Possible role of melatonin in precocious and accelerated puberty in females during the COVID-19 pandemic

Stefano Stagi (stefano.stagi@yahoo.it)
University of Florence, Anna Meyer Children's University Hospital

Marta Ferrari
Health Sciences Department, University of Florence, Anna Meyer Children's University Hospital, Florence

Giada Paiusco
Health Sciences Department, University of Florence, Anna Meyer Children's University Hospital, Florence

Maria Moriondo
Health Sciences Department, University of Florence, Anna Meyer Children's University Hospital, Florence

Chiara Azzari
Health Sciences Department, University of Florence, Anna Meyer Children's University Hospital, Florence

Research

Keywords: Children, Precocious puberty, Fast puberty, Melatonin, SARS-CoV-2, COVID-19, Puberty, Onset of Puberty, Public health, Epidemiology

DOI: https://doi.org/10.21203/rs.3.rs-855928/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

Background

Pubertal development is a complex process influenced by a multitude of factors; among these, the role of melatonin has only been partially investigated. During the COVID-19 pandemic an unexpected increase in cases of precocious and fast puberty was observed.

Aims

To evaluate the possible role of salivary melatonin in precocious and accelerated puberty during the COVID-19 lockdown.

Patients and Methods

Fifty-four females were evaluated from October 2020 to March 2021; of these, 39 children were diagnosed with central precocious puberty (CPP) and 15 with isolated premature thelarche (IPT). Thirty-three healthy children acted as controls. The enrolled patients were asked to take measures the day before the visit to avoid influencing readings of melatonin secretion. Salivary melatonin levels were analyzed using commercially available ELISA kit.

Results

There was no difference in the time from B2 to diagnosis (months) between IPT and CPP patients. However, the anamnestic and clinical data of our CPP patients confirmed an acceleration in the stages of pubertal development. As expected, subjects with CPP showed a significantly greater stature SDS and height velocity SDS. There were no differences in BMI SDS and Delta BMI SDS. Interestingly, we discovered a significant difference regarding the use of electronic devices between children with CPP, IPT and controls (p < 0.05). Children with CPP showed significantly lower values of salivary melatonin than patients with TPT and controls (p < 0.0001).

Discussion

Our results showed a marked reduction in salivary melatonin in children with CPP diagnosed during and after lockdown for COVID-19. Larger studies are needed to confirm and investigate these findings.

Introduction

Pubertal development is influenced by a multitude of factors, many of which are as yet only partially understood. In humans, nearly 70% of variations in pubertal timing can be explained by genetic factors, but the role of environmental factors is also significant and in recent years has received increasing attention.\textsuperscript{1,2}
Up to now, the role of melatonin has only been partially investigated. We know that melatonin production and release by the pineal gland are suppressed by exposure to natural and artificial light. The hormone also influences pubertal development by suppressing the concentration of kisspeptin, a protein encoded by the KISS-1 gene that, by interacting with GPR-54, a G protein-coupled receptor, induces the initiation of gonadotropin-releasing hormone (GnRH) secretion.

Increased exposure to light (whether natural or artificial) results in a lower production of melatonin, a consequent reduced suppression of kisspeptin and an increased production of GnRH.

During the early stages of the COVID-19 pandemic governments in several countries, including Italy, were forced to implement extraordinarily restrictive measures. For long periods of time many children followed online lessons from home instead of going to school and physical activity was severely limited.

These measures inevitably led to an increased use of televisions and electronic devices such as smartphones, tablets and computers resulting in an increased exposure to artificial light.

Interestingly, during this period an unexpected increase in cases of precocious and accelerated puberty was observed. The precise factors behind the long recognized secular trend for earlier puberty have yet to be clearly understood. We hypothesize that increased exposure to artificial light may have played a role in the unexpected increase in observed cases of precocious and accelerated puberty during lockdown in Italy and tentatively suggest that it may also be a factor in the continued trend towards earlier puberty in recent years.

