REVIEW

The Role of Soluble α-klotho Protein in Acromegaly

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Abstract

Soluble α-klotho (sαKl) is an isoform of the α-klotho protein that regulates calcium metabolism but also has anti-aging, anti-apoptotic, and tumor suppressor effects. The assumption that sαKl could inhibit the insulin-like growth factor 1 (IGF-1) signaling pathway has increased the interest of its research in acromegaly - a pathology characterized by an excess of growth hormone (GH) and IGF-1. This review aimed to identify the role of sαKl in acromegaly. Most studies have identified higher concentrations of sαKl in acromegaly versus non-GH pituitary adenomas (PAs) or healthy subjects. Also, the sαKl level was higher in active acromegaly versus controlled or cured disease. Moreover, sαKl positively correlated with GH, IGF-1, and GH-secreting PA size. The increased level of sαKl seems to be due to the autonomous secretion of GH, considering their drastic decrease after transsphenoidal surgery. The data regarding the relationship between sαKl, age, and gender in acromegaly are inconsistent. The latest research noted a correlation between sαKl and various parameters for monitoring the quality of life in acromegaly. In conclusion, sαKl could reflect the disease activity. Furthermore, sαKl would represent an alternative for monitoring acromegalic patients with discrepancies between IGF-1 and GH.

Keywords: soluble α-klotho protein, acromegaly, growth hormone-secreting pituitary adenoma

Rezumat

α-klotho solubilă (sαKl) este una dintre izoformele proteinei α-klotho, ce intervine în reglarea metabolismului calciului, având totodată și acțiune anti-îmbătrânire, antiapoptotică şi antitumorală. Ipoteza conform căreia sαKl ar inhiba calea de semnalizare a factorului de creștere de tip insulinic 1 (IGF-1) a crescut interesul cercetării acesteia în acromegalie - patologie caracterizată printr-un exces de hormon de creștere (GH) și IGF-1. Recenzia de față și-a propus să identifice rolul sαKl în acromegalie. Majoritatea studiilor au identificat concentrații înalte ale sαKl în acromegalie în comparație cu alte tipuri de adenoame hipofizare sau cu voluntarii sănătoși. Nivelul sαKl a fost mai înalt în acromegalia activă față de boala controlată sau vindecată. În plus, sαKl s-a corelat pozitiv cu GH-ul, IGF-1 și dimensiunea adenomului hipofizar. Concentrația înaltă a sαKl s-ar datora secreției autonome de GH, având în vedere scăderea drastică a acesteia după intervenția chirurgicală transsfenoidală. Datele privitoare la relația dintre sαKl, vârstă și sexul pacienților cu acromegalie au fost inconstante. Cele mai recente cercetări au identificat corelații între sαKl și diverși parametrii de monitorizare ai calității vieții în acromegalie. În concluzie, sαKl ar putea reflecta activitatea bolii şi ar reprezenta o alternativă a monitorizării pacienților acromegali ce prezintă discrepanțe între IGF-1 și GH.

Cuvinte cheie: proteina solubilă α-klotho, acromegalie, adenom hipofizar secretant de hormon de creştere

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INTRODUCTION

Acromegaly is a chronic illness generating metabolic, neoplastic, and cardiovascular complications. In general, acromegaly is due to a pituitary adenoma (PA) that secretes growth hormone (GH) in excess[1]. The diagnosis of acromegaly is established based on the specific phenotype, the lack of GH suppression (<0.4 ng/ml) during the oral glucose tolerance test (OGTT), and the detection of a high insulin-like growth factor-1 (IGF-1)[2]. Measurement of GH and IGF-1 is crucial in the monitoring of acromegaly, establishing the disease status. In some situations, these two biomarkers may be discordant[3,4] or their dosage may have technical limitations[5,6]. Therefore, the identification of new biomarkers in disease monitoring should be considered.

