

Study the Effect of Antioxidants and Oxidative Products and Their Genetic Effect on Methionine Synthase in People with Autism Spectrum Disorder

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ABSTRACT

Autism is a developmental disorder characterized by troubles with social interaction and communication by restricted repetitive behavior, Blood samples were taken for 60 people with autism and 30 healthy people as a control group. Both groups ranged age from 3 to 8 years .The laboratory and genetic analysis, (GSH & MDA) analysis were performed by ELISA technique, while Vit. D was performed by I-chroma II technique .The genetic aspect; it has been using the DNA separation kit then used PCR device, after that using nanoscope analyzer to determine the concentration of DNA extracted after the special primers were used to determine the presence of genetic defect. The results were a significant decrease in level of (GSH & Vit.D), a significant increase in level of (MDA), the genetic part, allele has been identified suffering from a significant change of methionine synthase where there is a significant relationship between this protein and autism disorder.

Keywords-- Autism, GSH, MDA & Methionine Synthase

One of the most important behaviors that characterize a person who suffers from autism is aggressive behavior and hyperactivity and attempt to destroy properties and tantrums (Chan, et al., 2019). In fact, autism is diagnosed at an early age of (24-36) months. This is the ideal age for learning the language. Therefore, the most important diagnosis of autism is communication. When a person's name is heard, it begins with a remarkable repetition. When he sees some of the children's games, he tries to get them to behave differently to ordinary children, the repetitive behavior he uses to repeat the words he quickly learns (Tomchek, et al., 2018).

The autistic person often appears physically normal and appears to be a layman and has control over his muscles but sometimes suffers from unusual repetitive movements. These movements are called self-stimulation or "stemming" movements (López-Doval, et al., 2016). Many symptoms of autism, such as difficulty in speech, fluttering of hands, movement of toes during walking, lack of attention, impulse, severe mood, perseverance, hyperactivity, involuntary activity disorders, difficulty of change, problems in following sequence of tasks and organization, A person facing autism is challenging society whether it is within the family or at school (Brown., 2018).

The degree of autism varies from one person to another according to established standards and agreed upon globally, such as the degree of intelligence of the autistic and the extent of his activity in the performance of duties entrusted to him, some of them suffer from functional disability and was mentally unstable where this type of the most severe spectrum of autism, Severity, others have a low intelligence level of up to 80%, and there are people with autism but they have a very high IQ, these ratios are measured on the proportion of the completion of the unit of activity and not according to what possessed of intelligence. As the term ASD is not a specific diagnosis but is a gateway to enter more than one direction, (Walsh, et al., 2017). Where the American Society of Psychiatry (APA, 2013) called the term an umbrella, as mentioned in the following figure (1).

I. INTRODUCTION

"Autism is a developmental disorder characterized by troubles with social interaction and communication by restricted repetitive behavior. Parents usually notice signs in the first two or three years of their child's life "(Landa., 2008).

Autism was defined by the American Journal of Madness in 1912 as a case in which thought was separate from logic and reality, the word autism is derived from Greek word autos that means self (LAM, Bick-har., 2019). Lack of attention, social disturbances and lack of intuition about people, these are the signs that appear on people who suffer from autism, become disabled since childhood and continue to go to puberty, that the autistic people do not interact with those who stimulate them through social exchange of speech and smiles , As well as respond to their queries or repeat their names, even communication through the eye is very little and weak or to have proactive positions such as playing with someone else's hand.



Figure 1: Types of Autism Spectrum Disorder

Chemically (Redza-Dutordoir, et al., 2016) were clarify that the oxygen-derived reactive yields like peroxide radicals ($\cdot\text{O}_2^-$), peroxide of hydrogen (H_2O_2), anion-superoxide (O_2^-), the singlet oxygen (${}_1\text{O}_2$), and radicals of hydroxyl ($\cdot\text{OH}$), are yield of enduring aerobic metabolism thru mitochondrial oxidative phosphorylation pathway.

1) **Glutathione:** Glutathione exists in reduced (GSH) and oxidized (GSSG) conditions. The ratio which that reduced glutathione in order to oxidized glutathione inside cells is an amount of cell oxidative stress. Reported by (Gyamfi, et al., 2019).

