Role of melatonin in constitutional delayed puberty in boys

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Objective
The aim of this work to assess melatonin level in boys with constitutional delayed puberty (CDP) and study its correlation to follicle-stimulating hormone (FSH), total testosterone, and prolactin hormones.

Background
CDP is a common cause of pubertal delay. These boys have no underlying pathology and will progress normally. Melatonin hormone has an important role in pubertal onset. Before puberty, it is too high for hypothalamic activation. However, at puberty, it drop below threshold value, after which pubertal changes start occurring.

Patient and methods
This study was carried out on 50 boys aged 14–18 years who were divided into two groups: 25 boys with CDP as a patient group and another 25 age-matched boys with full pubertal development as a control group. All boys were subjected to full history taking, clinical examination, bone age determination, and laboratory investigations (testosterone, FSH, prolactin, and melatonin).

Results
Our results showed that in CDP, bone age is significantly delayed compared with their chronological age as well as the bone age of control group. Weight and height are significantly less in CDP than control. Both serum total testosterone and FSH are significantly lower in CDP compared with controls, whereas there were insignificant differences in serum prolactin. Melatonin is significantly higher in CDP compared with control. Melatonin is inversely correlated with both testosterone and FSH and has no correlation with prolactin.

Conclusion
Melatonin is significantly elevated in CDP boys, and it is negatively correlated with hormones of sexual maturation (testosterone) and reproduction (FSH).

Keywords:
delayed puberty, gonadotropins, melatonin, puberty

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Introduction

Delayed puberty (DP) is defined as the lack of pubertal development by an age that is 2–2.5 SD beyond the population mean. Although it generally represents a normal variant in pubertal timing, there is a concern that DP could be the initial presentation of a serious underlying disorder. This delay may be pathological as in cases of hypogonadotropic hypogonadism and hypergonadotropic hypogonadism or constitutional delay of puberty (CDP) [1].

The most common etiology of DP is the CDP observed in 2–2.5% of the population. It is more common in boys than in girls and represents up to 65% of DP in boys. It is a nonpathological state in which the maturation of the hypothalamic–pituitary–gonadal (HPG) axis is delayed and puberty will begin at an age at the extreme end of the normal spectrum. CDP is associated with positive family history and is established as a final diagnosis of DP and remains a diagnosis of exclusion [2].

As puberty is triggered by secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) under the effect of pulsative gonadotropin-releasing hormone (GnRH) secretion. The exact etiopathology of CDP is still unclear and mostly unknown, and many speculations exist in this regard such as genetic factors; metabolic, nutrition, hormonal, or environmental causes; etc., However, it has a strong genetic basis and accounts for ~50–75% of cases [3].

Melatonin or the hormone of darkness, an evolutionarily ancient derivative of serotonin with hormonal properties, is the main neuroendocrine secretory product of the pineal gland. It was ignored by the scientist and researchers for long decades, but has recently received much attention. Melatonin not only regulates the circadian rhythmicity and lowers vertebrate skin pigmentation, it also has very important functions such as acting a strong antioxidant and having immunomodulating, thermoregulatory, and antitumor properties, and it also regulates the HPG axis [4].

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The role of melatonin in the regulation of pubertal onset is unclear, and few research studies exist. It is proved that melatonin is very high in prepubertal boys and shows a sharp decline with the onset of puberty, which is followed by the release of the hypothalamic GnRH and pituitary FSH and LH with the start of cascade of pubertal changes [5].

Melatonin has been suggested as an inhibitory neurotransmitter for puberty onset. A human study suggested that a decrease in circulating melatonin before puberty appears to be owing to a slight increase in circulating LH, which stimulates gonadal steroids [6].

Puberty stage predicts melatonin amplitude better than the chronological age, and it is suggested that sexual maturation and the reactivation of the HPG axis may explain melatonin amplitude more precisely than age during this developmental period [5].

Pineal parenchymal tumor is a functioning tumor that stimulates secretion of melatonin and causes delayed or failed puberty. However, other tumors, like teratoma, hypothalamic hamartoma, or pineal cysts and other destructive tumors are associated with very low levels of melatonin and cause precocious puberty [7].

High nocturnal melatonin secretion has been reported in children with DP, whereas low levels of melatonin have been reported in children with precocious puberty [6]. So we assess serum melatonin level in boys with CDP and study its correlation to FSH, testosterone, and prolactin.

