



Association between hypoxia-inducible factor-1a levels in serum and synovial fluid with the radiographic severity of knee osteoarthritis

H. Chu, Z.M. Xu, H. Yu, K.J. Zhu and H. Huang

Department of Orthopaedics,
the 100th Hospital of Chinese People's Liberation Army, Suzhou,
Jiangsu Province, China

Corresponding author: H. Huang
E-mail: szhuanghong@126.com

Genet. Mol. Res. 13 (4): 10529-10536 (2014)

Received November 21, 2013

Accepted March 26, 2014

Published December 12, 2014

DOI <http://dx.doi.org/10.4238/2014.December.12.15>

ABSTRACT. Osteoarthritis (OA) is primarily characterized by articular cartilage degradation. Hypoxia-inducible factor-1a (HIF-1a), a subunit of the basic helix-loop-helix-containing PER-ARNT-SIM (PAS) domain transcription factors, plays a vital role in the survival of articular chondrocytes to the hostile hypoxic microenvironment and complicates the progression of OA. In this study, we examined whether HIF-1a levels in the serum and synovial fluid (SF) of patients with knee OA were increased and whether the increase was correlated with the radiographic severity of the disease. A total of 278 knee OA patients and 203 healthy controls were enrolled in this study. Knee OA radiographic grading was performed according to Kellgren-Lawrence (KL) grading system by evaluating X-ray changes observed on anteroposterior knee radiography. HIF-1a levels in the serum and SF were determined using an enzyme-linked immunosorbent assay. Serum HIF-1a levels in patients with knee OA were higher than those in healthy controls. Knee

OA patients with KL grade 4 showed significantly elevated HIF-1 α levels in the serum and SF compared with those with KL grades 2 and 3. Knee OA patients with KL grade 3 showed significantly higher SF levels of HIF-1 α than those with KL grade 2. HIF-1 α levels in the serum and SF of knee OA patients were significantly correlated with disease severity according to KL grading criteria. HIF-1 α levels in the serum and SF were closely related to the radiographic severity of OA and may serve as an alternative biomarker for the progression and prognosis of knee OA.

Key words: HIF-1 α ; Osteoarthritis; Radiographic severity; Synovial fluid

INTRODUCTION

Osteoarthritis (OA) is a common chronic, progressive, degenerative joint disorder that ultimately results in age-related regressive changes in articular cartilage, including joint-space narrowing, bony outgrowths at joint margins, subchondral sclerosis, and synovitis (Felson et al., 1995). OA is clinically characterized by severe pain, joint stiffness, reduced motion, swelling, and crepitation and is associated with a reduced quality of life and impaired mobility in the elderly population (Hill et al., 1999). Risk factors for OA include gender, age, obesity, smoking, genetics, joint injury, inflammation, and various mechanical and metabolic factors (Stannus et al., 2010). Among different areas in the body, the knee is the most common clinically significant site of primary OA involvement. Although a large number of basic and clinical studies examining OA have been carried out and it is now well-accepted that environmental, mechanical, and biochemical factors play vital roles in OA development, the exact cause of this disease still remains unclear (Felson, 2006). The most commonly used method for evaluating affected joints is radiological methods and the Kellgren-Lawrence (KL) grading system, which reflects disease severity by grading joint degeneration; however, the application of this method is limited due to its high cost and controversial standards. Thus, identifying biochemical parameters that could precisely reflect disease severity and OA progression would be advantageous (Ding et al., 2008).

Avascular articular cartilage obtains its oxygen and nutrients supply from the dynamic flow of synovial fluid (SF) during joint movement; however, the partial pressure of oxygen in the SF of normal joints is much below the general oxygen level. A previous study demonstrated that in the superficial zone of healthy cartilage, oxygen levels vary from 7-10%, while in the deep zone this value may be as low as 1% (Silver, 1975). In addition, Schneider et al. (1996) used *in vivo* measurement techniques to demonstrate that oxygen levels are further decreased in osteoarthritic joints. However, articular chondrocytes may adapt to and survive under this hostile microenvironmental condition by up-regulating a diverse set of genes, including erythropoietin, vascular endothelial growth factor, inducible nitric oxide synthase, and leptin, which are response for anaerobic energy metabolism and matrix synthesis (Strobel et al., 2010). This adaptive process is under the strict control of the transcription factor hypoxia-inducible factor-1 (HIF-1), which belongs to the family of the basic helix-loop-helix-containing PER-ARNT-SIM (PAS) domain transcription factors. HIF-1 is a heterodimer composed of 2 subunits, α and β (Gelse et al., 2008). The α -subunit is the primary and active component

