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FOCUSING ON METHIONINE CYCLE MECHANISMS AND FOLATE'S ROLE IN AUTISM SPECTRUM DISORDER: A NARRATIVE REVIEW

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Abstract

Background: Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by challenges in social communication and repetitive and restricted activities and behavior. According to etiology, it is a multifactorial disease where genetic and environmental factors are predominant. The pathogenesis of ASD involves disruptions in neurotransmitter production, DNA methylation, immune function, oxidation and antioxidant balance, and vitamin D metabolism. In addition, exposure to toxic chemicals, such as heavy metals and phthalates, is a major environmental risk factor in ASD. Folate is a water-soluble vitamin involved in the production of methyl groups through the methionine cycle. The methionine cycle produces methionine and metabolizes homocysteine, which is a neurotoxic substance, through transulfuration to cysteine. Cysteine is a structural component of glutathione, which is a crucial non-enzymatic antioxidant. The aim of this study is to observe the role of folate and its mechanism of action, through the methionine cycle, on the pathogenesis of ASD.

Methods: This study is a literature review that summarizes results of 40 research articles related to the keywords autism, folate, methionine cycle, and homocysteine, published in 2023 and 2024. Google Scholar, PubMed, and Science Direct databases are used for searching research articles, and all studies conducted on humans, written in the English language, and available in full text, are included in this review.

Results: As a result of this literature review, ASD pathogenesis is relevant to the activity of folate as a cofactor for pathways involved in the production of monoamine neurotransmitters such as dopamine. This study explains the major role of folate in the balance of excitatory and inhibitory neurotransmitters, which is crucial for neurodevelopment. Besides this, explore the importance of folate in vitamin D metabolism, which is vital for neuroprotection and neurotropism. This review sheds light on the role of folate in the methionine cycle, which produces methionine, an important amino acid for neurodevelopment. In addition, this study explicates the role of folate in ameliorating heavy metal toxicity because this vitamin increases antioxidant capacity by enhancing glutathione formation, and also has a direct role in methylation, which increases the excretion of toxic metals. The role of folate in DNA methylation, which is one of the epigenetic mechanisms involved in ASD pathogenesis, is also interpreted in this study.

Conclusion: It can be concluded that folate is important for neurodevelopment and lowering the incidence of ASD in children, because as a vital cofactor, it has a potential effect on the methionine cycle, synthetic pathways related to neurotransmitter and vitamin D metabolism. In addition, ameliorates the toxic effects of heavy metals on the brain.

Keywords: Autism spectrum disorder, Folate, methionine cycle, homocysteine.

Autism Spectrum Disorder (ASD) is a complex and heterogeneous neurodevelopmental disorder. Symptoms of autism include challenges in social communication, as well as repetitive and restricted activities and behaviors. In addition, many other disorders, such as sleep and eating disorders, motor problems, sensory deficits, and intellectual disabilities, can coexist [(1)]. According to a recent report of the American Centers for Disease Control and Prevention, the prevalence of ASD in 8-year-old children is one in every 36 children [(2)].

Introduction

Males are more prone to disease than females [(3)]. The etiology of ASD is multifactorial with genetic, environmental, neurobiological, neurodevelopmental, and psycho-affective components. Genetic factors are the major contributing factors for ASD [(1, 3)]. Environmental factors such as pregnancy complications, heavy and toxic metals, and receptor medications affect brain development during the prenatal stage [(1,4)]. Environmental factors trigger the modification of predisposing hereditary high-risk genes during fetal development through epigenetic mechanisms [(5)].

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One of the epigenetic mechanisms involved in ASD pathogenesis is DNA methylation. This process needs a methyl group provided by single-carbon metabolism, which needs folate as a cofactor [6]. In addition, there are brain-based and systematic biological abnormalities in ASD pathogenesis. Brain-based abnormalities are abnormalities in neurotransmitter production, imbalance in excitation and inhibition, and epilepsy, while Immune dysfunction, oxidative stress, and metabolic abnormalities are included in systemic abnormalities, one of which is folate metabolism [7].