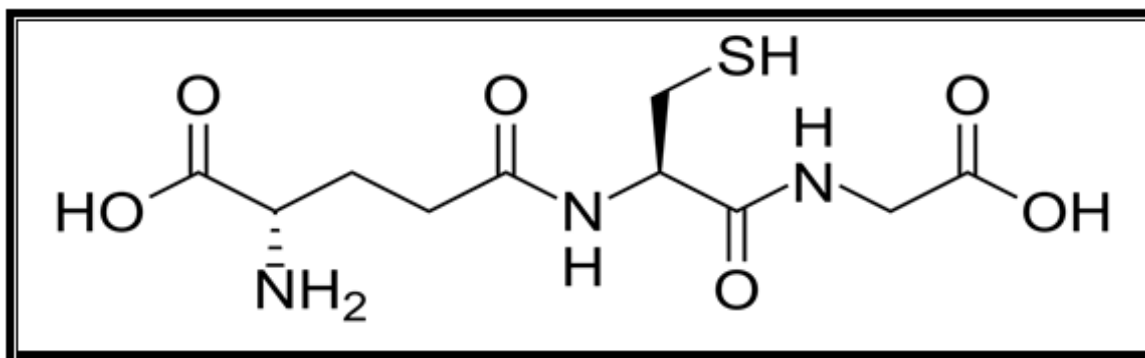


Figure 2: The Glutathione structure

(Rahbar, et al., 2018) were reported that the blood accrued from autistic kids illustrations low concentrations level of polyunsaturated lipids for membrane, upper phospholipase A2, and loss of the regular asymmetry of lipoproteins membrane, which might additionally indicate extended oxidative damage. (Gusev, et al., 2019) were stated that the value of endogenous Accretion of oxidized glutathione (GS-SG) in blood plasma are a sturdy sign of intracellular oxidative stress, as cells export the GS-SG to preserve redox equilibrium.

2) **Malondialdehyde (MDA):** Reactive oxygen species (ROS) can likewise reason the peroxidation of lipid to cell of organisms with the aid of attacking

polyunsaturated membrane lipid cause form Malondialdehyde (MDA) as end products of fatty acid polyunsaturated of cell (Kheradmand, et al., 2009) . The researchers were measured plasma MDA as a signal of lipid peroxidation station in all autistic kids as compared with control subjects consistent with the method described via (Chauhan, et al., 2004).

This research by (Hansen, et al., 2015) were talked about a vital part in autism investigation assessment stages of the antioxidant coenzyme Q10 and lipid peroxidation sign MDA in autistic kids and associates these effects with their conduct and language competencies with the intention to highlight the proper pathway for interposition. Maternal age that's may be

correlated with autism due to extended hazard of chromosomal irregularities in ova at augmented age.

3) **Vitamin D:** Vitamin D, a fats-soluble nutrition, is a preferred call for a group of steroid-like substances which comprises ergocalciferol (D2) and cholecalciferol (D3), reported by (Hajar, et al.,2016). Vitamin D is present inside the food restricted quantities and is obtained particularly the skin exposure to the UV radiation which coming from the sun. During solar exposure-7, dihydroxycholesterol that's gift particularly within the layers of epidermis and to a lesser amount in dermis, explained by (Markus, et al.,2017), so UVB radiation was absorbed and transformed to pre-Vit. D3. Vitamin D play significant function in fortification of DNA from damage, if it arise vit. D reparation it as soon as the harm was happened. In addition to Vitamin D can also reduce the hazard of autism thru its effect on immune machism thru, growing T- cells regulatory, Protecting by the aid of up-regulating glutathione, which also chelates heavy metals, this were explained by (Mathew, et al., 2019).

Study the genetics for knowledge of causes of autism is very complex. This because studies have shown that more than one gene involved in autism and there may be more than one gene in the same autistic person, may be involved different genes to cause autism in several people, these genes may interact with the environment to cause autism in a person. Genes may interact with each other to cause a new autism mutation. (Rozbroj, et al., 2019).

Autism may be genetic but not inherited. In other words, the genetic mutation that caused autism does not exist in the parental genome. (Raude,et al ,2019). The main enzymes involved in the metabolism of medium folic carbon by folate were methionine synthase or methylenetetrahydrofolate reductase (MTHFR), likewise methionine synthase redactase, these enzymes are also involved in DNA synthesis, DNA methylation, or DNA repair (Chen, et al., 2018) .

II. SUBJECTS AND METHODS

1) Subjects

1.1) **Patients:** 60 Iraqi children with Autism spectrum disorder (ASD) participated in the present study. All children (ASD) have completed all biochemical analysis tests. Their ages ranged between 3-8 years. These children (ASD) were registered in many specialized centers that care with autism in Iraq particularly in the governorates of Basra, Karbala, Babylon, Baghdad and

Najaf. Where the blood sample was taken from them after the approval of their parents.

