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A NARRATIVE REVIEW ON SALIVARY METABOLOMES AND DIETARY INTERVENTIONS IN AUTISM SPECTRUM DISORDER

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Abstract
Autism spectrum disorders (ASDs) are neurodevelopmental disorders with around 1% prevalence in children, posing challenges for early diagnosis and treatment. Genetic and environmental factors play crucial roles in ASD etiology. Identifying body fluid metabolome and proteome profiles has revealed metabolic anomalies in patients, offering potential ASD biomarkers. Currently, no definitive therapy exists, and conflicting information surrounds alternative treatments. Addressing abnormal metabolites related to oxidative stress, dietary modification to some extent may partially reverse autism. Dietary intervention helps in some cases but represents a small segment due to ASD's wide spectrum. Understanding ASD's pathophysiology becomes increasingly vital with rising patient numbers. Metabolomics biomarkers, especially salivary metabolomic changes, hold promise for early autism diagnosis.

Keywords: Autism spectrum disorders, Biomarkers, Saliva, Children, Metabolomics, Dietary Intervention

Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by social impairment, and alteration in social and communication skills of it's emergence within three years from birth [1]. There are concerns about the increasing prevalence of ASD in recent decades with the global prevalence of early-onset ASD by 1% [2]. There is no proven cure for ASD, and its underlying etiology is yet to be discovered. Numerous theories have indicated that various factors, including the most well-known genetic ones, may contribute to ASD. According to genetic testing based on advanced technologies, genes of ASD candidates are in abundance [3]. In the absence of specific laboratory tests, ASD is quite challenging to detect at an early stage and consequently restricts early intervention**.** However, recent research works have focused on identifying specific biological

abnormalities in ASD that could provide inputs for diagnosis and effective treatment on a larger scale [4]. Prior research has shown that individuals with ASD have biomarkers of oxidative stress, which could be used for early diagnosis and evaluation of ASD intervention [5]. Although there are no autism-defining metabolic biomarkers identified yet, however, examining the metabolites or proteins of pathways associated with ASD can point to potentially useful biomarkers [6]**.** Many promising biomarkers have been identified for ASD, and some might foretell responses to specific treatments [7]. Factors ranging from oxidative stress, advanced parental age, and environmental triggers have been suggested as potential causes of ASD, but consistent biomarkers have not been defined so far [8].

Recent studies have suggested that metabolomics, the study of small molecules in biological systems, may have the potential to identify biomarkers that could be added to the diagnosis and treatment of autism spectrum disorder [9]. Untargeted metabolomics is one of the newest and most promising techniques available for identifying such biomarkers [10]. Using several analytical techniques that have shown distinct metabolic signatures, potential metabolic biomarkers of ASD have been found, mostly in blood and urine. [11]. When comparing the metabolomic profiles of autistic children with typically developing children, metabolomics is a promising method. Interestingly, individuals with ASD have been found to have altered lipid, energy, and amino acid metabolisms [10]. The finding of possible biomarkers by metabolomics may be an important tool for the early detection and treatment of ASD. To validate these results and evaluate their clinical applicability in the management of ASD and its diagnosis, further extensive investigations need to be conducted.

Currently, there are no specific biomarkers or body fluid tests available to validate the diagnosis of ASD. This hinders early identification and hampers any kind of therapeutic and pharmacological intervention at an early stage. According to many studies, environmental, genetic, immunological, inflammatory, and metabolic factors play an important role in ASD. However, there is ongoing research to identify reliable biomarkers in genetics, epigenetics, treatment targets, neuroimaging, and other areas to better target the underlying roots of ASD for diagnosis and treatment [12]. These factors can influence brain development, neural connectivity, and synaptic function, which in turn can affect cognitive, social, and behavioral development. Identified Biomarkers in ASD could allow early diagnosis and proper dietary and therapeutic intervention, which could greatly help our society and minimize the challenges experienced by ASD children and their families.

Figure 1 shows the prenatal and postnatal factors associated with ASD. There are various factors related to the environment which contribute to the development of ASD. These factors include genetic predisposition, environmental exposures, immune dysregulation, and motor abnormalities [13,14]. Environmental risk factors for ASD include prenatal exposure to toxins, maternal infection, and nutritional deficiencies [15]. An increased risk for somatic and psychiatric illness and premature mortality shows the cumulative impact of ASD on health-related outcomes [16]. It is important to understand how these factors interact with a genetic predisposition to contribute to ASD etiology, as ASD is a multigenic and highly heterogeneous disease that often co-occurs with other conditions [17].

