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ORIGINAL ARTICLES

SCREENING FOR ACID-LABILE SUBUNIT DEFICIENCY IN PATIENTS WITH GROWTH HORMONE DEFICIENCY AT A TERTIARY PEDIATRIC ENDOCRINOLOGY CENTER

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ABSTRACT

INTRODUCTION: Acid-labile subunit (ALS) is a glycoprotein, which is produced in the liver in response to growth hormone (GH), with its main role being the formation of a complex with insulin-like growth factor 1 (IGF-1) and IGF-binding-protein-3 (IGFBP-3) in order to extend their circulating half-life and thus support the action of GH. Acid-labile subunit-deficient patients are of research interest because of the unclear incidence of the condition among the short-statured population and the need of specific therapeutic approach.

AIM: The aim of this study is to assess the prevalence of ALS deficiency in a cohort of patients with GH deficiency (GHD) followed up in a tertiary university pediatric endocrinology center.

DESIGN: The study participants were 71 children (76% boys, age range 2–18 years), diagnosed with GHD by 2 standard GH-stimulation tests (max GH <10 ng/mL), on GH therapy, and at mean age at the time of collection of samples: 11.6±3.3 years. Blood serum samples were collected from each patient during the routine visits at the center, and then were stored frozen at -80°C in 0.5 mL aliquots until analysis.

RESULTS: Acid-labile subunit deficiency screening identified serum ALS levels with range from 2.2 to 60 mg/L, with a mean of 17.4±8.7 mg/L. The mean ALS levels were significantly lower than the published ones from subjects without short stature but close to the levels in the referred for GHD patients (6.5±4.8 mg/L). Very low ALS levels (<4.0 mg/L) were detected in 3 (4.2%) of the patients. The low ALS levels corresponded with low SDS_{height} (-2.8±1.2) and low SDS_{IGF-1} (-1.4±1.0) before therapy. In one of the 3 patients, the ALS level (2.2 mg/L) was close to that in patients with IGFALS gene mutations (<1.0 mg/L).

CONCLUSION: The present results show the prevalence of ALS deficiency in the current GH treated cohort and support the evidence that investigation of ALS levels could be helpful in the differential diagnosis of growth disorders.

Keywords: *ALS screening, growth hormone deficiency, GH therapy*

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INTRODUCTION

Acid-labile subunit (ALS) is an 85 kDa glycoprotein, which is produced in the liver in response to growth hormone (GH) (1). The main role of ALS is to form a ternary complex with insulin-like growth factor 1 (IGF-1) and IGF binding proteins 3 or 5 (IGFBP-3 and IGFBP-5) in order to extend their circulating half-life from minutes to more than 12 hours (2). In this way the complex becomes an IGF-1 reservoir, regulating IGF-1 availability, thus supporting the action of GH (3,4). Acid-labile subunit could be bound to IGF-1-IGFBP-3/IGFBP-5 only when the complex is already formed, as ALS has no affinity towards free forms (3).

The structure and the function of ALS were first described by Baxter et al. in 1989 (5). In 1990 Baxter presented the circulating levels of ALS as mean ±SD and divided the results into several groups—levels in normal population, in GH-deficient (GHD) patients, and in acromegalic patients (6). More detailed model of ALS was described by David et al. (7), including analysis of the glycosylation and the effects of ALS mutations on protein structure. A recent study from Kim et al. (3) provided an insight of the IGF-IGFBP-ALS complex formation that revealed the structural basis of diseases associated with IGF-1 and ALS encoding genes (insulin-like growth factor binding protein acid labile subunit, or IGFALS) mutations.

Growth hormone is the main inducer of ALS synthesis in hepatocytes, therefore serum levels of ALS in GHD patients are quite reduced (1). Reduction of ALS serum levels is observed also in some conditions such as malnutrition, fasting, diabetes, catabolic diseases, and anorexia nervosa (1,4,8). Acid-labile subunit deficiency as a result of homozygous or heterozygous IGFALS gene mutations is characterized by very low serum levels of IGF-1 and especially IGFBP-3, normal or compensatory elevated levels of GH and clinical presentation of modest short stature (range -2 to -3 SD), delayed pubertal onset, insulin insensitivity, osteopenia and poor response to treatment with recombinant human GH (rhGH) (9, 10). To date, about 60 patients with complete ALS deficiency as a result of 27 different IGFALS mutations have been reported in the literature (3,11).