Folate is one of the B-group vitamins that is involved in one-carbon metabolism. The bioactive form of folate is tetrahydrofolate (THF) and serves as a methyl donor in one-carbon metabolism. The synthetic oxidized form of folate, termed folic acid, exists in fortified foods and supplements. The naturally occurring folate is 5-methyltetrahydrofolate. Folinic acid is the 5-formyl tetrahydrofolate and occurs naturally in some foods, including dark green leafy vegetables. Synthetic folinic acid is the formyl derivative and the reduced form of folate [8]. Factors such as malabsorption, poor diet, and an increase in demand, such as during pregnancy, lead to folate deficiency, impaired neurodevelopment, and the occurrence of ASD [9]. Folate uptake is dependent on folate receptors and folate receptor autoantibodies. There are three types of folate receptors: Folate receptor α (FRα) and folate receptors in hematopoietic cells. FRα is crucial during the repair and regeneration of the central nervous system. The 5-methyl tetrahydrofolate is transported from the blood by FRα to the choroid plexus. Folate receptor alpha autoantibodies have been detected in many neuropsychiatric diseases, such as ASD ([7, 9, 10, 11]).

Folate is involved in the folate methionine cycle, and the bioactive form (methyl THF) acts as a cofactor and methyl donor for methionine synthase to produce methionine from homocysteine (Figure 1). Homocysteine is a sulfur-containing amino acid, and its level higher than 15micromole/ liter is known as hyperhomocysteinemia, which is neurotoxic, hurts cognition, and causes loss of blood-brain barrier integrity [12]. One of the major causes of hyperhomocysteinemia is folate deficiency. Methionine in S-adenosyl methionine (SAM) form acts as a methyl donor for numerous molecules such as DNA, RNA, and proteins, and is important for neuron methylation in early life, which is considered vital for neurodevelopment [9,13].

Many studies focused on different mechanisms, such as exposure of the brain to toxic chemicals, alteration in immunity, DNA methylation, neurotransmitters, oxidant/antioxidant balance linked to ASD pathogenesis, and also correlated role of folate intake in altering these mechanisms [6,14].

In addition, for clarifying the role of folate on ASD systematic reviews also conducted on different frame time as Main et al in 2010 concluded that further researches required for about the role of disturbed folate methionine cycle in etiology of autism, in addition Bianka Hoxha et al in 2021 also emphasized on larger sample size studies with long-term observational periods for protective role of folic acid in ASD risk. Besides these, in 2023, Melissa Roufael et al explained the need for additional research related to the effect of folate supplementation on homocysteine level [15,16,17]. Therefore, this literature review was conducted to provide more detailed information about the role of folate as a cofactor in the mechanisms related to ASD pathogenesis.

Methods

This study is a narrative literature review. Google Scholar, PubMed, and Science Direct databases were used for downloading articles. Inclusion criteria for selecting articles were research articles that were written in the English language, available in full text, conducted on humans, and published in 2023 and 2024. In addition, keywords folate, autism, and homocysteine were considered for searching articles. The terms “folate and autism”, “folate and homocysteine”, “folic acid and vitamin D”, “autism and hyperhomocysteine", "autism and phthalates", and “folic acid and autism” were used during searching articles. All review articles were excluded, and after extracting the research articles, the full text was downloaded and observed for eligibility of this study and verified for duplication. The numbers of articles selected for this review are shown in Figure 1.

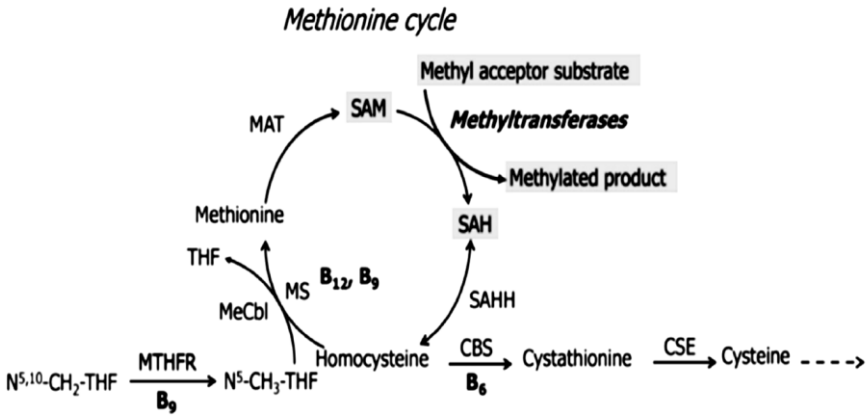


Figure1. Interference of the folate cycle with the methionine cycle. THF (Tetrahydrofolate), MTHFR (methyl tetrahydrofolate reductase), MS (methionine synthase), MeCbI (methylcobalamine), MAT (methionine adenosyl transferase), SAM (S-adenosyl methionine), SAH (S-adenosylhomocysteine), SAHH (S-adenosylhomocysteine hydratase), CBS (cystathionine beta synthase), CSE (cystathionine gamma lyase)[13]