α-Klotho protein (αKl), also known as "the protein of youth", was described more than 20 years ago by Kuru-o M, who found that a defect in αKl gene expression in the animal model produced features of aging such as atherosclerosis, vascular calcifications, osteoporosis, skin atrophy, infertility, etc[7]. Moreover, αKl gene overexpression has implications in inhibiting the aging process and prolonging the lifespan[8]. αKl gene, located on chromosome 13q2 in humans[7,9], is mainly expressed in distal renal tubules, placenta, and choroid plexus, but also in some endocrine glands such as the pituitary gland, the pancreas, the parathyroid glands, the adipose tissue, and the gonads[10-12]. αKl protein, encoded by αKl gene, contains at least two forms: (1) membrane-bound Klotho protein (mKl) that acts as a co-receptor/co-factor for fibroblast growth factor (FGF)-23, which determines the production of 1,25-$(OH)_2D_3$ and inhibition of phosphorus resorption in the kidney and (2) soluble α-Klotho (sαKl) that comes either from the proteolytic cleavage of mKl (via alpha secretase ADAM10 and ADAM17 or beta-secretase 1 (BACE1)) or through alternative RNA splicing[13,14] (Fig. 1). SαKl plays a decisive role in calcium metabolism by modifying the activity of transient receptor potential channels C5 and C6[15]. Studies conducted in the last years have shown that sαKl has multiple functions, such as anti-aging, anti-apoptotic, and tumor suppressor effects[16,17]. SαKl may influence several numbers of signaling pathways such as cAMP, Wnt, p53/21, protein kinase C, nuclear factor αB (NF-αB), transforming growth factor α (TGF-α), insulin, and IGF-1[9,10,18]. The crucial role of sαKl consists of inhibiting the IGF-1 signaling pathway, which acts as a

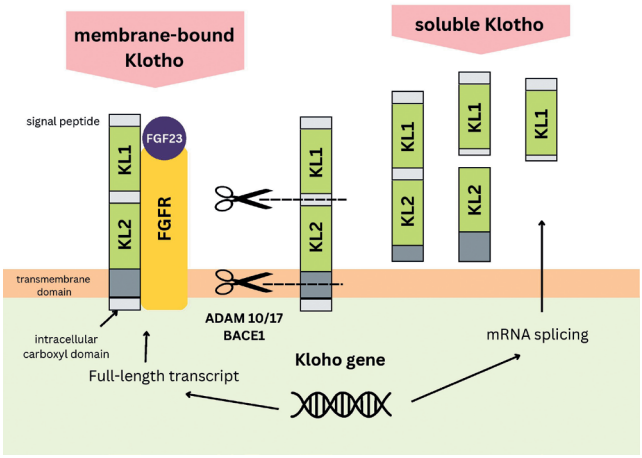

Figure 1 - The structure of α-Klotho protein: (1) membrane-bound Klotho (containing two extracellular domains: KL1 and KL2) acts as a co-receptor/co-factor for FGF-23; (2) soluble α-Klotho that comes from the proteolytic cleavage of membrane-bound Klotho (via ADAM10/ ADAM 17 or BACE1) or through mRNA splicing. FGFR: fibroblast growth factor receptor; ADAM: A Disintegrin and Metalloproteinase; BACE1: beta-secretase 1

negative regulator of GH secretion[10]. More than that, sαKl is a positive regulator of FGF2 signaling pathway, which is involved in the stimulation of GH release[10,19].

A recent study detected a significantly lower level of sαKl in children diagnosed with GH deficiency, which correlated with IGF-1[20]. As expected, a series of studies demonstrated a higher titer of sαKl in active acromegaly, which normalized after transsphenoidal surgery (TSS)[21-27].

This review focuses on the role of sαKl in acromegaly.

SAKL IN ACTIVE ACROMEGALY AND ITS POSTOPERATIVE BEHAVIOR

The promoter of sαKl research in acromegaly was Sze L[21], who performed in 2012, in a prospective manner, a study on 24 naïve acromegalic patients, before and 3 months after TSS. The author observed that naïve patients had a higher level of serum sαKl versus 26 healthy subjects (HS) (mean±SD 4.2±0.6 ng/ml versus 0.6±0.2 ng/ml) (Table 1). Moreover, sαKl correlated with PA diameter, GH, and IGF-1 ($r>0.5$, $p<0.01$). Postoperatively, sαKl decreased to normal for all included patients ($p<0.0001$) (Table 2). After seven years, Sze L[25] confirmed the same postoperative trend

of sαKl in a larger group, consisting of 42 naïve acromegalic patients (p<0.0001) (Table 2). In this research, the preoperative sαKl positively correlated with GH (r=0.5 p<0.01).

