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Biochemical studies in autistic patients of Iraq Salah Al-din treatment and use of pharmaceutical drugs and analysis of chemical parameters

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Abstract

Biochemical studies in autistic patients have mainly focused on monoamines (serotonin, norepinephrine, dopamine) and opioids (including, above all, ß-endorphins which belong to the group of endomorphins characterized by their central analgesic property). The results on which there is consensus relate to hyperserotoninemia and the existence of an abnormally increased stress response in infantile autism. The liver is an extraordinarily complex organ with a very wide range of tasks within our body: synthesis and destruction of carbohydrates, lipids and proteins, excretion of waste products through bile, modulation of the immune response. "Liver function tests" consist of the measurement in blood of the concentration of bilirubin and the activity of certain enzymes present in the liver (called GOT, GPT, FA and GGT). The elevation of its normal values indicates that there is a liver injury (although they can also be altered in non-hepatic processes). The results indicated that no sample had an abnormally increased level of 7-dehydrocholesterol reductase (7DHC), consistent with a diagnosis of SLOS. However, 19 of the 100 samples had a total cholesterol level of less than 100 mg/dL. The researchers demonstrated that SLOS is an uncommon cause of ASD, and additionally showed that at least 20% of ASD children have significant hypocholesterolemia.

Keywords: Autistic, Pharmaceutical Drugs, Chemical Parameters.

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دراسات كيميائية حيوية لدى مرضى التوحد في العراق صلاح الدين بمعالجة واستخدام الأدوية الصيدلانية وتحليل البارامترات الكيميائية

بشرى اسماعيل ابراهيم [1]

المستخلص

ركزت الدراسات الكيميائية الحيوية لدى مرضى التوحد بشكل أساسي على أحاديات الأمين (السيروتونين والنورإبينفرين والدوبامين) والمواد الأفيونية (بما في ذلك، قبل كل شيء، إندورفين بيتا الذي ينتمي إلى مجموعة الإندومورفين التي تتميز بخاصيتها المسكنة المركزية). النتائج التي تم الإجماع عليها تتعلق بفرط هرمون السيروتونين في الدم ووجود استجابة إجهاد متزايدة بشكل غير طبيعي في مرض التوحد الطفولي. الكبد هو عضو معقد للغاية وله مجموعة واسعة جدًا من المهام داخل الجسم: تخليق وتدمير الكربوهيدرات والدهون والبروتينات، وإفراز الفضلات من خلال الصفراء، وتعديل الاستجابة المناعية. تتكون "اختبارات وظائف الكبد" من قياس تركيز البيليروبين في الدم ونشاط بعض الإنزيمات الموجودة في الكبد (وتسمى GOT و GPTو FAو GGT) .يشير ارتفاع قيمه الطبيعية إلى وجود إصابة في الكبد (على الرغم من إمكانية تغييرها أيضًا في العمليات غير الكبدية). أشارت النتائج إلى أنه لم يكن لدى أي عينة زيادة غير طبيعية في مستوى إنزيم 7-ديهيدروكوليستيرول المختزل (DHC 7) ، وهو ما يتوافق مع تشخيص SLOS ومع ذلك، كان مستوى الكوليسترول الإجمالي في 19 من أصل 100 عينة أقل من 100 ملجم/ديسيلتر. أثبت الباحثون أن SLOS هو سبب غير شائع لاضطراب طيف التوحد، وأظهروا بالإضافة إلى ذلك أن ما لا يقل عن 20٪ من أطفال طيف التوحد يعانون من نقص كبير في نسبة الكولسترول في الدم.

الكلمات المفتاحية : التوحد، الادوية الصيدلانية، البارامترات الكيميائية

انتساب الباحث
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معلومات البحث
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Introduction

The liver is an extraordinarily complex organ with a very wide range of tasks within our body: synthesis and destruction of carbohydrates, lipids and proteins, excretion of waste products through bile, modulation of the immune response... "Liver function tests" consist of the measurement in blood of the concentration of bilirubin and the activity of certain enzymes present in the liver (called GOT, GPT, FA and GGT). The elevation of its normal values indicates that there is a liver injury (although they can also be altered in non-hepatic processes). This alteration occurs, in most patients, in one of the following forms, whose causes we will explain below: hyperbilirubinemia (with or without elevation of transaminases and/or cholestatic enzymes) [1].

