Improvement of sleep patterns and serum melatonin levels in children with autism spectrum disorders after consumption of beta-1,3/1,6-glucan in a pilot clinical study

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Short Report

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Abstract

Study objectives:

Poor sleep quality is a major problem that patients with autism spectrum disorders (ASD) face, which is attributed to their low melatonin levels. Melatonin supplementation is recommended, but its effectiveness is varied. Beta-glucans have previously been reported to improve melatonin levels in animal studies. Therefore, we examined the effectiveness of *Aureobasidium pullulans* (Nichi Glucan), a species of black yeast that contains beta-1,3/1,6-glucan, in a pilot study of children with ASD.

Methods

Thirteen children (age = 2.5 to 13 years) with ASD were recruited into the study. The control group consisted of four patients (Gr. 1), while nine patients were in the treatment group (Gr. 2). Gr. 2 received 1 g of Nichi Glucan along with conventional therapy, whereas Gr. 1 underwent conventional therapy alone for 90 days. The serum melatonin levels and sleep patterns, assessed using a subjective questionnaire, were evaluated before and after treatment.

Results

In Gr. 2, the average serum melatonin level increased from 238.85 ng/dl pre-intervention to 394.72 ng/dl post-intervention. Eight out of nine subjects (88%) in Gr. 2 group showed and improvement in their sleep pattern and quality, while this improvement was not observed in the control group.

Conclusions

Our study is the first in the literature to report that consumption of Nichi Glucan for 90 days showed visible improvement in sleep quality, sleep pattern, and serum melatonin levels. A larger multicentre study is warranted to validate our findings.

Introduction:

Sleep problems are reported in 50–80% of children with autism spectrum disorder (ASD) [1], and is most frequent in older children and adolescents. These problems include delayed sleep onset, shorter sleep duration, and daytime sleepiness; whereas in younger children, bedtime resistance, sleep anxiety, parasomnia, and night waking are predominant [2]. Sleep problems exacerbate other features of autism, such as tantrums, aggression, self-injury, inattention, hyperactivity, social interactions, and repetitive behaviours, thus adding to parental stress and negatively impacting the entire family's well-being [1, 3].

Melatonin, a neurohormone secreted by the pineal gland, regulates circadian rhythms, including sleep patterns, and has been shown to be released at lower levels in individuals with autism compared to that of healthy individuals. It has also been shown to have a positive effect on sleep in individuals with autism by relieving anxiety, improving sensory processing, possessing anti-nociceptive effects, and mitigating gastrointestinal (GIT) dysfunction or gut dysbiosis [3]. Indeed, a significant proportion of children with ASD have chronic GIT problems, such as diarrhoea, constipation, and irritable bowel syndrome. These GIT symptoms are related to the cortisol response to stress, and the gut dysbiosis–induced chronic inflammation [2], which in turn, are associated with the altered melatonin levels [2]. Thus, melatonin supplementation [2, 4, 5] is one of the main pharmacological approaches used to treat ASD. Clinical studies of melatonin supplementation in children with ASD have shown an improvement in the patients' sleep latency and quality [2, 4, 5], although the effectiveness varies [3]. It is also worth noting that although the side effects have been reported to be minimal, melatonin has been found to be effective mostly in the short-term treatment of sleep disorders, with clinical studies demonstrating that the positive effects wane during follow-up (6–12 months) [6].

A previous study showed that when rats were fed an extract consisting of a mix of rice bran and *Sarcodon aspratus* (mushroom), which contained beta-glucan, melatonin levels were upregulated in the blood serum of rats [7, 8]. In addition, clinical research reports have revealed the beneficial effects of Nichi Glucan, a beta-1,3/1,6-glucan food supplement derived from the *Aureobasidium pullulans* strain AFO-202, on metabolic disorders [9, 10], and cancer [11, 12], and as a suggested vaccine adjuvant for coronavirus disease (COVID-19) [13]. In our pilot clinical study, we explored the effects of Nichi Glucan on the sleep patterns and serum melatonin levels in children with ASD.

Methods:

This study was approved by the institutional ethics committee of the institute involved in the study and was registered in the National Clinical Trial Registry.

