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Altun, et.al. (2018); children with ASD have significant higher levels of total homocysteine and lower levels of B_{12} in plasma as compared to age matched controls.

A study of Omani children by Al- Farsi et.al. (2013) corresponded with this study and found that ASD have low dietary intake of B_{12} and thus have low serum B_{12} levels compared to neurotypical controls.

Another research on homocysteine levels in urine by Kałużna-Czaplińska et.al. (2011) found to be significantly higher in ASD children. While most studies on ASD children document B_{12} deficiency in children with ASD the results of Guo et.al. (2018) differed from this general trend as he finds that there were no significant interactions among vitamin B_{12} and autistic symptoms.

In the same line, Bala et.al 2022 analyzed and compared B_{12} levels in ASD (n= 34), ADHD (n= 16) and controls (n= 27) and noted that the ASD group had the lowest vitamin B_{12} levels, compared to the controls which agree with these results.

Similarly, Yetkas et.al. (2019) compared vitamin B_{12}, folate and homocysteine concentrations in ASD (n= 48), Attention deficit disorder ADHD (n= 35) and controls (n= 35). ASD had the lowest vitamin B_{12} and the highest homocysteine levels while ADHD had intermediate levels.

Hendren et.al. (2016) supported our results by their findings as they reported clinical improvement among children treated with methyl B_{12} was positively correlated with increases in plasma methionine, decreases in S-adenosyl- 1 homocysteine (SAH) and improvements in the ratio of S-adenosylmethionine (SAM) to SAH, indicating an improvement in cellular methylation capacity.

However, Bertoglio et.al. (2010) in a cross over trial did not find any statistically significant differences in the overall. However, nine (9 of 30) subjects (30%) demonstrated clinically significant improvement on the Clinical Global Impression Scale and at least two additional behavioral measures. More notably, these responders exhibited significantly increased plasma concentrations of GSH (glutathione stimulating hormone) or glutathione endpoints between active and placebo groups. This led the authors to conclude that methyl B_{12} may alleviate symptoms of ASD in a subgroup of children, possibly by reducing oxidative stress.

The other two studies primarily evaluated the impact of supplementation on biochemical parameters. James et.al. (2009) supplemented 40 ASD children with 75 µg/ kg Methyl cobalamin (2 times/ wk.) and 400 µg folinic acid (2 times/ d) for 3 months. Plasma concentrations of transmethylation/ transculturation metabolites and glutathione redox status in ASD children were measured as compared to controls. There were significant increases in cysteine, cysteinyl glycine, and glutathione concentrations. The oxidized disulfide form of glutathione was decreased, and the glutathione redox ratio increased after treatment.

Also, Guo, et.al. (2018) disagreed with this study, in China studied 371 children (274 ASD) aged (2- 7) years using the Autism Behavior Checklist and their Social responsiveness scale. No significant difference was found regarding vit B_{12} between ASD group and control group (p= >0.106).

Similarly, Yetkas et.al. (2019) reported that vitamin B_{12} was reduced while homocysteine was elevated among patients with ADHD and ASD. This finding was supported and extended to folate in another study by Altun et.al (2018).

ROC analysis in this study shows that MMA had highest sensitivity 88.46% and sensitivity 70.37% at a cutoff point 2.04 umol/ ml so, serum level of MMA can be considered as a good biomarker for ASD, there is a lot of studies that discuss the use of other metabolites rather than methylmalonic acid as a biomarkers for ASD but we need further studies to investigate this point.

Conclusion:

Serum level of MMA can be used for early detection of vitamin B_{12} deficiency in blood of ASD children; furthermore the level of MMA in blood could be used as a biomarker of ASD as the ROC curve shows that MMA had highest sensitivity (88.46%) and specificity (70.37%) at cut- off point (2.04).

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- Conflict of interest: None declared
- Ethical approval: The study was approved by the Institutional Ethics Committee.

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studies (IPGCS, 2014).

