Scholars LITERATURE

La Prensa Medica Argentina

Research Article

DOI: https://doi.org/10.47275/0032-745X-219
Volume 106 Issue 4

Measurement the Level of Dopamine Beta Hydroxylase Enzyme in Autistic Iraqi Children

Ahmed Hamed Jwaid*, Zainab Hassan Muslem and Elaf Basim Dawood

Department of Pharmacology and toxicology, University of Baghdad, Iraq

Abstract

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder that is characterized by persistent impairment of social communication and reciprocity across multiple contexts as well as restricted, repetitive, and stereotypic patterns of behavior, interests, and or activities. It is suggested that autism may result from an interaction between genetic, environmental, and immunological factors, with oxidative stress as a mechanism linking these risk factors. Dopamine beta-hydroxylase (DBH), also known as dopamine beta-monooxygenase, is an enzyme that is encoded by the DBH gene that converts dopamine to norepinephrine when this enzyme is inhibited by Clostridia bacteria, toxic dopamine metabolites are produced which lead to brain toxicity that may be a target in autism treatment. This study was designed to evaluate the level of dopamine beta-hydroxylase enzyme and its effect on autism in Iraqi children.

Method: Eighty children, their age range 3-10 years were involved in this study; a Performa was framed, and all the relevant information of each child was recorded. Children were classified into two groups (40 children each) as healthy children and children who were diagnosed as autism disease served as the autism patients' group.

Results: This study showed that there was a significant decrease in the level of serum dopamine beta- hydroxylase enzyme ($P<0.05$) in autism children their age range (3-10 years) compared to the corresponding level of serum dopamine beta-hydroxylase in control children at the same aged range. Moreover, there was no significant statistical association between DBH levels and age. According to the results obtained in this study, it could be concluded that alterations in level of serum dopamine beta- hydroxylase in autism patients were observed; this indicates that there is the imbalance between dopamine and Norepinephrine because of the inhibition of dopamine beta-hydroxylase enzyme that may be induced by the presence of a toxic metabolite of clostridia bacteria.

Keywords: Autism Spectrum Disorder; Dopamine beta-hydroxylase; Dopamine

Correspondence to: Ahmed Hamed Jwaid, Department of Pharmacology and toxicology, University of Baghdad, Iraq; E-Mail: ahmedataimish76@yahoo.co.uk

Citation: Jwaid AH, Muslem ZH, Dawood EB (2020) Measurement the Level of Dopamine Beta Hydroxylase Enzyme in Autistic Iraqi Children. Prensa Med Argent, Volume 106:4. 219. DOI: https://doi.org/10.47275/0032-745X-219.

Received: February 07, 2020; **Accepted:** March 03, 2020; **Published:** March 10, 2020

Introduction

Autism is a developmental disorder characterized by difficulties with social interaction and communication, and by restricted and repetitive behavior. Parents usually notice signs during the first three years of their child's life. These signs often develop gradually [1,2].

Autism is associated with a combination of genetic and environmental factors [3]. Risk factors during pregnancy include certain infections, such as rubella, toxins including valproic acid, alcohol, cocaine, pesticides and air pollution, fetal growth restriction, and autoimmune diseases. Controversies surround other proposed environmental causes; for example, the vaccine hypothesis, which has been disproven [4]. Autism affects information processing in the brain by altering connections and organization of nerve cells and their synapses. How this occurs is not well understood [5-7].

Globally, autism is estimated to affect 24.8 million people as of 2015[update]. In the 2000s, the number of people affected was estimated at 1-2 per 1,000 people worldwide. In the developed countries, about 1.5% of children are diagnosed with autism as of 2017 [update], from 0.7% in 2000 in the United States [8]. It occurs four-to-five times more often in males than females [9]. The number of people diagnosed has increased dramatically since the 1960s, partly due to changes in diagnostic practice [8]. The question of whether actual rates have increased is unresolved.

Autism Spectrum Disorder (ASD) refers to a group of neurodevelopmental disorders including autism, Asperger's syndrome (AS) and pervasive developmental disorder-not otherwise specified (PDD-NOS). The new diagnostic criteria of ASD focuses on two core domains: social communication impairment and restricted interests/ repetitive behaviors [10-12].

Autism spectrum disorder has no single known cause. Given the complexity of the disorder and the fact that symptoms and severity vary, there are probably many causes. Both genetics and the environment may play a role.

Genetics: Several different genes appear to be involved in autism spectrum disorder. For some children, autism spectrum disorder can be associated with a genetic disorder. For other children, genetic changes (mutations) may increase the risk of autism spectrum disorder. Still, other genes may affect brain development or the way

that brain cells communicate, or they may determine the severity of symptoms. Some genetic mutations seem to be inherited, while others occur spontaneously [13,14].

Environmental factors: Researchers are currently exploring whether factors such as viral infections, medications or complications during pregnancy, or air pollutants play a role in triggering autism spectrum disorder [14].

Dopamine is an organic chemical of the catecholamine and phenethylamine families. It functions both as a hormone and a neurotransmitter and plays several important roles in the brain and body. In the brain, dopamine functions as a neurotransmitter a chemical released by neurons (nerve cells) to send signals to other nerve cells [15-17]. The brain includes several distinct dopamine pathways, one of which plays a major role in the motivational component of reward-motivated behavior. The anticipation of most types of rewards increases the level of dopamine in the brain, and many addictive drugs increase dopamine release or block its reuptake into neurons following release. Other brain dopamine pathways are involved in motor control and in controlling the release of various hormones. These pathways and cell groups form a dopamine system which is neuromodulatory [18].