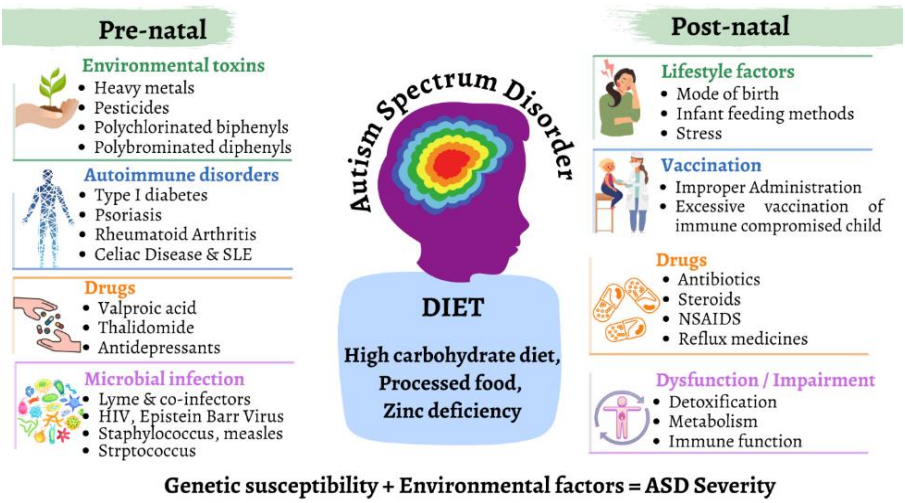

Figure 1*:*pre-natal and post-natal environmental factors related to Autism Spectrum Disorder

Due to the intricacies of ASD, omics studies, including metabolomics, are becoming the preferred method of diagnosis. Metabolomics studies, including those using whole saliva, are becoming a promising tool for identifying potential biomarkers in a multifactorial like ASD [18,19,20]. Metabolomics can quantify the metabolic profiles altered by environmental and genetic factors and can also help in by identification of those abnormal metabolic pathways, and thus identify biomarkers related to ASD. The whole saliva is a good specimen for diagnostic and prognostic purposes due to its convenience and noninvasive collection method. Furthermore, the method by which the collection of saliva is done brings less anxiety in the subject, especially in ASD children. The finding of metabolomics through biological fluids, including whole saliva, provides an extensive approach to identifying potential biomarkers in ASD [21, 22]. Salivary metabolites are equally functional and competent with blood metabolites making them an attractive alternative to blood testing [23]. Several studies have been conducted for the identification of biomarkers in saliva that could be used as a diagnostic tool for ASD children at the initial stage [24,25]. To enhance the capability of precisely identifying ASD in children in its early phases, a saliva-based biomarker panel and related algorithm have been developed. Although no specific and valid biomarker has been identified for ASD, which makes it a promising tool for ASD research [23]. This review article aims to examine the primary biomarkers found in saliva so that early diagnosis is possible in ASD children because of the significance of detecting ASD to implement intervention measures, which can improve the life of ASD children as well as their family members. Mainly different platforms are used for the metabolomics study: nuclear magnetic resonance spectroscopy (NMR), mass spectrometry (MS), Radioimmunoassay, Enzyme-linked Immunosorbent Assay (ELISA), etc. Each method has its own disadvantages and advantages.

Abnormal metabolites associated with ASD in saliva samples.

