DEVELOPMENTAL CHANGES IN CHILDREN WITH AUTISM SPECTRUM DISORDER FOLLOWING HIGH-SELENIUM FUNCTIONAL FOOD INTERVENTION: A ONE-GROUP PRETEST-POSTTEST STUDY

Neny Triana[1], Suwoyo[2], Sutiyah Heni[3]
[1,3]STIKES Karya Husada Kediri, East Java, indonesia
[2]Poltekkes Kemenkes Malang, East Java, indonesia

*Corresponding author: *nenytriana979797@gmail.com*

Abstract

One approach within Complementary and Alternative Medicine (CAM) involves the use of High Selenium Functional Food (HSFF) to improve immunity and antioxidant status in children with Autism Spectrum Disorder (ASD). This study aimed to assess improvements in the development of children with ASD following an intervention using HSFF.
The study employed a pre-experimental, one-group pretest-posttest design. Under this design, the participants' development was assessed initially, followed by a one-month intervention period, after which their development was re-assessed. The results were then compared with the pre-intervention baseline. The study population consisted of 33 children with ASD; a sample of 25 children was determined using Slovin's formula with a 10% margin of error. Inclusion criteria were a diagnosis of ASD, an age of 2–6 years, and receipt of both pharmacological and non-pharmacological therapies. Exclusion criteria included children with ASD who were not present at the study site or who had severe congenital abnormalities. Consecutive sampling was used, whereby subjects who presented sequentially and met the selection criteria were enrolled until the required sample size was reached. The HSFF intervention consisted of a daily 50g serving of a high-selenium beef liver dish, administered over a period of one month. Developmental progress in children with ASD was assessed using the ATEC score, a standardized instrument with established reliability, administered by teachers or therapists. Data were analyzed using a paired-samples t-test with a 95% significance level.
The study results showed significant improvement in the development (communication, social interaction, sensory, physical, and behavioral aspects) of children with ASD (p-value 0.036 < 0.05). The mean developmental score for children with ASD was 96.50 prior to the intervention and 76.00 following the intervention.
The HSFF intervention can significantly improve the development of children with ASD. It is recommended that parents maintain a Casein-Free, Gluten-Free, and Sugar-Free (CFGFSF) diet throughout the intervention period; adherence to the CFGFSF diet can help manage the child's behavior in addition to improving immunity and antioxidant status.

Keywords: Developmental Improvement, ASD, HSFF

1. INTRODUCTION

Autism Spectrum Disorder (ASD) is a developmental disorder characterized by two main symptoms: impairments in communication and social interaction, and restricted or repetitive behaviors (1). Parents typically first recognize and report ASD in their children around the age of 18 months, noting delays in verbal and non-verbal communication compared to peers. To identify children with ASD as early as possible, screening should be conducted at 18 and 24 months of age, prior to establishing a formal diagnosis based on the DSM-5 (Diagnostic and Statistical Manual of Mental Disorders, 5th Edition) (2). Early detection enables intensive early intervention to address the developmental disorder (3).

Children with ASD experience inflammation in the neurons (nerve cells) and glial cells of the hypothalamus, particularly in the anterior region. This triggers microglia to physiologically stimulate the production of pro-inflammatory cytokines—specifically IL-6, IL-β, and TNF-α—as a defense mechanism against invading foreign substances. These foreign substances may originate from toxins, microbes, or fungi in the digestive tract—stemming from abnormal gut flora and inefficient digestive enzymes—which leads to intestinal hyperpermeability. Intestinal hyperpermeability allows incompletely digested casein and gluten peptides to pass through the intestinal wall and bind to opioid receptors in the brain. Improving the methylation pathway is a strategy to enhance inter-neuronal signaling or facilitate the early formation of new neurons(4).

The methylation pathway, which boosts levels of S-Adenosylhomocysteine (SAH) and S-Adenosylmethionine (SAM), produces two amino acids: taurine and cysteine. Cysteine directly influences the production of glutathione peroxidase (GPx)—an antioxidant—as well as DNA/RNA protection, heavy metal detoxification, and immune support. Selenium-based amino acid components, specifically selenocysteine and selenomethionine, serve as a foundation for optimizing the methylation cycle to enhance SAH or SAM levels. Improving the methylation cycle reduces inflammation in neurons (nerve cells) and fosters developmental progress in children(5).

Development is closely linked to the nervous system, particularly the cerebrum. Abnormalities in children with ASD affect both the right and left hemispheres, with each hemisphere divided into four lobes corresponding to specific functional areas. The frontal lobe regulates behavior, language, and memory. The temporal lobe controls hearing and language comprehension. The parietal lobe interprets sensations (pain, temperature, and touch), while the occipital lobe interprets visual stimuli(6).

