Metabolic Therapies in Autism Spectrum Disorder: A Literature Review

By

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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
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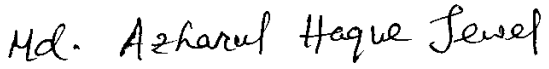

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Approval

The thesis/project titled “Metabolic therapies in autism spectrum disorder: A literature review” submitted by Md. Azharul Haque Jewel (13146048) of Spring, 2013 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons.) on February, 2022.

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Ethics Statement

This study does not involve any kind of animal trial or human trial.

Abstract

Autism spectrum disorder (ASD) is a neurodevelopmental condition that involves difficulty in social communication, emotions and regular behaviors. The pathogenesis of the ASD is poorly understood and the treatment option is still limited. Variety of drugs are being used to manage the medical and psychiatric comorbidities associated with ASD and numerous drugs are still in clinical trials. Currently, some conventional treatments such as speech therapy, occupational therapy, social skills training and counseling are still in the treatment of ASD in children. Scientists are considering metabolic therapy in the treatment of ASD as there is no direct treatment and pharmaceutical therapies are only able to treat the symptoms of ASD. Therefore, the aim of this review work was to compile the existing information about metabolic therapies and their effectiveness in ASD. Furthermore, this review also includes some popular diets with their evidences that are currently in under investigation for ASD.

Keywords: Autism spectrum disorder (ASD); nutrition in ASD; metabolism in ASD; metabolic therapy; evidences of metabolic therapy; special diets.

Dedication

Dedicated to my parents

Acknowledgement

Firstly, I am grateful to almighty Allah for making me able to choose this field and study Pharmacy. Without His blessings, I would not be able to continue this project paper and submit it for passing my Bachelor's degree in Pharmacy.

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List of Acronyms

ASD	Autism Spectrum Disorder
TMS	Transcranial Magnetic Stimulation
PET	Positron Emission Tomography
SPECT	Single-Photon Emission Computerized Tomography
MCT	Medium Chain Triglyceride
KD	Ketogenic Diet
MECP2	Methyl-CpG-Binding Protein 2
BTBR	Black and Tan Brachyury
EL	Epileptic anormaly strain of mice
VPA	Valproic Acid
GFCF	Gluten-Free and Casein-Free diet
DHA	docosahexaenoic acid
EPA	Eicosapentaenoic Acid
Hcy	Homocysteine
AA	Amino Acid
GABA	Gamma-Aminobutyric Acid

Chapter 1

Introduction

1.1 Autism spectrum disorder (ASD)

Autism spectrum disorder (ASD) is a neurodevelopmental condition that involves difficulty in social communication, emotions and regular behaviors. ASD can start in early childhood but it's effects may remain lifelong. According to the World Health Organization (WHO), 1 in 160 children suffer from ASD worldwide (WHO, 2017). The prevalence of ASD is increasing day by day. Scientists are trying to establish some factors behind the increase of Autism related disorders. First, modern diagnostic criteria and concepts may play a vital role in the prevalence. Second, awareness between general people and professional communities has increased. Third, the development of diagnostic instruments and their accuracy has been improved over the time (Takumi et al., 2020).

However, the pathogenesis of the ASD is poorly understood and studies suggest that along with environmental factors, genetic components act as triggering factors (Baronio et al., 2015). Most of the ASD cases are known as idiopathic ASD which is referred to as ASD without a known specific cause. The factors behind ASD can be multidimensional like genetic and/or environmental (Chen et al., 2021). Proposed environmental factors include maternal immune activation and paternal exposure to toxins. On the other hand, genetic factors are de novo mutations which can be single or polygenic mutations in nature (Varghese et al., 2017). The severity of ASD can vary from individual to individual that include a wide range of activities such as lack of interest in communication, difficulty in social interactions (Mierau & Neumeyer, 2019). Moreover, presence of restricted repetitive behavior or activity patterns can be observed in ASD individuals that

adversely affect social, occupational, or other areas (Kodak & Bergmann, 2020). ASD can also produce other neurodevelopmental conditions such as epilepsy, soft neurologic motor signs and non-neurologic conditions such as gastrointestinal and cardiac problems (Ramaswami & Geschwind, 2018).