Table 1 provides a list of the abnormal metabolites linked to ASD identified in saliva samples. These aberrations showed alterations in numerous metabolic processes. The severity of ASD is closely associated with changes in hormones, particularly cortisol, and proteins. ASD has also been associated with the dysfunction of inflammatory metabolic variables, including those connected to neuroinflammation and hormonal stress as well as enzymatic, mitochondrial, oxidative, and toxic alterations [26]. Some biomarkers, such as testosterone, GSH, and MT-2, are higher in saliva in comparison with blood, endorsing the use of saliva as the preferable body fluid for the measurement of biomarkers relevant to autism. Salivary RNAs and lipids have also been explored as potential biomarkers for ASD [22, 27, 28]. A few of the metabolites which have been identified in saliva are discussed below:

Lipids: essential to the appropriate brain growth and maintenance

Many lipids in saliva are from glandular systems, while some may also diffuse directly from serum. Low levels of cholesterol across the early stages of growth can result in mental dysfunction as cholesterol is necessary for the healthy development and maintenance of the brain. Cholesterol is also crucial for the normal development of embryos and fetuses and for synaptic fusion and myelin membrane growth in the central nervous system [29]. A study based on lipidomics has been used to explore the potential of salivary lipid-based cannabis-responsive biomarkers in children with ASD [28]. Studies have found a relationship between salivary and serum cholesterol, and low levels of cholesterol have been seen in the group of autistic people compared to the normal group. As cholesterol sulfate is amphiphilic and can penetrate the placental barrier more easily than cholesterol, it is transported from the mother to the fetus. Although higher cholesterol levels have also been seen in certain studies, the low amount of cholesterol in autism may be caused by the mother's inability to provide enough cholesterol sulfate to the fetus during gestation [30].

Cortisol: Important role in emotional development.

Cortisol plays a crucial role in human homeostasis, growth, neurodevelopment, stress response, and reactivity. In normal individuals, cortisol is a marker of a prominent and consistent rhythm, with peak production in the morning [31]. According to research, there is a positive correlation between cortisol levels and the presence of stereotyped and repetitive behaviors in individuals with autism [32]. Autistic children between the ages of 3 and 10 years have been found to show an increased serum cortisol response to stress compared with neurotypical controls [33, 34]. Dysregulation of the circadian rhythm of cortisol has also been observed in individuals with autism, with evening values showing a gradual decrease over the course of sampling done in the morning and consistently elevated levels in comparison with the neurotypical group in the evening [32, 35]. In another study of autistic children between 7 and 12 years of age, low-functioning autistic children had higher mean salivary cortisol levels than the high-functioning autistic group, which did not differ from neurotypical control children [36].

Testosterone: Contrast to the relationship with aggression

The prevalence of Autism is four times higher in males in comparison to females [37]. Salivary testosterone levels and autistic symptoms in adults are positively correlated, according to a study comprising 92 male and female individuals. Studies of testosterone levels in amniotic fluid reveal that fetal testosterone is associated with sexual dimorphic elements related to cognitive and behavioral changes in ASD. The effect of testosterone on autism begins at the fetus stage only. Elevated fetal steroidogenic activity has been associated with autism, with concentrations of sex steroids and cortisol being positively associated with each other and elevated in amniotic fluid samples of males later diagnosed with autism, Asperger syndrome, or pervasive developmental disorder not otherwise specified compared to typically developing controls [38]. There is growing evidence that steroid hormone levels, particularly androgen levels, are increased in autism [39]. According to one study, prepubescent boys and girls with ASD had considerably higher salivary testosterone levels than age-matched controls [40]. In contrast to boys who are typically developing, autistic boys had serum testosterone levels that were 2.23 times higher and salivary testosterone levels that were 2.1 times higher, according to another study [41,42]. Another study found no relationship between salivary testosterone levels and autistic traits in neurotypical men [38].

Uric Acid: Considered as a marker of Oxidative Stress.

Uric acid is a nitrogenous compound and generally saliva, plasma, and urine all contain uric acid. The primary byproduct of purine metabolism is uric acid, and the presence of uric acid in urine is a strong sign of metabolic disorders. Uric acid is also considered a strong antioxidant and the pathophysiology of various neurodegenerative illnesses is successfully treated with uric acid. Studies showed that uric acid level in saliva increases significantly when compared to healthy kids at their age and it could be the reason for children with autism were found to have a higher vulnerability to developing dental caries [43]. One study also revealed that autistic children's plasma has low levels of uric acid, which has been recognized as a marker of oxidative stress and suggested as a useful treatment for ASD [44]. In children and adolescents with ASDs, hyperuricemia may contribute to unfavorable metabolic effects as well as uric acid has also been studied as a potential biomarker for ASD [45].

