sciendo Revista Română de Medicină de Laborator 2023;31(3):205-212 RESEARCH ARTICLE
DOI:10.2478/rrlm-2023-0021

Levels and diagnostic values of serum visceral adipose tissue-derived serine protease inhibitor and secreted frizzle-related protein 5 in children with idiopathic short stature

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ABSTRACT

Background: Idiopathic short stature (ISS) is a common type of short stature. We aimed to analyze the diagnostic values of serum visceral adipose tissue-derived serine protease inhibitor (Vaspin) and secreted frizzle-related protein 5 (SFRP5) for ISS children.

Methods: Sixty-five ISS children treated from February 2019 to June 2022 were selected as an ISS group, while another 65 healthy children receiving physical examination in the same period were selected as a healthy group. Their general data, physical development status, levels of serum Vaspin and SFRP5, and levels of serum growth hormone-releasing hormone (GHRH)/growth hormone (GH)/insulin-like growth factor-1 (IGF-1) axis-related indicators were compared.

Results: The ISS group had lower body height, body weight, bone age, growth velocity and serum SFRP5 level and higher serum Vaspin level than those of the healthy group ($P<0.05$). The levels of serum GHRH, GH and IGF-1 were lower in the ISS group than those in the healthy group ($P<0.05$). Body height, body weight, bone age, growth velocity, and levels of GHRH, GH and IGF-1 were negatively correlated with serum Vaspin level ($r<0$, $P<0.05$) but positively correlated with serum SFRP5 level ($r>0$, $P<0.05$). The areas under the ROC curves of serum Vaspin and SFRP5 and their combination for the diagnosis of ISS were 0.871 [95% confidence interval (CI): 0.812-0.929], 0.880 (95% CI: 0.824-0.935) and 0.942 (95% CI: 0.907-0.977), respectively.

Conclusions: Children with ISS have a higher level of serum Vaspin but a lower level of SFRP5, and the combined detection has a higher diagnostic value for ISS.

Keywords: child; idiopathic short stature; secreted frizzle-related protein 5; visceral adipose tissue-derived serine protease inhibitor

Received: 19 April 2023; Accepted: 17 July 2023; Published: 20 July 2023

INTRODUCTION

Idiopathic short stature (ISS) is a common type of short stature and accounts for about 60-80% of short stature, with the morbidity rate increasing annually [1]. ISS refers to that the body height of children is more than two standard deviations lower than that of normal children of the same gender and age, and the growth velocity is less than 5 cm/year, but the birth length and weight are within the normal range, without obvious predisposing factors such as endocrine disease, growth hormone (GH) deficiency and genetic metabolic disease [2]. The pathogenesis of ISS has not been fully clarified, but it is mostly related to the growth hormone-releasing hormone (GHRH)/GH/insulin-like growth factor-1 (IGF-1) axis dysfunction, nutritional status and skeletal development disorder [3,4]. Insulin can accelerate glucose utilization and protein and lipid synthesis, thereby providing nutrients for growth and development. Visceral adipose tissue-derived serine protease inhibitor (Vaspin) is a cytokine with serine protease-regulatory activity, and secreted frizzle-related protein 5 (SFRP5) is a cytokine with a similar structural domain to that of Wnt transmembrane receptor frizzled protein [5]. Both Vaspin and SFRP5 are adipokines synthesized and secreted by adipose tissues, which can enhance insulin sensitivity and participate in the regulation of neuroendocrine, immune and metabolic activities [6,7]. Therefore, Vaspin and SFRP5 may be involved in growth and development by regulating the insulin level. However, the associations of Vaspin and SFRP5 with ISS have not been reported hitherto. Hence, the expressions of serum Vaspin and SFRP5 in ISS and healthy children were analyzed in this study, and their correlations with the physical development status and GHRH/GH/IGF-1 axis were explored.

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MATERIALS AND METHODS

General data

This prospective study has been approved by the ethics committee of our hospital. Sixty-five children with ISS treated in our hospital from February 2019 to June 2022 were selected as an ISS group. The inclusion criteria were as follows: 1) children who met the diagnostic criteria for ISS [8]: the body height was more than two standard deviations lower than that of normal children of the same gender and age, and the growth velocity was less than 5 cm/year, 2) those aged 3-14 years, 3) those with the maximum GH level >10 μg/L in the GH stimulation test, 4) those with normal cognitive function, 5) those without using drugs affecting bone metabolism, thyroid hormone or GH level in the last three months before enrollment, 6) those who cooperated in relevant examinations, 7) those without abnormalities in diet and psychological status, and 8) those whose parents voluntarily signed the informed consent form.

