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An analysis of acid-labile subunit (ALS) levels in children with short stature born with normal weight

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Abstract

Introduction: The acid-labile subunit (ALS) is a protein best known for its function in stabilising the insulin-like growth factor-1/2-insulin-like growth factor-1 binding protein 3/5 (IGF-1/2-IGFBP3/5) binary complex by creating the ternary complex and in consequence regulating the biological activity of IGF-1. The aim of the study was to assess ALS concentrations in a chosen population of children with short stature taking into account their clinical diagnosis.

Material and methods: A total of 109 prepubertal children were involved in the study — 85 children in the study group and 24 in controls. In all the children IGF-1, IGFBP3, and ALS were measured. The study group was divided according to diagnosis into groups: growth hormone deficiency (GHD), constitutional delay of growth and puberty (CDGP), idiopathic short stature (ISS), and familial short stature (FSS).

Results: In the control group the ALS concentration ranged from 4.81 to 13.66 μg/mL. In the whole study group the ALS concentration ranged from 2.73 to 15.81 μg/mL. The difference between both groups was statistically significant ($p < 0.0001$, $R = 0.39$). A strong, statistically significant correlation between ALS levels and age was observed, but only in the study group ($p < 0.0001$, $r = 0.59$). The ALS standard deviation score (SDS) was not significantly different between the control and CDGP children ($p = 0.0644$). The ALS concentration was significantly lower in children with short stature. There was, however, no difference between the subgroups of the study group.

Conclusion: There was no significant difference in ALS SDS between the control group and children with constitutional delay of growth and development. The usefulness of ALS in routine short stature diagnostics is uncertain, but it might play a role in the diagnosis of children with ISS and CDGP in the future. **(Endokrynol Pol 2024; 75 (5): 548–557)**

Key words: *growth hormone; IGF-1 protein, human; growth disorders; IGF-1 protein, acid-labile subunit; growth hormone; IGF-1-IGFBP3 complex*

Introduction

Short stature is defined as height below the third percentile or –2 standard deviations (SD) from the mean of the population, taking into account the age and sex [1]. Endocrine causes of short stature are relatively rare — 1.3 to 19.8% of cases [2–4]. Among the hormonal causes of growth failure, thyroid dysfunction and growth hormone deficiency are the most significant.

Acid-labile subunit (ALS) is a glycosylated protein with a molecular weight of 85 kDa. It belongs to the leucine-rich repeat (LRR) family of proteins. It is produced almost exclusively by the liver, and its production, as well as expression of messenger ribonucleid acid (mRNA) coding it, are regulated by growth hormone [5, 6]. Its name comes from how the ternary complex behaves in an acidic environment — the complex dissolves, with insulin-like growth factor 1 (IGF-1) still being able to bind to insulin-like growth factor 1 binding protein 3 (IGFBP3), but ALS function of binding with other proteins is permanently disrupted [7]. ALS protein is coded by the *IGFALS* gene, located on the short arm of chromosome 16 (16p13.3) [8]. Its mutations cause deficits of ALS in circulation, and as a consequence, mild postnatal growth impairment [8–12].

The most researched function of ALS is stabilising the binary complex IGF-1/2-IGFBP3/2 by creating a ternary complex. Free IGF-1 in circulation has a short half-life of about 12 minutes, which is extended to more than 12 hours by creating the ternary complex [13]. Only the free fraction of IGF-1 shows biological activity, which means the ternary complex is the main reservoir of IGF-1 in circulation for the target cells; it also regulates its bioavailability and bioactivity [14]. What confirms the stabilising role of ALS is the fact that, unlike free IGF-1 and the binary complex, the ternary complex cannot cross the endothelium. Hence, ALS is also responsible for regulation of metabolic properties of IGF-1 — mainly its insulin-like activity, which could cause hypoglycaemia [14].

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ALS synthesis takes place mostly in the hepatocytes; however, based on molecular studies it is known that cells with *IGFALS* gene expression are also present in kidneys, growing bones, mammary glands during lactation, thymus, and lungs [15]. It is believed that most of the ALS found in the extravascular space comes from the serum, but local synthesis of the ALS may play a role in regulating the auto- and paracrine activity of IGF-1 [16].

ALS deficiency may be caused by *IGFALS* mutations. The type of disorder is different in homo- and heterozygotic mutations and is associated with the number of the gene copies [13, 17]. The "gene dosage" effect is also present, where one functioning allele of the *IGFALS* gene is not sufficient to maintain normal ALS levels, endocrine IGF-1 action, full growth potential, muscle size, and periosteal expansion [18].

Total ALS deficiency causes concentrations of IGF-1 and IGFBP3 to be significantly lower as well. Ternary complex component deficiency is described as disproportionate to the growth retardation — short stature caused by total ALS deficiency is described as mild, with height between –2 and –3 SDS. The birth weight of children with ALS deficiency is usually below the mean (–2.23 to –0.08 SDS), which suggests a possible effect of ALS on prenatal growth; this mechanism is, however, still unknown [12, 13]. Growth hormone levels in patients with ALS deficiency is normal or elevated, which is probably caused by the lack of negative feedback loop caused by IGF-1 deficiency [19].

