Original Article

Short stature and insulin-like growth Factor-I in neurofibromatosis Type I

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Abstract

Aim: Certain NF1 patients have short stature due to unclear pathogenesis mechanisms. The aim of this study is to investigate endocrinology parameters which could affect height in NF1.

Materials and Methods: Clinical data of 48 NF1 patients aged 19 months to 18 years were collected by reviewing medical records. Current physical examination findings including height, weight, and pubertal status were recorded. Serum samples were obtained from all subjects for follicle stimulating hormone (FSH), luteinizing hormone (LH), thyroid stimulating hormone (TSH), free T4 (FT4), free T3 insulin-like growth factor (IGF1), and insulin-like growth binding protein3 (IGFBP3) levels.

Results: Of the 48 patients, 34 (70%) had height below 50 percentiles. Short stature, defined as height <-2 SD of mean for age and gender, was found in 25% of the patients. Pubertal children with short stature had significantly lower serum median IGF1 level than those with normal height. In the pre-pubertal group, serum median TSH level was significantly higher in those with short stature than those of normal height.

Conclusion: Short stature in NF1 might be related to TSH in the pre-pubertal period and to IGF1 after puberty. Investigation of these hormonal pathways in correlation with height appears warranted in children with NF1.

Keywords: Endocrinology; growth; height; IGF-1; neurofibromatosis type I; thyroid

INTRODUCTION

Neurofibromatosis 1 (NF1) is an autosomal dominant disorder affecting about 1 in 3000 people (1). Its clinical findings and degree of severity are highly variable even within the same family. Short stature is a well-recognized, but less well explained finding observed in 6.7-25% of NF1 patients (2-4). Growth hormone (GH) deficiency was suggested and lower levels of GH in brain and insulin-like growth factor (IGF1) in liver tissue have been observed (5,6). Some studies reported growth being nearly normal during childhood but slowing down after puberty (3,4,7,8). The reasons of short stature in patients with NF1 remain to be elucidated. The aim of this study was to investigate hormone levels likely to affect height in children and adolescents with NF1.

MATERIALS and METHODS

The study enrolled 48 patients, 30 male (62.5%) and 18 female (37.5%), who were diagnosed as NF1 in the Department of Pediatric Neurology, Çukurova University Hospital, according to the criteria of the National Institutes of Health Consensus Development Conference (9,10) between 2000-2010. Their ages at the time of study were 19 months-18 years (mean 9.57 years).

The study protocol was reviewed and approved by the Institutional Ethic Committee of Çukurova University, Faculty of Medicine in 19.01.2012 (Decision no:10-2/3). Written informed consent was obtained from all subjects or from their parents.

Clinical data including the diagnostic criteria and associated complications were collected by reviewing medical records. Missing data were completed during follow-up visits to the outpatient clinic. Height measurements were made by the same pediatrician and the same method, and compared to the percentile curves obtained from Turkish children by Neyzi (11) for children younger than 2 years old and to the percentile curves recommended by the World Health Organization for international use for older patients12. Short stature was

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defined as height below 2 SD of mean height for age and gender. Standard deviation scores (SDS) or Z scores for height were calculated by formula below;

SDS= [measured height (cm) - height 50. percentile for age and gender (cm)]: standard deviation for that age according to age and gender

Pubertal status was assessed by a pediatric endocrinologist according to Tanner staging. The commencement of puberty was accepted as breast stage 2 for females, and testis volume of 4 cm, measured by Prader orchiometer, for males (13). Cerebral and spinal magnetic resonance images (MRI) were evaluated by the radiology department of the same institution.

Blood samples were obtained from all patients between 9.00-12.00 am, centrifuged at 3000 cycle/minute for 3 minutes, and serum samples were stored at -700C until assay for: follicle stimulating hormone mIU/mL (FSH), luteinizing hormone mIU/mL (LH), thyroid stimulating hormone µIU/ml (TSH), free T4 µIU/ml (FT4), free T3 µIU/ml (FT3) (ECLIA method in Cobase 411, Roche Diagnostics GmbH kit, Germany), insulin-like growth factor ng/ml (IGF1) (IRMA method, Immunotech, Czech Republic); insulin-like growth binding protein3 (IGFBP3) (Immulite CLIA method, Siemens trademark kit, USA) in 2000 XPi device.

The [Short Stature and Insulin-like Growth Factor-I in Neurofibromatosis Type I] data used to support the findings of this study are included within the article.

Statistical Analysis
Data were described by statistical measures as mean, median, standard deviation, minimum and maximum, and summarized graphically as histograms, box plots and bar charts. Patients were classified into two groups as short stature (SDS<-2, n=12) and normal height (SDS>2, n=36), and patients whose puberty had started (n=30 patients) and those that were pre-pubertal (n=18). Endocrinology parameters were compared between groups with t-test or Mann Whitney U-test. Results were accepted as statistically significant for $p<0.05$ (IBM SPSS 19).