The most common laboratory assessment for measurement of ALS levels, when ALS deficiency is suspected, is an enzyme immunoassay (ELISA). Several studies (9,13,14) have reported low serum ALS levels in neonates then an increase until puberty and slow decrease in adulthood. Some publications have stated differences between genders but others found no significant differences between boys and girls (14,15).

The role of ALS as a diagnostic biomarker in GHD is still not well studied. Some studies (9,14,16) have published a comparison between serum levels of IGF-1, IGFBP-3, and ALS before and after initiation of rhGH. However, they showed no clinical advantage of ALS over the information gained from serum levels of IGF-1 and IGFBP-3.

On the other hand, measurement of serum levels of ALS as an additional biomarker could help pediatric endocrinologists in the differentiation of short stature. Moreover, ALS-deficient patients are bad responders to rhGH treatment and have low concentrations of IGF-1 and IGFBP-3 during rhGH therapy (12). Although the mechanism and the consequences of ALS deficiency are well studied, ALS-deficient patients are still of research interest because of the unclear incidence of the condition among the short-statured population and the need of specific therapeutic approach in these patients (10).

AIM

The aim of this study was to assess the prevalence of ALS deficiency in a cohort of patients with GHD, diagnosed and followed up in a tertiary university pediatric endocrinology center.

STUDY POPULATION AND METHODS

Study Population

In this study 71 children diagnosed with GHD (76% boys, n=54) were included. The diagnosis was based on two GH stimulation tests with GH secretion <10 ng/mL, after careful auxological assessment, biochemical and imaging studies according to international guidelines (17). In 55 (77.5%) of the patients, the GH peak during GH stimulation tests was ≤7.0 ng/mL in both provided tests, and in 16 (22.5%) of the patients, the levels of GH were >7.0 ng/mL at least in one of the tests. Height was measured with a Harpenden stadiometer following standard procedures. The results were presented as height gain

in cm/year and height SDS according to CDC standards (https://www.cdc.gov/growthcharts/index.htm). Bone age (BA) was calculated according to the Greulich and Pyle atlas. All patients were on rhGH therapy, followed up for about 2400 patient months at the clinic. The patients were between 2 and 18 years old with mean age at the time of collection of the samples 11.6±3.3 years. The selected blood samples were taken after rhGH treatment for a minimum of 3 months on rhGH starting dose over 0.028 mg/kg/d. More than two years of rhGH therapy and follow-up at the clinic had 41 (57.7%) of the patients.

ALS Assay

Blood serum samples were collected from each patient during the routine visits at the center. According to National Health System recommendations, all patients with GHD are being followed through the first year of rhGH treatment at 3rd, 9th, and 12th month and then they visit the clinic at every 6 months. The study sample was taken at a randomly selected visit that was performed in 2021. The patients were tested for common biochemical parameters after an overnight fasting period in the morning, according to international guidelines (17). After centrifugation, the serum was separated and serum samples were stored frozen at -80°C in 0.5 mL aliquots until assayed. The analysis was done on a single occasion with standard ALS ELISA kit (Reagent ELISA Genie) at an internationally accredited clinical laboratory. Before measurement all samples were diluted with standard solution as recommended by manufacturer. The sensitivity of the test was indicated as 0.938 ng/mL and the intra- and inter-assay coefficients of variation were <8% and <10%, respectively.

Serum IGF-1 and IGFBP-3 levels were measured from a blood sample from the same visit and on the same day of the blood draw, by Siemens Immulite 2000 automated chemiluminescent immunoassay. All the procedures were performed according to the manufacturers' instructions. Serum levels were presented in ng/mL.

Statistical Analysis

Statistical analysis was performed using SPSS ver. 21. Data were presented as percentages (%), standard deviation (SD) and some variables as mean (±SD) or median values. Statistical significance of the results was set at $p<0.05$.

Ethics

The study was approved by the local Ethics Committee of the Medical University of Varna. Informed consent was obtained from every parent after oral explanation and written information provided about the procedures and the purposes of the study.