The prevalence of ASD is increasing annually. Data from the Centers for Disease Control and Prevention (CDC) reported a prevalence of 1 in 68 in 2016—meaning one child in 68 had ASD—whereas in 2017, the figure rose to 1 in 36, meaning one child in 36 had ASD(7). The prevalence of ASD is four times higher in males than in females(8). Causative factors for ASD include neuroinflammation and neuroimmune issues(9),(10), as well as gastrointestinal disorders, particularly those affecting the small intestine(11),(12),(13). Immune system dysfunction and neuroinflammation in children with ASD lead to increased production of pro-inflammatory cytokines(14). The cytokines involved in ASD-related neurological processes include IL-1β, IL-6, and TNF-α. These cytokines can cross the blood-brain barrier, thereby influencing the hypothalamus to affect body temperature and behavior associated with ASD. Children with ASD also exhibit abnormalities in their fecal microbiota; research involving fecal bacterial cultures from children with ASD has shown significant findings regarding *Bifidobacterium*, *Escherichia coli*, *Lactobacillus*, and *Enterococcus*(12).

This alteration in fecal flora indicates the presence of intestinal inflammation in children with ASD; this disrupts the blood-brain barrier, as intestinal function—which supplies nutrients to neurons—is compromised. Consequently, the functioning of the nervous system is affected, triggering neuroinflammation(9). Another causative factor for ASD is heavy metal toxicity. In ASD cases, elevated mercury (Hg) concentrations in hair are significantly associated with ASD symptoms, and a significant positive correlation exists between lead content in the hair of individuals with ASD and verbal communication development ($p = 0.020$)(15). Genetics also play a role; currently, approximately 15% of ASD cases are linked to genetic mutations, specifically involving *de novo* genetic variations or mutations. Research estimates indicate that 37%–90% of siblings of individuals with ASD are at risk of developing the condition themselves(16).

Interprofessional management of ASD involves collaboration among pediatricians, psychologists, nurses, medical rehabilitation specialists, and nutritionists working as a team to assist families with children who have ASD(17). Early intervention—prior to the age of three—is highly recommended(7). The goal of ASD management is to maximize the child's independence and quality of life by minimizing symptoms associated with the condition's development. Complementary and Alternative Medicine (CAM) is utilized for prevention, treatment, and health promotion in the context of ASD. CAM is categorized into two types: biological therapies and non-biological therapies. Biological therapies include dietary interventions—such as casein-free, gluten-free, and sugar-free (CFGFSF) diets—and supplementation. Non-biological therapies include social therapy, auditory integration training, sensory integration therapy, drama therapy, dance therapy, acupuncture, massage therapy, yoga, and animal-assisted therapy(18), (19).

One approach to managing Autism Spectrum Disorder (ASD) using biological therapy involves selenium supplementation(20). Selenium is essential for specific and non-specific immune responses

as well as antioxidant activity(18). When administered for ASD, selenium functions as a component of selenoproteins, specifically the enzyme glutathione peroxidase (GPx). GPx acts to reduce hydrogen peroxide (H_2O_2) into water (H_2O)(21), repair neuronal degeneration, and eliminate reactive oxygen species (ROS) caused by oxidative stress(22). Selenium acts as an antioxidant compound; it functions by donating an electron to an oxidizing agent, thereby inhibiting the latter's activity(12).

This study proposes a selenium intervention using a natural alternative rich in selenium. This alternative source is expected to operate similarly and target brain growth factors, serving as an antioxidant and immune system booster; one such source is beef liver, a protein-rich food(23). Beef liver is an excellent alternative source of selenium as it is easily digested, readily available, and easily consumed by the Indonesian population. The body adapts well to digesting beef liver, making it a viable alternative for improving development in individuals with ASD. Beef liver contains 59.19 µg (micrograms) of selenium per 100 g(23). The daily selenium requirement for children with ASD aged 4 to under 7 years is 20 µg(24).

Based on the background above, the researcher is interested in conducting a study on the intervention of High Selenium Functional Food (HSFF) to improve development in children with Autism Spectrum Disorder (ASD).

2. METHODOLOGY

This study employed a pre-experimental, one-group pretest-posttest design. In this design, a group of participants underwent an initial developmental assessment, followed by a one-month intervention. After the designated one-month intervention period, their development was assessed again, and the results were compared with the pre-intervention baseline.

