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Original Communication

Mean Corpuscular Volume and Risk of Autism Spectrum Disorder

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Abstract

Background: Autism spectrum disorder (ASD) can be diagnosed as early as 18 months old, but more reliably after two years. Notably, no laboratory test exists to identify mothers at higher risk of having a child who will later be diagnosed with ASD or to identify at-risk infants before the manifestation of symptoms. One frequently described risk factor for neurodevelopmental disorders is vitamin B12 and folate deficiency, which results in macrocytic anemias. **Methods**: We evaluated whether increased mean corpuscular volume (MCV), an indicator of macrocytic anemias in the mother or child, is associated with increased odds of a subsequent ASD diagnosis. Maternal mean MCV (mMCV) was calculated from any value in the year before birth, and the mMCV for the child was calculated from any MCV value from birth until the end of the follow-up time. Odds ratios with 95% confidence intervals were estimated from logistic regression models. **Results**: A total of 3798 mothers (984 cases—ASD/2814 controls) and 9633 children (3206 cases—ASD/6427 controls) had at least one MCV value. The mMCV for the mother one year before birth was not associated with a later diagnosis of ASD in their children. In children, compared to the reference group (mMCV 76 femtoliters (fL)), an mMCV of 81 fL, 84 fL, and 91 fL was increased odds of ASD of 26%, 38%, and 32%, respectively. **Conclusion**: The MCV can be a potential inexpensive biomarker to identify a subset of children at risk of ASD or other developmental disorders; this exploratory study can inform larger studies to determine the clinical utility of MCV.

Keywords: autism spectrum disorder; folate; vitamin B12; mean corpuscular volume; neurodevelopmental disorders; nutrition

1. Introduction

Autism Spectrum Disorder (ASD) is a developmental disability that causes a wide array of deficits, including significant difficulty in social communication and interaction, repetitive behaviors, and restricted interests [1]. Over the last 20 years, the prevalence of ASD in the United States has been steadily increasing, with a new study published in April 2025 reporting a prevalence of 3.22‰ (one in 31) in 2022 [2], up from 23.0 per 1000 children in the surveillance year 2018 [3] and approximately 4.8 times higher than the first ADDM estimate between 2000 to 2002 at 6.7 per 1000 (one in 150) [2]. Currently, ASD can be diagnosed as early as 18 months of age and more reliably after two years based on signs and symptoms showing behavioral or developmental delays [4,5]. No laboratory test exists to identify mothers at higher risk of having a child later diagnosed with ASD or to identify at-risk infants before the manifestation of symptoms. Studies have assessed potential biomarkers for early diagnosis of ASD, specifically biomarkers of oxidative stress, which include folate and vitamin B12 (vB12) and its metabolites [6].

The multifactorial theory of ASD risk is broadly accepted and is complex, including genetic and environmental risk factors (e.g., maternal health, medications, and pollutants), to name a few [7–9]. One risk factor for neurodevelopmental disorders that has been described is maternal nutritional status, particularly during the critical period for developing ASD, in utero or early infancy [10–14]. Over the past six decades, case reports and literature reviews have reported developmental regression and negative consequences on brain development during infancy associated with maternal nutritional deficiencies, including folate and vB12 [11–15]. The use of medications that reduce folate or vB12 levels during pregnancy, such as valproic acid and metformin, as well as maternal hyperemesis [16], possibly a surrogate for maternal low nutritional status, have also been linked to increased risk of ASD [16–18]. The Methylenetetrahydrofolate-reductase (MTHFR) 677CT polymorphism, which impairs the efficiency of the one-carbon metabolic pathway and affects the ability to efficiently utilize folate and vB12 [19], is more common among children with ASD and their mothers [20] and may be associated with more severe ASD symptoms [21]. In addition, this polymorphism had shown to be a moderate predictor of ASD with synergistic interaction with hyperhomocysteinemia [22], a marker for both vB12 and/or folate deficiency.

In adults, screening for vB12 and folate deficiency is only recommended for high-risk individuals such as those with a vegetarian diet, with disorders of the intestines that may affect absorption, or those taking medications that cause drug-induced depletion [23,24]. While there are currently no published guidelines for antenatal screening for vB12 and folate deficiency, it has been recommended [24]. VB12 deficiency in women of childbearing age and pregnant women worldwide is fairly common [18]. In a multinational meta-analysis, the overall prevalence of vB12 insufficiency in pregnant women across all trimesters was estimated at 25% [25], and an Italian study found low vB12 levels during 48% of randomly selected pregnancies in healthy women [18].

