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دراسة بعض المنغيرات الدموية في أطفال النودد الباحثة.نور تحريرعبد سلطان جامعة بغداد/كلية العلوم للبنات استلام البحث: ٢٠٢٣/٩/١٩ قبول النشر: ٢٠٢٢/١/١ تاريخ النشر : ٢٠٢٤/٤/١

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الملخص

اضطراب طيف التوحد (ASD) هو مصطلح يستخدم لوصف الأشخاص الذين لديهم مجموعة محددة من مشاكل التواصل الاجتماعي والسلوكيات المتكررة، بالإضافة إلى الاهتمامات المقيدة للغاية و/أو النشاط الحسي الذي يبدأ في وقت مبكر من الحياة. في السنوات الأخيرة، ارتفع معدل انتشار اضطراب طيف التوحد بشكل كبير. إن المسار الفسيولوجي لاضطرابات طيف التوحد غير معروف حتى الآن؛ ومع ذلك، فقد تم ربط الوراثة غير الطبيعية والأسباب المتعددة العوامل بالمشاكل الوراثية. الغرض من هذه الدراسة فقد تم ربط الوراثة غير معروف حتى الآن؛ ومع ذلك، مو تحد بشكل كبير. إن المسار الفسيولوجي لاضطرابات طيف التوحد غير معروف حتى الآن؛ ومع ذلك، فقد تم ربط الوراثة غير الطبيعية والأسباب المتعددة العوامل بالمشاكل الوراثية. الغرض من هذه الدراسة هو تحليل التغيرات في العديد من مؤشرات الدم لدى الأطفال المصابين بالتوحد. تناولت الدراسة حالات مرضى التوحد مركز الصفا للتوحد الخاص والمركز الحكومي للإعاقة العقلية مائة وعشرين عينة دم من مرضى التوحد مركز الصفا للتوحد الخاص والمركز الحكومي للإعاقة العقلية مائة وعشرين عينة دم من

(۲۰ ذکراً و ۲۰ أنثى) تتراوح أعمارهم بين ٥ سنوات إلى ١٥ سنة. يعتمد تشخيص مرضى التوحد اخصائي نفسي وموشرات فحص الدم، كما تم إجراء تعداد الدم الكامل (CBC)، بما في ذلك إجمالي خلايا الدم البيضاء (۱۰^ ٣/ميكرولتر)، وتم قياس كريات الدم البيضاء التفاضلية، وعدد الصفائح الدموية، وHGB، وMCHC، ومستويات RDW واختبار فصيلة الدم. أظهرت النتائج ارتفاعا كبيرا (>۰,۰۱۹) في مستوى RDW، حيث انخفض مستوى HGB بشكل ملحوظ (<۰,۰۱۹) في حين أظهر مستوى PLT ارتفاعا كبيرا (>۹۰۰). و WBC بشكل ملحوظ \* (>۰,۰۰)، \* \* (>۰,۰۱۹).

الكلمات المفتاحية: اضطراب طيف التوحد، عرض توزيع خلايا الدم الحمراء، متوسط تركيز الهيموجلوبين الكروي، تعداد الصفائح الدموية، تعداد الدم الكامل.

# Study of some Hematological Parameters in Autistic Children

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### Abstract

Autism Spectrum Disorder (ASD) is a term used to describe people who have a specific combination of problems with social communication and repetitive behaviors, as well as very restricted interests and/or sensory activities that start early in life. In recent years, the prevalence of ASD has risen dramatically. The pathophysiology of ASDs is yet unknown; however, genetic abnormalities and multifactorial reasons have been linked to genetic problems. The purpose of this study is to analyze changes in several hematological parameters in autistic children. The study dealt with cases of autistic patients at the Al-Safa Center for Private Autism and the Governmental Center for Mental Disability. One hundred twenty blood samples were taken from autistic patients (60 males and 20 females) and a group of healthy volunteers (20 males and 20 females) aged between 5 and 15 years old. The diagnosis of autistic patients was based on the psychologist, and parameters of the blood test were also performed, including the complete blood count (CBC), including total WBC (10<sup>3</sup>/µL) cells, differential WBC, platelet count, HGB, MCHC, RDW levels, and blood group test. Results revealed a substantial rise (P<0.001) in the level of RDW, where the decreased level of HGB significantly (P>0.001), while the PLT level exhibited a major rise (p<0.05), and WBC is significantly \* ( $P \le 0.05$ ), \*\* ( $P \le 0.01$ ).

