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Original Article

Possible effects of N-acetylcysteine in autism spectrum disorders: major clinical aspects, eating behaviors, and sleeping habits

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ABSTRACT

Background. N-acetylcysteine (NAC) is a promising agent for reducing irritability and hyperactivity and enhancing social responsiveness in children with autism spectrum disorders (ASD). This study aims to examine the effects of NAC on cardinal symptoms, eating, and sleeping habits in preschool children with autism.

Methods. The medical records of ASD patients were investigated retrospectively. 37 children with ASD who regularly received oral NAC in two divided doses per day (400-600 mg/day) for 8 weeks were included as the study group. The control group consisted of 21 children with ASD who were recommended NAC but never used it. The initial and second assessment scores after 8 weeks of regular use of the NAC group and control group on the Childhood Autism Rating Scale (CARS), Aberrant Behavior Checklist (ABC), Children Eating Behavior Questionnaire (CEBQ), and the Sleep Habits Questionnaire (CSHQ) were compared.

Results. Our findings suggested that oral NAC alleviated the intensity of cardinal autistic symptoms in areas of social withdrawal, interpersonal relationships, body use, listening response, and verbal communication. Corresponding problem behaviors such as irritability, stereotypic behavior, and hyperactivity were reduced. It was determined that there was no difference between the two groups in terms of eating behaviors and sleeping habits.

Conclusions. According to the results, NAC alleviated the severity of cardinal symptoms and reduced problem behaviors in autism. Additional trials with more systematic planning, controlling for confounding effects, and long-term follow-up should be provided in future studies.

Key words: autism spectrum disorder, child and adolescent psychiatry, N-acetylcysteine, eating behavior, sleep habits.

Autism spectrum disorder (ASD) is accompanied by adversities in social interaction and communication, limited interests, and stereotypical behaviors.[1] The prevalence of autism has increased in recent years and affects approximately 2% of children.[2] Although there is no definite explanation for the etiopathogenesis of autism, it is thought that the interaction of environmental and genetic variables play a role.[3,4] It is known that oxidative stress, which can increase in response to both genetic and environmental variables, triggers many different diseases[5,6] and is considered to have a prominent role in the etiopathogenesis of autism.[7-9] The deficiency of effective treatments and the widespread diagnosis of autism indicate that more studies are needed.

Glutathione (L-γ-glutamyl-L-cysteinyl-glycine) is a constitutional antioxidant that helps scavenge free radicals and buffer the reactive products of oxidative reactions.[10] It presents in two forms, the oxidized form (glutathione disulfide/GSSG) and

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the reduced form (GSH). GSH gives its electrons to free radicals and prevents cell damage. It also plays a role in other critical functions such as protein and prostaglandin synthesis, transport of amino acids, and enzyme activation.[11,12] Furthermore, glutathione acts as a depot for neuronal glutamate.[13] The dysregulation of the glutamate-glutamine cycle between glial cells and glutaminergic neurons affects synaptic excitability.[13] In research exploring the role of glutathione in the etiopathogenesis of autism, it has been noted that people with autism tend to have low glutathione reserves and low plasma and cellular glutathione levels.[8] A study emphasized that the glutamate-glutamine ratio was elevated in the amygdala–hippocampus areas in individuals with autism[14], and it has been claimed that raised glutamate levels may result in neuronal excitotoxicity, which may lead to inadequate inhibition of the prefrontal cortex and hypersensitivity of the amygdala.[8,9] Additionally, it has been shown that oxidative stress in the brain may affect the pathogenesis of autism by decreasing glutathione levels in the temporal cortex and cerebellum.[15,16]

