ORIGINAL ARTICLE

Evaluation of asprosin levels in growth hormone-deficient children

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Abstract

Background: Growth Hormone Deficiency (GHD) in children is a rare endocrine condition characterized by a low GH secretion that minimizes the secretion of growth factors such as Insulin-Like Growth Factor-I (IGF-I). Asprosin (ASP), a novel adipokine predominantly secreted by white adipose tissue, plays a crucial role in the liver's production of glucose. Elevated levels of ASP are observed in metabolic diseases. *Aim and Objectives*: This study aimed to assess the levels of ASP in pediatric patients with GHD. *Material and Methods:* This research included 40 subjects (children) with GHD and 50 healthy control subjects, with measurements conducted using ELISA kits for ASP. *Results:* We found the levels of ASP to be significantly higher in the patient group (mean 5.7, range 2.28-17.79 ng/ml) compared to the control group (mean 4.1, range 3.12-5.21 ng/ml) ($p < 0.001$). *Conclusion*: This finding suggests a potential link between GHD and altered ASP secretion. Elevated levels of ASP in GHD patients may contribute to metabolic dysregulation, insulin resistance, and other associated complications.

Keywords: Asprosin, Growth hormone, FBN1, IGF-1, Insulin resistance

Introduction

Growth Hormone (GH) is a peptide hormone secreted by somatotrophs in the anterior pituitary gland with diverse tissue-specific activities such as anabolic effects on muscle and bone or catabolic action on white adipose tissue [1]. Growth Hormone Deficiency (GHD) represents an endocrine condition characterized by diminished GH synthesis, which results in decreased production of growth factors and GH-dependent hormones. It may be due to disorder within the pituitary glands or at any level of hypothalamic and brain structures that regulate its release cascade [2-3].

Asprosin (ASP) is a new adipokine fasting glucogenic and orexigenic protein identified in 2016. ASP causes the liver to release glucose quickly. However, ASP's molecular mechanisms and function are yet to be discovered. Apart from other tissues and organs, ASP is involved in various Central Nervous System (CNS) processes. It plays a role in appetite, glucose metabolism, Insulin Resistance (IR), apoptotic death of cells, and other processes [4-5]. High ASP levels are pathologically associated with IR, which stimulates the production of Insulin-Like Growth Factor-I (IGF-I), leading to the inhibition of GH secretion [6]. We hypothesized that the ASP can modulate and have important prognostic implications for GHD in children [7]. Since the FBN1 gene is widely expressed in human tissues, white adipose is likely not the only one responsible for plasma ASP. ASP secretion from wild-type human dermal fibroblasts suggests that it may be secreted from the skin [8-9]. ASP was derived from adipokine, cleaved from the C-terminal portion of pro-fibrillin. This hormone is regulated by fasting, can cause a rapid release of hepatic glucose by activating the G protein-cAMP-PKA pathway in the liver, and is found in the blood at nano-molar levels [10].

Recent studies proved there is a correlation (positive) between glycated hemoglobin (HbA1c), plasma ASP levels, Waist Circumference (WC), Fasting Plasma Glucose (FPG), IR, and Triglyceride (TG) levels [11]. Overnight fasting increases ASP levels, while feeding decreases ASP levels.

Furthermore, serum ASP concentrations are associated with mice and humans' IR and insulin levels [12]. Fibrillin-1 gene provides instructions for producing fibrillin-1, a protein crucial for the formation of elastic fibers in connective tissue. *FBN1* is helpful in maintaining the structure and elasticity of tissues such as the lungs, blood vessels, and skin. The C-terminal pro-fibrillin-1 propeptide asprosin is described as a white adipose tissue-derived hormone that stimulates hunger and activates glucose-release from hypothalamic neurons. Moreover, patients with a truncated mutation of *FBN1* coding pro-fibrillin have an impact on plasma ASP and reduce the levels of insulin [13]. There is no definitive proof linking ASP and GH directly, yet both play a role in metabolism and energy regulation. Research indicates that ASP might indirectly govern GH release through its impact on insulin production and glucose utilization. Conversely, GH can also affect fat tissue function, potentially indirectly influencing ASP generation or activity. Seravel studies proposed correlations between clinical parameters and ASP levels [14-15].

