Author's response to reviews

Title: Dickkopf-1 (Dkk-1) in plasma and synovial fluid is inversely correlated with radiographic severity of knee osteoarthritis patients

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Author's response to reviews: see over
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Prof Dr. David Hunter
Editor, BMC Musculoskeletal Disorders

Ref.: Manuscript No.: 5868130014439416
Title: Dickkopf-1 (Dkk-1) in plasma and synovial fluid is inversely correlated with radiographic severity of knee osteoarthritis patients

Dear Editor,

Thank you very much for your mail and the reviewers’ comments on our manuscript entitled "Dickkopf-1 (Dkk-1) in plasma and synovial fluid is inversely correlated with radiographic severity of knee osteoarthritis patients". We have revised accordingly for all the reviewers’ comments. We also have done following on the check list.

Reviewer’s comments:

Reviewer #1:

Comment:
Dr. Honsawek et al. present their findings on the amount of Dickkopf-1 (DKK) measurable in the plasma and synovial fluid of patients with radiographic knee osteoarthritis and the plasma level of DKK in normal control patients. The manuscript is well presented.
There are several points, which would improve the clarity of the manuscript.

- Major Compulsory Revisions
1. The authors state that their data suggest that low levels of DKK-1 correlate with progressive arthritis. The only data presented are for a single time point in a cross sectional study and cannot be extrapolated to determining progression (conclusion page 13).
Response: We clarified that point in:
-Abstract, page 2, line 21-22:
Dkk-1 levels in plasma and synovial fluid are inversely related to the severity of joint damage in knee OA.
-Conclusion, page 13, line 12-14:
“This study has revealed a significant decrease in plasma Dkk-1 of OA patients and illustrated a pronounced inverse correlation with the degree of radiographic severity in patients with primary knee OA.”

2. In the methods on page 5 there is a statement that none of the participants had diabetes or inflammatory disease and later in the same paragraph it reiterates that arthritis, cancer and other chronic diseases were excluded. Were there two stages of exclusion? The controls are described after the exclusion criteria. Were the same exclusion criteria applied?
Response: The same exclusion criteria were applied in both patient and control groups. We addressed that point in:
- Methods, page 5, line 20-22:
  “None of the participants had underlying diseases such as diabetes, histories of corticosteroid medication, other forms of arthritis, cancer, or other chronic inflammatory diseases.”

3. Please clarify if the plasma was drawn at the same time as the synovial fluid aspirate was performed.
Response: We clarified that point in:
- Methods, page 5, line 24-25 and page 6, line 1-4:
  “Synovial fluid was aspirated from the affected knee using sterile knee puncture just prior to surgery, when a total knee replacement was performed, centrifuged to remove cells and joint debris and stored immediately at –80ºC until the day of measurement. No synovial fluid was extracted from the controls due to ethical concerns. Venous blood samples collected from the same patients on the day of surgery were centrifuged and stored at –80ºC until utilized.”

4. As there are a limited number of patients in this study Table 1 should be expanded to include the gender, age, and BMI by Kellgren and Lawrence classification. Counting the data points there are 17 K/L 4 and 8 K/L 2 patients. Including the stratification in the table would clarify this point.
Response: We included the gender, age, and BMI by Kellgren and Lawrence classification in Table 1 in page 20.

Table 1 Characteristics of knee osteoarthritis patients and controls. Data are expressed as mean and SD.

<table>
<thead>
<tr>
<th></th>
<th>Controls (95%CI)</th>
<th>OA patients (95%CI)</th>
<th>KL grade 2</th>
<th>KL grade 3</th>
<th>KL grade 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>15</td>
<td>35</td>
<td>10</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>Age (years)</td>
<td>67.5±4.6  (65.3-70.3)</td>
<td>68.8±8.2 (66.3-70.5)</td>
<td>68.8±6.8 (63.9-73.6)</td>
<td>68.1±8.3 (61.5-74.6)</td>
<td>69.5±7.5 (64.9-73.9)</td>
</tr>
<tr>
<td>Female/Male</td>
<td>10/5</td>
<td>26/9</td>
<td>7/3</td>
<td>9/3</td>
<td>10/3</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.5±1.3 (24.6-26.4)</td>
<td>26.6±3.8 (25.3-28.0)</td>
<td>26.2±1.4 (25.2-27.2)</td>
<td>26.4±3.1 (24.5-28.4)</td>
<td>27.2±4.8 (23.7-30.7)</td>
</tr>
</tbody>
</table>