of HIF-1, conferring the hypoxic adaptation responsiveness of HIF-1, whereas the β -subunit, also known as aryl hydrocarbon receptor nuclear translocator, is constitutively expressed. Under normoxic conditions, hydroxylation of 2 proline residues and acetylation of a lysine residue in the oxygen-dependent degradation domain of HIF-1 α promote tight interactions with the von Hippel-Lindau tumor suppressor and is degraded rapidly by the ubiquitin-proteasome system (Huang et al., 1998; Masson et al., 2001). Under hypoxic conditions, HIF-1 α is not degraded and translocates to the nucleus, functioning as a complex with the β -subunit to initiate the transcription of hypoxic-associated genes (Hofer et al., 2001).

Recently, 2 independent groups observed HIF-1 α mRNA expression in articular chondrocytes *in vivo* and indicated that HIF-1 α is of critical importance in cartilage development and homeostasis (Aigner et al., 2001; Stokes et al., 2002). Coimbra et al. (2004) detected HIF-1 α expression in human normal and OA cartilage chondrocytes under normoxic conditions. Hypoxic condition or catabolic stress was found to result in a significant increase in steady state levels of the HIF-1 α protein and is associated with the progression of articular cartilage degeneration, suggesting that HIF-1 α plays an important role in survival in the hypoxic microenvironment of cartilage (Yudoh et al., 2005; Pfander and Gelse, 2007). Another study demonstrated that the expression level of HIF-1 α , together with its target genes, glucose transporter-1 and phosphoglycerate kinase-1 in OA cartilage was higher than that in normal cartilage and increased with the severity of OA, indicating that chondrocytes depend on the adaptive functions of HIF-1 α to maintain anaerobic energy metabolism and matrix synthesis during OA (Pfander et al., 2005). Therefore, HIF-1 α may be important in the pathogenesis of OA.

There is a large amount of clinical data regarding the association between circulating and SF levels of cytokines and growth factors, including many target genes of HIF-1 α , during various stages of primary knee OA. Previous reports found increased leptin levels in cartilage specimens and SF from OA joints and that the SF leptin level is significantly increased and related to the radiographic severity of OA, which can be used as a quantitative marker of OA (Dumond et al., 2003; Ku et al., 2009). However, studies examining the association between serum and SF HIF-1 α levels and severity of OA have not been conducted. Accordingly, we hypothesized that the levels of HIF-1 α in the serum and SF are also increased. In this study, we examined whether HIF-1 α levels in the serum and SF of patients with knee OA are increased and whether this increase is correlated with the radiographic severity of the disease.

MATERIAL AND METHODS

Subjects

This study was approved by the research Ethics Committee of our hospital and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all subjects. A total of 278 patients diagnosed with knee OA according to the clinical symptomatic criteria of American College of Rheumatology and radiographic criteria for OA in at least 1 knee were enrolled in the present study. Additionally, 203 gender- and age-matched healthy controls with normal knee radiographs were recruited. Subjects with histories of corticosteroid medication, bilateral knee replacements, other forms of arthritis, cancer, or other chronic inflammation diseases were excluded from the study.

Radiographic assessment of OA

A knee OA radiographic severity test, performed by an experienced observer who was blinded to information about the subjects, was assessed according to the KL grading system by evaluating X-ray changes observed on anteroposterior knee radiography (Kellgren and Lawrence, 1957): grade 0, no radiological changes; grade 1, doubtful narrowing of joint space and possible osteophytic lipping; grade 2, definite osteophytes and possible narrowing of joint space; grade 3, moderate multiple osteophytes, definite narrowing of joint space, some sclerosis, and possible deformity of bone contour; and grade 4, large osteophytes, marked narrowing of joint space, severe sclerosis, and definite deformity of bone contour. Subjects with radiographic knee OA of KL grade 2 or higher in at least 1 knee were defined as OA patients and those who had KL grades of 0 in both knees were defined as healthy controls.

