

Determination of Some Biomarkers that affect in Behaviors of Autism Spectrum Disorder Individuals in Iraq

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Abstract

Autism is a developmental disorder characterized by troubles with social interaction and communication by restricted and repetitive behavior. The parents usually notice signs in the first two or three years of their child's life. These signs often develop gradually. 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. After completion of number, required tests were carried out on samples. The tests were three biochemical markers. The first is neurotransmitters (γ ABA), the other enzymes of happiness (Serotonin & Melatonin), all this biochemical markers were measured by elisa technique, the results were a decrease in level of γ ABA for ASD as compare with control group, A significant increases in serotonin in ASD. relative to control group, and no change in level of Melatonin in both ASD and healthy group these results consistent with many research and another research inconsistent with these results have shown the reasons that led to differences in results

Key word: Autism, GABA, Serotonin & Melatonin

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. These signs often develop gradually, though some children with autism reach their developmental milestones at a normal pace and then worsen”^{1,2} so researchers were characterized the “**Autism spectrum disorders** by vast lack of social communication skills and skill of attraction or care between the patient and society According to recent studies carried out at one of the American centers that deal with diseases and methods of prevention³, a statistical study showed (ASD) affects (1) child from (68) less than eight years old,”⁴, the great challenges facing developmental neuroscience is to understand how complex social interactions can shape brain development during ontogeny to affect adult functioning in social and behavioral contexts. Additionally the type of autism Spectrum Disorders (ASDs), including Autistic Disorder (AD), Asperger's Syndrome AS Childhood disintegrative disorder (CDD), Rett's Syndrome (RS) and Pervasive Developmental Disorder Not Otherwise Specified (PDD-NOS) represent a cluster of instability in the growth of nervous which suffers from this cluster as a result is the inability to social communication at all

levels, the excessive behavior repeated since the third year age⁵.

So research was connect between (ASD) and neurotransmitter when they explain gamma-Aminobutyric acid, γ -aminobutyric acid, GABA which is the chief inhibitory neurotransmitter in the developmentally mature mammalian central nervous system. Its principal role is reducing neuronal excitability throughout the nervous system. In humans, GABA is also directly responsible for the regulation of muscle tone⁶.

One of the factors that has revolved around the information on autism and Serotonin level in autistic children and assess the reliability of serum serotonin levels as a biomarker for diagnosis of ASD. Serotonin influences many physiological activities in the human body⁷.

Another studies that deals with Melatonin and its relative with (ASD) This hormone is primarily produced by the pineal gland, several factors have been shown to alter circadian rhythms and melatonin levels, abnormal melatonin secretion has been implicated in circadian disturbances and neurodevelopmental abnormalities including autism spectrum disorder (ASD)⁸.

Subjects & Method

1- Subjects

1-1-Patients: 60 Iraqi children with Autism spectrum disorder (ASD) and 30 apparently healthy Adult were selected as the control group. 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.

The present study excluded the children (ASD) from apparent other mental disease .

1-2- Blood Samples

A Five milliliters of venous blood samples were collected from each children of (ASD) and control subject. then serum was separated and transported

