

Impact of Auditory Integrative Training on Transforming Growth Factor- β_1 and Its Effect on Behavioral and Social Emotions in Children with Autism Spectrum Disorder

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Significance of the Study

- This study investigated the impact of auditory integrative training (AIT) on transforming growth factor (TGF)- β_1 and its effect on behavioral and social emotions in children with autism spectrum disorder (ASD). The increased plasma levels of TGF- β_1 after AIT support the therapeutic effect of AIT on TGF- β_1 followed by improvement in social awareness, social cognition, and social communication in ASD children. TGF- β_1 may potentially serve as a predictive biomarker of clinical symptoms of ASD and therapeutic efficacy.

Keywords

Autism spectrum disorder · Short Sensory Profile · Transforming growth factor- β_1 · Childhood Autism Rating Scale · Social Responsiveness Scale

Abstract

Objective: To explore the impact of auditory integrative training (AIT) on the inflammatory biomarker transforming growth factor (TGF)- β_1 and to assess its effect on social behavior in children with autism spectrum disorder (ASD). **Subjects and Methods:** In this cross-sectional study, 15 patients (14 males and 1 female) with ASD aged 3–12 years were recruited. All were screened for autism using the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV). Plasma levels of TGF- β_1 were measured in all patients using a sandwich enzyme-linked immunoassay (ELISA) immediately and 1 and 3 months after the AIT sessions. Pre- and post-AIT behavioral scores were also

calculated for each child using the Childhood Autism Rating Scale (CARS), the Social Responsiveness Scale (SRS), and the Short Sensory Profile (SSP). Data were analyzed using the Statistical Package for the Social Sciences (SPSS 21.0 for Windows). **Results:** Plasma levels of TGF- β_1 significantly increased to 85% immediately after AIT (20.13 ± 12 ng/mL, $p < 0.05$), to 95% 1 month after AIT (21.2 ± 11 ng/mL, $p < 0.01$), and to 105% 3 months after AIT (22.25 ± 16 ng/mL, $p < 0.01$) compared to before AIT (10.85 ± 8 ng/mL). Results also revealed that behavioral rating scales (CARS, SRS, and SSP) improved in terms of disease severity after AIT. **Conclusion:** Increased plasma levels of TGF- β_1 support the therapeutic effect of AIT on TGF- β_1 followed by improvement in social awareness, social cognition, and social communication in children with ASD. Furthermore, TGF- β_1 was associated with severity in all scores tested (CARS, SRS, and SSP); if confirmed in studies with larger sample sizes, TGF- β_1 may be considered as a marker of ASD severity and to assess the efficacy of therapeutic interventions.

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Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by impairments of social interactions, repetitive behavior, and sensory abnormalities [1] with various levels of severity occurring before 3 years of age. ASD is an important cause of childhood disability that imposes significant burden on the parents and society [2].

Although the exact cause of this disorder remains poorly understood, immunological factors have been suggested to have a major role in its pathophysiology [3]. Several candidate molecules are emerging as promising biomarkers of autism and may pave the way to better biological understanding of this condition. It is also possible that the identification of reliable and robust biomarkers may facilitate early diagnosis and personalized treatment, and improve outcome of patients with autism [4].

Several ASD screening and diagnostic procedures have been developed, including the Childhood Autism Rating Scale (CARS) [5], Social Responsiveness Scale (SRS) [6], and Short Sensory Profile (SSP) [7]. According to several recent theories, sensory processing and integration abnormalities may play important roles in impairments of perception, cognition, and behavior in patients with autism. Among these sensory abnormalities, distortion of auditory perception could contribute to many typical symptoms of autism [8]. Impairment in sensory processing has been reported in 42–88% of children with autism; however, observational research examining the existence of sensory processing dysfunction in autistic children is rare. Furthermore, little attention has been given to research on the relationship between sensory processing dysfunction and biomarkers that are measured in autistic patients [4].

Early intervention has been shown to improve the prognosis of children with ASD [9], but the most beneficial method of intervention remains unclear [10]. Tapping into the auditory strengths and preferences of children with ASD may well lead to an improved option for early language intervention as audition bears great significance in the acquisition of verbal speech, a lingering challenge for about 30% of children with ASD [11].

Auditory integrative training (AIT) was developed as a technique for improving abnormal sound sensitivity in individuals with behavioral disorders including autism [12]. Berard [13] suggested that the abnormal sensitivity or insensitivity to certain sound wave frequencies, regardless of overall hearing ability, is associated with a range of behavior and learning problems, and that

AIT would bring about a “reeducation” of the hearing process.

