Assessment of Serum Afamin Level as a Diagnostic Biomarker for Growth Hormone Deficiency in Children with short stature

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Abstract

Background: Growth hormone deficiency is a common cause of short stature. Currently, for the accurate diagnosis of growth hormone deficiency, there is no single biochemical parameter used. Afamin is a glycoprotein generated from the liver, and it's a novel metabolic biomarker, Age, gender, prandial state, or circadian rhythms do not affect the circulating levels of Afamin, which is rather stable and can be tested in serum or plasma.

Objectives: To assess whether serum Afamin levels (both basal and stimulated) could serve as an alternative or complementary diagnostic marker for growth hormone deficiency in children with short stature.

Methods: A case- control study involve a total of 80 children,40 short stature with GHD and 40 control also short stature without GHD, aged 6-15 years attended the the National Diabetic Center for Treatment and Research/Al-Mustansiriya University in Baghdad/Iraq for the period from October 2024 to January 2025. Blood samples were collected from the studied subjects to determine levels of basal GH, after 60 mins and 90 mins of provocation with clonidine. The study also included the measurement of the levels of basal insulin like growth factor (IGF-1) and Afamin both basal and after stimulation

Results: Growth hormone deficiency patients demonstrate higher Afamin levels compared to controls ($P < 0.01$), even after stimulation serum Afamin levels remain significantly higher.

Conclusions: serum Afamin level might be useful biomarker for the diagnosis of growth hormone deficiency in children. Further large studies are needed to confirm the diagnostic utility of the serum Afamin level.

Keywords: Afamin, growth hormone deficiency, IGF-1, short stature

INTRODUCTION

Anterior pituitary produces and secretes growth hormone, a polypeptide hormone that promotes the synthesis of insulin-like growth factor (IGF)-I in peripheral tissues such as cartilage and the liver. [1, 2] In addition to carrying out metabolic activities like glucose synthesis, protein anabolism, fat metabolism, and bone metabolism, IGF-I promotes the growth and division of cartilage tissues. [3]

The definition of growth hormone deficiency (GHD) is growth failure due to the inability to produce enough GH by the pituitary gland. [4] Assessment of the patient's medical history, physical examination, and proper interpretation of longitudinal growth which has distinct characteristics at every stage of life is necessary for the diagnosis. [5] Because pituitary growth hormone (GH) release is pulsatile, has a relatively short half-life 10–20 min, and can often remain undetectable for significant periods of the day, measuring GH production is challenging. [6, 7] Somatostatin and growth hormone releasing hormone (GHRH) are the two most significant hypothalamic proteins in the complex regulation of GH production, which involves several peptides and neurotransmitters. [8]This implies that in order to test for GHD, GH secretion must be provoked by physiological or pharmacological stimulation. [9]

To differentiate GHD from non-GHD participants, GH stimulation tests employ a predetermined cut-off concentration for peak GH. Due to the absence of a "gold standard" test for diagnosing GHD, relatively arbitrary cut-off levels have been developed. [6] As a result, straightforward GH measures are not an option, and there is not a single biochemical parameter or combination of them that can be used as a GHD marker with accuracy, longevity, and affordability. [10] Given all of these controversies, further study is required to provide a reliable diagnostic standard for GHD. The likelihood of reaching final adult height may be improved by starting therapy early. Therefore, it is crucial to diagnose growth hormone deficiency as soon as possible. [11] However, overdose of growth hormone can lead to aortic and mitral valve calcifications, hypertension, and arrhythmia. [12]

