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Serum cholesterol in children with ASD

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ABSTRACT

Objectives. Because the brain contains one-quarter of all cholesterol in the body, cholesterol has a significant effect on the brain. Cholesterol may also have an impact on autism, which is associated with poor brain development. Damage to neurons can lead to the emergence of autism spectrum disorders (ASD). This is a case-control study conducted to explore the potential association between serum cholesterol level and ASD in children's patients.

Material and methods. It involved collecting data from medical records, performing interviews, and taking blood samples from patients and a control group of children. The samples consisted of 60 child patients with ASD and an equal number of controls, for a total of 120 participants.

Outcomes. There was a significant inverse relationship between cholesterol levels and ASD. The children diagnosed with ASD displayed significantly lower cholesterol levels when compared to healthy children (p-value = 0.004). The age distribution of the participants showed a non-significant difference between the ASD patient group and the control group (p-value = 0.085). This supports the existing knowledge that ASD is more common in children under the age of 18.

Conclusions. Children with ASD had a lower total serum cholesterol level than healthy children. This may suggest that there is an inverse relationship between serum cholesterol levels and disease state in children diagnosed with ASD who have lower cholesterol levels. Also, significant differences of gender and BMI were found among study groups, suggesting their association with the disease's development and consequences.
The results of the study also highlight some significant differences in the main basic characteristics of ASD in patients, evaluating their possible roles with the disease's roots and with serum cholesterol, on the other hand. These findings contribute to a deeper understanding of the sophisticated relationship between cholesterol level and ASD development, potentially opening up new ways for the update of intervention, treatment, and follow-up strategies.

Keywords: autism, autism spectrum disorders, cholesterol, children

INTRODUCTION

Autism spectrum disorders

This group is characterized by core impairments in reciprocal social contact and communication, as well as a prevalence of limited or stereotyped interests and behaviors. The majority of the time, exact underlying causes cannot be determined [1]. Autism spectrum disorders refer to any abnormality in nervous system development that leads to neurological/neuropsychiatric disorders. Although Asperger's disease is classified as an ASD, it is a more homogeneous group of patients who do not require special care once they achieve adulthood. In contrast, there are examples of ASD with severe neurological abnormalities, such as self-injury, that require lifetime care [2].

Cholesterol

It is essential for the biosynthesis of steroid hormones (adrenal gland hormones, cortisol, and aldosterone), as well as sex hormones, bile acid, and vitamin D. This implies that cholesterol acts as a precursor to these molecules. It also participates in a variety of biological reactions; cholesterol makes cell membranes more flexible and resistant to harm. It also improves nerve conduction, making it faster; a myelin sheath, which is high in cholesterol, isolates impulses [3].

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Evidence of a link between cholesterol and ASD

Because the brain contains one-quarter of all cholesterol in the body, cholesterol has a significant effect on the brain. Cholesterol may also have an impact on autism, which is associated with poor brain development.

As a result, if neurons are damaged, ASD may arise. Because cholesterol is found in cell membranes, it plays an important role in neuron development and is essential for neurons and synapses. The oligodendrocyte cells are glial cells that are found in the nervous system. They are responsible for producing myelin coating for neurons in the central nervous system, and they require a large amount of cholesterol to do so. As a result, high cholesterol levels are required for myelin sheaths, and low cholesterol levels can cause structural defects in neurons, which may lead to autism [4].

MATERIAL AND METHODS

Study design and ethical consideration

This is a case-control study, and it was carried out because there is growing evidence that this single gene condition of aberrant cholesterol synthesis may be a model for studying the genetic bases of autism and the role of cholesterol in ASD. The research methods were ethically approved by the Thi-Qar Health Directorate, research committee no. 327/2022; informed consents were also filled out (Appendix I). The study was conducted from 8th November 2022 to 7th March 2023 at Thi-Qar Rehabilitation Center for autistic diseases. It involved extracting data from the center's patient records. In addition, interviews with the children and their parents were carried out at the center to complete the data collection form. A research plan had been constructed to perform this research.

The study was applied to 60 ASD children and included reviewing their case sheets. It included all autistic child patients of both sexes and of a range of ages (below 18 years old). The excluded patients were those aged more than 18 years. The aim of this study was to determine the association between serum cholesterol level and autism spectrum disorders in children. In the Thi-Qar Rehabilitation Center for Autistic Disorders, a set of questions containing the required information about the children patients and about controls were structured and evaluated by expert specialists.

The blood samples from sixty autistic children patients, as well as samples from sixty control children, were collected in the central laboratory and analyzed calorimetrically for the measurement of total serum cholesterol.

