Finally the plate was washed four times and 100 μL of 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) liquid substrate was added to the wells and color developed in proportion to the amounts of bound CTGF. Absorbance was detected by spectrophotometry, at 405 nm, with wavelength correction set at 650 nm. The CTGF concentration was examined by a standard optical density-concentration curve (range 31.5–4000 pg/mL). The intra- and interassay coefficient of variations (CVs) of this ELISA test were 6 and 20%, respectively. The detection limit was 4.0 pg/mL. For the technical validity of measurements, plasma and synovial fluid samples were selected and analyzed for human CTGF at serial two fold dilutions. The recovery ranged between 88% and

102%. The recovery of CTGF spiked to levels throughout the range of the assay was 90–109% (Dendooven et al. 2011).

Statistical analysis

Statistical analysis was carried out using the statistical package for social sciences (SPSS) software, version 16.0 for Windows. Tests of normality and test of homogeneity of variances were employed to analyze the subject's age, BMI, CTGF concentration in the plasma and synovial fluid. When the populations from which the samples were normally or approximate normal distribution and the variances of the populations were equal, Student's *t*-test was performed to compare the means of two independent groups and one way analysis of variance (ANOVA) was employed to compare the means of more than two independent groups. Comparisons between groups were made using Mann-Whitney *U* test (for two groups) or Kruskal-Wallis test (for more than two groups) when the variances were not equal among the groups. Correlations between plasma and synovial fluid CTGF with disease severity, age, or BMI were calculated using Pearson's correlation coefficient (r). Data were expressed as mean ± standard deviation (SD). *P*-values < 0.05 were considered statistically significant for differences and correlations.

Results

Baseline clinical characteristics

The baseline clinical characteristics of the subjects are shown in Table 1. There was no significant difference in age, gender, and BMI between OA patients and controls. As demonstrated in Figure 1, OA patients had higher plasma CTGF concentrations compared to controls (483.1 ± 462.8 vs. 180.2 ± 149.5 pg/mL, $P < 0.05$). CTGF levels in synovial fluid were substantially higher than in paired plasma samples ($P < 0.001$).

Table 1. Baseline clinical characteristics of knee OA patients and controls. Data presented as mean ± SD.

	Knee OA patients	Controls	*P*
N	75	25	
Age (years)	68.9 ± 8.1	67.8 ± 7.5	0.5
Gender (F/M)	60/15	20/5	0.5
BMI (kg/m^2)	25.9 ± 3.4	25.1 ± 2.5	0.4

BMI, Body mass index; F/M, female/male.

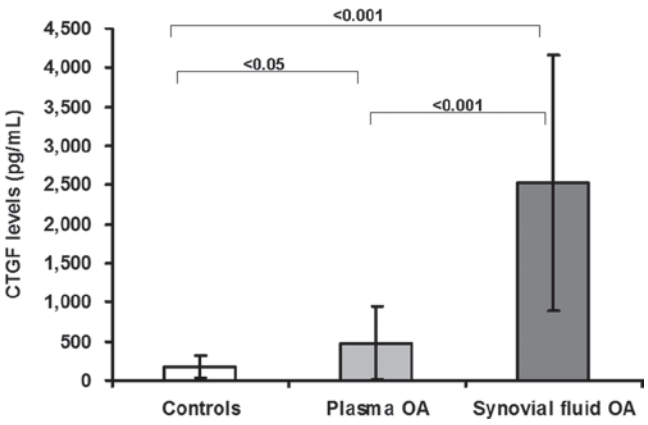

Figure 1. CTGF levels in plasma and synovial fluid of OA patients and healthy controls.

CTGF levels in knee OA patients with different KL subgroups

The CTGF levels of plasma and synovial fluid in OA patients with different KL subgroups are present in Table 2. Knee OA patients with higher radiographic severity had significantly more pronounced CTGF levels in both plasma and synovial fluid ($P < 0.001$). Plasma CTGF levels in KL grade 4 were significantly elevated compared with those of KL grade 2 and 3 (Table 2). Although plasma CTGF levels in KL grade 3 were higher than those in KL grade 2, the difference was not significant ($P = 0.3$). Moreover, knee OA patients with KL grade 4 had significantly greater synovial fluid CTGF levels than those with KL grade 2 and 3 (Table 2). Synovial fluid CTGF levels in OA patients with grade 3 were higher than those in KL grade 2.

Correlation between plasma and synovial fluid CTGF and disease severity

There was no correlation of plasma and synovial fluid CTGF levels with age, BMI and gender distribution. Subsequent analysis revealed that plasma CTGF levels were positively correlated with the knee OA severity ($r = 0.708$, $P < 0.001$) (Figure 2). Synovial fluid levels of CTGF were strongly associated with the radiographic severity of knee OA ($r = 0.885$, $P < 0.001$) (Figure 3). Additionally, plasma CTGF levels showed a positive correlation with synovial fluid CTGF levels ($r = 0.759$, $P < 0.001$) (Figure 4).

