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Circulating levels of IL-6 and TNF-α are associated with knee radiographic osteoarthritis and knee cartilage loss in older adults

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SUMMARY

Objective: The role of inflammation in osteoarthritis (OA) pathogenesis is unclear, and the associations between inflammatory cytokines and cartilage loss have not been reported. We determined the associations between serum levels of interleukin (IL)-6 and tumor necrosis factor-α (TNF-α), knee radiographic OA (ROA) and cartilage loss over 2.9 years in older adults.

Methods: A total of 172 randomly selected subjects (mean 63 years, range 52–78, 47% female) were studied at baseline and approximately 3 (range 2.6–3.3) years later. IL-6 and TNF-α were assessed by radioimmunoassay. T1-weighted fat-suppressed magnetic resonance imaging of the right knee was performed at baseline and follow-up to determine knee cartilage volume. Knee ROA of both knees was assessed at baseline.

Results: At baseline, quartiles of IL-6 and TNF-α were associated with increased prevalence of medial tibiofemoral joint space narrowing (OARSI grade ≥1) in multivariate analyses [odds ratio (OR): 1.42 and 1.47 per quartile, respectively, both $P < 0.05$]. Longitudinally, baseline IL-6 predicted loss of both medial and lateral tibial cartilage volume (β: −1.19% and −1.35% per annum per quartile, $P < 0.05$ and $P < 0.01$, respectively), independently of TNF-α. Change in IL-6 was associated with increased loss of medial and lateral tibial cartilage volume (β: −1.18% and −1.06% per annum per quartile, both $P < 0.05$) and change in TNF-α was also negatively associated with change in medial cartilage volume (β: −1.27% per annum per quartile, $P < 0.05$).

Conclusions: Serum levels of IL-6 and TNF-α are associated with knee cartilage loss in older people suggesting low level inflammation plays a role in the pathogenesis of knee OA.

Introduction

Osteoarthritis (OA) is one of the most common diseases among older people and is a leading cause of disability. It affects the whole joint structure including articular cartilage, synovial membrane, subchondral bone, meniscus, and periarticular muscles[1]. The structural changes of OA are due to a combination of risk factors, ranging from common factors such as aging, obesity, being female, smoking, genetics and joint injury, to mechanical and metabolic factors[1–4]. Inflammation has been implicated in the pathogenesis of OA. Synovitis is common in early[5] and advanced[6] OA, and it has been associated with knee pain[7] and progression of cartilage degeneration[8]. The inflammatory changes in OA synovium include synovial hypertrophy and hyperplasia with an increased number of lining cells[9], and an infiltration of the sublining tissue with a mixed population of inflammatory cells including synovial macrophages[10], activated B and T lymphocytes[6]. Local levels of pro-inflammatory cytokines such as interleukin (IL)-1β, tumor necrosis factor (TNF) α, and IL-6 produced by these cells are detectable even in early OA but generally at lower levels than in rheumatoid arthritis[11–13], and *in vitro* and animal studies have documented that these cytokines can enhance cartilage degradation or induce bone resorption[9,14,15].

Multiple studies have demonstrated that circulating levels of C-reactive protein (CRP), a marker of low-grade systemic inflammation, are modestly elevated in OA and are associated with decreased cartilage volume and disease progression[16–18]; however, the associations between IL-6 and IL-1, the primary regulators of CRP, and severity and progression of OA have been rarely reported. A recent

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study in women reported that prevalent radiographic OA (ROA) was significantly associated with both IL-6 and CRP, and that incident ROA was significantly predicted by IL-6[19]. The same study found no significant associations between TNF-α and prevalent or incident OA[19], while a recent Dutch study suggested that *ex vivo* production of TNF-α from whole blood samples upon lipopolysaccharide stimulation was associated with radiological progression of knee OA over 2 years[20].

While OA disease progression is commonly measured by radiographs, such means have been proven to have limited sensitivity due to their two-dimensional nature and measurement error. Magnetic resonance imaging (MRI) can visualize whole joint structure directly and is recognised as a more sensitive, accurate and reproducible tool than radiographic assessment to monitor OA disease progression[21]. The aim of this study was to describe the associations between circulating levels of inflammatory markers (CRP, IL-1, TNF-α and IL-6), and both ROA prevalence and MRI-detected knee cartilage loss over approximately 3 years in older adults.