Dopamine Receptors

There are five subtypes of dopamine receptors, D1, D2, D3, D4, and D5, which are members of the large G-protein coupled receptor superfamily [19]. Dopamine receptors are implicated in many neurological processes, including motivation, pleasure, cognition, memory, learning, and fine motor control, as well as modulation of neuroendocrine signaling. Abnormal dopamine receptor signaling, and dopaminergic nerve function are implicated in several neuropsychiatric disorders. Thus, dopamine receptors are common neurologic drug targets; antipsychotics are often dopamine receptor antagonists while psychostimulants are typically indirect agonists of dopamine receptors [20]. Due to extensive localization of dopamine receptor to brain areas and its role in a wide range of functions, dopaminergic dysfunction has been implicated in the pathophysiology of schizophrenia, mood disorders, obsessive-compulsive disorder (OCD), and autism spectrum disorders, attention deficit–hyperactivity disorder (ADHD), substance dependency, Parkinson's disease and other disorders [19].

Dopamine Catecholamine, Biosynthesis and Release

Dopamine is synthesized in a restricted set of cell types, mainly neurons, and cells in the medulla of the adrenal glands [21]. The direct precursor of dopamine, L-DOPA, can be synthesized indirectly from the essential amino acid phenylalanine or directly from the non-essential amino acid tyrosine. These amino acids are found in nearly every protein and so are readily available in food, with tyrosine being the most common. Although dopamine is also found in many types of food, it is incapable of crossing the blood- brain barrier that surrounds and protects the brain. It must, therefore, be synthesized inside the brain to perform its neuronal activity [22]. The most fundamental building block of the three is the essential amino acid L-phenylalanine. Essential amino acids cannot be made in the body and must be supplied in the diet. L-tyrosine is the next step in the dopamine pathway. Since Tyrosine can be synthesized from L- phenylalanine, it is considered conditionally essential [23]. It is not essential in the same way L- phenylalanine would be because it can be synthesized in the body. But there are circumstances (e.g., illness, high stress, increased cognitive demands) where the body might not be able to make enough to meet demands. Under these conditions or circumstances, it becomes essential to get it from the diet [24].

Dopamine degradation

Dopamine metabolites are the products following the breakdown of Dopamine. Dopamine is inactivated by reuptake via the dopamine transporter, then enzymatic breakdown by catechol-O-methyl transferase (COMT) and monoamine oxidase (MAO). Dopamine that is not broken down by enzymes is repackaged into vesicles for reuse [25]. Different breakdown pathways exist but the main end-product is homovanillic acid (HVA), which has no known biological activity. From the bloodstream, homovanillic acid is filtered out by the kidneys and then excreted in the urine. The two primary metabolic routes that convert dopamine into HVA are [26,27]:

Dopamine → DOPAL → DOPAC → HVA – catalyzed by MAO, ALDH, and COMT respectively Dopamine → 3-Methoxytyramine → HVA – catalyzed by COMT and MAO+ALDH respectively

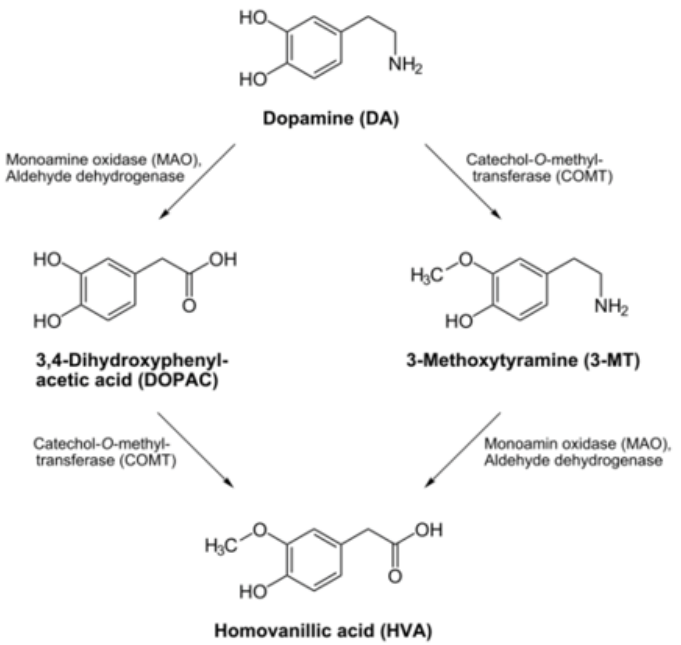

Figure 1: The dopaminergic pathway [27].

Excessive Dopamine neurotransmitter

Dopamine is a reactive molecule compared with other neurotransmitters and dopamine degradation naturally produces oxidative species. More than 90% of dopamine in dopamine neurons is stored in abundant terminal vesicles and is protected from degradation.