In newborns, vB12 deficiency is most often of maternal origin [18]. Newborns are normally born with 25 ug of vB12 in the liver, which is thought to be enough for the first year of life [26]; however, fetal vB12 storage is significantly decreased in mothers with vB12 deficiency [27], especially those who are breastfed from deficient mothers [18]. Currently, in newborns, screening for vB12 and folate deficiencies is not the standard of care; infants are only screened after becoming symptomatic. While infants usually become symptomatic between 4–6 months of life, diagnosis is often not made until 6 months or later [18].

In this study, we aimed to find a laboratory biomarker that is routinely ordered during prenatal and well-baby visits. The complete blood count (CBC) is an inexpensive clinical laboratory test that can be routinely applied in clinical practice to screen for anemias in pregnancy [28]. The mean corpuscular volume (MCV), an index of the CBC, is used in conjunction with hemoglobin and hematocrit to assist with elucidating the type of anemia the patient may have based on the MCV value. In this study, we evaluated whether an increased mean MCV in the mother in the year before birth is associated with increased odds of having a child with ASD and whether the child's mean MCV is associated with increased odds of ASD diagnosis.

2. Materials and Methods

2.1 Participants

Data came from a previously created retrospective matched case-control study using the Military Healthcare System (MHS) Data Repository (MDR). It is comprised of inpatient and outpatient records of low to no-cost healthcare provided to over 4 million uniformed service members, military retirees, and dependent family members, who demographically generally mirror the U.S. population, with some over-representation of minority groups [29]. A previously validated methodology [30] which found two or more International Classification of Disease, Ninth Revision (ICD-9) diagnoses was consistent with chart review validation, was used to identify cases of ASD with ICD-9 diagnostic codes between ages 2 and 18 at two separate inpatient or outpatient encounters between 01 October 2000 and 30 September 2013. All cases and controls have a birth record in the Military Health System. Three controls were matched to each case by the child's date of birth, sex, and length of time the children were followed. For both cases and controls, children with only one diagnostic code were excluded, as were children diagnosed with ICD-9 code 330.8 (childhood disintegrative disorder), which includes Rett syndrome. The mothers of cases and controls were followed for at least one year before the child's birth. All children were followed from birth. Included children had at least six months of documented care before the first diagnosis of ASD and at least 6 months of care following their second ASD diagnosis. Controls had at least 12 months of follow-up. In this present study, mothers and children were included if either the mother or child had at least one MCV lab value in their medical records, which could only be extracted for care provided at Military Treatment Facilities. The matched format from the original study was not maintained.

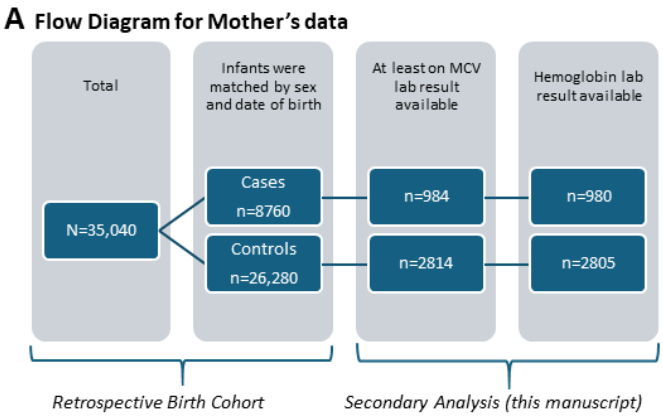
2.2 Analysis

2.2.1 Mean Corpuscular Volume Method of Detection

The MCV test is performed as part of the automated CBC in the laboratory. The whole blood is collected in a lavender top tube, avoiding hemolysis, and is transported to the hematology laboratory, in which the blood is passed through automated machines that calculate the red blood cell (RBC) indices. The MCV is a measure of the average volume, or size, of a single RBC and is therefore used in classifying anemias. MCV is expressed as femtoliters (fL) and is derived by dividing the hematocrit by the total RBC count: MCV = Hematocrit (%) $\times$ 10 $\div$ RBC (million/mm^3) [31,32]. VB12 and folate deficiencies result in megaloblastic macrocytic anemias with MCV values $\geq$100 fL, whereas iron deficiency anemias result in microcytic anemias, with MCV values $<$80 fL [23,33].