Available literature also describes glucose metabolism (GM) disorders in children with total ALS deficiency. The mechanism of those is still not precisely described. A decrease in insulin sensitivity is assumed, caused by elevated GH and lower IGF-1 levels, which may lead to glucose metabolism disorders, including type 2 diabetes [13, 14, 20].

Studies indicate that ALS not only affects linear growth of the bones, but also their mineral density, which means that ALS deficient patients may be at higher risk of osteopaenia and osteoporosis [18].

In patients with ALS deficiency, delayed puberty was observed more often in boys than girls. The mechanism behind this is still unknown [20].

As of this writing, about 30 patients with total ALS deficiency have been described [14], but families with new, unknown mutations are still being found. The correlation with the type of mutation and number of copies of the affected gene causes the phenotype of ALS-deficient patients to be heterogeneous [12, 21, 22].

Measuring ALS levels, until recently, was expensive and technically difficult. With the introduction of an ELISA test, the availability has significantly increased [23]. In 2012, 7% of surveyed European Society of Paediatric Endocrinology members declared that they measure ALS levels during the IGF-1-generation test [24]. It is still not part of routine diagnostics of short stature, however, and its usability is still uncertain. Researchers focusing on ALS emphasise the need for more ALS level assessments in groups of children divided by age [25].

In this paper we aimed to assess the following, based on a prospective study:

— the levels of ALS in a chosen population of children with short stature, born apropriate for gestational age (AGA), according to their diagnosis — growth hormone deficiency (GHD), constitutional delay of growth and puberty (CDGP), familial short stature (FSS), idiopathic short stature (ISS);
— the correlation between ALS levels and anthropometric measurements;
— the correlation between ALS levels and other GH-IGF-1 axis elements;
— the usefulness of ALS measurement in routine short stature diagnostics.

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Material and methods

The study was allowed by the Pomeranian Medical University Bioethics Committee, decision number KB-0012/102/15 (issued 5 October 2015). Informed consent to participate in the study was obtained from the legal guardians of the subjects.

The study group consisted of children with short stature diagnosed in the referential Paediatric Endocrinology Clinic for the region of Western Pomerania, inhabited by 1.7 million people. The control group consistent of healthy children qualified for minor surgical interventions or taking part in population health check-ups. The study was performed between March 2016 and November 2017.

Inclusion criteria:

— height < 3 pc for age and sex, according to Polish percentile charts [26];
— birth weight and/or length ≥ –2 SDS for gestational age;
— puberty stage I according to the Tanner scale [27].

Exclusion criteria:

— birth weight and/or length < –2 SDS for gestational age [small for gestational age (SGA), intrauterine growth restriction (IUGR)];
— puberty (Tanner scale stage II and above);
— secondary causes of short stature found during the diagnostic process [an magnetic resonance imaging (MRI) scan of the pituitary gland, assessing the girls' karyotypes, exclusion of children with oncologic history, eating disorders, treated with glucocorticoids, as well as children with confirmed genetic disorders associated with short stature];
— primary IGF-1 deficiency.

Children qualified to the control group had normal height, normal birth weight (defined as ≥ –2 SDS for the gestational age), and no chronic diseases.

All children's heights were measured with Harpenden type stadiometer. Each measurement was performed 3 times, with a mean value derived from all the measurements. Body weight was measured using a medical scale. The results were assessed according to the Polish norms [26, 28].

The diagnosis of GHD was given based on the result of an assessment of spontaneous GH secretion during the night and 2 stimulation tests (L-DOPA and clonidine), with a cutoff point of 10 ng/ml. FSS was diagnosed in children with normal GH secretion with short parents and normal bone age. CDGP was diagnosed in children with normal GH secretion, significant delay in bone age,

a family history of CDGP, predicted final height within the normal range, and based on further observation of the patients in the Outpatient Clinic. ISS was a diagnosis in children for whom no other cause of short stature could be identified.
In all children, fasting levels of IGF-1, IGFBP3, and ALS were measured. Children from the study group continued their diagnostics in the clinic to find out the cause of short stature.
The whole study consisted of 109 children – 85 in the study group and 24 in the control group. The study group consisted of 34 girls (41.18%) and 51 boys (58.82%), and the control group comprised 8 girls (33.33%) and 16 boys (66.67%).
IGF-1 levels were measured on a Siemens IMMULITE®2000 using the ECLIA method. The results were then presented as SDS, using a mathematic formula created by Bedogni et al. [29]. IGFBP3 levels were measured on the same device, and the results were presented as SDS using a formula proposed by Löfqvist et al. [30].
ALS levels were measured using the Mediagnost ELISA kit on a BIOTEK device. The manufacturer did not propose any normative values for the paediatric population. The results were presented as SDS based on the data from the study by Barrios et al. [31].