Study group description

The group of patients involved sixty children diagnosed with ASD under the supervision of pediatric specialists. They were selected from different age ranges (2-18 years); the ages below and higher than this range were excluded. This group involved fifty males and ten females. The control group involved sixty healthy children with the same age range (2-18 years), and it involved thirty-three males and twenty-seven females. The main anthropometric and basic characteristic data of children in both study groups were collected in questionnaire lists along with the blood samples required to accomplish this study.

Materials and instrumentation

It involves cholesterol kits, gel tubes, micropipettes, yellow disposable tips, syringes (3 cc), cotton, alcohol 70%, centrifuge instruments, and reagents. All from USA origin (Cell Biolabs Inc.), and UV-VIS spectrophotometer (Pg instruments, UK).

Principle and procedure of cholesterol assay

The serum from collected blood samples of the patients and controls was analyzed by the colorimetric method. The cholesterol esterase enzyme hydrolyzes cholesterol esters to release free cholesterol, while cholesterol oxidase oxidizes the free cholesterol to create H_2O_2. The peroxidase enzyme combines H_2O_2 with 4-aminophenazone and phenol to produce quinoamine (a chromogen molecule). The assay procedure involves mixing well and incubation of constituents at 37°C for 10 minutes. A pink color sample has been observed. Set the UV-colorimeter to zero using a blank at 500 nm and measure the absorbance of the standard and test. The results are calculated using the following formula:

$$\text{Cholesterol (mg/dl)} = \frac{[\text{A(sample)}]}{[\text{A(standard)}]} \times \text{n},$$

n = standard concentration (200 (mg/ dl)

Statistical analysis

The resulting data were analyzed statistically using IBM-SPSS software and Microsoft Excel version 2019; Pearson's correlation and Chi-squared test for assessing data significance and association. The results were expressed as mean ±SD and were considered significant only when $P \leq 0.05$.

OUTCOMES

Table 1 includes different sociodemographic data for child patients and controls like age, body mass index (BMI), gender, address, economic states, and duration of ASD disease.

TABLE 1. Some anthropometric and basic characteristics of the study groups

Categories		Patients	Control	P-value
		Mean ± SD		
Age (years)		6.92 ± 3.17	6.15 ± 3.92	0.085
BMI (Kg/m2)		21.0 ± 4.04	16.7 ± 1.91	0.0001*
Duration of disease (years)		3.52 ± 2.24	None	None
		No. (%)	No. (%)	
Gender	Male	50 (83.3%)	33 (55%)	0.001*
	Female	10 (16.7%)	27 (45%)	
Home address	City	52 (86%)	48 (80%)	0.327
	Periphery	8 (13.3%)	12 (20%)	
Socioeconomic state	Poor	15 (25%)	21 (35%)	0.460
	Medium	40 (66.7%)	35 (58.3%)	
	Good	5 (8.3%)	4 (6.7%)	

The data were expressed as mean ± SD and % of values. The superscript (*) represents significant difference (P ≤0.05).

The results in Table 1 revealed non-significant age differences between patients and control groups (P = 0.085), consistent with Baio et al. [5].

Given the prevalence of ASD in children under 18 years, this finding was anticipated. However, a highly significant BMI difference emerged (P = 0.0001), aligning with Curtin et al. [6]. The overweight and obesity prevalence are similar to that of typically developing children, potentially affected by medication, sleep, and dietary habits. According to Chiarotti et al., the average age of diagnosis was 3.5 years. [7]. The ASD's neurodevelopmental nature, rather than a defined duration or onset, was evident, affected by environmental and genetic factors. Gender-wise, 83.3% of patients were males, which is consistent with Werling et al. [8].

One theory proposes that females with ASD are more protected against some of the symptoms of ASD (it's called the female protective effect, or FPE) [9]. According to this theory, a higher rate of ASD risk factors should be observed in the affected females compared to their affected male counterparts. These additional risk factors are required for females to surpass the higher clinical or diagnostic threshold achieved by the FPE.

Furthermore, certain gene mutations were more strongly associated with ASD and had greater severity in boys than girls. A non-significant address disparity was found (P = 0.327). Lauritsen et al. suggest that geographic locations have a limited impact on ASD diagnosis and treatment [10]. The socioeconomic status revealed a non-significant difference (P = 0.460), concomitant with the finding of Durkin et al. [11], but lower SES backgrounds might hinder interventions, as explained by Barnard-Brak et al. [12].

Table 2, it also describes ASD patients' cholesterol levels as subgroups that include gender, age, BMI, address, economic status, disease duration, family history, disease severity, disease symptoms, and other comorbidities.