1.2 Diagnosis of ASD

Despite many ongoing research, diagnosis of ASD at an early age is still an obstacle. Various studies found out some broad range of immunologic, toxicologic, oxidative stress and neurological biomarkers to interpret the condition with ASD. It is a matter of fact that no reliable biomarker has been identified yet to properly diagnose ASD. Another recent perspective to identify ASD is to examine different parts of the brain by magnetic resonance imaging (MRI). For example, one study suggests that the cortical surface area may enlarge between 6 to 12 months of age for infants with ASD (Hazlett et al., 2017). Again, transcranial magnetic stimulation (TMS), positron emission tomography (PET) and single-photon emission computerized tomography (SPECT) are other modern scientific approaches to diagnose ASD (Choueiri & Zimmerman, 2017).

1.3 Treatment Options for ASD

The physicians and psychiatrists are using a variety of drugs to manage the medical and psychiatric comorbidities associated with ASD such as seizures, anxiety, attentional deficits, sleep disturbances, and gastrointestinal problems (Neumeyer et al., 2019). There are numerous drugs still in clinical trials such as oxytocin, arbaclofen for improved social behavior and only few drugs are FDA-approved for example risperidone, aripiprazole for the treatment of aggression and irritability in ASD. However, these pharmaceutical agents are responsible only for treating the symptoms of ASD and careful consideration must be taken as they can produce adverse effects

which can vary patient to patient (Peñagarikano, 2015). They said, there is no direct treatment option available for autism spectrum disorder at this moment. Treatment options for ASD are still limited due to its complex etiology and multidimensional development process. At present, some conventional treatments such as speech therapy, occupational therapy, social skills training and counseling are used in the treatment of ASD in children (Choueiri & Zimmerman, 2017).

1.4 Mitochondria, Metabolism and ASD

Metabolism is a biochemical process that can be defined as the process of converting food into energy and eliminating the waste. Metabolism starts with digestion and absorption which continues at cellular and organ level (López-Otín et al., 2016). According to various studies, any disturbances in these processes of metabolism can contribute to ASD. Since, ASD has extreme etiological diversity, evidence suggests that mitochondrial function can be one possible factor to explain metabolism process as mitochondria is known as the powerhouse of the cell. Mitochondria is responsible for cellular metabolism, intracellular calcium signaling and production of reactive oxidative species. It also regulates both adaptive and innate immunity of the individuals (Cheng et al., 2017). Furthermore, human brain consumes 20% of the energy as Adenosine Triphosphate (ATP) produced by mitochondria while the brain is only 2% of the total body weight (Raichle & Gusnard, 2013). Moreover, mitochondria is responsible for proliferation, survival and development of natural stem cells (Cheng et al., 2017). Therefore, in general, mitochondrial dysfunction can cause abnormal metabolism. Studies suggests that, 30% of the children with ASD may experience metabolic abnormalities and one-third of them reported dissimilarities in mitochondrial biomarkers compared to control groups (Rossignol & Frye, 2012).

As mentioned before, ASD can result in some co-morbidities such as epilepsy, seizures and those co-morbidities also explained the mitochondrial and metabolic dysfunctions. Various studies found seizures as biochemically confirmed mitochondrial disorder (Rahman, 2012) and gastrointestinal dysfunction which is a common ASD co-morbidity as mitochondrial disease (Richard E. Frye & Rossignol, 2016). Moreover, several animal model also suggest that linkage between ASD and mitochondrial dysfunction is present (Correia et al., 2006) (S. Rose et al., 2014).

