Longitudinal analysis of the relationship between serum insulin-like growth factor-I and radiographic knee osteoarthritis


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Summary

Objective: To examine the relation between serum insulin-like growth factor I (IGF-I) levels and both incident and progressive radiographic knee osteoarthritis (OA) in the Framingham Osteoarthritis Study.

Design: Subjects had bilateral weight-bearing, anterior–posterior knee radiographs performed in 1983–1985 and again in 1992–1993. IGF-I levels were measured from blood specimens obtained in 1988–1989 by a competitive binding radio-immunoassay (RIA) after separation with octadecasilyl-silica cartridges of serum IGF-I from binding proteins. Participants without baseline radiographic OA (Kellgren and Lawrence grades (K&L) = 0–1) were classified as having incident disease if they had K&L ≥ 2 grades at follow-up. Progressive OA was defined as an increase in K&L score of ≥ 1 in knees with baseline OA (K&L ≥ 2). All analyses were knee-based and sex-specific. We examined IGF-I tertiles in relation to the risk of incident and progressive radiographic OA separately, adjusting for age, body mass index (BMI), and baseline K&L score, and used generalized estimating equations to adjust for the correlation between fellow knees.

Results: Four hundred and forty-one participants had knee radiographs and serum IGF-I levels measured. No associations were found for serum IGF-I levels and incident [women: OR = 0.9 (0.6–1.7), men OR = 1.2 (0.6–2.6)] or progressive [women OR = 0.9 (0.6–1.6), men OR = 0.9 (0.3–3.0)] radiographic knee OA in either sex. Neither did we observe any association between IGF-I and worsening of individual radiographic features of OA (i.e., osteophyte growth and joint space loss).

Conclusion: In summary, this longitudinal study did not demonstrate any association of serum IGF-I and incident or progressive radiographic knee OA. Further studies are needed to clarify the role of IGF-I in OA.

Key words: IGF-I, Osteoarthritis, Knee, Radiograph.

Introduction

Cartilage matrix integrity is normally maintained by a balance between the effects of catabolic cytokines and growth factors. A disruption in this equilibrium resulting in increased cartilage breakdown is thought to contribute to the pathogenesis of osteoarthritis (OA) [1]. Insulin-like growth factor (IGF-I) has been shown to promote type II collagen and proteoglycan synthesis [2–5], to inhibit matrix degradation in vitro [6], and to be anabolic for articular cartilage in vivo [7].

Previous studies of serum IGF-I levels and OA have demonstrated conflicting results. Cross-sectional studies have reported high [8], low [9], and normal levels of IGF-I [10, 11] in subjects with OA compared to controls. The only longitudinal study published to date found a positive correlation between IGF-I levels and OA progression in subjects with pre-existing OA [12]. The development of new disease (incident OA) has not been addressed in prior studies.

Recent studies suggest that risk factors for incident and progressive OA might differ. Knee OA progression, and not incidence, has been linked to both low bone mineral density [13] and low serum levels of vitamin D [14], whereas knee pain, injury, and physical activity were found to influence incident but not progressive OA [15].
Based on the anabolic actions of IGF-I on chondrocytes, we hypothesized that decreased serum IGF-I is associated with an increased risk of knee OA. To test this hypothesis, we examined the relation of serum IGF-I and (1) incident and (2) progressive radiographic knee OA in a large population based cohort.

Methods

Participants

The study participants were members of the Framingham Osteoarthritis Study, which has been extensively described in previous publications [16, 17]. In brief, the Framingham Osteoarthritis Study includes members of the Framingham Heart Study, a population based cohort followed since 1948, who were evaluated for radiographic knee OA at biennial examinations 18 (1983–1985) and 22 (1992–1993).

We performed a nested case–control study in which all participants with incident and progressive OA (defined below) were included as cases. For the incident case group we randomly drew up to three controls from all subjects without knee OA at baseline. For the OA progression analysis all those with baseline OA were included as controls. As part of their routine exam, blood was taken from the participants at biennial exam 20 (1988–1989) which was centrifuged and stored at $-70^\circ$F. All people participating in this study were healthy enough to attend the Framingham clinical exams from 1983 through 1993.