Hyperbilirubinemia

Hyperbilirubinemia consists of an increase in the concentration of bilirubin in the blood, which may or may not be accompanied by other alterations in liver function tests, depending on the process in question; when it is of sufficient intensity, it gives rise to a yellowish coloration of the skin and mucous membranes called "jaundice". Bilirubin is a waste product (specifically, it comes from the degradation of red blood cells, degradation from which indirect "or" unconjugated" bilirubin arises in the first instance) that the liver is responsible for eliminating through the bile to the intestine. To do this, the liver submits bilirubin to the processes of conjugation (in which "direct" or "conjugated" bilirubin is obtained) and excretion (process by which "conjugated" bilirubin passes into bile) and, Next, the bile travels through a series of tubes called the "bile duct" that are responsible for leading it to the intestine. The increase in bilirubin in the blood may be due to an excess of bilirubin production that exceeds the elimination capacity of the liver, to a failure in the hepatic processes of conjugation and/or excretion or to some problem that prevents the arrival of bile to the intestine [2]. The production of bilirubin is increased in all those processes that occur with an increase in the destruction of red blood cells, such as hemolysis, among other entities. Failure in conjugation or excretion processes can occur in isolation in congenital Gilbert and Crigler-Najjar (conjugation) and Dubin-Johnson and Rotor (excretion) syndromes, or as part of a much broader liver problem. for example within acute hepatitis or in the course of cirrhosis. Finally, the obstruction to the flow of bile that prevents its elimination to the intestine occurs in disorders such as primary biliary cirrhosis, tumors of the head of the pancreas, the presence of stones in the bile duct, etc [3] .

Cytolysis Pattern

In this case there is an increase in the blood of transaminases mainly: AST and ALT. Aspartate aminotransferase (AST or GOT) and alanine aminotransferase (ALT or GPT) are enzymes whose function is to transfer molecules called "amino groups". The destruction of the cells that contain transaminases causes the release of these enzymes into the blood, so that the increase in their concentration in the blood results in an injury to those tissues in which they are found: therein lies their usefulness. While AST is found within the cells of various organs and tissues such as liver, kidney, cardiac and skeletal muscle, pancreas or brain, ALT is located predominantly (although not exclusively) in the liver [4] . Thus, and given that ALT is found predominantly in the liver, a

significant increase (≥ 1. 000 IU/L) of ALT (which will normally be accompanied by an equally important increase in AST, constituting a pattern of cytolysis) will almost always come from the liver, indicating destruction of liver cells; which is characteristic of processes such as acute viral hepatitis, acute ischemic hepatitis or toxic hepatitis. Moderate increases in transaminases, on the other hand, can be due to both hepatic diseases (acute alcoholic hepatitis, in which AST typically rises more than ALT, chronic viral hepatitis, hepatic steatosis, etc.) and extrahepatic diseases (hyperthyroidism, celiac disease , adrenal insufficiency, etc.). indicating liver cell destruction; which is characteristic of processes such as acute viral hepatitis, acute ischemic hepatitis or toxic hepatitis. Moderate increases in transaminases, on the other hand, can be due to both hepatic diseases (acute alcoholic hepatitis, in which AST typically rises more than ALT, chronic viral hepatitis, hepatic steatosis, etc.) and extrahepatic diseases (hyperthyroidism, celiac disease , adrenal insufficiency, etc.). indicating liver cell destruction; which is characteristic of processes such as acute viral hepatitis, acute ischemic hepatitis or toxic hepatitis. Moderate increases in transaminases, on the other hand, can be due to both hepatic diseases (acute alcoholic hepatitis, in which AST typically rises more than ALT, chronic viral hepatitis, hepatic steatosis, etc.) and extrahepatic diseases (hyperthyroidism, celiac disease , adrenal insufficiency, etc.) [5] .

