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ORIGINAL ARTICLE

Leptin, ghrelin, nesfatin-1, and orexin-A plasma levels in girls with premature thelarche

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Abstract

Purpose Reducing the mean age of puberty onset in recent years has crucial public health, clinical, and social implications. This study aimed to evaluate the serum levels of appetite-related peptides (leptin, ghrelin, nesfatin-1, and orexin-A) and anthropometric data in girls with premature thelarche (PT).

Methods We enrolled 44 girls aged 4–8 years diagnosed with PT and 33 age-matched healthy girls as controls. The demographic data of the girls were obtained using a questionnaire. Anthropometric data were measured and fasting blood samples were collected.

Results Body weight, height, body mass index (BMI), body fat mass, and basal metabolic rate (BMR) were higher in the PT group than in the control group ($p < 0.05$). Serum leptin ($p < 0.001$), nesfatin-1 ($p = 0.001$), and orxein-A ($p < 0.001$) levels were significantly higher in the PT group than in healthy controls. However, there were no significant differences in the serum ghrelin levels between the groups ($p > 0.05$). The results of multivariate logistic regression revealed that serum leptin level (OR (95% CI): 42.0 (10.91, 173.06), $p < 0.001$), orexin-A (OR (95% CI): 1.14 (1.04, 1.24), $p = 0.006$), and BMI for age z-score (OR (95% CI): 6.97 (1.47, 33.4), $p = 0.014$) elevated the risk of incidence of PT at 4–8 girls.

Conclusion These results suggest that in addition to serum leptin levels, serum orexin-A and nesaftin-1 can take part in the initiation of PT. Few studies have investigated the relationship between nesfatin-1 and orexin-A levels and age at onset of puberty; hence, it should be a subject for future studies.

Keywords Premature thelarche · Leptin · Nesfatin-1 · Orexin-A

Background

Premature thelarche (PT) is defined as unilateral or bilateral breast development without other signs of puberty (accelerated growth rate, enhanced bone maturation, axillary and pubic hair growth) in girls younger than 8 years. PT is one of the most common forms of early pubertal development in girls. In recent years, breast development in girls occurs at an earlier mean age and the prevalence of PT is rising [2, 17]. The etiology of PT remains unclear, but some possible mechanisms include increased sensitivity of breast tissue to estradiol, temporary estradiol secretion from ovarian follicular cysts, dietary estrogen intake, and temporary activation of the hypothalamic-pituitary–gonadal (HPG) system because of the release of excessive follicle-stimulating hormone (FSH) have been proposed before [38, 49, 51].

Studies have demonstrated the crucial role of energy homeostasis of the organism in the regulation of puberty onset, especially in girls [20, 48]. Among the endocrine regulators involved in the integrative control of metabolism, energy stores, and reproduction, the adipose-derived hormone, leptin, has been identified as an important signal for the integration of reproduction and metabolism. Observational studies in humans and animals have shown that leptin plays an important role in the initiation of puberty, but the mechanisms involved have not been fully understood [10, 14, 37]. Ghrelin, another hormone that has a crucial role in

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the control of metabolism and energy balance, is primarily produced by stomach [47]. Ghrelin regulates endocrine and non-endocrine functions such as stimulating the release of growth hormone (GH), food intake, and control of energy hemostasis and adipose tissue [9]. Recent studies have suggested that ghrelin also plays a role in reproductive function [7, 29]. Ghrelin stimulates appetite and creates a positive energy balance, which promotes fat storage. Ghrelin also decreases GnRH secretion during the pre-pubertal period [19]. It has been reported that ghrelin has a role in the inhibition of gonadotropin secretion in animals and humans [32]. Nesfatin-1 is defined as a neurohormone or a satiety molecule derived from nucleobindin2 (NUCB2) protein, which is expressed especially in the hypothalamus, adipose tissue, pancreas, gastric mucosa, and brain in humans and rats. It has been demonstrated that nesfatin-1 suppresses food intake and has anorexigenic effects [6]. Due to its multifarious actions in the homeostatic control of energy balance and its related bodily functions, the latest experimental surveys have revealed a probable role for NUCB2/nesfatin-1 in central networks that initiate puberty and maintain reproductive function [3, 23]. Orexin-A is a neuropeptide that plays a role in regulating both food intake and the sleep–wake state. Orexin neurons are expressed in different parts of the brain and are associated with sleep patterns, food intake, energy metabolism, neuroendocrine function, and fluid homeostasis [36]. In addition, it is thought that orexin has a regulatory role in the reproductive system and releases some hormones such as insulin, leptin, and catecholamines. Orexin is known as a neuromodulator in food [28]. It has been reported that in short-term fasting, orexin increases food intake and protects body weight. On the other hand, orexin can promote energy expenditure to defend against obesity [25]. It is thought that orexins, especially orexin-A, increase plasma luteinizing hormone (LH) secretion by acting on the HPG axis to regulate reproductive function, thereby affecting estrogen and progesterone levels [28].