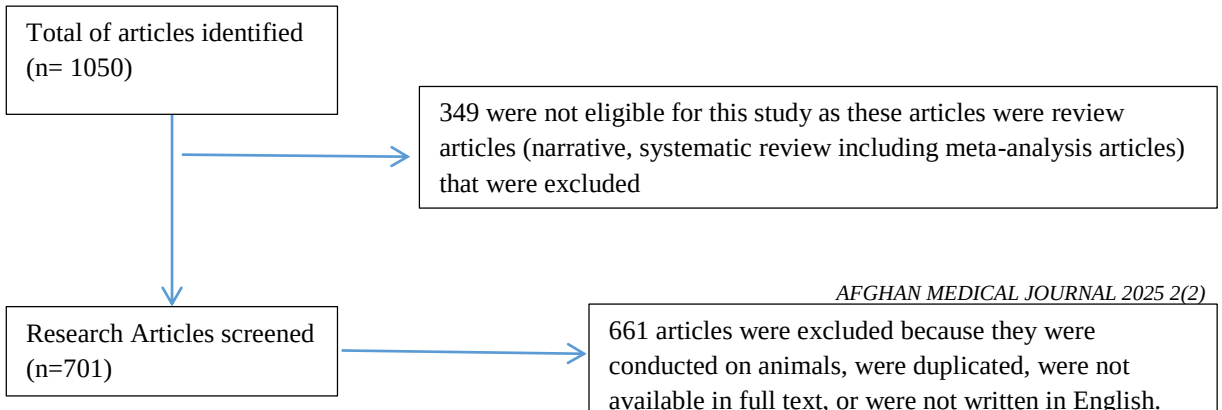


Figure 2. Schematic diagram of the articles selected for this review

Results

Result of this study express the role of folate as a vital cofactor in proceeding methionine cycle and pathways related to vitamin D metabolism and neurotransmitter formation, therefore emphasize on folate involvement in pathogenesis of ASD through its effect on oxidative stress, DNA methylation, neuroinflammation, neurotransmitter balance, metabolism of vitamin D and toxic effects of

Environmental pollutions and heavy metals on neurodevelopment.

Role of folate in DNA methylation

DNA methylation is one of the important epigenetic mechanisms that influence gene activity. Methylation in the DNA sequence causes gene silencing and in the promoter region inhibits transcription. This process is crucial for fundamental biological processes such as X chromosome inactivation and genomic imprinting during intrauterine development. DNA methylation alters gene expression according to developmental timing, as specific genes are methylated during childhood and hypomethylated during adulthood. Altered DNA methylation styles were located in people with ASD, specifically in genes associated with neurodevelopment and synaptic function.

DNA methylation involves several steps catalyzed by the enzyme DNA methyltransferase with the presence of S-adenosyl methionine as a cofactor. DNA methyltransferase transfers a methyl group provided by SAM to the fifth carbon of cytosine, thus forming 5-methylcytosine that is connected to guanine nucleotide. Therefore, deficiency in folate results in SAM depletion and finally dysregulation of DNA methylation that ends in neurodevelopmental disorder [18,19,20].

Role of folate in neurotransmitters

Neurotransmitters are chemical substances released from nerve cells and act as messengers between neurons. There are three categories of neurotransmitters: excitatory, inhibitory, and modulatory. Besides their role on synapses, these neurotransmitters are involved in learning and memory and are important for brain development and cognition. Imbalance of excitation and inhibition is one of the mechanisms involved in autism. Hyperhomocysteinemia, due to its excitotoxic effect, increases glutamate, which is an excitatory neurotransmitter. Homocysteine is a stimulant for the GluN2A subunit of NMDA (N-methyl D-aspartate) receptor, causing a progressive increase in intracellular calcium. This increase in calcium causes an increase in extracellular signal-regulated kinase, a member of the mitogen-activated protein kinase family, phosphorylation, which leads to neural cell death and excitotoxicity. As Folate intake enhances the metabolism of homocysteine through the synthesis of methionine, it decreases the excitotoxic effects of hyperhomocysteinemia and prevents the imbalance of excitation and inhibition, resulting in a positive effect on brain development [7,21,22,23].