A small fraction of dopamine is cytosolic, and it is the major source of dopamine metabolism and presumed toxicity. Cytosolic dopamine undergoes degradation to form a compound called 3, 4-dihydroxyphenylacetic acid (DOPAC) and Homovanillic Acid (HVA) as well as hydrogen peroxide via the monoamine oxidase [28]. The HVA is a marker measured on the Organic Acids Test. Dopamine also undergoes oxidation to form superoxide, hydrogen peroxide and o-quinone and reacts with cysteine residues on glutathione, thus rendering glutathione ineffective. Dopamine oxidation can also form cysteinyl-dopamine and cysteinyl-DOPAC conjugates which are neurotoxic. These biochemical abnormalities caused by excess dopamine may cause severe neurodegeneration of neural pathways that utilize dopamine as a neurotransmitter [29].

Dopamine beta-hydroxylase

Dopamine beta-hydroxylase (DBH), also known as dopamine beta-monooxygenase, is an enzyme that in humans is encoded by the DBH gene [30]. Dopamine beta-hydroxylase catalyzes the chemical reaction:

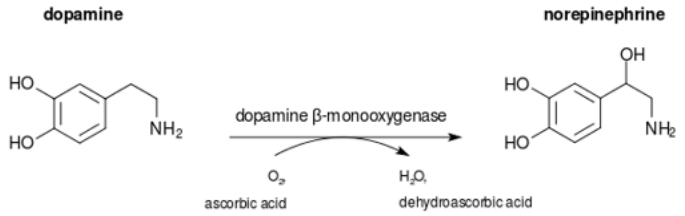

Figure 2: Dopamine is converted to norepinephrine by the enzyme dopamine β-hydroxylase (30).

The three substrates of this enzyme are Dopamine (3,4-dihydroxyphenethylamine), Vitamin C (ascorbate) which serves as a cofactor, and O_2, whereas its three products are norepinephrine, dehydroascorbate, and H_2O. It is the only enzyme involved in the synthesis of small-molecule neurotransmitters that is membrane-bound, making norepinephrine the only known transmitter synthesized inside vesicles. It is expressed in noradrenergic nerve terminals of the central and peripheral nervous systems, as well as in chromaffin cells of the adrenal medulla [31].

Clostridia difficile and Autism

Clostridia difficile Bacteria is a species of Gram-positive spore-forming bacterium [32]. It is anaerobic, motile bacteria, ubiquitous in nature, and especially prevalent in soil. Clostridia difficile cells are Gram- positive and show optimum growth on blood agar at human body temperatures in the absence of oxygen [33]. Clostridia difficile is catalase and superoxide dismutase negative and produces two types of toxins: enterotoxin A and cytotoxin B, which disrupts cytoskeleton signal transductions in the host. C. difficile may become established in the human colon; it is present in 2-5% of the adult population. Sometimes antibiotic therapy for various infections has the adverse effect of disrupting the normal balance of the gut microbiota, in which case Clostridia difficile may opportunistically dominate, causing Clostridia difficile infection [34]. The increase in phenolic Clostridia metabolites common in autism significantly decreases brain dopamine beta-hydroxylase activity [35]. This leads to overproduction of brain dopamine and reduced concentrations of brain norepinephrine and can cause obsessive, compulsive, stereotypical behaviors associated with brain dopamine excess and reduced exploratory behavior and learning in novel environments that are associated with brain norepinephrine deficiency [36].

1. Aim

This study was designed to evaluate the level of Dopamine Beta Hydroxylase enzyme in autistic patients and compare it with healthy Iraqi children.

2. Chemical and its supplier

Chemical utilized in this study was of the highest available purity. Specific chemical used was human Dopamine-β-Hydroxylase (DβH) Kit, its supplier is Novus Biologicals Company, America. This ELISA kit applies to the in vitro quantitative determination of Human DβH concentrations in serum, plasma and other biological fluids.

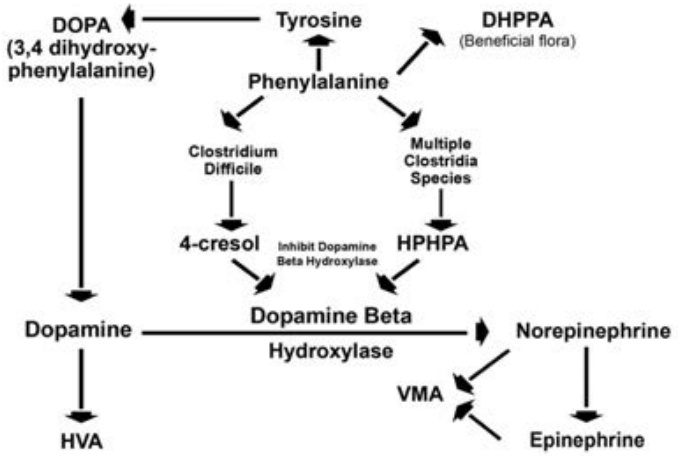

Figure 3: Effect of Clostridia metabolites on human catecholamine metabolism. DHPPA, 4-cresol, HPHPA, HVA, and VMA [37].

3. Subjects and methods

This study was carried out on eighty (80) children, with age range 3-10years; a performa was framed and all the relevant information of each child was recorded.

This study was approved by the Scientific Committee of the Department of Pharmacology and Toxicology, College of Pharmacy, University of Baghdad. Children were classified into two groups (each is 40) as follows:

Group I: Healthy children who were non-autism, and with clinically normal behavior were served as controls.