This study aimed to investigate the appetite-related peptide levels in premature thelarche girls aged 4–8 years compared to age-matched healthy children.

Materials and methods

Subjects

From February 2019 to March 2020, 44 girls aged 4–8 years diagnosed with PT and 33 age-matched healthy girls as controls were enrolled in this study. This study was endorsed by the local ethics committee (2019-008). The participants were included in the study after their parents confirmed the informed consent form statements. Laboratory tests, direct radiography of the left hand and wrist, and anthropometric measurements were performed in girls with a complaint of breast enlargement before the age of 8 years. Pubertal staging was evaluated according to Tanner staging [34]. Girls diagnosed with PT underwent pelvic uterine and ovarian sonographic and GnRH stimulation test. Girls with onset of breast development before the age of 8 years, bone age/chronological age ratio < 1, peak LH < 5 IU/L during the exogenous GnRH stimulation test and girls who were followed up for at least 6 months and did not show any progression of precocious puberty were included in PT group. We did not include the girls with chronic diseases, any organic brain disease, adrenal abnormalities, endocrine pathology (Cushing syndrome and hypothyroidism), and history of drug use (steroids and antipsychotics). The control group was recruited from girls who applied to the pediatrics outpatient clinic for routine health control. In the control group, girls had not breast development and no girls had any sign of chronic disease or used any kind of medication.

Anthropometric and body composition measurements

The measurements were collected after overnight fasting. Height and weight were measured in all girls using a Harpenden stadiometer with a sensitivity of 0.1 cm and a weight electronic scale with a sensitivity of 0.1 kg. Waist and hip circumferences were measured to the nearest 0.1 cm with a fiber-glass tape. The girls wore minimal clothing without shoes during measurements. Body mass index (BMI) was calculated as dividing weight (kg) by the square of height (m2), and BMI-z score was calculated using WHO Anthro 3.2.2.1 (for < 5 years girl) and WHO Anthro-Plus 1.0.4 (for ≥ 5 years girl) software [4, 52]. Girls with BMI z-score greater than or equal to 2 SDS were considered obese and those with BMI z-score between 1 and less than 2 SDS were considered overweight. For determining the body composition of the girls, arm-leg bioimpedance (right-sided) was measured using a Bodystat 1500 bioimpedance analyzer (Bodystat Ltd., Isle of Man, UK).

Measurement of leptin, ghrelin, nesfatin-1, and orexin-A serum levels

Following an overnight fast, 5-mL venous blood samples were taken from each girl. Blood samples were centrifuged and serum samples were collected in three Eppendorf tubes and stored at −80 ºC until analyzed. Serum leptin, ghrelin, nesfatin-1, and orexin-A (catalog no respectively: SEA084Hu, CEA991Hu, CEA242Hu, and CEA607Hu Cloud-Clone Corp., Wuhan, China) levels were determined using enzyme-linked immunosorbent assay commercial kit (ELIZA) according to the manufacturer's protocols.

Statistical analyses

Statistical evaluation of results was performed using IBM SPSS 22. package program. Data normality was assessed by Kolmogorov–Smirnov test. An independent Student's *t*-test was used to compare parametric data. Nonparametric data were compared using the Mann–Whitney *U* test. Univariate logistic regression analysis was used to determine that anthropometric measurement and appetite-related peptides levels were associated with PT. A multivariate regression model was used to re-evaluate factors statistically significant in univariate analysis. Hosmer–Lemeshow goodness-of-fit statistics were used to assess the model fit. Odds ratio (OR) with 95% confidence intervals (CIs) were calculated to illustrate the relative risk. The level of statistical significance was considered as p value of 0.05 or less.

Results

The mean chronological age of PT and the control group were 6.97 ± 0.93 and 6.52 ± 1.35 years, respectively and there was no significant difference between the two groups ($p > 0.05$). Additionally, there was no significant difference in birth weight between the groups. Weight, height, BMI, and BMI for age z-score and BMR in the PT group were significantly higher compared to the control group ($p < 0.05$). However, body fat mass was higher in PT girls ($p < 0.05$), and there were no significant differences between the groups in body fat percentage ($p > 0.05$) (Table 1).