Neidert MC[22] obtained similar results with the mentioned above studies, evaluating 46 naïve acromegalic patients, before and after about 2 months postoperatively. The sαKl concentration before TSS was four times higher than the upper limit of normal. The author noticed a decrease of sαKl after TSS from an average of 4053±447 pg/ml to 770±66.66 pg/ml (Table 2). Twelve acromegalic patients, 26 HS, and 7 patients with non-functioning PAs (NFPAs) benefited from the short-term postoperative assessment (average of 5 days). The low value of postoperative sαKl of the twelve acromegalic patients was similar to that of NFPAs and HS (average 889 pg/ml versus 585 pg/ml versus 624 pg/ml). A year later, Neidert MC[28] noted increased levels of sαKl in 14 active acromegalic patients compared to the control group (CG) consisting of 9 operated prolactin-secreting PAs and 13 operated NFPAs (p<0.001) (Table 1). Postoperatively, sαKl decreased significantly in the acromegalic group compared to the baseline (p<0.001) (Table 2). In contrast, the TSS did not significantly change the level of sαKl in acromegaly in comparison with CG. Immunohistochemical staining for sαKl in GH-secreting PAs was weaker than in CG. This last aspect suggested that the high level of sαKl was not due to the overexpression of sαKl in GH-secreting PAs, but more likely due to autonomous GH secretion[28]. In the last research, Neidert MC[29] monitored 55 naïve acromegalic patients before and 7-16 years after TSS. Acromegalic patients were included in three groups: cured disease (n=39), persistent disease after initial remission (n=7), and active disease (n=9). The preoperative level of sαKl was higher in patients with active disease versus those with cured disease (p=0.032). Postoperatively, the levels of GH, IGF-1 and sαKl significantly decreased in all three study groups compared to the baseline (p<0.0001) (Table 2). Despite this aspect, active disease still showed an increased level of sαKl and IGF-1. After TSS, the patients with active disease showed significantly higher levels of sαKl (p<0.0007), IGF-1 (p<0.0001), and GH (p=0.003) compared to cured patients. Before TSS, sαKl positively correlated with GH (r=0.66, p<0.001), IGF-1 (r=0.41, p=0.002), and PA volume (r=0.52, p<0.001). The correlation between sαKl and IGF-1 was maintained even postoperatively (r=0.36, p=0.006).

Schmid C[14] detected increased serum and urinary sαKl in the active acromegaly group (n=9) versus NFPAs (n=6) (Table 1). Also, the nine acromegalic patients manifested a postoperative decrease in sαKl in both biological samples.

Varewijck AJ[23] observed a higher concentration of sαKl in 15 active acromegalic patients versus 11 patients with panhypopituitarism under complete replacement treatment (p<0.001) (Table 1). SαKl levels in CG were comparable to the healthy population[30]. Age-adjusted sαKl positively correlated with total and bioactive IGF-1 (r>0.62, p<0.05). However, there were no significant correlations between sαKl and random GH, IGF-binding protein (IGFBP) 1 or 3.

Jawiarczyk-Przybyłowska A[24] evaluated the serum level of sαKl in 55 acromegalic patients versus 29 HS. The acromegaly group included active (n=20), controlled (n=17), and cured (n=18) patients. The active group showed significantly higher values of sαKl versus HS (p=0.001), cured (p=0.000), and controlled (p=0.002) patients (Table 1). SαKl positively correlated with GH in the controlled group (r=0.66, p<0.01) and with IGF-1 in the active group (r=0.58, p<0.05).

Schweizer JROL[27] investigated 109 acromegalic patients versus CG consisting of 20 NFPAs and 31 HS. The acromegaly group included 29 naïve patients, 47 surgically treated patients, and 33 patients treated by surgery and somatostatin analogs (SSAs). The last two subgroups included controlled, uncontrolled and discordant patients. The research team identified higher concentrations of serum sαKl in patients with naïve acromegaly versus CG (p<0.0001) (Table 1). The sαKl cutoff at 1641 pg/ml accurately discriminated the naïve acromegalic patients from HS (93% specificity, 100% sensitivity). However, no significant differences in sαKl were observed in controlled acromegalic patients versus CG (p>0.99). After TSS, the uncontrolled group had increased sαKl levels compared to controlled acromegalic patients and CG (p<0.01). The postoperative value of sαKl of 1548 pg/ml was considered the cutoff that discriminated the controlled from the uncontrolled acromegalic patients (97.8% specificity, 100% sensitivity). In addition to that, the patients belonging to the discordant groups had significantly lower levels of sαKl than the naïve patients (p<0.02). SαKl positively correlated with random GH (r=0.68, p<0.01), IGF-1 (r=0.8, p<0.01), and IGFBP3 (r=0.72, p<0.01) in the acromegalic group. Instead, sαKl did not correlate with the gender or the PA size (p>0.05).

