MEASUREMENT OF URINARY SUGARS BY HPLC AS A NON-INVASIVE TEST OF INCREASED INTESTINAL PERMEABILITY IN AUTISTIC CHILDREN

Thesis

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By

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List of Abbreviations

^{51}Cr-EDTA	: Chromium-labeled ethylenediaminetetraacetate
5-FU	: 5-fluorouracil
^{99m}Tc-DTPA	: ^{99m}Tc-diethylenetriaminopentaacetate
ABC	: The Autism Behavior Checklist
a-CGH	: Array comparative genomic hybridization
ADHD	: Attention deficit hyperactivity disorder
ADI-R	: Autism Diagnostic Interview-Revised
AJs	: Adherens junctions
ASDs	: Autistic spectrum disorders
ASDSQ	: Autism Spectrum Disorder Screening Questionnaire
ASQ	: Autism Screening Questionnaire
ATSDR	: Agency for Toxic Substances and Disease Registry
BBB	: Blood Brain Barrier
BDNF	: Brain derived neurotrophic factor
CARS	: Childhood Autism Rating Scales
CD	: Cluster of differentiation
CE	: Capillary electrophoresis
CGRP	: Calcitonin gene-related peptide
CHAT	: Checklist for Autism in Toddlers
CNS	: Central nervous system
DHA	: Docosahexaenoic acid
DPT	: Diphtheria, Pertussis and Tetanus
DSM	: The Diagnostics and Statistics Manual of Mental Disorders
DTA	: Docosatetraenoic acid
EEG	: Electroencephalogram
EFAs	: Essential fatty acids
EPA	: Environment protection agency
FDA	: The Food and Drug Administration
FISH	: Fluorescent in situ hybridization
fMRI	: functional magnetic resonance imaging
FXS	: Fragile X syndrome
GABA	: γ-amino butyric acid
GC	: Gas chromatography
GI	: Gastrointestinal
GluR6	: Glutamate receptor 6
HCL	: Hydrochloric acid
HIV	: Human immune deficiency virus
HPLC	: High-performance liquid chromatography
HRP	: Horseradish peroxidase
IGF	: Insulin like growth factor
IL-2	: Interleukin 2
INF- γ	: Interferon-γ
INF-α	: Interferon-α

List of Abbreviations (Cont.)

IPT	: Intestinal permeability test
IQs	: *Intelligence Quotient*s
JAM-1	: Junctional adhesion molecule-1
L/M	: Lactulose/Mannitol
LNH	: Lymphoid nodular hyperplasia
MLPA	: Multiple ligation-dependent probe amplification
MMR	: Measles, Mumps and Rubella
MR	: Mental retardation
MT	: Metallothioneins
MtD	: Mitochondrial Dysfunctions
MTX	: Methotrexate
NADPH	: Nicotinamide adenine dinucleotide phosphate hydrogen
NSAIDs	: Non-Steroidal Anti-Inflamatory Drugs
NSRIs	: None-selective serotonine reuptake inhibitors
NT 4/5	: Neurotrophin 4/5
PDD-NOS	: Pervasive Developmental Disorder-Not Otherwise Specified
PDDs	: Pervasive Developmental Disorders
PEGs	: Polyethyleneglycols
PET	: Positron emission tomography
PL-ADOS	: Pre-Linguistic Autism Diagnostic Observation Schedule
PST	: phenylsulfotransferase
PUFAs	: Polyunsaturated fatty acids
RNA	: Ribonucleic acid
ROC	: Receiver Operating Characteristic
SD	: Standard deviation
SFAs	: Saturated fatty acids
SIP	: Small intestinal permeability
SNPs	: Single-nucleotide polymorphisms
SPECT	: Single photon emission computed tomography
SSRIs	: Selective serotonin reuptake inhibitors
TDC	: Transcephalic direct current
TJs	: Tight junctions
TNF-α	: Tumor necrosis factor-α
TSC	: Tuberous sclerosis complex
VIP	: Vasoactive intestinal peptide
ZO-1	: Zonula occludens-1

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INTRODUCTION

Autism is the most prevalent of a subset of disorders organized under the umbrella of pervasive developmental disorders (PDDs) usually presented within the first three years of infancy. It is a life long neurological disorder affecting as many as 1 in 500 children, primarily strikes males. Male to female ratio is about 4: 1. On the other hand, severer forms of autism are more prevalent in females ***(White, 2003)***. It has been found now to be more prevalent than childhood cancer, diabetes and Down syndrome ***(Geier and Geier, 2005)***.