1.5 Rationale of the Study

A wide number of studies showed that there is no direct treatment of ASD. Only few drugs are in clinical trials and the use of antipsychotic drugs in ASD is limited due to their adverse effects and the complexity of autism. Generally, the physicians and psychiatrists are using some medicines such as risperidone, aripiprazole to control the behavior and comorbidities in ASD. As mentioned earlier, some of the conventional therapies like speech therapy, occupational therapy is being used as treatment option of ASD. However, these treatments are time consuming and the results are not impressive as expected. Early diagnosis of ASD is important to choose the treatment option. Metabolic therapy can be an impressive addition to the diagnosis and treatment of ASD. Metabolic therapy has less adverse effects compare to the pharmaceutical agents which is significant in case of ASD. As a result, metabolic therapy should be considered as a significant treatment option in autism spectrum disorder to improve the quality of life.

1.6 Aim of the Study

This review aims to compile the existing information and studies about metabolic therapy and its effectiveness in autism spectrum disorder. It will also represent different types of diets in ASD.

1.7 Objectives of the study

The objectives of this study were to:

1) evaluate the metabolic abnormalities associated in autism spectrum disorder.

2) evaluate the existing metabolic therapies in ASD,

3) find out the effectiveness of metabolic therapy as a treatment option of ASD.

Chapter 2

2. Methodology

To conduct this literature review, key words are searched in a structured way at different databases such as PubMed, Elsevier. Most used keywords are ASD, autism spectrum disorder, metabolism, metabolic therapy, treatment of ASD, metabolic intervention, special diets, nutrition in ASD. During searching, the time period of the published article has been customized from 2005 to 2021 to find the most recent publications. Furthermore, cross-referencing technique is used to find additional information and journals.

Chapter 3

Findings and Discussion

3.1 Nutrition in ASD

Nutritional deficiencies or inappropriate choice of nutrition could be a risk factor in autism. Several studies suggests that maternal nutrition can affect the neurodevelopment of progeny (Sullivan et al., 2014). Literature evidences also suggests that during pregnancy, metabolic status and diet choices are positively connected with the mental health disorder development of the children. At the same time, some literature demonstrates ASD based on deficiency and excess of nutrients. Most of the cases, ASD patients show food selectivity and habitual eating behaviors (Peretti et al., 2019). Food selectivity and sensitivity can cause intolerance to a specific diet thus resulted inflame to the brain that affects the physical and emotional feelings of the ASD children (Buehler, 2011). Some studies showed that iron concentration is connected to the symptoms of ASD (Dosman et al., 2007) while another study found no association (Reynolds et al., 2012). Similarly, contradictory result has been found in case of keto diets, fatty acids and gluten-free diets in ASD patients (Peretti et al., 2019). In sum, there is a scarcity of literature to clarify the effects of diets on ASD. Further study and investigation are needed in this area to overcome the limitations and improve the results.

3.1.1 Maternal Diet and Risk of ASD

As mentioned earlier, maternal food habit, choices and metabolic activity can negatively affect the development of neonate's mental health. Some studies found mothers' diets during pregnancy as a determinant of neural development (Schmidt et al., 2012). A case-control study found that during

pregnancy, methanol containing food has a connection to the children with ASD and high fat diets are associated with the risk of autism in offspring (Sullivan et al., 2014). Due to the growing placenta and fetus, nutritional deficiency is common during pregnancy which affects the neural development of the children. Other study found that vitamins and folic acid is associated with the minimization of ASD risk (Schmidt et al.,2011). One study found negative result while searching correlation of maternal fish oil intake and ASD risk (Suren et al., 2013). However, scientists are recommending to conduct more research in this regard as ASD is complex and has unknown etiology. But it is proven that mental health of pregnant mothers can affect the neurodevelopment of the fetus which is a risk factor of ASD development.

3.1.2 Diet in Children with Autism

In ASD, feeding the patients is one of the common reported problems due to the food sensitivity, selectivity, metabolic abnormality and gastrointestinal problems. In some cases, food colors, shape and temperature can also cause food sensitivity to the ASD patients (Postorino et al., 2015). Parents faces difficulty introducing new foods such as vegetables, fruits and due to the feeding difficulties, it is found that most of the children with ASD shows nutritional deficiencies (Evans et al., 2012). Literature evidence found vitamins, minerals and fatty acids as most common deficiency in ASD children (Ranjan & Nasser, 2015). As consequence, parents are intended to use supplement to compensate the nutritional deficiencies of children and most supplements are multivitamin and mineral supplements (Nuttall, 2017).