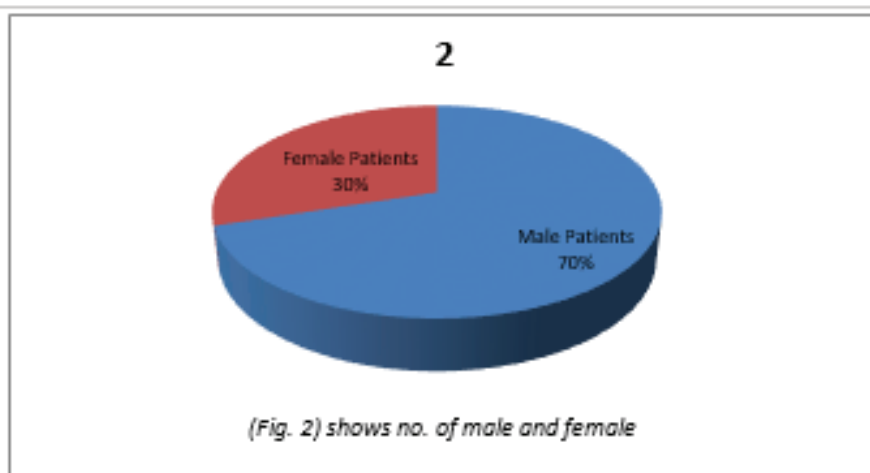
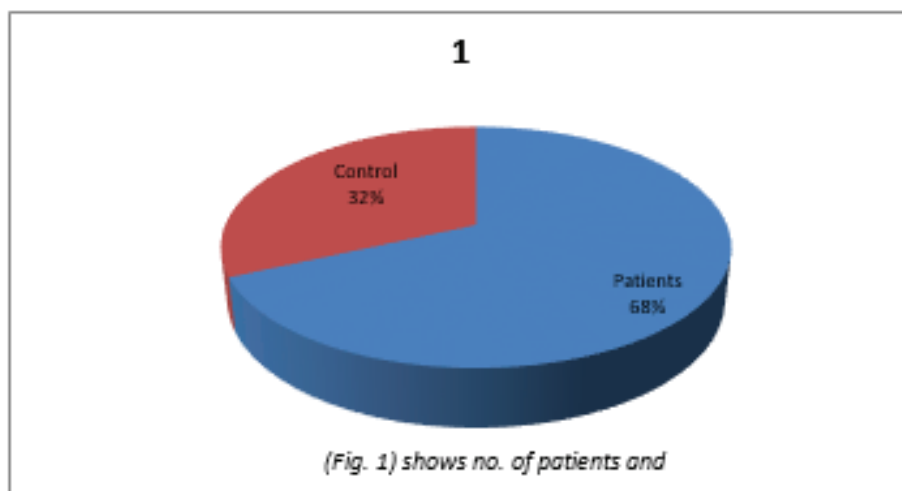
into new disposable tubes and freezes at (-70 c°) until assayed.

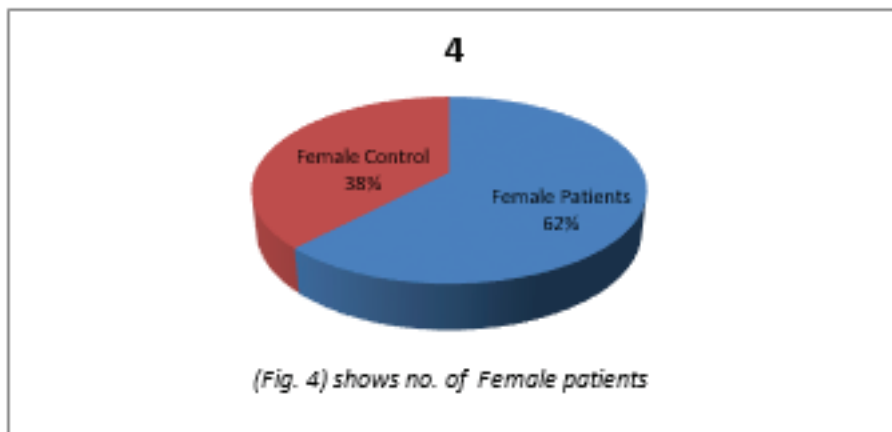
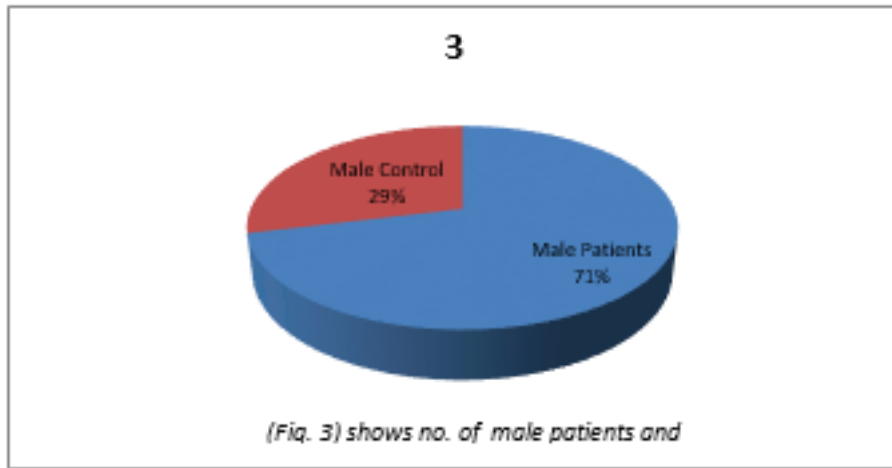
2- Methods : Measuring each of (Gama amino butyric acid (γ ABA) , Serotonin and Melatonin) markers by ELISA ElabSciences kit .