N-acetylcysteine (NAC) contains the amino acid cysteine, which is required in glutathione synthesis.[17,18] NAC is clinically used in the treatment of glutathione deficiency such as some genetic and metabolic disorders. It is further reported that NAC is useful as adjuvant therapy in different medical conditions such as chronic lung diseases, sleep apnea, parkinsonism, multiple sclerosis, acquired immune deficiency syndrome, schizophrenia, bipolar affective disorder, and obsessive-compulsive disorder.[19] NAC is thought to be a potential drug for alleviating autistic symptoms due to its functions overlapping with both glutaminergic and oxidative stress hypotheses, which are thought to be related to the pathophysiology of autism.[20] In an animal study investigating the effectiveness of an NAC valproate-induced model of autism, it was reported that the administration of NAC in male rats increased GSH levels and reduced repetitive and stereotypical behavior of the rats.[21] In another study, valproate-induced autism rat models were divided into two groups and NAC was given to one group and saline to the other for 10 days starting from day 21 postpartum. At the conclusion of the study, it was noted that the period and frequency of social interactions increased and anxiety-like attitudes decreased in rats given NAC.[22] When human studies researching the effect of oral NAC supplementation in autism were reviewed, it was seen that few studies were conducted in this field. In a meta-analysis of randomized placebo-controlled studies, it was found that NAC reduced hyperactivity and irritability and improved social responsiveness in children with ASD.[20] In addition, it was emphasized that NAC could be considered as an off-label drug because it was a well-tolerated and cheap drug with limited adverse effects.[20]

In this study, the possible effect of oral NAC on the eating behavior and sleeping habits of children with autism was also examined. When studies investigating the relationship between NAC and eating behaviors were reviewed, no study investigating this relationship was found in the literature. When studies exploring the connection between NAC and sleep habits were reviewed; a study reported that NAC had an effect on the central processes associated with obstructive sleep apnea and positively affected sleep[23], which led us to investigate the possible effect of NAC on the relationship between sleep and autism.

Although studies have reported the oxidative stress hypothesis and dysregulation in the glutaminergic system in the pathophysiology of autism, this study was planned because the effects of oral NAC on different clinical aspects in children with autism remain unclear and there are limited studies in this regard, the objective of this study was to assess the impact of oral NAC on cardinal autistic symptoms, and to investigate its possible effects on eating behaviors and sleeping habits.

Material and Methods

Study center, sample

The files of 318 children who presented to the outpatient clinic at the Necmettin Erbakan University Meram Faculty of Medicine Department of Child and Adolescent Psychiatry and were diagnosed as having ASD according to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM 5)[24] between February 2018 and February 2019 were included in the study.

The inclusion criteria for the NAC group and control group at initial assessment were being between the ages of 3 and 6 years and recommended NAC supplementation for one or more of the following symptoms: very short attention span, hyperactivity, irritability, restlessness, and sleep or eating problems. The inclusion criteria in the NAC group at the second assessment were to use oral NAC 400-600 mg/day regularly for eight weeks. The inclusion criteria in the control group at the second assessment were children who were recommended NAC but whose family did not use NAC for various reasons. The exclusion criteria were as follows: receiving any other treatment other than individualized special education, current or past use of any psychotropic medication, any individualized special education program change, routine rating scales not completed or missing at the initial assessment, and follow-up, the presence of any additional chronic disease (e.g., epilepsy, neurometabolic disorder), a genetic syndrome associated with autism (e.g., fragile X syndrome, tuberous sclerosis), and active infectious diseases or obesity.

It was determined that 53 of the children met the inclusion and exclusion criteria and used oral NAC 400-600 mg/day regularly. Of the 53 children with ASD who used NAC regularly, 16 more files were excluded from the study; eight files due to incomplete screening scales in the 8th-week evaluation, six files due to additional antipsychotic medication, and two files due to additional methylphenidate treatment. Finally, the files of 37 children with ASD who received 400-600 mg/day NAC for 8 weeks, who had no changes in individualized special education programs during the study period, and who did not use any additional drugs/agents were included in the study. It was determined that 25 of the children met the inclusion and exclusion criteria for control group. Of the 25 children with ASD who were recommended NAC but whose family did not use NAC, 4 more files were excluded from study; three files due to incomplete screening scales in the 8th-week evaluation and one file due to use of additional antipsychotic medication.