Our study focuses on the correlation between precocious and accelerated pubertal development during and after the COVID-19 lockdown and levels of salivary melatonin.

**Patients And Methods**

Eighty-seven females followed by Meyer Children's Hospital between October 2020 and March 2021 were enrolled. Of these, 54 children were evaluated for suspected precocious puberty (PP) and 33 children were enrolled as controls.

The study was conducted according to the Declaration of Helsinki and European Guidelines on Good Clinical Practice. Ethical approval was obtained from the Meyer Children's University Hospital Ethics Committee (Paediatric Ethics Committee – Tuscany Region: number 228/2020 of 29 September 2020). Written informed consent was obtained from the parents of the participating patients after they had acknowledged their full understanding of the objectives of the research.

Subjects with melatonin-secreting tumors, genetic pathologies and/or established neurological or neuro-oncological pathologies were excluded from the study. We also excluded subjects under drug therapy with melatonin or drugs capable of interfering with the secretion or metabolism of melatonin.
Controls were selected and matched for age and sex from children investigated by the Allergology Unit of our Hospital. Only children who tested negative to tests for allergies were included.

Enrolled subjects were asked to take certain measures the day before the salivary test to avoid influencing melatonin levels. They were required to be in bed before 10.00 pm, not to use electronic devices (tablets, computers, cell phones, television, etc.) after 8 p.m and not to sleep with a light next to their bed or in the bedroom. All patients underwent an auxological evaluation and complete physical examination to record their height, weight, body mass index (BMI), growth velocity (HV), change in BMI in the last year, stage of pubertal development and rate of pubertal progression (according to Tanner's rating scale).

At the time of the visit a sample of 3 ml of saliva using a Salivette® cotton swab was collected. The sample was taken at least 30 minutes after a meal and the time was noted. Saliva samples were directly collected in clear sterile plastic tubes using sterile plastic straws. A 1-mL aliquot of each saliva sample was pipetted onto a Salivette® cotton swab (cotton saliva collection) and into clear sterile plastic tubes (passive saliva collection). All saliva samples were centrifuged at 1500× g for 5 min at room temperature and then frozen at -30°C until they were assayed.

Melatonin levels were analyzed in duplicate using commercially available ELISA kits (Direct Saliva Melatonin ELISA; Bühlmann Laboratories, Allschwil, Switzerland), and the mean values of the duplicates were used for analyzing the results. The kit sensitivity was 0.5 pg/mL. The intra- and inter-assay coefficients of variation were 12.6% and 22.9%, respectively.

Family and personal clinical data relevant to the study were collected (e.g., age at diagnosis of CPP, personal and family history for the presence of major diseases, family history for CPP).

Skeletal maturation (BA) was determined by radiographs of the left hand and wrist and then calculated according to the method of Greulich and Pyle. Pelvic ultrasound was also performed and the area (S) of the ovaries was calculated as follows: S = length x width x 0.8. Normal ovarian surface area is < 2 cm² in prepubertal and 2 to 6 cm² in pubertal subjects.

All laboratory measurements were performed on blood samples collected after overnight fasting. Precocious puberty was defined as the development of pubertal changes before 8 years of age. We considered peak LH values of > 5 IU/L on GnRH in the presence of pubertal signs or a basal LH value of > 1.1 IU/L and a ratio of stimulated LH to stimulated FSH of > 1.0 combined with isolated and/or axillary hair growth accompanied by breast development, as indicative of activation of the hypothalamic GnRH pulse generator.

Statistical analysis

Data analysis was performed using the “Statistical Package for the Social Sciences for Windows” (SPSS, Inc, Chicago, IL, USA), version 13.0. Descriptive statistics are presented as numbers (percentages),
median and range and mean ± SD values. Histograms and Shapiro-Wilk tests were used to verify the normality of continuous data. The variation of continuous variables at the beginning and end of observation was analysed by paired Student's T for parametric data, while the Wilcoxon signed-rank test was used for nonparametric analysis. Categorical variables were compared by the χ² test or Fisher exact test. The Pearson correlation test was used to determine correlation coefficients. All tests were two-sided, and values of p < 0.05 were considered statistically significant.