1.2) **Exclusion Criteria:** The present study excluded the children (ASD) from apparent other mental disease.

1.3) **Blood Samples:** A Five milliliters of venous blood samples had been drawn using a disposable needle and plastic syringes from every kid of (ASD) and control situation. Blood became divided in to two elements,the primary approximately 1.5ml which become installed EDTA tube and transported for freezes at (-70c°) until assayed the relaxation of blood became about three.5ml installed serum tube then left at room temperature for 10 mins for clotting, centrifuged 3000 Xg for 10 minutes, and then serum become separated and transported into new disposable tubes and freezes at (-70c°) till assayed.

2) Methods

The level of glutathione, malondialdehyde and vitamin D were measured by the ELISA apparatus using kits from elabscience www.elabscience.com. While the **genitic measurements** that's done by use of the DNA extraction Mini Kit (Geneaid Company) Human genomic DNA became extracted from all clean blood samples.

III. STATISTICAL ANALYSIS

The data collated after biochemical analysis were subjected to statistical calculation using statistical software (Megastat). The mean, standard deviation of mean, F-distribution test were obtained. Critical value or test of probability less than 0.05 ($p < 0.05$) was regarded significant in addition, if the probality less than 0.001 ($p < 0.001$) was regarded as a highly significant also we use Microsoft Excel (2010), SPSS17 and Minitab v. 14 (Basim., 2016).

IV. RESULT AND DISCUSSION

The level of **Glutathione** (GSH) in the table (1) shows a significant increase (p value = 0.00267) in case of ASD (28.85) as compare with their control group (41.23) , while the same table displays a significant increase in level of Malondialdehyde in ASD sample (451.78) as associate with its healthy group (349.1), additionally the level of vitamin D which is appear a significant decrease (P. Value = 0.000184) in level of Vitamin D for autism individual (Mean= 26.04) as compare with the healthy group (Mean= 35.29).

Table 1: Level of parameters (Glutathione & Malondialdehyde) for autism spectrum disorder and their control group

Item	Patients=60	Controls=28	P. Value
	Mean± SD	Mean± SD	
GSH µg/ml	28.85 ±12.76	41.23 ± 17.71	0.00267
MDA ng/ml	451.78 ± 274.75	349.1 ±186.58	0.0478
V.D	26.04 ± 9.9	35.29 ± 10.1	0.000184

Antioxidants are known as chemical compounds that inhibit the oxidation processes that occur within the human body, even though their concentration is low. Therefore, antioxidants have an important physiological role in the body. (Chang, et al., 2019).also reported that children are more susceptible to stress than adults because of the low concentration of antioxidants. (Elena, et al., 2013). also said that autistic children are deficient in antioxidants, including

1) **Glutathione:** Glutathione, by comparing them with their healthy counterparts. So, (Saghazadeh, et al., 2017). proved that glutathione deficiency affects the metabolism of fatty acids within the cell, leading eventually to a disorder of behavior through neurological and biochemical deformities.

2) **Malondialdehyde:** Malondialdehyde is the final product for the oxidation of polyunsaturated fats. MDA is one of the important signs of lipid peroxidation and oxidative stress processes (AL Zurfi, et al., 2016). Many studies confirm a significant relationship between the peroxidation of polyunsaturated fatty acids, lipid peroxidation and autism.

As seen in the research done by (Carrascosa, et al., 2017). They said that the oxidative stress affects the cell membranes functions, so the most important cell membranes role is permeability, in this study they proved that the cell membrane's red blood cell fluid had difficulty in permeability for autistic children compared to healthy

children. (Herbert., 2009) explained the relationship between oxidative stress and psychological and neurological disorders, This was a mechanical suggestion that showed effect of oxidative stress on the membrane of the nerve cell in particular, leading to an abnormality of the membrane lipid, then begin the fluidity of the membrane decline, eventually suffering the neuronal membrane dysfunction due to those distortions.

3) **Vitamin D:** Vitamin D is a group of fat-soluble secosteroids responsible for increasing intestinal absorption of calcium, magnesium, and phosphate, and multiple other biological effects. Reported by (Holick., 2004). (Kerley, et al., 2018) showed in a study illustrate the level of vitamin D in autistic people is very low compared with a group of healthy people of the same age, where they said that vitamin D deficiency increases the incidence of autism.