It was determined that the included children used effervescent tablets containing NAC in two divided doses per day, children under 15 kg used 2x200 mg/day, and children above 15 kg 2x300 mg/day of NAC. In the outpatient clinic of the researcher, clinical rating scales are routinely used for tracking each child with ASD in terms of cardinal symptoms, behavior, speech, eating habits, and sleep monitoring. Of these scales, the Turkish Version of the Childhood Autism Rating Scale (CARS) was applied by the researcher, and the Aberrant Behavior Checklist (ABC), the Children Eating Behavior Questionnaire (CEBQ), and the Sleep Habits Questionnaire (CSHQ) was completed by the parents. Approval for the study was acquired from the ethics committee by the Ethics Committee of the Necmettin Erbakan University Meram Faculty of Medicine Non-Pharmaceutical and Medical Device Research on April 16th, 2021 (Decision No: 2021/3202).

Instruments and measures

Demographic data and clinical history

All data were retrieved from Sociodemographic and Clinical Information files.

Childhood Autism Rating Scale (CARS)

The validation and reliability study of CARS was carried out by Schopler et al.[25] CARS

comprises 15 items, and each of these items contributes equally to the calculation of the total score. Each of the 15 items is rated using half-point increments, ranging from 1 to 4. CARS is typically assessed based on information gathered from both family interviews and direct observations of the child by physicians. The items assessed in the CARS are as follows:: 1. interpersonal relationships, 2. imitation, 3. emotional response, 4. body use, 5. object use, 6. adaptation to change, 7. visual response, 8. listening response, 9. taste and smell responses, 10. use of touch, 11. fear/nervousness, 12. verbal communication, 13. nonverbal communication, 14. activity level, 15. level of intellectual response, and 16. general impressions. A total score in the range of 15-29 typically suggests that the child does not have autism. A score falling between 30-36.5 indicates mild-to-moderate autism. A score ranging from 37-60 is indicative of severe autism. The adaptation of CARS to the Turkish language was first performed by Sucuoglu et al.[26], followed by Gassaloğlu et al. who extended the validity and reliability analysis.[27] The Cronbach's alpha coefficient for the total score of the scale was determined to be 0.95.[27]

Aberrant Behavior Checklist (ABC)

ABC is rated by the parent (or primary caretaker) and confirmed by a physician. The ABC tool is employed to characterize and quantify behavioral challenges commonly observed in children diagnosed with ASD. ABC has 58 items that range from 0 = no problem at all, to 3 = the problem is of a significant or intense magnitude.[28] ABC items are categorized and scored into five different subscales, which are as follows (1) Irritability, agitation, crying; (2) Lethargy, social withdrawal; (3) Stereotypical behavior; (4) Hyperactivity/incompatibility; (5) Inappropriate speech. The Turkish adaptation and validity and reliability study was performed by Karabekiroğlu and Aman.[29] The Turkish version of the ABC demonstrated satisfactory internal consistency. The Cronbach's alpha values were calculated as follows: Irritability, 0.94; Lethargy/Social Withdrawal, 0.92; Stereotypic Behavior, 0.87; Hyperactivity, 0.65; and Inappropriate Speech, 0.87.[29]

Children's Eating Behavior Questionnaire (CEBQ)

The CEBQ was designed to classify children's eating behaviors, with a particular focus on identifying early signs related to obesity and eating disorders.[30] The Turkish adaptation, as well as the assessment of validity and reliability, were carried out by Yılmaz et al.[31] Cronbach's alpha coefficients ranged from 0.61 to 0.84.[31] The CEBQ consists of 35 items. The CEBQ is formed by eight sub-scales. These subscales are responsiveness to food, emotional overeating, enjoyment of food, desire for drinks, satiety responsiveness, slowness in eating, emotional undereating, and food fussiness. These subscales are collected in two groups under the headings 'positive eating responsive' and 'negative eating responsive.' The positive eating-responsive subscales include food responsiveness, enjoyment of food, emotional overeating, and desire to drink, and the negative eating-responsive subscales include satiety responsiveness, slowness in eating, emotional undereating, and food fussiness.

