Evaluation of some biomarkers of autistic children in Thi-Qar province / Iraq

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Abstract:

Several studies have been carried out to find a candidate biomarkers linked to the development of autism disorder. The aim of this study is to provide an overview of the various biomarkers and discuss it is roles in the pathogenesis of autism. Because of, early analysis may lead to early estimation and prevention for the autistic neurodevelopment disorders rather than only to a symptom category. This study investigates some blood standers and concentrations of trace elements and toxic metals as bio marker for autism disorder indicators.

The study was involved 95 autistic children aged 1–14 years, included 77 males (81%) and 18(19%) females. The results were showed the age group of 3-5 years recorded the highest percentage (41.05%). Blood analysis results showed blood groups that were RH+ recorded the highest percentage for B+, A+, O+ and AB+ with 30%, 28%, 26% , and 5% respectively. While that were RH- noted the lower percentage for O- (7%), A- (4%), AB- and B- didn't record any percentage. Blood cell counts (CBC) of WBC in autistic children were not significantly different from those of the control group, with the sole exception of a significant trend for neutrophil and lymphocyte, slightly lower in the autistic group ($p \leq 0.05$). While, RBC variables showed significant differences regarding HGB, HCT, MCV and MCH in autistic children compare with control group($p \leq 0.05$).

The study included measurements of trace elements (Copper and Zinc) in whole blood and toxic element (Lead) in serum. The results were showed elevation in level of this elements in all age groups with significand deference between patients and control ($p \leq 0.05$). These result could be correlated with the severity of autism.

These findings suggest that biomarkers for neurodevelopment may be critical and improve early diagnosis and probably for therapy. More biochemical investigation, or any other test that helps diagnose the abnormality disorder in children.

Key words: Autism ; CBC ; Biomarkers ; Trace elements

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Introduction:

Autism spectrum disorders (ASD) defines a group of neurodevelopmental disorders characterized by impaired verbal and nonverbal communication and social interactions associated with stereotyped patterns of behavior and mannerisms [(1)]. ASDs continue to increase in prevalence approximately 1% of the population affecting with disorder which is usually diagnosed in early childhood [(2)]. It is unknown etiology, but genetic and wide range of environmental factors have been implicated in the disorder [(3)]. The diagnosis of autism is uniquely based on the patient's history and the observation of behavioral criteria, rather than physical examination findings or laboratory tests.

Since there are no definitive biological markers of autism for a majority of cases, diagnosis depends on a range of behavioral signs, although endogenous factors interest needs to be evaluated [(4)]. A dependable biomarker, could significantly contribute to an early and more exact autism diagnosis, a crucial requirement for an early behavior modifying therapeutic intervention.

Biomarkers have been defined as a specific measurements to evaluated normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention[(5)]. In other words, a biomarker is any neurophysiological, neuropsychological, neuro- anatomical, endocrinological, biochemical, cognitive and genetic marker that is analytic of the presence of disease [(6)]. New biomarkers that can be used in identifying and validating population that are considered as symptoms of an underlying cause of autism such as ABO or Rh incompatibility [(7)], abnormal blood levels and changes in trace element metabolism are commonly found in autistic patients as neurotrophic factors have also been reported [(8; 9)]. Therefore, in this study focuses on three main objectives:

(1) Estimate the blood standards of autistic children and control.

(2) Measure Pb, Cu and Zn concentration in children with autism and typically developing children .

(3) Evaluate relationship between biomarker in this study and main symptoms of ASD .

Methodology:

The diagnosis of ASD was made in accordance with the standardized criteria provided in the American Psychiatric Association's Diagnostic and Statistical

Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria [10]. This study involved the autistic children attending Poison consultation Center in Habboubi Teaching Hospital / Thi-Qar province / Iraq, from January to November, 2016. The study was include 145 children with the mean age (1-14 years). 5 ml from blood of veins were collected in a tube with EDTA, to make the following tests:

1.Blood groups

2.Complete Blood Count (CBC)

3.Minerals Estimate

Blood groups test was performed for detect type of blood for autistic children by agglutination method on slid [11]. CBC test were done to count the amount of blood cells in circulating blood by using Colter(MODEL GFA-7000 CELL-DYN /Germany) according to manufacture company. While, metal analysis were measured with Atomic absorption Spectrophotometers (Shimazdu/ Japan). On the other hand, minerals were assured in peripheral blood according to the manufacturer's recommendations. The present study reports on blood metal results expressed as µg toxic metal per dl blood for Cu, Pb, and Zn.