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 also we use Microsoft Excel (2010) , SPSS₁₇ and Minitab v. 14 .

Results and Discussion

This study involved 60 patients with autism (42 males and 18 females) and 28 controls (17 males and 11 female).





The statistical analysis was divided into two sections ,so the first was divided into three parts the first was deal with all Samples , the second was covenant with male Samples, finally the rerst part was deals with female Samples . the second section was deals with the relation ship between all items which was measured in patients sample only ²⁵.

Table (1) level of parameters(γ ABA , Serotonin & Melatonin) for patients with ASD as comparison as control group.

Items	Patients = 60	Control = 28	P Value
	Mean \pm SD	Mean \pm SD	
γ ABA	275.3 \pm 101.7	371.9 \pm 173.7	0.009685
Serotonin	160.7 \pm 75.6	124.8 \pm 73.9	0.04031
Melatonin	121.1 \pm 52.7	149.7 \pm 68.2	0.05623

The table (1) shows a high significant decrease (p value = 0.0097) in level of γ ABA for the case of ASD(275.3) as compare with the control group (371.9) , In this paper, study γ ABA , serotonin and melatonin hormones . The result in table (1) shows a high significant decrease in level of γ ABA for the case of ASD as compare with the control group , as well the results in table (2&3) were congruous with the results of the table (1) which were a significant decrease in level of γ ABA as compare with their healthy group , many papers were agreement with these results , a significant reduction in the level of γ ABA in people with autism ⁹ , the level of γ ABA was very low in people with autism ¹⁰ , in addition scientists were demonstrated with all confidence that the association of autism has an inverse relationship with γ ABA , where the lower the level of γ ABA lead to increase the emergence of symptoms of autism¹¹ , In a second demonstration, so the γ ABA level was low in those with autism at the beginning of the diagnosis ⁹ .

On the other hand, there are other studies that contradict the results of current study , the research about persons suffering from autism were different groups in the ages , that the level of γ ABA was within the normal level , the discrepancy between current paper and the researchers' It may be due to differences in the age of the studied cases for both studies¹² . The age of the new research is 3-8 years, while the research whose results are opposed was about 12-16 years old. A rise in the level of γ ABA the attribution of reason for contrast ages of autism individuals , they said the study of autism must be for all age groups, so, the cause of different levels of γ ABA was attribute gap ages in people with ASD¹³ .

In contrast to γ ABA the level of serotonin in the table (1) shows a significant elevation (p value = 0.04) in case of ASD (160.7) as compare with their control group (124.8), Several previous studies have demonstrated a common phenomena in persons with autism is the high level of serotonin , so in this current study as shwn as in table (1) a significant elevation for level of serotonin in case of ASD as compare with their control group , In a research paper on autism, Levitt P.(2011) explained the high level of serotonin in autism patients ¹⁴ ,in addition researcher was concluded that the level of serotonin increases in people with autism , so the reason for increasing the concentration of serotonin comes from a decrease in turnover of serotonin in the body and to support the results conducted experiments on a type of mice treated mice with a substance that increases the level of serotonin and found that The rate of rotation

is inversely proportional to the level of serotonin in the body¹⁵.