Results

A total of 54 patients underwent GnRH testing for suspected precocious puberty: 39 females with a diagnosis of central precocious puberty (CPP) and 15 with a diagnosis of isolated premature telarche (IPT) were enrolled and compared with 33 age-matched controls. Table 1 describes clinical and hormonal characteristics of subjects with CPP, IPT and controls (Table 1).

There was no difference in time from B2 to diagnosis (months) between IPT and CPP patients. However, the anamnestic and clinical data regarding the appearance of secondary sexual characteristics in patients with CPP confirmed our previous data⁷ and pointed to an acceleration in the stages of pubertal development. Despite the short time between the onset of secondary sexual characteristics and our clinical evaluation, we observed a significant difference in the Tanner stage between females with CPP and IPT at the time of evaluation.

Subjects diagnosed with CPP showed a significantly greater stature SDS and height velocity SDS than IPT subjects and controls. However, the BMI SDS and the Delta of the BMI SDS (in respect to the value of the previous year) was not significantly different in the three groups. Children with CPP also had a significantly advanced bone age and delta between bone and chronological age than the IPT subjects, as well as increased basal LH, LH peaks after GnRH test, estradiol values, uterine length and ovarian volumes. Interestingly, we discovered a significant difference regarding the use of electronic device between children with CPP, IPT and controls (p < 0.05).

Salivary melatonin assay showed that patients with a diagnosis of CPP had significantly lower values (1.03 ± 0.91 pg/mL) than patients with idiopathic telarche (3.51 ± 2.49 pg/mL; p < 0.0001). Significantly higher values were found in the control group (4.76 pg/mL ± 4.15; p < 0.0001 vs CPP group). Evaluating the correlations among salivary melatonin and age, sex, Tanner stages, height SDS, BMI SDS, estradiol, LH, FSH, uterine length and ovarian volume, we noted that salivary melatonin inversely correlated with age (r = −0.51, p < 0.005) Tanner stages (r = −0.43, p < 0.005), tempo of puberty (r = −0.41, p < 0.005) and the use of electronic devices (r = −0.39, p < 0.05).

Discussion

During the lockdown period, we observed a marked increase in cases of CPP with a probable increase in the rate of pubertal progression. We also observed that salivary melatonin values were significantly lower
in patients with pubertal developmental disorders compared to controls.

We suspect that the profound changes in lifestyle brought about by the lockdown played a role in triggering pubertal development. Increased exposure to artificial light from electronic devices, whose use by children increased greatly during lockdown because of distance learning and personal use, may have caused a reduction in endogenous melatonin production and promoted early pubertal development.

A study by Salti et al.\textsuperscript{11} showed an overall, age-dependent decrease in urinary melatonin concentration associated with children's exposure to a television screen.\textsuperscript{11} Many other recent studies have investigated the effects of electromagnetic field (EMF) exposure on melatonin levels.\textsuperscript{11–13} Exposure to simulated EMF has been associated with a decrease in melatonin production by isolated pinealocytes in vitro. Nocturnal serum melatonin levels are highest in very young children and decline progressively by 80% during childhood, adolescence and young adulthood. Animal studies show that a reduction in melatonin can accelerate pubertal development\textsuperscript{14} and that exogenous melatonin can suppress GnRH secretion.\textsuperscript{15}

The time children spent in front of cell phones, television screens, computers, and tablets increased by a factor of 2.5 during lockdown and it is plausible that this led to a reduction in melatonin levels, which in turn triggered endocrine changes leading to a greater incidence of early pubertal development,\textsuperscript{16} although we acknowledge that other factors, such as overnutrition and psychological stress may also have been at play. Our study has several limitations, including a small sample size, the impossibility of assessing changes in physical activity, psychological wellbeing, and the reliance on self-reporting to determine patients' use of electronic devices. Clearly, further investigations are needed to correlate the observed increase in precocious puberty with specific triggers. We emphasize the need for studies involving a larger number of patients with central precocious puberty and a completely healthy control population enrolled outside the hospital setting.