The exclusion criteria involved: 1) children with a recent history of fracture or bone metabolism-related diseases, 2) those who used exogenous vitamin D preparations in the last three months before enrollment, 3) those with ISS caused by chromosomal diseases, intrauterine growth retardation or Turner syndrome, or 4) those with severe mental disorders, 5) those complicated with chronic organic diseases, 6) those complicated with major organ dysfunction, or 7) those with intrauterine growth retardation.

Another 65 healthy children receiving physical examination in our hospital in the same period were selected as a healthy group, and they had normal organ function and development status.

GH stimulation test

This test was conducted for fasting children who lay down quietly in the morning. On the 1st day, 10 g/kg levodopa was given orally. Venous blood samples were collected before and 30, 60, and 90 minutes after administration, and sent for examination immediately. On the 2nd day, 0.5 g/kg arginine (maximum dose: 30 g) was dissolved in normal saline and infused intravenously within 30 minutes. Venous blood samples were collected 30, 60, 90, and 120 minutes after administration, and sent for examination immediately.

General data and physical indicators

The gender, age, and physical indicators were recorded, specifically as follows:

1. Body height: The children were instructed to stand bareheaded and barefooted on the floor, with their eyes looking straight ahead and their bodies close to HW-700 ultrasonic height and weight measuring machine (Henan Lejia Electronic Technology Co., Ltd., China). Then the researcher moved the horizontal plate to the top of the child's head and read the value. The same machine was used for all children.
2. Body weight: After the above-mentioned measuring machine was placed on a flat surface and calibrated, the children were instructed to stand barefooted scantily clad in the middle of the scale, and the researcher read the value. The same scale was used for all children.
3. Bone age: The bone age was measured at the bone in the middle of the left wrist joint on the palmar side by radiologists using Philips digital X-ray machine following the Skeletal Development Standards of Hand and Wrist for Chinese Children (CHN method) [9].
4. Body mass index (BMI): BMI = body weight (kg)/ body height^2 (m).
5. Growth velocity: Growth velocity = (body height during follow-up - body height at enrollment)/time interval (month) × 12.

Detection of serum laboratory indicators

Fasting venous blood (5 mL/patient) was collected from the healthy group on the day of physical examination and from the ISS group before treatment, left still at room temperature (20-26°C) for 30 min, and centrifuged at a radius of 10 cm and 2,500 r/min for 10 min. Then the upper serum was harvested, placed into a centrifuge tube, and stored at -80°C for later testing. The levels of serum Vaspin, SFRP5, GHRH, GH and IGF-1 were measured by enzyme-linked immunosorbent assay according to the instructions of corresponding kits (Kebang Xingye (Beijing) Technology Co., Ltd., China).

Statistical analysis

SPSS 23.0 software was used. All measurement data were tested for normality, and those of normal distribution were described by ($\overline{x} \pm$ SD) and analyzed by the *t*-test. The non-normally distributed measurement data were expressed as median and interquartile range and analyzed by the Mann-Whitney U test. A Bivariate Pearson linear correlation test was performed to detect the correlations between indicators. The effects of serum Vaspin and SFRP5 levels on ISS were assessed by general linear regression analysis. The diagnostic values of serum Vaspin and SFRP5 levels and their combination for ISS were evaluated using receiver operating characteristic (ROC) curves. $P<0.05$ was considered statistically significant.

RESULTS

General data and levels of serum Vaspin and SFRP5 of ISS and healthy groups

There were no significant differences in gender or age between ISS and healthy groups (P>0.05). The two groups were age- and sex-matched. The ISS group had smaller body height and body weight, lower bone age, growth velocity and serum SFRP5 level and higher serum Vaspin level than those of the healthy group (P<0.05) (**Table 1**).

GHRH/GH/IGF-1 axis in ISS and healthy groups

The levels of serum GHRH, GH and IGF-1 in the ISS group were lower than those of the healthy group (P<0.05) (**Table 2**).

Correlations of serum Vaspin and SFRP5 levels with physical development and GHRH/GH/IGF-1 in ISS group

The results of Pearson correlation analysis showed that body height, body weight, bone age, growth velocity, and levels of GHRH, GH and IGF-1 were negatively correlated with the serum Vaspin level (r<0, P<0.05) but positively correlated with the serum SFRP5 level (r>0, P<0.05) (**Table 3**).

Results of linear regression analysis of influencing factors for ISS

As shown by linear regression analysis, body height, body weight, bone age, growth velocity, and levels of Vaspin, SFRP5, GHRH, GH and IGF-1 were all associated with ISS (P<0.05) (**Table 4**).