Oxytocin: Association with social behavior

According to recent reviews and meta-analyses, oxytocin has an important role in social behavior and cognition in humans and may also be associated with the pathophysiology of illnesses linked to social deficits, such as autism spectrum disorder. Nowadays oxytocin is a new biomarker associated with mental illness and Autism Spectrum Disorder (ASD)'s pathophysiology is thought to be significantly influenced by changes to the brain's oxytocinergic system [46]. However, there was no correlation between salivary oxytocin and ASD severity. Salivary oxytocin level estimation is gaining popularity in autism research, and some studies have suggested that administering oxytocin to individuals with ASD may reduce their symptoms. A pilot study found that salivary oxytocin concentrations increased

in seven kids and their mothers had higher salivary oxytocin levels in response to touch therapy [47]. Children with autism spectrum disorder ASD have lower amounts of salivary oxytocin than neurotypical children, according to other studies. According to a study, people with an ASD diagnosis have decreased morning levels of salivary oxytocin. However, oxytocin administration has been found to enhance brain function in children with ASD [48, 49].

Zinc: Associated with Cognitive Development:

Essential minerals and trace elements are known to contribute to several health issues and may play a role in the development of ASDs [50]. According to research zinc deficiency in early childhood may cause ASD [51]. Zinc deficiency is probably prevalent in ASD and may play a significant role in the etiology of behavioral and mood disorders. In neurological disorders and ASD, zinc interacts with the gut and brain, and zinc deficiency may be a significant factor in the development of ASD. The role of zinc in ASD needs to be established. While investigating the relationship between zinc and growing neurons there may be hope for new therapies and new understandings of the potential mechanisms underlying ASD [52]. Low salivary zinc levels may contribute to the development of autism and serve as a biomarker for early autism diagnosis [53].

Table 1 Summarizes a comparative picture of biomarkers within the saliva.

Biomarker	Metabolomic technique	Category	Function in ASD	Conclusion	Authors and year of publication
Diurnal variation of cortisol (Cortisol VAR),	radioimmunoassay (RIA)	Hormone	Low level associated with aggression	Decreased salivary levels of cortisol VAR	[54]
Glutathione (GSH), GSSG MT2 Testosterone GABA Cortisol	Mass spectrometry, Enzyme-linked Immunosorbent Assay (ELISA)	Peptide Peptide Peptide Hormone Amino acid Hormone	Low levels of glutathione and MT-2 associated with oxidative stress	Lower levels of GABA, cortisol, GSH (glutathione), and MT-2 were found, and increased levels of testosterone and total glutathione (GSSG) were found	[55]
Salivary proteins	Nano liquid chromatography-tandem mass spectrometry (Nano LC-MS/MS)	Protein	Lactoferrin is Involved with leaky gut, and it has antimicrobial activity. Thus, it is a component of the immune responses of the digestive system. Prolactin has immune system regulatory functions	High levels of lactoferrin prolactin inducible protein Ig kappa chain C region, Ig gamma1 chain C region, Ig lambda-2 chain C regions, neutrophil elastase, and polymeric immunoglobulin receptor	[56]
Alpha-amylase, CREB-binding protein, p532, Transferrin, Zn alpha2 glycoprotein, Zymogen granule protein 16, cystatin D, plasminogen	Nano LC-MS/MS	proteomes	Low levels of CREB binding protein involved in seizer and cognitive impairment in Autistic children. Transferrin is a major antioxidant	Decreased levels of CREB-binding protein, p532, Transferrin, Zn alpha2 glycoprotein, Zymogen granule protein 16, cystatin D, Alpha-amylase, and plasminogen	[57]
Oxytocin, Cortisol and Testosterone	Radioimmunoassay (RIA)	Hormone	Impairment in social interaction is associated with a low level of Oxytocin and a higher level of testosterone. Reactive aggression is related to higher cortisol level.	Lower oxytocin concentration and unchanged cortisol and testosterone concentrations.	[58]
Zinc	Atomic spectroscopy	Mineral	Low Zn concentrations are related to oxidative stress and decreased cognitive ability.	Decreased salivary zinc concentration.	[53]

Dietary Approach: For proper growth development and functioning diet plays a crucial role from the beginning of life [59]**.** There are several dietary approaches for addressing abnormal metabolites related to ASD. The dietary approach is based on three theories, 1. Gut-brain axis theory, 2. Impaired methylation theory 3. Mitochondrial dysfunction theory. (Figure 2)

