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Soluble α-Klotho — a new marker of acromegaly activity?

Rozpuszczalne białko α-Klotho — nowy marker aktywności akromegalii?

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Abstract

Introduction: Klotho is a transmembrane protein that attenuates insulin/insulin-like growth factor-1 (IGF-1) signalling and appears to be involved in ageing. Recent data suggest that soluble α-Klotho (sKlotho) is also elevated in acromegaly.
The aim of this study was to assess serum levels of sKlotho in patients in relation to the activity of the disease and to compare with the control group.
Material and methods: We studied 55 patients with acromegaly and 29 healthy controls (CG). Patients were divided into three subgroups according to minimal GH (growth hormone) concentration during the oral glucose tolerance test (OGTT) and the IGF-1 concentration: a surgically cured acromegalic group (SCA), well-controlled acromegalic group (WCA), and active acromegaly group (AA). In all subjects, blood samples were taken to assess the concentration of sKlotho, GH, IGF-1, and biochemical markers.
Results: Soluble α-Klotho was highest in the AA group and lowest in the SCA group. The differences in sKlotho levels were statistically significant when the AA group was compared to the SCA, WCA, and CG groups ($p = 0.000$, $p = 0.002$, $p = 0.001$, respectively). There were no significant differences in sKlotho levels among the SCA, WCA, and CG groups. sKlotho positively correlated with GH levels in the WCA and WCA + SCA groups ($r = 0.666$, $p = 0.009$; $r = 0.366$, $p = 0.047$, respectively) and with the IGF-1 level in the AA group ($r = 0.589$, $p = 0.021$).
Conclusions: sKlotho is increased in active acromegaly and normalises after successful treatment. It could be a new biomarker of acromegaly activity. **(Endokrynol Pol 2016; 67 (4): 390–396)**

Key words: *growth hormone; IGF-1; activity of disease; acromegaly; α-Klotho*

Streszczenie

Wstęp: Klotho to białko transbłonowe, osłabiające sygnał na szlaku insulina/insulinopodobny czynnik wzrostu-1 (IGF-1), biorące prawdopodobnie udział w procesie starzenia się organizmu. Najnowsze dane wskazują, że stężenie rozpuszczalnego białka Klotho (sKlotho) jest również podwyższone w akromegalii.
Celem badania była ocena stężenia rozpuszczalnej formy białka Klotho w surowicy pacjentów chorujących na akromegalię w zależności od aktywności choroby w porównaniu z grupą kontrolną.
Materiał i metody: W badaniu wzięło udział 55 pacjentów z akromegalią i 29 zdrowych pacjentów tworzących grupę kontrolną (CG). Pacjentów z akromegalią podzielono na trzy podgrupy na podstawie minimalnego stężenia hormonu wzrostu (GH) podczas testu doustnego obciążenia glukozą (OGTT), oraz stężenia IGF-1: grupa wyleczonej operacyjnie akromegalii (SCA), grupa dobrze kontrolowanej akromegalii (WCA) i grupa aktywnej akromegalii (AA). U wszystkich osób biorących udział w badaniu pobrano krew celem oznaczenia stężenia sKlotho, GH, IGF-1 i parametrów biochemicznych.
Wyniki: Średnie stężenie rozpuszczalnego białka α-Klotho było największe w grupie AA, a najmniejsze w grupie SCA. Różnice stężeń sKlotho były istotne statystycznie, gdy grupę AA porównano z grupą SCA, WCA i CG ($p = 0{,}000$, $p = 0{,}002$, $p = 0{,}001$, odpowiednio). Nie stwierdzono istotnych różnic stężenia sKlotho pomiędzy pozostałymi grupami, tj. SCA, WCA i CG. sKlotho korelowało dodatnio ze stężeniem GH w grupie WCA i WCA + SCA ($r = 0{,}666$, $p = 0{,}009$; $r = 0{,}366$, $p = 0{,}047$, odpowiednio) i stężeniem IGF-1 w grupie AA ($r = 0{,}589$, $p = 0{,}021$).
Wnioski: Stężenie sKlotho jest podwyższone w aktywnej akromegalii i ulega normalizacji po skutecznym leczeniu. Białko Klotho może być nowym markerem aktywności akromegalii. **(Endokrynol Pol 2016; 67 (4): 390–396)**