The high-selenium functional food (HSFF) intervention consisted of a variety of side dishes made from high-selenium beef liver, rotated on a weekly basis; a 50-gram portion was consumed once daily. The daily selenium requirement for children with ASD aged 4 to under 7 years is 20 µg. The target population comprised all children with ASD (both male and female). The accessible population consisted of children with ASD attending the Cahaya Harapan Clinic (Mrican, Ngronggo), AGCA Center (Kediri City), the Medical Rehabilitation units at Gambiran Regional General Hospital (RSUD) and Baptis Hospital (Kediri City), and Adib Center (Pare, Kediri) who met the inclusion and exclusion criteria. Consecutive sampling was used, meaning subjects were enrolled sequentially as they presented and met the selection criteria until the required sample size was reached. From a population of 33 children with ASD, a sample of 25 was determined using Slovin's formula with a 10% margin of error. Participants were recruited from ASD clinics in Kediri City and Regency. Inclusion criteria were a diagnosis of ASD, age 2–6 years, and receipt of both pharmacological and non-pharmacological therapies. Exclusion criteria were absence from the study site and the presence of severe congenital abnormalities. The HSFF intervention consisted of a 50-gram serving of a high-selenium beef liver dish administered daily for one month. Developmental progress was measured using the Autism Treatment Evaluation Checklist (ATEC) score, assessed by teachers or therapists. Data were analyzed using a paired-samples t-test to determine improvements in the children's development, with a 95% confidence interval. This study has undergone ethical review by the Ethical Clearance team at STIKES Karya Husada Kediri, under reference number 158/EC/LPPM/STIKES/KH/VIII/2026, dated August 1, 2026.

3. RESULTS

Based on the research findings regarding the 25 child respondents with ASD, the following respondent characteristics were identified:

Table 1. Characteristics of respondents based on the age of the child with ASD

Age	Frequency	%
3 Tahun	3	12
4 Tahun	5	20
5 Tahun	12	48
6 Tahun	5	20
Jumlah	25	100

Based on the research findings involving 25 respondents with ASD, nearly half were 5 years old (48%), and a small proportion were 3 years old (12%).

Table 2. Characteristics of respondents based on the gender of the child with ASD

Gender	Frequency	%
Laki-laki	21	84
Perempuan	4	16
Jumlah	25	100

Based on the research findings involving 25 child respondents with ASD, the vast majority were male (84%), while a small minority were female (16%).

Table 3. Results of the paired-sample t-test on the development of children with ASD (pre- and post-intervention)

Development score ATEC 0-179	
Pre	96,68±13,88; 96,50(72,00-122,00)
Post	76,40±15,21; 76,00(45,00-102,00)
ρ	0,036
Comunication (0-28)	
Pre	23,86±5,81; 25,00(5,00-32,00)
Post	16,31±6,83; 16,50(2,00-28,00)
ρ	0,000
Social interaction (0-40)	
Pre	24,18±9,42; 22,50(9,00-45,00)
Post	18,68±8,43; 19,00(2,00-34,00)
ρ	0,001
Sensori (0-36)	
Pre	21,13±4,68; 22,00(11,00-32,00)
Post	16,18±4,85; 16,50(6,00-27,00)
ρ	0,001

Fisical, behavior (0-75)	
Pre	29,68±10,76; 29,00(14,00-49,00)
Post	23,18±8,57; 22,00(12,00-41,00)
ρ	0,000

P paired sample t-test

4. DISCUSION

Based on the study results, nearly half of the respondents were 5 years old (48%), while a small proportion were 3 years old (12%). Consistent with established theory, the prevalence of ASD was higher in males than in females (a 4:1 ratio), with 21 male respondents (84%) and 4 female respondents (16%)(1),(2). Improvements were observed in ATEC scores across the subscales of communication, social interaction, sensory/health, and physical/behavioral functioning. The HSFF intervention in this study consisted of a beef liver dish (50g/day) providing 20 µg of selenium—meeting the selenium requirements for children with ASD aged 4 to under 7 years—though the children experienced boredom with the HSFF despite variations in the dish's presentation. The intervention for the HSFF group was based on the methylation cycle. Improving the methylation pathway can enhance the activity of S-Adenosylhomocysteine (SAH) and S-Adenosylmethionine (SAM), thereby reducing inflammation in brain nerve cells or tissue, as selenium supplementation influences ASD progression(5). SAH and SAM facilitate the production of taurine and cysteine; these two amino acids support antioxidant activity, immune function, and protection against DNA/RNA damage(4).