Children's Sleep Habits Questionnaire (CSHQ)

The CSHQ was developed by Owens et al. with the aim of assessing the typical sleep patterns and sleep-related issues in children between the ages of 4 and 10 years.[32] The CSHQ comprised of a total 33 items. There are eight subscales that screen sleep disorders in children according to the international sleep classification. The subscales in the scale are listed as bedtime resistance, sleep onset delay, sleep duration, sleep anxiety, night wakings, parasomnias, sleep-disordered breathing, and daytime sleepiness. A cumulative score of 41 points is established as the threshold, with scores surpassing this value regarded as 'clinically significant'. The validity and reliability study was performed by Perdahlı Fiş et al.[33]

The Cronbach's alpha coefficient for the internal consistency of the scale was found to be 0.78.[33]

Data analyses

Statistical analysis of the data was conducted using the Statistical Package for the Social Sciences, SPSS 21.0 software. The normality of the data was assessed through the utilization of the Kolmogorov-Smirnov test and an examination of Skewness and Kurtosis statistics. The Mann-Whitney U test and Spearman correlation tests were used for non-parametric data. Before and after analyses of the scales were compared using the paired-samples T-test or Wilcoxon signed rank test according to whether parametric or non-parametric test assumptions were met, respectively. P-values less than 0.05 were accepted for statistical significance except for the CARS items. The CARS items Bonferroni corrected significance level P-value was 0.002.

Results

When the NAC group and the control group were compared in terms of sociodemographic data, no statistical difference was found between the two groups. Sociodemographic data and clinical features are given in Table I.

When the NAC group and control group were compared according to the scale scores in the initial assessment, it was seen that there was no statistically significant difference between each group (Table II).

In the NAC group, the scale scores in the initial assessment and second assessment were compared. A statistically significant decrease was found in the children's CARS total score ($p<0.001$) and C1 (interpersonal relationships) ($p<0.001$), C4 (body use) ($p<0.001$), C8 (listening response) ($p<0.001$), and C11 (verbal communication) ($p<0.001$) items score (Table III). A statistically significant decrease was found in the ABC subscale irritability ($p=0.001$), social withdrawal ($p<0.001$), stereotypic behavior ($p<0.001$), hyperactivity ($p<0.001$), inappropriate speech ($p=0.006$), and total scores ($p<0.001$) (Table IV). In the CEBQ, only a statistically significant increase was found in the emotional undereating subscale score ($p=0.003$) (Table IV). In the CSHQ, only a statistically significant increase was found in the 'sleep onset delay' subscale score ($p=0.012$) (Table IV).

When the scale scores were compared between the NAC group and control group, a statistically significant decrease was found in the children's CARS total score ($p<0.001$) (Table V). Also, a statistically significant decrease was found in the ABC subscale irritability ($p=0.001$), stereotypic behavior ($p<0.001$), and hyperactivity ($p<0.001$) and, total score ($p= 0.010$) (Table V).

In this study, when the initial assessments and second assessments of the CEBQ subscale scores were compared in the NAC group, a significant increase in the 'emotional undereating' subscale scores was found ($p=0.003$) (Table III). The relationship of this subscale with other scales and subscales scores was examined, no relationship was found. Also, no significant difference was found when the NAC group was compared with the control group (Table V).

Table I. Demographic and clinical characteristics of children with autism.

	NAC group (n=37)	Control group (n=21)	p
Age (month), mean (SD)	59.81 (23.26)	64.24 (21.88)	0.48
Gender, male/ female	30 / 7	18 / 3	
Age of diagnosis (month), mean (SD)	27.68 (7.43)	26.24 (4.21)	0.35
Beginning of individualized special education training time (month), median (min-max)	26 (2-93)	34 (3-70)	0.39
NAC usage dose (mg/day), median (min-max)	500 (400-600)	-	-

NAC: N-aceylcysteine, SD: standard deviation.

Table II. Aberrant Behavior Checklist, Children's Eating Behavior Questionnaire and Children's Sleep Habits Questionnaire scores at initial assessment in NAC and control groups.