Study design:

The enrolled participants were clinically diagnosed with ASD by developmental paediatricians using standard assessments that involved clinical interviews incorporating the childhood autism rating scale (CARS).

Eighteen subjects (n = 18) with ASD were enrolled in this prospective, open-label pilot clinical trial comprising of two groups:

Gr. 1: A control group of six subjects with ASD (n = 6) who underwent conventional treatment comprising of remedial behavioural therapies and 500 mg of L-carnosine per day.

Gr. 2: The treatment arm with twelve subjects (n = 12) who underwent supplementation with the Nichi Glucan food supplement along with conventional treatment. Each subject consumed two sachets (0.5 g

each) of Nichi Glucan daily—with meals twice daily—for a period of 90 days.

Inclusion criteria:

1. Age: 3 to 18 years
2. Sex: Male and female
3. ASD criteria based on cumulative score obtained on the CARS scale
4. Parents willing to consent for their children to actively participate in the study

Exclusion criteria:

1. Subjects older than 18 years old
2. Any child with acute general illness or any antibiotic, anti-inflammatory, or antioxidant treatment in the two weeks prior to enrolment in the study
3. Being hyperallergic to any of the investigational products
4. Having chronic infections

Outcome measures

(1) Sleep pattern assessment by questionnaire:

Parents or caregivers completed a survey questionnaire. Sleep problems were assessed using the Children's Sleep Habits Questionnaire (CSHQ-A) that consisted of 22 questions (NICHD SECCYD—Wisconsin), with adaptations made to suit the local cultural and social conditions.

(2) Evaluation of serum melatonin:

Melatonin levels in the serum were measured in peripheral blood collected during the daytime, and analysis was performed using the human melatonin ELISA Kit (BT- LAB – Bioassay Technology Laboratory kit, China).

Data analysis:

All data were analysed using the Excel statistics package analysis software (Microsoft Office Excel). Student's paired t-tests were also calculated using this package; P-values < 0.05 were considered significant.

Data availability statement

No new data were generated or analysed in support of this research.

Results:

Six participants with ASD were initially enrolled in Gr. 1, with one drop-out before the start of the study, and another lost to follow-up (participant relocated to another city). In Gr. 2, 11 participants were initially enrolled, and two were lost to follow-up (one individual reported social problems in the family, and the other relocated to another city). A total of thirteen subjects (four in the Gr. 1 and nine in Gr. 2) completed the study. Both Gr. 1 and Gr. 2 had one female participant each.

Improvement in sleep pattern:

In the CSHQ, a reduction in the total score was observed in Gr. 2 compared to that of Gr. 1 (Fig. 1), particularly in the measures of bedtime resistance and the time of onset of sleep (Table 1). At the baseline measurements, the mean total sleep score (range) (Mean ± SD) was 66.25 ± 0.5 (66–67) and 72 ± 5.02 (62–75) in Gr. 1 and Gr. 2, respectively. Post-treatment, the total sleep score was 64 ± 4 (58–66) and 64.22 ± 7.47 (51–70) in Gr. 1 and Gr. 2, respectively. The reduction in the sleep score after the intervention, which indicated an improvement in sleep behaviour, was statistically significant in Gr. 2 (paired student's t-test, $p = 0.009$), suggesting a significant improvement in the sleep patterns when subjects were treated with Nichi Glucan. Meanwhile, no statistically significant difference was observed in the control group (paired student's t-test, $p = 0.153$).

Table 1
Results of the children's sleep habits questionnaire (CSHQ), which show a reduction of the overall sleep score in Gr. 2 compared to that of Gr. 1, resulting from the decrease in the scores of bedtime resistance and time of the onset of sleep.

	Gr. 1 (Mean values)		Gr. 2 (Mean values)	
Parameters	Baseline	End of Study	Baseline	End of Study
Bedtime resistance	28	25.75	28.55	23.22
Time of onset of sleep	20.25	20.25	21.88	19.44
Duration of sleep	6	6	6.44	5.55
Night waking	6	6	6	6
Day-time sleepiness	6	6	9.11	10

Serum melatonin levels:

Following treatment, the average serum melatonin level of the participants in Gr. 1 increased from 110.585 ng/dl to 114.11 ng/dl (Fig. 2A), whereas for those in Gr. 2, the average level increased from 238.85 ng/dl to 394.72 ng/dl (Fig. 2B). The increase in the Nichi Glucan group was 2.29, compared with 1 in the control group (Fig. 2C), although this was not statistically significant ($p = 0.06$).

