Evaluation Serum Gamma-Aminobutyric Acid (Gaba) Receptor Levels In Autistic Children And Correlated With Behavior

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Abstract

Autism is described by impaired functioning on three behavior domains: qualitative impairments in social interaction, qualitative impairments in communication, and the occurrence of stereotyped behaviors or restricted interests. Experimental evidence of GABAergic mechanism in Autistic Disorders is limited. Few reports have demonstrated abnormalities involving the GABA receptor in the brain, blood, and platelets of subjects with autism in compared to nonautistics. Serum from 50 individuals with autism (36 males, 14 females;, mean age 7.80 years) and 25 non-autistic group (21 males, 4 females; mean age 8.46years) was assessed for GABA receptor concentration using ELISAs. Personality ,severity, language and ability to learning) was assessed in these autistic individuals and compared with GABA receptor. In this novel study, individuals with Autistic had significantly significant decrease serum level of GABA ($p < 0.05$) (33.10±5.12 ng/ml) compared with the healthy control (144.04±9 ng/ml). In addition, the negative correlation was found between low GABA receptor levels and speaking, learning, symptoms severity. While positive correlation between low GABA receptor levels and personality among autistic patients. These results concluded correlation between GABA and Autism.

Keyword: GABA receptor , Autism, Behavior, communication, .

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تقييم مستويات مستقبل Gamma-aminobutyric acid (الكابا) في مصل اطفال التوحد وعلاقته بسلوكهم

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الخلاصة

يوصف التوحد بضعف مجالات السلوك والمتمثلة بالضعف النوعي في التفاعل الاجتماعي والتواصل وحدوث السلوكيات النمطية أو وظائف محدودة. الأدلة التجريبية لفهم آلية اضطرابات التوحد محدودة. وقد أظهرت بعض التقارير الاختلال في مستوى الكابا في الدماغ والدم والصفائح مع مرضى التوحد بالمقارنة مع غير المصابين بالتوحد(الاصحاء). جمعت خمسين عينة مصل من مرضى التوحد(36 ذكور و 4 اناث) و خمس وعشرين مصل من الاصحاء. قيمت مستويات الكابا بطريقة الاليزا وكذلك قيم سلوك مرضى التوحد المتمثلة بالشخصية وشدة المرضى والنطق وقابلية التعلم وعلاقتها بمستقبل الكابا. في هذه الدراسة الجديدة لوحظ انخفاض معنوي في مستوى مستقبلات لدى (33و1±12نانوغرام/ملليتر) (P<0,05) مرضى التوحد مقارنة مع مجموعة السيطر(144و04±9نانوغرام/ملليتر).اضافة الى ذلك ، وجدت علاقة سلبية بين مستوى الكابا المنخفض والتحدث والقابلية على التعلم وشدة المرض .بينما كانت هنالك علاقة ايجابية بين مستوى الكابا المنخفض وشخصية مرضى التوحد.

الكلمات المفتاحية: مستقبل الكابا، التوحد،السلوك ،التواصل.

Introduction

Gamma-aminobutyric acid (GABA) is the most abundant inhibitory neurotransmitter in the mammalian brain, where it is widely distributed [1]. In the mature brain, GABA are the main excitatory and inhibitory neurotransmitters in the human brain and both have important roles during early development of the nervous system, an ontological stage when the evidence indicates that autism begins.In addition, GABA during the embryonic and the perinatal period, it depolarizes targeted cells and triggers calcium influx, regulating different developmental processes from cell proliferation migration, differentiation, synapse maturation, and cell death [2]. Gamma-aminobutyric acid A ($GABA_A$) receptors are ligand-gated ion channels responsible