Afamin is a glycoprotein generated from t he liver that was identified in 1994,With a molecular weight of 87 kDa and a 55% amino acid sequence similarity to albumin, However, it is much more complexly and heavily glycosylated than albumin. [13,14] Since then, it has been recognized as a possible biomarker for neurological disorders, certain forms of cancer, and pregnancy-related issues. [13] Type 2 diabetes mellitus (T2DM), insulin resistance (IR), and metabolic syndrome (MS) have all been linked to elevated serum afamin levels. [15] Afamin is mostly produced by the liver, but it is also found in considerable quantities in human cerebrospinal fluid and ovarian follicles. [16] However there have been reports of minor AFM expressions in the human brain, testis, heart, kidney, and ovary. [17] Both α-tocopherol and γ-tocopherol, two of the most significant forms of vitamin E, exhibit a particular binding affinity for afamin. It has been calculated that each afamin molecule has up to 18 binding sites for vitamin E. [14] Because afamin has a high binding capacity for vitamin E, it is believed to play a role in transporting of vitamin E in bodily fluids. [18]

Only lately have we discovered that afamin may function as a chemokine generated from osteoclasts in the field of bone biology. [17] It is interesting to note that mesenchymal stem cells, preosteoblasts, and mature osteoblasts all produced afamin in addition to differentiated osteoclasts. These findings

imply that afamin might have other roles in the bone microenvironment as a coupling factor. [19] Age, gender, menstrual cycle, prandial state, or circadian rhythms do not affect the circulating levels of afamin, which is rather stable and can be tested in serum or plasma. [20]

METHODS

This case-control study was carried out in the National Diabetic Center for Treatment and Research/Al-Mustansiriya University in Baghdad/Iraq for the period from October 2024 to January 2025. A total of 80 patients(48 male, 32 female), aged 6-15 years were selected 40 short stature children with growth hormone deficiency, and 40 controls, also short stature without growth hormone deficiency. Subjects were chosen after they were admitted and diagnosed as short stature based on the CDC growth charts, which were developed by the National Centre for Health Statistics in collaboration with the National Centre for Chronic Health Promotion and Disease Prevention (2000).[21]

The GHD diagnosis has been established based on assessment of the patient's medical history, physical examination, as well as biochemical tests of the GH-IGF-1 axis and proper interpretation of longitudinal growth which has distinct characteristics at every stage of life. Growth hormone levels (<7 ng/ml) after clonidine stimulation tests are considered indicative of GHD. [22]

The Inclusion criteria: short-statured children aged 6-15 years old, and Exclusion criteria: this study excluded patients having any type of chronic disease such as diabetes or hypothyroidism, Recent major surgery or trauma, Acute infectious diseases, Who had a fracture , children on cortisone, thyroxin, or oestrogen drugs.

Blood samples (2–5 ml) were collected from each participant via venepuncture using disposable syringes and transferred into gel tubes. Sampling was done between 8:00 and 11:00 AM after an overnight fast. A baseline sample was taken before administering clonidine orally at a dose of 0.15 mg/m². Follow-up samples were collected at 1 hour and 1.5 hours after administration to measure growth hormone (GH) levels. All blood samples were centrifuged at 3000 rpm for 10 minutes, and the resulting serum was stored at -20°C until analysis. Also The basal blood sample was taken for Afamin measurement, and only the serum sample corresponding to the higher GH level after stimulation (either at 1 hour or 1.5 hours) was used, while the remaining sample was discarded.

Human growth hormone (HGH) is determined using a sandwich chemiluminescence immunoassay technique, the method for the quantitative determination of IGF-1 is based on a one-step sandwich chemiluminescence immunoassay technique, and afamin was detected using enzyme-linked immune sorbent assay (ELISA) kite.

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki .After ethical approval obtained from the local ethics committee (number: 52 on 31/10/2024).

Statistical analyses were performed using: IBM SPSS Statistics (Version 26.0, IBM Corp., Armonk, NY, USA) and GraphPad Prism (Version 9.0, GraphPad Software, San Diego, CA, USA) Statistical significance was set at $p<0.001$. Continuous variables were reported as means ± SD; categorical variables as frequencies and percentages. Parametric tests (Student's t-test) and non-parametric tests (Mann-Whitney U test) were used as

appropriate. Chi-square and Fisher's exact tests were applied for categorical data. Paired t-tests test analyzed paired data. Biomarker and Diagnostic Analysis: ROC curve analysis determined sensitivity, specificity, and optimal cutoffs using Youden's index.

