



Relationship Between Pineal Gland Volume And Autistic Patients

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ABSTRACT

Aims And Objectives: Abnormalities in melatonin physiology and circadian rhythm are detected in patients with autism. Melatonin is produced predominantly in the pineal gland and the amount of melatonin released is proportional to the pineal gland volume. This study aimed to examine whether the pineal gland volume in children with autism is different from that in healthy children.

Material and methods: Brain magnetic resonance images (MRI) of 120 paediatric patients with autism and 82 control paediatric subjects were examined; pineal parenchymal volume (PPV), pineal cyst rate (PCR), and total pineal gland volume (TPGV) were measured using a multimodality viewer (MMV), but only the TPGVs were measured using a tumour tracking (TT) method. Measurements were taken by 2 separate radiologists

Results: The PCR was substantially greater in autistic patients, but the PPV and TPGV according to MMV and the TPGV according to TT were significantly lower. Additionally, the autistic patient group's PPV to TPGV ratio was noticeably lower. In both studies, the autism patient group's TPGVs were noticeably lower than the controls in every age range.

Conclusions: Using two distinct approaches, our study was the first to thoroughly investigate TPGVs in pediatric autistic individuals. Autism has been found to have high PCR and low PPV-TPGV. Comparable reference values for pineal gland size in healthy and autistic children ages 2–17 are also provided by this study.

Keywords: pineal cyst, autism children, , pineal gland volume.

INTRODUCTION

One percent of the general population suffers from autism spectrum disorder (ASD), a complex neurodevelopmental disorder that can be congenital or develop during the first three years of life. Restrictive and repetitive behavior, along with communication and social interaction disorders, are the hallmarks of the disease [1].

Patients with ASD are found to have abnormalities in their circadian rhythm and melatonin physiology. There is a correlation between autistic behavior and physiological levels of melatonin and/or melatonin derivatives, which are typically below average in ASD. It has been reported that differences in the melatonin production pathway and genetic pathways are the cause of these abnormalities in melatonin concentrations in children with autism. Children with ASD who received exogenous treatment with melatonin supplements showed improved nighttime management in relation to their daytime behaviors.

It has been noted that the mean melatonin level is less than half in about 65% of patients with ASD. Additionally, sleep disturbance is one of the most prevalent symptoms in pediatric ASD patients, with a prevalence of 50–80% [4], although it is observed in 9–50% of normal children. The pineal gland produces melatonin, an endogenous neuro-hormone, primarily at night and in the dark. The volume of the pineal gland determines how much melatonin is released [5].

The pineal gland is a photo-neuro-endocrine gland that emerges from the third ventricle's posterior wall in the brain's center. Because of its location, it can secrete hormones into the bloodstream and directly into the cerebrospinal fluid [1].

The quantity and total volume of pinealocytes can be thought of as proportional to the pineal parenchyma volume (PPV) (corrected pineal volume for people with pineal cysts), which is the portion that releases melatonin. To get the right result when analyzing the function of this gland, it is also necessary to take into account pineal cysts, which are quite common but whose effects on pineal function are unknown [7,8].

AIMS AND OBJECTIVES:

- This study aimed to examine whether the total or parenchymal volume of the pineal gland in children with ASD is different from that in healthy children.
- To examine whether there is a difference between patients with ASD and control individuals in terms of the presence of a pineal gland cyst.
- To investigate whether there is a change in pineal gland volume (PGV) based on increasing age groups in ASD children and normal children.
- Finally, in light of the fact that knowing the normal pineal gland volume can help radiologists to identify

abnormal cases, we also aimed to reveal normal pineal gland volumes in children, which could be used to compare MRI findings.

MATERIALS AND METHODS

SOURCE OF DATA:

This is retrospective study used data from MVJ medical college and research hospital, Bengaluru on 120 children with a definitive diagnosis of ASD and 82 control pediatric subjects, both of whom were between the ages of 3 and 16. This study was approved by the Ethics Committee of MVJ hospital and research hospital.

The study period was two years starting from July 2022 to June 2024.

METHOD OF COLLECTING DATA:

MRI was performed using a 1.5 Tesla MR scanner and comprised axial T1-weighted dual-echo, axial T2-weighted single-shot fast-spin echo sequences (SS-FSE), and diffusion-weighted imaging (DWI), succeeded by a dynamic contrast-enhanced investigation.