Patients and methods

The study was approved from the ethical committee of Faculty of Medicine Menoufia University, and the patient gave an informed consent. This is a case–control study that included 50 boys aged 14–18 years who were attending the Menoufia Skin and Andrology Outpatient Clinic. DP is considered when the boys reach 14 years of age and do not start pubertal manifestation, or are 18 years without pubertal completion.

They were classified into two groups:

- Group 1 (CDP group): it included 25 boys with CDP (Tanner's stages I, II, and III)
- Group 2 (control group): it included 25 boys with full puberty (Tanner's stage V).

All study participants were subjected to the following after taking written consent: thorough history tacking focusing on family history, nutritional state, history of chronic diseases, trauma, operations, or drug intake.

Moreover, all patients and control were subjected to thorough general examination: general condition, body built, secondary sexual characters, gynecomastia, weight, height, span, upper/lower body segments and signs of systemic diseases, and local examination with emphasis on testicular size, scrotum, penis, and pubic hair distribution and determination of Tanner's stage of puberty.

- Tanner I: no pubic hair, and penis and testes with childhood size
- Tanner II: minimal brown hair at base of penis, testes have grown, and scrotal texture changes
- Tanner III: darker and coarse hair over pubic region, increased penile length, and testes continue to grow
- Tanner IV: adult-type hair distributed across pubic region, increased penile length and girth, and testes continue to growth with darkening of the scrotum
- Tanner V: adult-type hair extends to medial surface of thighs and umbilicus and adult-size penis and testes.

Radiography for hand and wrist bones was done to determine the bone age of the boy. It was done for left hand for those who are right-handed and vice versa.

Overall, 5 ml of blood sample was collected from each individual at 8 a.m.–10 a.m. after 12 h fasting, under aseptic condition by clean venipuncture without venous stasis. Blood sample was added to a sterile plain tube. The blood was left to clot at 37°C (for 30 min) and rapidly centrifuged at 4000 rpm for 10 min and then stored at −80° C for assessment of serum total testosterone, FSH, and prolactin as well as serum melatonin levels.

Inclusion criteria were as follows:

Boys with DP proved to be of CDP.

Exclusion criteria were as follows:

(1) Boys aged less than 14 years or more than 18 years
(2) DP owing to other causes, for example, hypogonadotropic or hypergonadotropic hypogonadism
(3) Any diseases, operation, or drugs that may affect the onset of puberty.

The results were statistically analyzed by statistical package for the social sciences, version 20 (SPSS Inc., Chicago, Illinois, USA). Statistics were calculated in terms of percentage, mean, SD, Student's t test, Mann–Whitney test, χ^2 test, Spearman and Pearson tests, P values, and Wilcoxon test [8].

Results

A total of 50 boys were studied: 25 boys with CDP as a patient group (Tanner's I, II, and III) and a similar number of normal pubertal boys (Tanner's V) as a control group. Both groups were age matched (14–18 years).

There was no significant difference in chronological age between both groups (age-matched groups). However, patients with CDP had significantly lower bone age compared with the control group (Table 1).

There were no significant differences between chronological and bone age of the control group, whereas in the CDP group, bone age is significantly delayed compared with their chronological age as well as the bone age of the control group (Table 2).

The patients had significantly lower weight and height compared with the control group (Table 3). This is may be owing to the malnourishment or the delayed testosterone secretion (anabolic hormone). Malnutrition has a suppressive effect on gonadotropin secretion and gonads by inhibiting secretion of hypothalamic GnRH and decreased response of the pituitary to GnRH.

The patients had significantly lower level of serum total testosterone and significantly lower level of serum FSH level compared with the control group. Prolactin showed no significant difference between both groups (Table 4).

The patients had significantly higher serum melatonin level compared with the control group (Table 4).

In CDP group, serum melatonin is significantly negatively correlated with both total testosterone and FSH and has a positive correlation with prolactin (Table 5).

Discussion

DP is defined as no signs of start of puberty by the age of 14 years or not completed by the age of 18 years. CDP is defined as a disorder that occurs in healthy adolescents and is a common variant of normal that results from a delayed onset of normal puberty. It is considered as a temporary state of hypogonadotropic hypogonadism and diagnosed after careful evaluation and exclusion of the other potential underlying causes of hypogonadotropic hypogonadism. Uptill now, CDP remains a diagnosis of exclusion [9].

