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A Study of Plasma Homocysteine Level in Children with Autism

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Autism is a neurodevelopmental disorder that appears in early life. B6 and B12, and folate support homocysteine (Hcy) metabolism. Hence, any defect to them leads to Hcy accumulation in the body. Hcy, vitamins B12, B6 and folate are important in psychiatric disorders such dementia, schizophrenia, and autism. This study intended to compare the plasma Hcy levels in normal and autistic cases to find out whether there is difference between the levels of Hcy in plasma of autistic and normal children.
Methods: This case control study enrolled 60 children who were allocated in two groups 30 with autism and other 30 as controls. All patients were exposed to full history taking, physical examination, mental status examination, hearing test, routine lab investigations and specific lab investigations (serum folic acid, plasma vitamin B12 and Hcy level).
Results: Childhood Autism Rating Scale (CARS) level among autistic children was 49.400 ± 5.840. The mean plasma Hcy level was considerably higher in children with autism than controls (P value< 0. 01). The mean serum folic acid level and vit B12 level was significantly reduced in autistic cases than controls (P value< 0. 01). There was a statistically significant positive correlation between plasma Hcy level (µmol/L) and CARS level in children with autism (P value< 0. 01).
Conclusions: Children with autism have increased levels of Hcy, and decreased levels of folic acid and vit B12. So, Hcy may be used as a clinical biomarker for early diagnosis and managing of autistic children.

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Keywords: Homocysteine; autism; CARS; folate; vitamin B12.

1. INTRODUCTION

Autism is a severe neurodevelopmental condition that starts before 3 years of age with difficulties in communications, social relationships, and interest/Behaviour patterns [1].

Autism is thought to be a complex condition that is impacted by immunological, environmental, genetic, and oxidative stress susceptibility factors. No one gene has been discovered to be connected with autism, and numerous genes have been hypothesised to be involved. Autism's aetiology may incorporate environmental variables such as measles, lead, mercury, rubella virus, and the usage of alcohol during pregnancy. Autoimmune, immune and infectious factors have also been implicated in the cause of autism [2].

In addition to behavioural issues, some people with autism have been characterised as having sleeping disturbances, gastrointestinal disorders, and neurological illnesses such as epilepsy [3]. Homocysteine (Hcy) is a sulphur amino acid that undergoes two metabolic pathways: transsulfuration to cystathionine, that demands pyridoxal-50 -phosphate and remethylation to methionine, which needs folate and vitamin B12 (or betaine in an alternate reaction) [4].

Hcy serves several crucial functions in human physiology. Increased Hcy levels in bodily fluids are connected with a variety of diseases, including neuropsychiatric illnesses and autism [5].

The enzymes involved in Hcy metabolism need several B vitamins as cofactors, including and B9 (folic acid), B12 (cobalamin) and B6 (pyridoxine). Their deficiency causes the build-up and elevation of Hcy. Monitoring Hcy levels in the body fluids of autistic children may thus give information on hereditary and physiological disorders, poor lifestyle (including dietary habits), and a number of pathological conditions [6].

This research was conducted to examine the plasma Hcy concentrations of healthy and autistic children to determine whether there was a difference between the plasma Hcy concentrations of healthy and autistic children.

2. PATIENTS AND METHODS

This case control study enrolled 60 children who were admitted to Pediatric Department, Neurology Unit, Tanta University Hospital, outpatient clinic from June 2021 to June 2022. Patients were categorized into two equal groups thirty children with autism with mean age 4.500±0.919 years who were diagnosed depending on the American psychiatric association criteria, diagnostic and statistical manual of mental disorders, fifth edition (DSM-V), and thirty control subjects aged 4.467±0.890 years.

Inclusion criteria were children newly diagnosed as autism aged 3-6 years, no medication intake or vitamin supplementation at least last six months before the study. no sex predilection.

Exclusion criteria were children with clinical criteria of fragile X-disorder, tuberous sclerosis, and phenylketonuria, epilepsy or other neurological disorders, pervasive developmental disorder-not otherwise specified (PDD-NOS) or Asperger syndrome and children with known cardiovascular, endocrine, liver, pulmonary or kidney disease or evidence of malabsorption diseases.

