Research Square

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Association between Haemoglobin and Growth Hormone peak in Chinese Children and Adolescents with Short Stature: A Cross-Sectional Study

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Abstract

Background: This research aimed to investigate the relationship between haemoglobin (Hb) and growth hormone (GH) peak in children and adolescents with short stature.

Methods: This cross-sectional study included a total of 787 children and adolescents with short stature. Anthropometric and biochemical indicators were measured at baseline. All patients underwent GH provocation tests with L-dopa and insulin to assess GH peak levels.

Results: The univariate analysis results showed that Hb was positively associated with GH peak (β 0.07, P=0.001). Furthermore, a non-linear relationship was detected between Hb and GH peak in multivariate piecewise linear regression analysis, and the breakpoint was 123 g/L. GH peak increased with Hb elevation when the Hb level was greater than 123 g/L (β 0.08, 95% CI 0.01, 0.14; P=0.0207). However, when the Hb level was lower than 123 g/L, there was no significant relationship between Hb and GH peak (β -0.12, 95% CI -0.30, -0.06; P = 0.1867).

Conclusion: In children and adolescents with short stature, we found a non-linear association between Hb and GH peak. These findings suggest that clinicians should pay more attention to Hb levels in patients with short stature.

Introduction

Short stature refers to individuals in a similar living environment and of the same race, same sex and age who are 2 standard deviations lower than the average height of the normal population[1]. Short stature can be caused by many factors, such as endocrine, genetic, environmental, nutritional, psychological, metabolic, and clinical factors. The GH/insulin-like growth factor-1 (IGF-1) axis plays an important role during critical periods of child growth and development[2, 3].

GH is secreted in pulses, with obvious fluctuations occurring during day and night. Thus, GH levels at a single time point cannot accurately reflect GH secretion status in the body, and peak-stimulated GH after provocation testing is therefore used to determine GH status. Many studies have shown that GH level in children is closely related to the nutritional status of the body[4, 5]. Nutrition plays a critical role in programming and shaping linear growth through influencing the neuroendocrine somatotropic (GH/IGF-1) axis [6].

Hb is an indicator of the nutritional status of the human body, and Hb levels are largely affected by diet [7, 8]. Partial eating, picky diet, and single nutrient intake, which are detrimental eating habits common among patients with short stature, may lead to low Hb levels. In recent years, Hb has been found to be associated with linear growth[9–11]. Previous studies have shown that low Hb levels are associated with growth hormone deficiency (GHD)[12], but the association between Hb and GH peak after a growth hormone stimulation test is not clear. This study retrospectively explored the relationship between Hb and GH peak.

Participants And Methods

Study population

Participant data were collected from the Department of Endocrinology, Affiliated Hospital of Jining Medical University. To protect patient privacy, identifiable data were not included. This study was approved by the Human Ethics Committee of the Affiliated Hospital of Jining Medical University (Shandong, China). All procedures were performed in accordance with the ethical standards laid out in the Declaration of Helsinki. Written informed consent was obtained from each participant before data collection.

This cross-sectional study analysed data collected from a total of 787 participants between March 1, 2013 and February 28, 2019. Inclusion criteria were as follows: each participant was completely in accordance with short stature; body weight and length at birth were in the normal range; and all patients agreed to undergo two GH provocation tests with L-dopa and insulin. Exclusion criteria included patients with any of the following: thyroid dysfunction, brain tumour, Turner syndrome, congenital heart disease, chronic liver or kidney disease, malnutrition, congenital metabolic disease, chromosome abnormalities, and blood system diseases except anaemia. All tests were performed in a short-stay ward by experienced nurses according to established criteria[13].

Anthropomorphic measurements

All participants were measured with the same height-measuring instrument (Nantong Best Industrial Co. Ltd. Jiangsu, China), with an allowable error range of 0.1 cm. Participant height was measured after participants removed their hat and shoes. Height SDS was calculated based on the normal range for Chinese children[14]. Weight of all participants was measured with the same electronic scale (Xiangshan Weighing Apparatus Co. Ltd. Guangdong, China) and was accurate within ± 0.1 kg. Participant weight was measured in a fasting state. Body mass index (BMI) was calculated as weight (kilograms)/height (metres squared). The stage of puberty was assessed by physical examination according to the Tanner stages[15]. The following criteria were used to define prepuberty: for boys, a testicular volume of less than 4 mL and no pubic hair; for girls, no breast development and no pubic hair[16–18].

