

## Fibroblast Growth-factor 23 and Calcium-binding Proteins are not Associated with Chronic Itch in Patients on Haemodialysis

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Chronic itch (CI) in haemodialysis, often termed uraemic pruritus, is a frequently experienced, tormenting and challenging symptom and a burden in afflicted patients (1).

There has been an ongoing discussion about whether CI in chronic kidney disease is brought about by the common disturbance of calcium/phosphate homeostasis. This notion is supported by a number of observations. In the late 1960s, itch was reported to be resolved by parathyroidectomy in patients with both severe hyperparathyroidism and CI (2). Later Blachley et al. (3) reported an increased calcium/phosphate load in the skin of patients with CI in haemodialysis and Momose et al. (4) were able to demonstrate a disrupted calcium gradient in the skin of patients with CI.

An observational study found that patients with CI had higher serum-calcium levels (5). It has been proposed recently that a vicious circle of metabolic derangements (malnourishment, inflammation, arteriosclerosis) may explain the exaggerated morbidity and mortality in a subset of haemodialysis patients. Inflammation might be the most deleterious factor in this scenario, contributing, among other factors, to the occurrence of CI in haemodialysis (6, 7). It has also been shown that Fetuin-A, an important calcium-binding circulating protein, is down-regulated by progressive inflammation in haemodialysis patients, thus promoting tissue-calcification (8). Similarly, matrix Gla protein (MGP) has been shown experimentally to inhibit vascular calcifications (9), whereas high levels of fibroblast growth-factor 23 (FGF23), a potent player in calcium/phosphate-regulation, seem to be associated with increased inflammation and morbidity and mortality in patients with chronic kidney disease (CDK) (10).

In a previous study we measured Fetuin-A levels in a small number of patients on haemodialysis and did not find any significant differences between patients with and without CI (11). In the present study a larger number of patients was investigated for CI and a potential decrease in serum levels of calcium-binding proteins, such as Fetuin-A and MGP, or other factors known to influence calcium-phosphate regulation (e.g. FGF23) potentially leading to an increased tissue deposition of calcium salts.

## PATIENTS AND METHODS

In a hospital-based single centre patients were screened for chronic itch (duration >6 weeks). Eighteen patients reported CI, while another 18 randomly selected patients on haemodialysis did not report having had CI during at least 6 months prior to the investigation. None of the patients had been treated with ultraviolet (UV) phototherapy in the 6 months prior to this study. After having obtained informed consent, patients with CI were asked to score the intensity of current itch using a visual analogue scale (VAS) ranging from 0 (no itch) to 10 (worst imaginable itch). In both groups 10 ml blood was taken immediately after puncture of the arterio-venous fistula for haemodialysis.

The following laboratory measurements were performed: Fetuin-A, 25-hydroxyvitamin D3 (25[OH]D3) total protein, albumin, calcium (corrected for serum albumin), phosphate, and high-sensitivity C-reactive protein (hsCRP). Fetuin-A (ELISA; Epitope Diagnostics, San Diego, CA, USA), MGP (ELISA BI 2062 Biomedica, Vienna, Austria) and FGF 23(C-terminal, ELISA, Biomedica Austria) were determined by means of an enzyme-linked immunosorbent assay (ELISA). An electrochemiluminescence immunoassay (ECLIA) was used for measurement of 25(OH)D3. Total protein, calcium and phosphate were measured by means of the automated clinical chemistry analyser (MODULAR ANALYTICS®P-Module (Roche Diagnostics, Penzberg, Germany). Albumin and hsCRP were determined by immunonephelometric measurement (BNII System Siemens Healthcare Diagnostics, Eschborn, Germany).

Statistical analyses were carried out using R 3.1.3®. Descriptive statistics were used to report medians and 1<sup>st</sup> and 3<sup>rd</sup> quartiles for demographic data and the variables under study stratified by itch status. The distribution of sex and the use of cinacalcet and 1-hydroxyvitamin D (1[OH]D) were provided as absolute and relative frequencies. Continuous variables were tested for differences between the patients reporting CI and non-CI patients, using the non-parametric Mann-Whitney *U* test or, for nominal variables, the  $\chi^2$ -test. *p*-values below 0.05 were considered statistically significant. Due to the explorative nature of the analysis no adjustment for multiple testing was applied.

## RESULTS

The median itch intensity of patients with CI was 6.5 (Table S1<sup>1</sup>). Serum levels of hsCRP of Fetuin A and MGP were significantly higher in patients with CI, but no significant differences were found between the 2 groups in serum-calcium, serum-phosphate, 25[OH]D3 and FGF23. (Fig. S1<sup>1</sup>, Table S1<sup>1</sup>). Time on dialysis and hsCRP were

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associated with the intensity of CI, whereas other factors measured revealed no association with the intensity of CI. Use of 1[OH]D or cinacalcet was not different between patients with and without CI.

## DISCUSSION

In the present study patients with CI exhibited higher serum CRP levels compared with those without CI. This is in accordance with 2 previous experimental studies (6, 7), but in contrast to other observational studies (12). In addition, it could be demonstrated that the intensity of itch is associated with the level of CRP.