V. MOLECULAR MARKERS

1) **DNA Isolation Product:** For the purpose of making sure the presence of DNA after extracting from the frozen blood, two operations were carried out. first, measure its concentration through the device nanodrop.

The second operation performed on the DNA extract is agarose gel electrophoresis and testing under UV light. The results are as follows in figure (3).

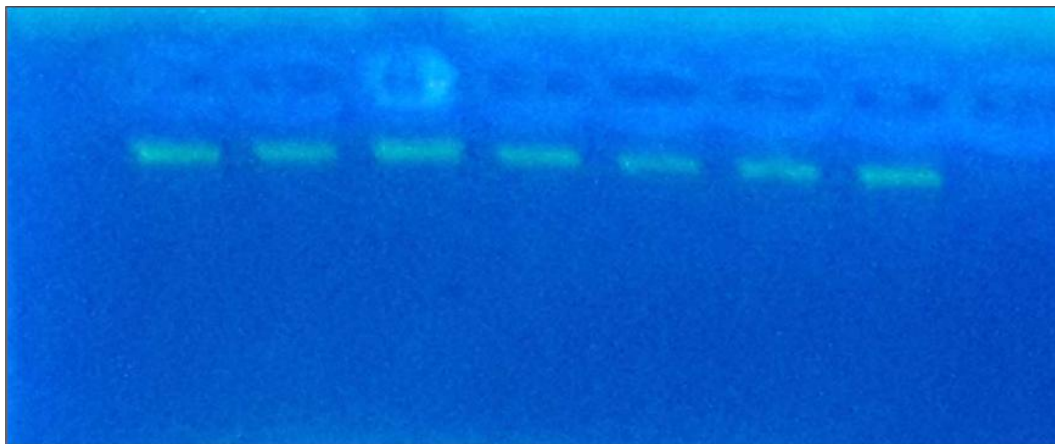


Figure 3: DNA on 0.8% agarose gel electrophoresis

2) **ARMS-PCR-Analysis:** ARMS - PCR technique was used for molecular screening a primer **MTRR A66G** studied type for autism disorder using a specific set of primers for each mutation and a set of internal control primers.

3) **ARMS-PCR-Screening:** The PCR products for MTRR A66G codon 22 A and G alleles was 401 and 367 bp respectively as shown as in figures (3-23) & (3-24).

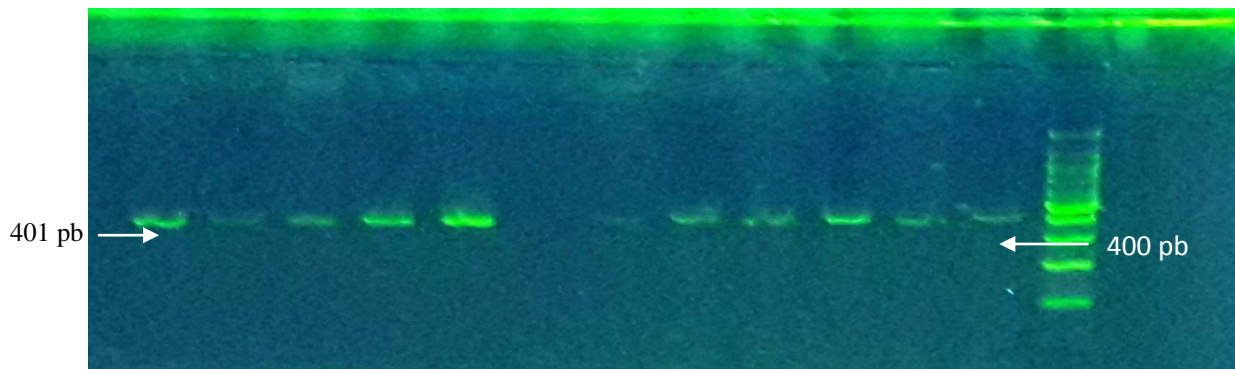


Figure 4: Agarose gel electrophoretogram showing the detection of MTRR ARMS-PCR. Products (401 bp amplicon, M = 100 bp ladder, molecular size marker).

