Author's response to reviews

Title: Fibroblast growth factor-23 and calcium phosphate product in young chronic kidney disease patients: A cross-sectional study

Authors:

Daisy Liu (dliu2012@meds.uwo.ca)
Luan Chau (luan@robarts.ca)
Joaquim Madrenas (joaquim.madrenas@mcgill.ca)
Guido Filler (guido.filler@lhsc.on.ca)

Version: 3 Date: 8 January 2013

Author's response to reviews: see over
Dear Dr. Henderson,

Thank you for the second review of our manuscript. We are glad that the statistical errors were identified. The undersigned elected to approach the statistician in our group and requested a thorough re-analysis to eliminate any errors beyond any remote doubt. Indeed, the reviewers were correct, and we substantially changed the manuscript as a consequence. Without the reviewers’ concerns, we would not have identified that younger and older children actually differ.

We elected to change the authors and to include Abeer Yasin, PhD, applied mathematician, as first author, as she repeated all of the statistical analysis and owns the intellectual rights on the idea to separate children from adolescents and young adults.

You mentioned that you wanted a statistical review. Please advise whether you require the data file, which we obviously would have to de-identify any personal information.

Please, find below our responses to the reviewer’s concerns. Rather than addressing both reviewers separately, both of whom identified the same weaknesses, we provide a list of the changes made in the manuscript. The entire data were reanalyzed. The message slightly changed, and we now do believe that there is a considerably improved manuscript. Please find the itemized list of changes below. New text is given in *italics*.

Specifically, the following changes were made:

- Abstract: The abstract is now 349 words long, allowed are up to 350 words. All the relevant statistics were double checked and updated. The novel information about children versus adolescents and young adults was incorporated. The new abstract reads:

  **Abstract**

  **Background:** Fibroblast growth factor-23 (FGF-23), a novel marker of bone disease in chronic kidney disease (CKD) has been shown to correlate with vascular calcifications. We aimed to describe the effect of the calcium phosphate product (Ca*P) on FGF-23 concentrations in children and young adults without confounding cardiovascular disease. **Methods:** Paediatric and young adult patients with CKD stages I-V were recruited in this cross sectional study to measure FGF-23, cystatin C, vitamin D-metabolites and other serum markers of bone metabolism. FGF-23 levels were determined with an enzyme-linked immunosorbent assay. The association between FGF-23 and (Ca*P) was
assessed using non-parametric methods. Patients were divided into two age groups, less than 12 years of age and greater than 12 years of age.

Results: This cross-sectional study measured serum FGF-23, in 81 patients (42 females, 51.9%) at London Health Sciences Centre, aged 2 to 44 years, with various stages of CKD (Cystatin C estimated glomerular filtration rate, eGFR=10.7-213.0 ml/min). For the whole entire group of patients, FGF-23 levels were found to correlate significantly with age (Spearman r= 0.26, p=0.0198), Cystatin C eGFR (Spearman r=-0.40 p=0.0002), CKD stage (Spearman r=0.457, p<0.0001), PTH (Spearman r=0.330, p=0.0039), ionized calcium (Spearman r=-0.330, p=0.0049), CysC (Spearman r= 0.404, p=0.0002) and 1,25-dihydroxyvitamin D (Spearman r=-0.345, p=0.0034) concentrations. No significant correlation was found between FGF-23 levels and calcium phosphate product (Spearman r= 0.164, p=0.142). Upon classification of patients into two age groups, less 12 years of age and more than 12 years of age, correlational results differed significantly. FGF-23 correlated with CysC eGFR (Spearman r= -0.633, p<0.0001), CKD stage (Spearman r=0.731, p<0.0001), phosphate (Spearman r= 0.557, p<0.0001), calcium phosphate product (Spearman r=0.534, p<0.0001), 125(OH)2 Vit D (Spearman r=-0.631, p<0.0001), PTH (Spearman r= 0.475, p=0.0017) and ionized calcium (Spearman r= -0.503, p=0.0015) only in the older group. The relationship between FGF-23 and Ca*P for the older group could be expressed by the exponential model FGF-23= 38.15 e 0.4625Ca*P.

Conclusion: Abnormal values of FGF-23 in adolescents and young adults with CKD correlate with Ca*P in the absence of vascular calcifications, and may serve as a biomarker for the risk of cardiovascular calcifications.

Errors occurred because one data set was entered as text and not as a number and therefore was excluded from the calculations in Excel. This slightly changed the age and percentage of females. The correct results read:

A total of 81 patients were included in the study. Median age was 13.0 years (25th percentile 8.0, 75th percentile 17 years), and 42 patients (51.9%) were female.

The text was checked for consistency and for introducing every abbreviation and then using it consistently. As a consequence, the following sentence changed to:

Forty seven patients had CKD stage I (Cystatin C-based estimated glomerular filtration rate (eGFR) >90 ml/min/1.73 m²), 9 patients had CKD stage II (eGFR 60 to 89.9 mL/min/1.73 m²), 13 patients had CKD stage III (eGFR 30 to 59.9 mL/min/1.73 m²) and 13 patients had an eGFR <30 mL/min/1.73 m².

The statistical analysis was repeated using the cleaned data file. All statistics were verified. The main section of the results now reads:

To assess for any correlations between eGFR and other variables under study for the entire group of patients we performed the non-parametric spearman rank correlation analysis. Similar analyses were conducted for the two age groups of less than 12 years and greater than 12 years of age. Interestingly, FGF-23 correlated with CysC eGFR
negatively and significantly) but did not correlate with either phosphate or calcium phosphate product for the entire group of patients. This was despite of a similar eGFR range. For the younger group of age less than 12 years, FGF-23 did not correlate with CysC eGFR, Phosphate or Ca*P while it correlated negatively and significantly with CysC eGFR, positively and significantly with Phosphate and calcium phosphate product for the older group of patients aging 13 years and above. CysC eGFR was found to correlate positively and significantly with Calcium, 1,25-OH Vitamin D levels, ionized Ca and negatively with FGF-23, Phosphate, 25-OH, alkaline phosphate and PTH for the entire group of patients. CysC eGFR correlations with bone markers differed when considering age groups. For the younger group of patients CysC eGFR correlated only with 1,25-OH Vitamin D levels, PTH and ionized Ca and for the older group it correlated with FGF-23, Phosphate, Ca*P, 1,25-OH Vitamin D levels, Alkine Phosphate, PTH and ionized Ca only. Table 3 summarizes the correlational analysis results.

We believe that we have adequately addressed the reviewers’ concerns and wish to resubmit the manuscript for your kind consideration.

Subsequently, we obviously had to change the main conclusion. It now reads:

Conclusion
In conclusion, the current study describes FGF-23 in relationship to other markers of renal osteodystrophy in 81 CKD patients. As expected, the prevalence of abnormal findings and medians for 1,25-dihydroxyvitamin D, serum phosphate, PTH, serum calcium and FGF-23 changed with worsening kidney function. The study confirms the association of FGF-23 levels with the Ca*P only for patients older than 12 years of age.

We also corrected numerous formatting and spelling mistakes. We apologize for sending an obviously insufficiently edited version. We hope that the revised manuscript is now suitable for publication.

Sincerely

Guido Filler, MD, PhD, FRCPC
Professor and Chair
Department of Pediatrics
Schulich School of Medicine & Dentistry
Chief of Pediatrics
Children’s Hospital