Cholestasis Pattern

The pattern of cholestasis is characterized by an increase in alkaline phosphatase (AP) and gamma-glutamyl transpeptidase (GGT) (hence called "cholestasis enzymes") with or without an associated increase in bilirubin. FA and GGT are two enzymes present in numerous tissues, whose function is, respectively, to break certain bonds between molecules and to transfer "gamma-glutamyl groups". The cause of the cholestasis pattern is the impediment to the arrival of bile from the liver cells to the intestine, either due to inability to form it or due to obstruction to its flow. Examples of pathologies that cause cholestasis are the consumption of certain drugs (anabolic steroids, amoxicillin-clavulanate, chlorpromazine, etc.), severe bacterial infections, processes that obstruct the main bile duct (such as the presence of stones or pancreatic tumors), or pathologies that cause obstruction and destruction of the small bile ducts of the liver, such as tumor infiltration of the liver, amyloidosis, primary biliary cirrhosis , etc. It should not be forgotten, however, that both AF and GGT are not only found in the liver, but have different sources of origin, so their increase is not always of hepatic origin. Thus, for example, in growing adolescents an increase in AF of bone origin is typical and in pregnant women an increase in AF of placental origin; Similarly, GGT can be elevated in numerous extrahepatic pathologies such as diabetes mellitus, acute myocardial infarction or chronic renal failure [6] .

Etiopathogenesis in primary Autism Spectrum Disorder Inflammation

Inflammation is the response caused by the immune system to an attack caused by a microorganism or its products, or by any other biological, mechanical, physical or chemical agent capable of generating tissue damage. It is therefore an innate defense mechanism and nonspecific, which originates as a reparative response to an injury, despite the fact that it can cause tissue

damage by itself, as we will see below. Various cell types and a wide group of proinflammatory mediators and inducers are involved in inflammation.

Inflammation and neurological diseases

In recent years, inflammation is gaining great interest in the etiopathogenic studies of mental illnesses, as in the case of major depression. Compared with healthy individuals, subjects with major depression exhibit all the cardinal features of inflammation, including elevations of inflammatory cytokines and their soluble receptors in blood and cerebrospinal fluid (CSF), as well as elevations in blood. of the levels of acute phase proteins, adhesion molecules and inflammatory mediators such as prostaglandin. The association between inflammatory markers and some depressive symptoms [7] such as fatigue, cognitive dysfunction and sleep disturbances have been related to elevated levels of IL-6 and increased nuclear factor kappa B (NF-KB) activity [8] . On the other hand, there are several lines of evidence indicating that the administration of cytokines or inducers of cytokines such as liposaccharides or vaccination in healthy subjects can cause symptoms related to depression such as fatigue, confusion, anguish or sadness [9] .

Inflammation and Autism Spectrum Disorder

In recent years, different works have been developed that support the theory that there is an inflammatory component in a group of autistic patients, and that this proinflammatory situation could contribute to the pathophysiology of ASD [10] found an increase in proinflammatory cytokines in the brain and CSF of children with ASD. The authors found an active inflammatory pattern in the cerebral cortex, white matter, and particularly in the cerebellum. The authors pointed out that the microglial activation found may play a fundamental role in the pathogenesis of the autistic brain. They found significantly more microglia in the fronto-insular and visual cortex than controls.

[11] determined the levels of Brain Glial Acidic Protein (GFAP) in the CSF of autistic children, finding an average level three times higher than that of the control group. The authors determined that this situation could indicate gliosis and brain damage. For their part quantified GFAP levels in post-mortem brain tissue samples from patients with autism, finding higher levels than control samples in the frontal, parietal and cerebellum cortex, concluding that the elevation of GFAP could confirm microglial and astroglial activation in autism, and would indicate gliosis, reactive injury and disturbance of neuronal migration processes. [12] found a significant increase in autistic patients in astrocytic markers aquaporin 4 and connexin 43 in areas such as the cerebellum, Brodmann's area 40 and the superior frontal cortex, which would indicate astrocyte activation.

There seems to be evidence of an increase in inflammatory cytokines in the brain and CSF of autistic children. [13] stated that the levels of TNF-α, IL-6, IL-8, GM-CSF and IFN-γ were significantly higher in the brains of ASD patients than in controls.