3.2 Metabolic Therapy in ASD

Due to the adverse effects of pharmaceutical treatment strategy and less direct efficacy against ASD, scientists are considering alternative treatment option for autism. Metabolic therapy in ASD is being investigated for past decades. According to some studies, parents are considering metabolic therapies in ASD to improve the comorbidities associated with ASD by eliminating or adding different types of foods into the diets (Chidambaram et al., 2020). For example, one study found ketogenic diet (KD), a high-fat and low carbohydrate diet effective against epilepsy which is a common comorbidity of ASD (Neal et al., 2008). The mechanism of metabolic therapy in ASD is still not clear properly due the complex etiology of ASD, but it showed promising result against ASD, sleep disorder, brain trauma, stroke, Parkinson's disease and multiple sclerosis. However, clinical models for metabolic theory in ASD is limited and further research is necessary to show the promise of the therapy (Cheng et al., 2017).

3.2.1 Metabolic Therapy for ASD: Clinical Evidence

Clinical study of metabolic therapy in the ASD treatment is limited. One study was conducted on 18 children with autism age ranges from 4 to 10 years (Evangeliou et al., 2003). They investigated with medium chain triglyceride (MCT) diet and found improvement for most of the children on the autism rating scale. Among 18 children with autism, 2 showed significant, 8 showed average and 8 showed minor improvement in Childhood autism rating scale. Another study was conducted with Ketogenic diet in a group of 6 autistic child and the result was promising. Out of 6 participants, significant, average and minor improvement was noticed in one, two and three participants respectively (Spilioti et al., 2013). Ketogenic diet is also found effective against comorbidities and to improve the cognitive behavior.

One control study was conducted for 6 months where children are divided into two groups; the ketogenic diet (KD) group and KD was stopped two months prior in the non KD group. The researchers examined the outcomes based on the standard diet protocol that are available globally. On that research, they selected children whether they are autistic only, cognitively impaired only or both. After statistical analysis using student t-test, Pearson test and chi-square test, among 20 children where 11 children were in the KD group and they showed improved behavioral pattern compare to rest of the 9 children who had stopped ketogenic diet. They found difference in seizure frequency among the groups which was 16±8 for KD group and 99±52 for non-KD group. However, they suggested further research in this area to find better and more retrospective and prospective results (Eapen, 2012).

Another study on a 4 years old child was done with gluten and casein-free for several years and the result was extraordinary. The child had less eye contact while talking, unexplained tantrums, less social interest, stereotypies and hypersensitivity. She also had irregular bowel movements with diarrhea, moaned every morning and continuously ill. In autism rating scale, her scored was 49 where 30-37 represent mild autism and 38-60 represent severe autism. After, introducing the gluten and casein-free diet, the researchers found that her speaking ability and auditory sensitivity improved. They also noticed that her tantrums and morning moaning was reduced. After two years of study, they found 50-points improvement in her intelligence quotient. Furthermore, at the age of 11, the researchers performed another assessment and found 70-points intelligence quotient compare to the time of her autism diagnosis and her autism rating scale reduced to 17 which is in the non-autistic range. However, they suggested that their study might influenced by her age and puberty as they conducted the study from age 4 to 12 years (Herbert & Buckley, 2013). In summary, although the researches are limited, however, the results from available evidence are

promising. Metabolic therapy such as KD, gluten-free diets can be used to improve the cognition behavior. As mentioned earlier, most of the ASD patient showed significant clinical improvements when received metabolic therapy. However, it would be important to know the relation between effects of diets and individuals' metabolic profile of ASD patients.