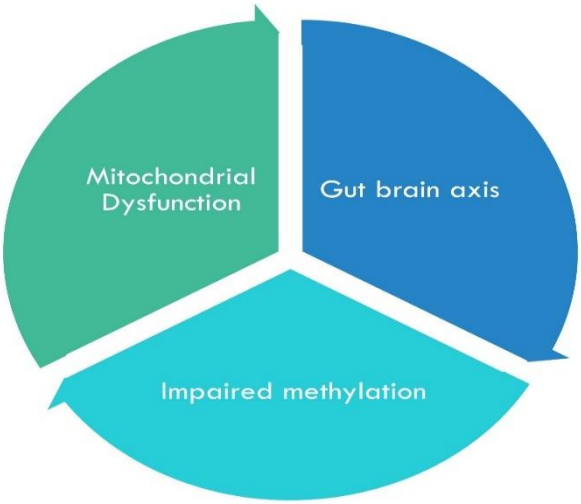

Figure2: Theories related to dietary Intervention

Mitochondrial dysfunction theory: In the population with ASD, mitochondrial disease is thought to be present in around 5.0% of cases. About 30% of kids with ASD may have metabolic variations such as elevated lactate levels or a high lactate-to-pyruvate ratio. Other mitochondrial biomarkers (pyruvate, carnitine, and ubiquinone) are significantly different between ASD and controls [60]. The central nervous system is dependent on mitochondria for several functions. Physiological consequences of Mitochondrial dysfunction in ASD are Increased oxidative stress, reduced Gamma-Aminobutyric Acid (GABA) interneuron activity, Abnormal calcium regulation, and reduced synaptic plasticity [61].

Dietary intervention for mitochondrial dysfunction: Modified ketogenic diet with MCT supplementation which produces ketone bodies is a potential dietary therapy for mitochondrial dysfunction because ketone bodies protect the brain from damage. These ketone bodies cross the blood–brain barrier and play a part in enhancing energy metabolism by the generation of Adenosine Triphosphate (ATP). It also promotes mitochondrial biogenesis and reduces oxidative stress, which contributes to restoring normal mitochondrial function and decreasing neuronal death. Ketone bodies raise levels of GABA which helps in the regulation of neurotransmitters. Mitochondrial activity is enhanced by ketone bodies that alter the gut microbiota by promoting mitochondrial biogenesis and preventing the mTOR signalling pathway that reduces the incidence of seizures [62].

Impaired methylation theory: There are two facts related to impaired methylation. according to the first fact, autistic children have low levels of metabolites like SAM (S-adenosylmethionine) and glutathione related to the methylation cycle and oxidative stress. According to the second fact, intake of casein and gluten in autistic children synthesizes opioid peptides as these children are not able to digest them properly. These opioid peptides can affect neurotransmission by crossing the blood-brain barrier. They can also pass through permeable intestinal membranes. Physiological consequences of Impaired methylation in ASD include Reduced glutathione levels in the blood and increased oxidative stress, leading to neurodevelopmental defects. Reduced level of SAM decreases the synthesis of carnitine, phosphatidylcholine, and serotonin. Low levels of carnitine alter the metabolism of Polyunsaturated Fatty Acid (PUFA) which affects language, social and cognitive development. Low levels of phosphatidylcholine damage the cell membrane leading to sensory and motor development issues. Low levels of serotonin lead to reduced levels of melatonin which causes sleep impairment [63,64]

Dietary Therapy for Impaired methylation: Gluten free casein free (GFCF) diet is commonly used for impaired methylation in ASD, as metabolic pathways providing GSH and SAM are supported by

specific nutritional factors e.g., sulfur amino acids cysteine (CYS) and methionine (MET), folate, vitamins B12, and B6, which are critical for ASD diets [65, 66, 67].