[14] carried out an interesting review on brain inflammation and perinatal stress associated with ASD. The authors raise the possibility of the existence of an endophenotype of autism with autoimmune dysregulation, as various authors have reported [15] , and that this could participate in the pathogenesis of autistic symptoms in these patients. In a cohort of Egyptian autistic children,

54.5% had anti-neuron antibodies, which may indicate a loss of self-tolerance to neuronal antigens during the early phases of neurodevelopment, although its significance is currently unknown. exact role in the pathogenesis of autistic symptomatology. On the other hand, the authors valued the importance of the role of TGF-β1 in ASD.

There are authors who have detected low levels of TGF-β1 in ASD children [16] , although the importance of this finding is not clear based on animal studies, since an overexpression of TGF-β1 after birth could be related to with decreased social interaction in mice, while a chronic overexpression of TGF-β1 in adult mice conditions an opposite behavior, which could be in line with the overexpression of TGF-beta1 found in autistic patients.

NF-KB expression could participate in neuroinflammation in some patients with ASD. [17] stated that NF-KB is aberrantly expressed in the orbitofrontal cortex in patients with ASD, which could be part of an inflammatory molecular cascade that would take place fundamentally in brain regions involved in autistic symptomatology. [9] examined peripheral blood samples from 67 autistic children and 29 control children, and found that the autistic group had a statistically significant increase in NF-KB activity compared to the control group.

Materials and Methods

Determination of oxidized glutathione and reduced glutathione

For GSH measurement, 10µl of saliva with 10µl of an ethanol ophthalaldehyde solution (OPT, 1mg/ml) and 180µl of phosphate buffer (sodium phosphate 100mM, 2.5mM EDTA-Na 2 , pH 8.0) for 15min at room temperature. Next, the fluorescence of the samples was read in a plate fluorimeter. To quantify GSH, the fluorescence emitted by the samples was compared with that of a curve of standard solutions with known concentrations of GSH [18] .

Blood Specimens Collection by (venipuncture):

Blood samples were collected by venipuncture from 100 patients and 30 controls (five milliliters of venous blood) were drawn by disposable syringe under aseptic technique. each blood sample was divided into two parts:

a- Two milliliters were put directly in a sterile tube containing EDTA for WBC deferential count.

b- Eight milliliters were placed in a two sterile plane tube and allowed to clot, then serum was separated by centrifugation at 4000 rpm for 15 minutes. The serum was stored at -20 C° freezing. These sera (100 ASD patients and 30 controls) were used for estimating:

The concentration of Immunoglobulin's (IgG, IgG, IgA).

Measurement of some biochemical tests (SGOT, SGPT, ALK, CRP. And GSH)

Liver function tests: AST, ALT, AP and GGT

Within the so-called liver function tests (LFT) several biochemical parameters are included, such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutaryl transpeptidase (GGT), alkaline phosphatase (AP), bilirubin, albumin and prothrombin activity. However, this terminology is not entirely accurate since of all of them, only the last three measure the functional capacity of the liver, the rest being potential indicators of liver damage. Due to the high frequency with which these tests are included in screening programs for both symptomatic and

asymptomatic patients, a common problem in clinical practice is to correctly interpret the results obtained. To do this, first of all, it must be taken into account that the normal range for any laboratory test is in the interval between ± 2 standard deviations in relation to the mean value obtained from a healthy population with similar characteristics. According to this criterion, up to 5% of the healthy population in whom liver function tests are requested present at least one altered value. On the other hand, normal results do not completely exclude the existence of liver disease. Two examples of this are chronic hepatitis due to HCV, in which the existence of a fluctuating pattern in transaminase values over time is characteristic, or advanced cirrhosis, where we can find a liver profile within normal values.