Laboratory measurements

To measure GH peak, two types of GH provocation tests were performed in sequence. On the first day, a provocation test with L-dopa was administered; participants with a body weight < 30 kg received 0.25 g orally and those with a body weight ≥ 30 kg received 0.5 g orally. Blood was collected at 0, 30, 60, 90, 120 min, and GH level was measured by radioimmunoassay. On the second day, a second GH provocation test was performed with an intravenous bolus of ordinary insulin. The insulin dose was 0.1–0.15 U/kg, and both blood glucose and GH levels were monitored at 0, 15, 30, 60, 90, and 120 min. The

success rate of the test was determined by the minimum value of blood glucose lowered by 50% or ≤ 2.8 mmol/L. Before the trial, all participants and their guardians received health education, in which they were informed that the participant would undergo the tests while sedated. All participants fasted for 8 hours before the test. Both tests were performed while the participant was in a quiet state.

GH was measured using a chemiluminescence method (ACCESS2, Beckman Coulter; USA) with an analytical sensitivity of 0.010 ug/L. We obtained Hb through an automatic blood analyser (XN-20 125 (Al), SYSMEX; Japan). Liver function measurements, including alanine aminotransferase (ALT) and aspartate aminotransferase (AST), renal function measurements, including creatinine (CR) and uric acid (UA), lipid profiles, including total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C), and fasting plasma glucose (FPG) were assessed using a biochemical autoanalyser (Cobas c 702, Roche; Shanghai, China).

Statistical analysis.

All analyses were performed with the statistical software packages R (http://www.R-project.org, The R Foundation) and EmpowerStats (http://www.empowerstats.com, X&Y Solutions, Inc, Boston, MA). Continuous variables were presented as mean ± SD and categorical variables were presented as a percentage (%). A univariate analysis model (Table 3) was used to determine the association between GH peak and Hb, as well as any association with other independent variables. We then investigated the relationship between Hb and GH peak using smooth curve fitting after adjustment for potential confounders (Fig. 1). P values less than 0.05 (two-sided) were considered statistically significant.

Results

Baseline characteristics

Data of 787 participants were selected for the final analysis. Table 1 shows baseline characteristics of participant anthropometric measurements and biomarkers. The mean age of participants was 10.3 ± 3.8 years old. A total of 558 (70.90%) males and 229 (29.10%) females were included. The mean height SDS of participants was − 2.66 ± 0.58. GH peak value was defined as the highest level of GH, regardless of time point or provocation test. We divided GH peak levels into three groups: GH < 5 ng/mL; GH 5 ng/mL-10 ng/mL; and GH ≥ 10 ng/mL (Table 2). BMI decreased across the tertiles ($P < 0.001$), but E2, T, TC, IGF-1 and Hb increased in the three groups (all $P < 0.05$). However, there were no obvious significant differences in age, CR, UA, or TG (all $P > 0.05$).

Table 1

Clinical and biochemical characteristics.

Variables	All
Number	787
Sex (male, n, %)	558 (70.90%)
Age (years)	10.3 ± 3.5
Height (cm)	125.79 ± 17.69
Height SDS	-2.66 ± 0.58
Body weight	27.74 ± 10.78
BMI (kg/m2)	16.84 ± 2.97
IGF-1 (ng/ml)	188.83 ± 122.57
IGF-1 SDS	-0.96 ± 1.28
IGFBP-3 (ug/ml)	4.51 ± 1.32
Hb (g/L)	130.57 ± 9.53
GH peak (ng/mL)	8.17 ± 5.56
CR (umol/L)	39.77 ± 12.51
BUN (umol/L)	4.92 ± 6.40
UA (umol/L)	261.58 ± 74.67
TG (mmol/L)	1.19 ± 9.12
TC (mmol/L)	3.85 ± 0.75
Pubertal stage	583 (74.74%)
In prepuberty (%)	197 (25.26%)
In puberty (%)	

Abbreviations: Height SDS, height standard deviation scores; BMI, body mass index; IGF-1, insulin-like growth factor-1; IGF-1 SDS, insulin-like growth factor-1 standard deviation scores; IGFBP-3, insulin-like growth factor-binding protein-3; Hb, haemoglobin; CR, creatinine; BUN, blood urea nitrogen; UA, uric acid; TG, triglyceride; TC, total cholesterol.