In contrast to our initial hypothesis serum levels of the calcium-binding proteins Fetuin-A and MGP were slightly increased in patients with CI; however, without showing an association with the intensity of CI. Thus, the assumption that a potential inflammation-associated decrease in calcium-binding proteins may lead to a more pronounced calcium-deposition in the skin, which in turn provokes itch, could not be corroborated.

FGF23, another important player in the regulation of calcium, phosphate and mineral bone homeostasis (13), was determined in our study. However, levels of FGF23 were not noticeably different in patients with CI compared with those without CI and did not show any association with CI in our patients. Thus, it is unlikely that this protein is involved in the pathogenesis of CI.

Serum-calcium, serum-phosphate and 25(OH)D3 values were not significantly different between the 2 groups in our study, which is, at least in part, in accordance with previous findings (12). As UVB therapy is still a mainstay in the treatment of CI, and theoretically leads to augmented vitamin D supply, we were interested to ascertain whether patients with CI (but without previous UVB treatment) have lower levels of vitamin D. As there were no differences in 25(OH)D3 values between the groups, it is unlikely that vitamin D deficiency is more pronounced in patients with CI and therefore related to their symptoms. This is in accordance with results from Shirazian et al. (14), who showed that administration of high doses of ergocalciferol (50,000 IU per week for a period of 12 weeks) did not result in improvement of CI in 50 patients with CKD.

Taking the limitations of the present analysis into account (small number of patients, no matched pairs, MGP was not differentiated in carboxylated [active] form and uncarboxylated [inactive] form), there is no evidence that CI in haemodialysis patients is related to an inflammation-driven lowering of calcium-binding proteins. The only factor found to be associated with the intensity of CI is CRP, a marker for inflammation and

time on dialysis, which is, at least in part, in accordance with our and others' previous findings (6, 7, 11). Further studies are needed to determine whether inflammation plays a causative role in CI in haemodialysis patients, and which processes might be involved.

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## REFERENCES

- Weiss M, Mettang T, Tschulena U, Passlick-Deetjen J, Weishaar E. Prevalence of chronic itch and associated factors in haemodialysis patients: a representative cross-sectional study. *Acta Derm Venereol* 2015; 95: 816–821.
- Hampers CL, Katz AI, Wilson RE, Merrill JP. Disappearance of "uremic" itching after subtotal parathyroidectomy. *N Engl J Med* 1968; 279: 695–697.
- Blachley JD, Blankenship DM, Menter A, Parker TF, 3rd, Knochel JP. Uremic pruritus: skin divalent ion content and response to ultraviolet phototherapy. *Am J Kidney Dis* 1985; 5: 237–241.
- Momose A, Kudo S, Sato M, Saito H, Nagai K, Katabira Y, et al. Calcium ions are abnormally distributed in the skin of haemodialysis patients with uraemic pruritus. *Nephrol Dial Transplant* 2004; 19: 2061–2066.
- Duque MI, Thevarajah S, Chan YH, Tuttle AB, Freedman BI, Yosipovitch G. Uremic pruritus is associated with higher kt/V and serum calcium concentration. *Clin Nephrol* 2006; 66: 184–191.
- Kimmel M, Alschner DM, Dunst R, Braun N, Machleidt C, Kiefer T, et al. The role of micro-inflammation in the pathogenesis of uraemic pruritus in haemodialysis patients. *Nephrol Dial Transplant* 2006; 21: 749–755.
- Virga G, Visentin I, La Milia V, Bonadonna A. Inflammation and pruritus in haemodialysis patients. *Nephrol Dial Transplant* 2002; 17: 2164–2169.
- Metry G, Stenvinkel P, Qureshi AR, Carrero JJ, Yilmaz MI, Bárány P, et al. Low serum fetuin-A concentration predicts poor outcome only in the presence of inflammation in prevalent haemodialysis patients. *Eur J Clin Invest* 2008; 38: 804–811.
- Schlieper G, Aretz A, Verberckmoes SC, Kruger T, Behets GJ, Ghadimi R, et al. Ultrastructural analysis of vascular calcifications in uremia. *J Am Soc Nephrol* 2010; 21: 689–696.
- Hanks LJ, Casazza K, Judd SE, Jenny NS, Gutiérrez OM, Sanchez-Margalet V. Associations of fibroblast growth factor-23 with markers of inflammation, insulin resistance and obesity in adults. *PLoS ONE* 2015; 10: e0122885.
- Mettang T, Mattered U, Roth HJ, Weishaar E. Lacking evidence for calcium-binding protein Fetuin-A to be linked with chronic kidney disease-related pruritus (CKD-rP). *NDT Plus* 2010; 3: 104–105.
- Weishaar E, Weiss M, Passlick-Deetjen J, Tschulena U, Maleki K, Mettang T. Laboratory and dialysis characteristics in hemodialysis patients suffering from chronic itch – results from a representative cross-sectional study. *BMC Nephrol* 2015; 16: 291.
- Olauson H, Vervloet MG, Cozzolino M, Massy ZA, Ureña Torres P, Larsson TE. New insights into the FGF23-Klotho axis. *Semin Nephrol* 2014; 34: 586–597.
- Shirazian S, Schanler M, Shastry S, Dwivedi S, Kumar M, Rice K, et al. The effect of ergocalciferol on uremic pruritus severity: a randomized controlled trial. *J Ren Nutr* 2013; 23: 308–314.