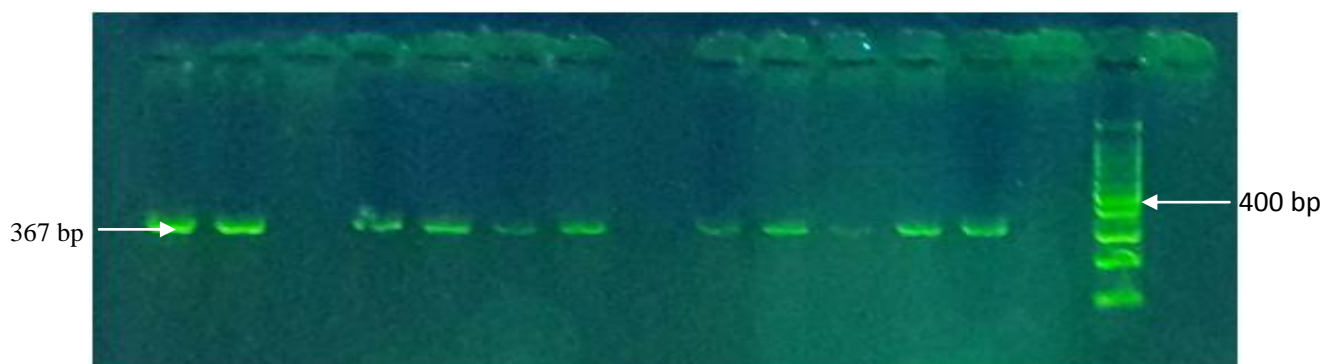


Figure 5: Agarose gel electrophoretogram showing the detection of MTRR ARMS-PCR. Products (367 bp amplicon, M = 100 bp ladder, molecular size marker).

VI. GENOTYPE AND ALLELE FREQUENCIES

The results in table (3-9) appear The genotype frequencies of AA= 17 (28%), AG = 23(39%) and GG= 20(33%) in the children with autism were represent to the total = 60(100%) , in addition the control group =

28(100%) the genotype frequencies of AA= 13(46.3%), AG= 13(46.3%) and GG= 2 (6.3%), in the same table appear total no. of allele for autism individuals = 120, the allele incidences of A= 57(47%) and G allele was 63(53%) , in the other hand that table perform the total no. of allele for control group=56, A allele was 39(70%) and allele of G was 17(30%) .

Table 2: Shows the Genotype and allele frequencies of MTRR A66G polymorphism in ASD. And its control groups.

rs1801394	Patients	Control	
Genotype	n = 60(%)	n = 28(%)	P Value
A/A	17(28%)	13(46.3%)	0.001
A/G	23(39%)	13(46.3%)	0.59
G/G	20(33%)	2 (6.3%)	0.001
Allele	n = 120	n = 56	Odd ratio
A	57(47%)	39(70%)	0.0219
G	63(53%)	17(30%)	0.008

This table clarify a significant difference between individual of autism and its control group. There are many studies that deal with genetic studies on people with autism, since many genetic and hormonal effects have a significant relationship to this disorder. Most gene scientists have devoted their research to genetic aspects.

(Au, et al ., 2017; Zhu, et al., 2003) conducted a genetic study of mutations on methionine synthase

reductase (MTRR), a genetic code that activates an enzyme essential to activate methionine synthase (MTR). The study found that there is a significant relationship between these mutations and mental illnesses in general, Congenital defects may occur in the neural tube, leading to many ills, including mental retardation and autism, even some types of cancers. this is in line with the findings of the genetic part of current research.

(Padmanabhan, et al., 2018 ; Zavadakova., 2002) showed that individuals with a deficiency in the methionine synthase gene had variable levels of homocysteine and methionine and were therefore affected by nervous weakness and possibly mental retardation.

One of the most important things that (Hudler., 2018) has done with his research on the genes that contribute to the formation of folate is the discovery of the strong relationship between these genes and some of the serious diseases that afflict humans. In addition, Hudler, explained the relationship between the genetic defects of those genes that create folate with mental illness and autism.

The mechanical process by which folate is produced produces glutathione, which has been previously studied in the current research and clarifies its moral relationship with autism. perhaps the lack of folate synthesis will lead to a significant reduction in glutathione level, Increase in symptoms of autism in individuals, as confirmed by the results of genetic testing as well as biochemical readings in the current research (Madhusudhan, et al., 2017).

VII. CONCLUSIONS

In the present research, there is a strong correlation between autism and low glutathione level, which in turn helps explain the decrease in vitamin D level on the one hand and also explains the rise in autism in malondaldehyde level, from the results in this recent research that concluded the clearly significant relation between vitamin D and autistic individuals, the consequences confirmed considerable affiliation in the genotype distribution of the MTRR 66A > G polymorphism among autism sufferers and controls.

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