Statistical analysis methods

Using the results, a database was created in Microsoft Excel, which was then further analysed in STATA 11.
The Kolmogorov-Smirnov test was used to verify the normality of distributions of continuous variables. Those variables were then described as mean values, standard deviations, medians, quartiles, and minimum and maximum values.
Statistical differences between 2 groups were then checked with Student's t-test and the Mann-Whitney test.
Non-continuous variables were described as quantity and frequency. The analysis of relationships between non-continuous variables was carried out using Pearson's X^2 test or Fisher's test.
To analyse the relationships between continuous variables Pearson's correlation was used. The results were described as probability p, correlation coefficient r, and regression lines.
To choose factors best describing dependent variables in the regression model, multivariate analysis and stepwise estimation were used. The results were described by partial correlation coefficient r and probability p. For the whole model, values of R (multivariate correlation coefficient) and p (probability) are presented.
Tests were considered statistically significant with values of $p \leq 0.05$.

Results

Group characteristics

The control group consisted of 24 children with normal height, including 8 girls (33.33%) and 16 boys (66.67%). The study group consisted of 85 children diagnosed because of short stature, including 34 girls (41.18%) and 51 boys (58.82%). The age of children in this group ranged from 2.42 to 14 years, on average 6.78 ± 2.43 years. The auxological data of both groups are presented in Table 1.

For the statistical analysis, the study group was divided further according to the final diagnosis:
— GDH: n = 17;
— CDGP: n = 27;
— FSS: n = 18
— ISS: n = 23.

The control group did not differ from the study group significantly in terms of children's age (p = 0.4360, R = 0.07). Statistically significant differences were described between the control and study group in the context of auxological parameters: height (p < 0.0001, R = 0.42), height SDS (p < 0.0001, R = 0.84), body weight (p < 0.0001, R = 0.38), body weight SDS (p < 0.0001, R = 0.38), body mass index (BMI) (p = 0.0340, R = 0.17), and body mass index (BMI) SDS (p = 0.0265, R = 0.22).

Laboratory test results

IGF-1

In the control group the IGF-1 concentration ranged from 56.9 to 315 ng/mL, on average 137.5 ± 76.9 ng/mL. In the whole study group, it ranged from 25 to 231 ng/mL, on average 91.4±48.3 ng/mL. The difference between the control group and the study group was statistically significant (p = 0.0005, R = 0.33).

IGF-1 SDS ranged from –2.36 to –0.28 in the control group, on average —1.52 ± 0.52. In the study group it ranged from –2.6 to –0.72, on average −1.93 ± 0.39. The difference was statistically significant (p = 0.0001, R = 0.37). The results in subgroups of the study group were also significantly different from the control group. The results are presented in Figure 1.

Table 1. *Auxologic data of study and control groups*

Parameter	Group	n	$\bar{x}$	SD	Min.	Max.	Me	p
Age [years]	Control	24	7.17	2.38	4.00	12.00	6.58	0.4952
	Study	85	6.78	2.43	2.42	14.00	6.25	
Height SDS	Control	24	−0.07	1.20	−1.95	2.17	−0.09	< 0.0001
	Study	85	−2.83	0.54	−4.74	−2.01	−2.77	
Body weight SDS	Control	24	0.00	1.72	−3.05	5.11	0.02	< 0.0001
	Study	85	−2.26	0.83	−3.75	0.80	−2.30	
BMI SDS	Control	24	−0.01	1.80	−3.00	4.50	−0.20	0.0245
	Study	85	−0.82	1.45	−2.98	4.49	−1.09	

$\bar{x}$ — mean value, p — probability, SD — standard deviation, Min. — minimal value, Max. — maximal value, Me — median value

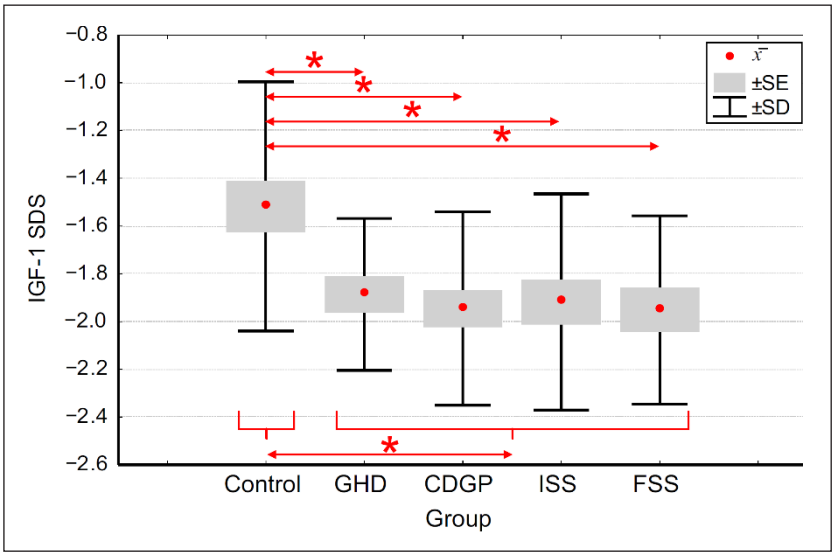

Figure 1. *Insulin-like growth factor 1 (IGF-1) standard deviation score (SDS) in all examined children. $\bar{x}$ — mean value; *p < 0.05; SE — standard error; SD — standard deviation*; *GHD — growth hormone deficiency; CDGP — constitutional delay of growth and puberty; ISS — idiopathic short stature; FSS — familial short stature*