Radiographs

Each participant had bilateral weight-bearing, anterior–posterior knee radiographs. Knee radiographs were scored using a modified Kellgren–Lawrence Scale (K&L) based on the presence of joint space narrowing and/or osteophytes, where 0 = no osteophyte or joint space narrowing, 1 = questionable osteophyte(s) and/or joint space narrowing, 2 = definite small osteophyte(s) and/or mild joint space narrowing, 3 = definite moderate osteophyte(s) and/or moderate joint space narrowing of at least 50%, and 4 = definite large osteophyte(s) and severe joint space narrowing. Total knee replacements were scored as 4. In addition, the presence and severity of osteophytes and joint space narrowing were scored separately for each joint (0 = normal, 1 = mild, 2 = moderate, 3 = severe) based on an atlas of standard radiographic features. We did not measure joint space narrowing using a ruler based on the findings of Spector et al. [18] which found that this method did not offer any advantage over qualitative measures using a 0–3 scale.

All radiographs were scored by one of two radiologists specializing in musculoskeletal radiology. Intraobserver agreement for radiographic knee OA based on K&L $\geq 2$, the presence of osteophytes, and the presence of joint space narrowing was kappa ($k$) = 0.91, 0.72, and 0.79 (all $P < 0.001$), respectively. The interobserver reliability for these measures was moderate ($k$ = 0.50, $P < 0.025$, k = 0.50, $P < 0.001$, and $k$ = 0.65, $P < 0.001$). Because of the modest interobserver reliability obtained, knee radiographs were subsequently reread by a second set of readers, using a strategy described elsewhere in detail [17]. A consensus reading was used in the analyses reported here.

Classification of OA

Incident OA was defined as subjects without baseline radiographic OA (K&L = 0 or 1 in both knees) who developed radiographic OA in either knee at follow-up (K&L $\geq 2$). Patients with baseline OA (K&L = 2 or 3) and a follow-up score which increased by $\geq 1$ grade in the same knee were classified as having progressive OA. Participants with baseline K&L = 4 were excluded from the analyses since they were not eligible to develop incident or progressive OA. Osteophyte growth was defined as an increase of $\geq 1$ grade between the baseline and follow-up films. Joint-space loss was also defined as an increase in $\geq 1$ grade except for patients with baseline grade = 0, for which the follow-up grade had to be at least 2. These definitions are based on previous analyses used to validate definitions of OA changes over time, in which the above cut-off points were determined to maximize the association between definitions of radiographic OA changes and known risk factors for radiographic knee OA [19].

IGF-I Measurements

Before serum IGF-I measurement by competitive binding radio-immunoassay (RIA), IGF binding proteins (IGFBPs) were removed using an octadecasylsilica-silica technique, as previously described, to prevent their interference in the assay [20]. This method has been shown to remove more than 99% of IGF BPs [20]. Briefly, 50 $\mu$L serum was incubated for 1-2 h with 150 $\mu$L of 0.5 N HCL. Octadecasylsilica cartridges (C-18 Sep-Pak, Millipore/Waters, Milford, MA, U.S.A.) were pretreated sequentially with 2-propanol, methanol,
and 4% glacial acetic acid. The 200 µl acidified serum sample was applied to the cartridge, incubated at room temperature for 3 min, and drawn through the cartridge under vacuum. Each cartridge was washed with 4% acetic acid solution and the IGF-I eluted with methanol. The resulting IGF-I/methanol solution was dried overnight under vacuum centrifugation and reconstituted 30:1 with phosphate-buffered saline. Subdilutions of the 30:1 solution were prepared to provide three final dilution levels for RIA (30:1, 75:1, and 150:1).

IGF-I was measured by competitive binding RIA (Nichols Institute Diagnostics, San Juan Capistrano, CA, U.S.A.) according to the manufacturer's specifications. Briefly, extracted serum samples, standards, and controls were mixed with primary IGF-I antibody (rabbit anti-human) and incubated for 1 h at room temperature. 32P-I-IGF-I antibody was then added and incubation continued for 16-18 h at 4°C. A second antibody, (goat anti-rabbit), was added and incubated for 20 min at room temperature. Samples were centrifuged, decanted, and precipitant pellets counted in a gamma counter. IGF-I concentrations, corrected for dilutions, were calculated by reference to a standard curve generated for each RIA kit. The mean IGF-I concentration from the three dilutions was calculated for each subject. The inter-assay and intra-assay variance were 9.8–11.8% and 2.3–2.9% respectively.