RESULTS

The study included 80 paediatric subjects (48 male, 32 female) divided into two groups: Growth Hormone Deficiency (GHD group; n=40) and non-GHD (n=40) as control group. The GHD group comprised 25 males (62.5%) and 15 females (37.5%), while the Control group included 23 males (57.5%) and 17 females (42.5%). Table1 present the frequency and percentages of the sex distributions.

Table 1: Bassline Characteristic of the study groups

Parameter		GHD (n=40)	Control (n=40)	p-value
Sex	Male, n (%) femal, n (%)	25 (62.5%) 15 (37.5%)	23 (57.5%) 17 (42.5%)	0.614
Age (years)		11.77 ± 2.56	11.45 ± 2.69	0.58
Height (cm)		133.53 ± 13.35	131.21 ± 14.93	0.457
Weight (kg)		35.63 ± 14.28	27.75 ± 7.69	<0.001*
BMI (kg/m²)	mean ± SD	19.30 ± 4.81	15.77 ± 1.59	<0.001*
	Underweight Normal Overweight Obese	12.5% (5) 40% (16) 37.5% (15) 10% (4)	27.5% (11) 65.0% (26) 7.5% (3) 0% (0)	<0.0001*

*Values are presented as mean ± SD. *Statistically significant (p<0.001)*

The comparative analysis of anthropometric parameters between the GHD and control groups highlights notable differences. (Table 1) The age distribution in both groups was well-matched, with no significant difference (GHD group: 11.77 ± 2.56 years, control group: 11.45 ± 2.69 years; p = 0.58), ensuring that age is not a confounding factor in the analysis.

Height measurements between the two groups showed no significant difference (GHD group: 133.53 ± 13.35 cm, control group: 131.21 ± 14.93 cm; p = 0.457), suggesting comparable linear growth. In contrast, weight was significantly higher in the GHD group (35.63 ± 14.28 kg)

compared to the control group (27.75 ± 7.69 kg; $p < 0.001$). The GHD group also exhibited a much larger standard deviation in weight (14.28 vs. 7.69), indicating a more heterogeneous body weight distribution among GHD patients. Similarly, body mass index (BMI) was significantly higher in the GHD group (19.30 ± 4.81 kg/m²) compared to the control group (15.77 ± 1.59 kg/m²; $p < 0.001$). The larger variation in BMI within the GHD group (SD: 4.81 vs. 1.59) further underscores the diversity in body composition patterns among these patients.

The analysis of BMI categories reveals significant differences in the distribution of BMI classifications between the GHD and control groups ($p < 0.001$ for all categories). Among the GHD group (n=40), 12.5% (5 individuals) were classified as underweight, compared to 27.5% (11 individuals) in the control group, indicating a significantly lower prevalence of underweight cases in the GHD group. Conversely, a higher percentage of individuals in the control group fell within the normal BMI range (65.0% or 26 individuals) compared to the GHD group (40% or 16 individuals) Table 1.

The distribution shifts notably for the overweight and obese categories. In the GHD group, 37.5% (15 individuals) were classified as overweight, compared to only 7.5% (3 individuals) in the control group. Furthermore, 10% (4 individuals) in the GHD group were classified as obese, whereas no individuals in the control group fell into this category. These findings demonstrate a clear trend toward higher BMI classifications in the GHD group.

As shown in Table 2, the analysis of GH before and after GH stimulation revealed a significant difference in basal GH levels between the GHD and control groups. However, IGF1 levels remained comparable between the two groups.

Table 2 Growth Hormone and IGF1 Levels among studied participants.