3.2.2 Metabolic Therapy for ASD: Animal Models

Numerous animal models are showing positive results for metabolic therapies with restricted diets. To explain, Rett syndrome is a neurodevelopmental disorder characterized by impairment of motor functions, and intellectual disability and it is caused by the mutation of methyl-CpG-binding protein 2 (MECP2) gene. Rett syndrome in children can show autism like behavior (Percey, 2010). One study found that MECP2 mutant mice shows impaired motor function and anxiety compare to controlled mice group. In that study, anxiety and motor impairment of the mutant group was treated with restricted diet or KD. In Rett syndrome, the mice usually show motor deficiency to hang on a wire and interaction deficiency. The study found that both KD and restricted diet improves the motor activity and behavior of the mice. Furthermore, the study also found increase activity of the mice in the open field study after introducing KD and restricted diets (Mantis et al., 2009).

Animal models for ASD have been characterized by behavioral tests. One common mice breed for ASD is BTBR (Black and Tan Brachyury) strain which is characterize by deficits social interaction and communication and repetitive behaviors. The BTBR mice showed improved social behavior in three chamber test in a food preference assay in the present of KD (Ruskin et al., 2013). Another study measures the amount of dietary carbohydrates in a food item that affects the blood glucose level of the BTBR mice and alter the biochemical behavior of the mice. This data explains that

diet can express the autism spectrum disorder (Currais et al., 2016). Epilepic anormaly strain of mice (EL) is another breed of mice characterized by ASD- associated behaviors and epilepsy. One study was conducted on EL mice group where KD was fed for 3 weeks and improvement of social behavior and decreased repetitive behavior was noticed (Ruskin et al., 2017). To prove the environmental factor of ASD, effects of valproic acid (VPA) exposure during pregnancy was tested in some studies which is one of the common animal models of ASD. One study was conducted on this matter. The study suggested that exposure to VPA during pregnancy as an antiepileptic drug in case of epilepsy during pregnancy, increased the risk of ASD in the children. However, the study was conducted on 628 pregnant women and found that for untreated epilepsy during pregnancy resulted zero neurodevelopmental disorders. (Bromley et al., 2013). It is found that KD treatment can improve the behavior pattern in juvenile rats that were exposed to VPA during pregnancy.

Furthermore, the study also found that KD can improve the mitochondrial respiration caused by VPA exposure during pregnancy (Ahn et al., 2014). To sum up, it is clear that ketogenic diet or other metabolic therapy are producing promising results in animal models of ASD. However, the mechanism of metabolic therapy in ASD is still unclear and only few number of animal models and human tissue models are conducted in this area (Cheng et al., 2017). In this study, some of the proposed mechanisms of metabolic diet in ASD will be provided later. More studies are required to utilize the animal models of ASD by incorporating different behavioral tests to prove the effectiveness of metabolic therapy in ASD.

3.3 Special Diets in ASD

Due to the food sensitivity, selectivity and difficulty feeding, parents and caregivers are interested in special diets in ASD. As mentioned earlier, food habit has an impact in neurodevelopment of the children and scientists are considering metabolic therapies as an alternative treatment option for autism and its comorbidities. However, metabolic therapy may produce additional expense to the family. Appropriate cautions must be taken before eliminating or adding of any kinds of diet to avoid nutritional deficiencies or excessiveness'. Table 1 summarized the popular diets in ASD.

Special diets & supplements	**Proposed target of improvement**
Gluten-free and casein free diets	Social and autistic like behavior
Vitamin D	Core ASD symptoms
Ketogenic diet	Cognitive and social skills
Iron diet	Cognitive behavior
Fatty acid	Core ASD symptoms
Folic acid	Language skills
Probiotics	Gastrointestinal tract function