Recently, Anand G[31] carried out a prospective research on a group of 29 acromegalic patients, before and after TSS (n=21) or TSS combined with medical treatment (n=8). As in the mentioned above studies, serum sαKl was significantly reduced after TSS (p<0.001) (Table 2). Also, the medical-surgical treatment contributed to the significant decrease of sαKl (p<0.01). Furthermore, sαKl had a more significant decrease than IGF-1 in both groups.

Sato T[32] retrospectively investigated seven acromegalic patients and 14 non-GH-secreting PAs before and after TSS. The preoperative sαKl in the acromegaly group was double versus CG (p<0.01) (Table 1). Although, after TSS, the decrease of sαKl occurred in both groups, it was more drastic in acromegaly (p<0.05) (Table 2) and was accompanied by the reduction of GH (p<0.05) and IGF-1 (p<0.001).

Kohler S[33] monitored 48 operated acromegalic patients. Twenty-nine of these patients, with disease remission, had low levels of sαKl, IGF-1, and adequate GH suppression during OGTT (Table 2). At long-term follow-up, two of the 29 patients presented symptoms of recurrence and also associated increases in IGF-1 and sαKl.

Helvaci N[34] evaluated the serum level of sαKl in 49 acromegalic patients compared with 47 HS. The author confirmed that sαKl was significantly increased in acromegaly versus HS (p<0.0001) (Table 1), its level having a strong correlation with IGF-1 (r=0.5) and GH (r=0.51).

Takir M[35] investigated the sαKl level in 54 (42 active and 12 controlled) acromegalic patients and 31 HS. Contrasting with all other studies, the author identified lower concentrations of sαKl in active acromegaly compared to controlled patients and HS but without a statistically significant difference (p>0.05) (Table 1). The increased number of patients with hypertension, diabetes mellitus, and dyslipidemia in the active group could justify the low sαKl level.

Table 1 – SαKl trend in active acromegaly

Reference	Acromegaly group	Control group	Biological sample	Laboratory technique	sαKl trend	P value
Sze L et al, 2012[21]	active disease (n=24), 15M/9F, 28-76 y	HS (n=26)	Serum	ELISA	↑	ns
Schmid C et al, 2013[14]	active disease (n=9), 6M/3F	NFPAs (n=6)	Serum, urine	ELISA	↑	ns
Neidert MC et al, 2013[28]	active disease (n=14), 6M/8F, 41-53 y	operated prolactin-secreting PAs (n=9) and NFPAs (n=13)	Serum	ELISA	↑	<0.001
Varewijck AJ et al, 2014[23]	active disease (n=15), 12M/3F, 53.8±13.9 y	treated panhypopituitarism (n=11)	Serum	ELISA	↑	<0.001
Jawiarczyk-Przybyłowska A et al, 2016[24]	active disease (n=20), 16M/39F, 53.70±13.6 y	controlled acromegaly (n=17), cured acromegaly (n=18), HS (n=29)	Serum	ELISA	↑	<0.01
Helvaci N et al, 2017[34]	active disease (n=49), 20M/29F, 46.7±11.8 y	HS (n=47)	Serum	ELISA	↑	p<0.0001
Sato T et al, 2018[32]	active disease (n=7), 5M/2F, 61.3±7.78 y	non-GH-secreting PAs (n=14)	Serum	ELISA	↑	<0.01
Takir M et al, 2019[35]	active disease (n=42), 19M/23F, 48.18±9.97 y	HS (n=31)	Serum	ELISA	↓	> 0.5
Schweizer JROL et al, 2021[27]	active disease (n=29), 36-62 y	NFPAs (n=20), HS (n=31)	Serum	ELISA	↑	<0.0001

ELISA: enzyme linked immunosorbent assay; F: female; GH: growth hormone; HS: healthy subjects; NFPAs: non-functioning pituitary adenomas; ns: not specified; M: male; PAs: pituitary adenomas; sαKl: soluble α-klotho protein; y: years