All patients were exposed to full history taken, physical consideration (complete neurological examination), mental status examination (evaluation of social interaction, language and communicative functions, childhood autism rating scale (CARS)), hearing test (auditory brain stem response (ABR), electroencephalography (EEG), brain magnetic resonance imaging (MRI)), routine lab investigations (CBC and renal and liver function tests) and specific lab tests (serum folic acid, plasma vitamin B12 and plasma Hcy level).

Childhood Autism Rating Scale (CARS): a 15-point scoring system for a child's conduct that helps distinguish between children with intellectual difficulties who aren't autistic and those who have autistic signs. When used to children, it is notably useful for identifying those with autism from those with mild intellectual disabilities. It also helps us classify cases of autism into mild-moderate and moderate-serious categories [7]. Using a scale that ranges from 1 to 4, each component is assigned a score in increments of half a point. It's possible to score from 15 to 60 points overall. Children with scores between 15 to 29.5 are not exhibiting autistic symptoms. Mild-moderate autism is diagnosed in children with scores between 30 and 36, while

severe autism is diagnosed in children with scores between 37 and 60 [7].

2.1 Statistical Analysis

For the statistical analysis, SPSS V.20 (IBM Inc., Chicago, IL, USA) was used. The mean and standard deviation (SD) were used to describe quantitative data, and the paired Student's t-test was used to compare the means of two groups with the same group. Qualitative analysis included numbers and percentages (%), whereas the Chi-square test was used to compare qualitative variables. Linear correlation coefficient test was used to compare two quantitative variables. A two tailed P value < 0.05 was considered significant.

3. RESULTS

The demographic data were insignificantly varied between children with autism and healthy controls as regard to the sex and age (P-value > 0.1). CARS level among children with autism was 49.400 ± 5.840 Table 1.

Regarding the specific lab investigation, the mean plasma Hcy level was considerably more elevated in autistic children than controls (P value< 0. 01). The mean serum concentration of folic acid was remarkably lower in children with autism than healthy children (P value< 0. 01). The mean plasma vit B12 level was significantly reduced in autistic children than controls (P value< 0. 01) Table 2.

The plasma homocysteine level (µmol/L) was insignificantly different between male and female participants (P value =0.204).

There was a statistically significant positive correlation between plasma Hcy level (µmol/L) and CARS level in autistic children. The more the elevated plasma concentration of Hcy, the higher the CARS level, (r =0.925), (p- value <0.001) Fig. 1.

Table 1. Demographic data and CARS level in participants children

		Patients (n=30)	Control (n=30)	P value
Age (year)		4.5±0.919	4.467±0.89	0.887
Sex	Male	24 (80%)	22 (73.33%)	0.542
	Female	6 (20 %)	8 (26.67%)	
CARS		49.400 ± 5.840	-	-

Data were presented as mean ± SD or frequency (%)

Table 2. Serum folic acid, plasma vitamin B12 and plasma Hcy level in children with autism and the healthy control children

	Patients (n=30)	Control (n=30)	P value
Hcy level (µmol/L)	20.377±2.223	10.033±1.710	<0.001*
Folic acid level (ng/mL)	1.937± 0.494	12.597±1.868	<0.001*
VIT B 12 level (pg/mL)	138.133 ± 10.244	345.133 ± 31.556	<0.001*

Data were presented as mean ± SD or frequency (%). Hcy: Homocysteine

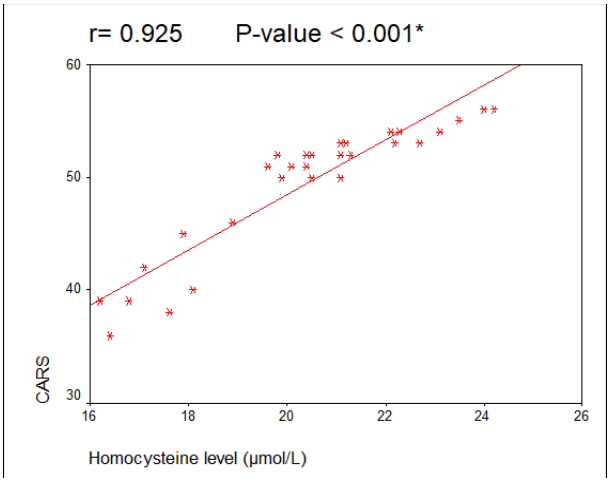

Fig. 1. Show correlation between plasma Hcy level (µmol/L) and CARS level in children with autism