Słowa kluczowa: *hormon wzrostu; IGF-1; aktywność choroby; akromegalia; α-Klotho*

Introduction

Acromegaly is a rare disease, characterised by excessive growth hormone (GH) and insulin-like growth factor-1 (IGF-1) secretion. GH excess is due to benign adenoma in 99% cases [1]. Currently GH and IGF-1 (a GH dependent, mainly liver-delivered hormone) are the biochemical markers for a confirmed disease and also for assessing the activity of the disease. According to recent guidelines in patients with typical symptoms of acromegaly, a test for IGF-1 concentration should be done. If the IGF-1 level is increased, it is obligatory to perform OGTT after an administration of 75 g of glucose [2]. The diagnosis and monitoring of the disease

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could be difficult when two parameters are discrepant. It is known that several factors can influence the relationship between the level of GH and IGF-1, such as gender, age, liver disease, and malnutrition. IGF-1 and GH are known to have biological and technical limitations [3, 4]. These facts influence long-term follow-up of acromegalic patients, as well as an early diagnosis of recurrence, and make them difficult. A new sensitive and specific biomarker is needed for the diagnosis and monitoring of acromegaly.

Klotho is a transmembrane protein, named after a Greek goddess. From Greek mythology "Klotho" was one of the three Fates, and she was responsible for spinning the thread of human life. It was shown that Klotho-deficient mice *(kl/kl)* had a shortened life and a lot of disorders caused by human ageing, including atherosclerosis, osteoporosis, skin atrophy, and pulmonary emphysema [5]. On the other hand, overexpression of Klotho prolongs the lifespan [6, 7]. Various functions of Klotho have been described until now. It is known that Klotho protein exists in two forms with different functions. Membrane-bound Klotho acts as a co-receptor for fibroblast growth factor-23 (FGF-23) and consequently has an important role for the renal function of FGF-23, serving as a major regulator of phosphate homeostasis [8]. Klotho can be enzymatically cleaved, shed, and released as soluble α-Klotho into blood, urine, and cerebrospinal fluid. An association between sKlotho, GH, and IGF-1 secretion has been suggested. First, it was revealed that *(kl/kl)* mice were smaller and their somatotrophs showed atrophy and a reduced number of secretory granules [5]. Soluble α-Klotho could also intensify the activity of calcium channels TRVP5/6 and it could be a potential inhibitor of action of the insulin and IGF-1 track [6, 8]. Recently, decreased sKlotho levels have been observed in children with organic growth hormone deficiency (GHD) [9], and also a strong association between sKlotho and IGF-1 was revealed in a study comprising 159 healthy children [10]. On the other hand, it was also found that soluble α-Klotho is increased in patients with active acromegaly and normalised after successful removal of GH-producing pituitary adenoma, at least as quickly as IGF-1 [11, 12]. What is more, the level of sKlotho depends on gender and it is higher in women with acromegaly [13]. Up to this time, the mechanism that leads to the high level of sKlotho in active acromegaly is not clear. It is known that in acromegaly there are high levels of FGF-23, phosphate, and calcitriol and also FGF-23 resistance [13–14, 16]. On the other hand, it was shown that the high level of sKlotho is a consequence of increased pituitary GH secretion, and higher pituitary transmembrane Klotho expression was not observed [7, 12].

The aim of our case-control study was to assess the serum levels of soluble α-Klotho in acromegalic patients in relation to the activity of disease and to compare it with the control group (CG). We also analysed the relationship between GH and IGF-1 and serum levels of soluble α-Klotho in patients with acromegaly and the control group.

