INTRODUCTION

The autism spectrum disorder (ASD) describes a range of conditions classified as neurodevelopmental disorders in the fifth revision of the American Psychiatric Association’s Diagnostic and Statistical Manual of Mental Disorders 5th Edition (DSM-5). These disorders are characterized by social deficits and communication difficulties, stereotyped or repetitive behaviors and interests, sensory issues, and in some cases cognitive delays (American Psychiatric Association, 2013).

Historically, Kanner (1943) was the first to describe the case of an autistic individual who developed epilepsy. The relationship of epilepsy to ASDs has focused on the recognition and impact of epilepsy on ASD. That social-cognitive and communication deficits have an impact on epilepsy has received less attention and study (Rapin, 2014). The purpose of Tuchman’s study in 2015 is to emphasize the importance of early identification of ASD and social-cognitive deficits in individuals with epilepsy throughout the life span and to highlight the importance of implementing educational and behavioral strategies to ameliorate social-cognitive deficits as part of the comprehensive care of epilepsy (Tuchman, 2015).

The co-occurrence of ASD and epilepsy is well established. Epilepsy prevalence estimates in persons with ASD have ranged from 5% to 46% (Spence, 2009). The overlapping
prevalence suggests that epilepsy and ASD share at least some common biological mechanisms (Brooks-Kayal, 2010). Epilepsy accounts for increased morbidity and mortality in individuals with autism (Mouridsen et al., 2011). In a meta-analysis of 24 reports on autism and epilepsy published from 1963 to 2006, the pooled prevalence of epilepsy was 21.4% in 1485 individuals with autism and intellectual disability versus 8% in 627 persons with autism without intellectual disability (Amiet et al., 2008).

According to a 2013 study in the U.S., epilepsy hits around 13% of children with autism between ages 2 and 17 years, but among teenagers with autism, that rate doubles to 26 percent – far higher than 1 percent seen in the general population. Roughly, half of children with autism show unusual spikes suggestive of epilepsy on the electroencephalogram (EEG), which monitors brain activity, yet they do not actually have epilepsy (Perou et al., 2013).

Although genetic factors play an important role in the etiology of ASD (Rybakowski et al., 2016), the specific genetic causes remain obscure in the majority of cases. The branched chain ketoacid dehydrogenase (BCKDH) complex catalyzes the irreversible, rate-limiting step in the catabolism of branched chain amino acids (BCAAs) (Harris et al., 2005), and branched chain ketoacid dehydrogenase kinase (BCKDK) encodes a kinase that phosphorylates and thus inactivates the E1α subunit of this complex (Suryawan et al., 1998). Mutations in any of
the three subunits of the BCKDH complex lead to toxic accumulation of BCAAs and their metabolites (*Menkes et al., 1954*), characterized by severe neurological complications (*Novarino et al., 2012*). The first description of BCKDK-deficiency disease was that of a Mendelian form of autism with comorbid intellectual disability and epilepsy (*Novarino et al., 2012*).
Aim of the Work

1. To identify the metabolic abnormalities of BCAAs (leucine, isoleucine and valine) associated with ASD.

2. To study the correlation between ASD and the presence of Epileptiform discharges in EEG.

3. To study the correlation between ASD and the presence of seizures; their types and frequency.

In order to improve the management of cases.
Chapter 1

Autism Spectrum Disorder (ASD)

Background

Infantile Autism, first described in medical literature since 1943 by Leo Kanner, is a serious psychiatric disturbance of childhood which dooms a majority of its victims to lifetime disability. Early in life, these children have distorted relationships with their parents. The term autism (Greek for "self-ism") refers to their self-absorption and withdrawal from social transactions. Some such children remain totally mute while others fail to develop communicative speech. Stereotyped mannerisms and obsessive insistence on the preservation of sameness are other characteristics (Kanner, 1943; Kanner, 1944).