Analysis of appetite-related peptides revealed that leptin, nesfatin-1, and orexin-A level were significantly higher in PT girls than the control group ($p < 0.01$), but ghrelin level was similar in two groups ($p > 0.05$) (Table 1).

The factors affecting PT in the univariate logistic regression analysis are shown in Table 2. It was determined that in girls aged > 6 years, each year increase, increased the risk of PT by 4.15 times. Univariate analysis showed that body weight, height, BMI, BMI for age z-score, and BMR were associated with an increased odds of developing PT in girls. Because of the wide confidence interval of leptin levels, the median leptin level (2.62 ng/ml) was used. The risk of PT

Table 2 Factors affecting PT on univariate logistic regression analysis

	β	*p*	OR	95% GA	
				Min	Max
Age (≤ 6 and > 6 years)	1.42	**0.006**	4.15	1.50	11.46
Body weight (kg)	0.116	**0.005**	1.12	1.04	1.22
Height (cm)	0.067	**0.012**	1.07	1.02	1.13
BMI (kg/m^2)	0.292	**0.007**	1.34	1.08	1.65
BMI for age z-score	0.738	**0.001**	2.09	1.33	3.29
Body fat mass (kg)	0.130	0.066	1.14	0.992	1.31
BMR (kcal)	0.005	**0.002**	1.01	1.00	1.01
Leptin (2.62 ng/mL)	3.738	**< 0.001**	42.00	10.91	173.06
Ghrelin (pg/mL)	2.34	0.069	10.11	0.896	114.23
Nesfatin-1 (pg/mL)	0.224	**0.040**	1.25	1.01	1.55
Orexin -A (pg/mL)	0.159	**< 0.001**	1.17	1.08	1.27

Bold values indicate statistical significance at the $p < 0.05$ level

Table 1 The clinical characteristic of PT and control group

	PT ($n = 44$)			Control ($n = 33$)			p^*
	$\bar{x} \pm$ SD	Median (IQR)	min–max	$\bar{x} \pm$ SD	Median (IQR)	min–max	
Chronological age (years)	6.97 ± 0.93	7 (1)	4–8	6.52 ± 1.35	6 (3)	4–8	0.080
Birth weight (kg)	3.17 ± 0.59	3.22 (0.78)	1.40–4.28	3.07 ± 0.56	3 (0.8)	2.10–4.48	0.519
Body weight (kg)	27.85 ± 6.47	26.5 (8.08)	18.7–42.3	22.88 ± 7.21	21.5 (9.40)	12.2– 43	**0.001**
Height (cm)	125.11 ± 7.64	127 (9.53)	108.3–140.3	119.40 ± 10.98	117 (16.5)	98–140	**0.025**
BMI (kg/m^2)	17.60 ± 2.68	16.8 (2.71)	14.1–25.5	15.7 ± 2.73	14.7 (3.40)	12.1–24.3	**0.001**
BMI for age z-score	0.90 ± 1.06	0.7 (1.52)	−0.86–3.72	−0.13 ± 1.36	−0.45 (1.97)	−2.72–2.76	**< 0.001**
Waist circumference (cm)	59.11 ± 7.29	58 (7.36)	50–76	56.98 ± 7.47	57 (13)	45–77	0.208
Body fat (%)	22.84 ± 9.22	22.3 (11.95)	7.8–44.7	20.88 ± 8.13	17.9 (12.15)	9.23–39.3	0.338
Body fat mass (kg)	6.81 ± 3.78	5.9 (5.08)	1.5–17.9	5.17 ± 3.65	3.7 (4.05)	1.7–16.6	**0.019**
BMR (kcal)	1127.41 ± 145.41	1095 (181.50)	920–1451	1012.42 ± 162.61	963 (212)	774–1467	**< 0.001**
Leptin (ng/mL)	2.84 ± 0.81	2.62 (0.62)	1.73–5.32	1.65 ± 0.42	1.54 (0.35)	1.23–2.91	**< 0.001**
Ghrelin (pg/mL)	1.31 ± 0.23	1.27 (0.30)	0.91–1.93	1.23 ± 0.19	1.20 (0.24)	0.92–1.71	0.089
Nesfatin-1 (pg/mL)	7.34 ± 2.28	7.24 (2.16)	0.74–13.22	5.83 ± 2.55	5.79 (2.40)	1.15–16.04	**0.001**
Orexin A (pg/mL)	43.94 ± 10.82	41.21 (11.66)	22.64–80.93	33.47 ± 6.28	31.80 (8)	21.38–50.23	**< 0.001**