Table 2

Baseline Characteristics of participants.

Variables	< 5(n = 233)	GH peak (ng/mL) 5–10(n = 331)	≥ 10 (n = 223)	P-value
Age (years)	10.2 ± 3.3	10.1 ± 3.4	10.5 ± 3.7	0.369
BMI (kg/m2)	17.67 ± 3.61	16.53 ± 2.50	16.42 ± 2.70	< 0.001
Hb (g/L)	129.88 ± 9.11	130.10 ± 9.22	131.97 ± 10.28	0.03
CR (umol/L)	39.46 ± 8.32	40.16 ± 16.18	39.52 ± 9.64	0.761
UA (umol/L)	264.84 ± 69.50	253.30 ± 69.69	269.42 ± 84.80	0.056
TC (mmol/L)	3.93 ± 0.75	3.84 ± 0.75	3.76 ± 0.73	< 0.001
TG (umol/L)	0.78 ± 0.42	0.72 ± 0.34	2.30 ± 17.06	0.089
E2 (pmol/L)	20.65 ± 10.59	22.23 ± 16.32	25.27 ± 16.98	0.007
T (nmol/L)	0.37 ± 0.61	0.54 ± 0.86	0.85 ± 1.17	< 0.001
IGF-1 (ng/ml)	161.38 ± 95.56	187.03 ± 112.63	219.29 ± 151.23	< 0.001

Abbreviations: BMI, body mass index; Hb, haemoglobin; CR, creatinine; UA, uric acid; TC, total cholesterol; TG, triglyceride; E2, Estradiol; T, Testosterone; IGF-1, insulin-like growth factor-1.

Correlations between GH peak and anthropometrical and biochemical variables

The univariate analysis results of GH peak and all tested variables are shown in Table 3. BMI SDS (-0.94, -1.28, -0.61) and TC (-0.69, -1.22, -0.16) were negatively associated with GH peak. In contrast, univariate analysis showed that Hb (0.07, 0.03, 0.11), TG (0.05, 0.01, 0.10), E2 (0.06, 0.03, 0.08), and T (1.59, 1.16, 2.03) were positively correlated with GH peak. There was no significant correlation between GH peak and sex, age, height SDS, CR, or UA.

Table 3
Correlation between GH peak level and different variables

Covariate	β (ng/mL) (95%CI)	P-value
Hb (g/L)	0.07 (0.03, 0.11)	0.001
Sex		
Male	Reference	0.567
Female	-0.25 (-1.11, 0.61)	< 0.001
BMI.SDS	-0.94 (-1.28, -0.61)	< 0.001
Tanner stage	Refrence	0.844
In prepuberty	2.57 (1.69, 3.46)	0.462
In puberty	0.00 (-0.03, 0.03)	0.062
CR (umol/L)	0.02 (-0.04, 0.08)	0.011
BUN (umol/L)	0.01 (-0.00, 0.01)	0.019
UA (umol/L)	0.69 (-1.22, -0.16)	< 0.001
TC (mmol/L)	0.05 (0.01, 0.10)	< 0.001
TG (umol/L)	0.06 (0.03, 0.08)	
E2 (pmol/L)	1.59 (1.16, 2.03)	
T (nmol/L)		

Non-linear relationship between Hb and GH peak

In the present study, smooth curve fitting showed a non-linear relationship between Hb and GH peak after adjusting for age, sex, height SDS, BMI SDS, UA, TC, TG, CR, E2, T, and Tanner stage (Fig. 1). There was a two-stage change and one inflection point in this curve. When the Hb level was greater than the breakpoint, there was a positive relationship between Hb and GH peak; however, if the value was less than the breakpoint, there was a negative relationship between Hb and GH peak. Next, threshold saturation based on the curve fitting was analysed (Table 4), and we calculated the inflection point as 123 g/L. On the left side of the inflection point, the curve appeared to have a downward trend (β -0.12; 95% CI -0.30, 0.06; P = 0.1867); however, there was no statistical significance. On the right side of the inflection point, GH peak increased with Hb elevation (β 0.08; 95% CI 0.01, 0.14; P = 0.0207).

Table 4

The independent correlation between Hb and GH peak by multivariate piecewise linear regression.