Table 1 *Popular diets in autism spectrum disorder (ASD)*

3.3.1 Gluten-free and casein-free diet (GFCF)

One study was conducted on 14 children and found no significant relationship between GFCF diet in improving autistic behavior (Hyman et al., 2016). On the contrary, another study on 20 children

found improvement in social behavior after GFCF diet (Knivsberg et al., 2002). Another study was conducted for 12 months on 73 children with GFCF diets. They concluded the result by comparing the ASD group with a control group and the results were measured in Autism Diagnostic Observation Schedule (ADOS) and Gillium Autism Rating Scale (GARS). ADOS comprises of four modules to test language capabilities while GARS measure the symptoms of ASD by 56-item questionnaire. After the study, the researchers found that GFCF was able to improve the ASD symptoms (Whiteley et al., 2010). Again, another survey-based study was done among the children where food sensitivity, allergies and GI symptoms are reported by parents. The result was positive that showed improvement in social skills and behavior after GFCF diet (Pennesi & Klein, 2012). The mechanisms of GFCF in ASD is not clear yet, but there some proposals exist. For example, gluten sensitivity with or without intestinal pathology can be responsible for immune response allergic reaction to poor digestion of dietary products that can cause damage to the small intestine. This is known as gluten-sensitive enteropathy or celiac disease. (Catassi & Fasano, 2008).

Another approached mechanism is opioid-excess theory which referred autism as a result of metabolic disorder. According to this theory, opioid peptides is a metabolite from gluten-casein and affect the neurotransmitter after passing through permeable intestinal membrane and bind with the opioid receptors. Autistic children experience small bowel inflammation as are sensitive to gluten and allow opioid peptides to the brain (Baronio et al., 2015). To sum up, the evidence to support GFCF diet in ASD is incompatible. Further studies are necessary on this topic.

3.3.2 Ketogenic Diet (KD)

Ketogenic diet is consisting of low-carbohydrate, moderate protein, high-fat and characterized by metabolism of ketone. The goal of KD is to improve mitochondrial function, reduced blood

glucose and increase blood ketone (Ruskin et al., 2013). As mentioned earlier in chapter 4.2, one study showed that KD improved the intelligent quotient and reduced the score in autism rating scale of a girl (Herbert & Buckley, 2013). However, this was not a generalized data. Another study was conducted in 30 ASD children by feeding KD for six months. Among all the participants, only two showed significant improvement by reducing 5 points in autism rating scale while rest of the participants produce average improvement in social behavior. They also found consistent improved behavior for a long time even after the ketogenic diet termination. They also added that one possible hypothesis of ketogenic diet in ASD could be deficient glucose oxidation behavior in ASD where ketone is used as energy in the brain because they found four patient with abnormal ketone bodies after a glucose loading test (Evangeliou et al., 2003). Other proposed mechanism of KD includes direct actions on ion channels (Ma et al., 2007) and increase of gamma-Aminobutyric acid (GABA) (Yudkoff et al., 2007). Though KD showed some promising result in animal models of ASD, due to high fat diets, it can be problematic for ASD patients. Again, literature evidences of KD in ASD is still limited and more research is needed in this area.

3.3.3 Vitamin D

Vitamin D supplements in children with ASD showed great potential to improve symptoms of ASD. Some experiments produced extraordinary result regarding this. To begin, one study was conducted among 106 ASD children where vitamin D3 was given daily for three months and about 80 % patients showed significant result in autism rating scale (Saad et al., 2016). Another study was conducted on 32 months of child with ASD and vitamin D deficiency by intramuscularly administration of vitamin D for two months. After two months, extraordinary improvement of ASD symptoms was exhibited in the autism rating scale and reduced severity of illnesses (Cheng et al., 2017). However, this was on a single child. Randomized study of 109 ASD people by

providing 300 IU vitamin D/kg/day or placebo for four months was done in a study and improvement in the experimental group was recorded compare to the group without vitamin D3 (Moretti et al., 2005). Vitamin D is a steroidal hormone and little amount can be derived from diet and generally synthesized in the skin.

The link between vitamin D and autism is still not clear. However, some proposed theories are present to prove the association between vitamin D and autism. For example, one study stated that vitamin D receptors and metabolizing enzymes are found in the immune cells, placenta, brain, cortex and hippocampus and this theory indicates the link between vitamin D and brain development and function (Wang et al., 2021).

Another study stated that vitamin D has effects on neural differentiation, proliferation and apoptosis, and immune modulation (Bivona et al., 2019). Another possible theory is regulation of gene expression. One study found 223 ASD regulated gene are vitamin D3-sensitive genes. From this, the authors concluded that it is a possibility that ASD genes might be regulated by vitamin D (Trifonova et al., 2019).

