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Associations between serum ghrelin and knee symptoms, joint structures and cartilage or bone biomarkers in patients with knee osteoarthritis

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SUMMARY

Objective: The roles of ghrelin in knee osteoarthritis (OA) are unclear. This study aimed to examine cross-sectional associations of ghrelin with knee symptoms, joint structures and cartilage or bone biomarkers in patients with knee OA.

Methods: This study included 146 patients with symptomatic knee OA. Serum levels of ghrelin and cartilage or bone biomarkers including cartilage oligomeric matrix protein (COMP), cross linked C-telopeptide of type I collagen (CTXI), cross linked N-telopeptide of type I collagen (NTXI), N-terminal procollagen III propeptide (PIIINP), and matrix metalloproteinase (MMP)-3, 10, 13 were measured using Enzyme-linked immunosorbent assay (ELISA). Knee symptoms were assessed using the Western Ontario and McMaster Universities Arthritis Index (WOMAC). Infrapatellar fat pad (IPFP) volume, IPFP signal intensity alternation, cartilage defects, bone marrow lesions (BMLs) and effusion-synovitis were assessed using the (MRI). Osteophytes and joint space narrowing (JSN) were assessed using the Osteoarthritis Research Society International atlas.

Results: After adjustment for potential confounders, ghrelin quartiles were positively associated with knee symptoms including pain, stiffness, dysfunction and total score (quartile 4 vs 1: β 24.19, 95% CI 8.13 −40.25). Ghrelin quartiles were also significantly associated with increased IPFP signal intensity alteration (quartile 4 vs 1: OR 3.57, 95% CI 1.55−8.25) and NTXI, PIIINP, MMP3 and MMP13. Ghrelin was not significantly associated with other joint structures and biomarkers.

Conclusions: Serum levels of ghrelin were significantly associated with increased knee symptoms, IPFP signal intensity alteration and serum levels of MMP3, MMP13, NTXI and PIIINP, suggesting that ghrelin may have a role to play in knee OA.

Introduction

Ghrelin, the endogenous ligand for the growth-hormone secretagogue receptor (GHSR) composed of 28 amino acids, was first identified in the stomach of rats and humans in 1999 by Kojima[1]. The stomach is the major source of circulating ghrelin, although it is also expressed in many other organs, including the pituitary gland, hypothalamus, intestines, pancreas, heart, lung, kidneys, and gonads[2].

Ghrelin has multiple physiological effects such as promoting appetite, increasing adiposity, stimulating the release of growth

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hormone, regulating glucose and energy homeostasis, improving cardiovascular function, modulating immunological response and inflammatory processes, and affecting bone metabolism[3,4]. Data from animal experiments and *in vitro* studies indicated that ghrelin promoted osteoblast proliferation and differentiation, inhibited osteoblast apoptosis[5,6], and had dual actions in osteoclasts: inhibiting osteoclast progenitors directly but stimulating osteoclastogenesis systemically[7]. In addition, ghrelin may have a significant role in regulating chondrocyte metabolism[8], and has been detected both in rat and human cartilage tissues and chondrocytic cell lines, serving as a growth factor for chondrocytes[8]. Ghrelin has also been reported to affect pain, including suppression of the inflammatory pain[9], prevention of mechanical hyperalgesia[10], and reduction of chronic neuropathic pain[11]. The underlying mechanisms are still unclear, and this may be related to regulation of the central opioid system and pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α and interleukin (IL)-1β[9,12]. Two forms of ghrelin are found in the circulation: acyl-ghrelin, which is acylated with an n-octanoyl group on serine residue 3 and has multifarious biological effects through GHSR-1a, and des-acyl ghrelin. The majority of circulating ghrelin is des-acyl ghrelin. Although it was long believed to be inactive and reported not to bind GHSR, there is growing evidence that this form of ghrelin has biological effects as well[13].

Osteoarthritis (OA) is the most common form of joint disease characterized by joint structural changes, and is the leading cause of joint pain and physical disability, which causes a large socio-economic healthcare burden. There are no effective disease-modifying drugs approved for the treatment of OA currently. Although the pathogenesis of OA is still unclear, obesity is a well-recognized risk factor. Systemic and local inflammation is also reported to involve the pathogenesis of OA[14].