Gut-brain axis theory: The gut-microbiota-brain axis has been described as a multidirectional communication pathway between the three systems—the gut, gut microbes, and the brain—but it is still unknown whether these gastrointestinal microbes are involved in ASD and whether they can be used as a target for gastrointestinal therapies in ASD [68]**.** Abnormal gut metabolites have been linked to ASD symptoms and co-occurring GI abnormalities [69, 70]. ASD children have been found to display abnormal levels of metabolites compared to normal children, and these metabolites are majorly associated with the metabolism of amino acids, neurotransmitters, and gut bacteria [71]. Specifically, gut bacteria-derived metabolites indolyl-3-acetic acid and indolyl-lactate were more numerous in the ASD group compared to controls, according to one study [69]. Overall, abnormal levels of gut bacterial communities at the phylum level are related to ASD, while deeper studies suggest that specific metabolites produced by gut bacteria may play a role in the pathogenesis of ASD [72].

Dietary Therapy for Abnormal Gut in ASD: According to Figure 3, in the Gut-brain axis theory nutritional deficiency causes leaky gut problems and leads to behavioral problems in ASD, thus treating nutritional deficiency improves autistic behavior by improving leaky gut problems in ASD [73]. Abnormal gut in autism is associated with mitochondrial dysfunction and impaired methylation thus dietary interventions for abnormal gut include elimination diets and supplementation diets such as the GFCF diet, modified ketogenic diet, and inclusion of probiotics and nutritional supplements like Vitamin B12, folic acid, glutathione [74,75]. Supplementation with multiple probiotic strains and prebiotics showed a better result on behavioral symptoms of ASD [76]

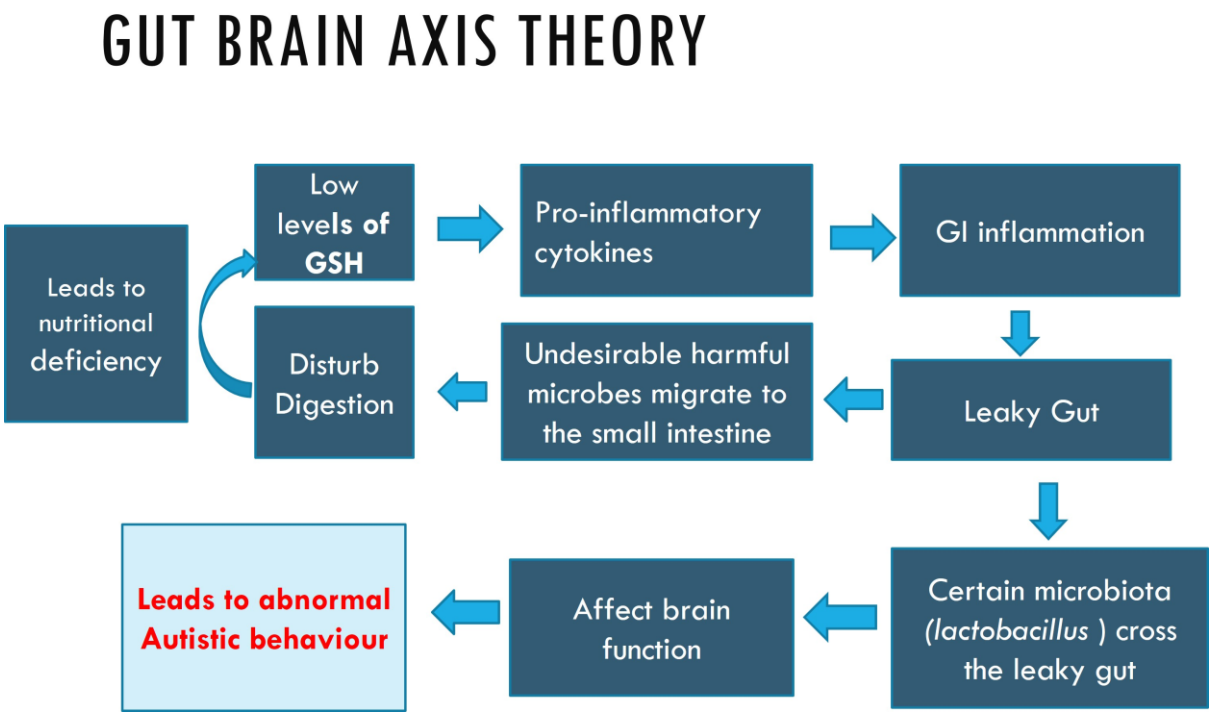

Figure3: Gut-Brain Axis Theory

Role of other dietary supplements in ASD: Dietary supplementations have been shown to improve behavioral symptoms in ASD such as vitamin D, Zinc, vitamin B6, vitamin B12, omega 3 fatty acid, and probiotics [77]. A study shows that omega-3 supplementation increases social communication and stereotyped behavior, which are two hallmarks of autism [78,79]. Another study shows

