Screening of Vitamin B12 in Children Diagnosed as Autism Spectrum Disorder

Original Article

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ABSTRACT

Background: A deficiency in Vitamin B12 leads to megaloblastic anemia and neurological disorders. In children with Autism Spectrum Disorder (ASD), gastrointestinal issues can impair the absorption of certain vitamins, including Vitamin B12, resulting in its deficiency.

Aim: This study aims to assess Vitamin B12 levels in children with ASD to identify potential etiological factors and explore their role in management if confirmed.

Patients and Methods: A case-control study was conducted involving 30 children diagnosed with Autism Spectrum Disorder (ASD) and 30 neurotypical children serving as a control group, all aged 3 - 8 years. All participants underwent the Modified Arabic Preschool Language Scale (PLS-4), the Stanford-Binet Intelligence Scales, Fifth Edition, and the Childhood Autism Rating Scale (CARS).

Results: Children with ASD had notably lower levels of Vitamin B12 than the control group.

Conclusion: The study found that children with ASD had lower Vitamin B12 levels compared to the control group and exhibited a reduced overall language level.

Key Words: Arabic PLS-4, autism spectrum disorder, CARS, language delay, vitamin B12.

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INTRODUCTION

A neurodevelopmental disorder refers to an impairment in the growth and development of the brain or central nervous system, particularly involving brain function disorders that affect emotions, learning abilities, and memory. These disorders often become progressively more noticeable as an individual develops[1]. While the term neurodevelopmental disorder is sometimes used interchangeably with Autism Spectrum Disorder (ASD), it encompasses a wider array of conditions. ASD specifically affects the brain's ability to process information, disrupting the organization and connections of nerve cells and synapses, though the exact mechanisms remain unclear[2].

Vitamin B12 plays a critical role in several biological processes, including cell formation, digestion, nutrient absorption, protein synthesis, and the breakdown and utilization of carbohydrates and fats for energy. Deficiency of Vitamin B12 is usually due to absorption problems, rather than insufficient dietary intake, except in strict vegetarians, as it is primarily obtained from animal sources[3].

Vitamin B12 deficiency is commonly observed in individuals with digestive disorders and is prevalent in children diagnosed with ASD[4]. Additionally, inadequate maternal intake of Vitamin B12 during pregnancy has been linked to severe disruptions in the myelination process of the nervous system, resulting in significant developmental delays[3].

Although existing research has explored Vitamin B12 role in neurodevelopmental disorders, the specific relationship between Vitamin B12 levels and ASD symptoms has not been fully established. Most studies have concentrated on maternal deficiency or general neurodevelopmental delays, with limited attention paid to the direct impact of Vitamin B12 deficiency on the severity of ASD symptoms in children. Therefore, this research aims to fill this gap by screening Vitamin B12 levels in children diagnosed with ASD and examining its potential role in influencing the severity of symptoms associated with ASD.

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This study seeks to provide a deeper understanding of the potential role of Vitamin B12 in ASD, exploring whether supplementation could offer benefits as part of a broader therapeutic strategy for managing ASD symptoms.

AIM OF THE WORK

The aim of this study is to assess Vitamin B12 levels in children with ASD to identify potential etiological factors and explore their role in management if confirmed.

PATIENTS AND METHODS

A case-control study was conducted on 60 patients attending the Phoniatric Outpatient Clinic at Ain Shams University and the Hearing and Speech Institute. These patients presented with concerns about delayed language development for their age. The study included 30 children diagnosed with Autism Spectrum Disorder (ASD) as the case group and 30 children without ASD as the control group.

Inclusion Criteria

1- Patient diagnosed as ASD.

2- Age 3-8 years old.

3- Both genders.

Exclusion Criteria

1. Patients on medications that affect level of vitamin B12
2. Patient on antiepileptic drugs (AED) (valproate and carbamazepine)
3. Hearing impairment patients.
4. Brain damage motor handicap (BDMH)

Procedures and clinical tools

A. Vitamin B12 serum level assessment.

We used IMMULITE® 2000 System Analyzer for the quantitative measurement of vitamin B12 in serum.

B. Language Assessment Protocol

All patients underwent the following comprehensive language assessment

Protocol at the start of therapy

I. Elementary Diagnostic Procedures:

Patient Interview:

a. Identification of the primary complaint and symptom analysis.