Diagnostic values of serum Vaspin and SFRP5 and their combination for ISS

The ROC curves were plotted with the presence or absence of ISS as a state variable (0 = Yes, 1 = No) and the serum Vaspin and SFRP5 levels as test variables. The areas under the ROC curves (AUCs) of serum Vaspin and SFRP5 and their combination for the diagnosis of ISS were 0.871, 0.880 and 0.942, respectively (**Table 5** and **Figure 1**), suggesting that the combined detection had the highest diagnostic efficiency.

Table 1. General data and levels of serum Vaspin and SFRP5 of ISS and healthy groups

Indicator		ISS group (n=65)	Healthy group (n=65)	Statistical value	P
Gender	Male	33	37	0.495	0.482
	Female	32	28		
Age (year)		7.32±1.75	7.52±1.68	0.665	0.507
Body height (cm)		113.03±6.01	129.02±7.48	13.434	0.000
Body weight (kg)		23.73±3.43	31.78±3.76	12.761	0.000
BMI (kg/m^2)		19.20±1.58	18.85±1.36	1.354	0.178
Bone age (year)		5.74±0.89	7.32±0.66	11.487	0.000
Growth velocity (cm/year)		3.82±0.42	6.32±0.68	15.515	0.000
Serum Vaspin (ng/mL)		28.22±2.77	24.04±2.21	9.523	0.000
Serum SFRP5 (pg/mL)		11.92±1.89	14.94±1.66	9.677	0.000

Except for gender, all data are presented as $\bar{x}$ ± SD. ISS: Idiopathic short stature; SFRP5: secreted frizzle-related protein 5; Vaspin: visceral adipose tissue-derived serine protease inhibitor.

Table 2. Levels of GHRH/GH/IGF-1 axis-related indicators in ISS and healthy groups ($\bar{x}$ ± SD, ng/mL)

Group	GHRH	GH	IGF-1
Healthy (n=65)	5.59±0.74	25.42±2.83	175.10±12.61
ISS (n=65)	3.84±0.80	17.03±2.77	158.84±10.34
t value	12.989	17.068	8.037
P	0.000	0.000	0.000

GH: Growth hormone; GHRH: growth hormone-releasing hormone; IGF-1: insulin-like growth factor-1; ISS: idiopathic short stature.

Table 3. Correlations of serum Vaspin and SFRP5 levels with physical development and GHRH/GH/IGF-1 in ISS group

Indicator	Vaspin	SFRP5	Body height	Body weight	Bone age	Growth velocity	GHRH	GH	IGF-1
Vaspin	-	-0.434**	-0.524**	-0.469**	-0.435**	-0.504**	-0.429**	-0.532**	-0.425**
SFRP5	-0.434**	-	0.481**	0.478**	0.451**	0.446**	0.525**	0.513**	0.474**

**P<0.01, with a significant correlation. GH: Growth hormone; GHRH: growth hormone-releasing hormone; IGF-1: insulin-like growth factor-1; SFRP5: secreted frizzle-related protein 5; Vaspin: visceral adipose tissue-derived serine protease inhibitor.

Table 4. Results of linear regression analysis of influencing factors for ISS

Item	β	Standard error	Standard regression coefficient	t	P	95% confidence interval
Constant	4.587	0.288	-	15.923	0.000	4.017-5.158
Body height	-0.006	0.002	-0.126	-3.428	0.001	-0.009 to-0.003
Body weight	-0.012	0.003	-0.147	-3.642	0.000	-0.018 to-0.005
Bone age	-0.064	0.015	-0.142	-4.320	0.000	-0.093 to-0.035
Growth velocity	-0.092	0.018	-0.197	-5.060	0.000	-0.128 to-0.056
Vaspin	0.015	0.005	0.096	3.103	0.002	0.005-0.024
SFRP5	-0.021	0.007	-0.097	-3.090	0.002	-0.034 to-0.008
GHRH	-0.085	0.015	-0.198	-5.879	0.000	-0.114 to-0.057
GH	-0.020	0.004	-0.199	-4.905	0.000	-0.028 to-0.012
IGF-1	-0.003	0.001	-0.071	-2.403	0.018	-0.005 to-0.000

GH: Growth hormone; GHRH: growth hormone-releasing hormone; IGF-1: insulin-like growth factor-1; ISS: idiopathic short stature; SFRP5: secreted frizzle-related protein 5; Vaspin: visceral adipose tissue-derived serine protease inhibitor.