Keywords: Autism spectrum disorders, red blood cell distribution width, mean corpuscular hemoglobin concentration, platelet count complete blood count

## Introduction

Autism Spectrum Disorder (ASD) is a neurodevelopment illness marked by speech and social interaction difficulties. There are also limited, recurring patterns of behaviour, hobbies, or activities. The scope and severity of these impairments vary and frequently fluctuate with the acquisition of other developmental skills. Autistic symptoms emerge in childhood and have a negative impact on social, occupational, and other domains [1]. ASD is now diagnosed in one out of every 44 babies, with a four-to-one male-to-female ratio [2]. The etiology of ASD is uncertain, however there is strong evidence that epigenetic, neurobiological, genetic, neurological, and hormonal variables play a role [3]. There are numerous hypotheses on the impact of genetic variants and anomalies in individual genes, neurotransmitter dysfunction, and abnormalities in brain connectivity. Toxin exposure or prenatal illnesses [4]. Furthermore, there is growing evidence that environmental (non-genetic) factors, such as prenatal factors (gestational diabetes, maternal bleeding and medications during pregnancy, intrauterine infections) and perinatal and neonatal factors (fetal presentation, umbilical cord complications, fetal distress, perinatal cyanosis, twin pregnancy, maternal internal bleeding, low birth weight, low Apgar score), may play a role in the etiology of ASD [5]. Metabolic problems, which are closely related to such variables, may contribute to the occurrence of ASD [6]. The aim of our casecontrol study is to investigate that what is change haematology parameters in healthy children and compared to Autistic children in (ASD) and Know the blood group in patients'.

#### materials and Methods

The current study included 120, 80 patients (20 females and 60 males) with autistic children, aged between (5-15 years), during the period from (March to June 2023), from Baghdad. Al-Safaa Center for Autism. All samples were taken with the patients' consent before inclusion in the study. Fourty samples from healthy subjects for comparison (3 ml) were collected and placed in a an anti coagulated tube with Ethylene Diamine Tetra acetic Acid (EDTA) to determine (CBC) and determine blood groups

#### Results

1.Distribution of sample study according to Gender

. There is a growing clinical recognition that a significant proportion of patients with gender is significant \*\* (P $\leq$ 0.01) in males and non significant in females. Table 1: Distribution of sample study according to Gender in patients and control

Factor		Patients (No=80)	Control (No= 40)	P-value
Gender:	Male	60 (75.00%)	20 (50.00%)	0.0001 **
No (%)	Female	20 (25.00%)	20 (50.00%)	1.00 NS



#### Figure

(1): Distribution of sample study according to Gender in patients group2.Distribution of sample study according to blood groups

Where to Perform Blood Group Testing (ABO and Rh) A simple blood test. Table( $\gamma$ ): Distribution of sample study according to Blood groups in patients.

Blood groups	No	Percentage (%)			
A	17	21.25			
В	30	37.5			
AB	4	5			
0	29	36.25			
Total	80	100%			
P-value		0.0001 **			
** (P≤0.01).					

In table (2) result shown percentage of blood group(B) 37.5% , blood group (O) 36.25% , blood group (A)21.25% and blood group (AB) 5% .

3.Comparison between patients and control groups in Age and BMI

In our study, we created a questionnaire for children with autism that included the children's age, gender, and Body Mass Index (BMI).

Table (3): Comparison between patients and control groups in Age and BMI

Group	Mean ± SE					
	Age (year)	BMI (kg/m <sup>2</sup> )				
Patients	8.71 ±0.29	20.21 ±0.42				
Control	9.70 ±0.45	17.36 ±0.11				
T-test	1.048 NS	1.188 **				
P-value	0.0648	0.0001				
	** (D<0.01)					
	<b>**</b> (P≤0.01).					

In table (3) results show is non significant in age and significant (BMI), calculated as weight in kilograms divided by height squared in meters  $(kg/m^2)$ , is the most common method for classifying obesity.



Figure (2): ROC curve for prediction of the disease activity by BMI

In Figure (2) and Table (4) the AUC of BMI 0.780 with a Sensitivity of 0.627% with cut off >18.6500, P<0.001.

Table (4) : ROC curve of BMI

	AUC	Sensitivity	Cut-off	P-value
BMI	0.780	0.627	18.6500 ( )	<0.001

The results of complete blood count

1.White Blood Cell Indices;The comparison of the white blood cell indices in the ASD group compared with the control group, including the total white blood cell numbers (WBCs) and their types: Lymphocytes (Lym), Monocytes (Mon), and Neutrophils (Neu) were shown in table(5).

Table(5): Comparison between patients and control groups in WBC and its

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			Mean	± SE			
Group	WBC	Neutrophil	Lymphocyte	Monocyte	Eosinophil	Basophil	
	(10^3/µL)	(%)	(%)	(%)	(%)	(%)	
Patients	8.63 ±0.32	48.29	$40.65 \pm 0.56$	8.91	$1.767 \pm 0.15$	0.251	
		$\pm 0.84$		±0.39		±0.36	

Control	$6.98 \pm 0.08$	56.76	34.57 ±0.49	7.63	$0.625 \pm 0.07$	0.162
		$\pm 0.47$		±0.15		$\pm 0.05$
T-test	0.907 **	2.185 **	1.712 **	1.118 *	0.443 **	0.161
						NS
P-value	0.0005	0.0001	0.0001	0.0267	0.0001	0.124
* (P≤0.05), ** (P≤0.01).						