Group II: Children who were diagnosed as autism disease, served as the autism patients' group.

Test principle.

This ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Human DβH. Standards or samples are added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Human DβH and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each microplate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Human DβH, biotinylated detection antibody and Avidin-HRP conjugate will appear blue. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of Human DβH. You can calculate the concentration of Human DβH in the samples by comparing the OD of the samples to the standard curve.

Kit components and storage

An unopened kit can be stored at 4℃ for 1 month. If the kit is not used within 1 month, store the items separately according to the following conditions once the kit is received [37].

Reagent preparation

The reagents used in this study are:

1. Bring all reagents to room temperature (18~25°C) before

use. Follow the Microplate reader manual for set-up and preheat it for 15 min before OD measurement.

2. Wash Buffer: Dilute 30 mL of Concentrated Wash Buffer with 720 mL of deionized or distilled water to prepare 750 mL of Wash Buffer. Note: if crystals have formed in the concentrate, warm it in a 40°C water bath and mix it gently until the crystals have completely dissolved

3. Standard working solution: Centrifuge the standard at 10,000×g for 1 min. Add 1.0 mL of Reference Standard &Sample Diluent, let it stand for 10 min and invert it gently several times. After it dissolves fully, mixes it thoroughly with a pipette. This reconstitution produces a working solution of 100 ng/mL. Then make serial dilutions as needed. The recommended dilution gradient is as follows: 100, 50, 25, 12.5, 6.25, 3.13, 1.56, and 0 ng/mL. Dilution method: Take 7 EP tubes, add 500uL of Reference Standard & Sample Diluent to each tube. Pipette 500uL of the 100 ng/mL working solution to the first tube and mix up to produce a 50 ng/mL working solution.

4. Biotinylated Detection Ab working solution: Calculate the required amount before the experiment (100 µL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the stock tube before use, dilute the 100×Concentrated Biotinylated Detection Ab to 1×working solution with Biotinylated Detection Ab Diluent.

5. Concentrated HRP Conjugate working solution: Calculate the required amount before the experiment (100µL/well). In preparation, slightly more than calculated should be prepared. Dilute the 100× Concentrated HRP Conjugate to 1×working solution with Concentrated HRP Conjugate Diluent [37].

Exclusion criteria

This study excludes the following:

1. Children older than 10 years of age and less than 3.

2. Patients with history of any recent systemic disease and those on medications.

Blood sampling

Venous blood (5 ml) was collected under aseptic precautions from the forearm of all children participated in this study by plastic disposable syringes. Centrifuge samples for 20 min at 2~8°C. Collect the serum to carry out the assay. Samples should be assayed within 7 days when stored at 4°C [37].

Biochemical assay

The following assay procedure was used in this study:

1. Add the Standard working solution to the first two columns: Each concentration of the solution is added in duplicate, to one well each, side by side (100 uL for each well). Add the samples to the other wells (100 uL for each well). Cover the plate with the sealer provided in the kit. Incubate for 90 min at 37°C. Note: solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and causing foaming as much as possible.

2. Remove the liquid out of each well, do not wash. Immediately add 100 µL of Biotinylated Detection Ab working solution to each well. Cover with the Plate sealer. Gently mix up. Incubate for 1 hour at 37°C.

3. Aspirate or decant the solution from each well, add 350 uL of wash buffer to each well. Soak for 1~2 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times.

4. Add 100 µL of HRP Conjugate working solution to each well. Cover with the Plate sealer. Incubate for 30 min at 37°C.

5. Aspirate or decant the solution from each well, repeat the wash process for five times as conducted in step 3.

6. Add 90 µL of Substrate Reagent to each well. Cover with a new plate sealer. Incubate for about 15 min at 37°C. Protect the plate from light. Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30 min.

7. Add 50 µL of Stop Solution to each well.

8. Determine the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm [37].

Statistical analysis

The significance of differences between the mean values was calculated by SPSS version 24. The numeric data were expressed as mean value. P-values less than 0.05 were considered significant for all data presented in this study. Furthermore, Mann-Whitney test used to compare between serum DBH level in patients and control. Kendall's tau test used to compare between age and DBH level.

Results

Gender and Age of Children

In (Table 1), according to the gender of children, it has been found there is a significant difference when compare between the healthy children and autistic children regarding to the gender of them (male- female) ($p<0.05$). According to the age of children, there are no significant differences when compare the age of female of the healthy children and autistic children ($p>0.05$), the same finding has been seen regarding to the age of male in which there are no significant differences when compare the age of male of the healthy children and autistic children ($p>0.05$).

Level of DBH in Normal and Autistic Children

In the (Table 2), the level of DBH of healthy male children was significantly higher when compare to the level of DBH of autistic male children ($p<0.05$), the same finding has been seen in which the level of DBH of healthy female children was significantly higher when compared to the level of DBH of autistic male children ($p<0.05$).

Correlation between the Age of Children and Level of DBH in (Table 3), the age of healthy male was not significantly correlated with level of DBH ($r = 0.229$, $P = 0.185$), this indicates that older subject,

Table 1: Gender and Age of Children. The gender was express as frequency;the age was express as mean ± standard deviation.