Statistical Analysis:

The comparison of blood standers and trace mineral levels between autistic and control children were statistically analyzed by using SPSS statistics program version 16. X^2 – test and $P \leq 0.05$ were measurement.

Results:

Distribution of children according to sex and age

The autistic children were included 77males (81%) and 18 females (19%) and 50 children as control (28males and 22 female), table (1).

Table (1): Distribution and percentages of autistic children according to gender

Gender	Patient		Control	
	n	%	n	%
Male	77	81	28	56
Female	18	19	22	44
Total	95	100	50	100

All the children whom infected with autism divided according to age as shown in table(2). The age group of 3-5 years recorded the highest percentage (41.05%), followed by age group of 6-8years (35.78%) when compared with the other age groups, while the lowest percentage shown in 12-14 years (4.21%).

Table (2): Distribution and percentages of autistic children and control according to age group.

Age group (Years)	Patients No.(%)	Control No.(%)
Less than 3	11(11.6)	1(2)
3 – 5	39(41.05)	11(22)
6 – 8	34(35.78)	13(26)
9 – 11	7(7.36)	13(26)
12 – 14	4(4.21)	12(24)
Total	95(100)	50(100)

Distribution of children according to blood groups

The results of distribution of autistic children according to blood groups showed blood group B^+ the highest percentage (30%), followed by blood groups A^+,O^+,O^-, AB^+ and A^- with percentage 28% , 26%,7%,5% and 4% respectively. While, blood group AB^- and B^- don't record any percentage, fig.(1).

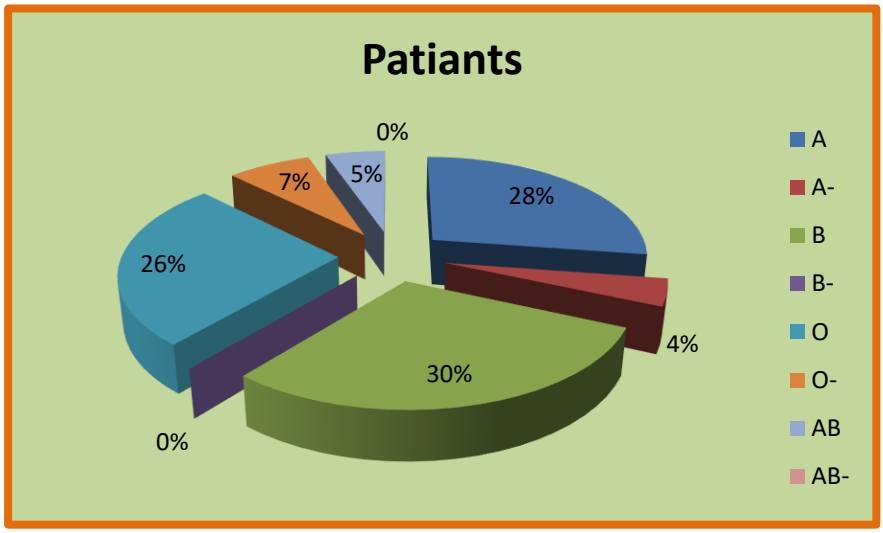

Fig.(1): Distribution of autistic children according to blood groups.

WBC and Differential Count

Blood cell counts in patients were not significantly different from those of the control groups, with the sole exception of a significant trend for Neutrophil and Lymphocyte, slightly lower in the autistic group ($p \leq 0.05$), table(4).