Bold values indicate statistical significance at the $p < 0.05$ level

*p values were calculated using independent Student's *t*-test and Mann–Whitney *U* test ($p < 0.05$ statistically significant)

incidence increased 42.0 times at ≥ 2.62 ng/ml leptin levels. In addition, it was observed that each unit increase in nesfatin-1 and orexin-A levels increased the risk of developing PT by 1.25 and 1.17, respectively.

The backward LR method in the multivariate regression model was used for the factors statistically significant in the univariate analysis to obtain the best model. The results of the multivariate logistic regression analysis are presented in Table 3. Furthermore, leptin levels were not included in this model because the effect of leptin levels was very high and adversely affected the other factors. It was observed that the BMI for age z-score and orexin-A level increased the odds of PT among girls (BMI z-score: OR (95% CI): 6.97 (1.47, 33.4), $p = 0.014$, orexin-A: OR (95% CI): 1.14 (1.04, 1.24), $p = 0.006$).

Discussion

This study set out with the aim of assessing the levels of appetite-related peptides in premature thelarche girls aged 4–8 years.

Many studies showed a relationship between childhood obesity and overweight with early puberty in girls; however, the mechanism of the association remains unclear. Cross-sectional [26, 33, 44] and prospective longitudinal [11, 30] studies demonstrated that overweight and obese girls show earlier pubertal development. Eckert-Lind et al. [18] reviewed 30 cross-sectional and longitudinal studies which assessed the relationship between obesity/overweight and body composition with PT in girls and concluded that higher BMI and obesity incidence decreased the age of thelarche by approximately 3 months per decade from 1977 to 2013. Similarly, we found that BMI ($p = 0.001$), BMI for age z-score ($p < 0.001$), and body fat mass ($p < 0.05$) were significantly higher in the PT group compared to the control group. Furthermore, our results revealed that the BMI for age z-score was a risk factor for the initiation of PT.

Leptin is mainly produced by white adipose tissue and has a pivotal role in the weight management. In addition to leptin's anorexigenic and metabolic effects, it acts as a permissive factor for the metabolic regulation of puberty [12]. Verrotti et al.[50] showed that serum leptin levels in girls with precocious puberty were not significantly different from those in healthy control girls at a similar stage of pubertal development. Matkovic et al. [35] indicated that each 1 ng/mL increase in serum leptin levels can delay the age of menarche by 1 month. In contrast to earlier findings, Gavela-Pérez et al. [24] demonstrated that high serum leptin levels in girls aged 6–8 years were associated with early age at menarche regardless of BMI. Another study [16] reported significantly high serum leptin levels in girls with PT compared to the age-matched control group. Çatlı et al. [13] also demonstrated that serum leptin levels were significantly higher in the PT group than the healthy controls. In the current study, our results were in accord with recent research which found the PT group had significantly higher serum leptin levels as compared to the healthy age-matched control group ($p < 0.001$). We also observed that serum leptin levels was a significant risk factor for PT. Taken together, these results suggest that serum leptin level may play a key role in the pathogenesis of PT.

It has been revealed that ghrelin hormone may have an inhibitory role in pubertal development [47]. Continuous reduction in ghrelin levels during puberty has suggested that the hormone may play a role at the onset of puberty [45]. On the other hand, Cheng et al. [15] and Bellone et al. [8] indicated that serum ghrelin levels were not associated with Tanner stages and puberty. Gavela-Pérez et al. [24] showed a decrease in ghrelin levels during puberty, however, it was not a significant relationship with age at menarche. Besides, Kurnaz et al.[32] and Zhu et al.[53] did not find any significant differences between the ghrelin levels of the PT group and the control group. Similarly, we found that there were not any differences between serum ghrelin levels of the PT group and the control group in the present study. Overall, these results strengthen the idea that serum ghrelin level has not any relationship with PT.