2.2.2 Statistical Analysis Method

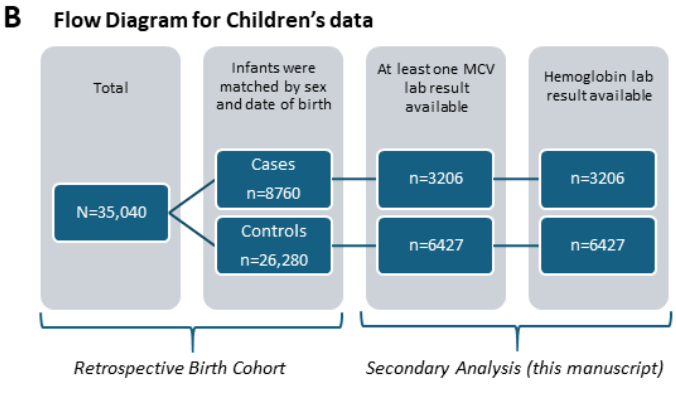
Maternal factors included in the analysis were the mother's age at the time of the child's birth, the total number of MCV results, mean MCV, and mean hemoglobin, calculated from any MCV or hemoglobin value at any point in the year before the child's birth. In our previous study, an MCV cutoff above 93 fL correctly identified abnormal methylmalonic acid (MMA) levels (defined as MMA $>$0.26 µmol/L and $<$0.376 µmol/L, the National Health and Nutrition Examination Survey (NHANES) published value at which MMA is abnormal but not a functional Vitamin B12 deficiency) [34] in pregnant women regardless of age or race with 81% sensitivity and 77% specificity [35]. Therefore, for mothers only, MCV was also analyzed as a dichotomous variable ($\geq$93 fL and $<$94 fL). Child factors included mean MCV and mean hemoglobin from birth until the end of the follow-up time, mean age at the time of lab values, and sex. Mean age was also analyzed as a categor-



Number of Labs Available	Cases (n, %)	Control (n, %)
1	220, 22%	640, 23%
2	199, 20%	643, 23%
3	202, 21%	692, 25%
4	167, 17%	395, 14%
5	83, 8%	196, 7%

Comparison between those with MCV data and those without MCV data

	Labs available	No labs available	*p* value
Mom's age at the child's birth Mean (SD)	28.69 (5.17)	29.37 (5.30)	<0.001
Age of ASD diagnosis of the child Median (min-max)	2.51 (2.44-2.56)	3.83 (3.77-3.88)	<0.001
Male Child n(%)	2,982 (79%)	24,998 (80%)	0.03



Number of Labs Available	Cases (n, %)	Control (n, %)
1	1762, 56%	4262, 66%
2	706, 22%	1222, 19%
3	334, 10%	497, 8%
4	158, 5%	203, 3%
5	84, 3%	107, 2%

Comparison between those with MCV data and those without MCV data

	Labs available	No labs available	*p* value
Mom's age at the child's birth Mean (SD)	28.87 (5.18)	29.45 (5.32)	<0.001
Age of ASD diagnosis of the child Median (min-max)	3.38 (3.13-3.44)	3.68 (3.60-3.76)	<0.001
Male Child n(%)	7,682 (80%)	20,298 (80%)	0.76

Fig. 1. Flow diagram of Mothers' and Children's data. MCV, mean corpuscular volume.

ical variable: ≤1-year-old, >1-year-old and ≤2, >2 years old and ≤3, >3 years old, and ≤4 and >4 years old. The oldest child's age that had an MCV lab value was 10.79 years old.

Multiple MCV and hemoglobin values were available for some of our subjects, and the age at which lab tests were performed varied among the children. Ideally, using the first lab value available or analyzing specific time frames would have been preferable, given that myelination is most significant in the earlier years of development. However, using a specific timeframe based on when the lab tests were performed (e.g., <6 months of age) would have drastically reduced the number of lab values available for analysis. Furthermore, using the first lab value available would result in data obtained at a wide range of ages. If these values change over time, the variability in the children's ages will introduce a source of meaningless information in the background. Therefore, mean MCV and mean hemoglobin were used to smooth out the data and make the values more comparable to each other, even though the lab values for different children were examined at different ages. Maternal mean MCV was calculated from any value one year before birth, and mean child MCV was calculated from any MCV value from birth until the end of follow-up time.