Table (4): Values of WBC and Differential Count for autistic children and control

Parameter	Patient	Control	P.value
WBC	8.19 ± 3.62	9.03 ± 3.43	0.178
NEU	3.60 ± 2.12	5.02 ± 3.27	0.007*
LYM	3.58 ± 1.69	2.83 ± 0.93	0.001*
MONO	0.61 ± 0.45	0.62 ± 0.35	0.849
ESO	0.34 ± 0.38	0.29 ± 0.24	0.339
BASO	0.11 ± 0.08	0.12 ± 0.09	0.313

WBC: White blood cell ; NEU: Neutrophil ;LYM: Lymphocyte ; MONO: Monocyte ; ESO: Eosinophil ; BASO: Basophil ; P.value ≤ 0.05 Significance

Red Blood Cell Counts

Blood analysis, explain changed RBCs shapes were present in the patients' group as compared to healthy control, the differences regarding HGB, HCT, MCV and MCH were detectable for the patient group, table(5). In particular, no laboratory signs of anemia in any of the two groups were evidenced. But, statistical analysis ($P \leq 0.05$) showed significantly between two groups.

Table(5) : Routine RBC variables in autistic children versus healthy controls.

parameter	Patient	Control	P.Value
RBC	4.89 ± 0.49	4.80 ± 0.57	0.317
HGB	12.34 ± 1.11	13.15 ± 1.33	0.000*
HCT	37.94 ± 4	41.58 ± 6.63	0.000*
MCV	77.83 ± 7.35	87.29 ± 5.76	0.000*
MCH	25.45 ± 3	34.56 ± 5.22	0.000*
MCHC	32.74 ± 3.14	38.93 ± 55.01	0.430
RDW	15.43 ± 2.26	14.96 ± 1.08	0.091

HGB: Hemoglobin; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hemoglobin ; MCHC: Mean Corpuscular Hemoglobin Concentration; RDW: Red cell Distribution Width. P.Value ≤ 0.05 Significance

However, the results of platelet count of the 95 ASD patients and control were mentioned in Table (6) which summarizes the overall mean ± standard deviation.

Table (6) : Platelet Count of Autistic and healthy children

Parameter	Patient	Control	P. value
PLT	288 ± 112.74	315.8 ± 86.76	0.131
MPV	7.89 ± 1.52	8.33 ± 1.96	0.137

P.value ≤ 0.05 Significance

The concentration rate of Copper , Zinc and Lead of Autistic Children

The concentration mean of trace elements Cu, Zn and toxic elements Pb in the blood of autistic children were measured according age and gender and compared with age and sex matched normal children .

The level of Cu in the blood of autistic children showed a significant elevation when compared to control, where the study was explained significant association($p \leq 0.05$) between the concentration of Cu in blood and age in most age groups, table (7) but non-significant association was found between the concentration of Cu in blood and gender of autistic children($p > 0.05$).

Table (7): Correlation between age and sex of autistic children and concentration of Cu in blood.

Age group (Year)	Concentration of Cu (µg / dL)		
	Male	Female	Mean
Less than 3 years	138.75	0	69.37
3-5	144.64	228.16	186.40
6-8	136.57	146.44	141.51
9-11	158.5	160.33	159.41
12-14	136	166	151
Mean	142.89	140.18	141.54

X2= 70.680 ; df= 4 ; p=.000

However, the level of Zn in patients showed a significant elevation when compared to control. The study displayed significant association between the concentration of Zn in blood and age in most age groups the table (8), but no significant association between the concentration of Zn in blood and gender ($p>0.05$).

Table (8): Correlation between age and sex of autistic children and concentration of Zn in blood

Age group(Year)	Concentration of Zn(µg / dL)		
	Male	Female	Mean
Less than 3 years	79.65	0	39.82
3-5	99.33	183.5	141.41
6-8	104.87	104	104.43
9-11	89.37	119.1	104.23
12-14	79	80	79.5
Mean	90.44	97.32	93.88

X^2= 35.659;df= 4;p=.000

Ultimately, the study was showed non-significant association between mean concentration of Pb in blood with age and gender of autistic children, table (9).