### COVARIATES

Baseline age and body mass index (BMI) were examined as potential confounders since these factors are associated both with OA and IGF-I. BMI was calculated as weight in kilograms divided by height in meters squared.

### STATISTICAL ANALYSES

All analyses were knee-based and sex-specific. Because age is very strongly related to both IGF-I and OA [10, 11], we created age-specific IGF-I tertiles, within 2 year age groups, to ensure that the effects of this confounder were adequately controlled for. We used multivariate logistic regression to examine age-adjusted IGF-I tertiles in relation to the risk of incident and progressive radiographic knee OA separately, adjusting for BMI and baseline K&L score. In each case the lowest IGF-I tertile was used as the referent category. Generalized estimating equations were used to adjust for the correlation between fellow knees.
Table II
Association of IGF-1 and incident* and progressive† OA

<table>
<thead>
<tr>
<th>Age-adjusted IGF-1 tertile</th>
<th>Women</th>
<th>Men</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>N of cases</td>
<td>Crude OR</td>
<td>Adjusted‡ OR</td>
<td>N of cases</td>
<td>Crude OR</td>
<td>Adjusted* OR</td>
</tr>
<tr>
<td>Incident OA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>33</td>
<td>1.0</td>
<td>1.0</td>
<td>10</td>
<td>1.0</td>
</tr>
<tr>
<td>Middle</td>
<td>17</td>
<td>1.1 (0.7–1.9)</td>
<td>1.3 (0.8–2.1)</td>
<td>7</td>
<td>0.9 (0.3–2.2)</td>
</tr>
<tr>
<td>Highest</td>
<td>23</td>
<td>1.0 (0.6–1.7)</td>
<td>0.9 (0.6–1.7)</td>
<td>14</td>
<td>1.5 (0.6–3.4)</td>
</tr>
<tr>
<td>Progressive OA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>19</td>
<td>1.0</td>
<td>1.0</td>
<td>7</td>
<td>1.0</td>
</tr>
<tr>
<td>Middle</td>
<td>12</td>
<td>0.9 (0.5–1.7)</td>
<td>1.1 (0.6–2.0)</td>
<td>8</td>
<td>1.0 (0.3–2.8)</td>
</tr>
<tr>
<td>Highest</td>
<td>19</td>
<td>0.9 (0.6–1.6)</td>
<td>0.9 (0.5–1.6)</td>
<td>5</td>
<td>0.7 (0.2–2.3)</td>
</tr>
</tbody>
</table>

*Baseline K&L score of 0-1 and a follow up x-ray of ≥ 2.
†Baseline K&L score of 2-3 and a follow-up score which increased by ≥ 1 grade.
‡Adjusted for: body mass index, and baseline K&L grade.

Results

IGF-I levels were measured in 293 women and 148 men. The descriptive characteristics for subjects with and without baseline OA are presented in Table I. As expected, IGF levels were negatively correlated with age (r = -0.2 for women and r = -0.1 for men), and positively correlated with BMI (r = 0.05 for women and r = 0.1 for men). There was no significant difference between IGF-I levels between those who survived versus those who died before obtaining repeat X-rays at exam 22. The mean IGF-I level in male survivors versus those who died before exam 22 were 201.5 ± 99.8 ng/ml versus 183.3 ± 100.1 ng/ml, P = 0.6. Similarly in women, the respective IGF-I levels were 170.5 ± 67.4 ng/ml, and 158.4 ± 89.7 ng/ml, P = 0.6.

For women, 73 knees were classified as having incident OA, and 50 were classified as having progressive OA. For men, 31 and 20 knees were classified as having incident and progressive disease respectively. No associations were observed for serum IGF-I levels with either incident or progressive radiographic knee OA in either sex (Table II). For example, among women without OA at baseline, those in the highest IGF-I tertile had an odds ratio of 0.9 (0.6-1.7) of developing incident knee OA. For women with OA at baseline, the odds of radiographic progression was 0.9 (0.5-1.6). In addition, we did not observe any association with IGF-I and individual radiographic features (i.e. osteophyte growth and joint space loss) in subjects with or without baseline OA (Tables III and IV).