Melatonin is known to inhibit pubertal onset in normal children. At pubertal time, its level shows sharp decline (75% of its level). It is believed that this decline allows the surge of the hypothalamic GnRH and the start of cascade of pubertal changes. This means that melatonin acts as a gonadostat allowing puberty to occur in its proper time. There are reports of cases that showed precocious puberty with destructive pineal gland tumors causing low or absent melatonin, and on the contrary, cases of failed puberty owing to functioning pineal gland tumors secreting excess melatonin [7].

Both CDP group and the control group are age matched, with no significant differences between both; this means good selection of the cases.

The results of this study also showed that the bone age in CDP is significantly delayed compared with the bone age of the control ($P > 0.0001$) and with their chronological age ($P > 0.0001$). However, in the control group, no significant differences were found between the bone and the chronological age. This is considered now as one of the criteria that must be considered in the diagnosis of CDP.

Consistent with our findings, Rogol *et al.* [10] reported that in the CDP group, the bone age does not advance 1 y for each calendar year, and it progressively deviates from the chronologic age.

Rosen and Foster [11] reported that bone age in patients with CDP retarded more than 2 years behind their chronological age. Attia *et al.* [12], El-Eshmawy and Aal [13], and Hasegawa [14] reported the same results that in CDP bone age retardation is more than 2 years and may reach up to 5 years behind chronological age.

Compared with the control group, our results showed that both weight and height of our CDP group were highly significantly lower than that of the control group ($P > 0.0001$). This may be owing to the malnourishment or delay testosterone secretion.

Table 1 Chronological age (years) and bone age (years) of the constitutional delayed puberty group compared with the control group

	Groups (mean±SD)		Mann-Whitney test	*P*
	Controls (*n*=25)	Patients (*n*=25)		
Chronological age	14.868±0.701	14.828±0.7144	0.278	0.781
Bone age	14.76±0.779	12.32±0.988	5.906	0.0001*

There is no significant difference regarding the chronological age between both group ($P>0.05$). The bone age is significantly delayed in constitutional delayed puberty group, compared with the control group ($P<0.001$). *Significant ($P < 0.001$).

Consistent with our findings, Attia *et al.* [12] reported that weight and height were lower in CDP than normal pubertal boys, and El-Eshmawy and Aal [13] reported the same result as CDP boys look younger than their biologic age.

Hasegawa [14], reported that CDP patients were significantly shorter and of lower weight than normal peers and this led to poor body image.

Comparing serum levels of total testosterone and FSH levels in CDP with the control, the results showed that both total testosterone and FSH are significantly lower in CDP compared with the controls ($P < 0.0001$ and <0.0001, respectively). This is acceptable as our patients were selected in Tanner's stages I, II, and III who were still sexually immature, and this type of pubertal delay is considered as a case of transient hypogonadotropic hypogonadism.

Consistent with our findings, Attia *et al.* [15] reported that CDP may be associated with low serum levels of total testosterone and FSH.

Harrington and Palmert [16] reported that CDP may occur as a result of a delay in the onset of pubertal gonadotropin stimulation of the gonads, and subsequently low testosterone.

Moreover, our results showed that there was insignificant difference in serum prolactin level ($P > 0.05$) between study groups which indicates that our groups were accurately selected, and DP in case group could be attributed to other causes in case group.

Consistent with our findings, Apter *et al.* [17] reported that in CDP serum prolactin did not show any significant changes. There was no correlation between serum prolactin and chronological or bone age.

Korth-Schutz and Grueters *et al.* [18] reported that basal and peak levels of prolactin were normal in CDP.

Ali and Adeel [19] reported that prolactin is within normal in CDP but patients with idiopathic hypogonadotropic hypogonadism had low basal and provocative serum prolactin levels as compared with CDP.

In our study, we have also found that melatonin is significantly higher in CDP compared with the control group. Few studies exist and reported elevated melatonin level in cases of CDP.

Cohen *et al.* [20] reported higher serum melatonin concentrations in single daytime samples in boys with DP. However, there was a significant fall of serum melatonin in these patients between mid and late puberty. The results suggest that melatonin may inhibit puberty. Moreover, among the boys with DP, there was a fall in melatonin concentrations between mid and late puberty, but not between prepuberty and early or mid-puberty.