One of the diagnostic criteria for autism is repetitive behavior that results from dopamine dysfunction. Dopamine deficiency in the substantia Nigra and ventral tegmental area dysregulates postsynaptic neurons and contributes to core behavioral features in ASD. Besides these, it has a vital role in neuronal migration and dendritic growth. Folate has a crucial role in monoamine neurotransmitters (dopamine, adrenaline, noradrenaline) synthesis through the formation of tetrahydrobiopterin, which is a cofactor for enzymes related to the synthesis of monoamine neurotransmitters [13, 24,25].

Role of folate on immunity

Immune and inflammatory abnormalities are involved in the pathogenesis of ASD. Activation of brain microglia is vital in brain inflammation and immune function. Reactive oxygen species generated in oxidative stress is a factor that activates microglial cells. As mentioned earlier, folate deficiency is one the causes of hyper-homocysteinemia. Hyperhomocysteinemia increases the level of Asymmetric Dimethyl Arginine (ADMA). A high level of ADMA stimulates the infiltration of immune cells and elicits a neuroinflammatory response. ADMA triggers immune-endothelial interaction since adhesion of primary monocytes and their extravasion across the endothelial monolayer is elevated [26]. Besides this, folate receptor autoantibodies that block the transfer of folate to the brain are also significant in ASD severity [7].

Role of folate in oxidative stress

Oxidative stress is caused by an increase in the level of reactive oxygen species or a decrease in the level of antioxidants, or it is an imbalance between the production of oxygen species and antioxidants [27]. Owing to the brain's high lipid content, oxygen usage, high energy demand, and low antioxidant ability, the organ is most prone to oxidative harm. Phospholipid content of the brain especially makes it more susceptible to peroxidation, which is mediated by Reactive oxygen species (ROS). ROS accumulation, which is a cellular hazard, can cause substantial neuronal damage. Therefore, plays an important role in the pathogenesis of ASD [28] One of the antioxidants is glutathione, which has cysteine in its composition. Cysteine is formed through the transulfuration pathway from cystathionine by the enzyme cystathionine beta synthase.

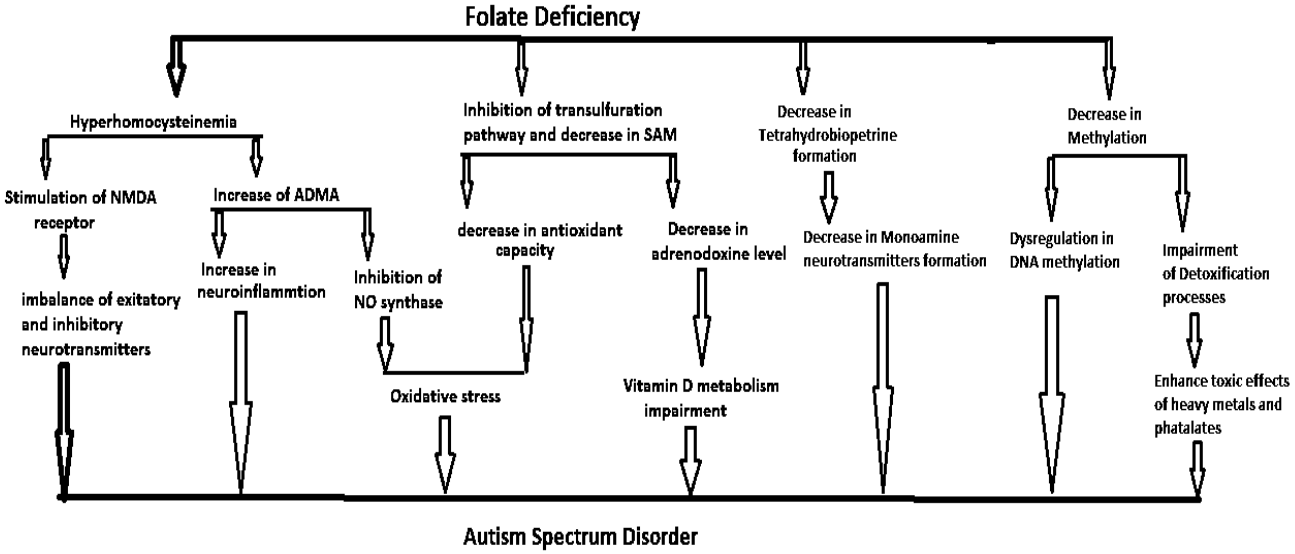
This enzyme is a rate-limiting enzyme for the transulfuration pathway, and SAM acts as an allosteric activator for this enzyme. Therefore, in case of folate deficiency, SAM level decreases and formation of cysteine through the transulfuration pathway declines [29]. On the other hand, hyperhomocysteinemia occurs, which enhances oxidative stress through inhibition of nitric oxide synthase by increasing the level of asymmetric dimethyl arginine. Inhibition of nitric oxide synthase has a negative effect on the microvasculature of the brain and is correlated with cognitive function decline [26].