Inclusion criteria:

Study population:

The study included patients with an ASD diagnosis who were between the ages of 3 and 16 and who were listed in the hospital database and who had had brain magnetic resonance imaging (MRI).

Control:

- 1) Healthy individuals who had undergone general health checkup.
- 2) As the control group, the PGVs of the pediatric patients whose brain MRIs were obtained for different purposes and found to be normal in comparable age groups were chosen.

Exclusion criteria:

- a. The study excluded patients and control subjects with pineal calcification, as well as those with a history of brain trauma, surgery, or tumor
- b. Cases of any known structural neurological disease.
- c. Patients who had metallic implants, pacemakers, hearing aids, insulin and medication pumps.
- d. Controls volunteers with first degree (blood relation) relative known to be suffering from psychiatric illness.
- e. Patients already on antipsychotic medication.

STUDY DESIGN: Hospital based Retrospective study.

EQUIPMENT AND PROTOCOL:

The imaging was done using a SIEMENS MAGNETOM ESSENZA 1.5 TESLA MRI machine and routine sequences were obtained.

STATISTICAL ANALYSIS:

The normality of the continuous variables was ascertained using the Shapiro-Wilk test. The Student's t test was utilized to ascertain the average differences between the ASD patients and the control group because the data displayed a normal distribution.

Total pineal gland volume (TPGV)-MMV (mm³) and TPGV-TT (mm³) in the patient and control groups were found to have a linear relationship using Pearson's correlation coefficients.

The in-class correlation coefficient was computed in order to assess the compatibility of the TT and MMV approaches. The cut-off point for TT and MMV was established, and the area under the two ROC curves was compared, using receiver operating characteristic (ROC) analysis. The number of patients with cysts and the gender of the patients by group were analyzed using the c2 test. The statistical significance level was set at 0.05, and the analyses were conducted using the demo versions of MedCalc 16 and SPSS 21.

RESULTS:

PARAMETER	ASD (n = 120), mean +/- SD	Control (n = 81), mean +/-SD	p
PPV-MMV	90.15 +/- 43.00	135.15 +/- 41.40	<0.001
TPGV-MMV	118.10 +/- 35.90	154.20 +/- 34.90	<0.001
PCV- MMV	55.8 +/- 23.30	56.20 +/- 26.31	0.941
Number of patients with a pineal cyst(n)	61 (50.0 %)	22 (24.%)	0.001
PPV-MMV/TPGV- MMV rate	0.76 +/- 0.25	0.90 +/-	<0.001

Table 1. parameter comparison among the groups.

ASD- autism spectrum disorder.
 PPV – pineal parenchymal volume.
 PCV – Pineal gland volume.
 TPGV – total pineal gland volume.
 MMV- multimodality viewer.

Parameter	ASD	Control	<i>p</i>
Age (mean $\pm$ SD)	8.50 $\pm$ 4.44	8.82 $\pm$ 4.54	0.688 ^a
Gender, <i>n</i> (%)			
Female	26 (21.7)		0.650 ^b
Male	94 (78.3)		

Table 2: Age and Gender distribution of cases.

		<i>TPGV-MMV</i> (mm ³)	<i>TPGV-TT</i> (mm ³)
ASD–Age	<i>r</i>	0.824	0.791
	<i>p</i>	< 0.001	< 0.001
Control–Age	<i>r</i>	0.735	0.742
	<i>p</i>	< 0.001	< 0.001

Table 3: Analysis of the relationship between total pineal gland volume and age.

r – Pearson's correlation coefficient,
 ASD – autism spectrum disorder,
 TPGV – total pineal gland volume,
 MMV – multimodality viewer,
 TT – tumour tracking

The two study groups had a homogeneous distribution of age and gender ($p = 0.650$ for gender and $p = 0.688$ for age). Despite the fact that the PPV mean was lower in the ASD patients compared to the controls.

The mean pineal cyst volume did not significantly differ between the ASD patient group and the control group ($p < 0.001$, $p = 0.940$, respectively). For both the MMV and TT techniques, the mean TPGV was lower in the ASD patients than in the control group ($p < 0.001$ for both).

Patients with ASD had a lower average PPV-MMV/TPGV-MMV ratio than the control group ($p < 0.001$). Patients with ASD had a higher incidence of pineal cysts than those in the control group ($p = 0.001$). According to both measurement methods, the mean TPGV volume was statistically significantly lower for ASD patients across all age groups than for the control group.