Table 3. The plasma Hcy level (µmol/L) in male and female participants

Sex	Hcy level (µmol/L)	P value
Male (n=24)	20.638±2.303	0.204
Female (n=6)	19.333± 1.622	

Data were presented as mean ± SD. HCY: homocysteine

4. DISCUSSION

In the current study, the results showed that the mean plasma Hcy concentration was remarkably higher in children with autism than healthy control children, the mean serum concentration of folic acid was significantly reduced in children with autism than healthy children, and the mean plasma concentration of vit B12 level was considerably lower in children with autism than healthy control children. It also showed that the more the increased plasma level of Hcy, the higher the CARS level.

Our findings agree with the results obtained by Ali Evren Tufan et al. [8] who had studied vit B12, folate and Hcy concentrations in autistic children and healthy controls. Children having autism had the lowest concentration of vitamin B12 and the highest Hcy levels. The study did not exhibit any change in serum levels of folate between autistic cases and controls.

The results of our work also agree with those by Hatice Altun et al. [9] who has examined the levels of complex B vitamins, vitamin D, vitamin D receptor and Hcy, in autistic children compared to normal, the Hcy concentration of autistic individuals were considerably higher in this research. Cases with autism had considerably reduced levels of folate, vitamin B6, and vitamin B12. The investigation revealed no significant variation in blood folate levels between autistic individuals and their normal samples.

The findings of this study also agree with Jan Jo´zefczuk, et al. [10] results who had studied Hcy as an etiopathogenic and diagnostic factor autistic patients. Their study showed that serum Hcy in autistics had statistically higher Hcy concentration than control healthy group.

Comparable to our results, Caihong Sun al. [11] had examined the effects of taking folic acid on autistic infants. Autistic infants had lower plasma concentrations of folic acid than controls, while adolescents with autism had higher Hcy values in their biological fluid than controls. Vitamin B12 concentrations were within the normal range before and after therapy, and there was no significant difference between pretreatment and controls.

The present results were comparable with the previously mentioned data by Yu Han, et al. [12] who had studied transsulfuration metabolism and lowered antioxidant capacity in Chinese autistic patients. They found that the Hcy serum concentrations were surprisingly higher in autistic cases than controls.

These results agree with Bianka Hoxha et al. [13] who had studied the relation between folic Acid and autism. They found that the Hcy concentration in autistic cases were remarkably higher contrasted to normal values, whereas serum folate and Vitamin B12 concentrations were much lower than the values identified as these nutrients deficiency levels.

The findings of this study were also comparable to Bao-Qiang Guo et al. [14] results who had studied blood levels of Hcy in autistic children. Hcy levels in autistic children's peripheral blood were found to be considerably higher than the normal individuals.

This research's findings also stand in contrast to those of Semih Erden, et al. [15] study who had studied serum B12 and Hcy levels in autistic children. The serum Hcy and B12 levels were significantly reduced in autistic cases than the controls.
The present findings were conflicting with Sharmistha Saha, et al. [16] who had studied the contribution between folate metabolic pathway and autism in eastern Indian populations. They discovered no significant statistical association between autistic patients and plasma Hcy levels. They speculated that the discrepancies may be due to variations in the research populations or the respondents' dietary preferences.

In contrary, K.A. BALA et al. [17] studied plasma amino acid profile in autism. They found that Hcy and methionine levels were lower in autistic children contrasted to controls.

Since Hcy is a potent excitotoxin, it is no wonder that the metabolic by-products of this compound also damage proteins and alter their structures, leading to the production of toxic proteins and the activation of the immune system [18]. Several research on Hcy's neurotoxicity show that it may

trigger cell death through excitotoxicity and apoptosis, as well as impair neurons by activating N-methyl-D-aspartate (NMDA) receptors. There is still much confusion around the causal connection between autism and Hcy.

The present results showed elevated plasma Hcy level in autistic children which could be explained by interaction of genetic and acquired factors.