Collection of personal history.

b. Evaluation of etiological factors, including prenatal, perinatal, and postnatal influences.

c. Assessment of developmental milestones and history of illnesses during early childhood.

II. Clinical Diagnostic Aids:

a. General examination.

b. Examination of the vocal tract, including assessment of lip and tongue movements and palatal mobility.

c. Examination of the ear and nose.

d. Re-evaluation of the vocal tract.

III. Additional Instruments:

a. Clinical Diagnostic Tools:

Stanford-Binet Intelligence Scale.

Childhood Autism Rating Scale (CARS).

Modified Preschool Language Scale (PLS-4), Arabic edition.

b. Audiological assessment.

Preschool Language Scale, Fourth Edition (PLS-4), Arabic Version[5]

The Arabic adaptation of the PLS-4, standardized for Egyptian children, is a developmental language assessment tool that evaluates syntactic profiles. It determines the child's language age through interactive testing.

Stanford-Binet Intelligence Scales, Fifth Edition[6]

This assessment measures five cognitive factors to evaluate mental age and intelligence levels.

Childhood Autism Rating Scale (CARS)[7]

CARS assesses the severity of autism symptoms by comparing a child's behaviors and abilities to those typical of neurotypical development.

Ethical Considerations

Informed consent was obtained from all participants prior to their inclusion in the study. Parents granted permission for the collection of 200 to 500 mL of blood from their children. The study received approval from the Ethics Board of Ain Shams University.

Statistical Analysis

The collected data were reviewed, coded, and input into a computer using the Statistical Package for the Social Sciences (SPSS)[8]. Parametric quantitative data were presented as means and standard deviations (±SD), while non-parametric data were expressed as medians and interquartile ranges. Qualitative data were reported as frequencies and percentages.

Statistical tests applied based on data type

Quantitative data: Analyzed with the Student's t-test or Mann-Whitney test.

Qualitative data: Evaluated using the chi-square test or Fisher's exact test.

A *p-value* of <0.05 was considered statistically significant.

RESULTS

The study included 30 children with Autism Spectrum Disorder (ASD) from the Phoniatric Clinic, along with 30 neurotypical children as a control group.

Description of clinical and medical data among cases group

Among cases group, IQ ranged from 28 to 65, with a mean of 42.20 + 11.23. The mean CARS were 36.30 and vitamin B12 level was 272.93. About half of cases had abnormal Vitamin B12 level (46.7%) (Table 1, Figures 1,2)

Table 1: clinical and medical data among cases group

		Mean	±SD	Minimum	Maximum
I.Q		42.20	11.23	28.00	65.00
CARS		36.30	2.93	31.00	41.00
Vitamin B12 Level		272.93	127.83	150.00	500.00
Vitamin B12	Normal	16	53.3%		
	Abnormal	14	46.7%		

±SD: Standard deviation

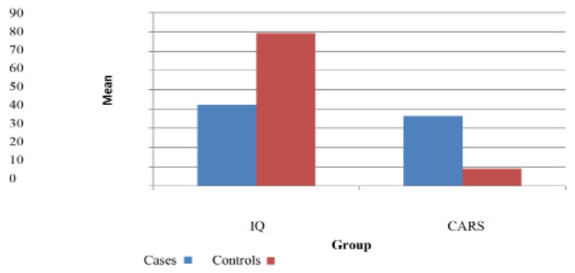

Fig. 1: Comparison between cases and control as regard IQ and CARS

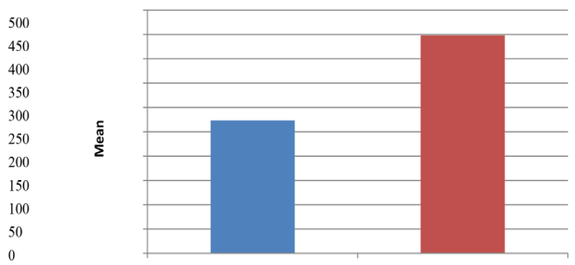

Fig. 2: Comparison between Cases and Control as regard Vitamin B12

Description of Total Language age and Total Standard Score among cases group

Among cases group, total language age ranged from 0.70 to 3 years with a mean of 1.23 ± 0.60 years. Total language standard score ranged from 50 to 54 with mean of 51.15 ±1.14. (Table 2, Figure 3).