A number of studies suggest that AIT plays a crucial role in social behavior and that it could greatly improve language disorders, difficulties in social interactions, typical behavior symptoms, and developmental levels [8, 14]. These studies report significant improvements in behavior and severity of autism in terms of verbal and IQ performance 3–12 months after an intervention. Furthermore, several studies have confirmed the effects of AIT on social communication and interaction in ASD [8, 12]. Russo et al. [15] also assessed the impact of auditory training on auditory function, and identified biological changes, including brainstem response timing, pitch tracking, and cortical response timing in children with ASD.

TGF- β_1 is an anti-inflammatory cytokine that performs many cellular functions, including the control of cell growth, cell proliferation, cell differentiation, and apoptosis [16]. TGF- β_1 has been found to play a crucial role in early central nervous system (CNS) development [17] and in the control of inflammatory and immune responses [18]; however, it can worsen brain inflammation when it is overexpressed in the brain [19]. At the same time, it is believed that TGF- β_1 protects the brain from neuronal degeneration during CNS inflammation [20].

Several studies have demonstrated altered TGF- β_1 levels in the brain and serum of autistic patients [21, 22]. Hashim et al. [23] found that TGF- β_1 levels were significantly decreased in the plasma of ASD children in comparison to controls. Similar results were reported by other studies in patients with ASD [22, 24].

Considering the key role of TGF- β_1 in brain development [17], it is of great interest to study the role of TGF- β_1 in the pathophysiology of autism. Therefore, the aim of this study was to test the possible effects of AIT on TGF- β_1 and the association between plasma TGF- β_1 levels and the severity of social and cognitive dysfunction in ASD children.

Methods

Participants

Subjects for this study were recruited from the Autism Research and Treatment Centre at the King Saud University, King Khalid University Hospital Riyadh, Kingdom of Saudi Arabia. Fifteen ASD subjects, 1 girl and 14 boys (ranging in age from 3 to 12 years), were enrolled in the study. All subjects were screened and assessed using the Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV. Scores were calculated before and after intervention (immediately and 1 and 3 months after AIT) for each child using CARS, SRS, and SSP. AIT was performed over 2 weeks, for a duration of 30 min, twice a day with a 3-h interval between sessions.

Children with a history of seizure were excluded from the study. Written consent was obtained from the parents of each subject, according to the guidelines of the Ethics Committee of the King Saud University King Khalid Hospital. During the study period, children were not allowed to begin any new therapies or stop any current therapies, including medications and supplements. Ethical approval was obtained for the study by the Institutional Review Board of the College of Medicine, King Saud University.

Childhood Autism Rating Scale

The CARS score was measured as a scale for autism severity. CARS assesses the child on a scale from 1 to 4 in each of 15 dimensions or symptoms (including the ability to relate to people, emotional response, imitation, body use, object use, listening response, fear or nervousness, verbal communication, nonverbal communication, activity level, level and reliability of intellectual response, adaptation to changes, visual response, taste, smell and touch responses and general impressions). A total score of at least 30 strongly suggests the presence of autism. Children who score between 30 and 36 have mild-to-moderate autism while those with scores between 37 and 60 have severe autism [25].

Social Responsiveness Scale

The SRS is a validated test of interpersonal behavior, communication, and stereotypical traits in autism [6, 26]. It is used as a diagnostic tool, distinguishing clinically significant ASD from varying levels of social impairment in other psychiatric disorders. It consists of 5 subscales: (1) social awareness, (2) social cognition, (3) social communication, (4) social motivation, and (5) autistic mannerisms. Total SRS raw scores range from 0 to 195, corresponding to significant social impairment as observed in individuals with ASD. A score of 76 or higher is considered severe and is strongly associated with a clinical diagnosis of autistic disorder. A score between 60 and 75 is in the mild-to-moderate range of social impairment [26].

The Short Sensory Profile

The SSP is a 38-item questionnaire designed for children aged 3–14 years; it provides quick information about the sensory processing skills of autistic children [4]. Each item on the SSP is measured on a 5-point Likert scale. Domain scores were measured in the areas of tactile, taste/smell, and movement sensitivity, seeking sensation, auditory filtering, low energy levels, and visual/auditory sensitivity. Domain scores and overall sensory responses were categorized as typical performance, probable difference from typical performance, or definite difference from typical performance. Scores less than 142 indicate severe performance (definite difference from typical performance), scores between 142 and 152 indicate mild-to-moderate performance (probable difference from typical performance), and scores between 153 and 190 indicate typical performance. The SSP has been used in many studies [7].