Inflection point of Hb (g/L)	Effect size(β) (ng/mL)	95%CI	P-value
< 123	-0.12	(-0.30, -0.06)	0.187
≥ 123	0.08	(0.01, 0.14)	0.020
Effect: GH peak			
Cause: Hb			
Adjusted: age; sex; BMI, body mass index; IGF-1, insulin-like growth factor-1; TG, triglyceride; TC, total cholesterol; CR, Creatinine; UA, Uric acid; E2, Estradiol; T, Testosterone; Tanner stage.			

Discussion

This cross-sectional study found a non-linear relationship between Hb and GH peak in children and adolescents with short stature; the Hb inflection point was 123 g/L. A positive relationship between Hb and GH peak was significant only when Hb levels reached this point.

GH regulates human growth through the GH/IGF axis, and GH levels reflect the nutritional status of the human body. This study found that, after the inflection point of 123 g/L, GH peak increased as Hb levels rose. Hb is the main index used to evaluate the presence of anaemia in humans. According to the 2001 WHO diagnostic criteria, anaemia is defined as an Hb level of less than the 5th percentile for age (< 110 g/L in children aged 6–59 months, < 115 g/L in children aged 5–11 years, < 120 g/L in children aged 12–14, and < 130 g/L in boys older than 14 years old[19]. According to this criteria, a total of 45 children in the present study had anaemia, including 19 (4.6%) children aged 5–11 years, 20 (7.3%) children aged 12–14 years, and 6 (21.4%) boys older than 14 years old. Notably, there were only 28 boys aged > 14 in this study, and the higher proportion (21.4%) with anaemia may be related to the small sample size. Generally, low Hb levels is a relevant clinical issue, particularly among children with short stature.

Our study found a non-linear relationship between Hb levels and GH peak. There have been few previous studies on the relationship between Hb and GH peak. Eugster et al., with a sample size of 100 participants, suggested that low Hb levels are associated with idiopathic GHD in children[12]. Similar findings were also reported in the another study, which reported that GH therapy can increase Hb levels [20]. These conclusions are consistent with our findings. Through the threshold effect analysis, we found the inflection point of Hb to be 123 g/L, and if the Hb value was less than this inflection point, the correlation between Hb and GH peak was not statistically significant; this shows that Hb is likely an important factor in GH secretion. The findings of the present study suggest that clinicians should pay more attention to improving nutrition in an effort to maintain optimal Hb levels in children and adolescents with short stature.

There are some limitations of this study. First, we could not determine causality due to the cross-sectional study design. Second, there are many factors that affect the level of Hb, such as diet and nutritional status. In the future, we intend to use dietary and exercise questionnaires to better assess the nutritional status of children and adolescents with short stature.

Conclusion

In conclusion, after adjusting for potential confounders, we identified a non-linear relationship between Hb and GH peak in children and adolescents with short stature. This finding suggests that, in this patient population, high levels of Hb may have a favourable effect on GH levels. Therefore, Hb levels should be considered in the diagnosis and treatment of short stature.

Abbreviations

Hb:haemoglobin; GH:growth hormone; Height SDS:height standard deviation scores; BMI:body mass index; BMI SDS:body mass index standard deviation scores; IGF-1:insulin-like growth factor-1; IGF-1 SDS:insulin-like growth factor-1 standard deviation scores; IGFBP-3:insulin-like growth factor-binding protein-3; GHD:growth hormone deficiency; ALT:alanine aminotransferase; AST:aspartate transaminase; CR:creatinine; BUN:blood urea nitrogen; UA:uric acid; TG:triglyceride; TC:total cholesterol; HDL-C:high-density lipoprotein cholesterol; LDL-C:low-density lipoprotein cholesterol; E2:Estradiol; T:Testosterone; FBG:fasting blood glucose

Declarations

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Availability of data and materials

The datasets used and/or analysed in the current study are available from the corresponding authors upon reasonable request.

Authors' contributions

Bing Sun and Hailing Sun conceived and designed the study. Tian Zhang participated in the design and drafted the manuscript. Baolan Ji performed the statistical analysis. Bo Ban revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Human Ethics Committee of the Affiliated Hospital of Jining Medical University approved the study. All procedures were performed in accordance with ethical standards laid out in the Declaration of Helsinki. All of the families of the patients were informed of the aims of the study, and written informed consent was obtained from the parents of the patients.