Table 4 explains the association between serum cholesterol with some characteristics like age, BMI and, duration of disease in ASD patients and healthy controls.

In Tables 2, 3, and 4, the total serum cholesterol was significantly lower in children with ASD (P = 0.004) as compared to the control group. This finding is consistent with Mazloumi et al. [13]. This suggests an inverse relationship may be found between serum cholesterol levels and ASD. Whereas the gender-based cholesterol differences were non-significant (P = 0.547), aligned with R. Arora et al [14]. Age-based cholesterol comparisons (<10 vs. >10 years) also found non-significant results (P = 0.943), similar to Stewart et al. [15]. No cholesterol-BMI correlation was seen among patients (P = 0.904), in line with Hu et al. [16]. Serum cholesterol wasn't significantly related to disease duration (P = 0.254), akin to Brinkman et al. [17]. It has been found that about 20% of ASD children had a family history of the condition, akin to Sandin et al. [18]. Aggressive behavior affects roughly 45%, correlating with Matson et al. [19]. The overlapping learning difficulties and autism were noted in approximately 85%, consistent with McNamara et al. [20, 21]. Language difficulties impacted around 55%, mirroring Arun et al. [22]. Behavioral issues and nervousness were common among patients (48.3% and 5%, respectively), congruent with Taylor et al. [23]. Around 11.7% experienced hallucinations and delusions, similar to Lugnegård et al. [24]. Communication difficulties, characteristic of ASD, were found in only 5%, contrasting Soorya et al. [25]. Stubbornness appeared in 28.3%, different from Uljarević [26], potentially related to language development delays. The autistic individuals experienced higher fear in social situations (5%), different from Kerns' findings [27]. Difficulty distinguishing places and times affected ap-

TABLE 2. Comparison of total serum cholesterol levels in patient and control children

Groups		Patients	Control	P-value
		Mean ± SD		
S. Cholesterol (mg/dL)		103.4 ± 25.1	120.3± 37.4	0.004*
Patients Only				
Gender male/female		104.5 ± 24.9	99.70 ± 21.7	0.547
Age < 10 years/ ≥ 10 years		103.5 ± 25.0	104.1 ± 21.9	0.943
BMI (Kg/m^2)	Underweight	103.8 ± 23.5		0.904
	Normal weight	102.5 ± 25.2		
	Over weight	106.7 ± 26.6		
Home address City/out city		104.2 ± 23.8	100.0 ± 29.0	0.648
Socioeconomic Status	Poor	110.7 ± 30.0		0.234
	Medium	102.8 ± 22.1		
	Good	89.80 ± 19.1		
Disease Duration ≤5 years/ >5 years		101.8 ± 24.5	110.9 ± 23.5	0.254
Family History		98.33 ± 27.9	105.0 ± 23.4	0.398
Severity of Disease	Mild	100.4 ± 20.2		0.582
	Moderate	109.3 ± 24.7		
	Sever	102.2 ± 25.6		
Aggression		107.2 ± 25.6	100.7 ± 23.2	0.310
Learning Problem		104.1 ± 23.1	101.2 ± 31.8	0.744
Distracted		102.0 ± 20.9	104.2 ± 25.5	0.767
Speech Disorder		99.84 ± 23.7	108.4 ± 24.6	0.178
Nervousness		102.6 ± 26.1	104.7 ± 22.8	0.745
Auditory and Visual Hallucination		96.42 ± 20.1	104.6 ± 24.8	0.405
Communication problems		101.6 ± 28.2	103.8 ± 23.3	0.884
Stubbornness		104.4 ± 24.4	103.3 ± 24.6	0.879
Fear		118.0 ± 13.1	102.9 ± 24.6	0.301
Inability to Distinguish Time and Place		105.0 ± 20.4	103.5 ± 24.9	0.882
Heart Abnormalities		125.0 ± 0.00	103.3 ± 24.3	0.382
Atrophy of Brain Cells		96.53 ± 28.2	105.6 ± 23.1	0.234
Jaundice		104.4 ± 24.6	103.4 ± 24.5	0.889
Epilepsy		99.10 ± 20.2	104.6 ± 24.1	0.517
Asthma		98.75 ± 24.9	104.0 ± 24.4	0.678
Rachitic		60.00 ± 0.00	104.4 ± 23.8	0.070

The data were expressed as mean ± SD. The superscript (*) represents significant difference (P ≤0.05).