RESULTS

Clinical Characteristics

The analysis of the participants' data showed mean SDS_{height} before rhGH treatment: -2.8±1.2 SD. The mean age of the start of rhGH treatment was 7.4±3.6 years, mean BA before therapy was 5.4±3.1 years, and the mean starting rhGH dose was 0.029±0.003 mg/kg/d. Mean SDS_{IGF1} before therapy was -1.4±1.0 SD. Serum levels of IGF-1 and IGFBP-3 at the time of collecting the samples for the ALS screening are presented in Table 1. All other blood and biochemical measurements that are, as explained above, part of the routine patients' follow-up at the Center were within reference ranges.

Table 1. Serum concentrations of IGF-1 and IGFBP-3 at time of collecting the samples.

IGF-1 ng/mL	224.3±111.9
IGFBP-3 ng/mL	5479±1362
IGF-1 SDS	0.29±1.0
IGFBP-3 SDS	1.41±0.93

ALS Screening

Acid-labile subunit deficiency screening identified serum ALS levels with range from 2.2 to 60 mg/L, with a mean of 17.4±8.7 mg/L (median 16.6 mg/L) (Fig. 1). The mean serum levels of ALS in boys were 18.0±9.1 mg/L, and in girls they were 15.3±6.3 mg/L (Fig. 2).

Very low ALS levels (<4.0 mg/L) were detected in 3 (4.2%) of the patients. In one of them (1.4%), the ALS level (2.2 mg/L) was close to that in patients with IGFALS gene mutations (<1.0 mg/L) (4,10). This was a 12-year-old boy, diagnosed with GHD with a start of rhGH treatment at the age of 7.6 years. Birth length was 49 cm and birth weight was 2700 g at 40th gestational week. No structural abnormali-

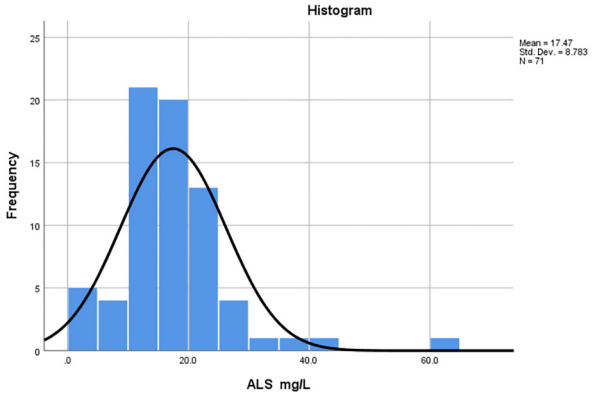

Fig. 1. Results of the ALS deficiency screening in the whole cohort.

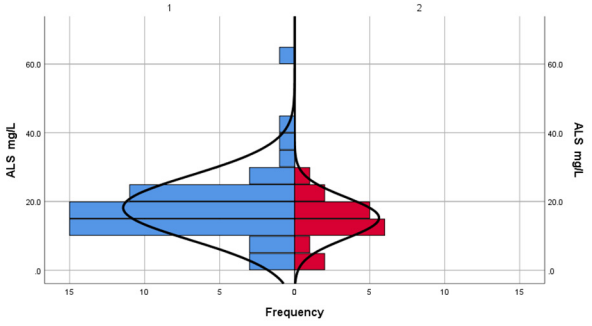

Fig. 2. Serum levels of ALS in boys (blue) and girls (red).

ties were detected on MRI of the hypothalamic-pituitary region. Before therapy with rhGH, the SDS_{height} was –3.1, SDS_{IGF-1} was –2.6, and the bone age was delayed in comparison to chronological age (6.6 years). The peak values of GH on GH stimulation tests were 9.53 ng/mL on the glucagon test and 6.17 ng/mL on the insulin test. The starting rhGH dose was 0.035 mg/kg/d and remained without change during the 1st year of therapy. For the first year of treatment with rhGH SDS_{height} of –2.1 and SDS_{IGF-1} of 0.67 were achieved. All biochemical measurements were within reference ranges. After 3 years of treatment, the SDS_{IGF-1} was 0.93, the $SDS_{IGFBP-3}$ was 1.8, and the IGF-1/IGFBP-3 ratio was 0.3 (18). The SDS_{height} was still very low (-1.4 SD) (Table 2).