For both techniques, a positive, strong linear relationship between age and TPGV was observed for both the control group and the ASD patient group. The TPGV rose in tandem with the patients' ages. In terms of calculating the TPGV, there was a high degree of agreement between the TT and MMV

approaches (intra class correlation [95% CI] 0.961; range: 0.948-0.970). The cut-off point for differentiating between ASD patients and healthy control subjects was $\leq 125 \text{ mm}^3$, as per the TPGV-MMV technique. A 53.33% of patients with ASD were detected, whereas 87.80% of children were found to be healthy. The MMV technique indicated that the TPGV's overall discrimination performance was 74.2%.

Using the TPGV-TT technique, the cut-off point to distinguish between patients with ASD and healthy patients was $\leq 150 \text{ mm}^3$. The detection rate of children who were healthy was 70.73%, whereas the detection rate of patients with ASD was 62.5%. The TPGV-TT technique's overall discrimination performance was 71.3%.

This value, though, lacked statistical significance. Regarding separating patients with ASD from healthy people, there was no difference in the TPGV measurements between the two approaches ($p = 0.056$).

MRI:

The 1.5 Tesla scanner was used for all MRI studies without the use of contrast. To define the pineal gland, we used the quadrigeminal cistern, the posterior portion of the third ventricle, the corpus callosum, and the upper colliculus as anatomical landmarks.

The pineal glands' volume was mainly marked and measured in the sagittal planes, and the results were corroborated in the axial and coronal planes. The volume analysis program was used to volumetry the pineal gland and pineal cysts, and e Portal software was used to perform 3-dimensional (3-D) volumetric analysis on a workstation. This program contains two distinct volume measurement tools: a tumor tracking tool and a multimodality viewer (MMV).

Only total PGVs were measured in T1-weighted sequences using the TT method because it is technically challenging to differentiate between a pineal cyst and the parenchyma using TT. Given that pineal cysts lack pinealocytes, the PPV was computed by deducting the cyst's volume from the total pineal volume. All volumetric data were presented as means $\pm$ standard deviation (SD) in cubic millimeters from two separate measurements separated by one week. The average was used to arrive at the final value.

The PPV was computed by deducting the cyst's volume from the total pineal volume, taking into account that pineal cysts are devoid of pinealocytes. All volumetric data were presented as means $\pm$ standard deviation (SD) in cubic millimeters from two distinct measurements separated by one week. The average was

used to arrive at the final value.

3-D Brainview T1W, TSE (turbo spin echo) T2W-coronal, TSE T2W-axial, T1WIR (inversion recovery)-coronal, and Flair-SPIR (spectral pre-saturation with inversion recovery)-axial were the sequences of the study protocol images used in the volumetric measurements. DICOM was the file format. The slices ranged in thickness from 1 to 3 mm. The following parameters made up the study protocol for 3-D structural T1-weighted gradient echo sequences: acquired voxel size = $0.99 \times 1.0 \times 1.0$ mm; 1.0 mm sagittal slice thickness without inter-slice gap; flip angle = 90° ; field of view = 240×240 mm; repetition time = 600 ms; echo time = 29 ms.

The following were specifics of the TSE (turbo spin echo) T2-weighted sequence parameters: Size of acquisition voxel = $0.70 \times 0.82 \times 3.0$ mm; thickness of sagittal slice without inter-slice = 3.0 mm gap; repetition time = 4.2 ms; echo time = 103 ms; flip angle = 90° ; field of view = 240×240 mm.