	NAC group (n=37) Mean (SD), Median (min-max)	Control group (n=21) Mean (SD), Median (min-max)	t or z	p
CARS Total	38.33 (4.53)	37.73 (2.34)	-0.333	0.73
ABC				
Total score	63.40 (34.43)	55.71 (15.80)	8.701	0.25
Irritability	15.32 (8.94)	16.19 (5.29)	5.632	0.64
Social withdrawal	16.00 (0 - 43)	14.00 (2 - 23)	-1.648	0.10
Stereotypic behavior	10 (1-19)	7.00 (0 - 15)	-1.709	0.09
Hyperactivity	16 (1-38)	11 (3-35)	-1.269	0.21
Inappropriate Speech	4 (0-12)	5 (0-9)	-1.062	0.28
CEBQ				
Food responsiveness	8 (5-24)	6 (5-22)	-0.973	0.33
Emotional overeating	5 (4-20)	5 (1-9)	-0.797	0.42
Enjoyment of food	15(5-25)	15 (11-20)	-0.122	0.90
Desire to drink	8 (3-14)	7 (2-10)	-0.984	0.32
Satiety responsiveness	21 (10-32)	16 (10-28)	-1.581	0.11
Slowness in eating	9 (4-20)	7 (5-18)	-0.952	0.34
Emotional undereating	9 (4-18)	7 (2-15)	-1.352	0.17
Food fussiness	7 (3-14)	7 (3-14)	-0.824	0.41
CSHQ				
Bedtime resistance	13 (9-16)	11 (9-16)	-1.614	0.10
Sleep onset delay	3 (1-3)	2 (1-3)	-1.662	0.09
Sleep duration	7 (5-9)	6 (5-8)	-1.890	0.059
Sleep anxiety	8 (4-12)	7 (4-12)	-0.838	0.40
Night wakings	4 (3-8)	5 (3-7)	-0.083	0.93
Parasomnias	9 (7-20)	8 (2-12)	-1.744	0.08
Sleep disordered breathing	3 (3-8)	3 (0-6)	-0.325	0.74
Daytime sleepiness	12 (10-19)	12 (10-17)	-0.534	0.59

ABC: Aberrant Behavior Checklist, CEBQ: Children's Eating Behavior Questionnaire, CSHQ: Children's Sleep Habits Questionnaire, NAC: N-acetylcysteine, SD: standard deviation.

Table III. Childhood Autism Rating Scale scores at initial and second assessment in NAC group.

	Initial assessment (NAC group) Mean (SD)	Second assessment (NAC group) Mean (SD)	t	p
CARS Total	38.33 (4.53)	33.21 (4.61)	11.298	<0.001
C1 (interpersonal relationships)	3.01 (0.32)	2.24 (0.48)	-4.850	<0.001
C4 (body use)	2.82 (0.37)	2.33 (0.45)	-4.225	<0.001
C8 (listening response)	2.62 (0.47)	2.29 (0.47)	-3.592	<0.001
C11 (verbal communication)	2.74 (0.56)	2.28 (0.57)	-3.756	<0.001

CARS: Childhood Autism Rating Scale, NAC: N-acetylcysteine, SD: standard deviation.

Table IV. Aberrant Behavior Checklist, Children's Eating Behavior Questionnaire and Children's Sleep Habits Questionnaire scores at initial and second assessment in NAC group.

	Initial assessment (n=37) Mean (SD) Median (min-max)	Second assessment (n=37) Mean (SD) Median (min-max)	t or z	p
ABC				
Total score	63.40 (34.43)	44.32 (22.08)	4.382	<0.001
Irritability	15.32 (8.94)	11.13 (5.62)	3.641	0.001
Social withdrawal	16.00 (0 - 43)	10.97 (6.37)	4.471	<0.001
Stereotypic behavior	10 (1-19)	6 (0-17)	-3.672	<0.001
Hyperactivity	16 (1-38)	12 (1-22)	-3.551	<0.001
Inappropriate Speech	4 (0-12)	3 (0-9)	2.951	0.006
CEBQ				
Food responsiveness	8 (5-24)	9 (5-25)	-1.032	0.30
Emotional overeating	5 (4-20)	5 (4-20)	-0.284	0.77
Enjoyment of food	15(5-25)	16 (6-25)	-0.777	0.44
Desire to drink	8 (3-14)	7 (3-14)	-0.312	0.75
Satiety responsiveness	20.52 (6.99)	20.36 (6.02)	0.295	0.76
Slowness in eating	9 (4-20)	9.50 (4-20)	-0.169	0.86
Emotional undereating	9 (4-18)	9 (4-17)	-2.990	0.003
Food fussiness	7 (3-14)	6.50 (3-13)	-0.035	0.97
CSHQ				
Bedtime resistance	13 (9-16)	13 (10-15)	-0.264	0.79
Sleep onset delay	3 (1-3)	3 (1-3)	-2.500	0.012
Sleep duration	7 (5-9)	7 (5-8)	-0.546	0.58
Sleep anxiety	8 (4-12)	8 (4-12)	0.437	0.66
Night wakings	4 (3-8)	4.50 (3-8)	-0.484	0.62
Parasomnias	9 (7-20)	9 (7-15)	-0.733	0.46
Sleep disordered breathing	3 (3-8)	3 (3-9)	-0.000	1.00
Daytime sleepiness	12 (10-19)	12.5 (10-18)	-1.064	0.28