**Declarations**

**Competing interests**

The authors declare that they have no competing interests.

**Financial competing interests**

The authors do not have any financial or non-financial competing interests in relation to this manuscript.

**Authors' contributions**

Stefano Stagi carried out endocrinological evaluations, conceived the study and participated in its design and coordination. stefano.stagi@unifi.it

Marta Ferrari carried out the endocrinological evaluations and data interpretation. Email: marta.ferrari0390@gmail.com
Giada Paiusco carried out the endocrinological evaluations, data interpretation and participated in the design of the study. Email: giada.paiusco@stud.unifi.it

Maria Moriondocarried out data interpretation and participated in the design of the study. Email: maria.moriondo@meyer.it

Chiara Azzari participated in the design and coordination of the study. chiara.azzari@uni.it

All authors read and approved the final manuscript.

Acknowledgements

Thanks to Tammy Ann Corkish BA MA for advice on the use of clear English.

References

1. Parent A-S, et al. The Timing of Normal Puberty and the Age Limits of Sexual Precocity: Variations around the World, Secular Trends, and Changes after Migration. Endocr Rev. 2003;24:668–93.
2. Sørensen K, et al. Recent Secular Trends in Pubertal Timing: Implications for Evaluation and Diagnosis of Precocious Puberty. Horm Res Paediatr. 2012;77:137–45.
3. Ganguly S, et al Role of a pineal cAMP-operated aryalkylamine N-acetyltransferase/14-3-3-binding switch in melatonin synthesis. Proc. Natl. Acad. Sci. 98, 8083–8088 (2001).
4. Hastings MH, Maywood ES, Reddy AB. Two Decades of Circadian Time. J Neuroendocrinol. 2008;20:812–9.
5. Skorupskaite K, George JT, Anderson RA. The kisspeptin-GnRH pathway in human reproductive health and disease. Hum Reprod Update. 2014;20:485–500.
6. Verzani M, et al. Impact of COVID-19 pandemic lockdown on early onset of puberty: experience of an Italian tertiary center. Ital J Pediatr. 2021;47:52.
7. Stagi S, et al. Increased incidence of precocious and accelerated puberty in females during and after the Italian lockdown for the coronavirus 2019 (COVID-19) pandemic. Ital J Pediatr. 2020;46:165.
8. Iannaccone GWW, Greulich, Pyle SI: Radiographic atlas of skeletal development of the hand and wrist. 2nd edition. I volume-atlante di 256 pagine. Stanford University Press, Stanford, California, 1959. Acta Genet. Med. Gemellol. (Roma) 8, 513–513 (1959).
9. Massin N, et al. Significance of ovarian histology in the management of patients presenting a premature ovarian failure. Hum Reprod. 2004;19:2555–60.
10. Carel J-C, et al. Consensus Statement on the Use of Gonadotropin-Releasing Hormone Analogs in Children. PEDIATRICS. 2009;123:e752–62.
11. Salti R, et al. Age-dependent association of exposure to television screen with children’s urinary melatonin excretion? 8 (2006).
12. Akkaya R, et al. Wi-Fi decreases melatonin protective effect and increases hippocampal neuronal damage in pentylenetetrazole induced model seizures in rats. Pathophysiology. 2019;26:375–9.

13. Lewczuk B, et al. Influence of Electric, Magnetic, and Electromagnetic Fields on the Circadian System: Current Stage of Knowledge. BioMed Res Int. 2014;2014:1–13.