A high level of serotonin in persons with autism is one of the important signs that a good candidates for the determination of the susceptibility to autism , Many of the studies are in line with the results of the new research¹⁶. serotonin plays an important role in their study of autistic patients¹⁷, in the other hand the researchers found that serotonin levels were low in serum while there was a significant increase in the level of serotonin in the whole blood or platelet-rich plasma, Therefore, this study is contrary to the results found in the current study , To know why the difference in results between the current study and the opposite study is that platelets is a place for the accumulation of serotonin in its ¹⁸, which leads to a decrease in level of serotonin this is what explained in their research, the relationship between the level of serotonin in people with autism in the serum and blood platelets. They also conducted a genetic study of serotonin generators¹⁹.

In this study they Clarify the relationship between levels of serotonin in platelets and serum , The difference may be due to the severity of case for subjects in the study, as there is a study by Amjad where the division of cases according to severity to three classes , the serotonin levels directly proportional to the increased severity of the disease , in addion scientists were proved there is no association between low levels of serotonin and autism ²⁰. while the same table (1) displays no change in level of melatonin in both ASD sample (121.1) and its control group (149.7) , The result in this current research shows no significant change in level of Melatonin between autism samples and its healthy group , perhaps most of the research that deals with the study of melatonin in people of autism, shed light on the relationship of this hormone with serotonin hormone , It is a daily synchronous hormone derived from serotonin , So serotonin hormone is inversely proportional to melatonin hormone This is dependable with the results in the current search , the relationship between serotonin and melatonin is negative and the level of melatonin is low while the level of serotonin is high in people with autism compared to control group ⁸.

The level of melatonin in people with autism should be low compared with the healthy people and the time when the blood sample was drawn ²¹. They showed that melatonin levels may give abnormal levels to people with autism or may have insignificant results

compared to Group healthy, where they attributed the abnormal results to many reasons including the situation of the ASD in a dark room or a room with a dim light, where the relationship of light with melatonin was an inverse relationship, so the lower light increased the level of melatonin in the other hand , The rise in levels of melatonin in the autistic may be caused by the use of monotherapy drugs containing a proportion of melatonin for giving them more sleep hours, leading to a

higher level of autistic compared to the standard group, this search also corresponds to the current search . additionally in their research that supplements and some drugs that increase the level of melatonin in the blood of the autistic, where they conducted a search on a group of autistic people taking drugs containing melatonin and found that the level of melatonin does not differ significantly from the control group²², these results was agreement with the current search.

Table (2) relationship between items for persons who are suffer from ASD.

	Melatonin	Serotonin
γABA	0.647	-0.292
	0.000	0.024
Serotonin	-0.306	
	0.017	

The second section of biostatistical analysis which was deal about the relative among items that appear in table no. (2), so the table illustrates a highly positive significant relationship between γABA and Melatonin in persons have ASD($r = 0.647$ & P value = 0.000) , while in the same table demonstrates a negative significant relationship between γABA and Serotonin in same cases of ASD ($r = -0.292$ & P value = 0.024), additionally the table appears a negative significant relationship between Serotonin and Melatonin with individuals who suffer from ASD ($r = -0.306$ & P value = 0.017) .

In recent years concern has risen about the increasing prevalence of developmental disorders. A striking example is Autism Spectrum Disorders (ASD). In this study which is interested in studying some of the biochemical factors and their effect on individuals who suffer from autism , neurodevelopmental disorders primarily diagnosed in childhood

Many researchs that agree with the recent study as the individual of ASD suffers from a lack of the level of γABA , at the same time also suffers from a deficiency in the level of melatonin, since melatonin is composed of serotonin, which means that the higher the level of melatonin decreased the level of serotonin and vice versa, This is agreed upon by²³ and²⁴

Ethical Clearance: Authors’ Contribution; All authors have made substantial, direct and intellectual contribution to the work and approved it for publication.

Ethics Statement: This article contain studies with human participants performed by the authors , the approval of collecting samples from the parents of the persons of the donors.

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Data availability; All datasets generated or analyzed during this study are included in the manuscript

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