Logistic regression analyses were used to calculate the unadjusted and adjusted odds ratio with a 95% confidence interval (CI) of ASD risk. For maternal data, mean MCV was analyzed per a 10 fL increase in MCV (mean MCV10 fL). To meet the linearity assumption, children's mean MCV was optimally transformed by creating a categorical variable for children's MCV by quartile. Trends across the categories were also assessed. In children, sensitivity analyses were performed using the first available MCV value and also the last available MCV value for the analysis. Lastly, in the children's analysis, MCV was analyzed using low MCV, normal MCV, and high MCV groups. Normal MCV was defined separately for <2-year-olds (73 fL to 85 fL) and ≥2–6-year-olds (75 fL to 86 fL) based on normal MCV clinical guidelines for children [36]. Children >6 years old were not included in this analysis since their clinically normal MCV range differs. Sensitivity analyses were performed to assess if the total number of MCV labs available differed between cases and controls. Interaction and confounding were assessed between MCV and all maternal and child factors listed above. Two-sample *t*-tests for mean differences, Wilcoxon rank sum for medians, and two-tailed Chi-square test for comparison of proportions were used to compare maternal and child factors between ASD cases and controls as appropriate. The Uniformed Services University institutional review board approved this study (Risk Factors and Co-Morbid Conditions, #20-10602). Data analyses were performed using Stata IC 16 statistical software (Stata Corp LLC, College Station, TX, USA).

3. Results

3.1 Flow Diagram of Mothers' and Children's Data

Of the 8760 mothers of cases, 984 (11.2%) had at least one MCV value, and of the 26,280 control mothers, 2814 (10.7%) had at least one MCV value. Of the mothers with at least one MCV lab value, four mothers of children with ASD and nine mothers from the control group did not have a hemoglobin lab value available. 3206 (36.6%) children with ASD and 6427 (25%) controls had at least one MCV value. These proportions represent a percentage of the original cases and controls from the 1:3 matched birth cohort. All children with an MCV value had at least one hemoglobin value. Mothers who had at least one MCV lab value were younger (28.69 *vs.* 29.37, $p < 0.001$), and the age of ASD diagnosis in the children was lower (2.51 *vs.* 3.83, $p < 0.001$) compared to mothers without an MCV lab. Children with ASD who had at least one lab value were diagnosed at a younger age (3.38 *vs.* 3.68, $p < 0.001$) and had younger mothers at their birth (28.87 *vs.* 29.45, $p < 0.001$). In both mothers' and children's data, 79–80% of the children in both groups, with and without labs, were male. 22% of the mothers in the case group had one MCV lab data point, and 23% of mothers in the control group had one MCV lab data point. 56% of children with ASD had one MCV data point, and 66% of the controls had one MCV data point (Fig. 1).

3.2 Descriptive Data of Mothers and Children With Available MCV Data in Our Study

The mean MCV for mothers of children with ASD was not significantly different from mothers of children without ASD (88.36 *vs.* 88.64, $p = 0.15$). The mean age for mothers at birth was slightly higher in moms with children with ASD compared to those with children without ASD (28.06 *vs.* 28.56, $p = 0.009$). The median age of ASD diagnosis of the child, in the mother's data, was 2.51 years old. No differences in the mean hemoglobin levels were found between the case and control groups (12.23 *vs.* 12.28 g/dL, $p = 0.25$), and when stratified by mean MCV levels, no differences were found. The mothers in the case group had similar mean hematocrit levels to the mothers in the control group (35.81 *vs.* 36.00, $p = 0.07$). When stratified by mean MCV levels, in mothers who had a mean MCV between ≥80 and <100 fL/L, the case group had lower hematocrit levels than the control group (35.95 *vs.* 36.17, $p = 0.04$). The mean gestational age (months pregnant) at the time of MCV test for mothers of children with ASD and mothers without children with ASD was 3 months ($p = 0.84$) (Table 1).