Adverse effects:

Only one patient in Gr. 2 exhibited a possible mild adverse effect. At one week after initiating Nichi Glucan supplementation, the patient had increased number of bowel movements, but this was resolved without treatment. No adverse effects were observed in any of the other children.

Discussion:

In this open-label clinical trial, we found that the majority of the children treated with Nichi Glucan (8/9; 88%) experienced an improvement in their sleep pattern and quality of sleep, which was indicated by a decrease in the sleep score. The serum melatonin level increased to a greater extent in Gr. 2 compared to that of Gr.1. The sleep score was also significantly decreased in Gr. 2 compared that that of Gr. 1 (Fig. 1). Furthermore, only a minimal number adverse effects were observed.

This is the first study of its kind—one in which a nutritional supplement that is not a pharmacological drug was able to improve sleep patterns based on evidence from the evaluation of serum melatonin levels in children with ASD.

Sleep difficulties are a major problem in children with ASD, with individuals often reporting difficulty with sleep onset (53%), restless sleep (40%), night-time awakening (34%), and difficulty with arousal from sleep (32%) [14]. Poor sleep impairs emotional and daily functioning, thus leading to the impairment of academic/social functioning and the ability to maintain relationships. Therefore, ensuring high-quality sleep is an essential part of therapy for children with ASD. Among the pharmacological interventions available, melatonin [2, 4, 5], trazodone, benzodiazepines, and SSRI antidepressants are the most commonly used medications in the paediatric population [15]. Melatonin supplementation remains the treatment of choice given the side effects of the other interventions and considering that clinical trials have shown positive outcomes from supplementation [15]. Nevertheless, some reports indicate that melatonin is more effective in the short term than it is in the long term, although these studies have mostly been conducted in cohorts of individuals without ASD [6].

A nutritional supplement that is easy to administer and has minimal to no adverse effects is an ideal alternative to melatonin. In the current study, Nichi Glucan, which has been consumed as a food supplement for several decades [16] and been proven to be beneficial for the treatment of diseases such as metabolic disorders and cancer [9–12], has been shown to be a promising alternative to melatonin. Specifically, it can help improve individuals' quality of sleep and increase their daytime serum melatonin levels.

It has been postulated that the aetiology of low melatonin levels in children with ASD arises from melatonin deficiency in their mothers, and that this melatonin exerts its effects on the embryo during neurodevelopment [17]. Another study reported a clear correlation between the gut microbiome profiles of children with ASD and their mothers, suggesting the importance of assessing the microbiome during the early stage of a mother's pregnancy, as well as the importance of planning personalised treatment and prevention of ASD via microbiota modulation [17]. Beta-glucans have also been shown to reduce the underlying chronic inflammation associated with gut dysbiosis, as well as help with fostering a healthy

microbiome. This is advantageous for individuals with ASD because chronic inflammation has been shown to be associated with the severity of their symptoms [18]. Thus, with the current study showing that beta-glucans can enhance melatonin and sleep quality in children with ASD, it is necessary to further investigate the ability of beta-glucans to modulate gut microbiota and reverse gut dysbiosis, which are the possible mechanisms behind increased melatonin levels [6, 19, 20], and thereby improving sleep.

As this was a pilot study, one of the limitations is the very low sample number, which can be overcome by further large-scale studies. In addition, future research should explore the possible beneficial effects of Nichi Glucan on the behavioural aspects of people with ASD, as well as other symptoms in patients with ASD.