Table (9): Correlation between age and sex of autistic children and concentration of Pb in blood

Age group(Year)	Concentration of Pb(µg / dL)		
	Male	Female	Mean
Less than 3 years	12.26	0	6.13
3-5	12.20	24.45	18.32
6-8	11.84	11.37	11.60
9-11	13.86	9.85	11.85
12-14	11.2	12.7	11.95
Mean	12.27	11.67	11.97

X^2= 2.237; df= 4; p=.692

Discussion:

Biomarkers play an important role in understanding the relationships between exposure to environmental chemicals, the development of chronic human diseases, and the identification of subgroups that are at increased risk of disease.

However, the results showed that males (81%) are more likely to have autism than females (19%) with a prevalence ratio of 4:1 this agree with many previously studies [12; 13; 14], the cause for this difference is not well understood but several theories have been proposed, among which the involvement of the sex chromosome in the etiology of ASD and the role of hormonal influences in utero [15].

The results of this study were associated with distributing of autistic children according to age documented appeared that the age group (3-5) years had the highest (41.05%), followed by the age groups of (6-8) years(35.78%), whereas the lower percentage (4.21%) which was recorded in the age group of (12-14) years. Despite of, the fact that ASD is a lifelong disorder that starts in early life, it can be recognized for the first time at any age. This may be explained by the difficulty or delay of spoken language. The child is not responsive to other people's facial expression or feelings, unable to social play or typical play purposefully near others and impairment in non-verbal communication. Preschool-aged children identified with ASD were more likely to have intellectual disability than school-aged children with ASD [16].

In current study, distributing of ASD patients according to blood group showed B+ group had the highest (30.5%) followed by the O+ group (29.47%) and A+ blood group (28.42%), whereas the lower percentage (2.35%) which was recorded in AB+ group. [7] identified several potential risk factors such as ABO or Rh incompatibility that are also considered as symptoms of an underlying cause of autism. Statistically no significant difference between blood type of ASD children, this agree with [17] study, this result adds evidence that there is no causal association between blood type and childhood ASDs.

Furthermore, a quantitative and leukocyte differential cell counters for diagnostic use in clinical laboratories. The purpose of CBC is to separate the normal member, with all normal system-generated parameters, from the member who needs additional studies. The results were given image for blood and detect measurements of cell size and cell distribution.

Nevertheless, the results showed non-significant different between autistic children and control groups in blood analysis with the sole exception of a significant trend for Neutrophil and Lymphocyte in autistic children. These findings would suggest abnormalities of the immune cells in autistic children. This agree with [18] who suggest differences in the peripheral immune system including cytokines, leukocytes, antibodies and brain cell have been reported in autism. And the analysis was explained significant different in some RBC variables (HGB, HCT, MCV and MCH) in autistic children compare with healthy control, the study suggested may qualified to anemia. The food selectivity may be caused for iron deficiency in children with autism that increase the severity of psychology and behavioral problems.

Moreover, the study was showed that mean concentration of trace elements Copper (Cu), Zinc(Zn) and toxic element Lead (Pb) was measured for autistic children compared with age and sex-matched normal children. The results showed elevated in concentration of Cu, Zn and toxic element Pb in the blood with a significant elevation ($p \leq 0.05$) when compared to control group.

To explain this result could be in two sides, the first include age-related changes in three elements may belong to a clear role of placenta, whereas fetuses depend on their mothers for nutrition, including essential elements such as zinc (Zn) and copper (Cu), also exposed their mothers to toxic elements such as lead (Pb) that transfer from mother to fetus during gestation [19].Second side involved environmental factors that considered one source in the pathogenesis of

neurodevelopment disorders by the epigenetic alteration in gene expression [20] and some toxic elements have been reported to be candidate factors that induce epigenetic disorders [21].

On the other hands, elevated Cu level was associated with excessive diet intake, infections, inflammation, trauma and autism [22]. The higher level of Cu toxicity in the autistic children correlated with the severity of symptoms [23]. Cu and Zn are antagonists, a low level of Zn strengthens Cu toxicity [24] on the other hand, the toxic effects of Zn are due to Cu deficiency [25]. Acute Zn intoxication is rare, as only exposure to high doses of Zn has toxic effects [25].