REPRESENTATIVE CASES

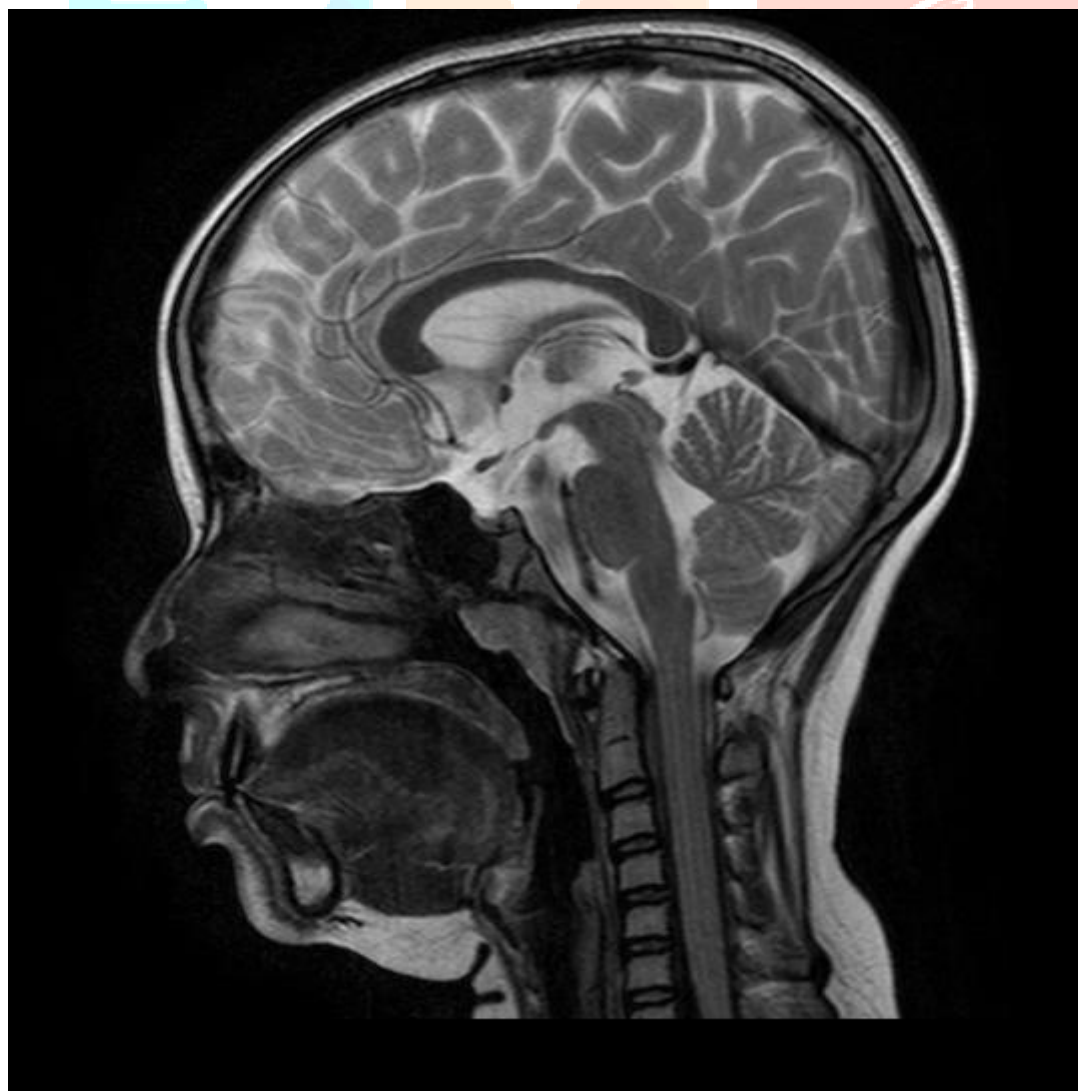


Figure 1: A Pineal gland volume of a 12 -year-old male patient measured using the tumour tracking method



Figure 2- axial image for pineal gland volum estimation.



Figure 3: A Pineal gland volume of a 14 year-old male patient measured using the tumour tracking method

DISCUSSION

Our MMV and TT measures showed that patients with ASD had a lower TPGV than the control group ($p < 0.001$).

Additionally, it was discovered that the ASD patients had a poorer PPV-MMV/TPGV-MMV ratio than the control group ($p < 0.001$ for both).

According to both measurement methods, the mean TPGV volume was statistically substantially lower for ASD patients across all age categories than for the control group. For both approaches, a positive, robust linear connection between age and TPGV was seen for both the control group and the ASD patient group. The TPGV rose in tandem with the patients' ages. In terms of calculating the TPGV, there was a high degree of agreement between the TT and MMV approaches (intraclass correlation [95% CI] 0.961; range: 0.948-0.970). The cut-off point for differentiating between ASD patients and healthy control subjects was $< 125 \text{ mm}^3$, as per the TPGV-MMV approach.

53.33% of patients with ASD were detected, whilst 87.80% of children were found to be healthy. The MMV method indicated that the TPGV's overall discrimination performance was 74.2%. Using the TPGV-TT approach, the cut-off point to distinguish between patients with ASD and healthy patients was $< 150 \text{ mm}^3$. The detection percentage of children who were healthy was 70.73%, but the detection rate of patients with ASD was 62.5%. The TPGV-TT technique's total discrimination performance was 71.3%. This value, though, lacked statistical significance.