Parameter	GHD (n=40)	Control (n=40)	p-value
Basal GH (ng/ml)	0.37 ± 0.46	1.09 ± 1.43	<0.001*
Stimulated GH (ng/ml)	4.15 ± 2.28	16.91 ± 5.99	<0.001*
p-value	<0.001*	<0.001*	
IGF1 (ng/ml)	203.02 ± 97.38	215.20 ± 128.38	1.315

Values are presented as mean ± SD. *Statistically significant (p<0.001).
Basal GH: Pre-GH stimulation test; **Stimulated GH:** Post-GH stimulation test; **GH:** Growth hormone; **IGF-1:** Insulin-like growth factor-1

Analysis of the novel biomarker revealed significant differences in AFM levels both at baseline and after stimulation Table 3.

Table 3: Comparison of Novel Biomarker between Groups

Parameter	GHD (n=40)	Control (n=40)	p-value
AFM Basal (µg/mL)	0.84 ± 0.12	0.60 ± 0.16	<0.001*
AFM Stimulated (µg/mL)	1.18 ± 0.11	0.79 ± 0.21	<0.001*
p-value	<0.001*	<0.001*	

Values presented as mean ± SD. *Statistically significant (p<0.001)

The analysis of stimulation-induced changes in AFM highlights the differential responses between the GHD and control groups. Biomarker responses were evaluated in terms of fold changes and percent changes following stimulation, providing insights into physiological adaptations and group-specific characteristics.

The basal measurements of AFM Table 4, emerged as a promising diagnostic marker with an AUC of 0.875 and optimal cutoff of 0.72 µg/mL Figur 1, showing robust sensitivity (81.40%) and specificity (82.50%). The curve demonstrates the relationship between sensitivity and specificity at various cutoff points

Table 4: Diagnostic Performance

Marker	**AUC**	**Optimal Cutoff**	**Sensitivity (%)**	**Specificity (%)**	**PPV (%)**	**NPV (%)**	**p-value**
GH basal ng/ml	0.767	≤0.29	69.77	75.00	75.0	69.8	<0.001*
AFM Basal (µg/mL)	0.875	0.72	81.40	82.50	83.33	80.49	<0.001*

*PPV (positive predictive value), NPV (negative predictive value).

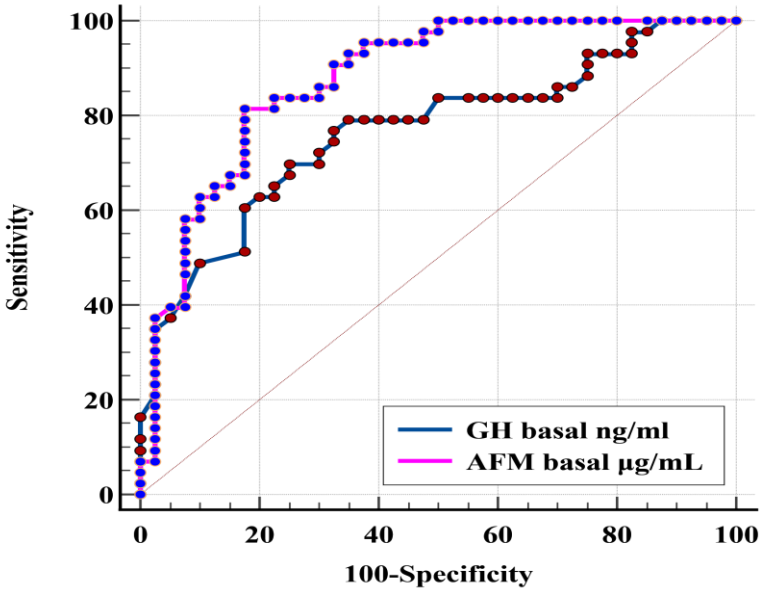

Figure 1: ROC curve analysis of basal AFM (µg/mL) and GH (ng/ml) as a diagnostic marker for GH deficiency in short stature children.