Material and methods

We enrolled 55 patients with acromegaly and 29 healthy subjects as the control group (CG). The acromegaly patients, 39 women and 16 men, were divided into three groups based on the clinical findings and biochemical evaluation (the minimal GH concentration during OGTT test and IGF-1 concentration). Normal, according to the age and sex, concentration of IGF-1 and GH level during OGTT test below 1 μg/L (ng/mL) was used as a criterion of cure or good control of the disease.

Eighteen patients with cured acromegaly were assigned to the surgically cured acromegalic group (SCA). Seventeen patients during the treatment with a long-acting somatostatin analogue were in the well-controlled acromegalic group (WCA). Twenty patients, who did not meet the criteria for the cure or good control of the disease, were in the active acromegalic group (AA). All subjects in the AA and WCA groups were treated with long-acting octreotide LAR. In the AA group two subjects received a 10 mg/dose, five subjects a 20 mg/dose, and 13 subjects a 30 mg/dose. Not all of the AA patients received the dose of 30 mg due to drug intolerance. In the WCA group six patients received a 20 mg/dose and 11 patients a 30 mg/dose. There was no statistically significant difference between the numbers of patients requiring hormonal replacement therapy in each subgroup. Among the study groups 23 acromegalic patients required the hydrocortisone and 22 the L-thyroxin replacement. Thirteen acromegalic patients with diabetes mellitus type 2 were treated by oral hypoglycaemic agents. None of the patients received insulin therapy. Nineteen subjects among the studied received statin therapy: five in the AA group, four in the WCA, and five in the SCA and the CG groups, respectively. None of them received substitution of calcium, or vitamin D. All patients had normal liver and kidney function-tests at the time of the study. All subjects were recruited from the patients of the Department of Endocrinology, Diabetes, and Isotope Therapy, Wroclaw Medical University. We selected a control group (CG) of 29 patients by matching subjects and controls by sex and age. The control group showed no clinical symptoms of acromegaly and had normal value of IGF-1 and GH. The protocol of the study

Table I. *General characteristics of patients with acromegaly and control group (all groups)*
Tabela I. *Charakterystyka pacjentów z akromegalią i grupy kontrolnej (wszystkie grupy)*

Group	No	Age (years)	Body mass [kg]	Height [m]	BMI [kg/m²]	Heart rate [beat/min]
AA	20	50.85 ± 5.24	88.1 ± 21.89*	1.73 ± 0.11#^v	29.31 ± 5.18	70.45 ± 7.27#
WCA	17	55.35 ± 12.45	81.47 ± 14.44	1.65 ± 0.09	29.73 ± 4.73*	63.35 ± 6.7*^
SCA	18	54.00 ± 11.52	88.0 ± 14.59*	1.65 ± 0.1	31.71 ± 4.48*	69.39 ± 9.75
CG	29	47.86 ± 15.76	74.78 ± 14.9	1.67 ± 0.09	26.83 ± 4.53	69.59 ± 5.2
SCA + WCA	35	54.66 ± 11.83	84.83 ± 14.68*	1.66 ± 0.09	30.75 ± 4.65*	66.46 ± 8.83
AA + SCA + WCA	55	53.70 ± 13.6	86.02 ± 17.52*	1.68 ± 0.11	30.23 ± 4.85 *	67.91 ± 8.46

*$p < 0.05$ compared to CG group; #$p < 0.05$ compared to WCA group; ^$p < 0.05$ compared to SCA group; ᵛ$p < 0.05$ compared to WCA + SCA group; AA — the active acromegalic group; WCA — the well-controlled acromegalic group; SCA — the surgically cured acromegalic group; CG — the control group

was approved by the local Bioethics Committee, and informed consent was obtained from all the subjects.