Selenoproteins aid in brain development and the prevention or repair of oxidative damage in brain tissue(21). Selenium acts as an antioxidant and supports the enhancement of brain neurochemistry (such as brain growth factors). It facilitates the proper functioning of the nervous and sensory systems, which operate through vision, hearing, smell, taste, and touch. The sensory component transmits information and stimuli from the body's surface and internal structures to the nervous system via spinal and cranial nerves. These signals are relayed to various parts of the nervous system, where the information is analyzed and processed. Motor functions involve controlling the contraction of all body muscles—including smooth muscles and internal organs—as well as exocrine and endocrine gland secretions. Motor activity is executed by transmitting information (neural signals) from the central nervous system to motor components throughout the body. The integration component relies on the coordination of memory, sensory stimuli, and motor activity(25). Regarding behavioral functions, the limbic cortex—a central structure of the basal cerebrum surrounded by the cerebral cortex—plays a key role in behavioral control. The limbic system comprises the hippocampus, amygdala, and thalamus; the amygdala works in conjunction with the hypothalamus to regulate behavior(25). Intellectual functions are mediated by the prefrontal cortex (the anterior part of the cerebral cortex), while communication functions are regulated and integrated within the cerebrum. The hippocampus, a component of the limbic system, plays a role in facilitating reciprocal communication(25).

Brain tissue consists of millions of interconnected nerve cells. As the central nervous system, the brain regulates all bodily activities. Various stimuli received by the body are transmitted to and processed by the brain, eliciting a bodily response. Brain cell dysfunction in children with ASD leads to developmental impairments—such as difficulties in communication, social interaction, and sensory and cognitive awareness, as well as physical and behavioral issues (26). The HSFF formulation, with a selenium content of 59.19 µg, is expected to support bodily metabolism and address mineral deficiencies—particularly selenium—in children with ASD (17, 18).

A paired-sample t-test regarding the development of children with ASD yielded a significant result (ρ = 0.036), indicating a reduction in developmental symptoms and, consequently, an improvement in the ASD developmental profile.

This developmental improvement was assessed using the Autism Treatment Evaluation Checklist (ATEC); the mean ATEC score was 70.2 prior to the intervention and 50.8 following the intervention (27).

Developmental improvements in children with ASD must be accompanied by a strict Casein-free, Gluten-free, and Sugar-free (CFGFSF) diet. The intervention for the HSFF group is based on the methylation cycle. Improving the methylation pathway can enhance the activity of S-Adenosylhomocysteine (SAH) and S-Adenosylmethionine (SAM), thereby reducing inflammation in brain nerve cells or tissue, as selenium supplementation influences ASD progression (5). SAH and SAM facilitate the production of taurine and cysteine; these two amino acids support antioxidant activity, immune function, and protection against DNA/RNA damage (4). Selenoproteins aid in brain development and help prevent or repair oxidative damage in brain tissue (17). Selenium acts as an antioxidant and modulates brain neurochemistry (specifically brain growth factors). The sensory function of the nervous system operates through vision, hearing, olfaction, gustation, and tactile sensation. The sensory component transmits information and stimuli from the entire body surface and internal structures to the nervous system via spinal and cranial nerves. These signals are relayed to the parts of the nervous system responsible for analyzing and processing the information. The motor function involves controlling the contraction of all body muscles—including smooth muscles and internal organs—as well as exocrine and endocrine gland secretions. This function is executed by transmitting information (neural signals) from the central nervous system to motor components throughout the body. The integration function relies on the coordination of memory, sensory stimuli, and motor activity(25)

5. CONCLUSIONS

There was an improvement in development (communication, social interaction, sensory, physical, and behavioral aspects) following the intervention involving a selenium-rich beef liver dish (p-value 0.036 < 0.05). Selenium acts as an antioxidant and enhances brain neurochemistry (specifically, brain growth factors). It supports key nervous system functions, including sensory processes involving vision, hearing, smell, taste, and touch. The sensory component transmits information and stimuli from the body's entire surface and internal structures to the nervous system via spinal and cranial nerves. These signals are relayed to the nervous system, which analyzes and processes the information. Motor functions involve controlling the contraction of all body muscles—including smooth muscles and internal organs—as well as exocrine and endocrine gland secretions. Motor activity is executed by transmitting information (nerve signals) from the central nervous system to motor components throughout the body. The integration component relies on the coordination of memory, sensory stimuli, and motor activity(25).

REFERENCES

[1] Nemeroff CB, Schatzberg AF, Rasgon N, Strakowski SM. The American Psychiatric Association publishing textbook of mood disorders. American Psychiatric Pub; 2022.

[2] Yu Y, Ozonoff S, Miller M. Assessment of autism spectrum disorder. Assessment. 2024;31(1):24–41.