Materials and methods

Subjects

The study was carried out in southern Tasmania from March until August 2002. The follow-up study was conducted approximately 3 years later (range 2.6–3.3 years). Subjects between ages 50 and 79 years were selected randomly from the roll of electors in southern Tasmania (population 229,000) with an equal number of men and women. Institutionalized persons were excluded. This study was conducted as part of the Tasmanian Older Adult Cohort Study, an ongoing, prospective, population-based study in 1100 subjects aimed at identifying the environmental, genetic, and biochemical factors associated with the development and progression of OA and osteoporosis (the overall response rate was 57% at baseline and 82% retention for follow-up). Subjects with rheumatoid arthritis were excluded from analyses. The first 172 subjects were selected to perform the measurements of serum inflammatory markers at baseline and follow-up. At follow-up, these measurements were not performed in nine subjects due to insufficient serum sample, leaving 163 subjects. The study was approved by the Southern Tasmanian Health and Medical Human Research Ethics Committee, and written informed consent was obtained from all participants.

Anthropometrics and questionnaire

Height was measured to the nearest 0.1 cm (with shoes, socks, and headgear removed) using a stadiometer. Weight was measured to the nearest 0.1 kg (with shoes, socks, and bulky clothing removed) using a single pair of electronic scales (Seca Delta Model 707, Bradford, MA) that were calibrated using a known weight at the beginning of each clinic. Body mass index [BMI; weight (kg)/height^2 (m^2)] was also calculated. Self-report of smoking status and diseases including asthma, cardiovascular disease, and diabetes were recorded by questionnaire. Steps per day were measured using a pedometer worn on their dominant side for seven consecutive days except during sleeping or water based activities.

Serum inflammatory markers measurement

Serum was isolated and refrigerated overnight in plastic tubes, at which time aliquots were prepared and stored at −80°C. The IL-1 β, IL-6 and TNF-α were measured at baseline and then at follow-up with a solid-phase, two-site chemiluminescent enzyme immunometric assay method by use of IMMULITE IL-1 β, IMMULITE IL-6 and IMMULITE TNF-α (all from EURO/DPC Llanberis, Gwynedd, United Kingdom). Samples with undetectable cytokine concentrations were assigned a value corresponding to the lower limit of detection of the assay (1.5 pg/ml for IL-1 β, 2 pg/mL for IL-6 and 1.7 pg/mL for TNF-α). The coefficients of variation (CVs) in our hands were 3% for IL-1 β, 8% for IL-6 and 6% for TNF-α[22].

Testing high-sensitivity CRP (hs-CRP) was performed by using the CRP-Latex (II) immunoturbidimetric assay (Abbott Diagnostic's c8000 Architect). The lower detection limit of the assay is 0.01 mg/L. The CV in our hands was of the order of 4.8%[22].

Changes in these markers were calculated as: change per annum = (follow-up value − baseline value)/(time between two visits in years).

Knee X-ray and knee pain assessment

A standing anteroposterior semiflexed view of the right and left knees with 15° of fixed knee flexion was performed in all subjects at baseline and scored individually for osteophytes and joint space narrowing (JSN) on a scale of 0–3 (0 = normal and 3 = severe) according to the Osteoarthritis Research Society International (OARSI) atlas as previously described[23]. The presence of medial or lateral tibiofemoral JSN or osteophytes was defined as any score of ≥1 in that compartment. The presence of JSN or osteophytes in the whole tibiofemoral compartment was defined as the presence of that feature in either of the medial or lateral compartments.

Knee pain (on flat surface, going up/down stairs, at night, sitting/lying and standing upright) was assessed by self-administered questionnaire using the Western Ontario McMaster Osteoarthritis Index (WOMAC) with a 10-point scale from 0 (no pain, stiffness or no function problems) to 9 (most severe pain, stiffness or severe function problems)[24]. Each component of joint pain was summed to create a total pain (0–45) score. Prevalent knee pain was defined as a total score of ≥1.

Knee cartilage volume and tibial bone area measurements

MRI scans of the right knee were performed at baseline and follow-up. Knees were imaged in the sagittal plane on a 1.5-T whole body magnetic resonance unit (Picker, Cleveland, OH) and a fat-saturated T1-weighted spoiled gradient echo sequence was used. Knee cartilage volume was determined by means of image processing on an independent workstation as previously described[2,25]. The volumes of individual cartilage plates (medial tibial, and lateral tibial) were isolated from the total volume by manually drawing disarticulation contours around the cartilage boundaries on a section-by-section basis. These data were then resampled by means of bilinear and cubic interpolation (area of 312 and 312 μm and 1.5 mm thickness, continuous sections) for the final 3-D rendering. The CVs for cartilage volume measures in our hands were 2.1–2.6%[25]. Rates of change in cartilage volume were calculated as: percentage change per annum = [100 × [(follow-up volume − baseline volume)/baseline volume]/(time between two scans in years)].

Tibial bone area at the medial and lateral compartments was determined as previously described[23].

Data analysis

T-tests or χ^2-tests (where appropriate) were used to compare means or proportions. Quartiles of IL-6 or TNF-α were used in analysis, because nearly a quarter of subjects had IL-6 levels under the lower limits of detection. The associations between quartiles of IL-6/TNF-α and presence of knee ROA (JSN or osteophytes), both