Role of folate in vitamin D metabolism

Vitamin D is a lipid-soluble vitamin. Besides its role in calcium and bone metabolism it's crucial for neuroprotection and neurotropism. Neuroplasticity and neurogeneration. Deficiency of this vitamin is associated with abnormal brain development. The three main steps in vitamin D metabolism, which are 25-hydroxylation, 1 α 1α-hydroxylation, and 24-hydroxylation are catalyzed by cytochrome P450 mixed-function oxidase (CYPs). These enzymes are located either in the endoplasmic reticulum or the mitochondria. Electron donors for cytochromes

located in mitochondria are adrenodoxin (iron-sulfur protein) and adrenodoxin reductase. Folic acid, through providing a methyl group for

vitamin B_{12}, is vital for the methionine homocysteine cycle. The produced homocysteine undergoes transulfuration and produces cysteine, a sulfur-containing amino acid that is used for the formation of adrenodoxin. Therefore, deficiency of folic acid is a cause for deficiency of adrenodoxin, which has a negative effect on vitamin D metabolism and neurodevelopment [30].



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Role

Heavy metals include Lead, Chromium, Cadmium, Mercury, and Arsenic. Heavy metals, also known as toxic metals, are one of the major environmental toxicants that damage the nervous system during the developmental stage. These metals have a toxic effect by enhancing oxidative stress and decreasing antioxidant capacity [31,32]. Therefore, folate mitigates the toxic effects of heavy metals by enhancing antioxidant capacity [33].In addition, folate has a positive effect on the excretion of some toxic metals through increasing methylation [34]. Phthalates are esters of phthalic acid, environmental pollutants and chemical plasticizers used in beverages, cosmetics, toys, packing materials, and infant clothing. Humans are exposed to it through inhalation, food intake, and skin absorption [35]. Phthalates, as endocrine-disrupting chemicals, disrupt the thyroid signaling pathway, also induce oxidant/antioxidant imbalance and enhance oxidative stress, dysregulate DNA methylation, and triggers neuroinflammation. All these events are linked to neurodevelopmental problems in offspring and trigger neuroinflammation [36]. Folate helps in the detoxification process of which is vital for detoxification. In addition, folate enhances antioxidant capacity, mitigates neuroinflammation, and regulates methylation of DNA to ameliorate the overall effect of phthalates in the pathogenesis of ASD [33].

Discussion

There are studies that revealed the preventive effect of folic acid for ASD A research study of Yan Jiang et al explained that supplementation with folic acid during the pre-conceptional and prenatal period decreases the risk of ASD in offspring [5]. In addition, the study of Prateek Kumar Panda et al supports the intake of folinic acid in improving the core features of ASD [10]. Updated research in 2025 also supports that folic acid has a crucial role in ASD. A study by Vasconcelos et al described that folic acid intervention improves socialization, behavior, and communication in individuals with ASD. Besides this, Farida Anggraini Soetedjo et al describe the role of folic acid in the reduction of ASD symptoms [37,38].

