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Urinary 6-sulfatoxymelatonin as a biomarker of melatonin secretion in children and adolescents with autism spectrum disorder: a systematic review

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ADMINISTRATIVE INFORMATION

Support - This research received no external funding.

Review Stage at time of this submission - Preliminary searches.

Conflicts of interest - None declared.

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Amendments - This protocol was registered with the International Platform of Registered Systematic Review and Meta-Analysis Protocols (INPLASY) on 12 April 2026 and was last updated on 12 April 2026.

INTRODUCTION

Review question / Objective Review question:
What is the relationship between urinary levels of 6-sulfatoxymelatonin and melatonin secretion, sleep disturbances, and behavioral symptoms in children and adolescents with autism spectrum disorder?

Objective:
The objective of this systematic review is to evaluate the role of urinary 6-sulfatoxymelatonin as a biomarker of melatonin secretion in children and adolescents with autism spectrum disorder. Specifically, this study aims to compare the urinary concentrations of 6-sulfatoxymelatonin between individuals with autism spectrum disorder and control groups, and to investigate the association between this biomarker and clinical outcomes such as sleep disturbances and behavioral symptoms related to autism spectrum disorder.

Using the PICOS framework:

Population (P): children and adolescents with autism spectrum disorder;
Exposure (E): measurement of urinary 6-sulfatoxymelatonin levels;
Comparison (C): individuals without autism spectrum disorder;
Outcomes (O): levels of urinary 6-sulfatoxymelatonin and their association with melatonin secretion, sleep disturbances, and behavioral symptoms;
Study design (S): observational studies including case-control, cohort, and cross-sectional studies.

Condition being studied Autism spectrum disorder (ASD) is a neurodevelopmental condition characterized by persistent deficits in social communication and social interaction, associated with restricted and repetitive patterns of behavior, interests, or activities. Symptoms typically emerge during early childhood and may vary in severity, leading to different levels of functional impairment in daily life. ASD affects approximately 1 in 100 children worldwide and is more frequently diagnosed in males than females.

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Individuals with ASD often present comorbid conditions, including sleep disturbances, behavioral dysregulation, anxiety, and cognitive impairments, which significantly affect quality of life for both patients and their families. Among these comorbidities, sleep disorders are particularly common, affecting a large proportion of children and adolescents with ASD. Alterations in circadian rhythm regulation and reduced melatonin secretion have been suggested as important mechanisms contributing to these sleep disturbances.

Melatonin is a hormone produced by the pineal gland that plays a central role in the regulation of circadian rhythms and the sleep–wake cycle. In individuals with ASD, several studies have reported reduced melatonin levels and abnormalities in melatonin metabolism. One of the most widely used biomarkers for assessing melatonin secretion is urinary 6-sulfatoxymelatonin (aMT6s), the principal metabolite of melatonin excreted in urine. Measurement of urinary aMT6s provides a noninvasive method for estimating nocturnal melatonin production.

Recent research has suggested that urinary aMT6s levels may be associated with sleep disturbances, behavioral symptoms, and overall clinical severity in individuals with ASD. However, the evidence regarding its role as a reliable biomarker remains inconsistent. Therefore, a systematic review of the available literature is necessary to synthesize current evidence regarding the association between urinary 6-sulfatoxymelatonin levels and clinical manifestations of autism spectrum disorder in children and adolescents.

METHODS

Search strategy A comprehensive literature search will be conducted in the following electronic databases: MEDLINE (via PubMed), LILACS, EMBASE, Cochrane Library, and Web of Science. No restrictions regarding publication date or language will be applied. Only studies involving human participants will be considered.

The search strategy will be developed using controlled vocabulary and free-text terms related to autism spectrum disorder and melatonin metabolism. Medical Subject Headings (MeSH) and equivalent terms will be used when applicable. Boolean operators (“AND” and “OR”) will be applied to combine search terms and ensure a broad and sensitive retrieval of relevant studies.

The primary search strategy will include the following terms and their variations:

("Autism Spectrum Disorder" OR "Autistic Disorder" OR autism)
AND
("Melatonin")
AND
("6-sulfatoxymelatonin" OR "6-sulphatoxymelatonin" OR "aMT6s")
AND
("Children" OR "Adolescents" OR pediatric)

Equivalent descriptors and synonyms identified through the MeSH database will also be incorporated into the search strategy when appropriate.

Additionally, the reference lists of all included studies and relevant reviews will be manually screened to identify potentially eligible studies not retrieved through the electronic database search.