combining omega-3 and vitamin D supplementation has a positive impact on social and behavioral symptoms in ASD patients [80]. The abnormal regulation of the body's response to stress in people with ASD may be a factor responsible for behavioral and increased anxiety symptoms. Cortisol, oxytocin, and vasopressin are just a few of the hormones crucial to the stress response and linked to the onset and maintenance of stress-related symptoms in ASD. A well-balanced and nutritious diet may enhance overall health and well-being, which may indirectly affect cortisol levels. However, no diet has been shown to reduce cortisol levels in people with ASD [81]. According to studies, testosterone levels may be higher in children with autism spectrum disorders (ASD) than in neurotypically developing children. Furthermore, no research has been done on the effect of nutrition on controlling testosterone levels in children with ASD. Low zinc levels have been linked to autism, according to research. Patients with autism may benefit from nutritional therapy using zinc supplements [82]. In mice models of autism spectrum disorders, dietary zinc supplementation has been demonstrated to restore fear-based learning and synaptic function [83]. In a different study, exon 13–16 mutant mice were given dietary zinc supplements to prevent autism-related symptoms and striatal synaptic dysfunction [84]. However, more research is needed to know the effect of dietary supplements in improving the behavioral symptoms of ASD.

Table 2 Summarizes different types of dietary interventions and their outcome

Type of Diet	Composition	Duration of Dietary Intervention	Outcome	Reference
Ketogenic Diet	No details were given by the author	6 Months	All subjects showed positive changes in CARS score.	[85]
Modified ketogenic Diet with MCT supplementation	Carbohydrates 20-25 g/day Protein Intake based on the child's age and weight maximum allowed two times of RDA. Fat, the rest energy MCT oil to comprise 20% of energy needs	3 Months	A modified gluten-free ketogenic diet with supplemental MCT is a potentially beneficial treatment option to improve the core features of autism spectrum disorder.	[86]
Ketogenic Diet	Ketogenic ratio (grams of fats: grams of proteins+ carbohydrates) from 4:1 to 2:1 in every meal	16 months	The study concluded that the ketone diet shows tremendous improvement in behavior, eating habits, and tantrums of Autistic children.	[87]
Modified Ketogenic Diet	Carbohydrates 20-25 g/day Protein Intake based on the child, s age and weight maximum allowed two times of RDA. Fat, the rest energy MCT oil to comprise 20% of energy needs	3 months	Showed positive behavioral outcomes in some, but not all, children with ASD.	[88]
Modified Ketogenic Diet	60% of the calories are from fat sources, 30% from proteins, and 10% from carbohydrates. This is less fat than a standard 4:1 KD (90% fat) but more than a typical diet (35% fat). Carbohydrates were limited to 8–10 g per day	6 months	Significant score changes were observed in CARS score after dietary intervention.	[89]
3 Month normal Diet and 3 months GFCF diet	Gluten and Casein rich food excluded from the diet	6 months	Study showed non-significant differences were observed in autistic behaviour and urinary peptides.	[90]
Gluten-Free, Casein-Free, Soy-Free Diet (HGCSF).	Gluten-free, casein-free, and soy-free diet with vitamin, mineral, fatty acid, and carnitine supplementation Adequate amount of calorie and Protein, variety of vegetables	6 months	Significant changes were observed in CARS score in the treatment group in comparison to the non-treatment group.	[91]
Gluten-free diet	Gluten-free diet consisted of gluten-free pasta and biscuits and gluten-free bread	6 Weeks	Gluten-free diet showed significant Behavioural improvement in autistic chidren	[92]
Gluten-Free, Casein-Free	Removal of casein and gluten-containing food items	12 weeks	Non-significant changes were observed in Autistic behaviour.	[93]
Gluten- and dairy-free	Suppl. gluten + milk powder for the control group vs. whole rice flour for the experimental group	6 weeks	Non-significant improvement in either behavioural or intestinal permeability levels.	[94]
Probiotic Supplementation	lyophilized powder mixtures contenting *Lactobacillus, Bifidobacterium, and Streptococcus thermophilus* twice a day	3 months	Significant improvement in autistic behaviour symptoms.	[95]
Probiotic supplementation	*Bifidobacterium* and *Lactobacillus* species 108 bacteria/g, 10 grams daily feeding product was prepared from whey powder (without casein) and some minced cooked yellow vegetables in adequate ratios fortified with the probiotic strains	3 months	Improvement on the autism scale, Reduced GI symptoms, improvement obsereved in communication skills, and reduction in hyperactivity.	[96]
Probiotic with oxytocin supplementation	Combination therapy of daily 2 capsules of lactobacillus plantarum (6×1010 CFUs) for 28weeks and oxytocin starting on week 16	28 weeks	Improvement in social and behavioral measurements of Autistic children who were on combination therapy of probiotics and oxytocin in comparison to the placebo group.	[97]