proximately 7%, differing from Nabila Cheour [28]. Moreover, an association between heart abnormalities and ASD was observed (1.7%), aligning with an increased cardiovascular risk in autistic patients. The study also revealed a significant relationship between disease duration and age in ASD patients (P = 0.000), as in Kohane et al. [29]. A non-significant BMI-age association was found in autistic patients (P = 0.765); a significant BMI-age link in healthy children was evident (P = 0.017), consistent with McConachie et al. [30]. The age-cholesterol (P = 0.609) and cholesterol-BMI (P = 0.749) relationships were non-significant in ASD children, consistent with Kamal et al. [31]. Researchers at Harvard Medical School, Massachusetts Institute of Technology, and Northwestern University identified a subtype of autism originating from a cluster of genes that regulate cholesterol metabolism and brain development in humans. Their findings can inform both the design of precision-targeted therapies for that specific form of autism and enhance screening efforts to diagnose this type of autism earlier. The scientific team identified the shared molecular basis between lipid dysfunction and autism through DNA analysis of brain samples, findings that they confirmed by examining medical records of individuals with autism. Actually, both children with autism and their parents had pronounced alterations in lipid blood, the analysis showed [32]. The variations in the results of this study highlight the need for further research in ASD medical fields.

CONCLUSIONS

Children with ASD had a lower total serum cholesterol level than healthy children. This may sug-

TABLE 3. Disease characteristics of ASD patients with their frequencies and percentages of occurrence.

Disease characteristic		No. (%)		Cal. X2	P. value
Family history	Yes	12 (20%)	36.0	< 0.001*	
	No	48 (80%)			
Severity of Disease	Mild	5 (8.3%)	36.14	< 0.001*	
	Moderate	21 (35%)			
	Sever	34 (56.7%)			
Characteristics of Autistic Patients	Aggression	Yes	27 (45%)	1.0	0.137
		No	33 (55%)		
	Learning problems	Yes	51 (85%)	49.0	<0.001*
		No	9 (15%)		
	Distracted	Yes	15 (25%)	25.0	<0.001*
		No	45 (75%)		
	Speech disorder	Yes	33 (55%)	1.0	0.317
		No	27 (45%)		
	Nervousness	Yes	29 (48.3%)	0.160	0.689
		No	31 (51.7%)		
	Auditory and visual hallucinations	Yes	7 (11.7%)	57.76	<0.001*
		No	53 (88.3%)		
	Communication problems	Yes	3 (5%)	81.0	<0.001*
		No	57 (95%)		
	Stubbornness	Yes	17 (28.3%)	19.3	<0.001*
		No	43 (71.7%)		
	Fear	Yes	3 (5%)	81.0	<0.001*
		No	57 (95%)		
	Inability to distinguish time and place	Yes	7 (11.7%)	57.76	<0.001*
		No	53 (88.3%)		
History of present illness	Heart abnormalities	Yes	1 (1.7%)	92.1	<0.001*
		No	59 (98.3%)		
	Atrophy of brain cells	Yes	13 (21.7%)	31.3	<0.001*
		No	47 (78.3%)		
	Jaundice	Yes	16 (26.7%)	21.1	<0.001*
		No	44 (73.3%)		
	Epilepsy	Yes	10 (16.7%)	43.5	<0.001*
		No	50 (83.3%)		
	Asthma	Yes	4 (6.7%)	73.9	<0.001*
		No	56 (93.3)		
	Rachitic	Yes	1 (1.7%)	92.1	<0.001*
		No	59 (98.3%)		

The data were expressed as % of values. The superscript (*) represents significant difference (P ≤0.05).

TABLE 4. Spearman's Correlations among study groups

Group / Characteristic			BMI (Kg/m^2)	Duration of disease (years)	Total serum cholesterol (mg/dL)
Patients	Age (year)	R.	-0.056	0.570*	0.067
		Sig.	0.672	0.000	0.609
	BMI (Kg/m^2)	R.		-0.039	-0.042
		Sig.		0.765	0.749
	Duration of disease (year)	R.			-0.047
		Sig.			0.722
Control	Age (year)	R.	0.308*		0.244
		Sig.	0.017		0.060
	BMI (Kg/m^2)	R.			0.220
		Sig.			0.091

R = regression or correlation coefficient. The superscript (*) represents significant difference (P ≤ 0.05).

gest that there is an inverse relationship between serum cholesterol levels and disease state in children diagnosed with ASD who have lower cholesterol levels. Also, significant differences of gender and BMI were found among study groups, suggesting their association with the disease's development and consequences.

The results of the study also highlight some significant differences in the main basic characteristics of ASD in patients, evaluating their possible roles with the disease's roots and with serum cholesterol, on the other hand. These findings contribute to a deeper understanding of the sophisticated relationship between cholesterol level and ASD development, potentially opening up new ways for the update of intervention, treatment, and follow-up strategies.

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