High dickkopf-1 levels in sera and leukocytes from children with 21-hydroxylase deficiency on chronic glucocorticoid treatment

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INTRODUCTION

Children with 21-hydroxylase deficiency (21-OHD) need chronic glucocorticoid (cGC) therapy to replace congenital deficit of cortisol synthesis, and this therapy is the most frequent and severe form of drug-induced osteoporosis. Therefore, 21-OHD patients are at risk of a great incidence of low bone mass. In this study, we enrolled 18 patients (9 females) and 18 sex- and age-matched controls and we evaluated the serum and leukocyte levels of dickkopf-1 (DKK1), a secreted antagonist of the Wnt/β-catenin signaling pathway known to be a key regulator of bone mass. We also studied the effects of dexamethasone on the expression of DKK1 in human leukocytes from controls in vitro. Finally, we examined the effects of the conditioned media by the serum of the patients on osteoblast (OB) differentiation and RANKL expression.

High DKK1 serum levels in 21-OHD patients

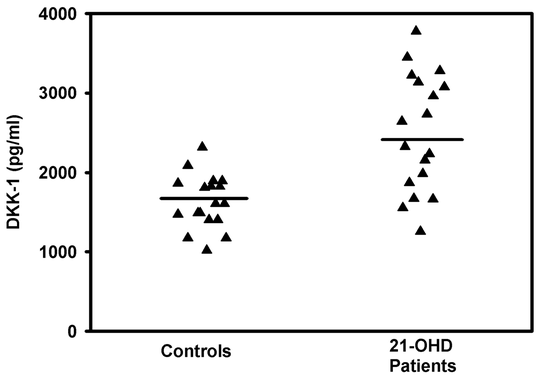


Figure 1. DKK1 serum concentrations were significantly elevated in patients with 21-OHD (2413 ± 218 pg/mL, range 1255–3776 pg/mL) compared to control subjects (1660 ± 88 pg/mL, range 1019–2318 pg/mL), p< 0.004.

High DKK1 expression in circulating leukocytes from 21-OHD patients

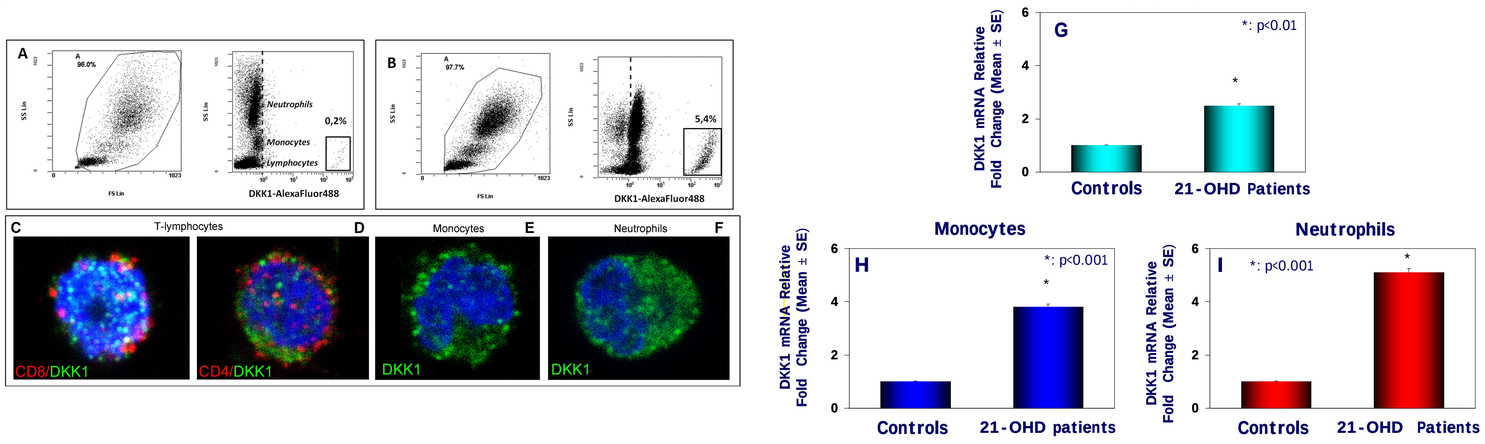


Figure 2. Flow cytometry analysis of DKK1 expression in permeabilized leukocytes revealed that DKK1 was significantly higher in all cell blood population from patients (B) compared with the age-matched normal controls (A), especially in lymphomonocytes. Data shown are gated on all blood cell populations and quadrants were established based on DKK1-intracellular staining versus cell side scatter (SS). Confocal micrographic images showing coimmunostaining of DKK1 (green) protein and CD8+ (red) or CD4+ (red) T lymphocytes (C-D) from patients. Strong DKK1 staining in monocytes and neutrophils were also observed (E-F). Nuclei were counterstained with TO-PRO-3 Iodide (blue). The high expression of DKK1 was also detected in T lymphocytes, monocytes and neutrophils from patients by real-time PCR (G-I).