Table 2 – Postsurgery sαKl trend in acromegaly

Reference	Number of patients	sαKl trend	P value
Neidert MC et al, 2012[22]	46	↓	ns
Sze L et al, 2012[21]	24	↓	<0.0001
Kohler S et al, 2013[33]	29	↓	ns
Neidert MC et al, 2013[28]	14	↓	<0.001
Schmid C et al, 2013[14]	9	↓	ns
Sato T et al, 2018[32]	7	↓	< 0.05
Sze L et al, 2019[25]	42	↓	< 0.0001
Anand G et al, 2019[31]	29	↓	< 0.001
Neidert MC et al, 2022[29]	55	↓	< 0.0001

ns: not specified; sαKl: soluble α-klotho protein

THE RELATIONSHIP BETWEEN SAKL, AGE, AND GENDER IN ACROMEGALIC PATIENTS

Previous studies found that sαKl gradually decreased with age, especially after 40 years[18,36]. Instead, the sαKl level did not differ according to gender in the healthy population[37].

Sze L[38] investigated the level of sαKl in 62 active acromegalic patients (31 male/31 female). Acromegalic women had a higher level of serum sαKl compared to acromegalic men (p=0.02). The level of sαKl did not vary significantly in women with estrogen deficiency compared to those with estrogen replacement treatment (p=0.27). SαKl positively correlated with gender (p=0.002) and GH (p=0.0001). This study did not observe a relationship between sαKl and age. Similarly, Neidert MC[29] noted a preoperative increased sαKl in acromegalic women versus men (p=0.045). In another study, although the sαKl level decreased postoperatively, it remained elevated in acromegalic women versus men (p=0.007)[39]. In 2019, Sze L[25] did not obtain a statistically significant difference in serum sαKl level between women and men (p=0.19), in a study performed on 42 naïve acromegalic patients.

A moderate negative correlation was observed between age and sαKl in acromegalic patients in two studies (r between -0.45 and -0.53, p<0.05)[21,29]. However, Schweizer JROL[27] detected a weak negative correlation between sαKl and age in 109 acromegalic patients (r=-0.22, p=0.02). The last author did not observe a causal relationship between the sαKl and gender[27]. Thus, the sαKl level might not be influenced by age or gender in acromegaly but by autonomous GH secretion.

SAKL AND THE QUALITY OF LIFE IN ACROMEGALY

Recently, Coopmans EC[26] investigated the relationship between sαKl and the quality of life (QoL) of 54 controlled acromegalic patients. At baseline, the patients were under treatment with first-generation SSAs and pegvisomant. The group was evaluated prospectively nine months after switching to Pasireotide-LAR alone (n=28) or combined with pegvisomant (n=26). SαKl increased significantly at nine months of treatment with Pasireotide-LAR and pegvisomant compared to baseline (p<0.001). Instead, this trend was not observed in patients treated for nine months

with Pasireotide-LAR alone compared to baseline (p>0.05). Two tools evaluated the QoL in this study group: the Acromegaly Quality of Life Questionnaire (AcroQoL)[40] and the Patient-Assessed Acromegaly Symptom Questionnaire (PASQ)[41]. The first questionnaire included 22 questions that investigated the physical and psychological function of acromegalic patients[40]. The second questionnaire included six questions that assessed symptoms of acromegaly such as osteoarthralgia, paresthesia, fatigue, headache, soft tissue swelling, and excessive sweating[41]. Acromegalic patients reported a significant improvement in QoL at nine months after switching to Pasireotide-LAR. Thus, sαKl negatively correlated with the scale of physical function and the global score of AcroQoL (r>-0.34, p<0.02). Also, sαKl positively correlated with the score of osteoarthralgia (r=0.46, p<0.001), soft tissue swelling (r=0.29, p<0.05), and headache (r=0.28, p<0.05) of the PASQ questionnaire, as well as with PASQ total score (r=0.35, p<0.02). No significant differences were noted in improving the QoL in patients treated only with Pasireoride-LAR compared to those treated with Pasireotide-LAR and pegvisomant. Considering that IGF-1 and GH did not correlate with the QoL, sαKl could be considered a helpful biomarker for assessing the QoL of acromegalic patients. At the opposite pole, the study by Varewijck AJ[23] did not identify correlations between sαKl and any parameter for evaluating the patient's QoL.

CONCLUSION

Considering the increased level of sαKl in active acromegaly and its positive correlations with GH, IGF-1, and PA size, it could represent a potential biomarker that reflects the disease activity. This aspect is reinforced by the notable decrease of sαKl following surgical or drug treatment. SαKl is a marker that can predict the QoL of acromegalic patients. In the future, sαKl could be a useful biomarker in monitoring patients with discrepancies between GH and IGF-1.

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