Conclusions:

Patients with ASD showed an improvement in their quality of sleep as well as improved levels of serum melatonin in this open-label pilot clinical study of nutritional supplementation with a beta-1,3/1,6-glucan food supplement (Nichi-Glucan), derived from the AFO-202 strain of black yeast *Aureobasidium pullulans*. Given that Nichi Glucan was found to be effective for the improvement of sleep and the other parameters observed in this pilot study of children with ASD, it is worth recommending as a supplementary food in such children if results from larger studies with longer-term follow-up are consistent with our findings. Further in-depth evaluation of the mechanisms and their correlation with other neurological parameters is recommended. These findings may shed light on novel solutions and other drug candidates.

Declarations

Financial disclosure:

No external funding was received for the study

Non-financial disclosure:

Author Samuel Abraham is a shareholder in GN Corporation, Japan which in turn is a shareholder in the manufacturing company of the AFO 202 Beta Glucan.

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Data availability statement:

The authors confirm that the data supporting the findings of this study are available within the article.

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Figures

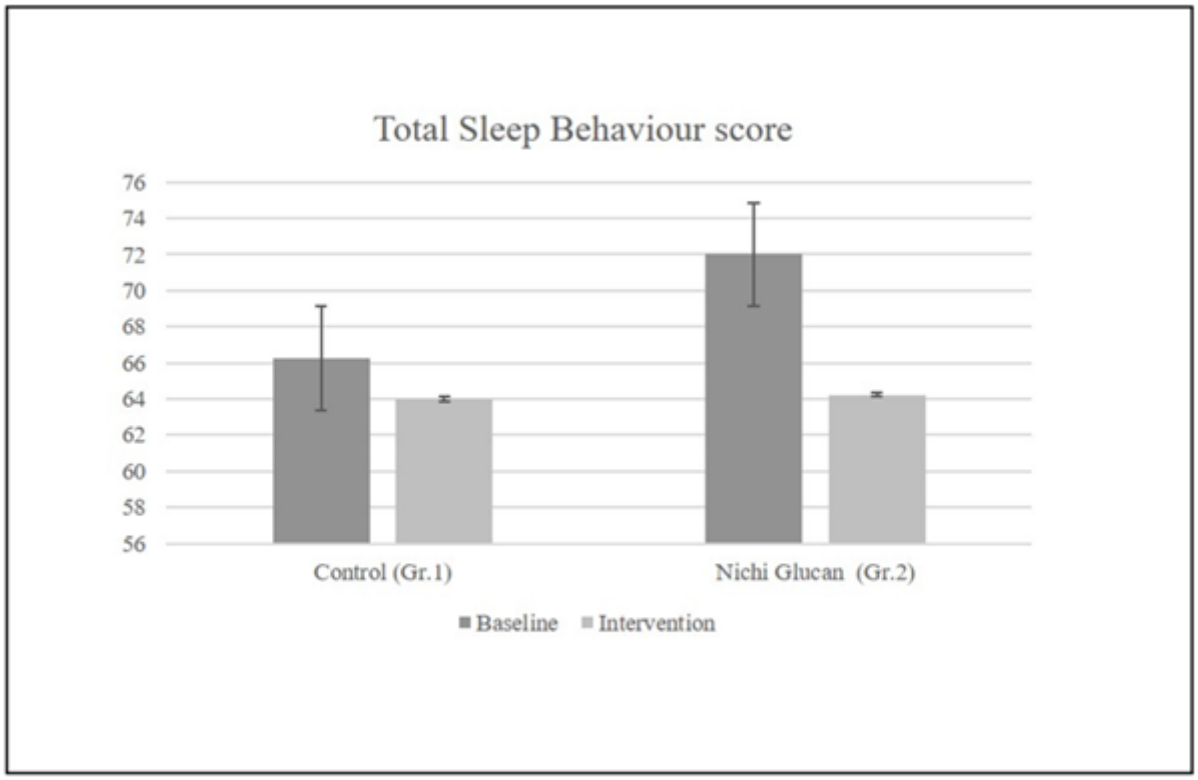

Figure 1

The total sleep pattern score of the Children's Sleep Habits Questionnaire–Abbreviated Form (CSHQ-A), which suggests that the Nichi Glucan treatment resulted in significant improvement in the sleep parameters, based on the decrease in the total score in individuals of Gr. 2 (Nichi Glucan) compared to that of those in Gr. 1 (control).