for mediation of fast inhibitory action of GABA in the brain [3]. Therefore, it is important to analyze the functional status of glutamatergic and GABAergic neurotransmission in the autistic brain [4]. $GABA_A$ receptors are divided into multiple subunits (α1-α6, β1-β4, γ1- γ4, δ, ε, π, θ, and ρ1-ρ2) producing multiple $GABA_A$ receptor iso-forms [5]. The $GABA_A$ receptor expression varies according to developmental time tables of the brain, suggesting different roles for different types of receptors, Glutamate and GABA are considered initial inhibitory, and excitatory, amino acid neurotransmitters. In spite of their distribution in brain function an sensual methods, neurotransmitters have extradited restricted concern in the autism research and associated developmental inabilities. Whereas GABA and glutamate pathways in the centeral nervous system proposes that all pathology will affect, amino acid neurotransmission, the conformation of special defects popular to autism might contribute to condense and cramped the concentrate of research [6]. GABAergic inactivation perhaps inhibited both by direct damage of GABA receptors, and by antagonism of GABAergic neurons carring receptors sentient to the glutamate analogue (N-methyl-D-aspartate). Decreased of inactivator control ,from GABAergic neurons, may initiate in high irritation of weakness target neurons, with discriminatory harm to large- to (mediumsized pyramidal and multipolar neurons)[7]. The hypothesis of suppressed GABAergic inhibition in autism is depend on two points.: I-pathology correlating to GABA receptors appears as a common factor in many suspected two etiologies of autism and II-both dis-inhibition of GABAergic effect and high activation of non-NMDA glutamate receptors, produce pathology which image that noted in autism [8]. Few number of researches have detected anomalies including the glutamate and GABAergic systems in brain, blood, and platelets of subjects with autism [9,10]. This study was aimed to estimate serum levels of GABA receptor and correlate with behavior in autism patients.

Materials and Methods

Patients and Controls

Fifty blood samples of autistic children (36 Male and 14 female ,age range was 2-10 years, Mean ± S.D.:7.80±2.34 years) and 25 non-autistic group (21 Male and 4 Female, Mean± S.D,: 8.46±2.11 years) were collected during the period December 2014-February 2015 in Al-Malak, Al-Sagheer center, Al-Safa center and Al-Rajaa center

Collection of Blood samples

A total of 3 ml venous blood was aspirated from each individual.The samples were brought to the laboratory in a well-insulated ice box. The clotted blood was centrifuged at 2000 rpm for 15 minutes; and by then, serum was collected and distributed into aliquots of 200 µl in eppendorf's tubes, which were frozen at -20°C until laboratory assessments.

Principle of estimation of GABA receptor

Immunoassay was used to measure serum GABA receptor in biological samples (Demeditec Diagnostic GmbH,Germany). Gamma-aminobutyric acid is quantitatively determined by ELISA. The competitive ELISA utilize the microtiter plate. The antigen is linked solid phase of the microtiter plate. The standards, controls and specimen and the solid phase join analyze compete for a steady number of antiserum binding sites. The antibody bound to the solid phase is discovered by uses an antirabbit IgG peroxidase conjugate using tetramethylbenzidine (substrate). The reaction is estimated at 450 nm. Assessment of unknown specimens is done by putting their absorbance on standard curve to obtain final concentrations (Fig.1).

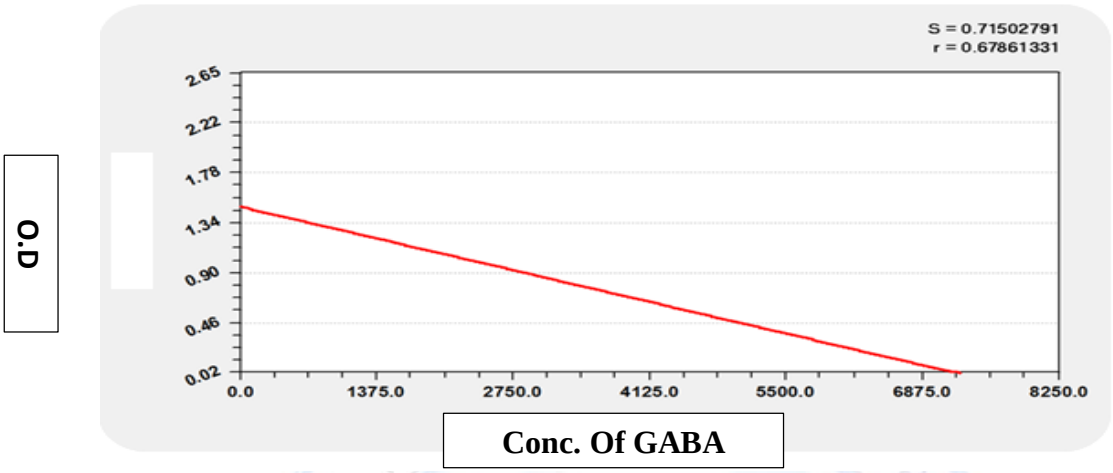

Figure 1: Standard Curve of GABA receptor Serum Level.