Auditory Integration Training

AIT was conducted according to a published protocol [13] and previously used by our group [27]. Subjects were first examined by a medical doctor to ensure that no excessive wax and/or fluid are present. The listener received 18–20 listening sessions lasting 30 min, over a 10- to 20-day period in most cases, and had a 1- or 2-day break after 5 days of listening. During the listening sessions, the child listened to processed music. That is, the AIT sound am-

plifier attenuated low and high frequencies at random from the compact disks and then sent this modified music through headphones to the listener. The intensity level (volume) during the AIT listening sessions did not exceed 80 dBA (low scale) and was set at much lower intensities depending on the individual's comfort level. Overall, the music was played at a moderately loud, but not uncomfortable, level. The 80-dBA level for a total of 1 h per day is well below the Occupational Safety and Health Act (OSHA) guidelines for nonhazardous noise levels. The OSHA Noise Standard permits exposure to an average noise exposure of 85 dBA for 8 continuous hours. Audiograms were obtained prior to, at the midpoint, and at the completion of the AIT listening session. The first and the midpoint audiograms were used to set filters on the AIT machines. These filters are used to dampen (40 dBA or more) those frequencies which the person hears too acutely (i.e., peaks).

Blood Sample Collection

After overnight fasting, a 3-mL blood sample was collected from each child in test tubes containing EDTA. Blood samples were immediately centrifuged at 3,000 rpm to collect plasma, which was then stored in a freezer at -80°C until analysis. All samples were assayed in duplicate and in a double-blind manner. Assay reproducibility error ranged generally from 5 to 10%.

TGF- β_1 concentrations were measured in the plasma of autistic subjects using a commercially available sandwich ELISA kit (Cusabio Biotech Co. Ltd., Wuhan, China). All biochemical analyses were performed in duplicate, and mean values were reported. No significant cross-reactivity or interference was observed.

Statistical Analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS 21.0 for Windows; SPSS, Chicago, IL, USA). Results are expressed as means \pm SD. Significant changes in the parameters measured were assessed with repeated-measure analysis of variance. Bonferroni multiple comparisons were also used to assess significant differences. The Pearson correlation coefficient was employed to determine correlations between TGF- β_1 levels before and after AIT. A value of $p < 0.05$ was considered significant.

Results

The changes in TGF- β_1 levels (means \pm SD) and the scores of the 3 behavioral rating scales (CARS, SRS, and SSP) before and immediately and 1 and 3 months after AIT are listed in Table 1. Plasma levels of TGF- β_1 significantly increased by 85% immediately after AIT (20.13 ± 12 ng/mL, $p < 0.05$), by 95% 1 month after AIT (21.2 ± 11 ng/mL, $p < 0.01$), and by 105% 3 months after AIT (22.25 ± 16 ng/mL, $p < 0.01$) compared to before AIT (10.85 ± 8). Scores of CARS, an indicator of autism severity, were decreased by 17% 1 month after AIT ($p < 0.05$) compared to before AIT. SRS total score significantly decreased (16%) and SSP total score increased (14%) 3 months after AIT ($p < 0.05$). The significant difference ($p = <0.01$) in SRS scores between 1 and 3 months after

Table 1. Effect of auditory integrative training (AIT) on transforming growth factor (TGF)- β_1 and social behavioral scales in children with autism ($n = 15$)

Variable	Before AIT	Immediately after AIT	1 month after AIT	3 months after AIT	Difference
TGF- β_1 , ng/mL	10.85 \pm 8	20.13 \pm 12	21.2 \pm 11	22.25 \pm 16	1 \neq 2*, 1 \neq 3**
CARS	38.4 \pm 8	-	31.7 \pm 5	31.5 \pm 7	1 \neq 3*
SRS	179 \pm 23	-	176 \pm 23	150 \pm 27	1 \neq 4*, 3 \neq 4**
SSP	136 \pm 22	-	150 \pm 26	155 \pm 24	1 \neq 4*

Means \pm SD. CARS, Childhood Autism Rating Scale; SRS, Social Responsiveness Scale; SSP, Short Sensory Profile. * $p < 0.05$; ** $p < 0.01$: before (1), immediately (2), and 1 month (3) and 3 months after AIT (4).