14. Hadinia SH, Carneiro PRO, Fitzsimmons CJ, Bédécarrats GY, Zuidhof MJ. Post-photostimulation energy intake accelerated pubertal development in broiler breeder pullets. Poult Sci. 2020;99:2215–29.

15. Roy D, Belsham DD. Melatonin Receptor Activation Regulates GnRH Gene Expression and Secretion in GT1–7 GnRH Neurons. J Biol Chem. 2002;277:251–8.

16. et al. Could long-term administration of melatonin to prepubertal children affect timing of puberty? A clinician's perspective. Nat. Sci. Sleep Volume 11, 1–10 (2019).

Tables
Table 1. Clinical data and laboratory results in the patients and controls

| Variable                                                                 | Central precocious puberty | Premature thelarche | Controls |
|--------------------------------------------------------------------------|----------------------------|---------------------|----------|
| Population number                                                        | 39                         | 15                  | 33       |
| Chronological age at diagnosis (yr)                                      | 7.41 ± 0.25                | 7.37 ± 0.27         | 7.43 ± 0.28 |
| Chronological age at B2 (as referred by parents or family pediatrician) | 7.13 ± 0.28                | 7.09 ± 0.29         | -        |
| Time from B2 to diagnosis (months)                                       | 2.83 ± 0.37                | 2.71 ± 0.34         | -        |
| Height, SDS                                                              | 0.89 ± 0.62                | 0.39 ± 0.40*        | 0.42 ± 0.41* |
| Height Velocity, SDS                                                     | 1.33 ± 0.66                | 0.41 ± 0.53***      | 0.43 ± 0.47*** |
| BMI, SDS                                                                 | 0.69 ± 0.52                | 0.57 ± 0.48         | 0.59 ± 0.50 |
| D BMI, SDS (last year)                                                   | 0.21 ± 0.11                | 0.24 ± 0.13         | 0.25 ± 0.15 |
| Tanner stage at diagnosis (percentage)                                   |                            |                     |          |
| II                                                                       | 51.3                       | 73.3***             | -        |
| III                                                                      | 48.7                       | 26.6***             | -        |
| IV                                                                       | -                          | -                   | -        |
| V                                                                        | -                          | -                   | -        |
| Bone age (yr)                                                            | 9.57 ± 1.23                | 7.89 ± 0.66***      | -        |
| Bone age minus chronological age (yr)                                    | 2.16 ± 0.98                | 0.52 ± 0.39***      | -        |
| Basal LH, IU/L                                                           | 1.30 ± 0.84                | 0.11 ± 0.31***      | -        |
| Basal FHS, IU/L                                                          | 1.14 ± 1.12                | 3.45 ± 1.48***      | -        |
| Peak LH at GnRH stimulation, IU/L                                        | 12.92 ± 5.18               | 3.43 ± 1.85***      | -        |
| LH peak/FSH peak ratio                                                   | 0.23 ± 0.11                | 1.83 ± 0.68***      | -        |
| Basal estradiol (females only), pmol/L                                   | 134.30 ± 32.55             | 49.93 ± 21.47***    | -        |
|                         | Value 1    | Value 2    | Value 3 |
|-------------------------|------------|------------|--------|
| Uterine length, cm      | 4.31 ± 0.37| 3.22 ± 0.42***| -      |
| Ovarian volume, cm³     | 3.43 ± 0.49| 2.24 ± 0.35***| -      |
| Electronic device use (h)| 3.64 ± 0.97| 3.01 ± 0.91*   | 3.05 ± 0.87*  |
| Salivary melatonin, pg/mL| 1.03 ± 0.91| 3.51 ± 2.49***| 4.76 ± 4.15***|

* = mean/yr. BA, bone age; CA, chronological age; SDS, standard deviation score; BMI, body mass index; LH, luteinizing hormone; FSH, follicle-stimulating hormone; GnRH, gonadotropin releasing hormone test.