The results indicated a notable increase (P $\leq$ 0.01) in the total WBC count and lymphocyte count within the serum of ASD children (8.063±0.32 and 40.65±0.56) when contrasted with the control group (6.98±0.08 and 34.57±0.49 x10). Conversely, the levels of Monocytes and Neutrophils displayed insignificant differences (P>0.05) between the ASD group (8.91±0.39 and 48.29± 0.84) and the control group (7.63±0.15 and 56.76±0.47 x10), as depicted in Table (5).

Table( 6):Relationship between Gender and WBC and its differential

in	patients	and	control	groups
	Patientes	unu	control	Sloups

		Patients		Control		
Parameters	Male	Female	P-	Male	Female	P-value
	(No=60)	(No=20)	value	(No=20)	(No=20)	
WBC	8.79	8.14 ±0.17	0.386	6.97	6.98	0.996
(10^3/mL)	±0.42		NS	±0.11	±0.13	NS
Neutrophil	47.74	$49.93 \pm 1.02$	0.204	56.29	57.08	0.304
(%)	±0.92		NS	±0.66	±0.66	NS
Lymphocyte	40.98	$39.62 \pm 1.01$	0.294	35.32	33.89	0.183
(%)	±0.66		NS	±0.57	$\pm 0.82$	NS
Monocyte	9.08	8.36 ±0.35	0.426	7.60	7.68	0.938
(%)	±0.51		NS	±0.19	±0.26	NS
Eosinophil	1.90	1.35 ±0.19	0.124	0.667	0.556	0.509
(%)	±0.20		NS	$\pm 0.08$	±0.11	NS
Basophil	0.261	0.217 ±0.6	0.621	0.143	0.194	0.765
(%)	±0.04		NS	±0.06	$\pm 0.08$	NS
		NS: Non-S	Significa	int.		

The difference between these cell numbers of patient and control groups was studied depending on the gender in table (6). However, there were no significant (*P*>0.05) differences between WBC, Lymphocytes, Monocytes, and Neutrophils numbers in the male group of control ( $6.97\pm0.11$ ,  $33.89\pm0.82$ ,  $7.60\pm0.19$  and  $56.29\pm0.6610^{\circ}$ /mL, respectively) compared to the female ( $6.98\pm0.13$ ,  $35.32\pm0.57$ ,  $7.68\pm0.26$  and  $57.08\pm0.6610^{\circ}$ /mL respectively) and male group of the patients ( $8.79\pm0.42$ ,  $40.98\pm0.66$ ,  $9.08\pm0.51$  and  $47.74\pm0.92$   $10^{\circ}$ /µL, respectively) compared to female ( $8.14\pm0.17$ ,  $39.62\pm1.01$ ,  $8.36\pm0.35$  and  $49.93\pm1.02$ ,  $10^{\circ}$ /mL respectively.

# المجلد (۲۱)

# 2.The Red Blood Cell Indices

The comparison of the red blood cell indices in the in ASD group compared with the control group, including the red blood cell numbers (RBCs), mean corpuscular hemoglobin concentration (MCHC)and Red cell distrubtion width (RDW), were shown in table (7).

Table(7): Comparison between patients and control groups in RBC, MCHC, RDW and PLT

G		Mear	$n \pm SE$				
Group	RBC (10 <sup>6</sup> /ml)	MCHC	RDW	PLT $(10^{3}/ml)$			
Patients	4.75 ±0.08	32.28 ±0.36	16.57 ±0.27	360.64 ±42.16			
Control	5.09 ±0.06	32.35 ±0.14	14.18 ±0.15	272.95 ±7.06			
T-test	0.230 **	1.049 NS	0.784 **	118.74 NS			
P-value	0.0044	0.906	0.0001	0.146			
	** (P≤0.01).						

This result in table (7) is non significant in PLT and MCHC between autistic children and healthy children, and is significant ( $P \le 0.01$ ) in RDW and RBC.

2.1 The platelet (PLT) indices in the blood of children with ASD and control groups. The comparison of the platelet numbers between control and patient according age (5-15)years of ASD children shown in table (8).