RESULTS

Demographic features of the patients are shown in Table 1. Most patients: 73.4% of pre-pubertal and 88.9% of pubertal group, had height <50 percentile, and 63.4% and 61.1%, below 25 percentile (Figure 1). There were 12 patients (25%) with short stature. These patients did not have precocious puberty or any suprasellar lesion, optic pathway glioma, or hydrocephalus on cranial MRI. There was no significant relationship between inheritance modality (familial vs sporadic) and short stature (p>0.05).

Patients with and without short stature were compared within their pre-pubertal or pubertal groups. In the pubertal group, median IGF1 values of children with short stature were significantly lower compared to those with normal height (Table 2, Figure 2). In the pre-pubertal group, median TSH levels of children with short stature

were significantly lower than those with normal stature (Table 3, Figure 3).

There was no significant relationship between short stature and other parameters measured (IGFBP3, FT3, FT4, LH, FSH) in pre-pubertal and pubertal patient groups.

| Table 1. Some Demographic Features of the Patients			
Parameters		n	%
Gender	male	30	67.5
	female	18	32.5
Age	<10	25	52
	>10	23	48
Inheritance modality	familial	32	66.7
	sporadic	16	33.3
Hereditary trait from	mother	9	28
	father	23	72
Consanguinity of parents	present	8	16.7
	not present	40	83.3
Scoliosis	present	2	4.2
	not present	46	95.8
Short stature	present	12	25
	not present	36	75
Signal abnormality on cerebral MRI	present	39	81
	not present	9	19
Presence of puberty	present	18	37.5
	not present	30	62.5
Precocious puberty	present	1	2.1
	not present	47	97.9
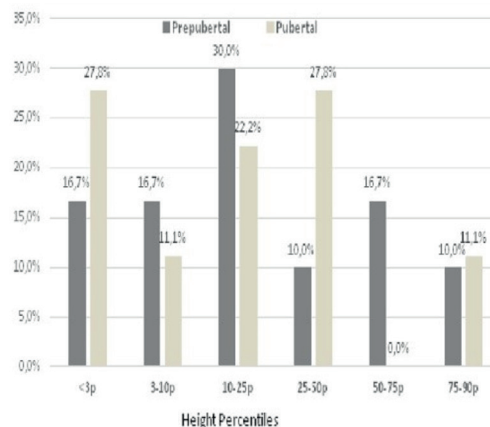
Figure 1. Distribution of Height by Age Percentiles of the Patients According to Pubertal Stage

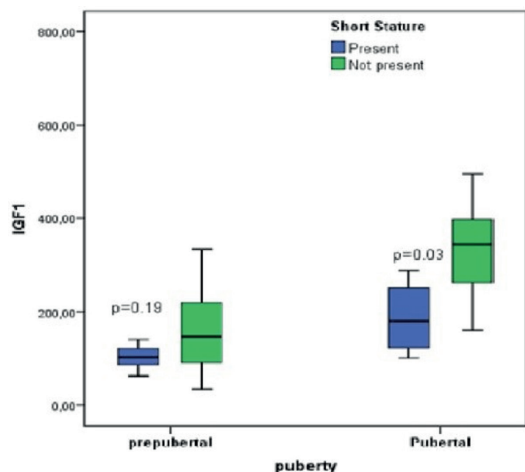| Table 2. The Relationship of Puberty and Short Stature with IGF1 Levels					
IGF1(ng/ml)		Median±SD	Min.	Max.	p
Pre-pubertal subjects	Short stature	102.5±26.9	62.5	140	0.192
	Normal stature	146±154.2	33.5	722	
Pubertal subjects	Short stature	179±82.0	101	288	0.032
	Normal stature	345±115.7	160	495	

Figure 2. The Relationship of Puberty and Short Stature with IGF1 Levels

Table 3. The Relationship of Puberty and Short Stature with TSH Levels

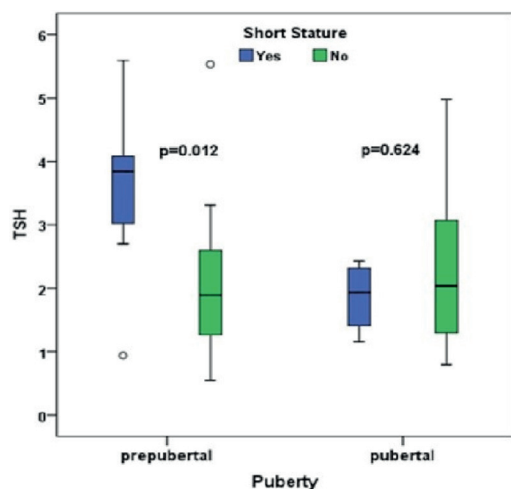| TSH (μIU/ml)		Median±SD	Min.	Max.	p
Pre-pubertal subjects	Short stature	3.84±1.43	0.95	5.59	0.012
	Normal stature	1.89±1.10	0.55	5.53	
Pubertal subjects	Short stature	1.83±0.56	1.16	2.43	0.062
	Normal stature	2.04±1.20	0.8	4.98	