According to O'Gorman an autistic child shows these essential features: (1) Withdrawal from or failure to become involved with reality; in particular, failure to form normal relationships with people. (2) Serious intellectual retardation with islets of normal, near normal, or exceptional intellectual function or skills. (3) Failure to acquire speech, or to maintain or improve on speech already learned, or to use what speech has been acquired for communication. (4) Abnormal response to one or more types of sensory stimulus (usually sound). (5) Gross and sustained exhibition of mannerisms or peculiarities of movement, including immobility and
hyperkinesis and excluding tics. (6) Pathological resistance to change. This may be shown by: (a) Insisting on observance of rituals in the patient's own behavior or in those around him. (b) Pathological attachment to the same surroundings, equipment and toys, and people (even though the relationship with the person involved may be purely mechanical and emotionally empty). (c) Excessive preoccupation with peculiar objects or certain characteristics of them without regard to their accepted functions. (d) Severe anger or terror or excitement, or increased withdrawal, when the sameness of the environment is threatened (e.g. by strangers) (O'Gorman, 1967).

Although the etiology of the condition remains obscure, certain information has emerged: (1) The prevalence of childhood autism is higher than previously suspected. (2) No genetic or familial relationship to schizophrenia has been demonstrated. (3) The parents of autistic children are likely to be of higher than average intelligence and from the upper middle class of society, but there is no greater incidence of mental illness among them than among the general population. (4) Epidemiologically, the pattern of childhood autism suggests an etiology that is organic rather than psychogenic. (5) The bizarre behavior and stereotypies such as twirling and smelling diminish in an environment that is stimulating and educational, and are reinforced in an environment that is understimulating. "Autistic children are particularly sensitive to lack of environmental stimulation. (6) The effectiveness of
psychoanalytic treatment has not been proved, but most authors agree that dynamically oriented personnel are effective in treating the autistic child (Charkes, 1968).

One of the major questions in ASD research is its etiology. Much ASD research concentrates on genetic causes (Rossignol and Frye, 2012a) even though inherited single gene and chromosomal defects only account for a minority of ASD cases (Schaefer et al., 2013). However, genetic etiologies may be overrepresented in children with ASD and epilepsy as many genetic syndromes and gene mutations associated with ASD include epilepsy as a common feature (Murdoch and State, 2013; Tuchman et al., 2013).

**Epidemiology**

By screening 78,000 schoolchildren of 8-10 years, Wing et al. (1967) found that; a rate of 4.5 per 10,000 of the child population were diagnosed clinically as autistics.

The Centers for Disease Control and Prevention (CDC) estimates that 1 in 68 children (or 14.7 per 1,000 eight-year-olds) in multiple communities in the United States has been identified with ASD (Centers for Disease Control and Prevention, 2016).

The increase of ASD prevalence can not be fully explained by advances in diagnostics or sudden genetic shifts. There is a growing consensus among scientists and clinicians
that ASDs ensue from an interaction between biological vulnerability factors and environmental or iatrogenic insults (Gillberg, 2009).

Recent statistics indicate that only about 50% of the observed increase in autism incidence can be accounted for by changes in diagnostic criteria, public awareness or other non-causal parameters. Given these alarming statistics, it is essential to identify the causes of this trend that is approaching epidemic proportions affecting many countries worldwide (Centers for Disease Control and Prevention, 2014).

Etiology

Intense scientific work has been performed in recent years to understand the potential origin of ASD, revealing that this disorder arises from both genetic and environmental factors, especially those influencing fetal and early-life development (Lai et al., 2013). Although ASD has been shown to be highly heritable (recent estimates 38–54%), several meta-analyses have highlighted that non-genetic prenatal causes of ASD exist, opening the door for further studies to investigate such mechanisms (Hallmayer et al., 2011).

Approximately 10% of ASD cases are linked to disorders of genetic etiology, such as fragile X syndrome, tuberous sclerosis, and Rett disorder. Supporting the idea of heterogeneity of ASD, single genetic mutations account for
only 1-2% of ASD cases, with the majority of cases remaining idiopathic (*Abrahams and Geschwind, 2008*).

Mutations identified by genetic studies have revealed that some affected genes are involved in brain development from in utero through infancy. Frequent aberrations in brain cytoarchitectural organization and neuronal connectivity have been observed in the brains of ASD patients, leading to the concept that ASD is a synaptopathy (*Won et al., 2013*).