Laboratory methods

Venous blood samples were collected from all subjects after overnight fasting. SF was obtained from the affected knee of OA patients using sterile knee puncture prior to receiving hyaluronic acid injection for the first time. No SF was extracted from controls because of ethical concerns. Blood and SF samples were centrifuged at 1600 g for 10 min and immediately stored at -80°C until conducting experiments. Serum and SF levels of HIF-1 α were measured using a commercially available enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, MN, USA) according to manufacturer instructions.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences software, version 16.0 for Windows (SPSS, Inc., Chicago, IL, USA). All data are reported as means \pm standard deviation of the mean (SD) or median (interquartile range). The Kolmogorov-Smirnov test was performed to analyze data normality, while the unpaired *t*-test, Mann-Whitney U test, or chi-square test was used to assess significance in clinical characteristics between patients with knee OA and healthy controls where appropriate. HIF-1 α levels in the serum and SF between OA patients with different KL grades were compared using one-way analysis of variance, while the correlation between HIF-1 α levels in the serum and SF with disease severity was determined using Spearman correlation coefficient (*r*) and multinomial logistical regression analysis. P values less than 0.05 were considered to be statistically significant.

RESULTS

Baseline clinical parameters

As shown in Table 1, there were no significant differences in baseline clinical parameters, including age, gender, and body mass index between knee OA patients and healthy controls.

Table 1. Characteristics of knee OA patients and healthy controls.

Characteristics	Knee OA patients (N = 278)	Healthy controls (N = 203)	P value
Age (years)	61.38 ± 8.75	60.96 ± 9.12	0.583
Gender (male/female)	107/171	89/114	0.258
BMI (kg/m ²)	24.75 ± 3.23	24.58 ± 3.17	0.565
HIF-1a in serum (pg/mL)	164.71 (99.58-225.26)	113.45 (53.78-168.46)	<0.001
HIF-1a in SF (pg/mL)	542.98 (466.38-648.21)		

The characteristics of age and BMI are reported as means ± SD; the characteristics of HIF-1a in serum and synovial fluid (SF) are reported as median (interquartile range).

HIF-1a levels in serum and SF

Two hundred and seventy-eight serum and SF samples from knee OA patients and 203 serum samples from healthy controls were collected for measurement of HIF-1a concentrations. Levels of HIF-1a in the serum and SF of healthy controls and serum level of knee OA patients are shown in Table 1. Serum HIF-1a levels were significantly elevated in knee OA patients compared with in healthy controls ($P < 0.001$).

HIF-1a levels in knee OA patients with different KL grades

The 278 knee OA patients were divided into KL grade 2 (N = 80), KL grade 3 (N = 106), and KL grade 4 (N = 92) according to the KL grading system. HIF-1a levels in the serum and SF of different KL grades are shown in Table 2. Serum levels of HIF-1a in patients with KL grade 4 were significantly higher than in patients with KL grades 2 and 3. Although the median serum levels of HIF-1a in KL grade 3 patients were higher than KL grade 2, the differences were not statistically significant. In addition, SF HIF-1a levels increased with KL grade. Particularly, SF levels of HIF-1a in KL grade 4 subjects were significantly higher than those in KL grade 2 and 3 subjects and patients with KL grade 3 had higher SF HIF-1a levels than subjects in the KL grade 2 groups.

Table 2. HIF-1a levels in serum and synovial fluid (SF) of knee OA patients with different KL grades.