One important study was performed during pregnancy of mothers of ASD children until next three years to see whether ASD was develop in the children or not. The result was evaluated at the age of 18 months of the child by using autism screening test and at the age of three by a questionnaire about autism. If the mother was treated with vitamin D during pregnancy's, it has been recorded as less risk for the development of ASD in children (Stubbs et al., 2016). However, there are some studies available that are failed to show connection of vitamin D with ASD (Cheng et al., 2017) Vitamin D approach looks like promising, but further investigation is needed to understand and validate the significance of vitamin D in ASD.

3.3.4 Iron Diet

Iron deficiency is more common in ASD patient than normal population and plays important role in cognitive development. One study was conducted in this matter on 122 children with ASD or without ASD and without development delay. The result was significant and the authors found about 7.5% children with ASD and 5.5% children without ASD has iron deficiency (Sidrak et al., 2014). ASD is associated with developmental delay and association of iron deficiency with delay development can be explained by some theories. Firstly, iron deficiency can cause disturbances in the neurotransmitter metabolism process. Secondly, it can alter the energy metabolism in the brain which can cause impaired cognitive development (Beard, 2003). Scientists are also suggesting that may be the altered neural network and chemistry of neurotransmitters in ASD is responsible for iron deficiency. Therefore, the alteration can result late neurodevelopment and behavioral problems (Hergüner et al., 2012). In order to identify the relationship between ferritin in the blood and sleep disturbance, a study was conducted for eight weeks with iron supplement. The result was extraordinary and 77% of children showed improvement in sleeping after iron supplement. From this, the authors concluded that iron deficiency can be related to sleep disturbance (Dosman et al., 2007). However, further research is needed in this manner to confirm the relationship between iron levels and ASD. On the contrary, another study did not found any relationship with iron deficiency and ASD (Reynolds et al., 2012).

3.3.5 Fatty Acid

There are very few studies that are showing significant result of using fatty acid supplements in ASD. Most of the evidences are conflicting the role of fatty acid in ASD (Cheng et al., 2017). However, one study conducted a population-based study of fatty acid to improve the

cardiovascular and cognitive function. The result showed effective at improving the brain health (Derbyshire, 2018). Another omega-3 supplement experiment showed positive response with autism , but the result was insignificant to show the differences between experimental and control group (Cheng et al., 2017). Similarly, another study was conducted to find the effectiveness of omega-3 in ASD children with supplement compare to ASD children with placebo. Again, the result was positive but insignificant due to the less sample size (Bent et al., 2011). The association of omega-3 fatty acid is not clear. However, the study found differences in fatty acid profiles in treated vs placebo groups. As evidence suggested, omega-3 fatty acid may contain effectiveness in major depressive disorder and can reduce the symptoms of depression upon taking the supplements (Turnbull et al., 2008).

Another study stated that omega-3 fatty acid can modulate the signal transduction pathways in the brain. They further added that omega-3 fatty acid consists of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) and they have inhibitory effect in the cell signaling pathway. So, omega-3 fatty acid can works as similar way to mood stabilizers (Marangell et al., 2006). Another randomized controlled study showed negative effects of omega-3 supplement in ASD where the authors did not find any significant differences between control group and placebo group (Mankad et al., 2015). To sum up, may be large trials are needed to find out the health benefits of fatty acid in ASD.

3.3.6 Folic Acid

Humans are unable to synthesize folic acid and solely dependent on dietary sources. Folic acid deficiency can resulted impair cellular methylation and unbalance redox homeostasis which can be a contributing factor in ASD. In autism children, reduced glutathione redox status, decreased

methylation capacity had been found. Thus, it is suggesting that there might be an association of folic acid with ASD. An extraordinary study of 66 ASD children was conducted by folic acid supplement two times daily for three months. The result was measured by Autism treatment evaluation technique and psychoeducational profile. The researchers found improvement at communication, language and expressions (Sun et al., 2016). One study suggested that folate deficiency can cause high level of homocysteine (Hcy) which is a glutamatergic receptor agonist that can alter the glutamatergic transmission in the brain and affect the communication skills (Puig-Alcaraz et al., 2015). Thus, it approaches association of Hcy metabolism and communication deficit in autism. The study of folic acid in ASD is also underway and could be beneficial to improve the cognitive function and behavior.

