Chapter 7

Determination of fibroblast growth factor 23 (FGF-23)

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Chapter 7: Determination of FGF-23

Abstract

Background: Fibroblast growth factor 23 (FGF-23) is a recently discovered hormone, that plays a key role in phosphate regulation. To investigate whether FGF-23 can be determined reliably, we validated the three available FGF-23 assays.

Methods: Currently, two intact FGF-23 assays (Kainos; Immutopics) and one C-terminal FGF-23 assay (Immutopics) are available. We determined intra- and inter-assay variation, linearity and matrix interference in these assays. Moreover, we compared healthy subjects to patient groups with expected high FGF-23 concentrations.

Results: Intra-assay variation was reasonably good in all three assays. Inter-assay variation and linearity were unacceptable for the intact-Immutopics assay, however reasonable for both other assays. Immutopics assays gave best results in EDTA-plasma, while Kainos assay showed comparable reproducibility in EDTA-plasma and serum. Although the manual of the Kainos assay states that an automatic washing machine could be used, acceptable results were only found by manual washing. Predialysis, dialysis patients and patients with hypophosphatemic osteomalacia had increased C-terminal FGF-23 concentrations compared to healthy controls.

Conclusion: There are two assays for FGF-23 of reasonable quality available, the intact FGF-23 assay (Kainos) provided proper attention to the washing procedure, and the C-terminal assay (Immutopics).
Chapter 7: Determination of FGF-23

Introduction

Fibroblast growth factor 23 (FGF-23) is a recently discovered growth factor produced by osteocytes and behaving as a hormone in phosphate homeostasis. FGF-23 decreases phosphate reabsorption in the kidney by down-regulating the expression of sodium-phosphate co-transporters in the proximal tubule. Moreover, FGF-23 inhibits 1α-hydroxylase expression, leading to decreased hydroxylation of 25-hydroxyvitaminD into 1,25-dihydroxyvitaminD.

Elevated FGF-23 concentrations, due to genetic mutations, cause excessive renal phosphate excretion and inappropriately low 1,25-dihydroxyvitaminD serum concentrations in several types of hereditary hypophosphatemic rickets. Tumour-induced osteomalacia (TIO) is a syndrome consisting of hypophosphatemia, decreased renal tubular phosphate reabsorption, inappropriately low 1,25-dihydroxyvitaminD, myopathy and osteomalacia, caused by tumour production of FGF-23.

Patients undergoing hemodialysis often have increased serum phosphate concentrations. Associated, they have increased FGF-23 concentrations as well. These FGF-23 concentrations appear to be independently associated with mortality.

Since its discovery, it has become clear that FGF-23 plays a pivotal role in numerous processes associated with phosphate homeostasis. Thus, it has become the central determinant in many studies. However, reliability of the available FGF-23 assays is unknown. In the present study, we evaluated the performance of all three commercially available assays.

Methods and Results

Assays

Three assays for measuring FGF-23 were evaluated. One assay was designed to measure C-terminal FGF-23 (cFGF-23) (Immutopics, San Clemente, California, USA), whereas two other assays were designed to measure iFGF-23 (iFGF-23) (Immutopics, Kainos, Tokyo, Japan). All three assays are sandwich enzyme-linked immunosorbant assays (ELISAs). According to the Immutopics package inserts, cFGF-23 and iFGF-23 (Immutopics) should be measured in EDTA (ethylenediaminetetraacetic acid)-plasma while Kainos recommends to measure iFGF-23 in serum. BioTek ELx50 (BioTek, Bad Friedrichshall, Germany) was used as automatic washing
machine for all three assays. All analyses were performed according to the manufacturers protocol, unless otherwise described.

**Intra- and inter-assay variation**

Intra- and inter assay variation were measured using human samples with low, normal and high concentrations of FGF-23. Results are shown in Table 1. For the Kainos intact assay, the results are shown for automatic washing (as recommended by Kainos) as well as manual washing. The high inter assay variation in the intact Immutopics assay was mainly caused by lot-to-lot variability.