The analyses were performed based on the division into groups. The first division was done on the basis of the activity of the disease (AA; WCA; SCA). The second classification was used to analyse the differences between the AA group, a group of patients with cured and well-controlled acromegaly (WCA + SCA), and controls (CG). The third classification compared the patients with acromegaly (AA + WCA + CA) and the control group (CG). Body weight (kg), height (m), and blood pressure (mmHg) were measured in all patients. The body mass index was calculated. The GH concentrations were measured by a chemiluminescent immunometric method (Immulite 2000, Siemens, USA or Germany). Serum IGF-1 level was assessed by radioimmunologic assay using an IGF-1-D-RIT-CT kit (BioSource S.A, Nivelles, Belgium), normal range: according to the sex and age. Vitamin D was assessed only in some patients (15 AA, 14 WCA, 16 SCA, and 24 CG) by radioimmunologic assay using a 25OH-Vit.D3-Ria-CT kit (DIAsource, Louvain-la-Neuve, Belgium). We used the following ranges of 25(OH)D concentrations indicating vitamin D deficiency (< 20 ng/mL [< 50 nmol/L]), suboptimal status (20–30 ng/mL [50–75 nmol/L]), and optimal status (30–50 ng/mL [75–125 nmol/L]). The intra- and inter-assay coefficients of variation (CVs) were 7.3% and 7.2%, respectively. Seasonality was based on the date of the blood sample collection. The October-April period corresponded to the winter season and the May-September period corresponded to the summer season. Serum calcium, inorganic phosphate, magnesium, and alkaline phosphatase were measured using colorimetric assays on an Architect c4000 (Abbott Laboratories, Abbott Park, IL, USA). Reference ranges were as follows: calcium 4.2–5.15 mEq/L; inorganic phosphate 2.7–4.5 mg/dL; magnesium 1.6–2.6 mg/dL; and alkaline phosphatase 40–150 IU/L. Human soluble α-Klotho levels were measured by commercially available immunoenzymatic method using the Human Soluble α-Klotho Assay Kit-IBL (Immuno-Biological Laboratories, Japan); measurement range: 93.75–6 000 pg/mL.

Statistical analysis

Statistical analysis was done by the computer program Statistica for Windows, version 7.0. Parameter distribution was assessed using Shapiro-Wilk's test. When a distribution was normal with equal statistical variance, Student's t test was taken for the assessment of the statistically significant differences. Mann-Whitney's U-test was used to assess statistically significant differences for other parameters. Pearson's test or the Spearman's rank correlation test R was used to assess the correlation between traits, depending on the kind of distribution. As a level a statistical significance a p value < 0.05 was used.

Results

The highest mean body mass was observed in the AA group and the lowest in the CG group (Table I). We found statistically significant differences between groups: AA and CG ($p = 0.014$); SCA and CG ($p = 0.005$); WCA + SCA and CG ($p = 0.009$); AA + WCA + SCA and CG ($p = 0.004$). The highest mean height was in the AA group, while WCA had the lowest. The comparison between the AA group and WCA, SCA, and WCA + SCA groups revealed statistically significant differences ($p = 0.023$, $p = 0.048$, $p = 0.13$, respectively). The SCA group had the highest BMI and the CG had the lowest. The difference was statistically significant when the CG was compared to the WCA, SCA, WCA + SCA, and AA + WCA + SCA groups ($p = 0.003$; $p = 0.001$; $p = 0.001$; $p = 0.003$, respectively). We did not find statistically significant differences between mean age and mean blood pressure among the groups (independent on

used the division), except a difference of mean systolic blood pressure between WCA + SCA *vs.* CG (p = 0.04). The highest mean heat beat was in the AA group and the lowest in the WCA group. We found a statistically significant difference between AA and WCA (p = 0.006) and between WCA *vs.* SCA and WCA *vs.* CG (p = 0.052; p = 0.004, respectively).