Results and Discussion

The present study was indicated significant decrease serum level of GABA receptor ($p < 0.05$) in autistic children (mean 33.10±5.12 ng/ml) compared with the healthy control (mean 144.04±9ng/ml) (Table 1 and Table 2).

Table (1): Mean of GABA in studied groups(Autism patients and non autistic).

Studied groups	Mean	SD.	SE	C.S
autism	33.10	36.26	5.12	P=0.000 P≤ 0.05 H S
Non autistic	144.04	45.00	9.00	

Table 2: Frequency of GABA in studied groups (Autistic patients and non autistic).

Studied groups	GABA ng/ml				Total
	Normal	%	Decrease	%	
Autism	15	30.0	35	70.0	50
non autistic	25	100	0	0	25

In autistic patients, negative correlation was found between GABA receptor and language (r = -0.286 , p=0.44), GABA and ability to learning (r= -134 , p=0.355), GABA and severity symptoms (r= -0.013, p=0.931). While positive correlation between GABA receptor and Personality (r=.0109,P=449) (Table 3).

Table (3): The correlation between GABA receptor and autistic behaviors.

		Personality	Language	learning	Severity
GABA receptor	Pearson Correlation	.109	-.286	-.134	-.013
	Sig. (2-tailed)	.449	.44	.355	.931
	N	50	50	50	50

In this respect, up to our knowledge, there is no study try to estimate of GABA receptor in Autistic and correlated with behavior. In the table (1) and table (2) showed a highly significant reduction in GABA receptor levels in (70%) of autistic children was agreement with ElBaz *et al.* (2014) [12]. Gaetz and his coworker have shown that GABA deficiency is common in autistic individuals, that may contribute to the poor inhibition that overstimulates their brains causing them to live in a constant state of anxiety [13]. According to a recent study (2014),which noted autistic children have lower GABA levels in brain regions correlated with motor control and sound processing. Gamma-aminobutyric acid mediated calcium signals

regulate a different of developmental processes from cell proliferation migration, synapse, maturation differentiation and neuronal wiring. Therefore, it is not surprising that some forms of neurodevelopmental disorders like ASDs are related with alterations of GABAergic signaling and impairment of the excitatory/inhibitory balance in selective neuronal circuits [14]. The reduction in GABA level in autistic children these due to (GABA is the most avaliable and multilateral neurotransmitter in the central nervous system. Gamma-aminobutyric acid is excitatory in the inhibitory in the mature one and immature brain).GABA in early stages of neural development has a paracrine action on immature neurons. It regulating migration of neuronal ,stimulating networks development and exerting a high range of trophic actions that lead to the correct establishment of neural circuits)[13]. to our knowledge, no further investigations have been published and the rare study for detection GABA levels by ELISA technique The results with respect to GABA level are incomparable to the results reported by Borgatti et al. [15], which were GABA levels of healthy groups (mean 18.8±4.5) were lower than the levels in controls in our results (mean 144.04±9). On the other hand , the previous study [16] revealed that mean serum GABA levels were lower (15.4± 6.2) than the levels noted in patients groups in our specimen (33.1± 5.12). These differences may be due to variation in samples of patient, status of medication and type of analytic method to estimate GABA concentraction. In addition, Our data adds to little evidence supporting the role of GABA in the etiology and pathophysiology of autism disorders. Others have been placed on the biological, anatomical, genetic results suggest some low or abnormal inhibition of GABA in autism [16][17].

Conclusion

These results conculded the etiology or pathophysiology of Autistic Disorders affected by concentration of GABA. In addition , the low serum GABA receptor levels postive related to personality in individuals with autism, while negative correlate with speaking and learning and severity.

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