Type of Diet	Composition	Duration of Dietary Intervention	Outcome	Reference
Probiotic supplementation	3×10^{10} CFUs of probiotics of lactobacillus plantarum (PS 128) daily for less than 30 kg and 6×10^{10} CFUs for higher weight	45-127 months	Clinical Global Impression (CGI) scores of Autistic children improve with the intake of probiotics. Subjects taking lactobacillus plantarum (PS 128) have fewer side effects and greater improvement compared to those who have taken other probiotics.	[98]
Probiotic supplementation	2 packets/day in the first month and 1packet/day in the following 5 months Each packet contained 450 billion of eight probiotic strains: Streptococcus thermophilus, Bifidobacterium breve, Bifidobacterium longum, Bifidobacterium infantis, Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus para-casei, Lactobacillus delbrueckii subsp. bulgaricus	72 Months	A significant improvement was observed in GI symptoms sensory profiles and autistic behavior in ASD children in comparison to the placebo group.	[99]
Probiotic supplementation	3 strains (Lactobacillus rhamnosus, Lactobacillus paracasei, and Bifidobacterium longum) of probiotics were given in the form of powder once daily. Doses: $20x10^9$ CFUs Lactobaillus rhamnosus: $6x10^9$ CFUs Lactobaillus paracasei: $10x10^9$ CFUs Bifibidobacterium longum: $4x10^9$ CFUs	6 weeks	The study showed significant changes in speech, social behavior, and sensory awareness.	[100]

Conclusion and future prospect:

Autism spectrum disorder (ASD) typically starts in childhood and lasts through adolescence and adulthood. During the first two years of life, the problems are frequently not noticeable. Early intervention during early childhood plays an important role in autistic children, hence the diagnosis of ASD at an early stage is very important to reduce the burden of ASD on the family as well as on society. This emphasizes the critical need for the development of easily available, non-invasive, and affordable diagnostic tools that aim to detect ASD on an early basis as well as also help in early treatment either with dietary intervention, or pharmacological intervention.

Saliva is a non-invasive biological fluid that can reveal important details regarding the underlying metabolic alterations in people with ASD. The development of focused therapeutics and individualized treatment regimens can benefit from the use of salivary metabolomic indicators in ASD research. As discussed, there is a research gap in the identification of salivary metabolomic biomarkers for ASD. To verify these indicators in larger clinical trials and to investigate the potential of salivary metabolomic biomarkers in ASD, more studies are required. Using biological fluids like saliva provides non-invasive tests for ASD indicators has great potential. The identification of biomarkers from saliva for ASD could be useful for neuro-physicians as a diagnostic tool as well as for their treatment, which currently lacks a definite therapy. Metabolomic biomarkers can also be a beneficial tool for dieticians for treating nutritional deficiencies / metabolic disorders. More research is required to properly comprehend the possible use of salivary metabolites as ASD biomarkers as there have been limited studies on salivary-based metabolomic biomarkers in ASD. Data from this review supports the idea that saliva is a suitable body fluid to assess metabolomic biomarkers related to autism as well as dietary intervention like ketogenic diet with probiotic supplementation can be used as a potential tool for the correction of these metabolites especially metabolites related to oxidative stress**.** In conclusion, analysis of salivary biomarkers has shown promise in revealing the possibility of biomarkers for ASD which can be utilized as a promising tool for dietary intervention.

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