The median age at the time of the lab test for children with ASD was higher than those without ASD (3.90 *vs.* 3.57, $p < 0.001$). The median age of ASD diagnosis in the child study was 3.38 years old. The mean MCV for children with ASD was higher than those without ASD (83.51 *vs.* 82.67, $p < 0.002$). In the last quartile, the mean MCV in

Table 1. Descriptive data of mothers and children with available MCV data in our study.

Mothers				
	All included†	Cases with ASD*	Controls, no ASD*	*p* value
	N = 3798	N = 984	N = 2814	
Mother's age at child's birth (years)				
Mean (SD)	28.43 (5.19)	28.06 (5.24)	28.56 (5.17)	0.009
MCV (fL)				
Mean (SD)	88.57 (5.18)	88.36 (5.29)	88.64 (5.14)	0.15
Gestational age of mother at time of MCV result (months)				
Mean (SD)	3.07 (3.28)	3.09 (3.28)	3.07 (3.27)	0.84
Age of child at ASD diagnosis				
Median (range)	–	2.51 (2.44–2.56)	–	
	All included†	Cases with ASD*	Controls, no ASD*	*p* value
	N = 3785	N = 980	N = 2805	
Hemoglobin (g/dL)				
Mean (SD)	12.27 (1.05)	12.23 (1.07)	12.28 (1.04)	0.25
Hemoglobin/MCV Level				
Mean MCV <80 fL	10.99 (1.31)	11.18 (1.59)	10.92 (1.18)	0.19
Mean MCV ≥80 to 100 fL	12.35 (0.98)	12.31 (0.99)	12.36 (0.98)	0.17
Mean MCV ≥100 fL	12.11 (1.10)	11.46 (1.07)	12.34 (1.04)	0.09
	All included†	Cases with ASD*	Controls, no ASD*	*p* value
	N = 3773	N = 973	N = 2800	
Hematocrit				
Mean (SD)	35.95 (2.88)	35.81 (2.93)	36.00 (2.85)	0.07
Hematocrit/MCV Level				
Mean MCV <80 fL	33.33 (3.70)	33.59 (4.40)	33.23 (3.43)	0.54
Mean MCV ≥80 to 100 fL	36.11 (2.74)	35.95 (2.76)	36.17 (2.73)	0.04
Mean MCV ≥100 fL	35.75 (2.88)	34.72 (1.51)	36.05 (3.14)	0.38
Number of MCV labs available				
Mean (SD)	3.01 (2.24)	3.23 (2.18)	3.04 (2.26)	0.02
Children				
	All included†	Cases with ASD*	Controls, no ASD*	*p* value
	N = 9633	N = 3206	N = 6427	
Mean child's age (years)				
Median (range)	3.70 (0.00–10.79)	3.90 (0.00–10.68)	3.57 (0.00–10.79)	<0.001
Mean age in categories (years)		N Median (range)		
≤1-year-old	N = 1170 0.34 (0.00–1.00)	N = 298 0.33 (0–1.00)	N = 872 0.34 (0.00–1.00)	<0.87
>1 to ≤2 years old	N = 1820 1.30 (1.00–2.00)	N = 507 1.42 (1.00–2.00)	N = 1313 1.26 (1.00–2.00)	<0.001
>2 to ≤3 years old	N = 1093 2.46 (2.00–3.00)	N = 426 2.47 (2.00–3.00)	N = 667 2.45 (2.00–3.00)	0.09
>3 to ≤4 years old	N = 1048 3.47 (3.00–4.00)	N = 414 3.47 (3.00–3.99)	N = 634 3.48 (3.00–4.00)	0.48
>4 years old	N = 4502 6.13 (4.00–10.79)	N = 1561 6.10 (4.00–10.68)	N = 2941 6.14 (4.00–10.79)	0.13
Age of child at ASD diagnosis				
Median (min–max)	–	3.38 (2.00–10.22)	–	
Male child				
N (%)	7677 (80%)	2526 (79%)	5151 (80%)	0.12

Table 1. Continued.