Biochemical factors

It has been shown that 25-33% of individuals with ASD have an increase in the level of serotonin (5-HT) in the blood. However, this situation is not specific for ASD [19] . It has been suggested that serotonin has a trophic effect on brain development, and that disruptions in the serotonin system may cause deterioration in the maturation of central nervous system neurons (such as neuronal differentiation, neuroblast division, cell migration, synapse formation). In addition, it has been reported that selective serotonin reuptake inhibitors reduce stereotypical behaviors and increase social interaction [20] . It has been reported that dopamine metabolism is also impaired in autism. Increased dopaminergic activity in the brain has been associated with hyperactivity and stereotypes seen in ASD [21] . The elevation of homovalinic acid (HVA), the major metabolite of dopamine, in the cerebrospinal fluid (CSF) has been found to be associated with increased intraction and stereotypical movements. This is consistent with the general observation that drugs that increase dopamine levels increase behavioral problems in autistic children. In addition, a decrease in symptom severity has been shown with an increase in the ratio of 5-hydroxy-indolacetic acid (5-HIAA; serotonin metabolite) level in CSF to HVA level. It has been thought that excessive secretion of brain opiate peptides, including beta-endorphins, may be important in ASD. However, studies with naltrexone, an opiate antagonist, in autistic individuals yielded conflicting results [22] .

[23] In studies on autism and oxidative stress, significant changes were found in antioxidant enzyme activity, lipid peroxidation products and NO (Nitric Oxide) levels in autistic children compared to controls. When the children diagnosed with ASD and the control group were compared, there was no significant difference in superoxide dismutase levels between the two groups, while NO and total nitrate levels were found to be higher in the patient group with ASD. Ideas have been put forward that changes in NO levels may be significant for autism. Significantly increased nitrite levels in autism indicate a possible role for NO in the pathogenesis. The sensitivity of the brain to oxidative damage also shows that NO may play a role in the neuropathophysiology of autism [23] reported that autistic children have higher plasma nitrate and nitrite levels than controls. In another study by Zoroğlu and his team with autistic patients, it was stated that serum nitrite and adrenomedullin levels increase in autistic children, total nitrite levels may be an indicator of NO activity in the CNS, and adrenomedullin may also play a role in the

pathogenesis of autism. In addition, the correlation between NO and lipid peroxidation products shows that NO and other molecules formed after it support the possible roles in the pathogenesis of autism.

Lipid metabolism

Phenylketonuria alters lipid metabolism because, as stated above, phenylalanine has the ability to inhibit HGM-CoA reductase, which is why in patients with PKU, HDL and LDL cholesterol levels in the blood have been decreased, as well as of apoproteins A-I, A-II and B. These patients also have very low levels of other lipids or lipid derivatives in our body, such as eicosanoids and arachidonic acid [24] .

Results and Discussion

Biochemical Parameters

Table 1 shows the descriptive statistics of the levels of the biochemical parameters analysed in patients with ASD and control, showing that the serum levels of Creatinine, Uric Acid, Triglycerides, Total Proteins, Albumin, Globulins, ALT, AST and Total Bilirubin, are very similar and did not present significant differences between both groups ($p > 0.05$). Similarly, it is observed that children with ASD have significantly higher levels of blood glucose ($p=0.001$), as well as significantly lower levels of total cholesterol ($p<0.05$) compared to the control group. In contrast to the usual range that is seen in youngsters, the levels of alkaline phosphatase were found to be quite elevated. There is a significant lack of vitamin D when there is a high level of serum alkaline phosphatase and low amounts of phosphorus. In addition, the control group had a mean phosphorus level that was lower than the ASD group as shown in Table (1).

Table (1): Descriptive statistics of the results of the biochemical parameters analysed in patients with ASD and control groups

	ASD Group						Control Group			P
Parameters	Group -1	Group -2	Group -3	Average(x)	±SD	n	n	Average(x)	±SD	
Glycemia	82.66	84.41	85.92	84.33	6.72	100	30	75.797	7.41	<0.001
Cholesterol	141.16	144.15	146.71	144.01	26.88	100	30	155.906	28.93	<0.05
creatinine (CRP)	0.49	0.50	0.51	0.50	0.12	100	30	0.459	0.10	>0.05
Uric acid	3.70	3.78	3.84	3.77	1.09	100	30	3.600	0.55	>0.05
Triglycerides	72.37	73.91	75.22	73.83	32.66	100	30	76.031	35.88	>0.05
Total proteins	7.10	7.25	7.38	7.24	0.41	100	30	7.061	0.34	>0.05
Albumin	4.08	4.17	4.24	4.16	0.41	100	30	4.148	0.41	>0.05
globulins	3.01	3.08	3.13	3.07	0.40	100	30	2.930	0.42	>0.05