Table 2. Pearson's correlation (r) between transforming growth factor (TGF)- β_1 before and after AIT

TGF- β_1	Before AIT	Immediately after AIT	1 month after AIT	3 months after AIT
Before AIT	1	0.65*	0.74**	0.591*
Immediately after AIT	0.65*	1	0.50	0.414
1 month after AIT	0.74**	0.50	1	0.514
3 months after AIT	0.59*	0.41	0.51	1

* $p < 0.05$; ** $p < 0.01$.

AIT also confirms the continuous improvement in SRS behaviors with time and duration of AIT intervention. Results revealed that these behavioral rating scales (CARS, SRS, and SSP) improved in terms of disease severity after AIT sessions. Changes in the 3 behavioral rating scales (SRS, CARS, and SSP) after AIT are shown in Figure 1.

Pearson correlation (r) values between TGF- β_1 levels before and after AIT are recorded in Table 2, showing strong and significant correlations between TGF- β_1 levels before AIT and immediately and 1 and 3 months after AIT.

Discussion

The findings of this study show a significant increase in plasma levels of TGF- β_1 and improvement in some aspects of ASD behaviors. This was demonstrated by significant changes in CARS, SRS, and SSP scores immediately and 1 and 3 months after versus before AIT sessions. Higher levels of TGF- β_1 and the lower scores of CARS and SRS indicate less severity of autism. ASD is a complex neurodevelopmental behavioral disorder with onset age

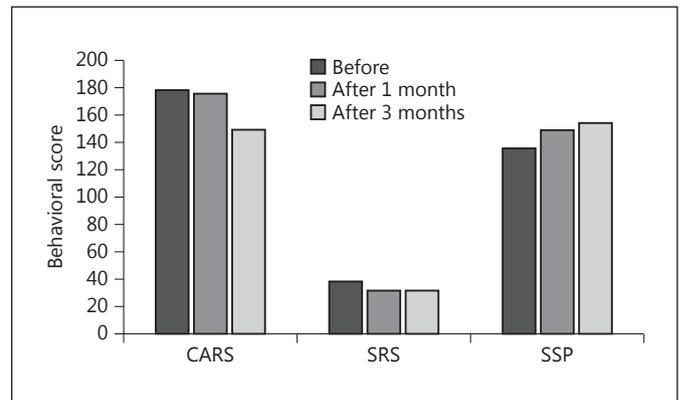


Fig. 1. Changes in the Childhood Autism Rating Scale (CARS), Social Responsiveness Scale (SRS), and the Short Sensory Profile (SSP) after auditory integrative training (AIT) in 3 different periods of time.

prior to 3 years [1]. While there are no concrete biological markers for this disorder, immune anomalies are frequently described among individuals with ASD [3].

Interaction between speech and language systems is severely compromised in ASD. Sensory dysfunction is a

common finding in ASD, including tactile sensation, smell, taste, visual, and auditory stimulation. Hypersensitivity to sensory stimuli is considered a disturbing feature in autism, especially hypersensitivity to auditory stimuli. This leads to communication difficulties which result in social isolation and consequently in difficulties in rehabilitation and learning [1]. AIT involves listening to music that has been computer modified to remove frequencies to which an individual demonstrates hypersensitivities and to reduce the predictability of auditory patterns. This treatment has been proposed to improve abnormal sound sensitivity in individuals with behavioral disorders, including ASD [13]. There is controversy in the literature regarding the effectiveness of AIT in reducing the auditory hypersensitivity. A Cochrane review was conducted with the objective to determine the effectiveness of AIT or other sound therapy methods in individuals with ASD [28]. Three out of 6 trials reported improvements after 3 months of AIT using the Aberrant Behavior Checklist (ABC) [29]. Results of the study conducted by our group [27] also supported previous studies suggesting that AIT improved behavior of ASD individuals [29, 30].

Our findings lead us to suggest that increased levels of TGF- β_1 following AIT in children with ASD may be implicated in the pathophysiology of autism although the result does not necessarily indicate causation. Furthermore, it is also of interest to measure plasma levels of TGF- β_1 in children without autism after AIT in order to determine the role of TGF- β_1 as a serological marker for children with ASD.