Attanasio *et al.* [21] reported that melatonin concentration shows higher day–night increments in four boys with CDP than in age-matched controls.

Cavallo [22] reported that nocturnal melatonin is significantly higher in CDP. He was also found that in puberty disorders, the chronological age is asynchronous from pubertal stages and bone age.

Table 2 Chronological age (years) compared with bone age in both control and constitutional delayed puberty groups

Groups	Age (years)	Mean±SD	Wilcoxon	*P*
Controls (*n*=25)	Chronological age	14.868±0.7010	2.294	0.22
	Bone age	14.76±0.779		
Patients (*n*=25)	Chronological age	14.828±0.7144	4.396	0.0001*
	Bone age	12.32±0.988		

In the control group, both the chronological age and the bone age are going together, with no significant difference ($P>0.05$), whereas in the constitutional delayed puberty group, the bone age is significantly delayed than the chronological age ($P<0.001$). *Significant ($P < 0.001$).

Table 3 Weight (kg) and height (cm) in the constitutional delayed puberty group compared with the control group

	Groups (mean±SD)		Mann-Whitney test	*P*
	Controls (*n*=25)	Patients (*n*=25)		
Weight	55.82±3.640	46.04±5.488	5.211	0.0001*
Height	166.52±3.513	152.68±6.88	5.586	0.0001*

The constitutional delayed puberty group is significantly underweight compared with the control group ($P<0.001$). The constitutional delayed puberty group is significantly shorter in height compared with the control group ($P<0.001$). *Significant ($P < 0.001$).

Table 4 Testosterone, follicle-stimulating hormone, prolactin, and melatonin levels in the constitutional delayed puberty group compared with the control group

	Groups (mean±SD)		Mann-Whitney test	*P*
	Controls (*n*=25)	Patients (*n*=25)		
Total testosterone	465.16±146.605	118.32±22.366	6.064	0.0001*
FSH	7.24±3.620	0.98±0.447	6.07	0.0001*
Prolactin	11.0818±2.19277	11.1455±2.49909	0.153	0.879
Melatonin	42.76±19.923	196.60±9.730	6.066	0.0001*

The constitutional delayed puberty group has significantly low total testosterone ($P<0.001$) and FSH levels ($P<0.001$) compared with the control group. There is no significant differences between both groups regarding prolactin level ($P>0.05$). Patients with constitutional delayed puberty have significantly higher melatonin level compared with the control group ($P<0.001$). FSH, follicle-stimulating hormone.*Significant ($P < 0.001$).

Table 5 Correlation between serum melatonin, total testosterone, follicle-stimulating hormone, and prolactin in constitutional delayed puberty

Melatonin	Mean±SD	*r*	*P*
Total testosterone	118.32±22.366	−0.652	0.0001*
FSH	0.98±0.447	−0.821	0.0001*
Prolactin	11.1455±2.49909	−0.028	0.885

This table shows that in constitutional delayed puberty group, serum melatonin is significantly negatively correlated with both total testosterone and FSH and has no correlation with prolactin. FSH, follicle-stimulating hormone. *Significant ($P < 0.001$).

Under such condition, the pineal–puberty relationship might be more readily detected, because the age factor is dissociated from the interaction of melatonin secretion with puberty.

Luboshitzky *et al.* [23] reported that male patients with CDP show GnRH deficiency and increased nocturnal melatonin secretion.

Ianas *et al.* [24] reported that melatonin secretion regulates the oscillation and temporal organization of maturity of hypothalamic gonadal axis and acts as an endocrine signal in resetting of gonadostat, and melatonin was higher in CDP.

Moreover, this study found that melatonin is inversely correlated with both testosterone and FSH. No such correlation was done before. This inverse correlation may point to the causal relation of melatonin to delay of puberty in CDP.

Conclusion

Melatonin is significantly elevated in CDP boys, and it is negatively correlated with the main hormones of sexual maturation (testosterone) and reproduction (FSH). This means that its elevation in these boys may be a cause and not a result of their delay. We think that melatonin is very alarming and a new entity that has entered the field of puberty, reproduction, and reproductive disorders. We suspect that 1 day it may become one of the routine parameters to be asked for in assessment of puberty disorders.

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Conflicts of interest

There are no conflicts of interest.

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