[3] Zwaigenbaum L, Bauman ML, Fein D, Pierce K, Buie T, Davis PA, et al. Early screening of autism spectrum disorder: recommendations for practice and research. Pediatrics. 2015;136(Supplement 1):S41--S59.

[4] Hernández DQ, Cruz AL, González SB, Vila DT, Robert YC. Variation of the REDOX Status in Patients with Primary Autism after Antioxidant Therapy. 2017.

[5] Deth R, Trivedi MS, Hodgson NW, Muratore CR, Waly MI. Redox/methylation theory and autism. Compr Guid to Autism. 2014;1389–410.

[6] Kowalak JP. Buku Ajar Patofisiologi (JP Kowalak, W. Welsh, \& B. Mayer. Buku Kedokt EGC. 2020.

[7] Sharma SR, Gonda X, Tarazi FI. Autism spectrum disorder: classification, diagnosis and therapy. Pharmacol Ther. 2018;190:91–104.

[8] Association AP, others. Diagnostic and statistical manual of mental disorders. BMC Med. 2013;17:133–7.

[9] Masi A, Glozier N, Dale R, Guastella AJ. The immune system, cytokines, and biomarkers in autism spectrum disorder. Neurosci Bull. 2017;33(2):194–204.

[10] Meltzer A, de Water J. The role of the immune system in autism spectrum disorder. Neuropsychopharmacology. 2017;42(1):284.

[11] Hsiao EY. Gastrointestinal issues in autism spectrum disorder. Harv Rev Psychiatry. 2014;22(2):104–11.

[12] Adams James B dan Johansen LJ dan PLD dan QD dan RRA. flora gastrointestinal dan status gastrointestinal pada anak autis - perbandingan dengan anak-anak tipikal dan korelasi dengan keparahan autisme. 11(1).

[13] Siniscalco D, Schultz S, Brigida AL, Antonucci N. Inflammation and neuro-immune dysregulations in autism spectrum disorders. Pharmaceuticals. 2018;11(2):56.

[14] Siniscalco D, Schultz S, Brigida AL, Antonucci N. Inflammation and Neuro-Immune Dysregulations in Autism Spectrum Disorders. pharmaceutical. 2018;1–14.

[15] Blaurock-Busch E, Amin OR, Dessoki HH, Rabah T. Toxic metals and essential elements in hair and severity of symptoms among children with autism. Maedica (Buchar). 2012;7(1):38.

[16] American Psychiatric Association. DSM-5 Update. Diagnostic Stat Man Ment Disord. 2017;Fifth Edit:1–42.

[17] Adams JB. Summary of Dietary, Nutritional, and Medical Treatments for Autism-based on over 150 published research studies. Autism Reasearch Institute Publication 40-Version. 2013.

[18] Brondino N, Fusar-Poli L, Rocchetti M, Provenzani U, Barale F, Politi P. Complementary and alternative therapies for autism spectrum disorder. Evidence-Based Complement Altern Med.2015.

[19] Abbas AK, Lichtman AH, Pillai S. Basic Immunology E-Book: Functions and Disorders of the Immune System. Elsevier Health Sciences; 2019.

[20] Li Q, Han Y, Dy ABC, Hagerman RJ. The Gut Microbiota and Autism Spectrum Disorders. Cell Neurosci. 2017;11(April).

[21] Raymond LJ, Deth RC, Ralston NVC. Potential role of selenoenzymes and antioxidant metabolism in relation to autism etiology and pathology. Autism Res Treat. 2014.

[22] Raymond LJ, Deth RC, Ralston NVC. Potential Role of Selenoenzymes and Antioxidant Metabolism in relation to Autism Etiology and Pathology. Autism Res Treat. 2014;2014:164938.

[23] Li C. The role of beef in human nutrition and health. Dikeman E, Ed Saf Qual Prod beef. 2017;2:329–38.

[24] Kipp AP, Strohm D, Brigelius-Flohé R, Schomburg L, Bechthold A, Leschik-Bonnet E, et al. Revised reference values for selenium intake. J Trace Elem Med Biol. 2015;32:195–9.

[25] Fetit R, Hillary RF, Price DJ, Lawrie SM. The neuropathology of autism: A systematic review of post-mortem studies of autism and related disorders. Neurosci \& Biobehav Rev. 2021.

[26] Donders J, Hunter SJ. Principles and practice of lifespan developmental neuropsychology. Cambridge University Press; 2010.

[27] Adams JB, Audhya T, Geis E, Gehn E, Fimbres V, Pollard EL, et al. Comprehensive nutritional and dietary intervention for autism spectrum disorder—a randomized, controlled 12-month trial. Nutrients. 2018;10(3):1–43.