To explain this increase of copper, may be due to drinking water which contains an amount of copper (copper sulfate is added to some drinking water supplies to kill yeast and fungi) [26] or cooking with copper cookware can also increase the copper content of foods. Industrial exposure to fumes, dust or mists may result in metal fume fever with atrophic changes in nasal mucous membranes [27]. Copper may be sprayed on fruits and vegetables to retard the growth of algae and fungus.

Thought, the concentration of Pb should be actually zero in biological fluids and tissues, but exposure to metals, such as lead one causes increased prevalence of autism [28]. Although, the previous studies on autistic children were reported unexposed results [29].

Mineral imbalance in bodies may play a main and epigenetic role as environmental factors in the pathogenesis of the neurodevelopment disorders such as autism [30]. Many studies suggest that children with autism have a decreased ability to excrete toxic metals leading to a higher body burden and toxic chemicals interact with the genome to produce changes in brain structure and function [31].

Overall, the practical agreement of the present results with those of the previous study lends confidence to the present results[32].

Conclusion:

More biochemical investigation, or any other test that helps diagnose the abnormality disorder in children.

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تقييم بعض المؤشرات الحيوية في أطفال التوحد في محافظة ذي قار

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الخلاصة:

أجريت العديد من الدراسات للعثور على مؤشرات حيوية ذات صلة بتطوير اضطراب التوحد. لذا هدفت الدراسة الحالية الى تقديم لمحة عامة عن المؤشرات الحيوية المختلفة ومناقشة دورها في التسبب في مرض التوحد. لكون التحليل المبكر يساعد في التقييم المبكر والوقاية من الاضطرابات التنموية العصبية التوحدية بدلاً من الاعتماد على الأعراض فقط. بحثت الدراسة الحالية بعض معايير الدم وتركيزات العناصر النزرة والمعادن السامة كمؤشر بيولوجي كمؤشرات حيوية لاضطراب التوحد.

وشملت الدراسة 95 طفلاً مصاباً بالتوحد تتراوح أعمارهم بين سنة واحدة و 14 سنة ، وشملت 77 من الذكور (81٪) و 18 (19٪) من الإناث. أظهرت النتائج أن الفئة العمرية 3-5 سنوات سجلت أعلى نسبة (41.05٪). أظهرت نتائج تحليل الدم أن مجموعة الدم التي كانت+ RH سجلت أعلى نسبة لـ +B و +Aو +Oو +AB بنسبة 30٪ و 28٪ و 26٪ و 5٪ على التوالي. في حين أن -RH لاحظت النسبة المئوية المنخفضة لـ -O 7 ٪ -A و 4 ٪ و -AB و -B لم تسجل أي نسبة مئوية. لم تكن تعداد خلايا الدم (CBC) من WBC في الأطفال الذين يعانون من التوحد مختلفة بشكل كبير عن تلك في مجموعة السيطرة ، باستثناء وحيد اتجاه الخلايا العدلات والخلايا اللمفاوية ، كانت أقل قليلا في مجموعة أطفال التوحد ($p\leq0.05$). في حين أظهرت لمتغيرات RBC فروق ذات دلالة معنوية فيما يتعلق HGB، HCT، MCV و MCH في الأطفال الذين يعانون من التوحد مقارنة مع مجموعة السيطرة ($p\leq0.05$).

شملت الدراسة قياسات العناصر النزرة (النحاس والزنك) في الدم والعنصر السام (الرصاص) في مصل الدم. أظهرت النتائج ارتفاعا في مستوى هذه العناصر في جميع الفئات العمرية وبفارق معنوي بين المرضى ومجموعة السيطرة ($p\leq0.05$). يمكن أن تكون هذه النتيجة مرتبطة مع شدة التوحد.

تشير هذه النتائج إلى أن المؤشرات الحيوية للنمو العصبي قد تكون حرجة وتحسن التشخيص المبكر وربما للعلاج. المزيد من التحريات البيوكيميائية ، أو أي اختبار آخر يساعد في تشخيص اضطراب الشذوذ لدى الأطفال.

.الكلمات المفتاحية: التوحد , المؤشرات الحيوية , فحص الدم الكامل , العناصر النزرة