Children				
	All included†	Cases with ASD*	Controls, no ASD*	*p* value
	N = 9633	N = 3206	N = 6427	
MCV (fL)				
Total Mean (SD)	82.83 (7.03)	83.15 (6.65)	82.67 (7.21)	<0.002
By Quartiles				
(Q1) N = 2402	76 (3.86)	76 (4.54)	75.65 (3.58)	0.96
(Q2) N = 2408	80.68 (0.87)	80.72 (0.86)	80.67 (0.88)	0.17
(Q3) N = 2383	84 (0.88)	83.61 (0.88)	83.63 (0.88)	0.69
(Q4) N = 2440	91 (7.24)	90.48 (6.48)	91.68 (7.61)	<0.001
	All included†	Cases with ASD*	Controls, no ASD*	*p* value
	N = 9629	N = 3206	N = 6423	
Hemoglobin (g/dL)				
Mean (SD)	12.65 (1.42)	12.73 (1.37)	12.61 (1.44)	<0.001
Hemoglobin/MCV Level				
Mean MCV <80 fL	12.10 (0.98)	12.19 (0.96)	12.06 (0.98)	0.001
Mean MCV ≥80 to 100 fL	12.69 (1.15)	12.76 (1.10)	12.65 (1.17)	<0.001
Mean MCV ≥100 fL	16.42 (2.41)	16.71 (2.80)	16.31 (2.26)	0.17
	All included†	Cases with ASD*	Controls, no ASD*	*p* value
	N = 9630	N = 3206	N = 6424	
Hematocrit				
Mean (SD)	37.03 (4.18)	37.20 (4.00)	36.94 (4.26)	0.004
Hematocrit/MCV Level				
Mean MCV <80 fL	35.48 (2.87)	35.69 (2.73)	35.40 (2.91)	0.01
Mean MCV ≥80 to 100 fL	37.11 (3.39)	37.26 (3.22)	37.03 (3.48)	0.008
Mean MCV ≥100 fL	48.00 (7.33)	48.92 (8.36)	47.67 (6.92)	0.15
Number of MCV labs available				
Mean (SD)	1.90 (3.29)	2.24 (4.32)	1.74 (2.62)	<0.001

* ASD, Autism Spectrum Disorder.

† at least one MCV value.

Table 2. Unadjusted and adjusted odds of ASD by maternal MCV and maternal factors.

Model 1: Unadjusted odds of ASD by maternal MCV and maternal factors *†				
N	Maternal factors	Odds ratio	95% Confidence interval	*p*
3798	Mean MCV (fL)	0.99	0.98–1.00	0.145
3798	Mean MCV (fL) Cutoff 93	0.91	0.76–1.11	0.36
3798	Maternal age at child's birth (years)	0.98	0.97–1.00	0.009
3785	Mean Hemoglobin (g/dL)	0.96	0.90–1.02	0.251
3798	Number of MCV labs per mother	1.04	1.01–1.07	0.02
Model 2: Adjusted odds of ASD by maternal MCV and maternal factors*				
N	Maternal factors	Odds ratio	95% Confidence interval	*p*
	Mean MCV (fL)	0.99	0.98–1.01	0.24
9629	Maternal age at child's birth (years)	0.98	0.97–1.00	0.02
	Number of MCV labs per mother	1.04	1.01–1.07	0.02

* Number of labs available is not a maternal factor; however, added to the model to adjust for confounding; odds ratios adjusted for other independent variables in the table.

† Model 1 Odds ratios are run separately for each independent variable in the table.

children with ASD was lower than in children without ASD, (90.48 *vs.* 91.68, $p < 0.001$). Mean hemoglobin was higher in children with ASD than controls and remained higher in children with a mean MCV ≤100 g/dL (12.73 *vs.* 12.61,

$p < 0.001$). Similarly, mean hematocrit was also higher in children with ASD and remained high in children with mean MCV <100 g/dL (37.20 *vs.* 36.94, $p = 0.004$). These results are summarized in Table 1.

Mothers of children with ASD had a slightly higher mean number of MCV lab data values available compared to the controls (3.23 *vs.* 3.04, $p = 0.02$). Similarly, the mean number of MCV labs available for children with ASD was also slightly higher than for the non-ASD group (2.24 *vs.* 1.74, $p < 0.001$) (Table 1).

3.3 Unadjusted and Adjusted Odds of ASD by Mean Maternal MCV and Maternal Factors

We did not find an association between mean maternal MCV, maternal factors, and ASD. The mother's age was associated with decreased odds of ASD (adjusted odds ratio (aOR) 0.98%, 95% CI [0.97–1.00]). An increase in the mean number of labs available was associated with 4% increased odds of ASD. These results are summarized in Table 2.