IGFBP3

In the control group the IGFBP3 concentration ranged from 2.45 to 7.39 mg/L, on average 4.56 ± 1.42 mg/L. In the whole study group, it ranged from 2.02 to 7.56 mg/L, on average 4.14 ± 1.12 ng/L. The difference between the control group and the study group was not statistically significant (p = 0.1277, R = 0.15).

IGFBP3 SDS ranged from –0.2 to 4.77 in the control group, on average 2.21 ± 1.3. In the study group it ranged from –1.45 to 5.46, on average 1.86 ± 1.26. The difference was not statistically significant (p = 0.2322, R = 0.12). There were also no statistically significant difference between the subgroups of the study group.

IGF-1/IGFBP3 ratio

The IGF-1/IGFBP3 ratio was calculated as SDS. In the control group the mean was -2.05 ± 1.39, and in the study group the mean was –3.18 ± 1.37, which was significantly different from the control group (p = 0.0002, R = 0.33). Values of IGF-1/IGFBP3 SDS were also significantly different between subgroups of the study group. The results are presented in Figure 2.

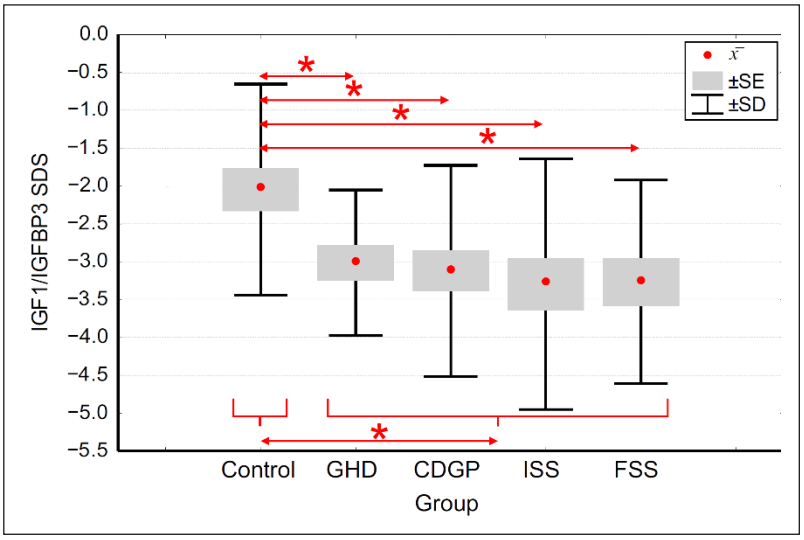

Figure 2. *Insulin-like growth factor 1/insulin-like growth factor-1 binding protein 3 (IGF-1/IGFBP3) standard deviation score (SDS) in all examined children. $\bar{x}$ — mean value; *p < 0.05; SE — standard error; SD — standard deviation*; *GHD — growth hormone deficiency; CDGP — constitutional delay of growth and puberty; ISS — idiopathic short stature; FSS — familial short stature*

Table 2. *Acid-labile subunit (ALS) and ALS SDS results in subgroups of the study group*

Parameter	Subgroup	$\bar{x}$	SD	Min.	Max.	p
ALS [μg/mL]	GHD	6.48	2.93	2.93	13.04	0.0075
	CDGP	6.2	2.91	3.53	15.81	0.0003
	FSS	6.29	1.73	4.04	10.66	0.0017
	ISS	5.64	1.92	2.73	9.42	0.0002
ALS SDS	GHD	–1.85	0.63	–2.68	0.25	0.0010
	CDGP	–1.5	0.7	–2.81	0.25	0.0644
	FSS	–1.83	0.55	–2.82	–0.99	0.0005
	ISS	–1.79	0.49	–2.95	–1.73	0.0003

SD — standard deviation; GHD — growth hormone deficiency; CDGP — constitutional delay of growth and puberty; ISS — idiopathic short stature; FSS — and familial short stature

ALS

In the control group the ALS levels ranged from 4.81 to 13.66 μg/mL, on average 8.57 ± 2.46 μg/mL. In the study group it ranged from 2.73 to 15.81 μg/ml, on average 6.12 ± 2.43 μg/mL. The difference between the groups was statistically significant (p = 0.0002, R = 0.35).