Soluble α-Klotho was highest in the AA group and lowest in the SCA group (Table II). The differences of soluble α-Klotho levels were statistically significant when the AA group was compared to the WCA (Fig. 1), SCA (Fig. 2), and CG groups (p = 0.002; p = 0.000; p = 0.001, respectively). The difference was also statistically significant when the AA group was compared to the SCA + WCA group (p = 0.000). There was no significant difference in the level of soluble α-Klotho among the SCA, WCA, and CG groups. Similarly, the levels of IGF-1 and IGF-1 ULN were highest in the AA group. The differences were statistically significant when the AA group was compared to the CG, WCA, SCA, and WCA + SCA groups (p = 0.000 for all). We did not observe a statistically significant difference between the WCA and SCA groups. GH levels were significantly higher in the AA group compared to the WCA, SCA, CG, and WCA + SCA groups (p = 0.000; p = 0.000; p = 0.000; p = 0.024, respectively). The same was observed when we compared nadir GH in the AA group to the WCA, SCA, CG, and WCA + SCA groups (p = 0.000 for all).

The highest 25(OH) D levels were found in the CG group and the lowest in the AA group (the difference was statistically significant, p = 0.012). In the WCA and SCA groups 25(OH) D concentrations were also lower compared to the CG group; however, the differences were not statistically significant (p = 0.109, p = 0.473; respectively). The differences in 25(OH)D levels among the AA, WCA, and SCA groups were not statistically significant. Based on the third classification (AA + WCA + SCA vs. CG), higher 25(OH)D concentrations were found in the CG group, and the difference was statistically significant (p = 0.047).

Calcium concentrations were highest in the AA group, but the differences compared to other groups were not statistically significant. We also did not find any significant differences in magnesium levels among the groups. The inorganic phosphate level was highest in the AA group and we noticed statistically significant differences when the AA group was compared to the CG, WCA, SCA, and WCA + SCA groups (p = 0.012; p = 0.029; p = 0.015; p = 0.006, respectively) (Table II).

Soluble α-Klotho positively correlated with GH levels in the WCA and WCA + SCA groups (r = 0.666, p = 0.009; r = 0.366, p = 0.047, respectively) and with nadir GH in the AA + WCA + SCA group (r = 0.51, p = 0.000). We observed a positive correlation between sKlotho

Table II. *Laboratory test results in the study and control groups*
Tabela II. *Wyniki analizy statystycznej w grupie badanej i grupie kontrolnej*

Group	GH 0 min [ng/mL] [μg/L]	GH 120 min [ng/mL] [μg/L]	IGF-1 [ng/mL]	Soluble α-Klotho [pg/mL]	25(OH)D [ng/mL] winter (10–60) summer (20–70)	Calcium [mEq/L] (n. 4.2–5.15)	Inorganic phosphate [mg/dL] (n. 2.7–4.5)	Alkaline phosphatase [U/L] (n. 40–150)	Magnesium [mg/dL] (n. 1.6–2.6)
AA	11.5 ± 14.93*#^v	9.65 ± 10.56*#^v	540.5 ± 263.9*#^v	1862 ± 1596.16*	11.06 ± 6.58*	4.88 ± 0.22	3.87 ± 0.65*#^v	129.17 ± 59.92	1.99 ± 0.25
WCA	2.21 ± 2.11*	1.44 ± 1.5*^	215.5 ± 129.7	693.03 ± 274.52*	12.95 ± 5.55	4.69 ± 0.72	3.36 ± 0.47	113.40 ± 91.13	1.98 ± 0.40
SCA	1.32 ± 2.04	0.98 ± 2.79	164.9 ± 86.1	599.69 ± 284.35*	16.03 ± 5.86	4.80 ± 0.29	3.43 ± 0.39	89.29 ± 22.32	1.93 ± 0.37
CG	1.51 ± 2.82	0.50 ± 1.09	153.9 ± 79.6	670.01 ± 255.39	18.70 ± 9.90	4.78 ± 0.29	3.37 ± 0.57	143.47 ± 158.26	2.04 ± 0.28
WCA + SCA	1.75 ± 2.09*	1.20 ± 2.84*	189.9 ± 110.9	643.25 ± 279.01*	14.59 ± 5.83	4.75 ± 0.53	3.40 ± 0.42	103.47 ± 70.77	1.95 ± 0.37
AA + WCA + SCA	5.32 ± 10.19*	4.27 ± 7.7	317.5 ± 247.4*	1049,82 ± 1095.47*	13.42 ± 6.25*	4.80 ± 0.44	3.59 ± 0.57	112.86 ± 66.35	1.96 ± 0.32