Stu	dy population	,	PLT		P value	
Stu	ay population	511	$((10^3/ml))$		$(p \le 0.05)$	
Age group	Туре	No.	Mean Std. Deviation			
4-6	Patient	19	329.20	38.89	.003*	
	Control	7	269.00	46.52		
7-9	Patient	34	326.14	64.01	$.007^{*}$	
	Control	11	269.45	23.67		
10-12	Patient	16	323.68	73.99	.109 <sup>NS</sup>	
	Control	13	283.69	50.63		
13-15	Patient	11	۳06.14	30.17	.323 <sup>NS</sup>	
	Control	9	264.77	57.25		
* Significant difference under $p \le 0.05$ by One way – ANOVA						
NS: Non- s	ignificant d	ifference				

Table (8):Evaluation of PLT in study population



Figure (3): ROC curve for prediction of the disease activity PLT The results of this study showed that there was a significant increase (P<0.05) in the numbers of PLT (x10<sup>3</sup>cell/ml) in the blood of ASD children when compared with the control group according. patient age 4-6 years (329.20±38.89),7-9 years(326.14±64.01),10-12years(323.68±73.99)and 13-15years (606.14±301.73) and control age 4-6(269.00±46.52),7-9 years(269.45±23.67),10-12years(283.69±50.63)and 13-15 years(264.77±57.25)..

Table (9) : ROC curve of PLT

PLT	AUC	Sensitivity	Cut-off	P-value
	<b>•</b> .776	0.821	277.5000	< 0.001

In Figure (3) and Table (9) the AUC was 0.776 with a Sensitivity 0.821 with cut off >277.5000, P<0.001.

#### Discussion

According to the research problem, its objectives, and the steps of the method used in it, and within the limits of the characteristics of the study group, its tools, and the statistical results that were reached, the research results can be discussed through a set of points that confirm the effectiveness of hematological parameters on to develop some life (autism) is as follows:

This results in table (1),figure(1) show ratio 3:1 that is of 3males is to1 females have concurrent (ASD), This study [7] approved a our study and confirmed that the percentage of males with autism is more than females.

In table (2) show results Where to Perform Blood Group Testing (ABO and Rh) A

simple blood test that aims to accurately determine a person's blood type, according

to the types of proteins or antigens that appear on the surface of his red blood cells

[8] finding in this study of autistic children, blood type B& o was detected in a

higher percentage, followed by blood group A, and finally blood group AB, this study agree with conducted [9] Where their found a similar result for the proportions of blood groups.

Also In this study, we created a questionnaire for children with autism that included the children's age, gender, Information about the psychological state of the mother during pregnancy, as well as the psychologist's diagnosis of cases of autism in children and knowledge of the severity of autism,

and Body Mass Index (BMI) in table(3,4) and figure(2) these reaults showed the BMI was strong predictor to analysis disease of ASD when increased in patients compared to healthy controls, the proof indicates which oral sensitivities may mediate food selectivity, food and nutrient intake, as well as other factors such as physical activity, ASD weight gain may be influenced to some degree by lack of sleep, genetics, and the consumption of medications, which may have a cumulative effect that is Highly significant , our study agree with [10][11] and confirmed that children with autism had a higher BMI than normal children.

In table (5) highlighting augmented lymphocyte and total WBC counts in ASD patients, Correspondingly, [12][13] identifies an elevated number of B lymphocytes among ASD children aged 5-15 years, diverging from [14], which shows an increase in lymphocytes within ASD and their integral role in the disorder's pathophysiology. A skewed distribution of helper and suppressor cells, contributing to an imbalance within ASD, is discussed in [15].

In table (6)The study delves into specific reactions among circulating monocytes within ASD children. In contrast to [16] and [17], where increased monocyte and neutrophil counts are reported, our investigation yields no discernible differences Agreement with [18]

accentuates the importance of accounting for factors that introduce heterogeneity, potentially contributing to divergent findings

in addation results in table (7) is higly significant ( $P \le 0.01$ ) between patient and control because had some food selectivity register and iron deficiency anemia showed this behaviour[21].

in the table (8) significant 4-9 and non significant 10-15 years. This study agree with [11] Due to hormonal changes and reaching the switching stage

and figure(3) ,table (9)These results showed PLT was strong predictor to analysis disease of ASD when increased in patients compared to healthy controls. Notably, our examination of platelet counts highlights an upswing in ASD as show in figure corroborating [22].Undoubtedly, platelets are intricately associated with immune responses, underscored by their diverse functional immune receptors and their capacity to store and release bioactive substances. Recent studies emphasize their role across various immunological functions. Interestingly, our investigation indicates substantial correlation between platelet no levels and the severity(mild,modrate, severe) of autism, in harmony with [23,24], as depicted in this study finding.

# Conclusion

The current investigations demonstrate that changes in haematology parameters increase the number of WBC and the number of PLT in autistic children when compared to control children, with a significant difference between them.

# Recommendations

We advise conducting a genetic study on autism to determine its causes and the reasons why male are more likely than female to get the condition. For a more accurate assessment of the parameter levels connected to gender and autism severity, the number of samples for males should be equal to the number of samples for females, as well as autism severity. Continually collect samples for the current investigation from more Iraqi cities.

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