ALS SDS

In the control group the ALS SDS ranged from –2.33 to –0.31, on average –1.19 ± 0.55, and in the study group the SDS values ranged from –2.95 to 0.25, on average –1.72 ± 0.61, which was significantly different from the control group (p = 0.0002, R = 0.35). After dividing the study group into subgroups, there was no significant difference in ALS SDS between the control group and CDGP subgroup of the study group (p = 0.0644, R = 0.24).

Both ALS and ALS SDS results in subsequent subgroups of the study group and p values representing their comparison to the control group are presented in Table 2.

Groups divided according to IGF-1 levels

Both the control and study group were divided according to IGF-1 concentration in groups with IGF-1 SDS < –2 and ≥ –2. ALS levels seem to be significantly higher in subgroups with normal IGF-1 levels, which is true for the control group (p = 0.0133), GHD (p = 0.0111), and CDGP (p = 0.0065). ALS SDS, however, differs between the 2 subgroups only in children with CDGP (p = 0.0130). The results are presented in Figure 3.

Correlations

In all examined children (n = 109) the analysis showed a significant correlation between IGF-1 and IGFBP3

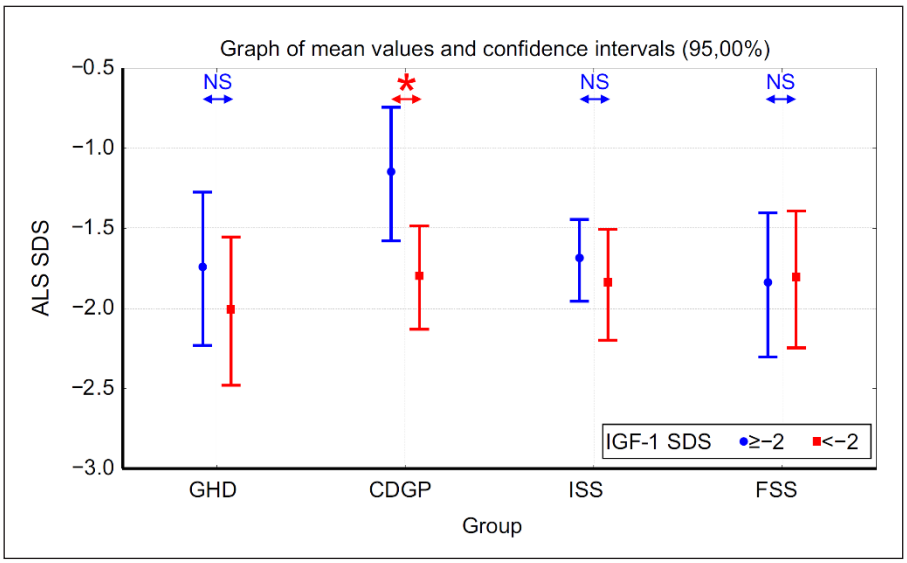

Figure 3. *Acid-labile subunit (ALS) standard deviation score (SDS) in children according to their insulin-like growth factor level (IGF-1). *p < 0.05; GHD — growth hormone deficiency; CDGP — constitutional delay of growth and puberty; ISS — idiopathic short stature; FSS — familial short stature; NS — non-significant*

Table 3. *Correlations between all examined children*

Parameter	r	p
IGF-1 and IGFBP3	0.53	< 0.0001
IGF-1 SDS and IGFBP3 SDS	0.50	< 0.0001
ALS and IGF-1	0.64	< 0.0001
IGF-1 and HSDS	0.27	0.0027
IGF-1 SDS and HSDS	0.30	0.0016
ALS SDS and IGF-1 SDS	0.32	0.0008
ALS and IGFBP3	0.43	< 0.0001
ALS and HSDS	0.41	< 0.0001
IGF-1 and age	0.67	< 0.0001

IGF-1 — insulin-like growth factor 1; SDS — standard deviation scores; HSDS — height standard deviation scores; ALS — acid-labile subunit; IGFBP3 — insulin-like growth factor 1 binding protein 3

levels ($p < 0.0001$, $r = 0.53$), as well as IGF-1 and ALS levels ($p < 0.0001$, $r = 0.63$). There was also a weak but statistically significant correlation between IGF-1 and height SDS ($p = 0.0027$, $r = 0.27$). IGF-1 levels changed significantly with children's age ($p < 0.0001$, $r = 0.67$).

IGF-1 SDS correlated significantly with IGFBP3 SDS ($p < 0.0001$, $r = 0.50$) and ALS SDS, although the strength of this correlation was lower ($p = 0.0008$, $r = 0.32$). A weak but statistically significant correlation between IGF-1 SDS and height SDS was shown ($p = 0.0016$, $r = 0.30$). Correlations between all examined children are shown in Table 3.