Discussion

In summary, this longitudinal study did not demonstrate an association of serum IGF-I levels and incident or progressive radiographic knee osteoarthritis. Our findings are consistent with the cross-sectional studies of McAlindon et al. [10] and Hochberg et al. [11]. Other cross-sectional studies have found both lower [9], and higher [8] mean serum IGF-I levels in subjects with OA compared to controls. Denko et al. [9] found that patients with both symptomatic and radiographic OA had lower serum levels than age, sex, race, height, and

Table III
Association of IGF-1 and osteophyte growth and joint space loss in subjects without baseline OA (K&L = 0-1)

<table>
<thead>
<tr>
<th>Age-adjusted IGF-1 tertile</th>
<th>Women</th>
<th>Men</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>N of cases</td>
<td>Crude OR</td>
<td>Adjusted* OR</td>
<td>N of cases</td>
<td>Crude OR</td>
<td>Adjusted* OR</td>
</tr>
<tr>
<td>Osteophyte growth</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>141</td>
<td>1.0</td>
<td>1.0</td>
<td>41</td>
<td>1.0</td>
</tr>
<tr>
<td>Middle</td>
<td>83</td>
<td>1.0 (0.6–1.9)</td>
<td>1.1 (0.8–1.6)</td>
<td>30</td>
<td>1.0 (0.4–2.3)</td>
</tr>
<tr>
<td>High</td>
<td>110</td>
<td>1.2 (0.7–2.1)</td>
<td>1.2 (0.8–1.7)</td>
<td>50</td>
<td>1.1 (0.5–2.4)</td>
</tr>
<tr>
<td>Joint space loss</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>13</td>
<td>1.0</td>
<td>1.0</td>
<td>16</td>
<td>1.0</td>
</tr>
<tr>
<td>Middle</td>
<td>2</td>
<td>1.2 (0.2–6.3)</td>
<td>1.5 (0.2–9.9)</td>
<td>7</td>
<td>1.3 (0.4–4.6)</td>
</tr>
<tr>
<td>High</td>
<td>12</td>
<td>1.7 (0.3–8.6)</td>
<td>1.6 (0.3–9.1)</td>
<td>10</td>
<td>0.7 (0.2–3.4)</td>
</tr>
</tbody>
</table>

*Adjusted for: body mass index, and baseline K&L grade.
weight matched controls. In contrast, Lloyd et al. [8] found a positive association between increased IGF-I levels and distal interphalangeal and bilateral knee OA, but not unilateral knee, spine or hip OA. We could not confirm the findings of Schouten et al. [12] who found that increased serum IGF-I levels were associated with osteophyte growth in subjects with baseline OA (examined 12 years after baseline). Several differences between this study and ours, including the mean age of the participants (older in our study), radiographic scoring techniques, IGF-I assays, and methods of analyses, might account for the contrasting results.

The relatively small size of our study sample (especially for men), might have prevented us from detecting statistically significant associations between incident and progressive OA with IGF-I. However, the adjusted odds ratios all approximated 1, suggesting that we were unlikely to miss an important association.

We measured IGF-I levels, using an assay which is capable of removing approximately 99% of IGF-I binding proteins, midway between baseline and follow-up radiographs. Although it is possible that some participants may have developed disease by the time IGF-I was assessed, it is doubtful that they would have been classified into different age-adjusted IGF-I tertiles had this exposure been measured at baseline, since serum IGF-I is unlikely to be influenced by local disease [8].

Our results do not discount a possible important local role of IGF-I in the pathophysiology of OA. IGF-I has been shown to have important anabolic effects on cartilage in vitro experiments. It increases chondrocyte collagen and proteoglycan synthesis, and inhibits proteoglycan degradation even in the presence of such potent catabolic cytokines as interleukin-1 beta and tumour necrosis factor alpha [1]. The lack of association between serum levels of IGF-I and OA might be explained by the interposition of other regulatory mechanisms between serum IGF-I levels and cellular access to this growth factor [21].

In conclusion, we did not find an association between serum IGF-I levels and either incident or progressive radiographic knee OA. Further studies are needed to clarify the role of IGF-I in OA.

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References