5. Figure 1 should be limited to a comparison of the plasma levels between OA patients and controls. Including the synovial fluid data here is redundant with figure 4 and there is no appropriate control.
Response: We limited to a comparison of the plasma levels between OA patients and controls, as shown in Figure 1.
6. The conclusion drawn from the Pearson correlation in Figure 3 should be tempered. There is a relatively low level of Dkk-1 in all of the synovial fluids and there are two “outliers” in the limited number of patients in the K/L 2 group (n=8). If these two points were excluded then there would be a relatively flat line through the groups. The small number of individuals in the K/L 2 group limits the emphasis that can be placed on interpreting this data.

Response: We addressed that point in:
-Results, page 9, line 15-17:
  “Synovial fluid levels of Dkk-1 were weakly associated with the radiographic severity of disease (r=-0.42, p=0.01) (Figure 3).”

7. In Figure 4 it is clear that overall the plasma levels correlate with synovial fluid levels, however it is equally clear that this is not uniformly proportional in all individuals. In the cluster of patients with DKK-1 plasma levels around 200 pg/ml a rough estimate of the spread of synovial levels is 25-70 pg/ml. This point should be clarified in the results.

Response: We clarified that point in:
-Results, page 9, line 17-20:
  “Figure 4 reveals that a rough estimate of plasma Dkk-1 levels in OA patients is 200-400 pg/ml whereas that of synovial fluid Dkk-1 levels is 25-70 pg/ml. However, plasma Dkk-1 levels showed a positive correlation with synovial fluid Dkk-1 levels (r=0.72, p<0.001).”

8. The discussion should also include the point that the daily variation of plasma DKK-1 has yet to be fully determined and it is unclear which activities might influence plasma levels (walking, jumping, eating etc). Only a single time point is shown.

Response: We stated that point in:
-Discussion, page 13, line 3-6:
  “Moreover, the daily variation of plasma Dkk-1 has yet to be fully determined and it is unclear which physical activities might influence plasma Dkk-1 levels (walking, jumping, eating, etc.). Therefore, diurnal and activity-related variations in plasma Dkk-1 will be needed to be further evaluated.”
9. On page 11 the authors conclude that there are differences in local and systemic production, but no data are provided on the location of synthesis.

**Response:** We addressed that point in:

- **Discussion, page 11, line 2-6 and line 8-11:**
  “The source of Dkk-1 could be derived from the local tissues (inflamed synovium, cartilage, and subchondral bone) and extra-articular tissues. Previous studies have shown that Dkk-1 was expressed in synovial cells, articular cartilage chondrocytes and subchondral bone osteoblasts in OA knees [10, 27, 28].”
  “This observation suggests a significant reduction in the systemic and local expression of Dkk-1 in patients with advanced knee OA. The mechanisms of Dkk-1 reduction in the circulation and synovial fluid of OA patients remain to be investigated further.”

**- Minor Essential Revisions**

1. Page 3 The statement: “More recent studies suggest a potential role for DKK-1 in malignant bone disease and arthritis. “Should be referenced. The next sentence should be changed to one report and not “Recent studies” as there is only one reference supplied.

**Response:** We corrected and added the references in:

- **Background, page 3, line 23-25 and page 4, line 1:**
  “More recent studies have suggested a potential role of Dkk-1 in malignant bone disease and arthritis [8-10]. Uderhardt et al. have shown that inhibition of Dkk-1 effectively reduces bone erosion of sacroiliac joints [11].”
- We also included three additional references in page 15, line 17-22 and page 18, line 10-12:

2. Page 12 the sentence “Moreover, the data derived from this study…. Does not make sense.

**Response:** We deleted that sentence in page 12.

**Reviewer #2:**

**Comment:** Authors explore the serum and synovial fluid Dkk-1 concentrations in osteoarthritic patients. They detect the Dkk-1 levels by ELISA and evaluate the correlation between Dkk-1 concentration and radiographic findings of osteoarthritic joint. The team demonstrates that Dkk-1 is a potent bio-marker for knee osteoarthritis progression. Wnt and Dkk-1 are emerging bio-active molecules participating in cartilage integrity and chondrocyte fate. In clinical vignettes, the pathomechanisms underlying osteoarthritis are complex. Dkk-1 reportedly regulates inflammation-mediated joint remodeling and accelerating osteoarthritic cartilage deterioration. In this article, linking secreted Dkk-1 levels to knee osteoarthritis severity is rational. However, the data interpretation and biological significance of Dkk-1 in various type of osteoarthritis remain further characterization.