Given that ghrelin and OA are both related to bone metabolism, obesity, inflammation and pain, we speculate that ghrelin may be involved in the initiation and progression of OA. As far as we know, there have been no epidemiological studies describing the association between ghrelin and OA so far. Only a study[15] reported that administration of ghrelin had no effect on mid-thigh muscle mass and functional performance in patients with hip OA undergoing elective total hip replacement. It is unknown whether serum levels of ghrelin are associated with knee symptoms, joint structures and cartilage or bone biomarkers in patients with knee OA. The aim of this study, therefore, was to investigate the cross-sectional associations of serum levels of ghrelin with knee symptoms that included pain, stiffness and dysfunction, joint structural changes that included joint radiographic changes, cartilage defects, bone marrow lesions (BMLs), effusion-synovitis, volume and signal intensity alternation of infrapatellar fat pad (IPFP) measured using magnetic resonance imaging (MRI), and serum cartilage or bone biomarkers in patients with symptomatic knee OA.

Methods

Subjects

This study was part of the Anhui Osteoarthritis (AHOA) Study, a clinical study of 205 patients aged 34–74 years, aiming to identify the environmental and biochemical factors associated with the progression of knee OA. Patients with symptomatic knee OA, diagnosed using American College of Rheumatology criteria[16], were consecutively recruited from the Department of Rheumatology and Immunology in the First Affiliated Hospital of Anhui Medical University, from January 2012 to November 2013. We excluded institutionalized patients, patients with rheumatoid arthritis or other inflammatory diseases, patients with diabetes mellitus, patients with severe OA planning to have knee arthroplasty within 2 years, patients with contraindications to MRI (including metal sutures, presence of shrapnel, iron filings in eye, and claustrophobia), and patients with incomplete data. 59 patients fulfilled the exclusion criteria and therefore were excluded from this study, leaving 146 patients. This study was conducted in accordance with the Declaration of Helsinki, and was approved by the First Affiliated Hospital Anhui Medical University Ethics Committee. Written informed consents were obtained from all participants.

Anthropometrics

Weight was measured to the nearest 0.1 kg (with shoes, socks and bulky clothing removed) by using a single pair of electronic scales that were calibrated using a known weight at the beginning. Height was measured to the nearest 0.1 cm (with shoes, socks and headgear removed) by using a stadiometer. Body mass index (BMI) was calculated [weight (kg)/height $(m)^2$].

Joint symptom assessments

Using the Western Ontario and McMaster Universities Arthritis Index (WOMAC)[17], the subscores of knee joint pain, stiffness, and dysfunction were self-reported. Knee joint pain was divided into weight-bearing pain (walking on flat surface, going up/down stairs, and when standing upright) and non-weight-bearing pain (at night while in bed and when sitting/lying).

Serum ghrelin and cartilage or bone biomarker measurements

Fasting blood was obtained from all patients in the morning. Serum was separated and aliquotted into plastic storage tubes. Aliquots were stored at −80°C till analyses. Serum levels of ghrelin, cartilage oligomeric matrix protein (COMP), cross linked C-telopeptide of type I collagen (CTXI), cross linked N-telopeptide of type I collagen (NTXI), N-Terminal procollagen III propeptide (PIIINP), and matrix metalloproteinase (MMP)-3, 10, 13 were measured by using enzyme-linked immunosorbent assay (ELISA; eBioscience, USA for ghrelin, and elabscience, China for others) kits, according to the manufacturer's instructions. The intra- and inter-assay coefficient of variations for ghrelin were 6.0% and 8.5%, and for others were all <10%.

Knee radiographic assessments

A standing anteroposterior semiflexed view of the diseased knee (the severer one if both were affected) with 15° of fixed knee flexion, was performed in all participants. Kellgren–Lawrence (KL) grading system (grades 0–4) was used to assess the radiographic severity of OA[18]. Radiographic OA (ROA) was defined as KL grade of ≥2. Joint space narrowing (JSN) and osteophytes were also assessed on a scale of 0–3 using the Osteoarthritis Research Society International atlas[19]. The inter- and intra-rater reliabilities for KL grading were all >0.80.

MRI assessments

Using a commercial transmit/receive extremity coil, MRI of symptomatic knee (the severer one if both were affected) was performed with a 3.0T whole-body magnetic resonance unit (GE Signa 3.0T HDXT). The following sequence and parameters were used: (1) a T1-weighted fat saturation 3-D spoiled gradient recall (SPGR) acquisition in the steady state; flip angle 30°; repetition time 31 ms; echo time 6.71 ms; field of view 16 cm; 60 partitions; 512 × 512 matrix; acquisition time 11 min 56 ms; one acquisition.