Statistical Analysis:

Collected data were computerized and statistically analyzed using IBM SPSS-23.0 for windows (SPSS Inc., Chicago, IL, USA) and the Jamovi project (2022) (Version 2.3). Qualitative data: represented in the form of number and percentages (N. %) while quantitative data after testing of normality using Shapiro- wilk test: normally distributed data presented with mean SD. At level of significance value (P value): P > 0.05 (Non- significant), P≤ 0.05 (Significant). For qualitative data: Chi- square test and Fisher's exact test were used, while quantitative data that was normally distributed: t- test. Correlation studies were done using Person's correlation coefficient. An ROC (Receiver operation Curve) curve is a plot of sensitivity on the y axis against (1–specificity) on the x axis for varying values of the threshold t. Area under the curve (AUC) were calculated and classified (9- 10: Excellent, 8- 9: good, 7- 8: fair, 6- 7: poor).

Results:

Table (1) Characteristics of the study participants

Variable		Autistic Group (N= 30)	Control Group (N= 30)	Test	P- Value
Age (Months)	Mean±Sd	110.2±39.6	94.6±28.4	1.75*	0.09
	(Range)	(36- 120)	(43- 114)		
Weight (Kg)	Mean±Sd	37±15.1	38±14.2	-0.92*	0.86
	(Range)	(16- 55)	(15-60)		

Independent sample t test

Table (1) shows no statistically significant difference between the studied groups as regards age or weight (P > 0.05).

Bar chart showing sex frequencies among studied groups which shows no statistical significance p value 0.19.

Table (2) Childhood Autism Rating Scale (CARS) among autistic patients

Variable		Autistic Group (N= 30)
CARS	Mean±Sd	33.4±4.59
	(Range)	(31- 45)
Mild To Moderate	(N. %)	28 (93.3%)
	Severe (N. %)	2 (6.7%)

Table (2) shows that 28 patients (93.3%) were mild to moderate and 2 patients (6.7%) severe autistic according to CARS.

Table (3) Methylmalonic acid (MMA) among studied groups

Variable		Autistic Group (N= 30)	Control Group (N= 30)	Test*	P- Value
Mma Levels	Mean±Sd	2.58±1.06	1.44±0.8	-4.81	<0.001
	(Range)	(0.22- 5.82)	(0.29- 2.98)		

Independent sample t test

Table (3) shows highly statistically significant difference between the 2 groups as regards MMA levels, which were higher among autistic group (2.58±1.06) in comparison to control group (1.44±0.8), (P <0.001).

Table (4) Correlation of MMA with age and CARS among autistic patients

Variable	MMA	
	R*	P- Value
Age	-0.206	0.12
CARS	0.208	0.11

Table (4) shows no significant correlation of MMA with age or CARS (P > 0.05).

Table (5) ROC analysis of MMA in discriminating between autistic patients and controls

Cut Point	Sensitivity (%)	Specificity (%)	PPV (%)	NPP (%)	AUC (%)
2.04	88.46%	70.37%	74.19%	86.36%	0.813

ROC analysis (Receiver operation Curve) was conducted to determine the optimal cutoff value to discriminate autistic patients from control group, analysis showed that MMA had highest sensitivity 88.46% and specificity 70.37% at cut- off point 2.04 umol/ml, with area under the curve 0.81, So MMA can be considered as a good marker in discriminating autistic patients from healthy controls Table (5).

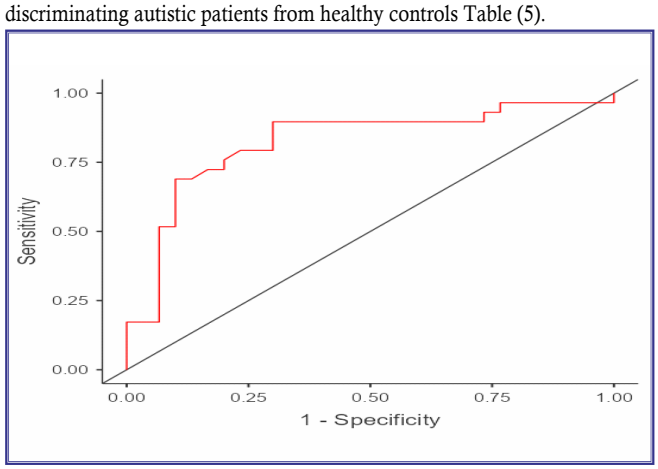

Figure (1) ROC analysis of MMA in discriminating between autistic patients and controls