All identified records will be imported into reference management software, and duplicates will be removed before the screening process. Study selection will be performed independently by two reviewers using the Rayyan platform, with disagreements resolved by a third reviewer.

Participant or population The population included in this systematic review will consist of children and adolescents aged 0 to 19 years diagnosed with autism spectrum disorder (ASD) according to recognized diagnostic criteria, such as the Diagnostic and Statistical Manual of Mental Disorders (DSM) or the International Classification of Diseases (ICD).

Studies including participants with a confirmed clinical diagnosis of ASD prior to the beginning of the study will be considered eligible. Both males and females will be included, regardless of ethnicity, geographic location, or socioeconomic status.

When available, studies including a control group composed of children and adolescents without autism spectrum disorder will also be included for comparative analysis. Participants may present different levels of ASD severity and may or may not present comorbid conditions such as sleep disturbances.

Studies involving adults, animal models, or cell-based experiments will be excluded from this review.

Intervention The intervention of interest in this systematic review is the measurement of urinary 6-sulfatoxymelatonin (aMT6s) levels, a major

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metabolite of melatonin excreted in urine and commonly used as a non-invasive biomarker of melatonin secretion.

Studies that evaluate the concentration of urinary 6-sulfatoxymelatonin in children and adolescents with autism spectrum disorder will be included. The measurement may be performed using different laboratory methods, such as immunoassay techniques (e.g., ELISA), and may be obtained from nocturnal urine samples, morning urine samples, or timed urine collections, depending on the methodology used in each study.

The review will assess studies that investigate urinary 6-sulfatoxymelatonin levels in relation to melatonin secretion and their potential association with clinical outcomes, particularly sleep disturbances and behavioral symptoms observed in individuals with autism spectrum disorder.

Comparator The comparator group will consist of children and adolescents without a diagnosis of autism spectrum disorder, when available in the included studies. These individuals will serve as a control group to allow comparison of urinary 6-sulfatoxymelatonin (aMT6s) levels between participants with autism spectrum disorder and those with typical neurodevelopment.

In addition, studies that compare different subgroups within the autism spectrum disorder population may also be considered, such as participants with and without sleep disturbances or participants with different levels of behavioral symptom severity.

The comparison aims to evaluate whether urinary levels of 6-sulfatoxymelatonin differ between individuals with autism spectrum disorder and control populations, and whether variations in this biomarker are associated with clinical manifestations such as sleep disorders or behavioral symptoms.

Study designs to be included Observational studies will be included, such as case-control, cohort, and cross-sectional studies, that evaluate urinary 6-sulfatoxymelatonin levels in children and adolescents with autism spectrum disorder and, when available, in comparison with control groups without autism spectrum disorder. Studies must report quantitative measurements of urinary 6-sulfatoxymelatonin and evaluate its association with clinical outcomes such as sleep disturbances or behavioral symptoms.

Eligibility criteria Studies will be included if they evaluate urinary levels of 6-sulfatoxymelatonin in children or adolescents diagnosed with autism spectrum disorder and report quantitative data on the biomarker. Eligible studies must assess the association between urinary 6-sulfatoxymelatonin levels and clinical outcomes such as sleep disturbances, behavioral symptoms, or melatonin secretion patterns.

Studies involving human participants aged 0–19 years will be considered, regardless of language or year of publication.

Studies will be excluded if they involve animal models or cell-based experiments, if the diagnosis of autism spectrum disorder was established after the start of the study, or if they measure melatonin metabolites only in serum without urinary assessment of 6-sulfatoxymelatonin. Review articles, editorials, conference abstracts without full data, and letters to the editor will also be excluded.

Information sources A comprehensive search will be conducted in the following electronic databases: MEDLINE (via PubMed), LILACS, EMBASE, Cochrane Library, and Web of Science. These databases will be searched to identify studies evaluating urinary levels of 6-sulfatoxymelatonin in children and adolescents with autism spectrum disorder.

No restrictions regarding language or publication year will be applied. Only studies involving human participants will be considered.

In addition to electronic database searches, the reference lists of all included articles and relevant review papers will be manually screened to identify additional potentially eligible studies not captured in the initial search. When necessary, the authors of included studies may be contacted to obtain missing or additional data.

All identified records will be exported to reference management software for organization and removal of duplicates prior to the screening process.