Autism is not a disease but a syndrome with multiple non- genetic and genetic causes. It is characterized by profound deficits in language, communication, and socialization, resistance to learning, and displays of stereotypical behavior. One out of three autistic children experiences epileptic seizures. Also, the disease is accompanied by mental retardation in three out of four patients ***(Rebecca et al., 2004)***.

The etiology of autism is still unknown. But it is generally accepted that it is caused by abnormalities in the brain structure or function. Abnormal electroencephalogram (EEG) results can be found in about 50% of individuals with autism, particularly in those with lower IQs. These abnormalities are nonspecific and usually are bilateral and diffuse. Some studies

postulated that measles, mumps and rubella (MMR) vaccination might be causally linked with autism ***(Kaye, 2001)*.**

One of the postulated causes of autism is increased intestinal permeability (Leaky gut), which was found in 43% of autistic patients. There are many reasons for this problem, such as, viral infection (measles), yeast infection (over growth of candida albicans), and a reduction in phenylsulfotransferase (PST) which lines intestinal tracts and protects it from leakiness. There is also some speculation that heavy metals in the intestinal tract lead to infection which in turn can cause leaky gut. As a result of the leaky gut, the digestion products of natural foods such as cow's milk and bread are able to enter the blood stream and induce antigenic responses. Moreover, they can pass through the blood brain barrier and produce a negative impact on the brain development ***(Horvath and Perman, 2002)*.**

The standard test for leaky gut syndrome is the mannitol and lactulose test. Both are water soluble molecules that the body can not use. Mannitol is easily absorbed by people with healthy intestinal lining. Lactulose is a larger molecule and is only slightly absorbed. A person drinks a solution containing both mannitol and lactulose. Urine is collected for six hours and the amount present in urine reflects how much was absorbed by the body. A healthy test shows high levels of mannitol and low levels of lactulose. If high levels of both molecules are found, it indicates a leaky gut condition ***(D'Eufemia et al., 1996)*.**

AIM OF THE WORK

The aim of the present study is to detect the increased intestinal permeability in autistic children by using the convenient and non-invasive lactulose/mannitol test which assesses mucosal integrity of the small bowel in children.

I. AUTISM

A. Historical Background:

In ***(1943) Kanner*** described a group of 11 children with a previously unrecognized disorder. He noted a number of characteristic features in these children, such as an inability to develop relationships with people, extreme aloneness, a delay in speech development, and non communicative use of speech. Other features included repeated simple patterns of play activities and islets of ability. He described these children as having "come into the world with innate inability to form the usual, biologically provided affective contact with people". Despite the variety of individual differences that appeared in the case descriptions**,** he believed that only two features were of diagnostic significance, autistic aloneness and obsessive insistence on sameness. He adopted the term early infantile autism to describe this disorder and drew attention to the fact that its symptoms were already evident in infancy**.** In describing these children, he used the word “autism” from the Greek word “autos” meaning “self”. Kanner’s choice of “autism” perhaps had only been used once before in the field of disabilities and that in reference to schizophrenia.

During the next decade, clinicians in the United States and in Europe reported patients with similar features. However, controversy continued over the definition of the disorder because the name autism was ill chosen ***(Despert, 1951 and***

Van Krevelen, 1952). It led to confusion with ***Bleuler (1950)*** who used the same term to describe schizophrenia in adults. This confusion led many clinicians to use terms such as childhood schizophrenia, borderline psychosis, symbiotic psychosis, and infantile psychosis as interchangeable diagnoses. In an attempt to clarify the confusion, ***Eisenberg and Kanner (1956)*** reduced the essential symptoms to two: extreme self-isolation and pre-occupation with the preservation of sameness. They also expanded the age of onset to the first 2 years of life.

In ***(1964) Rimland*** published his book "Infantile autism : The syndrome and its implications for a neural theory of behavior", which broke the ground in the area of autism because it debunked the assumption that autism was the result of bad parenting and helped establish autism as a true neurological disorder**.** It was not until 1971 that autism was finally distinguished from childhood schizophrenia. It is clear that autism and schizophrenia are two distinct disorders, but sometimes a child with autistic disorder can develop co-morbid schizophrenia ***(Kaplan and Sadock, 1998).***

B. Development of Diagnostic Criteria for Autism:

1- Diagnostic Statistical Manual:

The Diagnostics and Statistics Manual of Mental Disorders (DSM) is the standard by which autism spectrum disorders (ASDs) are diagnosed in the United States. Autism was not included as a separate diagnostic condition in the