**Linearity**

To determine linearity, samples were diluted in phosphate buffered saline (PBS) and FGF-23 was measured in all three assays. The recovery was calculated by dividing the result obtained by the expected value. A recovery of 100% represents a good linearity. For Immutopics, a recovery of around 100% was found for dilutions up to 10% for the C-terminal assay. In contrast, recovery for the intact assay was above 100% and increasing with dilutions of 60% and more. The Kainos assay showed a recovery of around 100% for the entire range.

**Matrix interference**

iFGF-23, cFGF-23 (Immutopics) and iFGF-23 (Kainos) were measured concurrently in paired drawn specimens in EDTA-plasma and serum. cFGF-23 and iFGF-23 (Immutopics) could only be determined in EDTA-plasma, since measurements in serum gave lower or even undetectable results. iFGF-23 by Kainos could be determined in both serum and EDTA plasma.

**Patients**

cFGF-23 was determined in 78 healthy subjects, 20 pre-dialysis patients GFR 34.6±10.7), 47 patients undergoing dialysis (samples taken prior to-dialysis) and four patients with hypophosphatemic osteomalacia, proven by non-decalcified iliac crest biopsy. cFGF-23 and
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<tr>
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<th>FGF-23 Intact (Immutopics)</th>
<th>FGF-23 Intact (Kainos) Automatic washer</th>
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Table 1: Intra- and inter-assay variation
*N is based on duplicate measurements; nd not determined
Chapter 7: Determination of FGF-23

iFGF-23 concentrations were significantly increased in pre-dialysis patients (397 ± 644 RU/mL; p<0.01) and patients undergoing dialysis (13915 ± 17782 RU/mL; p<0.01) compared with healthy subjects (39 ± 24 RU/mL; 34 ± 8 ng/l). A significant difference in concentrations of cFGF-23 and iFGF-23 between pre-dialysis and dialysis patients was seen as well (p<0.01). Patients with hypophosphatemic osteomalacia showed elevated cFGF-23 concentrations from 162 to 558 RU/mL compared with healthy subjects (p<0.01).

Statistics
Differences between groups were determined by Mann Whitney U test. A p-value below 0.05 was considered significant.

Discussion
The three assays evaluated in this study show reasonably good intra-assay variation. Inter-assay variation and linearity, however, was unacceptable in the intact (Immutopics) assay, but reasonable in the other two assays. The iFGF-23 assay by Immutopics was therefore discarded in further analysis.

The manual of the iFGF-23 assay by Kainos states that an automatic washing machine can be used when washing the plates. We started out by using an automatic washing machine, but were confronted with poor reproducibility of the assay. The effect of washing the plates manually was striking, and improved the CV from 43% to 8% (Table 1). We therefore recommend that manual washing is absolutely necessary to obtain acceptable results for the iFGF-23 assay by Kainos.

The obtained intra- and inter assay CVs show that both the C-terminal assay by Immutopics and the intact assay by Kainos give reproducible results. However, the question remains how accurate these assays are. The antibodies in the C-terminal assay are directed against the C-terminal region of FGF-23, and therefore detect both putative C-terminal fragments as well as intact FGF23. The antibodies in the intact assay are described to be directed against the C-terminal and the N-terminal region of FGF-23 and may therefore detect only iFGF-23.6

However, as long as there are no well defined standards available, we do not know the exact accuracy of these assays. In diseases with known pathological high concentrations of FGF-23 such as hypophosphatemic rickets and TIO, FGF-23 is increased when analysed with both assays.6 We observed grossly elevated concentrations of FGF-23 in four patients with
Chapter 7: Determination of FGF-23

hypophosphatemic osteomalacia. Moreover, pre-dialysis and dialysis patients showed increased FGF-23 concentrations. These results indicate that the assays probably detect FGF-23.

In analogy with PTH assays, it is important to know whether FGF-23 fragments are measured in these assays, since measurement of different fragments in patients with renal failure may cause problems\(^7,8\). More research has to be performed with regard to clearance of FGF-23 fragments to clarify this problem. Also the possibility that potential FGF-23 fragments might have unexpected biological activity should be addressed.

Conclusion

On the basis of this study, there are two assays for FGF-23 of reasonable quality on the market; the iFGF-23 assay by Kainos, provided proper attention is given to the washing procedure, and the cFGF-23 assay by Immutopics.

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Chapter 7: Determination of FGF-23

References