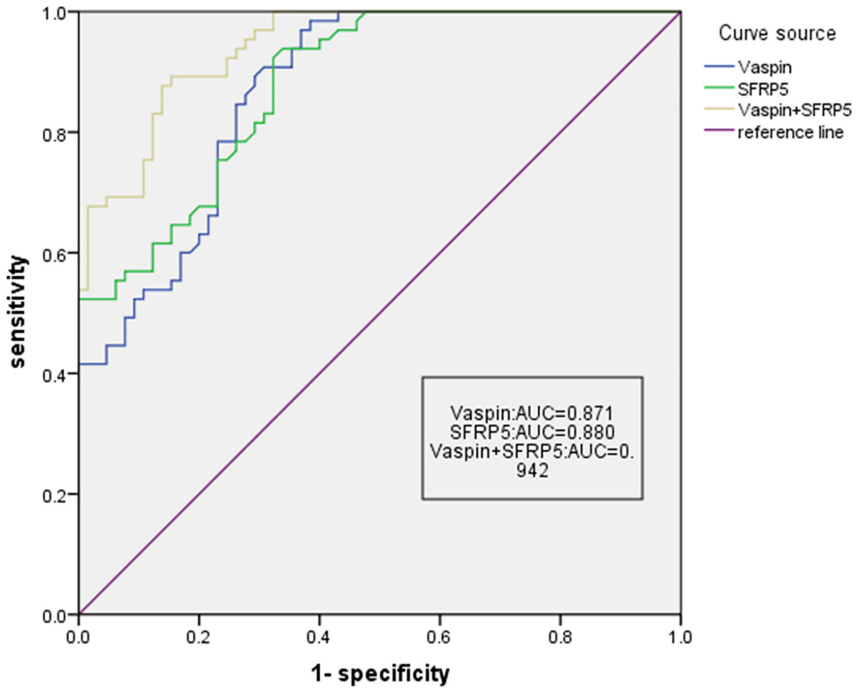

Fig. 1. ROC curves of serum Vaspin and SFRP5 and their combination for the diagnosis of ISS.

Table 5. Diagnostic values of serum Vaspin and SFRP5 and their combination for ISS

Indicator	Optimal cutoff value	AUC	Standard error	95% CI	P
Vaspin	26.352 ng/ml	0.871	0.030	0.812-0.929	0.000
SFRP5	12.932 ng/ml	0.880	0.028	0.824-0.935	0.000
Combination	-	0.942	0.018	0.907-0.977	0.000

AUC: Area under the curve; CI: confidence interval; ISS: idiopathic short stature; SFRP5: secreted frizzle-related protein 5; Vaspin: visceral adipose tissue-derived serine protease inhibitor.

DISCUSSION

The GHRH/GH/IGF-1 axis has been identified to play an important regulatory role in the skeletal growth and development of children [10]. GH promotes growth mainly through the following three mechanisms. First, after binding GH receptors (GHRs), GH can promote the proliferation of a variety of IGFs through IGF-1 transduction, thereby enhancing DNA and protein formation. Second, GH can directly bind GHRs on the surface of target cells to stimulate their growth. Moreover, GH can stimulate the release of IGFs by bones through paracrine and autocrine pathways, exerting a local growth effect [11,12]. Third, GH can stimulate the release of IGFs by liver cells, act on target cells through the endocrine pathway, and facilitate the transformation of pre-chondrocytes into chondrocytes and development into osteocytes, ultimately promoting skeletal growth and development. In addition, IGF-1 can contribute to GH synthesis and ac-

celerate chondrocyte division, thereby regulating human growth and development. In this study, the levels of serum GHRH, GH and IGF-1 in the ISS group were lower than those of the healthy group. The results are consistent with the pathogenesis of ISS that GHRH/GH/IGF-1 axis dysfunction is caused due to decreased GH levels and obstructed signal transmission, thus leading to growth and development disorders in children [13]. The inhibited function of the GH/IGF-1 axis can reduce the production of proteins related to cell activity and affect the growth and development of children, thus promoting the occurrence of ISS [14]. Adipose tissues not only consume and store energy but also work as an endocrine organ that can secrete free fatty acids, angiotensin, leptin and other adipocytokines, hormones and regulators, which can affect local or distant tissues and organs [15]. It has been confirmed that adipocytokines in adipose tissues are related to GH and IGF-1 levels, which may become new targets for regulating the axis function [16-18].