		Healthy children	Autistic children	P-value
Gender	Males	20 (50%)	30 (75%)	0.02092134
	Females	20 (50%)	10 (25%)	-
Age (years)	Males	6.65 ± 2.23	6.20 ± 2.31	0.4947151
	Females	6.95 ± 2.16	6.40 ± 1.83	0.475062

Table 2: Level of DBH in Normal and Autistic Children. The level of DBH was express as mean ± standard deviation.

		Healthy children	Autistic children	P-value
Level of DBH	Males	94.99 ± 4.8	33.43 ± 10.1	0.000125
	Females	95.57 ± 3.6	39.02 ± 10.9	0.000237

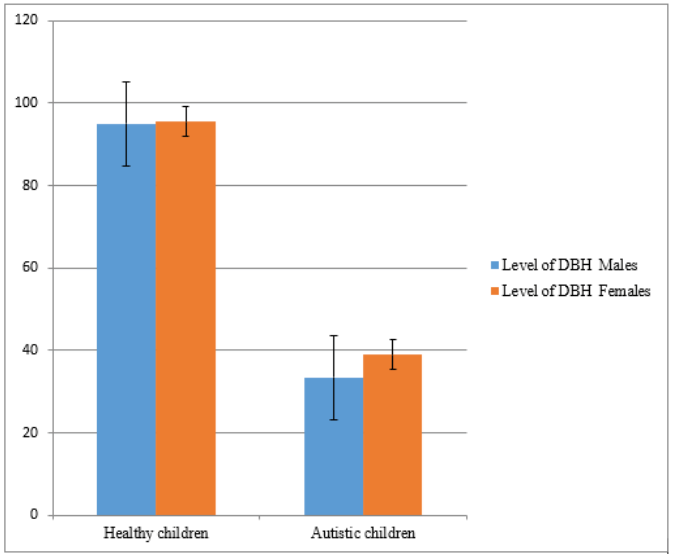

Figure 4: Level of DBH in Normal and Autistic Children.

Table 3: Correlation between Age and DBH levels in Different Groups. -(r) mean correlation coefficient.

			DBH	
			Healthy children	Autistic children
Age	Male	r	0.229	0.032
		P-value	0.185	0.814
	Female	r	0.398	-0.29
		P-value	0.019	0.264

higher level of DBH, the same results have been found in autistic male in which, the age of autistic male was not significantly correlated with level of DBH (r = 0.032, P = 0.814), this indicates that older subject, higher level of DBH (Figure 5).

The age of healthy female was significantly correlated with level of DBH (r = 0.398, P = 0.019), this indicates that older subject, higher level of DBH, meanwhile, the age of autistic female was not significantly negative correlated with level of DBH (r = -0.290, P = 0.264), this indicates that older subject, lower level of DBH (Figure 6).

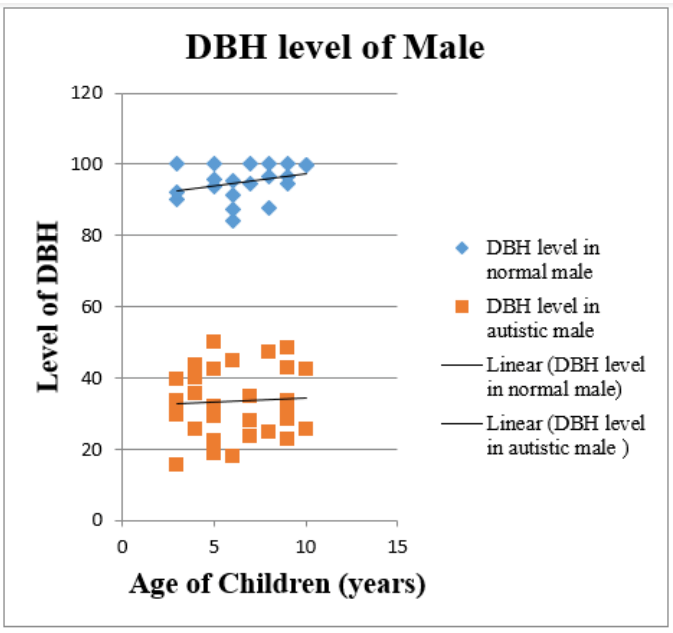

Figure 5: Correlation between Level of DBH and age in Normal and Autistic Male Children.

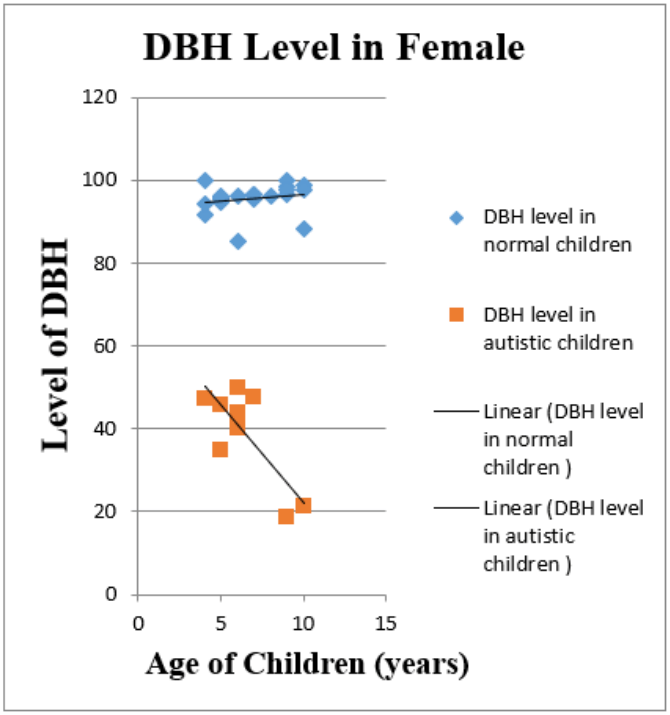

Figure 6: Correlation between Level of DBH and Age in Normal and Autistic Female Children.