DISCUSSION

The role of the circulating IGF-1 in mediating the GH effects on extrahepatic tissues is well studied (19). The absence of ALS represents a peculiar form of IGF-IGFBP deficiency (4). The inabili-

Table 2. Clinical, auxological, and biochemical characteristics of the cohort and the patient with very low level of ALS. Values are presented as mean ±SD.

	GHD Patients	Patient with Very Low Levels of ALS
n	**71**	**1**
Gender (M/F)	54/17	M
Age, years (at the time of collecting the samples)	11.6±3.3 (2 to 18)	12
Height, SDS, (before rhGH therapy) (n=71)	-2.8±1.2 (-6.0 to -0.10)	-3.1
Height, SDS after the 1st year of rhGH therapy (n=60)	-1.7±1.1	-2.1
ΔSDS_{height} for the 1st year	1.1	1.0
Starting rhGH dose, mg/kg/d	0.029±0.003	0.035
Mean rhGH dose for the 1st year	0.030±0.001	0.035
IGF-1, SDS before rhGH treatment (n=71)	-1.4 ±1.0	-2.6
IGF-1 SDS after the 1st year of rhGH therapy (n=60)	0.5±1.1	0.67
ΔSDS_{IGF-1} for the 1st year	1.9	3.27
Serum ALS levels, mg/L	17.4±8.7	2.2
IGFBP-3, SDS (at the time of collecting the samples)	1.41±0.93	1.8

ty of formation of a ternary complex leads to a decrease in the circulating IGF-1 and IGFBP-3 serum levels as a result of increased turnover (12). The low IGF-1 levels result in negative feedback on GH secretion, keeping levels of GH in ALS-deficient patients normal, or sometimes even high (1). Previous studies report predominant deficit of IGFBP-3 in comparison to IGF-1 (4,12). Despite the low serum levels of IGF-1, IGFBP-3, and ALS, the growth delay in ALS-deficient patients is relatively mild (20). Barrios et al. (9), in their study about the comparison between serum levels of ALS and the other components of IGF-system in patients with GHD and eating disorders, found that serum levels of IGF-1 remain lower than in the control group, the levels of IGFBP-3 were low at baseline but after 3 months of rhGH therapy reached the concentrations of the control group, and the levels of ALS were low at baseline, slightly increased during rhGH therapy, but showed significant dependence on other factors like some eating disorders. Domene et al. (4) reported SDS_{height} before rhGH therapy in patients with mutation of IGFALS gene between -2.0 and -3.0 SD and final height by 1.0 SD lower than midparental height.

In the present study, the mean SDS_{height} and the levels of IGF-1 of the participants before rhGH therapy were in concordance with the previous findings. Initiation of rhGH treatment led to an increase in the levels of IGF-1 and improved the height gain (Table 2). On the other hand, in the patient with low ALS levels, the SDS_{height} remained low despite the rhGH treatment

The mean levels of ALS in our study corresponded positively with the results from other studies in GHD patients ($p<0.05$) (6,14,21). Serum levels of ALS in the present study remained lower in the whole cohort in comparison with the levels in healthy subjects, even after rhGH therapy. This is in concordance with the statements that the increase in ALS levels during rhGH treatment is only partial (9).

The prevalence of boys in our cohort is seen also in the other studies (10,14). It could be explained by the fact that more attention is paid to growth in boys than in girls. This reveals the need of better growth monitoring and timely referrals in children (22).

The deficiency of ALS leads to multiple metabolic, bone, and pubertal consequences (1). First, the slightly elevated levels of GH in combination with low levels of IGF-1 are a precondition for a carbohydrate metabolism disorder resulting in insulin insensitivity (4). Second, the low levels of IGF-1 may cause reduction in bone mineral density (19). Moreover, almost 50% of the boys with IGFALS mutation show delayed puberty (23). In our cohort, 38 (53.5%) of the patients were in pubertal age, but none was described by delayed onset. In the case presented above, the puberty onset was at the age of 9.6. Decreased height gain is the most common finding in the majority of patients with ALS deficiency. Fofanova-Gambetti et al. identified in their study among 65 subjects with IGFALS mutation a basal SDS_{height} lower than -2.0 SD in most patients (24). Domene et al. reported decreased height velocity even after rhGH therapy in ALS-deficient patients (4–6 cm/year), but comparatively preserved growth spurt (4). In our study we observed mean height velocity for the 1st year of treatment with rhGH of 9.2±2.0 cm. These data reveal the need of more profound search for ALS deficiency among the patients with growth disorders.