Table 2: total language age and total standard score among cases group

	Mean	±SD	Minimum	Maximum
Total Language Age	1.23	.60	.70	3.00
Total Language Standard Score	51.15	1.14	50.00	54.00

±SD: Standard deviation

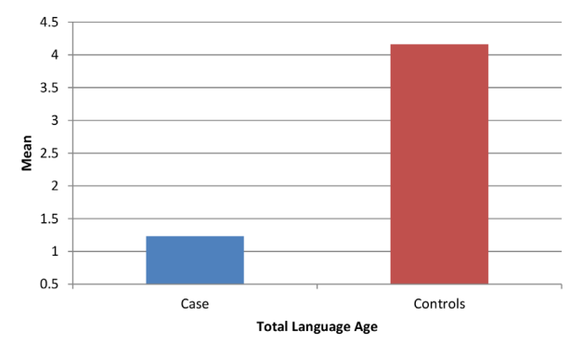

Fig. 3: Comparison between Cases and Control as Regard total language age

Description of clinical data among control group

Among control group, IQ ranged from 50 to 94, with a mean of 79.47 + 11.72. The mean vitamin B12 level was 447.93. Only 4 children of the control group had abnormal Vitamin B12 level (13.3%). (Table 3)

Table 3: clinical data among control group

		Mean	±SD	Minimum	Maximum
I.Q		79.47	11.72	50.00	94.00
Vitamin B12 Level		447.93	251.98	163.00	910.00
Vitamin B12	Normal	26	86.7%		
	Abnormal	4	13.3%		

±SD: Standard deviation

Description of Total Language age and Total Standard Score among control group

Among control group, total language age ranged from 2 to 7 with a mean of 4.16 ± 1.47 years. Total language score ranged from 50 to 114 with mean of 67 ±19.85. (Table 4)

Table 4: total language age and total standard score among control group

	Mean	±SD	Minimum	Maximum
Total Language Age	4.16	1.47	2.00	7.00
Total Language Standard Score	67.00	19.85	50.00	114.00

±SD: Standard deviation

Comparison between cases and control as regard clinical and lab data

There was a highly significant difference between the two study groups as regard IQ. Similarly, there was a significant difference between the two study groups as regard vitamin B12 level with lower levels among cases (Table 5)

Table 5: Comparison between cases and controls as regard clinical and lab data

	Group				*P*	Sig
	Cases		Controls			
	Mean	±SD	Mean	±SD		
I.Q	42.20	11.23	79.47	11.72	0.0001	HS
CARS	36.30	2.93	9.07	3.41	0.0001	HS
Vitamin B12 Level	272.93	127.83	447.9	251.98	0.023	S

*student t test **Fisher exact Tests

Table (6) there was a highly significant difference between the two study groups as regard Total language age and total language score.

Correlation between vitamin B12 level and each of receptive, expressive and total Standard Score among cases group

There was no significant correlation between vitamin B12 level and each of receptive, expressive and total language standard score among cases group (Table7)

Correlation between vitamin B12 level and each of CARS and IQ among cases group

There was no significant correlation between vitamin B12 level and each of I.Q and CARS among cases group (Table 8)

Table 6: Comparison between cases and controls as regard Total Language age and Total Language Standard Score

	Group				*P*	Sig
	Cases		Controls			
	Mean	±SD	Mean	±SD		
Total Language Age	1.23	.60	4.16	1.47	0.0001	HS
Total Language Standard Score	51.15	1.14	67.00	19.85	0.008	HS

*student t test **Fisher exact Tests

Table 7: correlation between vitamin B12 level and each of receptive, expressive and total standard score among cases group

		Vitamin B Level
Receptive Standard Score	*r**	-.042
	P	.893
	Sig	NS
Expressive Standard Score	*r**	.149
	P	.626
	Sig	NS
Total Language Standard Score	*r**	-.332
	P	.268
	Sig	NS

*Pearson correlation NS: Non-Significant

Table 8: correlation between vitamin B12 level and each of CARS and IQ among cases group

		Vitamin B Level
CARS	*r**	.316
	P	.251
	Sig	NS
I.Q	*r**	.075
	P	.791
	Sig	NS

*Pearson correlation NS: Non-Significant

DISCUSSION

This study aimed to examine the mental age (IQ), language development (PLS-4), severity of ASD (CARS), and Vitamin B12 concentrations in children diagnosed with Autism Spectrum Disorder (ASD). Our findings revealed significant differences between children with ASD and the control group, particularly regarding IQ, CARS scores, and Vitamin B12 levels.