Discussion

The Characteristics of Participating Healthy and Autistic Children

In this study, serum dopamine Beta hydroxylase (DBH) activity was measured in a total of 40 children with autism disorders (30 males and 10 female) and 40 healthy children (20 males and 20 female) to assess its role as a marker for specific molecular pathology in the autism disorders of childhood. According to the gender of children, it has been found there is a significant difference when compared between healthy children and autistic children as shown in (Table 1).

Regarding to the age of children, there are no significant differences when comparing the age of female of the healthy children (mean age: 6.95 ± 2.16 years) and female autistic children (mean age: 6.40 ± 1.83 years), the same finding have been seen regarding to the age of male in which there are no significant differences when compare the age of male of the healthy children (mean age: 6.65 ± 2.23 years) and male autistic children (mean age: 6.20 ± 2.31 years) as shown in (Table 1).

Level of DBH in Normal and Autistic Children

The result of this study shows that the levels of serum DBH in children with autism (their age range 3- 10 years) were significantly decrease (P<0.05) in both male and female (mean value = 33.43 ± 10.1,

39.02 ± 10.9 respectively), compared to the corresponding serum levels in healthy children in both male and female (mean value = 94.99 ± 4.8, 95.57 ± 3.6 respectively) as shown in (Table 2). The levels

of serum DBH in all children involved in this study were between the normal kit values range (1.56-100 ng/L). The result of the present study was an agreement with the study [38] which indicated that the level of serum DBH in autism children is low significantly as compared with control children. One of the possible explanations was the diminished serum levels of DBH activity in patients with autism syndrome may be due to the presence of Clostridia Bacteria in the gastrointestinal tract of autism patients that leads to inhibition of DBH enzyme which is responsible for converting dopamine to norepinephrine [39].

Correlation between the Age of Children and Level of DBH

In (Table 3), provides the relationship between DBH level and age amongst healthy and autistic children. It shows that there was no significant statistical association between DBH levels and age in male healthy children group ($r = 0.229$, $P = 0.185$), the same results have been found in the autistic male in which, the age of autistic male was not significantly correlated with the level of DBH ($r = 0.032$, $P = 0.814$) (Figure 5). On the other hand, there was a positive correlation between age and DBH levels in females healthy group which was statistically significant ($r = 0.398$, $P = 0.019$), meanwhile, the age of autistic female was not significantly negatively correlated with the level of DBH ($r = -0.290$, $P = 0.264$) as showed in the (Figure 6).

Serum DBH activity increases with age in childhood, but there is disagreement concerning the span of years involved. DBH activities were quite low during the 1st year of life in one study, and thereafter increased until they reached adult values at the 16-20-year age period [38]. The lack of relationship between age and serum DBH activity in autistic children is intriguing. It may reflect the smaller age range of autistic patients, or it may be related to the decrease in urinary free catecholamine [38]. The findings from the present study are consistent with the findings from other investigators [39-43] showed that there is an alteration in the composition of the fecal flora and metabolic products of the gut microbiome in patients with ASD that may cause alteration in DBH level. The gut microbiota influences brain development and behaviors through the neuroendocrine, neuro-immune and autonomic nervous systems.

The increase in phenolic Clostridia metabolites (3-(3-hydroxyphenyl)-3-hydroxy propionic acid (HPHPA) and 4-cresol) which were common in autism, significantly decreases brain dopamine beta- hydroxylase activity this leads to overproduction of brain dopamine and reduced concentrations of brain norepinephrine and can cause obsessive, compulsive, stereotypical behaviors associated with brain dopamine excess and reduced exploratory behavior and learning in novel environments that are associated with brain norepinephrine deficiency [36,39]. Such increases in dopamine in autism have been verified by finding marked increases in the major dopamine metabolite homovanillic acid (HVA) in urine, Moreover dopamine excess causes free radical damage to the nerve and tissues producing it, perhaps leading to permanent damage of the brain, adrenal glands, and sympathetic nervous system if the Clostridia metabolites persist for prolonged periods of time [39].In the same study of the gastrointestinal Clostridia bacteria have the ability to markedly alter behavior in autism and other neuropsychiatric diseases by the production of phenolic compounds that dramatically alter the balance of both dopamine and norepinephrine. Excess dopamine causes abnormal behavior and depletes the brain of glutathione and NADPH and causes a vicious cycle producing large quantities of oxygen superoxide that causes severe brain damage. Such alterations appear to be a major factor in the causation of autism. Previous issues related to autism disorder have been studied in different countries identifying main barriers and practices but none has investigated the relation between DBH enzyme and autism disorder in Iraq so study addressing the Dopamine beta-hydroxylase enzyme level and autism development are lacking; which makes this study unique.