Material and Methods

Forty (44.4%) pediatric patients with GHD and 50 (55.6%) healthy children in the age group of 5-14 years were recruited as patient and control group, respectively at the National Diabetes Center of Mustansiriyah University in Baghdad, Iraq after taking their parental consent. Their mean age and standard deviation are depicted in Table 1.

Table 1: Mean age in patient and control groups

Group	Mean ± SD (years)	N
Patient	11.06 ± 2.893	40
Control	9.4 ± 2.893	50

The inclusion criteria for controls were children with normal growth, free from chronic or endocrine diseases, while for patients, children diagnosed with GHD through clonidine stimulation tests were included. This test evaluates GH secretion in children suspected of GHD. Clonidine stimulates GH release by inhibiting somatostatin. Patients had not previously received growth hormone therapy, ensuring that the effects of untreated GHD were the focus. Both groups had to be in normal general health based on routine clinical and laboratory tests. Exclusion criteria eliminated individuals with chronic or acute illnesses, kidney dysfunction, endocrine, nutritional, cardiovascular, or autoimmune diseases, as well as genetic syndromes or malignancies, all of which could potentially affect hormone levels or metabolism.

The study was conducted from September 2023 to January 2024, during which eight milliliters of whole blood were collected from participants using disposable plastic syringes, placed in gel tubes, and centrifuged at 3000 rpm for 7 minutes to obtain serum for laboratory analysis. Participants also underwent medical assessments, including physical measurements like weight and height, and their BMI was calculated using the formula: BMI = weight (kg)/height^2 (m^2).

To assess hormonal levels, physical examination and patient histories were reviewed. Key diagnostic indicators of GHD in children, such as bone

age, growth velocity, and stature, were used. Auxological measurements, radiographic estimation of bone age, and IGF-I levels were also evaluated, alongside provocative GH tests. Vitamin D3, GH, and IGF-I levels were measured using the DiaSorin analyzer device with Elecsys kits for Vitamin D3, IGF-1, and hGH, respectively.

For ASP levels, an Enzyme-Linked Immunosorbent Assay (ELISA) was performed following the manufacturer's instructions. Serum samples were prepared according to the kit's protocol, and standards were created to generate a calibration curve. The assay involved coating ELISA plates with ASP capture antibodies, followed by incubation and blocking of non-specific binding. Serum samples and standards were added, and detection was achieved using a detection antibody and streptavidin-HRP conjugate. A substrate solution was then added to develop a colorimetric reaction, and the intensity, proportional to ASP concentration, was measured at 450 nm using a microplate reader. ASP levels were quantified based on the standard curve.

Ethical approval

All research involving human participants adhered to ethical guidelines established by the relevant institutional or national review board, ensuring acquiescence with the rules summarized in the 1964 Declaration of Helsinki and any subsequent revisions or comparable ethical standards. The institutional Ethics Committee of the University of Mustansiryah / National Diabetes Center approved the study on 28-08-2023, reference number EC/NDC/2023-012

Statistical analysis

The statistical software package used for the analysis was Statistical Package for Social Sciences (SPSS) version 26.0 and Microsoft Excel (2020). We considered the value of $p < 0.05$ to be statistically significant. The Kolmogorov-Smirnov test was utilized to assess the appropriate choice between parametric and non-parametric analyses, and all variables (except IGF-1) were determined to be non-parametric distributions. Thus, Mann-Whitney U test was used to evaluate the significance levels between the control and patient groups. These results are displayed as median, minimum, and maximum. In comparison, the Spearman test was used for correlation.