Discussion:

Vitamin B_{12}, also known as cobalamin, is a water- soluble vitamin that is derived from animal products such as red meat, dairy, and eggs. Intrinsic factor is a glycoprotein produced by parietal cells in the stomach and necessary for the absorption of vitamin B_{12} in the terminal ileum. (Kozyraki and Cases, 2020)

Methyl malonic acid is clearly more specific to vitamin B_{12} deficiency compared to Homocysteine (HC). A deficiency of vitamin B_{12} at the tissue level can lead to elevation of both MMA and HC even when serum vitamin B_{12} concentrations are within the reference values. (Chiang et.al., 2018)

Elevated MMA and HC levels together have been found to be 99.8% sensitive for diagnosing functional vitamin B_{12} deficiency, which is defined as elevated MMA and HC levels despite normal vitamin B_{12} levels in asymptomatic individuals. Therefore, using serum vitamin B_{12} testing alone may under- diagnose the deficiency of this vitamin. (Wolfenbüttel et.al., 2019)

The signs and symptoms of pediatric B_{12} deficiency frequently mimic those of autism spectrum disorders. Both autistic and brain- injured B_{12}-deficient children have obsessive- compulsive behaviors and difficulty with speech, language, writing, and comprehension. B_{12} deficiency can also cause aloofness and withdrawal. (Nnubia et.al., 2023)

In current study, there was highly statistically significant difference between the 2 groups as regards MMA levels, which were higher among autistic group (2.58±1.06) in comparison to control group (1.44± 0.8).

Several observational studies agreed with this study and have documented that children with ASD are deficient in B_{12} as reflected in the biochemical B_{12} parameters. As presented by Pasca et.al. (2006) and

Introduction:

Autism spectrum disorder (ASD) is a spectrum of complicated neurodevelopmental disorders that is typified by limited and repetitive behavior along with impaired social communication and/ or interaction. (Bhat, 2021)

There are multiple risk factors of autism spectrum disorder for example having an older parent, a history of the illness in the family, and specific genetic disorders are also risk factors. (Ferrara et.al., 2021)

According to the Autism and Developmental Disabilities Monitoring Network, 1 in 68 American children aged 8 have been diagnosed with ASD, reflecting the recent remarkable increase in the prevalence of the disorder globally (Christensen et.al., 2019). More recent study shows that about 1 in 36 children aged 8 years old in the United States has been identified with autism spectrum disorder (ASD) according to estimates from CDC's (centers for disease control and prevention), Autism and Developmental Disabilities Monitoring (ADDM) Network 2020. (Maenner et.al., 2023)

Co- morbidities that are most commonly observed in children diagnosed with ASD include autoimmune diseases, gastrointestinal disorders, sleep disorders, mental retardation, and sensory impairment. (Rydzewska et.al., 2021)

Vitamin B_{12} (water soluble vitamin) is an essential micronutrient. It functions as a cofactor for mitochondrial methyl malonyl- CoA mutase and cytosolic methionine synthase. (Mukherjee et.al., 2023)

Deficiency of vitamin B_{12}, either nutritional or due to inborn errors of metabolism, inactivate methionine synthase and methyl malonyl- CoA mutase leading to building up of homocysteine and methylmalonic acid (MMA) (Hannibal et.al., 2016). Accordingly, methylation is impaired as a result of vitamin B_{12} deficiency. (Tanwar et.al., 2020)

Anemia and the development of macrocytes can result from blood cell alterations brought on over time by a vitamin B_{12} shortage. Additionally, it may result in neuropathy symptoms including tingling and numbness in the hands and feet, and in more severe cases, mental or behavioral abnormalities like depression, anxiety, irritability, cognitive impairment, and speech delay. (Green, 2017)