HIF-1a (pg/mL)	Grade 2 (N = 80)	Grade 3 (N = 106)	Grade 4 (N = 92)	P value
Serum	152.15 (87.63-181.12)	160.62 (92.45-202.93)	181.43 (123.66-238.17) ^{a,b}	<0.001
SF	508.98 (448.55-603.72) ^b	530.36 (474.98-624.58) ^a	588.71 (502.48-681.15) ^{a,b}	<0.001

^a $P < 0.001$ vs KL grade 2. ^b $P < 0.001$ vs KL grade 3. The characteristics of HIF-1a in serum and SF are reported as median (interquartile range).

Association between HIF-1a levels with the radiographic severity of knee OA

We further analyzed the association between HIF-1a levels in the serum and SF and the radiographic severity of knee OA. Spearman correlation analysis showed that HIF-1a levels in the serum and SF were both positively correlated with radiographic severity of knee OA ($r = 0.258$, $P < 0.001$ and $r = 0.437$, $P < 0.001$, respectively). Multinomial logistical regression analysis showed that HIF-1a levels in the serum and SF were both positively correlated with KL grades ($P < 0.001$ and $P < 0.001$, respectively).

DISCUSSION

HIF-1 family members have been implicated in a diverse array of physiological and pathological processes such as tumorigenesis, inflammation, cell survival in ischemic tissues, and development of the growth plate as well as other organ systems (Semenza, 2001b; Cramer et al., 2003; Yudoh et al., 2005). The active subunit HIF-1 α is responsible for oxygen responsiveness and is hydroxylated by oxygen-sensitive prolylhydroxylases under normoxic conditions, followed by targeting of the von Hippel-Lindau tumor suppressor protein and degradation by the proteasome (Semenza, 2001a). Recent studies have showed that HIF-1 α is endogenously expressed in the cartilage and functions as an anabolic factor for chondrocytes to survive in a hypoxic microenvironment and maintain anaerobic energy metabolism and matrix synthesis of articular cartilage by stimulating various target genes (Yudoh et al., 2005; Pfander et al., 2006).

HIF-1 has been shown to be expressed in the synovial tissue of rheumatoid arthritis, OA patients, and 'normal' nonarthritic individuals and is related to both angiogenesis and inflammation in rheumatoid arthritis (Giatromanolaki et al., 2003; Brouwer et al., 2009). Additionally, HIF-1 α is also expressed in human normal and osteoarthritic chondrocytes (Coimbra et al., 2004), and there have been studies involving the correlation between OA severity and serum and SF cytokines such as thymosin b4 (Wei et al., 2013), CXCL12 (Xu et al., 2012), basic fibroblast growth factor (Honsawek et al., 2012), Dickkopf-1 (Honsawek et al., 2010), and even target genes of HIF-1 α (Dumond et al., 2003; Ku et al., 2009). However, there have been no studies regarding the relationship between HIF-1 α levels in the serum and SF of knee OA patients and disease severity. This is the first study to show that HIF-1 α was present in both serum and SF obtained from patients with primary knee OA and that HIF-1 α levels were positively correlated with radiographic grading of knee OA.

We found a significant increase in HIF-1 α levels in both the serum and SF of patients with knee OA compared to healthy controls. Our findings suggest enhanced local and systemic production of HIF-1 α in primary knee OA, but the level of HIF-1 α in SF was much higher than that in the paired serum sample. The mechanism of this increase may be related to either secretion of HIF-1 α residing in the extracellular matrix, increased HIF-1 α synthesis, or both. The HIF-1 α in SF may originate from synovial cells and chondrocytes in local tissues (such as the synovial membrane and articular cartilage), as previous studies have demonstrated that HIF-1 α is expressed in synovial cells, articular cartilage, and even connective tissues of synovial joints (Coimbra et al., 2004; Adesida et al., 2007; Brouwer et al., 2009). This indicates that systemic and local expression of HIF-1 α is significantly elevated in patients with advanced knee OA, but the mechanism of this increase in the serum and SF of knee OA patients requires further investigation. The increased levels of HIF-1 α in the serum and SF may be responsible for decreased oxygen levels in OA joints.