SFRP5 is an adipocytokine mainly secreted by white adipose tissues and mostly found in muscle cells and adipocytes, which can improve insulin sensitivity, relieve inflammatory injury and ameliorate glucolipid metabolism. Hu *et al.* reported that SFRP5 inhibited lipolysis, enhanced glucose utilization and uptake in skeletal muscles and improved insulin sensitivity, while the down-regulation of SFRP5 expression attenuated glycogenesis and peripheral glucose uptake in the liver, thus resulting in glucolipid metabolism disorders [19]. Carstensen-Kirberg *et al.* reported through *in vitro* experiments that SFRP5 enhanced glucose-stimulated insulin secretion in the rat pancreatic β-cell line INS-1E [20]. Rydzewska *et al.* also demonstrated that SFRP5 expression was associated with insulin sensitivity [21]. The associations of glucolipid metabolism and insulin sensitivity with ISS have been verified [22], so we herein postulated that SFRP5 was related to the occurrence and development of ISS. The results showed that the serum SFRP5 level in the ISS group was lower than that in the healthy group. The serum SFRP5 level was negatively correlated with the occurrence of ISS but positively correlated with body height, body weight, bone age, growth velocity, and levels of GHRH, GH and IGF-1. In other words, low SFRP5 expression may promote the occurrence of ISS, and its expression was related to the physical development and GHRH/GH/IGF-1 axis in children. The possible reasons are as follows. First, SFRP5 can suppress the endogenous Wnt signalling pathway by competitively binding Wnt5 protein, thereby weakening its downstream non-canonical signalling pathway, blocking the c-Jun N-terminal kinase pathway, and antagonizing the serine phosphorylation of insulin receptor substrate-1. As a result, insulin resistance is weakened and insulin sensitivity is enhanced [23]. Second, the GHRH/GH/IGF-1 axis plays a key role in the growth and development of children, and its dysfunction can affect the proliferation and differentiation of osteoblasts and cause growth disorders. SFRP5 may be indirectly involved in the pathogenesis of ISS through positive feedback regulation of the GHRH/GH/IGF-1 axis. Third, SFRP5 may affect osteoblasts and bone metabolism by inhibiting the activation of the Wnt signalling pathway. Chen *et al.* reported that the expression of SFRP5 in bone marrow fluid was negatively correlated with the levels of serum markers for bone formation (N-terminal propeptide, osteocalcin, alkaline phosphatase, *etc.*), and SFRP5 may be a potential negative regulator of bone mass by inhibiting bone formation [24].

As an adipocytokine mostly expressed in adipose tissues, Vaspin can reduce the expressions of pro-inflammatory adipocytokines (e.g. TNF-α, resistin, and leptin) and enhance glucose tolerance and insulin sensitivity [25]. Kurowska *et al.* found by immunochemistry that Vaspin was present in follicular theca, granulosa cells and adipocytes, and its level was increased by GHRH, IGF-1, insulin and steroids through the stimulation of JAK/STAT, ERK1/2, PI3K, AMPK and NF-κB [26]. Tarabeih *et al*. confirmed that the serum Vaspin level was closely related to skeletal muscle development and musculoskeletal pain [27]. However, no report is available on the correlation between Vaspin and short stature at present. In this study, the serum Vaspin level in the ISS group was higher than that in the healthy group, and it was negatively correlated with body height, body weight, bone age, growth velocity, and levels of GHRH, GH and IGF-1. It was verified for the first time that Vaspin level is associated with the occurrence and progression of ISS, but its specific regulatory mechanism remains to be further confirmed by *in vitro* or animal experiments. In addition, the AUCs of serum Vaspin and SFRP5 and their combination for the diagnosis of ISS were 0.871, 0.880 and 0.942, respectively, suggesting the diagnostic efficiency of the combined detection was the highest. Collectively, the expressions of serum Vaspin and SFRP5 in children should be closely monitored, and targeted therapy should be investigated and developed against Vaspin- and SFRP5-related signalling pathways, thereby preventing ISS and inhibiting disease progression.

In conclusion, children with ISS have a higher level of serum Vaspin but a lower level of SFRP5, which are closely related to the physical development status and the GHRH/GH/IGF-1 axis. The combined detection of Vaspin and SFRP5 has the highest diagnostic value for ISS. To our knowledge, this is the first article to establish a (predictive) relationship between SFRP5 (alone and together with Vaspin) and ISS. Nevertheless, this study

has limitations. The sample size is small. Additionally, the IGFBP-3 level was not measured. Moreover, most of the ISS children included in this study were 4-11 years old. Therefore, the impact of puberty (11-14 years old) on hormone changes and growth was not evaluated. In-depth studies with large sample sizes are ongoing in our group to further confirm our conclusion.

AUTHORS CONTRIBUTIONS

Ling Che and Lei Chen designed this study and significantly revised this manuscript; Binlan Hou, Fei Ouyang and Huimei Zhou performed this study and drafted this manuscript. All authors have approved the submission and publication of this paper.

CONFLICTS OF INTEREST

None to declare.

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