The present outcomes agreed with those of previous studies which are indicated that the level of serum DBH in autism children is lower than non-autism children, and the serum DBH levels are significantly decreased in patients with autism syndrome indicates that the diminished enzyme activity in these patients is due to the presence of bacteria in the gastrointestinal tract of the autism child, so these findings suggest that the level of dopamine b-hydroxylase may be a significant component in the etiology of autism in some affected individuals and can target in autism treatment [38,39].

Conclusion

According to the results obtained from this study, levels of serum DBH in children with autism (their age range 3-10 years) were significantly decreased ($P<0.05$) compared to the corresponding serum levels in control children, this indicates that there is an imbalance between dopamine and norepinephrine due to the inhibition of dopamine beta-hydroxylase enzyme that may be induced by the presence of a toxic metabolite of clostridia bacteria.

References

1. American Psychiatric Association (2013) Diagnostic and statistical manual of mental disorders. BMC Med 17: 133-137.
2. Landa RJ (2008) Diagnosis of autism spectrum disorders in the first 3 years of life. Nat Clin Pract Neurol 4: 138.https://doi.org/10.1038/ncpneuro0731
3. Stefanatos GA (2008) Regression in autistic spectrum disorders. Neuropsychol Rev 18: 305-319.https://doi.org/10.1007/s11065-008-9073-y
4. Ornoy A, Weinstein-Fudim L, Ergaz Z (2015) Prenatal factors associated with autism spectrum disorder (ASD). Reprod Toxicol 56: 155-169.https://doi.org/10.1016/j.reprotox.2015.05.007
5. Vohr BR, Davis EP, Wanke CA, Krebs NF (2017) Neurodevelopment: The impact of nutrition and inflammation during preconception and pregnancy in low-resource settings. Pediatrics 139: S38-S49.https://doi.org/10.1542/peds.2016-2828F
6. Samsam M, Ahangari R, Naser SA (2014) Pathophysiology of autism spectrum disorders: revisiting gastrointestinal involvement and immune imbalance. World J Gastroenterol 20: 9942.https://doi.org/10.3748/wjg.v20.i29.9942
7. Chaste P, Leboyer M (2012) Autism risk factors: genes, environment, and gene-environment interactions. Dialogues Clin Neurosci 14: 281.
8. Newschaffer CJ, Croen LA, Daniels J, Giarelli E, Grether JK, et al. (2007) The epidemiology of autism spectrum disorders. Annu Rev Public Health 28: 235-258. https://doi.org/10.1146/annurev.publhealth.28.021406.144007
9. Dhariyal RS, Kimothi V, Singh S (2019) A review on autism. Res J Pharmacol Pharmacodynam 11: 76-80.http://dx.doi.org/10.5958/2321-5836.2019.00013.2
10. Volkmar FR, Reichow B (2013) Autism in DSM-5: progress and challenges. Mol Autism 4: 13.https://doi.org/10.1186/2040-2392-4-13
11. Li G, Lee O, Rabitz H (2018) High efficiency classification of children with autism spectrum disorder. PLoS One 13: e0192867.https://doi.org/10.1371/journal.pone.0192867
12. Baker JK, Smith LE, Greenberg JS, Seltzer MM, Taylor JL (2011) Change in maternal criticism and behavior problems in adolescents and adults with autism across a 7-year period. J Abnorm Psychol 120: 465.https://psycnet.apa.org/doi/10.1037/a0021900
13. Kollia B, Kamowski-Shakibai MT, Basch CH, Clark A (2017) Sourcesand content of popular online videos about autism spectrum disorders. Health Promot Perspect 7: 238. https://dx.doi.org/10.15171/hpp.2017.41
14. Ratajczak HV (2011) Theoretical aspects of autism: Causes-Areview. J Immunotoxicol 8: 68-79.https://doi.org/10.3109/1547691X.2010.545086

15. Taylor LE, Swerdfeger AL, Eslick GD (2014) Vaccines are not associated with autism: an evidence-based meta-analysis of case-control and cohort studies. Vaccine 32: 3623-3629.https://doi.org/10.1016/j.vaccine.2014.04.085

16. Kushak RI, Winter HS (2018) Intestinal microbiota, metabolome and gender dimorphism in autism spectrum disorders. Res Autism Spectr Disord 49: 65-74.https://doi.org/10.1016/j.rasd.2018.01.009

17. Lampi KM, Lehtonen L, Tran PL, Suominen A, Lehti V, et al. (2012) Risk of autism spectrum disorders in low birth weight and small for gestational age infants. J Pediatr 161: 830-836.https://doi.org/10.1016/j.jpeds.2012.04.058

18. Berridge KC, Robinson TE, Aldridge JW (2009) Dissecting components of reward: 'liking', 'wanting', and learning. Curr Opin Pharmacol 9: 65-73.https://doi.org/10.1016/j.coph.2008.12.014

19. Seeman P (2010) Historical overview: introduction to the dopamine receptors. In: The dopamine receptors, Humana Press, Totowa, United States.