When considering the control and study groups separately, a significant correlation between IGF-1 and ALS levels was shown, which was stronger in the study group than in the control group ($p = 0.0155$, $r = 0.49$ for the control group, $p < 0.0001$, $r = 0.64$ for the study group). A similar correlation was found between IGF-1 and IGFBP3 ($p = 0.0017$, $r = 0.6$ for the control group; $p < 0.0001$, $r = 0.58$ for the study group). IGF-1 changed significantly with the children's age in both groups ($p = 0.0005$, $r = 0.66$ for the control group, $p < 0.0001$, $r = 0.66$ for the study group). Similarly, ALS levels changed significantly with children's age, but only in the study group ($p < 0.0001$, $r = 0.59$).

To check which independent variables affect dependent variables, models of multivariate analysis and stepwise estimation were used. This way it was determined that the only analysed factors significantly affecting ALS levels are height SDS and IGF-1, while variables affecting ALS SDS are height SDS, IGF-1 SDS, and sex. Body weight and BMI presented no significant correlations with either ALS or ALS SDS. Correlations in the study and control groups considered separately are shown in Table 4.

Table 4. *Correlations in study and control groups considered separately*

Parameters	Group	r	p
ALS and IGF-1	Study	0.64	< 0.0001
	Control	0.49	0.0155
ALS SDS and IGF-1 SDS	Study	0.22	0.0432
	Control	0.22	0.3044
IGF-1 and IGFBP3	Study	0.58	< 0.0001
	Control	0.6	< 0.0001
IGF-1 and age	Study	0.66	< 0.0001
	Control	0.66	0.0005
ALS and age	Study	0.59	< 0.0001
	Control	0.16	0.4504

ALS — acid-labile subunit; SDS — standard deviation scores; IGF-1 — insulin-like growth factor 1; IGFBP3 — insulin-like growth factor 1 binding protein 3

Discussion

The process of linear growth is one of the main qualities in the development of an organism. It depends on many factors — genetic, hormonal, and environmental. In the postnatal period the most important factors are the GH–IGF-1 axis and thyroid hormones [4, 32, 33]. ALS plays an important role in the functioning of the aforementioned axis by stabilising the ternary complex, which means its main function is to regulate the levels of circulating IGF-1 [34].

Despite the facts about ALS levels, such as them being lower in GH deficiency or states of catabolism [21], there are few studies comparing those data in age-matched groups, especially in children, which presents the greatest strength of our study. We have also divided the study children according to their diagnosis, which, to our knowledge, has only been done with ISS children before. Our control group is also well-matched to the study group, with no significant differences in regards of age. There was a higher number of boys (51, 58.82%) than girls (34, 41.18%) in the study group, which can be explained by boys being diagnosed with short stature more often — short height in girls is more accepted by both the parents and the children. Our study's main limitation is a small size of the sample group, and especially the control group. In the process of the study, no local norms were calculated, and norms and SDS values from other studies were used, which may have made the results less precise — keeping the correlations the same, nonetheless. Finally, the study was terminated before performing genetic testing on children with the lowest ALS concentrations, which we suggest should be the next step in our research.

The study group was divided into subgroups according to cause of short stature decided in the diagnostic process. The GHD group comprised of children diagnosed, meaning their GH levels were below 10 ng/mL in a spontaneous night secretion test as well as 2 stimulation tests. GHD was diagnosed in 17 children in the study group, which makes up 20% of that group — a percentage larger than the one found in the literature; for example, according to Sultan, GHD can be diagnosed in 6.1% of children, which makes for 39% of all hormonally caused cases of short stature [3]. It must be noted that Sultan's study was populational, while the group in our study was an artificially created, selected excerpt of the general population. It must also be remarked that GHD is, with a few exceptions, an arbitrary diagnosis – there is no clear, sharp cutoff point allowing the differentiation, with 100% certainty, of children with normal and deficient GH secretion. Guzzetti et al. [35] describe different values of optimal cutoff points, which are significantly lower than the arbitrary 10 ng/mL — a value which was the same for all the stimulation tests. According to the Italian study, in a clonidine stimulation test GH level of 6.8 ng/mL is sufficient to exclude GHD in children, with values for insulin and arginine tests being 6.2 and 6.5, respectively. Another difficulty comes in the form of different methods of GH measurements, which may lead to significant differences in results. As Bidlingmaier points out [36], despite the existing international consensus concerning GH measurements, there are still differences in methodology when it comes to different immunoassays. According to Shalet [37], the assessment of GH secretion in children is bound to be a certain continuum, where values in the lower norm are sometimes indiscernible from the incorrect values. One has to wonder though, if in a clinical setting such a distinction is absolutely necessary, and if the advantages the patient will gain from the treatment do not excuse the erroneous overdiagnosis of GHD by clinicians. In the latter parts of his paper, Shalet points to economic factors as the main cause of further therapeutic decisions in different countries. He also points to different cutoff values for GHD which are used historically or currently in different countries — 7 and 5 ng/mL (5 ng/mL is a value considered a severe GHD according to Polish criteria).

In the study group, 7 children reached peak GH levels < 7 ng/mL, which makes up 8.24% of the whole group; values < 5 ng/mL were measured in 3 children, which is 3.53% of the study group. Those values are closer to the percentages proposed in the literature. Statistical analysis included those 2 subgroups as well; however, their size does not really allow for any definitive conclusions.