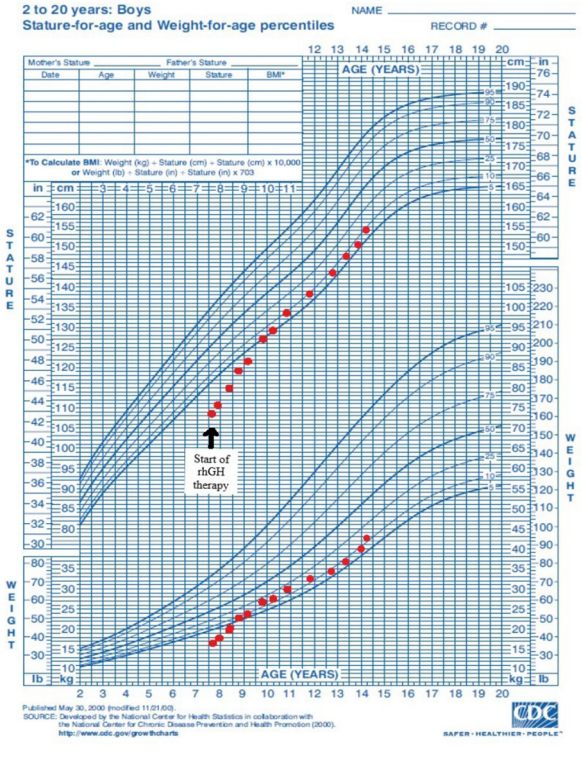

Fig. 3. Growth chart of the patient diagnosed with very low levels of ALS.

Very low serum levels of ALS and IGF-1, and great growth delay were established in our presented patient before treatment. On the other hand, the good height catch-up (9 cm for the 1st year) in conjunction with the GH peak on GH stimulation tests >7.0 ng/mL, lack of puberty delay, and normalization of the levels of IGF-1 and IGFBP-3 after treatment are more typical for GHD patients (4). Therefore, no further genetic testing was undertaken in this case. The growth chart of the patient is presented in Fig. 3.

The role of ALS as a diagnostic tool in short-statured patients is still controversial (15, 25, 26). Juul et al. (14) have stated the usefulness of serum levels of ALS only in adults with GHD. Fukuda et al. (27) and Barrios et al. (9) have reported lower diagnostic accuracy of ALS in comparison with IGF-1 and IGFBP-3. A recent study of Ertl et al. (16) concluded that neither serum levels of ALS, IGF-1, and IGFBP-3, alone or in combination, are sufficient for diagnosis of GHD and that the most reliable parameter is still GH testing. In addition, the results of the present study reveal that ALS screening after initiation of rhGH therapy is not particularly indicative.

However, ALS screening could be useful as an additional biochemical marker for identifying patients with IGFALS mutations in short-statured patients before initiating rhGH therapy where clinical characteristics, such as very low serum concentrations of IGF-1 and IGFBP-3, are present alongside mild growth failure, high insulin levels, and delayed onset of puberty (1,10,16). In addition, less severe cases of ALS deficiency in heterozygous children may remain hidden and undiagnosed correctly due to milder clinical presentation (4). The importance of correct diagnosis in these patients is related to the prevention of health consequences and initiation of appropriate therapy.

The strengths of this study include the well-defined cohort of patients with GHD who are followed up at a tertiary center. Furthermore, there are few studies about screenings for ALS deficiency in followed-up GHD patients. The lack of possibility to measure the ALS levels before the initiation of rhGH for better characterization of the patients is a limitation of the current study. The controversial clinical and laboratory findings in the only identified patient with low ALS in the current study underline the need of genetic analysis in cases with low ALS levels and typical clinical features.

CONCLUSION

Our results show the prevalence of ALS deficiency in the current rhGH-treated cohort and support the evidence that investigation of ALS levels could be helpful in the differential diagnosis of growth disorders.

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