Experimental studies showed that in addition to its anorexigenic effect, nesfatin-1 plays a pivotal role in the onset of puberty and maturation of the reproductive system [22, 41]. Abaci et al. [1] reported that there were no significant differences in the serum nesfatin-1 levels between puberty and prepuberty obese children. Similarly, Altıncık et al. [3], did not find significant differences between central precocious puberty and healthy control girls in terms of serum nesfatin-1 levels. In contrast to these studies, in the study conducted on rats, Şahin [41] reported that the age of puberty shifted forward significantly in the group administered intracerebroventricular nesfatin compared to the control group. Anwar et al. [5] showed that as the pubertal stage progressed, the level of nesfatin-1 increased in both obese and healthy children. In a study conducted

Table 3 Factors affecting PT on multivariate logistic regression analysis

	β	*p*	OR	95% GA	
				Min	Max
Body weight (kg)	0.382	0.065	0.07	0.46	1.02
Height (cm)	0.181	0.084	1.20	0.98	1.47
BMI for age z-score	1.94	**0.014**	6.97	1.47	33.04
Nesfatin-1 (pg/mL)	0.232	0.097	1.26	0.96	1.66
Orexin-A (pg/mL)	0.128	**0.006**	1.14	1.04	1.24

Bold values indicate statistical significance at the $p < 0.05$ level

on girls aged 2–8 years, serum nesfatin-1 level in the PT group was significantly higher compared to healthy controls [13]. Our results indicated that serum nesfatin-1 levels were significantly higher in the PT group than the control group ($p < 0.001$) which is in accordance with the findings of Çatlı et al. These results suggest that nesfatin-1 may affect the initiation of PT.

Orexin-A is a peptide thought to have a role in the regulation of reproductive functions; on the one hand, due to its neuromodulatory role in food intake and energy balance [28], and on the other hand, because it modulates the activity of GnRH neurons and gonadotropin-secreting pituitary cells [40]. However, the results of studies examining the role of orexin-A in the reproductive system, including the stimulation and secretion of GnRH and/or LH, in animal models are inconsistent [21, 31, 39, 42, 46]. Some in vivo studies demonstrated orexin- A's suppressive effect on LH secretion [21, 46], while others showed its stimulating effect on LH secretion [31, 39]. Small et al. [43] suggested that the effect of orexin-A may vary according to the injection site in female rats. Additionally, they reported that orexin-A had a stimulating effect on the rostral preoptic area (rPOA) in female rats and a suppressive effect on the medial preoptic area (mPOA) and median eminence (ME) hypothalamus. Sasson et al. [42] examined the effect of orexin-A on the expression of the GnRH gene in GT1-7 cell culture, and reported that orexin-A increased GnRH gene expression and secretion. Iqbal et al. [27] showed that orexin may affect the regulation of GnRH and LH secretion, because it has a crucial role in the regulation of GnRH cells. To the best of our knowledge, this is the first study to examine orexin-A levels in girls with PT. It is unique in providing evidence of differences in serum orexin-A levels between premature thelarche and healthy age-matched control girls. Our results in the current study indicated that serum orexin-A levels were significantly higher in the PT group than in the healthy controls ($p < 0.001$). Besides, we observed that each unit increase in serum orexin-A levels could increase the risk of PT by 1.14 times.

Conclusion

In conclusion, our results indicated that high BMI and overweight contribute to PT pathogenesis and pubertal development in girls. Our results provided further support for serum leptin levels' permissive role in the initiation of PT. Elevated serum levels of leptin, nesfatin-1, and orexin-A show that they can have an effect on the pathogenesis of PT. Additionally, we found that serum leptin and orexin-A level were significant risk factors for PT. The present study has number of limitations that need to be addressed. First; the prevalence of covid was an obstacle to continuing the study and collecting more samples, our study participants' numbers were small in each group, second; our study design did not allow us to identify the causal relationship between appetite-related peptides, body composition, and the timing of pubertal onset. Therefore, further research should be undertaken to investigate the relationship between nesfatin-1 and orexin-A levels with the age of onset of puberty and assesses these peptides' risk ratio in large-scale longitudinal studies.

Author contributions NA and MF designed the research. NA performed the research, analyzed, and interpreted the data. NA and HYZ performed statistical data analysis. NK, SAU and DİM contributed to sample collection in policlinics. NA wrote the manuscript, NHR revised it. MF had primary responsibility for the final content, and all authors reviewed the manuscript rigorously and approved the final version submitted for publication.

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Declarations

Conflict of interest The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical approval All procedures performed in this study which involving human participants were in accordance with the principle of the 1964 Helsinki Declaration and its later amendments. This study was approved by the Local Ethical Committee (18.02.2019, no: 2019-008).

Informed consent Informed consent was obtained from all parents of participants included in the study.

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