ABC: Aberrant Behavior Checklist, CEBQ: Children's Eating Behavior Questionnaire, CSHQ: Children's Sleep Habits Questionnaire, NAC: N-acetylcysteine, SD: standard deviation.

In the CSHQ, only a statistically significant increase was found in the 'sleep onset delay' subscale score (p=0.012) (Table III). When the relationship of this subscale with other scales and subscales scores was examined no relationship was found and, no significant difference was found when the NAC group was compared with the control group (Table V).

This study included only children whose special education conditions did not change during NAC use. However, we have seen that the total duration of special education hours that children with autism receive per month is not standard (duration of individualized special education training time, 26 hours/month (min-max 2-93). We analyzed whether there was any relationship between the total hours of special education the children received and our scale scores (CARS, ABC, CEBQ, and CSHQ) in the first and second evaluations. The study results indicated that there was no statistically significant correlation between the length of time a child received individualized special education and the outcomes assessed using our measurement scales.

Table V. Aberrant Behavior Checklist, Children's Eating Behavior Questionnaire, and Children's Sleep Habits Questionnaire scores at second assessment in NAC and Control groups.

	NAC group (n=37) Mean (SD) Median (min-max)	Control group NAC (n=21) Mean (SD) Median (min-max)	t or z	p
CARS Total	33.21 (4.61)	36.66 (2.22)	4.940	<0.001
ABC				
Total score	44.32 (22.08)	57.33 (14.86)	4.124	0.010
Irritability	11.13 (5.62)	15.09 (4.25)	1.754	0.007
Social withdrawal	10.97 (6.37)	14.00 (4.69)	1.900	0.063
Stereotypic behavior	6 (0-17)	8 (0-17)	-2.056	0.040
Hyperactivity	12.08 (6.93)	16.38 (7.55)	2.182	0.033
Inappropriate Speech	3.72 (2.11)	3.90 (2.49)	0.733	0.733
CEBQ				
Food responsiveness	9 (5-25)	10 (5-23)	-1.482	0.13
Emotional overeating	5 (4-20)	6 (1-17)	-0.431	0.66
Enjoyment of food	16 (6-25)	15 (12-20)	-0.233	0.81
Desire to drink	7 (3-14)	7 (2-12)	-0.548	0.58
Satiety responsiveness	20.50 (11-35)	20 (10-29))	-1.037	0.30
Slowness in eating	9.50 (4-20)	9.40 (5-18)	-1.097	0.27
Emotional undereating	9 (4-17)	8 (3-15)	-1.516	0.13
Food fussiness	6.50 (3-13)	6 (3-12)	-0.917	0.35
CSHQ				
Bedtime resistance	13 (10-15)	12 (9-14)	-1.428	0.15
Sleep onset delay	3 (1-3)	2 (1-3)	-0.893	0.37
Sleep duration	7 (5-8)	7 (5-8)	-1.021	0.30
Sleep anxiety	8 (4-12)	8 (4-12)	-0.336	0.73
Night wakings	4.50 (3-8)	4.70 (3-6)	-0.067	0.94
Parasomnias	9 (7-15)	7 (2-13)	-2.238	0.25
Sleep disordered breathing	3 (3-9)	3 (0-5)	-0.196	0.84

ABC: Aberrant Behavior Checklist, CEBQ: Children's Eating Behavior Questionnaire, CSHQ: Children's Sleep Habits Questionnaire, NAC: N-acetylcysteine, SD: standard deviation.