*p < 0.05 compared to CG group; #p < 0.05 compared to WCA group; ^p < 0.05 compared to SCA group; vp < 0.05 compared to WCA + SCA group; AA — the active acromegalic group; WCA — the well-controlled acromegalic group; SCA — the surgically cured acromegalic group; CG — the control group

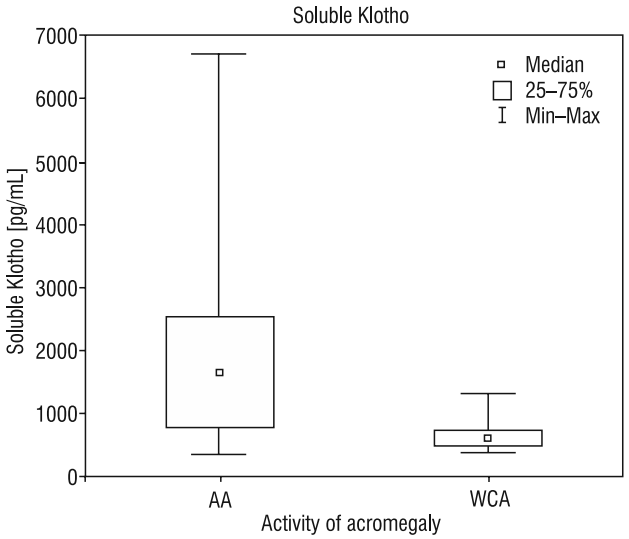

Figure 1. *Concentration of soluble α-Klotho in relation to the activity of acromegaly (AA — active acromegaly group, WCA — the well-controlled acromegalic group). AA vs. WCA, p = 0.002*

Rycina 1. *Stężenie rozpuszczalnego białka Klotho w zależności od aktywności akromegalii (AA — grupa aktywnej akromegali, WCA — grupa dobrze kontrolowanej akromegalii). AA vs. WCA, p = 0,002*

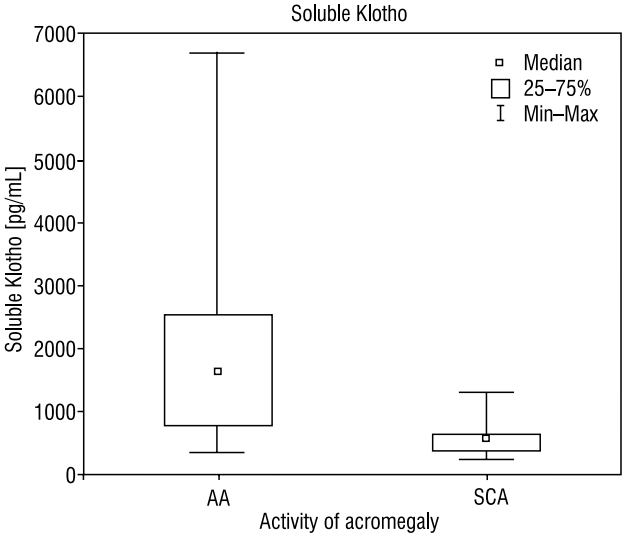

Figure 2. *Concentration of soluble α-Klotho in relation to the activity of acromegaly (AA — active acromegaly group, SCA — surgical cured acromegalic group). AA vs. SCA, p = 0.000*