Genetic, environmental and neuropathological factors play an important role in the etiology of ASD.

1. Genetic Factors

Genetic factors play an important role in the etiology of ASD. Risk of illness is significantly increased in first-degree relatives (siblings, children) and identical twins show a high concordance rate of the disorder (*Bailey et al., 1995*). Over 3,000 genes and many thousands of gene variants have been identified so far, from rare mutations to common polymorphisms, which may be associated with ASD (*Xu et al., 2012*).

The role of genetic factors in the risk of the disease, referred to as heritability, for ASD may be the highest among all psychiatric disorders – approx. 0.8-0.9 (*Bailey et al., 1995*), although some recent analyzes indicate values of the 0.4-0.7 range (*Hallmayer et al., 2011*). Genetic variability responsible
for the disease can be successfully identified in approximately 20–30% of cases. Those are mostly rare genetic defects: single gene mutations, chromosomal aberrations and microaberrations. They fit into the concept of the, so-called, common disease–rare variant (CD-RV) suggesting that the presence of a single, rare or very rare defect (mutation) of significant effect, or accumulation of such defects decides on development of symptoms (O’Roak and State, 2008; Awadalla et al., 2010).

It is now believed that ASD is a result of complex gene-environment interactions, with strong and clear genetic influences. Studies of twin pairs, high-risk infant siblings, families, and populations have estimated concordance rates and segregation of the disorder within families. The concordance rate was reported as 60–70% in monozygous twins and as 5-30% in siblings; this is in agreement with a recent large prospective study revealing a recurrence rate of 18% in infant siblings and of 33% in multiplex families (Bailey et al., 1995; Ozonoff et al., 2011).

An incomplete concordance of the disorder in monozygotic twins suggests an important role of environmental factors. Pre and perinatal factors are among them. Some authors treat those factors as: an independent noxious agent, being a cause of ASD, an effect of common pathogenic mechanism, leading both to gestational and perinatal complications, and to development of autism later in life, or as an expression of a different intrauterine development of a fetus that may lead to
gestational, perinatal and neonatal complications, and to development of ASD symptoms in later life (Gardener et al., 2011; Guinchat et al., 2012).

The known single-gene defects and the diagnosed medical conditions account for about 10% of the cases of autism (Chakrabarti et al., 2001). Between 21% and 50% of the boys with fragile X syndrome are on the autistic spectrum (Moss et al., 2009), and 0%-6% of the autism populations have fragile X syndrome (Fombonne et al., 1997).

The prevalence rates of ASD in tuberous sclerosis complex (TSC) consistently range from 24% to 60% (Moss et al., 2009). The incidence of autistic individuals with TSC complex has been estimated to be between 0.4% and 4% in epidemiologic studies (Chudley et al., 1998).

Copy number variants (CNVs) are DNA segments ranging in size from 50 base pairs to several megabases among individuals due to deletion, insertion, inversion, duplication, or complex recombination (Redon et al., 2006). It has been demonstrated that the CNV location and its functional relevance may play a more crucial role than the mean CNV number and size (Sung, 2015).

Two large datasets have been discovered: heterogeneous de novo copy-number variants collectively affecting several loci and presumably accounting for 5%-8% of the cases of
simplex forms of ASD (Sanders et al., 2011). The network-based functional analysis of these rare CNVs confirms the involvement of these loci in synapse development, axon targeting, and neuron motility (Gilman et al., 2011).

Several neuroligins and SHANK and neurexin genes encoding proteins crucial to synapse formation, maturation, and stabilization have been found to host mutations responsible for behavioral phenotypes, including autism (Betancur et al., 2009). At the extracellular level, postsynaptic neuroligins interact with presynaptic or neurexins stimulating the formation of the presynaptic bouton (Fabrichny et al., 2007). At the intracellular level, neuroligins associate with postsynaptic scaffolding proteins such as SHANK3 (Gerrow et al., 2006). Neuroligins are encoded by the NLGN1, NLGN2, NLGN3, NLGN4X, and NLGN5 genes. Among them, only the NLGN3, NLGN4, and NLGN4Y genes have been found to harbor mutations that could possibly cause autism (Sung, 2015).