Results

In this study, the BMI measurements of GHD in both patients and controls revealed no notable differences between the two groups. On the other hand, we examined the levels of ASP, GH, IGF-1, vitamin D3, and Haemoglobin (Hb) in both the patient and control groups. In the patient group, ASP levels and Hb were significantly increased compared to the healthy samples ($p = 0.000$ and $p = 0.026$, respectively). At the same time, GH and D_3 were significantly lower ($p = 0.000$ and 0,015, respectively) in the patient group. Gender distribution in the patient group shown in figure 1. The median and value of p of the results are shown in Table 2.

We found a significant inverse correlation between GH and ASP r = -0.312; $p = 0.015$) as shown in Figure 2. On the other hand, we found a significant positive correlation between IGF-1 and BMI (r = 0.266; $p = 0.040$), and between D_3 and Hb (r = 0.412; $p = 0.001$) as shown in Table 3.

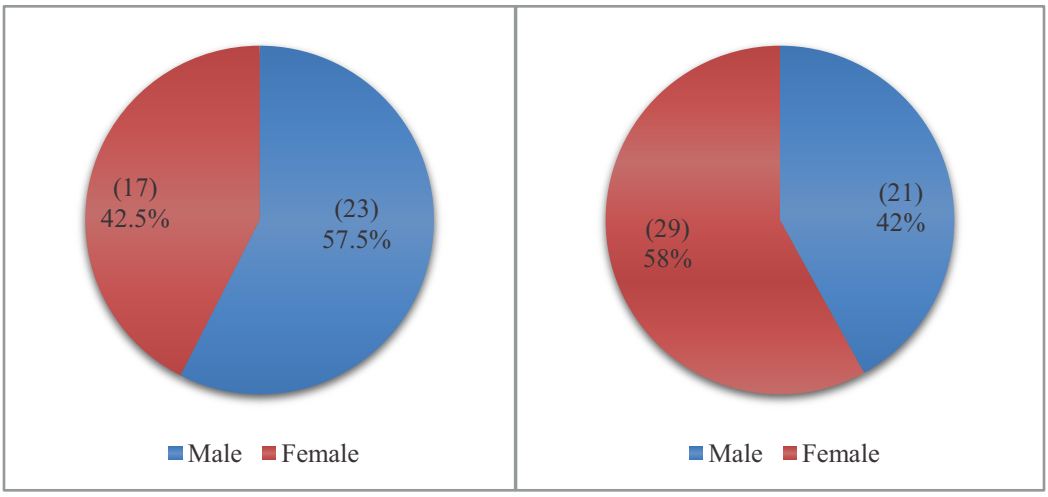

Figure 1: Gender distribution in the patient group [(23) 57.5% males, (17) 42.5% females] and control group [(21) 42% males, (29) 58% females]

Table 2: Study parameters levels in control and patient groups

Metabolites	Control	Patient	*p*
	[Median (Min - Max)]	[Median (Min - Max)]	
ASP (ng/mL)	[4.1 (3.12-5.21)]	[5.7 (2.28-17.79)]	0.000
GH (ng/mL)	[7.95 (6.9-19.1)]	[0.28 (0.1-3.5)]	0.000
IGF-1 (ng/mL)	[183 (25.38-541)]	[196 (47-396)]	0.760
BMI (Kg/m2)	[18.61 (12.25-21.33)]	[16.56 (9.6-31.64)]	0.172
D3 (ng/mL)	[11.4 (8.3-17.8)]	[10.65 (8.1-17.64)]	0.015
Hb (g/dl)	[10.65(9-13.3)]	[12.1 (9.1-14.8)]	0.026

ASP – Asprosin; GH – Growth Hormone; IGF-1 – Insulin-like Growth Factor 1; BMI – Body Mass Index; D3 – Vitamin D3; Hb - Hemoglobin