Therefore, measurements of serum and/or SF levels of HIF-1 α may serve as a biochemical parameter for determining disease severity and may be predictive of prognosis with respect to the progression of the osteoarthritic disease process. However, there were some limitations to our study. First, this was a cross-sectional study that included a relatively small number of subjects; further longitudinal studies with a larger population sample should be conducted. Second, we did not measure the HIF-1 α levels in the SF of healthy controls because of ethical concerns. Third, we did not analyze the relationship between HIF-1 α levels in the serum and SF and pain experienced by knee OA patients. Fourth, we did not account

for the effects of hyaluronic acid injection on changes of HIF-1a levels in the serum and SF in knee OA patients.

In conclusion, we found that serum and SF HIF-1a levels were significantly elevated in OA patients and we identified a positive correlation between serum and SF HIF-1a levels with the degree of radiographic severity in patients with knee OA. Measurement of HIF-1a levels in the serum and SF can be used as an alternative biomarker to assess the progression and prognosis of knee OA in addition to the use of traditional methods for assessing the risk and severity of knee OA.

Conflicts of interest

The authors declare no conflict of interest.

REFERENCES

- Adesida AB, Grady LM, Khan WS, Millward-Sadler SJ, et al. (2007). Human meniscus cells express hypoxia inducible factor-1alpha and increased SOX9 in response to low oxygen tension in cell aggregate culture. *Arthritis Res. Ther.* 9: R69.
- Aigner T, Zien A, Gehrsitz A, Gebhard PM, et al. (2001). Anabolic and catabolic gene expression pattern analysis in normal versus osteoarthritic cartilage using complementary DNA-array technology. *Arthritis Rheum.* 44: 2777-2789.
- Brouwer E, Gouw AS, Posthumus MD, van Leeuwen MA, et al. (2009). Hypoxia inducible factor-1-alpha (HIF-1alpha) is related to both angiogenesis and inflammation in rheumatoid arthritis. *Clin. Exp. Rheumatol.* 27: 945-951.
- Coimbra IB, Jimenez SA, Hawkins DF, Piera-Velazquez S, et al. (2004). Hypoxia inducible factor-1 alpha expression in human normal and osteoarthritic chondrocytes. *Osteoarthritis Cartilage* 12: 336-345.
- Cramer T, Yamanishi Y, Clausen BE, Förster I, et al. (2003). HIF-1alpha is essential for myeloid cell-mediated inflammation. *Cell* 112: 645-657.
- Ding C, Cicuttini F and Jones G (2008). How important is MRI for detecting early osteoarthritis? *Nat. Clin. Pract. Rheumatol.* 4: 4-5.
- Dumond H, Presle N, Terlain B, Mainard D, et al. (2003). Evidence for a key role of leptin in osteoarthritis. *Arthritis Rheum.* 48: 3118-3129.
- Felson DT (2006). Clinical practice. Osteoarthritis of the knee. *N. Engl. J. Med.* 354: 841-848.
- Felson DT, Zhang Y, Hannan MT, Naimark A, et al. (1995). The incidence and natural history of knee osteoarthritis in the elderly. The Framingham Osteoarthritis Study. *Arthritis Rheum.* 38: 1500-1505.
- Gelse K, Pfander D, Obier S, Knaup KX, et al. (2008). Role of hypoxia-inducible factor 1 alpha in the integrity of articular cartilage in murine knee joints. *Arthritis Res. Ther.* 10: R111.
- Giatromanolaki A, Sivridis E, Maltezos E, Athanassou N, et al. (2003). Upregulated hypoxia inducible factor-1alpha and -2alpha pathway in rheumatoid arthritis and osteoarthritis. *Arthritis Res. Ther.* 5: R193-R201.
- Hill CL, Parsons J, Taylor A and Leach G (1999). Health related quality of life in a population sample with arthritis. *J. Rheumatol.* 26: 2029-2035.
- Hofer T, Desbaillets I, Höpfl G, Gassmann M, et al. (2001). Dissecting hypoxia-dependent and hypoxia-independent steps in the HIF-1alpha activation cascade: implications for HIF-1alpha gene therapy. *FASEB J.* 15: 2715-2717.
- Honsawek S, Tanavalee A, Yuktanandana P, Ngarmukos S, et al. (2010). Dickkopf-1 (Dkk-1) in plasma and synovial fluid is inversely correlated with radiographic severity of knee osteoarthritis patients. *BMC Musculoskelet. Disord.* 11: 257.
- Honsawek S, Yuktanandana P, Tanavalee A, Saetan N, et al. (2012). Correlation between plasma and synovial fluid basic fibroblast growth factor with radiographic severity in primary knee osteoarthritis. *Int. Orthop.* 36: 981-985.
- Huang LE, Gu J, Schau M and Bunn HF (1998). Regulation of hypoxia-inducible factor 1alpha is mediated by an O2-dependent degradation domain via the ubiquitin-proteasome pathway. *Proc. Natl. Acad. Sci. U. S. A.* 95: 7987-7992.
- Kellgren JH and Lawrence JS (1957). Radiological assessment of osteo-arthrosis. *Ann. Rheum. Dis.* 16: 494-502.
- Ku JH, Lee CK, Joo BS, An BM, et al. (2009). Correlation of synovial fluid leptin concentrations with the severity of osteoarthritis. *Clin. Rheumatol.* 28: 1431-1435.
- Masson N, Willam C, Maxwell PH, Pugh CW, et al. (2001). Independent function of two destruction domains in hypoxia-