Consent for publication

All authors have read and approved the content, and they agree to submit it for consideration for publication in the journal.

Competing interests

The authors declare that they have no conflicts of interest.

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Figures

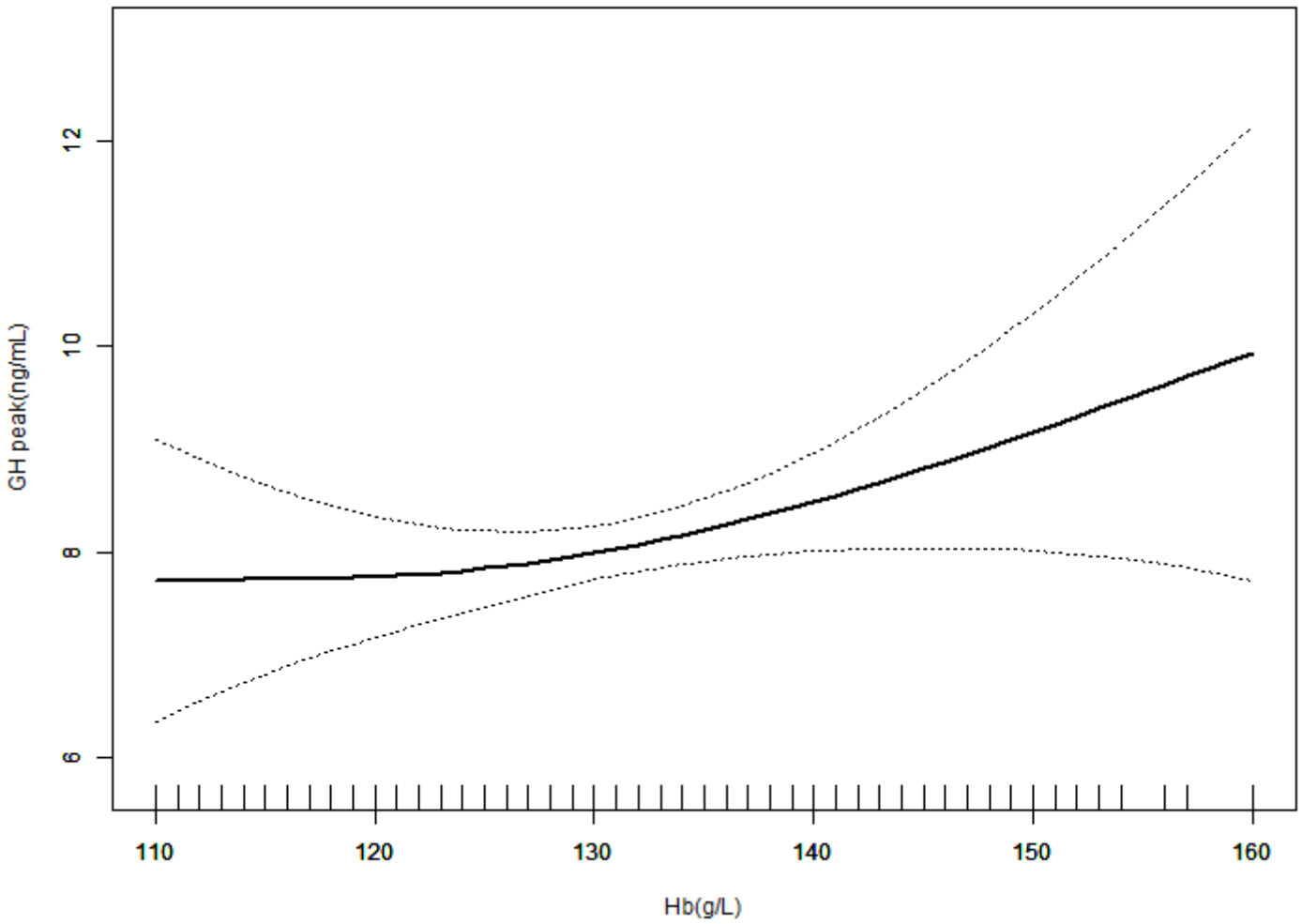

Figure 1

Smooth curve fitting showed a non-linear relationship between Hb and GH peak after adjusting for age, sex, height SDS, BMI SDS, UA, TC, TG, CR, E2, T, and Tanner stage