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Decreased synovial fluid ghrelin levels are linked with disease severity in primary knee osteoarthritis patients and are increased following laser therapy

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ABSTRACT

Background: Ghrelin has been proved to inhibit inflammation and promote cartilage growth. So far, its role in patients with primary knee osteoarthritis has not been investigated.
Objective: The current study was performed to explore the serum and synovial ghrelin levels as well as the relationship between ghrelin levels and disease severity in primary knee OA patients.
Methods: 52 primary knee OA patients were recruited in the study. 52 sex and age-matched patients visiting our hospital for regular body check were selected as controls. The serum and synovial fluid ghrelin levels were examined by enzyme linked immunosorbent assay (ELISA) before treatment, one week and four weeks after laser therapy, respectively. The inflammation markers IL-6 and TNF-α were also investigated. The radiographic progression was assessed by Kellgren-Lawrence (K-L) grade scale and the symptomatic severity was evaluated by visual analog scale (VAS), Lequesne index and Lysholm scores. The Receiver Operating Characteristic (ROC) analysis curve was conducted to test the diagnostic value of ghrelin, IL-6 and TNF-α for radiographic progression.
Results: No significant difference of serum ghrelin levels was found between knee OA patients and healthy controls. Synovial fluid ghrelin concentrations were significantly negatively correlated with K-L grading ($r = -0.591$, $P < 0.001$).Attenuated synovial fluid ghrelin levels were also related to clinical severity determined by Lequesne index ($r = -0.308$, $P = 0.025$),VAS scores ($r = -0.591$, $P < 0.001$) and Lysholm scores ($r = 0.381$, $P = 0.005$).In addition, ghrelin levels were also negatively associated with TNF-α ($r = -0.424$, $P = 0.002$) and IL-6 concentrations ($r = -0.428$, $P = 0.002$). ROC curve analysis demonstrated that ghrelin exhibited more diagnostic value than IL-6 and TNF-α for assessing radiographic progression in medium-late stage.
Conclusions: Decreased synovial fluid ghrelin levels are related to disease severity in patients with primary osteoarthritis and are increased following laser therapy. Local application of ghrelin may serve as an adjunctive therapy for knee OA.

1. Introduction

Osteoarthritis (OA) is a common chronic degenerative joint disease leading to physical disability, impaired quality of life as well as a large financial burden on health care systems and society [1]. OA can be present in major weight bearing joints and the knee is the most commonly affected. The main pathological process of knee OA involves progressive damage of articular cartilage, subchondral bone sclerosis, secondary synovial inflammation and osteophyte formation [2]. It affects > 37% of people whose age are over 60 years in US [3]. In China, one recent population-based longitudinal survey carried out between 2011 and 2012 showed the incidence of total symptomatic knee OA was 8.1%,with women 10.3% compared with men 5.7% [4,5], implicating a large number of OA patients suffering from knee OA worldwide.

So far, primary knee OA is mainly diagnosed according to the radiographic changes as well as complaints of pain and functional disability [5]. However, radiographic alternations including presence of osteophyte and joint space narrowing are almost signs of medium-late stage OA [6]. Structural molecules and fragments derived from bone,

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cartilage and the synovium in OA patients have been implicated as potential candidates for biomarkers of OA [7]. The analysis of joint serum and synovial fluid biomarkers could serve as a clinically relevant method to study markers of knee OA before signs of arthritis are visible by radiograph [8]. Therefore, it is an urgent task to seek for more effective, reliable methods to monitor early knee cartilage damage and treatment effects.

Ghrelin is a 28 amino acid peptide hormone first identified from the gut of both human and rat by targeting its endogenous ligand of the growth hormone secretagogue receptor (GHS-R) [9]. Ghrelin has been demonstrated to exert various biological effects on wide range of cells and organs [10]. Recent studies have shown that ghrelin plays a great role on bone or cartilage metabolism. Ghrelin was found by Caminos and his colleagues in rat, mice, and human chondrocytes, mainly expressed in proliferative and maturative zone of the epiphyseal growth plate [11]. Ghrelin could stimulate the proliferation, differentiation and migration of various cells including osteoblasts and chondrocytes [11,12].

Besides, ghrelin could significantly inhibit fatty acid uptake to further decrease the synthesis of types of eicosanoids, which play significant roles not only in joint physiology, but also in the pathogenesis of joint disorders [11]. Ghrelin was also shown to upregulate cAMP production in a dose-dependent manner in human and mice chondrocytes in vitro [11]. Ghrelin acts via GHS-R to specifically inhibit the expression of pro-inflammatory anorectic cytokines such as IL-1β, IL-6, and TNF-α, indicating the existence of a regulatory network by which ghrelin possibly controls immune cell activation and inflammation [13]. In addition, long-term treatment with ghrelin reduces chronic neuropathic pain in rat sciatic nerve injury model [14], implicating its potential role for pain alleviating [15].