Although some patients may have some degree of neuropathy, an elevated concentration of MMA is frequently visible before blood cell alterations and before full- blown symptoms appears. Elevated MMA levels in the blood or urine can be measured to provide a sensitive and early indication of vitamin B_{12} insufficiency. (Raghavan et.al., 2018)

Adjusted serum MMA concentrations were not significantly different between males and females ($p< 0.11$). (Ganji and Kafai, 2018)

Results from earlier research have validated the protective benefits of sufficient vitamin B_{12} intake for expectant mothers against the risk of autism spectrum disorder (ASD) in their offspring. (Okocha et.al., 2023)

Objective:

Measuring the level of MMA (as an indicator of vitamin B_{12} level) in blood of autistic children, aiming to early detection of vitamin B_{12} deficiency and it s correlation with autism spectrum disorder (ASD).

Methodology:

This case control study that was conducted at Special Need Center of Faculty of Postgraduate Childhood Studies, Ain Shams University on children who visited the center from January 2023 till October 2023. This study included 60 individuals, 30 children diagnosed as ASD, and 30 healthy children who were the controls. Both groups are equivalent in age, sex and socioeconomic status.

Inclusion criteria were children aged from 3- 10 years old diagnosed as autism according to DSM- 5 criteria for diagnosis. Children with no known neurodevelopmental, psychiatric, neurological disorder, infection, and/or no record of vitamins and mineral supplements were selected as healthy controls.

Exclusion criteria included any patient referred with genetic syndrome or taking supplements with vitamin B_{12} containing preparations in the last 6 months; vegetarian patients were excluded.

ASD was diagnosed using the Diagnostic and Statistical Manual of Mental Disorders, fifth edition, (DSM- 5) criteria.

Childhood Autism Rating Scale (CARS) was applied. It is the most common standardized tool used in the ASD assessment process that was originally developed by Schepler and Reichle. (Schopler et.al., 2010)

All children were subjected to full detailed history, physical examination, CARS, and assessing methylmalonic level in blood.

Socioeconomic status evaluated through the application of scale for determining social and economic level of all families contriting in this study. (Ayman Salem, 2018)

Venous blood was collected for measuring serum level of methylmalonic acid and samples were put the in-20c until analysis. All samples were analyzed using enzyme immunoassay technique (ELISA).

Final Assessment and evaluation were made through using all the information supplied from the history, the physical examination, investigations and CARS results.

Expected limitations of the study were number of patients, cost of investigations and refusal of parents to participate in the study.

- Ethical Consent: The study was done after the approval of ethical committee of the faculty of postgraduate childhood studies. Written informed consent was obtained from care givers after explanation of the nature, aims of the study, its benefits for their children and for all the community and expected risks for their children if participated in the study. All children with vitamin B_{12} deficiency were treated.
- Privacy And Confidentiality Of Subjects: The patient entire data recorded was highly confidential by working staff. Patients' laboratory samples were discarded after performing required labs and weren't used for any purpose.
- Verbal Assent: Informed verbal assent was taken from the care givers of all children after a simplified explanation of the aim and benefits of the study. The study complied according to the instruction of the research ethics committee in the faculty of postgraduate Childhood

Serum Level of Methylmalonic Acid in Children with Autism Spectrum Disorder

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المركز القومي للبحوث

Summary

Background: Vitamin B_{12} is required for proper red blood cell formation, neurological function. The methylmalonic acid (MMA) serum test is more sensitive than the vitamin B_{12} serum level. A deficiency of serum level of vitamin B_{12} is the most common cause of raised levels of MMA.

Objective: Measuring the level of MMA (as an indicator of vitamin B_{12} level) in blood of autistic children, aiming to early detection of vitamin B_{12} deficiency.

Methodology: this case control study that was conducted at Special Need Center of Faculty of Postgraduate Childhood Studies, Ain Shams University on children who visited the center from January 2023 till October 2023. A total of 60 children were enrolled in this study and divided into 2 groups, group I (ASD group); included 30 children and group (control group); included 30 healthy children. The 2 groups were equivalent in age (3- 10) years, sex, and socio- economic class. Childhood Autism Rating Scale (CARS) was applied. Socio economic status was evaluated through the application of scale for determining social and economic level.