20. Stoof JC, Kebabian JW (1984) Two dopamine receptors: biochemistry, physiology and pharmacology. Life Sci 35: 2281-2296.https://doi.org/10.1016/0024-3205(84)90519-8

21. Daubner SC, Le T, Wang S (2011) Tyrosine hydroxylase and regulation of dopamine synthesis. Arch Biochem Biophys 508: 1-2.https://doi.org/10.1016/j.abb.2010.12.017

22. Lightman SL, Forsling M (1980) Evidence for dopamine as an inhibitor of vasoprotein release in man. Clin Endocrinol (Oxf) 12: 39-46.https://doi.org/10.1111/j.1365-2265.1980.tb03130.x

23. Best JA, Nijhout HF, Reed MC (2009) Homeostatic mechanisms in dopamine synthesis and release: a mathematical model. Theor Biol Med Model 6: 21.https://doi.org/10.1186/1742-4682-6-21

24. Cosentino M, Marino F, Maestroni GJ (2015) Sympathoadrenergic modulation of hematopoiesis: a review of available evidence and of therapeutic perspectives. Front Cell Neurosci 9: 302.https://doi.org/10.3389/fncel.2015.00302

25. Girault JA, Greengard P (2004) The neurobiology ofdopamine signaling. Arch Neurol 61: 641-644.https://doi.org/10.1001/archneur.61.5.641

26. Asanuma M, Miyazaki I, Ogawa N (2003) Dopamine-or L-DOPA-induced neurotoxicity: the role of dopamine quinone formation and tyrosinase in a model of Parkinson's disease. Neurotox Res 5: 165-176.https://doi.org/10.1007/BF03033137

27. Eisenhofer G, Kopin IJ, Goldstein DS (2004) Catecholamine metabolism: a contemporary view with implications for physiology and medicine. Pharmacol Rev 56: 331-349.https://doi.org/10.1124/pr.56.3.1

28. Alonso ER, León I, Kolesniková L, Cabezas C, Alonso JL (2018) The Rotational Study of Dopac, a Neural Metabolite. 73rd International Symposium on Molecular Spectroscopy, United States.

29. Valko-Rokytovská M, Očenáš P, Salayová A, Kostecká Z (2018) New Developed UHPLC Method for Selected Urine Metabolites. J Chromatogr Sep Tech 9: 2.https://doi.org/10.4172/2157-7064.1000404

30. Schamp J, Florang V, Doorn J (2014) Toxic dopamine metabolites and oxidative stress as key contributors to neurotoxicity (LB641). FASEB J 28: LB641.https://doi.org/10.1096/fasebj.28.1_supplement.lb641

31. Rush RA, Geffen LB (1980) Dopamine β-hydroxylase in health and disease. Crit Rev Clin Lab Sci 12: 241-277.https://doi.org/10.3109/10408368009108731

32. Di Bella S, Ascenzi P, Siarakas S, Petrosillo N, Di Masi A (2016) Clostridium difficile toxins A and B: insights into pathogenic properties and extraintestinal effects. Toxins 8: 134.https://doi.org/10.3390/toxins8050134

33. Ryan KJ, Ray CG (2004) Medical microbiology. McGraw Hill, United States.

34. Kuehne SA, Cartman ST, Heap JT, Kelly ML, Cockayne A, et al. (2010) The role of toxin A and toxin B in Clostridium difficile infection. Nature 467: 711.https://doi.org/10.1038/nature09397

35. Shaw W (2010) Increased urinary excretion of a 3-(3-hydroxyphenyl)-3-hydroxypropionic acid (HPHPA), an abnormal phenylalanine metabolite of Clostridia spp. in the gastrointestinal tract, in urine samples from patients with autism and schizophrenia. Nutr Neurosci 13: 135-143.https://doi.org/10.1179/147683010X12611460763968

36. Goodhart PJ, DeWolf WE Jr, Kruse LI (1987) Mechanism-based inactivation of dopamine beta-hydroxylase by p-cresol and related alkylphenols. Biochemistry 22: 3091-3096.https://doi.org/10.1021/bi00383a025

37. Novus Biologicals (2019) Human Dopamine beta-Hydroxylase ELISA Kit (Colorimetric), United States.

38. Young JG, Kyprie RM, Ross NT, Cohen DJ (1980) Serum dopamine-beta-hydroxylase activity: Clinical applications in child psychiatry. J Autism Dev Disord 10: 1-4.https://doi.org/10.1007/BF02408428

39. Shaw W (2017) Elevated urinary glyphosate and clostridia metabolites with altered dopamine metabolism in triplets with autistic spectrum disorder or suspected seizure disorder: A case study. Integr Med 16: 50-57.

40. Sandler RH, Finegold SM, Bolte ER, Buchanan CP, Maxwell AP, et al. (2000) Short-term benefit from oral vancomycin treatmentof regressive-onset autism. J Child Neurol 15: 429-435.https://doi.org/10.1177/088307380001500701

41. Persico AM, Napolioni V (2013) Urinary p-cresol in autism spectrum disorder. Neurotoxicol Teratol 36: 82-90.https://doi.org/10.1016/j.ntt.2012.09.002

42. Srikantha P, Mohajeri MH (2019) The Possible Role of the Microbiota-Gut-Brain-Axis in Autism Spectrum Disorder. Int J Mol Sci 20: 2115.https://doi.org/10.3390/ijms20092115

43. De Angelis M, Francavilla R, Piccolo M, De Giacomo A, Gobbetti M(2015) Autism spectrum disorders and intestinal microbiota. Gut Microbes 6: 207-213.https://doi.org/10.1080/19490976.2015.1035855