Rycina 2. *Stężenie rozpuszczalnego białka Klotho w zależności od aktywności akromegalii (AA — grupa aktywnej akromegalii, SCA — grupa wyleczonej operacyjnie akromegalii). AA vs. SCA, p = 0,000*

and IGF-1 in the AA group (r = 0.589, p = 0.021) (Fig. 3) and IGF-1 ULN in the WCA + SCA and AA + WCA + SCA groups (r = 0.46, p = 0.009; r = 0.73, p = 0.000, respectively). We did not observe any correlation be-

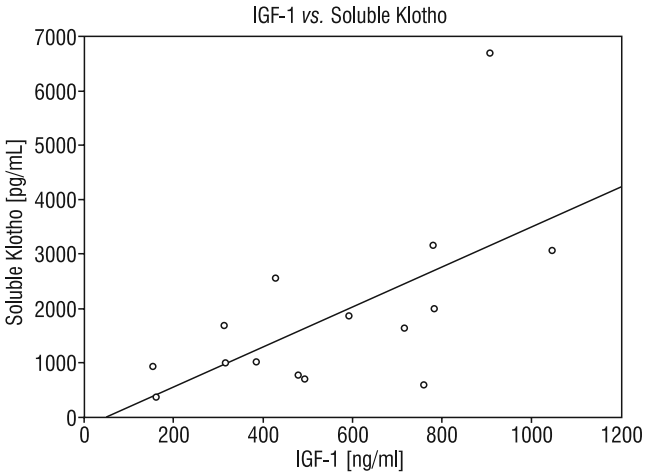

Figure 3. *Correlation between soluble α-Klotho and IGF-1 in the active acromegaly (AA) group (r = 0.589, p = 0.021)*

Rycina 3. *Korelacja stężenia białka Klotho ze stężeniem IGF-1 w aktywnej akromegalii (AA) (r = 0,589, p = 0,021)*

tween sKlotho and anthropometric parameters, but we observed a correlation between sKlotho and vitamin D (25OHD). sKlotho correlated positively with vitamin D in SCA and SCA + WCA groups (r = 0.514, p = 0.020; r = 0.439, p = 0.015, respectively). We also found significant positive correlation between inorganic phosphate levels and IGF-1 in the AA group (r = 0.582, p = 0.014) and GH concentrations at 0′ in the CG (r = 0.516, p = 0.028). Calcium levels correlated negatively with GH at 0′ in CG (r = –0.641, p = 0.014.). There was no correlation between sKlotho and calcium and magnesium levels. Soluble Klotho correlated positively with inorganic phosphate in the AA + WCA + SCA groups (r = 0.419, p = 0.013).

Discussion

Recent studies suggest that soluble α-Klotho is elevated in patients with active acromegaly and decreases towards the normal after successful surgical treatment. Our present study confirms the earlier findings of highly significant difference in sKlotho levels in patients with active acromegaly to other groups [11–13]. The difference was statistically significant when we compared the AA group to the SCA and WCA groups as well as to the CG group, so it was independent of methods of therapy or health conditions. The lowest level of sKlotho was observed in patients with surgically cured acromegaly. It had been suggested before that elevated sKlotho is specific for GH-positive adenomas as a consequence of systemic action of GH rather than local sKlotho expression by the adenoma. Neidert et al. presented patients with acromegaly having signifi-

cantly higher levels of sKlotho compared to the control group patients with other adenomas, and the level of sKlotho declined after surgery during early as well as late follow-up [11]. The mechanism by which sKlotho increased in active acromegaly is unclear. It could be a consequence of distinct transcript or a result of ectodomain shedding of membrane Klotho (mKlotho). Two members of the 'A Disintegrin and Metalloproteinase' (ADAM) family, ADAM10 and ADAM17 (α-secretases), have been suggested as responsible enzymes. It is speculated that the activity of these enzymes could by regulated directly by GH or indirectly by factors or proteolytic activity induced by GH [15–17]. Varewijck et al. observed a positive-correlation between sKlotho and IGF-1 in the active acromegaly group. The same correlation was observed in our study. They suggest two potential causes for the relation between IGF-1 and sKlotho. Firstly, sKlotho could be an independent marker of the activity of acromegaly as IGF-1. On the other hand, some researchers tried to explain that that high level of sKlotho is a consequence of the adaptation mechanism, by which the body attenuates increased IGF-1 action. It has been observed that sKlotho may suppress the activation of IGF-1 receptor (IGF1R) [6, 18].