Discussion

The results demonstrate that the number of males was higher than that of females in both groups. The same results were reported in previous study. [22] While other study demonstrate that multiple pituitary hormone deficiencies (MPHD) were detected in a higher percentage of girls than boys, even though males were more likely to present to paediatric endocrinology clinics for short stature evaluation and GH provocation testing. [23] Other study suggested that it is possible that males are more vulnerable than females to pituitary function, specifically GH secretion. [24] There are several factors that explains the above results, one factor may be that both the Physician and parent are more concerned about short stature in boys than in girls due to societal expectations about male height and masculinity specially in the developing country, other factor is that growth patterns differ slightly by sex**,** with boys potentially having more pronounced growth delays or conditions that manifest more noticeably. However, true sex-based biological differences in the prevalence of GHD are not well established. Most evidence suggests the condition occurs with roughly equal frequency in both sexes.

The significantly higher BMI in the GHD group suggests alterations in body composition, possibly due to an imbalance in growth hormone (GH)-regulated metabolic processes. These findings are similar to previous study, [22] and contrasts with previous research. [25] The greater variability in both weight and BMI within the GHD group could reflect diverse responses to GH deficiency or variations in associated factors such as diet, activity level, and comorbidities.

The Serum Afamin levels were found to be significantly higher in GHD patients than in controls ($P < 0.01$), and this

difference persists even after stimulation. There are no similar studies that investigate the serum afamin level in growth hormone deficient children, although Serum afamin in adult growth hormone deficiency was investigated, and also AGHD group demonstrated significantly higher serum afamin levels compared to controls. [11] Growth hormone has numerous metabolic impacts that last a lifetime. [26] If GH levels are kept within a physiological range, clinical and animal research indicates that GH and IGF1 mediate these metabolic effects by improving whole-body nutrient utilization and lowering inflammation. This would ultimately improve insulin sensitivity and shift nutrients away from the liver. [27] GH can influence bone metabolism and body composition, the significance of GH in regarding to bone mass has long been established. [28]

On the other hand, afamin is highly linked to MS, type 2 diabetes, non-alcoholic fatty liver disease, and other IR-related disorders, according to a large-scale epidemiological investigation, even though the physiological characteristics of this hepatokine are not entirely understood. [13] Afamin acts as an antioxidant against the onset of oxidative stress in neurons and protects cortical neurons from apoptosis. This impact can be seen on its own or in combination with vitamin E. [29] Additionally, osteoclasts can control bone remodeling by directly secreting biological substances like Afamin. [30]

From the above, we can conclude the similarities between growth hormone and afamin and their relationship in metabolic processes. And the increase in afamin serum level in GHD is likely a complex interaction of hormonal change, metabolic shifts, and compensatory mechanisms in the body. Thus afamin might emerge as a promising diagnostic marker with an AUC of 0.875 and optimal cutoff of 0.72 μg/mL, The finding showsrobust sensitivity (81.40%) and specificity (82.50%). This is only relevant, though, if the patient has no additional conditions that could impact the serum afamin level, such as active cancer, kidney failure, liver illness, or children using cortisone, thyroxin, or estrogen medications. The literature discusses several potential uses in addition to the diagnosis of GHD in children. These include the potential use of serum afamin as a biomarker for GH therapy complications and safety as well as the diagnostic utility of GHD in adults. Lastly, we showed how the serum Afamin level can be used to diagnose pediatric GHD. However, there are also some limitations in our study. First, some participant groups were excluded from our study, including persons with conditions that potentially impact serum levels of Afamin and small children under the age of six. Second, the National Diabetic Center was the source of all participant recruitment. As a result, our findings might not apply to different groups.

In Conclusion

Children without conditions that potentially impact serum AFM, the serum AFM is a helpful diagnostic marker for the diagnosis of GHD. To establish the serum AFM's diagnostic utility, more extensive research in different countries is required and research included other stimulation tests to compare with.

Conflicts of interest; There are no conflicts of interest.

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