The MECP2 gene is important for the correct brain function and development: loss of MECP2 has been shown to delay neuronal maturation and synaptogenesis, and MECP2 de novo loss-of-function mutations cause Rett syndrome in approximately 70% of the affected females, while they are generally found to be lethal in males (Amir et al., 1999).

Children with idiopathic autism often display minor facial dysmorphisms and abnormal head/body growth rates
Macrocephaly is seen in approximately 14% of children with autism (Meguid et al., 2014).

Phosphatase and tensin homolog (PTEN) is a tumor suppressor gene that favors cell cycle arrest in G1 and apoptosis. Genetic syndromes linked to PTEN germline haploinsufficiency are often associated with autism or mental retardation (Goffin et al., 2001).

Genetic evidence proposes that at least some ASD cases may result from abnormal Ca2+ homeostasis during neurodevelopment (Krey et al., 2007). Moreover, several genetic studies have found autism-related genes encoding proteins either directly or indirectly, controlling intracellular Ca2+ levels or regulated by cytosolic Ca2+ transients. The biochemical parameters linked to the mitochondrial function are frequently abnormal in autism (Giulivi et al., 2010).

It is only in rare instances that mutations in mitochondrial DNA or in nuclear DNA heavily involved in the mitochondrial function explain the disease. Children with mitochondrial disease thus, represent a small percentage (<1%) of all autistic patients (Sung, 2015). In reality, mitochondrial ASD forms are rare, as mitochondrial dysfunction appears to be secondary in most patients-i.e., downstream of other pathophysiological abnormalities such as excessive oxidative stress (Rose et al., 2014a).
2. Environmental Factors

The expression of the autism gene may be influenced by environmental factors (Lawler et al., 2004). According to the epigenetic theory, environmental factors may modulate the already existing genetic factors responsible for the manifestation of ASDs in individual children. The increasing age of mothers is a risk factor of ASD, both independently and in combination with the increasing age of fathers (Durkin et al., 2008).

There may also be mutagens in the environment, such as mercury, cadmium, nickel, trichloroethylene, and vinyl chloride. The factors associated with vitamin D deficiency may cause mutations, as vitamin D contributes to the repair of DNA damage (Kinney et al., 2010).

A case-controlled cross-sectional study reflects the fundamental importance of vitamin D in autism. Results showed that circulating vitamin D levels (both 25-hydroxy and 1, 25-Dihydroxy vitamin D) are significantly lower in children with autism than in healthy controls (Meguid et al., 2010). Results suggesting that vitamin D insufficiency may play a role in the etiology of autism. Supplementation with vitamin D3 may be extremely important in treating children with autism since their diets tend to be very restricted and they may not be consuming enough vitamin D (Meguid et al., 2010).
Prenatal exposure to harmful chemicals, or occurrence of an infection during the primary gestation period of a maturing fetus, can detrimentally affect the development of the immune system (Landrigan, 2010).

The occurrence and exposure to harmful chemicals or infection can increase the incidence of autoimmune deficiencies in young children. Thus, research has linked autoimmune deficiencies as one of the many trademarks of ASD. There are various examples of autoimmune abnormalities observed in autistic children, such as a higher influx in pro-inflammatory cytokines, a decrease in plasma IL-23, and an elevated count of plasma leptin levels (Hertz-Picciotto et al., 2008). This strong correlation of exemplifying irregular immune system development in autism demonstrates that damaging events are possibly occurring during certain stages of the gestation period. In theory, if certain parts of the immune system development are altered, adverse life lasting effects may persist. There is assumption that during prenatal and initial postnatal stages of fetal development that neurodevelopment and immune system developments have an association to one another. Research suggests that during development of the fetus there is an influx of the Type 1 T helper (TH1) response, which aids in pro-inflammatory response (Hertz-Picciotto et al., 2008). Thus, association of the occurrence of an infection can induce certain immunological changes, such as an inflammatory response (Hertz-Picciotto et al., 2008).