IQ and CARS Scores

A highly significant difference was observed between the ASD group and the control group concerning IQ and CARS scores. These findings align with previous studies highlighting the cognitive and developmental challenges faced by children with ASD. However, these results do not necessarily contradict the findings of Estes *et al*. 2010[9] who emphasized that many children with ASD possess academic abilities higher than traditionally recognized. One possible explanation is that children with ASD may show discrepancies between their IQ scores and their performance in academic areas such as reading, spelling, and math. This is a common phenomenon where children with high IQs may struggle with academic tasks due to the core symptoms of ASD.

Additionally, the clinical presentation of children with ASD often correlates with their IQ levels. Severely impaired children may have limited or no language skills, while those with higher IQs may exhibit language abilities, albeit with a tendency for repetitive speech and routines. As noted by Pacholok (2014)[10], such children might also show areas of exceptional talent in specific domains, further complicating the relationship between IQ and language development in ASD.

Language Development (PLS-4)

The total language age and scores were significantly higher in the control group compared to the ASD group. This finding is consistent with previous studies (Crowley, 2009; Hertz-Piciotto, 2004)[11,12] that report delays in language development as a hallmark of ASD. Language impairments in ASD are commonly described as part of a triad of behavioral deficits, which include difficulties in social interaction, delayed language development, and restricted behavioral patterns. These findings underscore the need for early intervention in ASD to address language development challenges.

Vitamin B12 Deficiency

In this study, Vitamin B12 levels were significantly lower in the ASD group compared to the control group. This result supports previous findings by Hodgson *et al*. (2016)[13], who observed abnormally low levels of both cobalamin (Cbl) and methylcobalamin (MeCbl) in individuals with ASD and schizophrenia. Vitamin B12 is essential for proper neurological function, and deficiencies are known to have a profound impact on brain development, which may contribute to the neurodevelopmental delays observed in children with ASD.

Methylcobalamin, a crucial form of Vitamin B12, is frequently deficient in children with ASD. Potential causes of this deficiency include selective eating habits, gut dysbiosis affecting B12 absorption, neurodegenerative damage from autoimmune antibodies, and exposure to

neurotoxins or heavy metals (George & Austin, 2019)[14]. These factors may hinder the body's ability to effectively utilize Vitamin B12, exacerbating the challenges faced by children with ASD.

Lack of Correlation between Vitamin B12 Levels and IQ/CARS

Although lower Vitamin B12 levels were observed in the ASD group, no significant correlation was found between Vitamin B12 levels and IQ or CARS scores. This finding contrasts with the work of Duthie *et al*. (2002)[15], who identified significant correlations between vitamin B12 levels and cognitive abilities in older adults. However, the absence of a correlation in our study may be explained by several factors, including the different age group studied (children versus adults) and the relatively small sample size. While our study met the minimum statistical requirements, larger sample sizes may provide more definitive insights into this potential link.

Implications for Treatment

Despite the lack of a significant correlation in this study, there is evidence suggesting that Vitamin B12 supplementation may benefit children with ASD. For instance, Rossignol and Frye (2021)[16] reported improvements in core ASD symptoms, including expressive language and social skills, following Vitamin B12 treatment. This suggests that, while the relationship between Vitamin B12 levels and ASD symptoms may not be immediately clear in our study, supplementation could still have therapeutic potential, particularly in addressing the metabolic abnormalities associated with ASD.

LIMITATIONS

Small sample size of the study, lack of long-term follow-up, and potential biases in Vitamin B12 measurement or ASD diagnosis.

CONCLUSION

- Vitamin B12 deficiency is common in children with ASD.
- There was no significant relation observed between vitamin B12 levels and IQ or CARS scores in children with ASD.
- No meaningful relationship was detected between Vitamin B12 levels and receptive, expressive, or total language standard scores in children with ASD.

RECOMMENDATIONS

- Additional studies with a larger sample size are needed to validate the effect of vitamin B12 deficiency in individuals with ASD.
- Vitamin B12 supplementation with methylcobalamin (750-2500 mcg injectables, 64.5 mcg/kg) administered twice a week, with daily injections, has shown to help 40% of children. A positive response to this trial may qualify as a successful treatment.
- Routine assessment of serum vitamin B12 levels should be mandatory for all children with ASD.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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