Discussion

Proinflammatory cytokine mediators and growth factors have been known to play a potential role in the pathophysiological process of osteoarthritis. CTGF is a member of a family of growth factors termed the CCN [cysteine-rich 61 (Cyr61), connective tissue growth factor (CTGF), nephroblastoma overexpressed (Nov)] gene family that is characterized by a high degree of amino acid sequence homology ranging from 50% to 90% (Takigawa et al., 2003). This family consists of six distinct members: CYR61 (CCN1), CTGF (CCN2), NOV (CCN3), WISP-1 (wnt-1-inducible gene, CCN4), WISP-2 (CCN5) and WISP-3 (CCN6) (Perbal, 2004). Each member comprises four distinct structural modules as follows: insulin-like growth factor-binding protein module, von Willebrand factor type C repeat module, thrombospondin type 1 repeat module, and carboxy-terminal cysteine-knot module (Bork, 1993). These distinct modules are believed to interact with other regulatory molecules to accomplish the pleuritropic functionality of CCN proteins. In fact, CTGF exerts diverse cellular functions including proliferation and differentiation of articular chondrocytes

Table 2. Plasma and synovial fluid CTGF in osteoarthritis patients. *P*-values for differences among Kellgren and Lawrence subgroups. Data presented as mean ± SD.

	Total	KL Grade 2	KL Grade 3	KL Grade 4	*P*
N	75	22	25	28	
Age (years)	68.9 ± 8.1	68.9 ± 6.8	68.5 ± 8.2	69.2 ± 7.8	0.5
Gender (F/M)	60/15	18/5	20/5	22/5	0.5
BMI (kg/m^2)	25.9 ± 3.4	25.4 ± 2.8	25.9 ± 3.2	26.2 ± 4.0	0.4
SF CTGF (pg/mL)	2525.0 ± 1636.9	711.7 ± 402.8	2186.4 ± 647.0	4251.9 ± 1004.1	<0.001
Plasma CTGF (pg/mL)	483.1 ± 462.8	143.3 ± 137.6	282.4 ± 193.6	929.3 ± 446.6	<0.001

CTGF, connective tissue growth factor; KL, Kellgren and Lawrence; SF, synovial fluid; BMI, Body mass index; F/M, female/male.

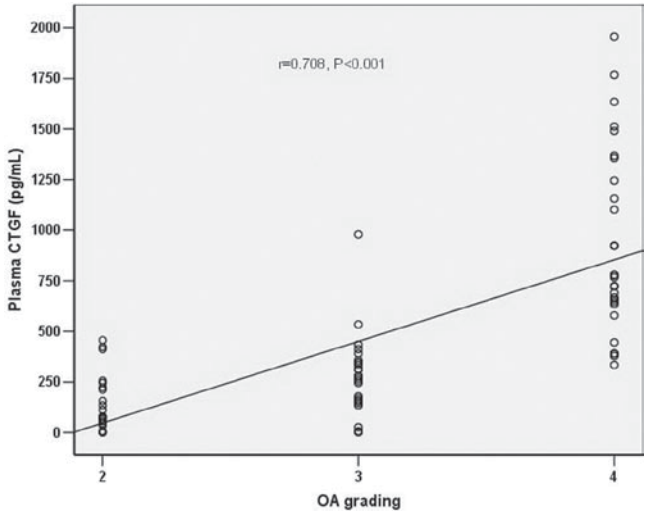

Figure 2. Scattergram showing the positive correlation between plasma CTGF levels in OA patients and severity classified according to Kellgren and Lawrence grading scale ($r = 0.708$, $P < 0.001$).

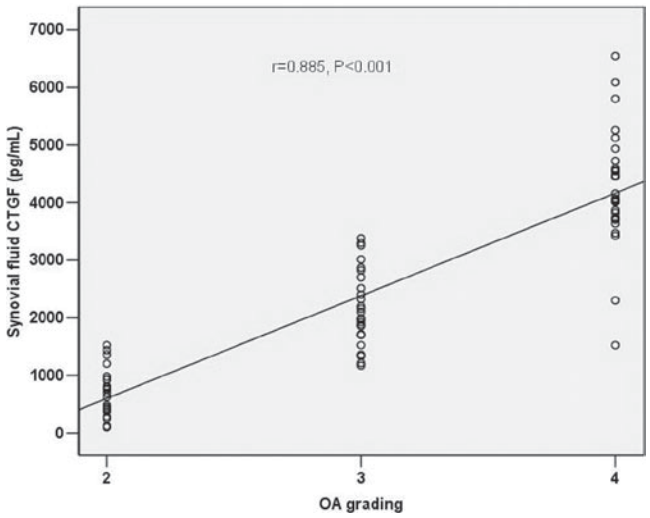

Figure 3. Scattergram showing the positive correlation between synovial fluid CTGF levels in OA patients and severity classified according to Kellgren and Lawrence grading scale ($r = 0.885$, $P < 0.001$).