Figure 3. The Relationship of Puberty and Short Stature with TSH Levels

DISCUSSION

The frequency of short stature in our patients was 25%, at the upper end of the range of 6.7-25% reported in children with NF1. This figure may be affected by the median age of patients in various series, as true short stature (<3rd percentile) increases from childhood to late puberty in NF1 (2,3). Even among children within the normal height range, the frequency of height <50 and 25 percentiles clearly indicates a shift of the normal distribution towards the left (Figure 1). Previous studies found NF1 patients of all age groups had a mean height 7 cm shorter than age- and gender-matched control groups. The 3rd and 50th height centiles of children with NF1 are lower than normal children after age 7 years in girls and 12 years in boys. Adult height tends to be shorter in NF1 (3,4,7). In a series of 170 NF1 patients, Soucy et al (14) found no difference between the height z scores of girls younger and older than 10 years, nor between boys younger and older than 12 years. Several reports, including a longitudinal study, show normal growth rate in pre-pubertal NF1 patients (4,7,15). These suggest different mechanisms and pathways taking part in growth in NF1 at different ages. Our results indicate growth is affected in the pre-pubertal as well as pubertal periods.

The pattern of inheritance has been reported to affect height: in familial cases where NF1 is inherited from the father, short stature in the father was associated with lower adult height in the offspring (13). We did not observe any difference between familial and sporadic cases, but the size of our series did not allow a separate analysis of paternally inherited cases.

Several pathophysiological mechanisms have been proposed for short stature in patients with NF1. IGF-1 is one possible variable. Experimental studies in genetically engineered mouse strains lacking NF1 expression in central nervous system neurons showed dysfunction of the hypothalamic-pituitary axis and lower IGF1 levels (6). The mRNAs of growth hormone releasing hormone (GHRH), gonadotropin releasing hormone (GNRH) and TSH-releasing hormone (TRH) are 40-60% lower in NF1BLBP CKO mice* who have a 75% reduction in the number of neurofibromin-expressing cells compared to wild-type control mice. GH production in the pituitary gland and serum IGF1 levels is reduced (6). These findings suggest that absence of neurofibromin might cause GH deficiency due to neurofibromin's regulatory role on the hypothalamo-pituitary axis. Our results support IGF1-related mechanisms affecting growth in NF1 in the pubertal period. In fact, IGF1 concentrations rise two- to three fold during the process of puberty, reaching concentrations observed in adults. The rise in sex steroids during the pubertal period stimulates IGF1 production through GH secretion and also through a direct effect on IGF1 production (16). However, we found no difference in FSH and LH levels between NF1 patients with and without short stature. Therefore we think the lower levels of IGF1 in pubertal patients with short stature are GH-mediated, with no direct effect of sex steroids on IGF1 production. Neurofibromin protein regulates the function of the hypothalamopituitary axis. Loss of neurofibromin in the brain leads to decreased GH and IGF1 levels (6). Vassilopoulos-Sellin et al (5) found that 80% of the patients with NF1 and short stature have GH deficiency without any related abnormality on neuroimaging. These point to GH as one of the direct players in short stature.

In our study, the relationship between endocrine parameters and short stature was investigated and found to be compatible with the literature. However, when we divided our patients into two groups as short stature

and not, a significant difference was found between the averages only in the prepubertal period TSH and pubertal IGF1 levels. IGF1 was found to be low where TSH was high. In addition, its relationship with malnutrition, a parameter affecting IGF1 level, was investigated and no significant result was found. In other words, malnutrition had no effect on the significant difference in pubertal IGF1. A borderline result was also obtained between puberty and short stature and FT4 distributions. It was thought that this could be related to the pubertal peak of IGF1. In other words, IGF1 level normally starts to increase during puberty. It suggests that it is effective in patients with NF whose height is short. In addition, while the rate of short stature in patients with NF1 is 25% in puberty in the literature, this rate increases to 40% in adult age. We can say that this information has an important role in IGF1's short stature in NF1.

A growth hormone stimulation test could clarify the GH status in our patients, but was not included in our study due to its invasive nature and lack of clinical indication. We did not find any changes in IGFBP3 levels between pre-pubertal or pubertal patients with and without short stature. This discrepancy might be due to IGFBP3 reflecting not only IGF1, but IGF2 concentrations as well, and also its age-related expression being less consistent than IGF1(16).

LIMITATIONS

Short stature is noticeable during childhood including prepubertal and pubertal periods in patients with NF1. It appears to be related to IGF1 levels. The pathway linking neurofibromin to IGF1 needs to be studied as it might point to treatment targets both for stature and possibly other complications of NF1.

CONCLUSION

Median TSH level of pre-pubertal patients with short stature was higher than those with normal stature. Although all TSH levels were within normal limits, this finding in prepubertal children with NF1 may suggest thyroid hormone-related mechanisms, possibly a subclinical thyroid hormone deficiency or ineffectiveness, or a secondary elevation of TSH, in this group. This pathway is another area to be explored in young children with NF1.

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