All the other diagnoses (CDGP, FSS, ISS) are thus given to children with normal GH levels, based on the auxological data of the parents, the history of their growth and development, as well as further observation in the Outpatient Clinic and bone age; other causes of short stature were excluded. Among children with GH levels > 10 ng/mL, one could expect several children with GH resistance. However, analysis of max GH secretion proved no significant differences between children in those 3 groups, which means there were probably no such children in the study group.

Interestingly, in our study there were no statistically significant differences in IGFBP3 levels of children from the study and control group, as well as between the subgroups of the study group. What is also curious, while calculating IGFBP3 SDS using the mathematic model proposed by Löfqvist [30], none of the children had IGFBP3 SDS lower than –2. The first thing that should to be taken into consideration in this case is the difference in methodology between our study and the study by Löfqvist, who measured IGFBP3 using radioimmunoassay (RIA) kit, while our laboratory used chemiluminescent immunoassay (CLIA) kits. While comparing the results with the kit's manufacturer's suggested reference values, it was confirmed that none of the children had IGFBP3 values below the lower norm, and many of them exceeded the upper norm. To explain this, an interference of IGFBP3 proteolysis products was taken into account — a study by Cianfarani et al. [38] showed a presence of 18kDA fragment of IGFBP3, which may disrupt immunoassays measuring IGFBP3 levels. This suggests that we cannot exclude a laboratory error. However, as expected, there was a statistically significant correlation between IGF-1 and IGFP3, as well as IGF-1 SDS and IGFP3 SDS ($p < 0.0001$, $r = 0.63$ and $p < 0.0001$, $r = 0.50$. respectively).

The study most similar to ours is a study by Domené et al. from 2013 [35], in which IGF-1, IGFBP3, and ALS levels in children with ISS were measured, with genetic studies being performed in all the children. ALS levels in ISS children were significantly lower than in the control group, and the genetic studies revealed 22 polymorphisms in the *IGFALS* gene, 6 of which were described for the first time. The study was performed again on a larger group in 2017, with lower ALS levels being confirmed in 5% of the children with ISS. Our study confirmed those results, with ALS levels being significantly lower in the ISS group than in the control group [36].

ALS measurements in the paediatric population require certain compromises to be made — there are no reference values set for this parameter, including the kit used in our study, in which the manufacturer

only proposes reference values for adults. As has been shown in numerous studies, ALS levels change significantly in childhood, along with the progression of puberty, reaching relatively steady values in adulthood [12, 15].

While analysing the results of our study, auxological parameters need to be taken into account first. Only prepubertal children were included in the study, which means there was no effect of sex hormones on the linear growth. It also means no patients presented a significant, rapid rise in IGF-1 and ALS levels, as described in the literature [31, 34, 37–40].

One of the inclusion criteria was being born with adequate body weight for gestational age. In the recent years many endocrine consequences of SGA have been reported, one of which is discrepancies in the GH–IGF-1 axis, which further lets us believe that ALS levels will be significantly different in those children [41–43]. In the study by Renes et al., SGA children had significantly lower ALS levels than AGA children [44, 45]. Tseng et al. described a significant correlation between ALS levels in the umbilical blood and body weight, head circumference, and placenta weight at birth [45]. Interestingly, a study by Iñiguez et al. suggested higher ALS levels and ALS mRNA expression in placentas of SGA children [47]. All this suggests that a separate study should be performed on those children, considering all the differences in their endocrine status.

The control group was significantly different to the study group when comparing auxological measurements. There was, however, no significant difference in BMI SDS between the control group and GHD group, both of which, however, were different from all the other subgroups of the study group. Those findings are consistent with past data — children with GHD do not have low body weight and very low GH levels are even associated with central obesity [48]. Domené also points to significantly lower BMI of children with ISS [35].

In our study, ALS levels were significantly different between the study and control groups ($p < 0.0001$, $r = 0.39$), as well as between the control group and each subgroup of the study group. Similarly, ALS SDS was significantly different between the control and study groups ($p = 0.0002$, $r = 0.35$); there were, however, no differences between the subgroups of the study group. After further diving the groups according to IGF-1 levels into subgroups with IGF-1 SDS ≥ -2 and < 2, ALS levels were significantly different in the control group ($p = 0.0133$), GHD group ($p = 0.0026$), and CDGP group ($p = 0.0014$).

ALS levels changed significantly with children's age ($p < 0.0001$, $r = 0.48$); this correlation was stronger in the study group. This confirms previous findings – according to Barrios et al ALS levels grow significantly with age reaching a plateau in Tanner stage III puberty [31]. According to Nimura et al., girls reach their highest ALS levels 2 years faster than boys, which is probably related to girls experiencing a pubertal growth spurt earlier [48].