Table 1. Summary of statistical analysis and type of study of articles that relate folate to ASD pathogenesis

Author	Country	Year of publication	Type of study	Sample size and statistics
Yan Jiang et al	China	2024	Cross sectional	N 6049 toddlers, age 16-30 months. Supplementation with folic acid during the periconceptional and prenatal period decreases the risk of ASD in offspring (OR=2.47)
Prateek Kumar Panda et al	India	2024	double-blind, placebo-controlled RCT**	N 40 participants in each group (placebo and folinic acid group), 2-10 years old children Changes in CBCL* total score were better in the folinic acid group (15.4 ± 7.8 vs. 8.5 ± 5.7, $p < 0.001$ for both).
Ali A. Alshamrani et al.	Saudi arabia	2023	Experimental study	N 28 ASD group and 24 normal, age: 8.45 ± 3.15 years. Data show that the neutrophils of ASD subjects have hypomethylated DNA levels.

Li et al	USA	2024	cohort	N 110 ASD clinic patients. Communication SRS-2*** score worsened by 1.6 (0.75) for those not on leucovorin and improved by 1.2 (1.00) for each 1 unit of binding titer. The effect of leucovorin on the Cognition score was affected by the binding FRAA**** titer [F (1,417.0) = 4.80, p = 0.03] such that the Cognition worsened by 1.8 (0.82) for those not on leucovorin and improved by 0.86 (0.90) for each 1 unit of binding titer.
Barbara Carpita et al			Comparative study	N=24 ASD, 24 Broad autism phenotype, and 24 Control .29.17% (n = 7) of ASD subjects, and 0% (n = 0) of the healthy subjects showed HCY values above 15 μM,
Tetyana P. Buzhdygan et al	USA	2024	Experimental study	N 6 donors of brain microvascular endothelial cells. Overall, our data suggest that ADMA can impair BBB functions, disrupting the endothelial barrier and eliciting neuroinflammatory and neuroimmune responses.
Masahito Morimoto et al	Japan	2023	Case control	N=61 typical development children and 199 children with ASD stress-related substances, and antioxidants are altered in ASD under oxidative stress, and Reactive oxygen metabolites (ROM) are considered as a measure of oxidative stress level, which is high in ASD
Karam Radwan et al	USA	2023	Case series	N= 6 patients An imbalance in redox reactions is only one of the many factors contributing to ASD. Oral glutathione supplementation was generally well-tolerated throughout the course of the study.
Allegra J. Johnson et al	USA	2023	Case control	N=30 ASD, 30 Sensory deficit children without ASD, and 37 typical development children. A greater imbalance in cerebellar GABA+/Glu was correlated with more severe social impairment as measured by the ADOS-2.
Amira Mansour et al	Egypt	2024	Cross sectional	N=80 Egyptian children with ASD, 2-6 years old. 63.8% of ASD children have vitamin D insufficiency, 28.8 % have vitamin D deficiency, and 7.4% have normal serum levels
Hao-Hsuan Lin et al	Taiwan	2023	cohort	N=168062 live births. The Cox proportional hazard model with a distributed lag nonlinear model (DLNM) was used to estimate the association between heavy metals in $PM_{2.5}$ and ASD. A positive association between heavy metals and ASD was found at 9 months after birth (Hazard Ratio: 1.63; 95% CI: 1.13-2.36).
Joshua D. Alamp	canada	2024	cohort	N=601 pregnancies. Third-trimester blood lead levels were associated with increased social responsive scale-2 scores [$\beta_{ad\times j}$=3.3; 95% confidence interval (CI): 1.1, 5.5. plasma total folate concentrations modified first-trimester blood lead level–social responsive scale associations.
by Feng-Chieh Su et al	Taiwan	2024	cohort	N=67 participants. Logistic regression analysis underscored a significant positive association between high phthalate levels and higher AD-8 scores (odds ratio: 1.217, p = 0.006).
Xingchen He et al	China	2024	Cross sectional	N= 2100 participants In multiple linear regression models, for participants in the highest tertile of phthalates, total serum folate concentrations were 1.566 [β: −1.566; 95% confidence interval: −2.935, −0.196], 1.423 (−1.423; −2.689, −0.157), 1.309 (−1.309; −2.573, −0.044), 1.530 (−1.530; −2.918, −0.142), and 1.381 (−1.381; −2.641, −0.122) ng/mL lower than those in the lowest tertile. The phthalate mixture showed a strong inverse correlation with serum folate.