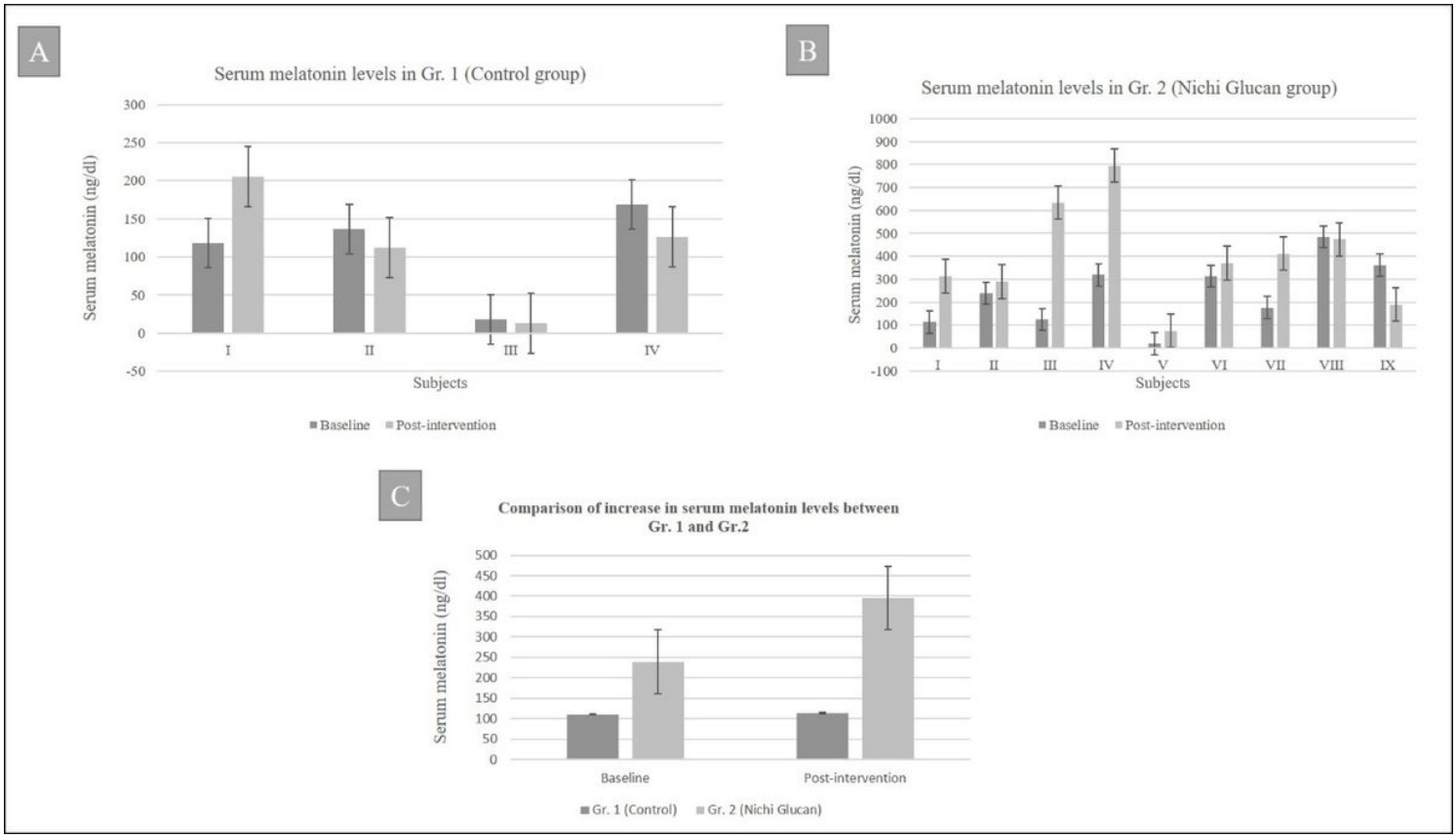

Figure 2

(A) Serum melatonin levels in Gr. 1 (control) pre- and post-intervention; (B) Same as (A), only for Gr. 2 (Nichi Glucan); (C) Increase in serum melatonin of patients in Gr. 2 was greater than that of patients in Gr. 1.