Main outcome(s) The primary outcome of this systematic review will be the urinary concentration of 6-sulfatoxymelatonin (aMT6s) in children and adolescents with autism spectrum disorder. This metabolite represents the main urinary excretion product of melatonin and is widely used as a biomarker of endogenous melatonin secretion.

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The review will evaluate quantitative measurements of urinary aMT6s reported in the included studies and compare these levels between individuals with autism spectrum disorder and control groups without the disorder, when available. The effect measure will be the difference in mean urinary aMT6s concentrations between groups, reported in units such as ng/mL, µg/L, or equivalent standardized measures.

When sufficient data are available, pooled effect estimates will be calculated using standardized mean differences with 95% confidence intervals to assess the association between urinary 6-sulfatoxymelatonin levels and autism spectrum disorder.

Quality assessment / Risk of bias analysis The methodological quality and risk of bias of the included studies will be assessed independently by two reviewers using the Newcastle-Ottawa Scale (NOS) for observational studies. This tool evaluates study quality based on three main domains: selection of study groups, comparability between groups, and ascertainment of exposure or outcomes.

Each study will be awarded a maximum of nine stars according to the NOS criteria. Studies will then be classified according to their methodological quality as low, moderate, or high risk of bias, based on the total score obtained.

Any disagreements between reviewers during the quality assessment process will be resolved through discussion, and when necessary, by consultation with a third reviewer.

Strategy of data synthesis A qualitative synthesis will initially be performed to summarize the characteristics and findings of the included studies, including study design, sample size, participant characteristics, and reported urinary 6-sulfatoxymelatonin levels.

When sufficient and comparable data are available, a meta-analysis will be conducted to quantitatively synthesize the results. Continuous outcomes related to urinary 6-sulfatoxymelatonin concentrations will be analyzed using standardized mean differences (SMD) with 95% confidence intervals.

Statistical heterogeneity among studies will be evaluated using the I^2 statistic. An I^2 value below 40% will be considered low heterogeneity, while higher values will indicate moderate to high heterogeneity. A fixed-effects model will be used when heterogeneity is low, and a random-effects model will be applied when heterogeneity is substantial.

All statistical analyses will be conducted using Review Manager (RevMan) software. When meta-analysis is not possible due to methodological or clinical heterogeneity, the findings will be presented through a narrative synthesis.

Subgroup analysis When sufficient data are available, subgroup analyses will be performed to explore potential sources of heterogeneity among studies. Subgroup analyses may include comparisons according to age groups (children versus adolescents), presence or absence of sleep disturbances, and severity of autism spectrum disorder symptoms when reported by the included studies.

Additional subgroup analyses may also be conducted based on study design, laboratory methods used to measure urinary 6-sulfatoxymelatonin (such as ELISA or other immunoassay techniques), and timing of urine sample collection (e.g., morning urine or overnight samples).

These analyses will aim to investigate whether variations in urinary 6-sulfatoxymelatonin levels differ across specific participant characteristics or methodological aspects of the included studies.

Sensitivity analysis Sensitivity analyses will be conducted to evaluate the robustness and stability of the pooled results when sufficient studies are available. This analysis will involve repeating the meta-analysis after excluding studies with a high risk of bias, as identified through the methodological quality assessment.

Additional sensitivity analyses may be performed by removing studies with extreme effect sizes or small sample sizes, as well as studies presenting methodological differences that could influence the results, such as variations in the measurement methods of urinary 6-sulfatoxymelatonin.

These procedures will allow assessment of whether the overall results are substantially affected by specific studies. If major changes in the pooled estimates are observed after exclusion of certain studies, the potential reasons for these differences will be discussed.

Language restriction There will be no language restriction.

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Country(ies) involved The study is being conducted in Brazil, with all authors aliated with the University of the Extreme South of Santa Catarina (UNESC), Criciúma, Santa Catarina, Brazil.

Keywords Autism spectrum disorder; Melatonin; 6-sulfatoxymelatonin; Biomarker; Sleep disorders; Children; Adolescents.

Contributions of each author

Author 1 - Amanda Dagostin - Amanda Ceron Dagostin conceived the study, developed the research question, conducted the literature search strategy, and drafted the initial version of the manuscript.
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Author 2 - Ingrid Freitas - Ingrid Gonçalves de Freitas contributed to the study design, assisted in the development of the search strategy, and will participate in study selection and data extraction.
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Author 3 - Gabriele Prestes - Gabriele da Silveira Prestes supervised the study, contributed to the methodological design, risk of bias assessment strategy, and provided critical revision of the manuscript.
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