- inducible factor- α chains activated by prolyl hydroxylation. *EMBO J.* 20: 5197-5206.
- Pfander D and Gelse K (2007). Hypoxia and osteoarthritis: how chondrocytes survive hypoxic environments. *Curr. Opin. Rheumatol.* 19: 457-462.
- Pfander D, Cramer T and Swoboda B (2005). Hypoxia and HIF-1 α in osteoarthritis. *Int. Orthop.* 29: 6-9.
- Pfander D, Swoboda B and Cramer T (2006). The role of HIF-1 α in maintaining cartilage homeostasis and during the pathogenesis of osteoarthritis. *Arthritis Res. Ther.* 8: 104.
- Schneider U, Miltner O, Thomsen M, Graf J, et al. (1996). Intra-articular oxygen partial pressure measurements under functional conditions. *Z. Orthop. Ihre. Grenzgeb* 134: 422-425.
- Semenza GL (2001a). HIF-1, O(2), and the 3 PHDs: how animal cells signal hypoxia to the nucleus. *Cell* 107: 1-3.
- Semenza GL (2001b). Regulation of hypoxia-induced angiogenesis: a chaperone escorts VEGF to the dance. *J. Clin. Invest.* 108: 39-40.
- Silver IA (1975). Measurement of pH and ionic composition of pericellular sites. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 271: 261-272.
- Stannus O, Jones G, Cicuttini F, Parameswaran V, et al. (2010). Circulating levels of IL-6 and TNF- α are associated with knee radiographic osteoarthritis and knee cartilage loss in older adults. *Osteoarthritis Cartilage* 18: 1441-1447.
- Stokes DG, Liu G, Coimbra IB, Piera-Velazquez S, et al. (2002). Assessment of the gene expression profile of differentiated and dedifferentiated human fetal chondrocytes by microarray analysis. *Arthritis Rheum.* 46: 404-419.
- Strobel S, Loparic M, Wendt D, Schenk AD, et al. (2010). Anabolic and catabolic responses of human articular chondrocytes to varying oxygen percentages. *Arthritis Res. Ther.* 12: R34.
- Wei M, Duan D, Liu Y, Wang Z, et al. (2013). Increased thymosin beta4 levels in the serum and SF of knee osteoarthritis patients correlate with disease severity. *Regul. Pept.* 185: 34-36.
- Xu Q, Sun XC, Shang XP and Jiang HS (2012). Association of CXCL12 levels in synovial fluid with the radiographic severity of knee osteoarthritis. *J. Investig. Med.* 60: 898-901.
- Yudoh K, Nakamura H, Masuko-Hongo K, Kato T, et al. (2005). Catabolic stress induces expression of hypoxia-inducible factor (HIF)-1 α in articular chondrocytes: involvement of HIF-1 α in the pathogenesis of osteoarthritis. *Arthritis Res. Ther.* 7: R904-R914.