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René Descartes described the pineal gland as the "organ of the psychophysiological control" or the "seat of the soul" in the 16th century [9].

The production of melatonin at night and serotonin and N-N-dimethyltryptamine during the day is controlled by the pineal gland, with the rhythm of the circadian cycle [10]. Melatonin is well-known for its ability to regulate the circadian rhythm, but it also has anti-inflammatory, potent antioxidant, and synaptic plasticity and immune response regulation capabilities [11,12].

The pineal gland is located between the dorsal habenular commissure and the posterior commissure on the sagittal line. It is solid, pinecone-shaped, reddish-gray in color, and is roughly 5–9 mm in length and 1–5 mm in breadth [9,13].

The geographic location of the clithe and the activity of the light stimulus determine the size of the pineal gland in animals [14]. Follicles, pinealocytes, and glial tissue make up the pineal gland. The astrocytes predominate in the glial tissue, while the pinealocytes constitute the pineal parenchyma [15]. The amount of melatonin released rises in direct proportion to the PPV. Pinealocytes are the only cells found in the PPV. The gland's secretory functions are more dysfunctional when there are more parenchymal calcifications, gliosis, and cysts [16].

Physiological melatonin and/or melatonin derivative levels are typically below normal in ASD, and they are linked to autistic behavior. This may be brought on by anomalies in the melatonin-related genes in ASD. Melatonin supplementation dramatically reduces sleep delay and length of sleep in individuals with ASD [17, 18]. According to Maruani et al. [19], patients with ASD had lower pineal gland volume and early-morning melatonin levels than the controls. A different investigation discovered a linear correlation between the PPV and human melatonin plasma concentrations [16]. Similar variations in 24-hour melatonin levels per pineal tissue volume were seen in that study [16].

Characterizing the pineal gland's secretory capability is made easier by looking at the correlation between MRI volume and melatonin levels. This is particularly evident in conditions like schizophrenia, Parkinson's disease, sleep disorders, and aging that involve a disrupted circadian melatonin rhythm [20–22]. Using both the MMV and TT techniques, we discovered that the TPGV in patients with ASD was low, in line with the literature. An increase in total brain volume and cerebellar aberration were among the anomalies found in a brain imaging investigation of autistic people [23].

PGV has been the subject of numerous thorough radiological investigations in both normal and aberrant

cases. Mild anesthesia can be used to relax autistic children during noninvasive imaging procedures such as brain MRIs [24].

According to a thorough investigation on pineal volume in young, healthy individuals, the real pineal volume in healthy adults was 94.2 ± 40.65 mm, while the mean pineal volume in male adults without cysts was 89.21 ± 28.78 mm³ [3,25]. According to a statistically significant study, the age group aged 31–40 had the highest mean pineal volume (158.34 ± 43.17 mm³), followed by the 41–50 age group (109.90 ± 37.50 mm³), the under-30 age group (131.47 ± 41.42 mm³), and the over-50 age group (109.90 ± 37.50 mm³).

This is prevalent in computed tomography and autopsy, occurring in over 66% of instances. There is debate on how pineal calcifications affect melatonin secretion.

Kunz et al. [30] discovered a link between non-calcified pineal tissue and 24-hour sulphatoxymelatonin, but no correlation between the degree of calcification and 24-hour sulphatoxymelatonin. We excluded patients with calcification from our study because of this contentious circumstance. It was discovered that the frequency of pineal cysts was 2.6% in adults and 0.6% in children [31]. Using 3-T MRI, pineal cysts can be accidentally found. Additionally, PGV tends to rise as people age [32].

In our study, patients with ASD had a considerably greater rate of pineal cysts (50% vs. 25.6%), although there was no difference in cyst volumes between the ASD and control groups ($p = 0.001$, $p = 0.940$, respectively). In children with ASD, we also found that the pineal volume grew with age.

Limitation of the study:

1. There are several restrictions on this study. First, because we were unable to enroll patients with pineal calcification, the study population was small.
2. Second, we did not take into account the connection between PGV and melatonin; instead, the study solely comprised a volumetric analysis. But we also assessed the volume of parenchyma linked to direct melatonin release.
3. Thirdly, patients' use of antipsychotic medications was not taken into account. Lastly, although we did include patients with pineal cysts, it was helpful to evaluate the amount and presence of cysts between the people in the control group and those with ASD.