According to genetic factors, it is observed in autistic cases that higher frequency of methylenetetrahydrofolate reductase (MTHFR) allele variants favours the increase in plasma Hcy [19], reduced ileal absorption of B12 in conjunction with a confirmed intestinal pathology which includes diarrhea, abdominal pain, abdominal distention and food intolerance. Decreased B12 level impairs Hcy transmethylation to methionine resulting in increased Hcy levels [20]. So elevated Hcy contribute to autism pathogenesis [21].

Another explanation for increased homocysteine level in autistic children was that 5-MTHFR polymorphism was substantially more prevalent among autistic cases. During low folate status, the presence of the polymorphic form of this enzyme is associated with greater plasma Hcy concentrations than normal, as shown by many investigations. In addition, meta-analysis of secondary preventive studies shown that B vitamin intake reduced plasma Hcy levels [22].

Another genetic explanation for increased Hcy in autistic children is the theory of inadequate remethylation. This idea was validated by the neurofuzzy model's demonstration of synergistic interactions between MTRR A66G and MTHFR C677T that increased Hcy levels [23].

Another explanation for increased Hcy levels is magnesium shortage in autistic children that decreases methylation of Hcy and reduces adenosine triphosphate (ATP) levels leading to decreased genome transcription and increases the quantity of histones and noncoding RNA, and so slows regeneration of Hcy to methionine, resulting in an increase in Hcy level [24].

According to acquired causes of increased Hcy level in autistic cases is the low levels of folic acid and vitamin B12 observed in those children.

Low blood vitamin B12 levels in autistic individuals were interpreted to suggest elevated levels of oxidative stress and decreased DNA methylation, both of which may play essential roles in the pathogenesis of autism. Vitamin B12 deficiency may be linked to autism because of its potential to result in DNA hypomethylation, which in turn interrupts the normal CNS development [8].

Evidence indicates that autistic youngsters eat selectively. Inadequate nutrition, significant food selectivity, and/or gastrointestinal issues are associated with decreased dietary intake and micronutrient difficulties [25]. According to the studies conducted by Xia et al. [26], the majority of autistic children had inadequate intakes of vitamins B6, folate, A, C, and zinc according to their dietary preferences. The levels of Hcy and some autistic symptoms are inversely linked to the levels of folate and B vitamins, since decreasing vit B12 and folic acid hinder transmethylation of Hcy to methionine, resulting in higher Hcy levels.

An explanation for vitamin B12 and folic acid deficiency and subsequent elevated Hcy level is that children with autism eat selectively and often have feeding problems during meals contrast to normal individuals [27]. Pica, a condition in which children tend to be picky eaters, prefer to consume non-edible things, and avoid swallowing solid food, makes it difficult for parents of autistic children to feed their children. Although the underlying causes are poorly understood, some autistic children have motor impairments that lead to difficulty with eating and swallowing, while others have gastrointestinal issues (constipation or diarrhea). So deficits in many nutrients, including but not limited to vitamins B9, B6, and B12, have been linked to autism [27].

According to the findings of Ali et al., supplementation with vitamins B12,B6, and folate decreased blood Hcy levels [28].

Some studies propose supplementing autistic children's diets with critical nutrients such as vitamin B12, B6 and folate to improve their feeding issues [29]. This may enhance their hyperactivity, conduct, and receptive language abilities [30]. In addition, supplementation of folate to autistic children has been reported to regulate folate levels and alleviate autistic symptoms [31].

Supplementing the diets of autistic children with vitamins and minerals orally has been shown to improve their nutritional and metabolic condition (including lowering their blood levels of Hcy) and

alleviate some of the disorder's symptoms [30]. A substantial decrease in blood Hcy concentrations was seen in an open-label study of folic acid supplementation for autism, as measured by the autism treatment evaluation checklist (ATEC) [14].

5. CONCLUSIONS

Higher levels of Hcy and decreased concentrations of folic acid and vit B12 are seen in autistic children. As a result, Hcy has potential as a clinical biomarker for the early detection and treatment of autism in children.

6. STUDY LIMITATION

The current research has certain limitations, such as a limited sample size and the fact that it was conducted in just one location. Furthermore, identification of the probable cutoff prediction of Hcy in autistic individuals was absent.

CONSENT AND ETHICAL APPROVAL

The Research Ethical Committee, Faculty of Medicine, Tanta University approved this study. Signed consent was obtained from all enrolled cases or their parents

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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