Discussion

This was a retrospective file review study researching the effects of oral NAC on autism symptoms, problem behaviors, eating behaviors, and sleep habits. The study compared children with autism aged 3-6 years whose parents reported that they used oral NAC for at least 8 weeks, and children with autism who were recommended oral NAC but did not use NAC for any reason.

When the results of our study were reviewed regarding the difference between the first and the second assessments in the NAC group, it was found that there was a statistically significant progression in interpersonal relationships, body use, listening response, and verbal communication scores and total score of CARS. Also, there was a statistically significant progression in all subscales scores and total scores of ABC. When the results about the difference between the NAC group and the control group, it was shown that there

was a progression in the total score of CARS and, there was a progression in the irritability, stereotypic behavior, and hyperactivity subscales scores and total score of ABC. In addition, it was determined that there was an increase in 'emotional undereating' in terms of eating behaviors, and a rise in the 'sleep onset delay' in terms of sleep habits. However, no significant difference was found when the NAC group was compared with the control group.

When the studies were reviewed in this area, in a randomized, placebo-controlled, double-blind study researching the effects of NAC in children with ASD, ABC, the Repetitive Behavior Scale-Revised, and the Social Responsiveness Scale were evaluated at baseline, 4th, 8th, and 12th weeks. It was reported that there were 14 children in the NAC group and 15 children in the placebo group aged 3-10 years in which oral NAC had few adverse effects and was well tolerated, and the ABC irritability subscale improved remarkably in the NAC group compared with placebo.[34] In our study, a substantial improvement was found in all of the ABC scale's total scores and subscale scores. In our study, the average age of the participants was 4.5 years, and a more homogeneous group was formed in our study in terms of the age range. However, investigator or parent bias could not be controlled due to the design of our study. Nevertheless, these results suggested that the effect of NAC use at younger ages on autism symptoms should be examined in more detail to benefit from NAC.

In another randomized, placebo-controlled double-blind research examining the augmentation of NAC in risperidone treatment, the effect of using 2x600 mg/day NAC + risperidone for 8 weeks was investigated using the ABC scale. Upon concluding the study, it was found that risperidone + NAC reduced irritability more than risperidone + placebo. It has been reported that the adverse effects of NAC are not common and are generally well tolerated, but do not alter the core symptoms of autism.[35] In another study by Nikoo et al., it was reported that there was a meaningful improvement in the irritability and hyperactivity/incompatibility subscale scores of the ABC scale and that NAC could be considered an adjuvant treatment in the treatment of autism.[36] In another randomized, placebo-controlled, double-blind, 12-week follow-up research involving 31 children (aged 4-12 years) with autism that evaluated the effectiveness, safety, and tolerability of NAC, the effectiveness of NAC was demonstrated using Clinical Global Impression (CGI) and venous blood samples were collected at baseline and at 12 weeks to explore the effect of NAC on markers of oxidative stress in blood. At the conclusion of the study, it was reported that NAC therapy was well tolerated and had the expected effect in increasing reduced form glutathione (GSH) output, but had no discernable effect on social difficulties in children with ASD.[37]

When all these studies were reviewed, in four studies, except for the study by Wink et al., it was observed that irritability scores on the ABC scale improved. In our research, it was found that there was a statistically significant advancement in interpersonal relationships, body use, listening response, and verbal communication item scores and total score of CARS, and there was a statistically significant progression in all subscale scores and total score of ABC. In our study, it was found that the irritability score, which was 14.15 (SD 9.15) at baseline, decreased to 10.23 (SD 6.21) in the 8th week. The CGI-I scale is a superficial assessment tool compared with CARS, where improvement is scored based on the physician's observation. In our study, autism symptoms were compared using CARS, enabling us to make a more detailed and autism-specific assessment in terms of comparison of change. However, the interpretation of the results should be made in the terms of the limitations of the study. The most obvious limitation of our study is the retrospective review of data and

the absence of a placebo-controlled comparison group. Although this situation makes it difficult to generalize the results of the study to all children with autism, it is thought that it will be more appropriate to use evaluation tools that screen symptoms in detail when investigating the effect of NAC on autism symptoms in future studies.