CONCLUSION:

Using two distinct measuring techniques (MMV and TT), autistic children's total and parenchymal pineal gland volumes were considerably smaller than those of normal children. We discovered that children with ASD had a much greater incidence of pineal cysts than did the healthy controls. We demonstrated that age affects the pineal volume in both healthy and ASD children.

The typical distribution rate for the volumes of normal pineal glands in children with autism and healthy children between the ages of 2 and 17 is also provided by this study. We think that greater explanation and research into the anomalous findings we found in ASD are necessary; in the future, more thorough and in-depth research will yield useful insights regarding the pathophysiology of ASD patients.

BIBLIOGRAPHY

1. Yenkovyan K, Grigoryan A, Fereshetyan K, et al. Advances in understanding the pathophysiology of autism spectrum disorders. *Behav Brain Res* 2017; 331: 92-101.
2. Cohen S, Conduit R, Lockley SW, et al. The relationship between sleep and behavior in autism spectrum disorder (ASD): a review. *J Neurodev Disord* 2014; 6: 44.
3. Rossignol DA, Frye RE. Melatonin in autism spectrum disorders: a systematic review and meta-analysis. *Dev Med Child Neurol* 2011; 53: 783-792.
4. Geoffroy MM, Nicolas A, Speranza M, et al. Are circadian rhythms new pathways to understand Autism Spectrum Disorder? *J Physiol Paris* 2016; 110: 434-438.
5. Emet M, Ozcan H, Ozel L, et al. A review of melatonin, its receptors and drugs. *Eurasian J Med* 2016; 48: 135-141.
6. Shen MD, Kim SH, McKinstry RC, et al. Increased extra-axial cerebrospinal fluid in high-risk infants who later develop autism. *Biol Psychiatry* 2017; 82: 186-193.
7. Sumida M, Barkovich AJ, Newton TH. Development of the pineal gland: measurement with MR. *AJNR Am J Neuroradiol* 1996; 17: 233-236.
8. Pu Y, Mahankali S, Hou J, et al. High prevalence of pineal cysts in healthy adults demonstrated by high-resolution, noncontrast brain MR imaging. *AJNR Am J Neuroradiol* 2007; 28: 1706-1709.
9. Nichols DE. N,N-dimethyltryptamine and the pineal gland: separating fact from myth. *J Psychopharmacol* 2018; 32: 30-36.
10. Pfeffer M, Korf HW, Wicht H. Synchronizing effects of melatonin on diurnal and circadian rhythms. *Gen Comp Endocrinol* 2018; 258:

11. Malow BA, Findling RL, Schroder CM, et al. Sleep, growth, and puberty after two years of prolonged-release melatonin in children with autism spectrum disorder. *J Am Acad Child Adolesc Psychiatry* 2021; 60: 252-261.
12. El-Ansary A, Hassan WM, Daghestani M, et al. Preliminary evaluation of a novel nine-biomarker profile for the prediction of autism spectrum disorder. *PLoS One* 2020; 15: e0227626.
13. Patel S, Rahmani B, Gandhi J, et al. Revisiting the pineal gland: a review of calcification, masses, precocious puberty, and melatonin functions. *Int J Neurosci* 2020; 130: 464-475.
14. Tan DX, Xu B, Zhou X, et al. Pineal calcification, melatonin production, aging, associated health consequences and rejuvenation of the pineal gland. *Molecules* 2018; 23: E301.
15. Koshy S, Vettivel SK. Varying appearances of calcification in human pineal gland: a light microscopic study. *J Anat Soc India* 2001; 50: 17-18.
16. Nölte I, Lütkehoff AT, Stuck BA, et al. Pineal volume and circadian melatonin profile in healthy volunteers: an interdisciplinary approach. *J Magn Reson Imaging* 2009; 30: 499-505.
17. Jenabi E, Ataei S, Bashirian S. Evaluation of drug interventions for the treatment of sleep disorders in children with autism spectrum disorders: a systematic review. *Korean J Pediatr* 2019; 62: 405-409.
18. Cuomo BM, Vaz S, Lee EAL, et al. Effectiveness of sleep-based interventions for children with autism spectrum disorder: a meta-synthesis. *Pharmacotherapy* 2017; 37: 555-578.
19. Maruani A, Dumas G, Beggato A, et al. Morning plasma melatonin differences in autism: beyond the impact of pineal gland volume. *Front Psychiatry* 2019; 10: 11.