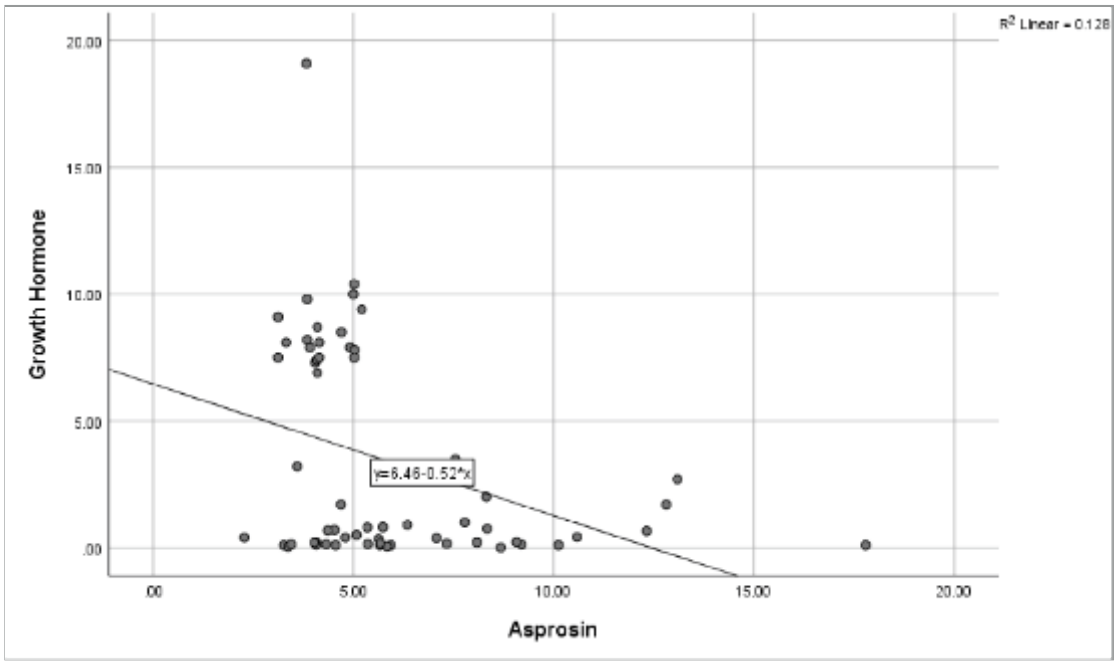

Figure 2: Correlation between GH and ASP

Table 3: Correlation of metabolite levels in patient and control groups

Metabolites		GH	BMI	IGF-1	ASP	Hb	D_3
GH	**Correlation Coefficient**	1.000	0.148	-0.081	-0.312*	-0.169	0.248
	Sig. (2-tailed)	.	0.258	0.536	0.015	0.197	0.056
BMI	**Correlation Coefficient**	0.148	1.000	0.266*	-0.025	-0.112	0.180
	Sig. (2-tailed)	0.258	.	0.040	0.847	0.394	0.169
IGF-1	**Correlation Coefficient**	-0.081	0.266*	1.000	-0.121	0.104	-0.138
	Sig. (2-tailed)	0.536	0.040	.	0.359	0.431	0.295
ASP	**Correlation Coefficient**	-0.312*	-0.025	-0.121	1.000	0.134	-0.042
	Sig. (2-tailed)	0.015	0.847	0.359	.	0.307	0.752
Hb	**Correlation Coefficient**	-0.169	-0.112	0.104	0.134	1.000	0.412**
	Sig. (2-tailed)	0.197	0.394	0.431	0.307	.	0.001
D_3	**Correlation Coefficient**	0.248	0.180	-0.138	-0.042	0.412**	1.000
	Sig. (2-tailed)	0.056	0.169	0.295	0.752	0.001	.

**Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level (2-tailed)*

ASP – Asprosin; GH – Growth Hormone; IGF-1 – Insulin-like Growth Factor 1; BMI – Body Mass Index; D3 – Vitamin D3; Hb - Hemoglobin

Discussion

Generally, puberty starts later and extends over a more extended period for boys than girls. Consequently, conditions linked to growth, like GHD, tend to be more apparent in males than females, prompting an earlier consultation with endocrinology specialists. Certain conditions impacting the pituitary gland or genetic factors might be more prevalent in males. BMI includes two main parameters, height and weight of the body, and is raised in overweight/obese children [16].