In our study, the possible effect of oral NAC on the eating behavior of children with autism was also examined. It has been reported that children with autism are extremely selective in their eating habits.[38-40] In a meta-analysis, it was reported that children with autism had a 5-times greater risk of nutritional problems compared with children without autism, and lack of nutritional diversity put individuals at risk for nutritional deficiencies.[40] Although food selectivity is considered to be associated with sensory hypersensitivity, the exact reason is unknown.[41] In a recent study investigating emotional eating behavior in autism, it was reported that children with autism were more prone to emotional overeating and emotional undereating behavior compared with their typically developing peers.[42] Therefore, in our study, it was planned to examine the possible effect of NAC on eating behaviors. In our research, a notable increase in scores was found in the emotional undereating subscale. However, no significant difference was found when the NAC group was compared with the control group. To the best of our knowledge, there has been no study that has specifically investigated the connection between NAC and eating behaviors. It is therefore important to investigate the relationship between NAC and emotional undereating in future studies to establish a cause-effect relationship.

In our study, we also explored the impact of oral NAC on the sleep patterns of children with autism. It is known that children with autism experience sleep disorders, especially insomnia, at much higher rates than the typical population, and the etiopathogenesis of this condition has not been fully elucidated.[43] A study reported that NAC had an effect on the central processes associated with obstructive sleep apnea and positively affects sleep[23], which led us to investigate the possible effect of NAC on the relationship between sleep and autism. When the literature was reviewed, no study was found that explored the relationship between NAC and sleep patterns in autism. In the present study, it was determined that children with autism in the NAC group fell asleep significantly later in their second evaluation compared with their first evaluation. Considering that there is no previous study in this area, it is thought that research with larger samples is needed to enlighten whether the prolongation of time to fall asleep was associated with the NAC group. However, no significant difference was found when the NAC group was compared with the control group.

The strongest aspect of our study is that the effects of oral NAC use on eating behaviors and sleeping habits in autism were also investigated. As far as we know, there is no study investigating the effects of NAC on the eating behaviors and sleep habits of children with autism. Another strength of our study is the indication of the severity of autism symptoms using CARS in the evaluations and the comparison between the control group. Previous studies were conducted in a wider age range in this area. In our study, a more homogeneous group was formed by including only children with autism in the 3-6 years age group because autism is an early-onset disorder, and oxidative stress, which can increase in response to both environmental and genetic factors, is considered to be the trigger of various diseases.[5,6] Compared with other animals, human neuronal development continues to progress rapidly after birth, increasing the impact of environmental factors on neuronal development.[44] Therefore, it is important to uncover the possible role of environmental agents in autism at younger ages because many environmental agents can be modified, regulated, and configured.

The most essential limitations of this study were having a retrospective chart review design, no placebo control group, and including only children who attended a tertiary psychiatric clinic. These limitations prevent generalizing the findings to all children with autism. Also, using self-report scales involve disadvantages such as presuming that parents understand the evaluation method and include bias in the responses. Follow-up studies on a larger sample will help enhance the levelevidence.

As a result, our findings suggested that oral NAC might reduce cardinal autistic symptoms and problematic behaviors. It is known that the emotional difficulties of children with autism are associated with behavioral problems and social difficulties, and also negatively affect vegetative symptoms such as sleep and eating patterns. Therefore, based on our findings, it was thought that the effect of NAC on the cardinal symptoms of autism should be examined in more detail in future studies. Aside from its limitations, this study offers important data for future studies in this field. NAC may be considered an adjuvant therapy with helpful therapeutic results for preschool children with autism. Larger samples, randomized controlled, and longer follow-up studies are needed to research the possible effects of NAC on autism.

Ethical approval

This research was approved by the Ethics Committee of the Necmettin Erbakan University Meram Faculty of Medicine Non-Pharmaceutical and Medical Device Research on April 16th, 2021 (Decision No: 2021/3202).

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: KN, SE; data collection: SE; analysis and interpretation of results: KN, SE; draft manuscript preparation: KN, SE. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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