Effect of dexamethasone on DKK1 expression in human leukocytes

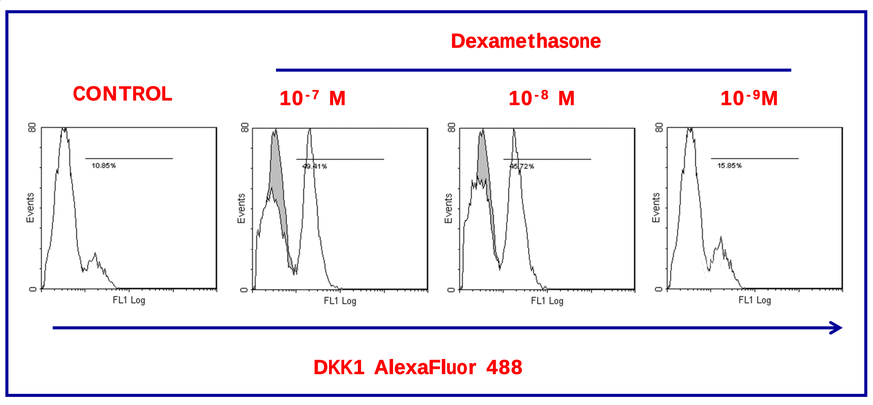


Figure 3. Flow cytometry analysis of DKK1 expression in 6 hour dexamethasone stimulated heparinized blood from controls showed that in permeabilized leukocytes DKK1 expression increase, reached the maximum amounts at 10^{-7} M dexamethasone, maintained elevated levels at 10^{-8} M, and thus returned at control levels at 10^{-9} M. The grey histograms signify staining with isotype control, the white histograms represent the staining with anti-DKK1 monoclonal antibody.

Effect of serum from 21-OHD patients on osteoblast differentiation in vitro

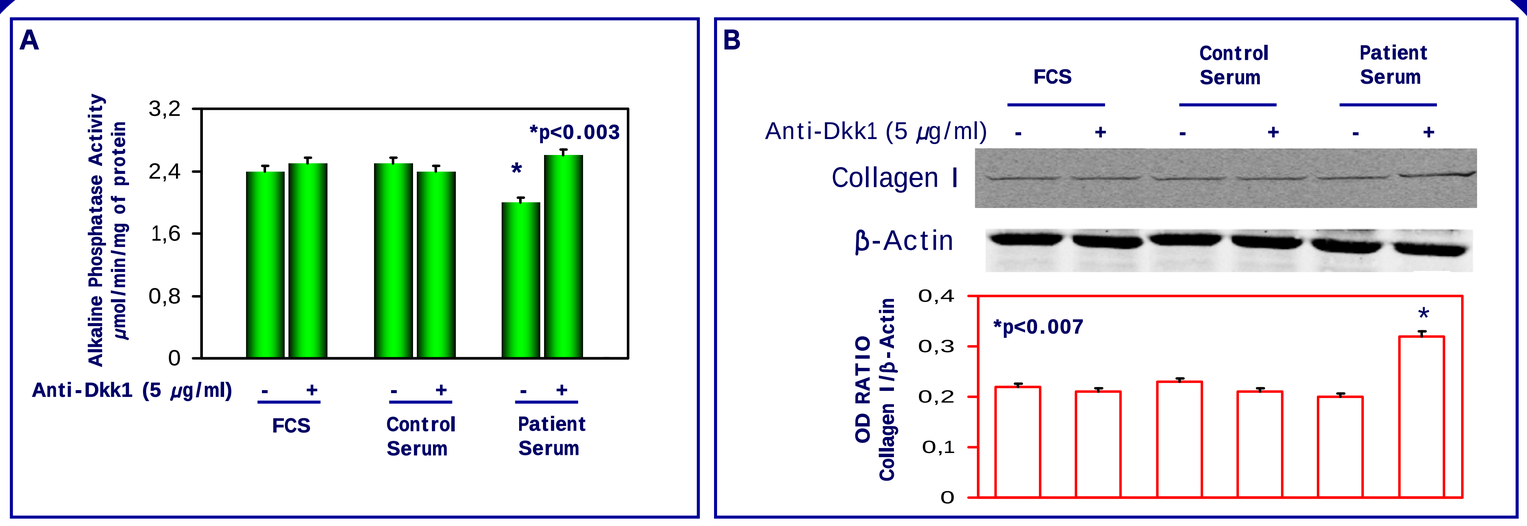


Figure 4. Human OBs were cultured with BMP2 in basal condition (medium + 10% FCS), in conditioned media with sera from patients or controls, in presence or in absence of anti-DKK1 neutralizing antibody. In all the described condition the activity of alkaline phosphatase was evaluated (A) as well as collagen I expression by western blot analysis (B). In OBs cultured in the presence of sera from patients, anti-DKK1 antibody positively affect both alkaline phosphatase activity and collagen I expression. The intensity of the bands obtained by western blot was quantified by densitometry (histogram) and normalized to β-Actin.