In this study, BMI showed no significant differences between the patient 16.56 (9.6-31.64) kg/m^2 and control 18.01 (12.25-21.33) kg/m^2 groups (p = 0.075). This might be due to the multiple metabolic effects seen in GHD including impaired bone growth, and fat to muscle composition in the body [17]. Multiple hormones, such as GH, are secreted by the pituitary gland. Generally, there is a connection between IGF-1 and GH levels, as GH stimulates the production of IGF-1 in tissues and the liver. IGF-1 plays a key role in the growth and development of muscles, bones, and the skeletal system [18-19].

When comparing GHD children to the control group, a noticeable reduction in stature among patients aligns with the recognized outcomes of GHD, often presenting as diminished growth in minors. This reduction in stature suggests that GHD notably influenced the patients' linear development. IGF-1 is separately linked with the lowering of the mass of skeletal muscle, alongwith BMI. Since our results showed no significant difference in IGF-1 levels between groups, this may explain the normal results of BMI among the two groups [20-21].

There was a notable negative correlation between levels of ASP and GH. These results differ from those of Al-Jubawi *et al.* (2022), who found that growth hormone was linked to ASP, with no significant change observed between ASP and growth hormone levels. They attributed these findings to a genetic defect in the gene responsible for ASP [22].

We found that ASP was significantly increased (p = 0.000) in the patient group 5.7 (2.28-17.79) ng/mL as compared to the control group 4.1 (3.12-5.21) ng/mL. Studies show that IR is pathologically associated with high ASP levels, which increase glucose levels in serum, resulting in higher insulin levels. This may stimulate the production of IGF-1, which leads to the inhibition of GH secretion through feedback inhibition [23]. ASP plays a critical role in IR, glucose metabolism, and obesity in children; frequently, GHD characterized by dysregulation and changed metabolic work of ASP might occur due to the metabolic deviations linked with GH insufficiency [24]. Furthermore, recent studies have proven the anabolic action of GH in most tissues except adipose (where ASP is synthesis), which converts the stored triglycerides into Free Fatty Acids (FFA) due to the catabolic influence of GH [24]. Insulin action and GH are antagonized via different pathways [25]. This includes the restriction of the anti-lipolytic effect of insulin in the systemic circulation, which then raises FFA and stimulates lipotoxicity, thus leading to pathophysiological problems such as IR. Several studies suggest ASP is associated with IR, which can lead to raised ASP concentration and change the metabolic profile of the body [19, 25-26].

According to these results, alterations in the endocrine environment of patients, marked by hormonal imbalances, might be impacting ASP levels. The idea of a feedback loop between GH and ASP levels is intriguing, suggesting that changes in GH activity or levels could influence ASP concentrations [27].

This study had a strong, significant positive correlation between D_3 and Hb ($p = 0.001$). Vitamin D_3 may increase iron subcategory of absorption for hemoglobin synthesis and erythropoiesis, probably improving anemia in people with vitamin D_3 deficiency. Overall, while there is evidence to suggest a correlation between vitamin D_3 and Hb levels, the exact nature of this relationship and the mechanisms involved are still being studied [28-29]. Impact on immune function, anemia, regulation of red blood cell production, and bone health could be the primary causes of a significant correlation between Vitamin D_3 and Hb [30].

Conclusion

Our study demonstrates a significant elevation in ASP levels in children with GHD as compared to the control group, thereby suggesting a potential link between GHD and altered ASP secretion. Elevated levels of ASP in GHD patients may contribute to metabolic dysregulation, IR, and other associated complications. Further research is warranted to elucidate the precise mechanisms underlying the relationship between GHD and ASP, as well as the implications of elevated ASP levels in this patient population. Understanding the role of ASP in GHD could offer insights into novel therapeutic targets for managing metabolic disturbances in affected children. Overall, the findings of our study suggest complex interactions between GH, IGF-1 and ASP highlighting the importance of understanding the interplay between these hormones in various physiological processes and diseases. Further research is needed to fully elucidate the mechanisms underlying these relationships and their implications for clinical practice.

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