Table 1, the gender of subjects is significantly different. Authors can not exclude the possibility that menopause is an underlying etiologic cause for different plasma Dkk-1 level, because the average age of female patients is more than 65 years. The data is needed to statistically re-analyze by age and gender correction.
Response: We reanalyzed and addressed that point in:
- Results, page 8, line 6-8:
  “In addition, the female/male ratio was 26/9 in patients and 10/5 in controls (p=0.1). The study population was adjusted for age and gender.”
- and we also included the gender, age, and BMI by Kellgren and Lawrence classification in Table 1 in page 20.

Table 1 Characteristics of knee osteoarthritis patients and controls. Data are expressed as mean and SD.

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</tr>
</tbody>
</table>

Comment: Figure 1, authors compare plasma Dkk-1 and synovial fluid Dkk-1 in osteoarthritic patients. They may need to discuss the aim of this comparison.

Response: We limited to a comparison of the plasma levels between OA patients and controls, as shown in Figure 1.

Figure 1 Plasma Dkk-1 levels of patients with osteoarthritis (n=35) and healthy controls (n=15).
Comment: The reviewer concedes that various tissue source may have different Dkk-1 levels. Without providing synovial fluid Dkk-1 levels in normal subject, the role of synovial fluid Dkk-1 in diagnosis of osteoarthritis severity seems dispensable.

Response: Synovial fluid aspiration of controls was not included in the protocol of this study when the protocol was submitted and approved by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University. However, we addressed that point in:
- Methods, page 6, line 2-3:
  “No synovial fluid was extracted from the controls due to ethical concerns,”

Comment: Again in the discussion, many sentences describing “a significant decrease in the systemic and local expression of Dkk-1 in osteoarthritic patients” is not justified, because they do not provide synovial fluid Dkk-1 levels in normal subjects. While plasma Dkk-1 concentration correlated with osteoarthritis progression, whether the Dkk-1 in peripheral blood can reflect the local change in osteoarthritic joint microenvironment remains additional evidence to support their conclusion.

Response: We corrected that point in:
- Discussion, page 11, line 8-11:
  “This observation suggests a significant reduction in the systemic and local expression of Dkk-1 in patient with advanced knee OA. The mechanisms of Dkk-1 reduction in the circulation and synovial fluid of OA patients remain to be investigated further.”

Comment: Authors emphasize that “Dkk-1 may be a useful prognostic parameter to reflect the disease severity of primary knee OA”. It will be nice to provide additional evidence of the cutoff values and sensitivity of Dkk-1 vs. various types of osteoarthritis to support this conclusion. Besides, it will be more comprehensive to describe how to use this secreted Dkk-1 for prognosis of primary knee OA.

Response: We have provided additional information in:
- Results, page 9, line 20-25:
  “Using ROC curves drawn with data of our results, the cutoff value was set to provide optimal diagnostic accuracy and likelihood ratios for Dkk-1 protein. A plasma level of 459.5 pg/ml was with a sensitivity of 90% and a specificity of 92% for moderate knee OA (KL grade 3). A plasma level of 303.5 pg/ml was with a sensitivity of 77% and a specificity of 85% for advanced knee OA (KL grade 4),”

-The manuscript was also copyedited by a native English speaking colleague.

We agree with the entire reviewers’ comments. We hope that all revisions will meet all the reviewers’ recommendation.

Should you have any queries, please do not hesitate to contact me. Along with this letter, we have enclosed the revised manuscript with all changes marked.

Once again, we would like to thank you very much in advance and we are looking forward to hearing from you.

Sincerely yours,
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