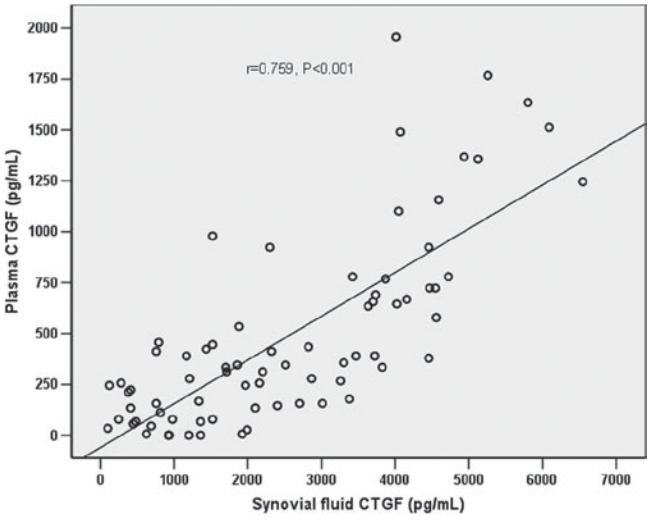

Figure 4. Scattergram showing the positive correlation between plasma and synovial fluid CTGF concentrations in OA patients ($r = 0.759$, $P < 0.001$).

(Fujisawa et al., 2008; Kanaan et al., 2006; Nishida et al., 2002). Recently, CTGF was shown to regenerate the damaged articular cartilage in rat OA model (Nakao et al., 2005; Nishida et al., 2004).

Previous investigation has demonstrated that CTGF was the most intensely expressed growth factor present in chondrocytes of human patients with severe osteoarthritis (Zhang et al., 2002). However, plasma and synovial fluid CTGF levels of OA patients have not been explored and their correlations with disease severity have never been specifically evaluated in knee OA patients. The present study has been the first to show that CTGF was detected in both plasma and synovial fluid obtained from patients with primary knee OA, and that CTGF was positively correlated to radiographic grading of knee OA patients.

This study illustrated a significant elevation of CTGF concentrations in both plasma and synovial fluid of knee OA patients compared to the control plasma concentrations. In addition, CTGF levels were more pronounced in end-stage OA patients compared with early OA patients. Our results indicate that there is increased local and systemic production of CTGF in knee OA. It should be noted that CTGF levels in synovial fluid were considerably higher than those observed in paired plasma samples. Remarkably high CTGF levels in synovial fluid are presumably attributable to either the secretion of CTGF residing in extracellular matrix, or the increased synthesis of CTGF, or both. The source of CTGF in the synovial fluid could be originated from synovial cells and chondrocytes in the local tissues (inflamed synovium, articular cartilage, and subchondral bone) and extra-articular tissues.

Previous studies have shown that CTGF was expressed in synovial lining cells (Blaney et al., 2006), fibroblast-like synovial cells (Lee et al., 2010) and articular cartilage chondrocytes in osteoarthritis (Omoto et al., 2004; Masuko et al., 2010). Further researches will be required to verify whether the concentrations of CTGF in synovial fluid and plasma are related to the local expression of CTGF in joint tissues.

Recent studies have documented lactate dehydrogenase (LDH) and albumin levels in serum and synovial fluid of osteoarthritic patients (Hurter et al., 2005; Krachler and Domej, 2001). LDH is an enzyme that catalyses the conversion of pyruvate to lactate and was found to be elevated in the synovial fluid of osteoarthritic joints. The presence of LDH was demonstrated in chondrocytes and in the interterritorial matrix of degenerative stifle joints, suggesting that LDH in synovial fluid of degenerative joints originates from cartilage (Walter et al., 2007). Additional studies on the measurement of LDH or albumin in both plasma and synovial fluid and their comparison might be useful to argue the origin of synovial CTGF.

The potential limitations of the present study should be mentioned. First, this investigation was administered as a single-center trial with relatively small sample size. Prospective study conducted on a random sample of multiple centers with larger sample sizes is necessary to validate our data. Second, only CTGF level has been measured in both plasma and synovial fluid. Further immunohistochemical studies of CTGF expression in local tissue could render more valuable information on the pathogenic role of CTGF in OA. Third, synovial fluid samples from healthy controls were not taken for ethical reasons. Finally, we did not determine functional impairment in these patients. Future investigation is needed to assess whether CTGF correlates with functional impairment (WOMAC or Lequesne score). As this study has been designed as a cross-sectional study; therefore, absolute cause and effect relationships may not be possible. However, more studies are warranted to demonstrate disease progression and clarify the precise role of CTGF in knee osteoarthritis.

In conclusion, plasma CTGF in patients with OA was significantly elevated compared with that of healthy controls. CTGF concentrations in synovial fluid were remarkably higher with regard to paired plasma CTGF. Both plasma and synovial fluid CTGF levels were shown to be positively correlated with the degree of radiographic severity in patients with primary knee OA. These findings suggest that CTGF could be a useful biochemical marker to reflect the disease severity and progression of knee OA.

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Declaration of interest

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