One study found effective improvement in cognitive function on a child who did not have ASD but had seizures, developmental delay and psychomotor regression (Moretti et al., 2005). Another 12 weeks long control based study found improvement at verbal communication in treated group with folic acid supplement compare to the placebo group (R. E. Frye et al., 2018). Most of the studies showed significant result at improving behaviors and cognitive skills in ASD. May be folic acid can play a promising role in the neural development of ASD children. However, it will be interesting to see how this diet works in a large population of sample in the future research.

3.3.7 Probiotics

Dietician and caregivers are experimenting probiotics to find out the effectiveness at improving gastrointestinal symptoms or cognitive symptoms. Mainly, scientists are considering probiotics to improve the gastrointestinal disturbances like gut permeability which is a potential problem in ASD (D. R. Rose et al., 2018). Many studies reported the presence of gastrointestinal symptoms

such as pain, diarrhea, bloating in ASD children (Munasinghe et al., 2010). There is scarcity of literature evidence about probiotics in ASD and may be they are effective at improving the core symptoms of ASD (Li et al., 2017). A control-based study was conducted on 22 children for 12 months aged between 4-16 years and result showed improvement in total behavior problem score compare to the placebo group (Mccartney et al., 2010). A study of combined dietary approach of prebiotic and exclusion diet showed improvement in antisocial behavior. The study was conducted on 41 autistic children in a double blind, placebo-controlled experiment and prebiotic mixture was fed to the children. They found lower abundance of bifidobacterial and veillonellaceae in the faecal sample of the participants and amino acids (AA) as main metabolites (Grimaldi et al., 2018). AA is known as precursor for neurotransmitters. However, how diet can modulate the AA metabolite is still unknown and further study is needed. One hypothesis of increased AA is that it can be produced by microorganism in the gut (Tuohy et al., 2014). On the contrary, another study did not find any improvement while experimented a placebo- controlled trail on 43 children aged 3-8 years for 6 months (Munasinghe et al., 2010). Therefore, advanced control design and more study is needed to evaluate the role probiotics in ASD.

Chapter 4

4.1 Conclusion

Metabolic therapy is showing promising results at improving some of the core symptoms of ASD. Scientists, dieticians, caregivers and parents considering metabolic therapies as an option for the treatment of ASD and its comorbidities. However, the animal models, trials and literature evidences are scarce and further study and research is necessary in this aspect immediately. Proper observation at nutritional status has potential to produce improvement in ASD associated symptoms such as aggressive behavior, sleep disturbances, self-injury or gastrointestinal disturbance. As a matter of fact, nutritional or metabolic therapy could be a non-pharmacological option to improve quality of life, overall health by increasing the level of functioning. However, the caregivers and dieticians must aware the parents or families that these metabolic therapies are not establish yet as a recommended treatment option for people with ASD.

4.2 Future Directions

Even though metabolic therapy in ASD showing promising results, there is still scarcity of clinical and animal model evidence. In future, more study and clinical trial must be arranged, so that metabolic therapy can be established as a treatment option of ASD. This literature review indicates that metabolic therapy in ASD might be effective on treating the core symptoms and improving the behavior. Nutritional status and metabolic intervention can result profound improvement in ASD. Therefore, in near future, the scientists and caregivers should consider metabolic therapy in ASD and more clinical based study is needed in this area.

4.3 Limitations of the study

This study has some limitations. This review paper was conducted solely based on previously published papers and their availability on the sources mentioned in the methodology. Sometimes access to some of the papers was denied by the publishers. As other coursework was running, complete attention and time couldn’t be given while writing this paper.

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