Full detailed history was taken. CARS was performed to autistic children only. Venous blood was collected for measuring serum level of methylmalonic acid. All samples were analyzed using enzyme immunoassay technique (ELISA).

Results: There was highly statistically significant difference between the 2 groups as regards MMA levels, which were higher among autistic group (2.58±1.06) in comparison to control group (1.44± 0.8). ROC (Receiver operation curve) analysis of MMA in discriminating between autistic children and controls revealed that our cut off point is 2.04 umol/ml, sensitivity of the test is 88.46%, specificity of the test is 70.37%.

Conclusion: Serum level of MMA can be used for early detection of vitamin B_{12} deficiency in blood of ASD children, furthermore the level of MMA in blood could be used as a biomarker of ASD.

Keywords: Methylmalonic Acid; Autism Spectrum Disorder.

قياس مستوي حمض الميثيل مالونيك في دم الاطفال المصابين بطيف التوحد

الخلفية: فيتامين ب١٢ ضروري لتكوين خلايا لدم الحمراء بشكل سليم، والوظيفة العصبية، و تخليقا لحمض النووي. حمض الميثيل مالونيك (قاعدة ميثيل مالونات المترافقة) هو حمض ثنائي الكربوكسيل هو مشتق C− ميثيلمنالمالونات. كلما زاد مستوي حمض المثيل مالونيك في الدم قل مستوي فيتامين ب١٢ في الدم.

الهدف: قياس مستوي حمض المثيل مالونيك في الدم كمؤشر لمستوي فيتامين ب ١٢ في دم الاطفال المصابين بطيف التوحد بهدف الكشف المبكر عن نقص مستوي فيتامين ب ١٢ في الدم) والارتباط بين اعراض نقص فيتامين ب ١٢ ومستوي حمض المثيل مالونيك عند اطفال طيف التوحد.

طرق البحث: أجريت هذه الدراسة في مركز ذوي الاحتياجات الخاصة بكلية الدراسات العليا للطفولة جامعة عين شمس على الأطفال الذين ترددوا علي المركز في الفترة من يناير ٢٠٢٣حتي اكتوبر ٢٠٢٣. وقد تضمنت الدراسة ٦٠ طفلا وتم تقسيمهم إلي مجموعتين، المجموعة الأولى مجموعة الاطفال المصابين بطيف التوحد؛ شملت ٣٠ طفلا تم تشخيصهم على أنهم مصابون بطيف التوحد من خلال DSM5 والمجموعة الثانية المجموعة الضابطة؛ شملت ٣٠ طفلا أصحاء والذين كانوا بمثابة الضوابط. تم تطبيق مقياس تصنيف التوحد لدى الأطفال. تم إجراء CARS لتحديد شدة الاضطراب لدي الاطفال المصابين بطيف التوحد وتم قياس المستوى الاقتصادى للاسر المشاركة عن طريق تطبيق مقياس د.ايمن سالم وتم اخذ تاريخ طبي مفصل من جميع افراد العينة وتم عمل فحص طبي شامل وتم اخذ عينة دم من جميع الاطفال لقياس مستوي حمض المثيل مالونيك في دم الاطفال المشاركين في الدراسة وتم حفظ العينات في درجة حرارة -٢٠° وتم تحليل العينات باستخدام ELISA.

النتائج: كان هناك فرق ذو دلالة إحصائية عالية بين المجموعتين فيما يتعلق بمستوي حمض الميثيل مالونيك، والتي كانت بين مجموعةا الاطفال المصابين بطيف التوحد (٢,٥٨ ±١,٠٦) مقارنة بالمجموعة الضابطة (١,٤٤± ٠,٨).

الاستنتاج: يمكن استخدام مستوى حمض الميثيل مالونيك في الدم للكشف المبكر للأطفال المصابين بطيف التوحد.

الكلمات المفتاحية: حمض الميثيل مالونيك؛ اضطراب طيف التوحد.

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