It is possible that high TGF- β_1 levels may result in immune regulation after AIT and thus possibly improve symptoms and behaviors associated with ASD. Furthermore, peripheral immune markers may reflect biological factors that could affect behavior in ASD children; however, further work is necessary to study the precise role of TGF- β_1 and how AIT is specifically linked to core autism. As a major role of TGF- β_1 is to control inflammation [18], the positive correlations observed between TGF- β_1 levels and AIT may suggest that there is decreased inflammation in children who exhibit improved behavioral scores. Further investigation is warranted on the use of TGF- β_1 as a serological marker in children who have recently been diagnosed with ASD, as well as its use as a biological marker to monitor potential efficacies of therapies that target behavioral outcome.

Several behavioral studies [8, 12, 14] have demonstrated the effects AIT on deficits in social communication and interaction in ASD and significant improvements in behavior and severity in autistic patients, but the effects

of AIT on biochemical markers have not been studied in ASD. Depino et al. [31] described a central role of TGF- β_1 in the programming and modulation of social interaction and repetitive and depression-related behavior. They also suggested a role for TGF- β_1 and early-life neuroinflammation in the development of behavioral alterations observed in ASD patients. These reports suggest that immune system aberrations may lead to abnormal immune responses, autoimmunity, or adverse neuroimmune interactions during brain development.

Given the key role of TGF- β_1 in brain development and inflammation, serum levels of TGF- β_1 were reported to be significantly lower in autistics than in age- and gender-matched controls [21, 23]. The reduced TGF- β_1 levels may lead to inappropriate regulation of immune responses as well as the development of neuroinflammation in ASD. However, the mechanisms underlying these processes have not been elucidated.

Our TGF- β_1 levels found before AIT intervention are similar to those reported earlier [21, 23] in typically developing children with autism, which significantly increased up to 105% 3 months after AIT with improvement in social behavioral functions. Taken together, these findings suggest that TGF- β_1 may play a role in the pathophysiology of ASD, although further work is needed to confirm these reports. It is further recommended to measure plasma levels of proinflammatory cytokines, such as IL-6 and TNF, that have actions opposite of TGF- β_1 to ascertain whether AIT is associated with changes in TNF as well as IL-6, and thus the proinflammatory milieu is changed to an anti-inflammatory status in ASD patients following treatment.

One of the potential limitations of the present study is the small sample size; this may have resulted in specific effects of AIT on TGF- β_1 being missed because of lack of statistical power to detect significant changes between the TGF- β_1 and behavioral scores (CARS, SRS, and SSP). We measured plasma TGF- β_1 levels before and after AIT, which might not accurately reflect levels in the cerebrospinal fluid or in brain regions, whereas cytokines readily cross the blood-brain barrier, suggesting that plasma levels should correlate well with cerebrospinal fluid levels [32]. However, a disrupted blood-brain barrier has been demonstrated in autism [33]. The data from the present study pave the way for a larger, more focused study on a wider range of cytokines. A further potential limitation of the present study is that the exact mechanism of action of AIT remains to be elucidated. Finally, an additional potential limitation of the present study is the fact that the duration of AIT used may not have been optimal.

These findings suggest that AIT could clinically alleviate an ASD core symptom about social reciprocity with enhancement of brain activity and functional coordination in ASD children. The current results may have a significant influence on future biological studies and clinical trials of AIT effects on children with ASD. We proposed that exposure to AIT sessions would result in improved behavioral evaluation scores and positively influence TGF- β_1 levels in autistic children. However, these data should be treated with caution until further investigations are performed in a larger study cohort, to determine whether the increase in TGF- β_1 levels is a mere consequence of autism or has a pathogenic role in the disease.

Conclusions

Our findings provide evidence for altered TGF- β_1 protein levels in subjects with ASD, which may contribute to the early pathogenesis of ASD, serve as a valuable biomarker, and could be even predictive for the ameliorative effects of the treatment, as studied here. Furthermore, in children with ASD there were correlations between TGF- β_1 levels before and after AIT. This finding suggests that inflammatory responses may be linked to AIT. Over-

all, the results of this study support the therapeutic effect of AIT resulting in increased TGF- β_1 plasma levels and improvements in clinical ASD severity scores (CARS, SRS, and SSP).

Future studies in larger sample sizes including TGF- β_1 determinations in normally developing children (controls) are strongly recommended to assess the exact beneficial effect of AIT and to ensure the greatest level of validity and reliability.

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Disclosure Statement

The authors have no conflict of interest.

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