* Child Behavior Checklist ** Randomized Controlled Trial ***Social Responsiveness Scale Second Editon ****Folate receptor Autoantibody

DNA methylation represents one of the potential epigenetic mechanisms that may contribute to the risk of ASD due to interactions with genetic elements during the development of normal brain function. Samuel Perini et al reported the correlation of DNA methylation with gene expression [39]. A study conducted by Ali A. Alshamrani revealed differences in the DNA methylation pattern in neutrophils of ASD patients and normal subjects [18].

In addition, a study on placental DNA methylation by Sanne D. van Otterdijk explained the effect of the availability of folate and SAM on placental DNA methylation. SAM levels were positively correlated, and SAH levels were negatively correlated with overall DNA methylation [19]. Li et al explained in a research study that the elevated level of IL-17 in ASD patients shows the involvement of inflammation in the pathogenesis of ASD also, it was shown that folate levels were more decreased in severe ASD patients than in mild and moderate ASD patients [7]. Barbara Carpita et al, in a study conducted on adult ASD patients, explained that ruminative thinking, which is a feature frequently associated with ASD, is linked to a high level of interleukin 6. Besides this, a high level of homocysteine was also reported in ASD [14].

A study conducted by Tetyana P. Buzhdygan et al explained that ADMA, stimulated by a high level of homocysteine, triggers inflammatory cytokines [2 Reducing oxidative burden improves symptoms and social functioning in patients with ASD [40]. A report of research conducted by Masahito Morimoto et al explained that the Oxidative stress level is higher in ASD children with ASD than in normal children [27]. One of the enzymatic defense mechanisms against oxidative stress is glutathione, a tripeptide of cysteine-glutamate and glycine. Cysteine is formed through the transsulfuration pathway. Karam Radwan et al explained the neuroprotective effect of glutathione and demonstrated general glutathione tolerability and some efficacy in decreasing problematic behaviors observed in children with ASD [40].

Neurotransmitters serve as significant indicators in ASD [41]. Excitatory and inhibitory neurotransmitters are involved in the pathogenesis of ASD. A study conducted by Masaki Oya et al revealed an increased level of glutamate in ASD patients [23]. Allegra J. Johnson et al in a case-control research study reported a higher concentration of Glutamate than GABA in the cerebellum of ASD patients than in the control group [42].

Vitamin D is important for brain homeostasis, immune modulation, and neurodevelopment. Assessment of serum vitamin D in ASD patients by Amira Mansour et al revealed that about 63.8% of autistic children have vitamin-D- D insufficiency, and about 28.8% % of autistic children have vitamin-D- D deficiency; therefore, there is a low level of vitamin D in ASD patient[s] [43]. A study conducted by Gregory John Russell-Jones explains the functional need for B12, which is dependent on folate, for processing homocysteine to generate cysteine and sulfur for iron sulfur clusters of adrenodoxin for proceeding vitamin D metabolism [30]. Heavy metals disrupt the developmental process and are considered important in the pathogenesis of ASD [4]. A study conducted by Hao-Hsuan Lin et al concluded that exposure to heavy metals has a positive association with ASD incidence [31]. Joshua D. Alampi et al explained the importance of folate on the neurotoxic effects of prenatal lead exposure and explained that the association between gestational lead exposure and child autistic like behavior is modified by folate [33]. In addition, Irene Martinez-Morata et al explained that folate enhances methylation of Arsenic, therefore mitigating Arsenic's toxic effect [34].

A study conducted by Feng-Chieh Su et al concluded that High concentrations of phthalate compounds are positively associated with impairment of cognition [44]. Research by T. Peter Stein et al explained the reduction of detoxification efficiency of phthalates in ASD patients [45]. Besides these, research conducted by Xingchen He et al showed that higher concentrations of urinary phthalate metabolites were associated with lower serum folate concentrations in children. [35] Table (1) summarizes the sample size, study design, and statistical analysis of studies related to folate and the pathogenesis of ASD. **Conclusion**

According to this literature review, it can be concluded that the methionine cycle is important for the production of the methyl group, which is important for neurodevelopment, and folate is crucial for the progression of the methionine cycle. In addition, brain-based and system-based abnormalities, which are included in ASD pathogenesis, are related to the activity of the methionine cycle and the production of methyl groups that are coordinated with the existence of sufficient folate during neurodevelopment. This review did not emphasize the dose of folate intake and its efficacy on the exact age of gestation, and these points are considered limitations. Further research is needed to explain these limitations and the role of folate in all aspects of ASD pathogenesis in more detail.

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