Effect of serum from 21-OHD patients on osteoblast expression of RANKL and OPG

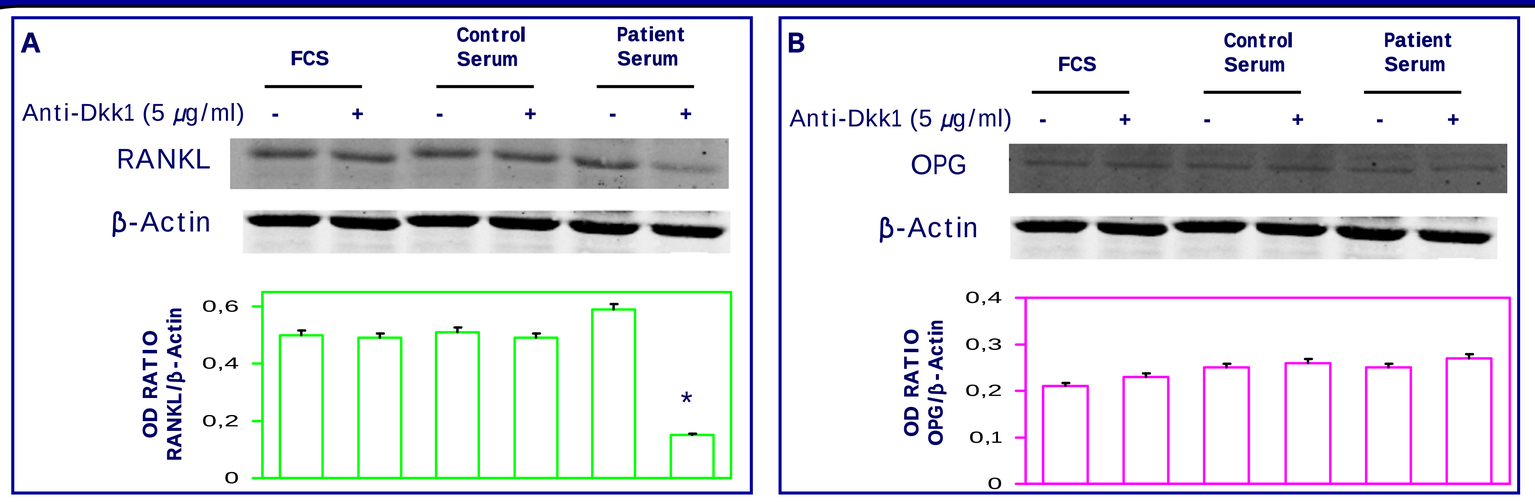
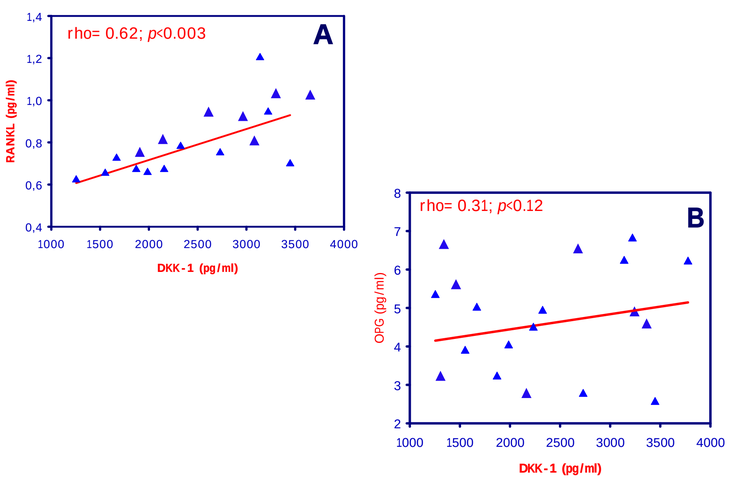


Figure 5. Human OBs were cultured with BMP2 in basal condition (medium + 10% FCS), in conditioned media with sera from patients or controls, in presence or in absence of anti-DKK1 neutralizing antibody. In all the described condition RANKL (A) as well as OPG (B) expression was evaluated by western blot analysis. In OBs cultured with FCS or serum from controls, the anti-DKK1-neutralizing mAb did not exert effects on RANKL or OPG expression. On the contrary, the anti-DKK1 mAb strongly down-regulated the RANKL expression and did not affect the OPG expression by OBs The intensity of the bands obtained by western blot was quantified by densitometry (histogram) and normalized to β-Actin. *p<0.003

High DKK1 and RANKL serum levels significantly correlate



The graph showed the direct correlation between DKK1 and RANKL serum levels (rho= 0.62; p<0.003) in sera from 21-OHD patients (A). No significant correlation were found between DKK1 and OPG serum levels (B)

CONCLUSIONS

Our data indicated that DKK1, produced by leukocytes, may contribute to the alteration of bone remodeling in 21-OHD patients on cGC treatment.