All these studies above implicated that ghrelin may act as a protective role in OA progression. However, to our knowledge, there have been no detailed studies available exploring ghrelin levels in serum or synovial fluid of patients with various stages of knee OA. We hypothesized that attenuated ghrelin expression may be related to disease severity in patients with OA. Therefore, the present study was to investigate ghrelin expression in the serum and synovial fluid of patients with primary OA of the knee and identify the possible correlations with the radiographic and symptomatic severity of OA, which may serve as a useful tool for implicating the disease severity and progression of knee OA. Laser therapy including low-level laser therapy (LLLT) and high intensity laser therapy (HILT) has both been proved to be effective intervention methods to treat various diseases, mainly due to their potential roles on tissue healing, capacity of the inflammation inhibition and positive effect on pain relief [16]. A series of studies have demonstrated both LLLT and HILT are effective on treating OA in experimental models and clinical studies [17–20]. Hence, in addition to the cross-sectional study, we further applicate laser therapy to test whether it can increase synovial ghrelin levels.

2. Methods

2.1. Patients and samples

This study was approved by the medical ethics committee of The Third Affiliated Hospital of Southern Medical University, and all patients had signed the informed consent. These patients were diagnosed with knee OA according to the criteria of the American College of Rheumatology [5]. Patients were excluded if they had gout, rheumatoid arthritis, meniscal injury, previous knee surgery, intraarticular glucocorticoid or hyaluronic acid injection malignant tumors, systemic and autoimmune diseases, and corticosteroids intake inner two months. The control group was randomly selected from 52 age and sex matched healthy individuals who received regular body check-up in our hospital. They were confirmed with normal knee radiography and had no knee symptoms.

2.2. Laboratory examination

Venous blood samples collected from all participants were centrifuged and stored immediately at − 80 °C until analysis. Synovial fluid was taken from the most affected knee for further examination. We failed to obtain synovial fluid ghrelin levels from healthy controls due to ethical reasons. The specimen was then centrifuged to remove cells and joint debris and stored at − 80 °C prior to analysis. Serum and SF ghrelin levels were determined with commercially available enzyme-linked immunosorbent assay (ELISA) kits (R & D Systems, Minneapolis, MN, USA) according to the manufacturer's protocol. The inflammation markers TNF-α and IL-6 were also examined (R & D Systems, Minneapolis, MN, USA).The Intra- and interassay coefficients of variation (CV) for ghrelin, TNF-α and IL-6 were 7.8% and 11.3%, 4.5% and 8.6%, 6.0% and 7.7%, respectively. The experiments were triplicated and the results were averaged.

2.3. Assessment of radiographic severity

Knee X-ray radiography plain was taken for each subject standing with both legs fully extended knee. The X-ray beam was centered right at the level of the joint. The Kellgren and Lawrence (KL) grading system was applied to evaluate the radiographic progression of knee OA [21]. In which osteoarthritis was classified into five grades (0 to 4) as defined below: grade 0 (normal findings), no X-ray changes; grade 1 (questionable), doubtful narrowing of joint space and possible osteophyte lipping; grade 2 (mild), definite osteophytes and possible joint space narrowing; grade 3 (moderate), multiple moderate osteophytes, definite narrowing of joint space, bone sclerosis and possible deformity of bone contour; grade 4 (severe), large osteophytes, marked joint space narrowing, severe sclerosis, and deformity of bone contour. In the present study, the enrolled patients were identified as knee OA of KL grade ≥ 2 in at least one knee. Controls had no signs of radiographic OA nor symptomatic knee OA. The grading scale used for analysis was the one found higher upon comparison between both knees. The X-ray images were evaluated by two experienced radiologists who were blinded to the enrollment of patients.

2.4. Pain assessment

Pain was evaluated by visual analog scale (VAS) [22] consisting of a 10-cm rule 0 cm at the left side means “no pain” and 10 on the right side, indicates “extremely pain” is written. The patients were instructed to mark a line to represent their extent of pain. A ruler was used to measure the distance from the beginning of the line to the marked point, with higher values of centimetres indicating more severe pain.

2.5. Definition of functional disability

The clinical severity of knee OA patients was determined using Lequesne index and Lysholm score. The Lequesne index is comprised of 11 items about pain, discomfort and function [23]. One of the questions is designated for hip OA, so this question will be excluded. Each answer has an equivalent score. The total score ranges from 0 to 24 and is classified into five categories of functional impairment: no = 0, bit = 1–4, moderate = 5–7, severe = 8–10, very severe = 11–13 and extremely severe ≥ 14, suggesting the higher the score, the greater the impairment of function. The Lysholm score [24] ranges from 0 to 100 points including 8 items: limp, locking, pain, stair-climbing, support, instability, swelling, and squatting. Where higher scores represents mild pain or good physical function, lower scores represent more severe pain or poorer function. The Lequesne index and Lysholm score were both validated tools designed for the assessment of knee OA and were widely used based on clinical evidence and experience [25,26].