As expected, ALS levels significantly correlated with IGF-1 levels ($p < 0.0001$, $r = 0.63$), which was also true for ALS SDS and IGF-1 SDS (0.0008, $r = 0.32$). This correlation was stronger in the study group than in the control group. This finding is consistent with previous studies by Nimura and Barrios [31, 49]. After dividing the study group into subgroups, CDGP children were the only group not significantly different from the controls. This is an interesting finding, considering that half of the patients with total ALS deficiency (especially boys) are said to have delayed puberty [21]. The mechanism behind this remains unknown. The first patient with an *IGFALS* gene mutation described by Domené et al. was a 14.6-year-old prepubertal boy with height SDS -2.08, body weight SDS -2.34, and bone age delayed by 2 years. GH levels were normal, but IGF-1 SDS was –5.3. Eventually the boy reached his final height of –0.9SD at the age of 19 years [14]. CDGP children were further investigated by Banerjee et al. — 90 children were analysed, comprising 80 boys and 10 girls. All the patients had the IGFALS gene sequenced; no mutations were found. The authors thus do not recommend IGFALS sequencing in the routine diagnostics of CDGP [49].

What are the possible clinical uses of ALS levels assessment? Domené et al. suggest that ALS levels should only be checked in children with suspicion of total ALS deficiency, which are characterised by normal levels of GH in stimulation tests, low IGF-1 levels with equally low (or lower) IGFBP3 levels, short stature disproportionally mild considering the IGF-1 and IGFBP3 levels, insulin resistance, especially elevated insulin levels with normal glucose levels, and delayed puberty, especially in boys [15]. They also point out the most important uncertainties in ALS deficiency diagnosis and potential deficiency treatment: the mechanism of linear growth in the situation of severe IGF-1 deficiency, indications for insulin resistance treatment in total ALS deficiency, the long-term effects of severe IGF-1 deficiency on the mineral structure of the bones, and the exact effect of heterozygous IGFALS mutations on the GH-IGF-1 axis in children with ISS. It is also unknown if all the pathological findings in ALS deficiency are caused by IGF-1 deficiency or if they are a direct effect of ALS deficiency itself. Finally, the potential treatment is still undecided — it is not known if ALS deficient patients should be treated with GH, IGF-1, or a combined preparation [15, 35, 36].

The mechanisms of short stature in ALS deficiency are still not fully known. There have been cases of children with severe IGF-1 and IGFBP3 deficiency caused by ALS deficiency, who later had a normal pubertal growth spur and reached their predicted final height [51]. While testing the parents of children with IGFALS mutations, heterozygotes were found, with normal, albeit slightly lower than the population mean, height [35]. This suggests that a significant fraction of the patients with ALS deficiency may never seek an endocrinologist's help.

All the authors of studies assessing ALS levels treat their results with scepticism: Barrios notes that ALS provides little more information than routine assessment of IGF-1 and IGFBP3 levels [52]. David et al., the authors of the current ALS molecule model, point to the necessity to further study the physiology and biochemistry of its activity; despite knowing about 90% of the structure of the molecule, the mechanism of ternary complex creation is still uncertain [53].

Our study is another step in the possible assessment of clinical uses of ALS levels. However, we are able to present conclusions and suggest further steps in the research area:

— the ALS concentration was significantly lower in children with short stature than in the control group. There was however no difference between the subgroups of the study group (GHD, CDGP, ISS, FSS), as well as between the children with growth hormone deficiency and children with short stature and normal growth hormone levels. There was no significant difference in ALS SDS between the control group and children with constitutional delay of growth and development;
— ALS concentration shows a significant correlation with height and weight, as well as their standard deviation scores;
— the ALS concentration shows a significant correlation with other GH-IGF-1 axis compounds. There was a significant correlation between ALS and IGF-1, IGFBP3 and the IGF-1/IGFBP3 ratio. There was however no significant correlation between ALS and maximal growth hormone concentration in spontaneous or provocation tests.

In the future, the children in our study with the lowest ALS levels should have their *IGFALS* gene sequenced to check for any mutations and polymorphisms. A study on a larger group is also necessary to create local reference values.

Usefulness of ALS assessment in routine short stature diagnostics is, at this point, still uncertain. It is possible that in the future it might play a role in the diagnosis of children with idiopathic short stature and constitutional delay of growth and development.

Data availability statement

Data available upon inquiry.

Ethics statement

The study was allowed by the Pomeranian Medical University Bioethics Committee, decision number KB-0012/102/15 (issued 5 October 2015). Informed consent to participate in the study was obtained from the legal guardians of the subjects.

Author contributions

Conceptualisation: T.J., M.W., and E.P.; methodology: T.J. and E.P.; validation: T.J., M.W., and E.P.; formal analysis: T.J., E.B, A.H-J., M.W., and E.P.; investigation: T.J. and E.P.; writing — original draft preparation: T.J. and E.P.; writing — review and editing: T.J., A.H-J., E.B., M.W., and E.P.; supervision, M.W. and E.P. All authors have read and agreed to the published version of the manuscript.

Conflict of interests

Authors declare no conflict of interests.

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