High levels of sKlotho can be observed without changes in mKlotho expression. In this study we observed a statistically significant increased level of phosphate in the AA group compared to the CG, WCA, SCA, and WCA + SCA groups and a positive correlation between sKlotho and phosphate in the AA + SCA + WCA groups. Acromegaly has unique biochemical and endocrine characteristics. High serum phosphate and FGF23 levels are observed despite the enhanced GFR, and it also leads to insulin resistance (IR) and hyperglycaemia. What is more, a high level of calcitriol is also detected. It is suggested that increased mKlotho-proteolytic enzymes activity could lead to decreasing mKlotho and to impaired FGF23 signalling, which explains FGF23 resistance. As a consequence of increased activity of enzymes, sKlotho is increased and contributes to IR [7]. As we observed in our previous study 25(OH)D correlated positively with IGF-1 only in AA group [19]. It confirmed that the mechanism of regulation of FGF-23-membrane-Klotho complex and sKlotho excess is different in acromegaly.

The Klotho gene was identified as an aging suppressor gene in mice, so it suggests that the level of sKlotho should be decreased with age in healthy subjects. We did not observe any negative correlation between sKlotho and age in our groups. These results are consistent with earlier ones and confirm an autonomous GH secretion of sKlotho [11].

It seems that sKlotho could be a good indicator of activity of acromegaly but there are some questions related to its use as a marker in acromegaly. First of all, different assays were used to determine sKlotho in human serum. The Elisa system seems to be the best option but the three commercial assays differ in quality. Additionally, the normal range for serum levels of sKlotho cannot be known. Neidert et al. measured the level of sKlotho in the sera of 26 volunteers and the median was 596 pg/mL (506–734) [12]. On the other hand, Yamazaki et al. reported the reference serum of sKlotho levels in 142 volunteers: the range of values was from 239 to 1266 pg/mL (mean ± SD: 526 ± 146 pg/mL). In their study the levels were not influenced by gender or by skeletal metabolism but correlated negatively with serum creatinine level and age. What is more, additional analysis with Asiatic children (23 boys and 16 girls) revealed significantly greater concentrations of sKlotho (952 ± 282 pg/mL) [20]. In another study it was shown that sKlotho has a circadian rhythm and the lowest concentration is around midnight and the highest is in the early morning [21]. Some factors such as age, gender, BMI, and renal function can influence the level of soluble α-Klotho. In our study we did not observe any correlation between soluble α-Klotho and any of the anthropometric measurements. The previous study showed no influence of age, and this was explained by the autonomy of GH adenoma [11]. The impact of BMI is unclear. Semba et al. presented no influence of BMI on the concentration of sKlotho [22]. In another small study it was noticed that anorexia nervosa as well as obesity could be responsible for lower levels of sKlotho [23]. It is very important to remember that acromegaly is also associated with a decrease in fat mass and an increase in lean body mass. This is a result of the lipolytic effect of GH. In this situation BMI does not reflect an increased fat mass as in the normal population [24]. In fact, attenuation of daily activity could be connected with the decrease in sKlotho [25]. It was also observed that levels of IGF-1 and soluble α-Klotho are different with regard to gender. Sze at al. showed that IGF-1 had a tendency to be higher in men whereas soluble α-Klotho levels were greater in females. Lower levels of IGF-1 are caused by the influence of oestrogens in premenopausal women [13].

To sum up, soluble α-Klotho is increased in patients with active acromegaly and normalises after successful treatment. It could be an independent new biomarker of the activity of acromegaly. More study is needed to analyse the influence of gender, BMI, age, and other factors on the level of sKlotho. Moreover, the standardisation of sKlotho assays and normal range is required.

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