ALT (SGPT)	29.31	29.93	30.47	29.90	8.13	100	30	31.143	4.87	>0.05
AST (SGOT)	31.84	32.51	33.09	32.48	9.14	100	30	29.828	5.67	>0.05
Total bilirubin	0.47	0.48	0.49	0.48	0.25	100	30	0.432	0.17	>0.05
Alkaline phosphatase (U/L)	227.93	232.76	236.90	232.53	83	100	30	204	58	0.011
GSH	80.90	82.61	84.08	82.53	36.1	100	30	96.67	16.95	0.036

Reference : Significantly lower amounts of both total glutathione and the reduced or active form of glutathione are found in children with ASD [25] . Given the importance of this system for normal cellular function, the considerable declines in total and free plasma glutathione and the redox ratio (active reduced: inactive oxidised glutathione) in adolescents with ASD is of special concern.

Biochemical parameters

According to our results, 76.6% of diagnosed children are male, with a ratio of 4:1, that is, for every female there are approximately 4 males with ASD. This increased male prevalence has been frequently reported in various neurodevelopmental disorders, suggesting the fact of a female protective model. In favor of this conclusion, a study has been published in the "American Journal of Human Genetics", where the genes of 1,614 people with autism were analysed. Identifying a gene known as SHANK [26] that, when mutated, may be responsible for generating alterations in the proper functioning of the synapse, triggering difficulties in the ability to communicate and social interaction typical of autism, and although the reason is not yet fully known, it is observed that in women this mutation does not seem to affect the synapse, creating a certain level of resistance. However, when the cases of the mutation are more severe, female immunity is broken, and that is when they are capable of presenting alterations in the synapse [27] .

In favor of female immunity, another study was published in the "American Journal of Human Genetics", in which they identify a new candidate gene for autism, the orphan alpha receptor (RORA) related to retinoic acid (RAR), which is a sex hormone dependent transcription factor. In summary, they indicate that patients with autism have a reduction in protein expression of RORA, the latter being involved in several key processes, including Purkinje cell differentiation, protection of neurons against oxidative stress, suppression of inflammation and regulation of the circadian rhythm. It was observed in this research that testosterone levels (androgens) generated lower protein expression of RORA, however, increases in estrogen levels in turn generated increases in RORA. In conclusion, women, by physiologically presenting higher levels of estrogen, generated a protective effect of neurons against oxidative stress and thus less risk of presenting ASD [28] .

Regarding diagnosis, we know that there is currently no specific biomarker for ASD capable of generating measurable differences, either intracellularly or extracellularly, between

physiological and pathological states [28] . In this study we analyzed all routine biochemical parameters in both children with ASD and controls; showing that children with ASD have significantly lower levels of total cholesterol compared to healthy children.

[29] published a study that reflects the importance of cholesterol and what its deficit can contribute to ASD, as observed in the SLOS. The researchers indicate that people with SLOS treated with cholesterol have fewer autistic behaviors, as well as fewer infections and symptoms of irritability and hyperactivity. Studies indicate that cholesterol should definitely be considered as a useful treatment approach while awaiting a better understanding of cholesterol metabolism and ASD. For their part, [30] studied the possible biomarkers that could be significant in neurological disorders. The authors concluded that a relevant candidate in the profile of biomarkers in ASD patients was cholesterol, as well as its associated molecules, due to its great importance in the development and maintenance of the Central Nervous System. This study indicated that the determination of cholesterol can generate relevant clues for the diagnosis and early intervention in childhood of children with Autism Spectrum Disorder. In addition to hypocholesterolemia, in our research we found significantly higher blood glucose levels in patients with ASD compared to healthy controls.

Conclusions

The present study highlights the relationship between some chemical parameters and ASD. Further study must carry out with large number of cases to investigate this relationship more deeply in these patients.

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