3.4 Unadjusted and Adjusted Odds of ASD by Mean Child MCV and Child Factors

Overall, there were significant interactions between child MCV and sex ($p = 0.02$) and between MCV and categorical age ($p = 0.002$); therefore, stratified odds ratios for MCV were reported. The mean MCV in quartile 2 (Q2), quartile 3 (Q3) and quartile 4 (Q4) was 81 fL, 84 fL and 91 fL. The mean MCV in the referent group, quartile 1 (Q1), was 76 fL (Table 3A,3B). In children, compared to the referent group, Q2, Q3, and Q4 were associated with 26%, 38%, and 32% increased odds of ASD, (aOR 1.26, 95% CI [1.11–1.43], aOR 1.38, 95% CI [1.22–1.57] and aOR 1.32, 95% CI [1.15–1.51]). When stratified by age and sex, compared to the referent group (mean MCV 76 fL), Q4 was associated with 83% and 96% increased odds of ASD in boys ≤1-year-old and the >3 years old to ≤4 years old age group (OR 1.83, 95% CI [1.20–2.82] and OR 1.96, 95% CI [1.24–3.10]), respectively. Among boys in the >1-year-old to ≤2 years old and >2 years old to ≤3 years old, compared to the referent group, Q4 was associated with 2.35- and 2.00-times higher odds of ASD (OR 2.35, 95% CI [1.57–3.51] and OR 2.00, 95% CI [1.18–3.38]), respectively. In boys between the ages of >2 years old to ≤3 years old, compared to the referent group, Q2 was associated with 44% increased odds of ASD (OR 1.44, 95% CI [1.03–2.01]). Compared to the referent group, Q3 was associated with 2.05 times higher odds of ASD in the >1-year-old to ≤2 years old and 63% increased odds of ASD in the >3 years old to ≤4 years old (OR 2.05, 95% CI [1.48–2.83] and OR 1.63, 95% CI [1.10–2.44]) respectively. Compared to the referent group, we did not find an association between mean MCV and ASD for Q2 in boys ≤1-year-old, >1-year-old to ≤2 years old, >3 years old to ≤4 years old and >4 years old (OR 1.60, 95% CI [0.87–2.92], OR 1.29, 95% CI [0.97–1.72], OR 1.14, 95% CI [0.77–1.66] and OR 1.07, 95% CI [0.85–1.33]), for Q3 in boys ≤1-year-old, >2 years old to ≤3 years old and >4 years old (OR 1.75, 95% CI [0.94–3.23], OR 1.43, 95% CI [0.97–2.10] and OR 1.14, 95% CI [0.93–1.14]) and Q4 in boys >4 years old (OR 1.17, 95% CI [0.94–1.45]).

Among female children compared to the referent group, Q4 is associated with a 4.02 and 2.05 times higher odds of ASD for those >1-year-old to ≤2 years old and >4 years old (OR 4.02, 95% CI [2.03–7.95] and OR 2.05, 95% CI [1.28–3.29]), respectively and Q2 was associated with a 4.58 times higher odds of ASD in the <1-year-old age group (OR 4.58, 95% CI [1.08–19.38]) compared to the referent group. Compared to the referent group, we did not find an association between mean MCV and ASD for Q2 in girls >1-year-old to ≤2 years old, >2 years old to ≤3 years old, >3 years old to ≤4 years old and >4 years old (OR 1.39, 95% CI [0.78–2.46], OR 0.85, 95% CI [0.44–1.69], OR 0.84, 95% CI [0.39–1.81] and OR 1.41, 95% CI [0.84–2.37]), for Q3 in girls ≤1-year-old, >1-year-old to ≤2 years old, >2 years old to ≤3 years old, >3 years old to ≤4 years old and >4 years old (OR 1.67, 95% CI [0.35–7.84], OR 1.82, 95% CI [0.98–3.36], OR 1.18, 95% CI [0.59–2.34], OR 1.08, 95% CI [0.51–2.29], and OR 1.34, 95% CI [0.82–2.19]) and for Q4 in girls ≤1-year-old, >2 years old to ≤3 years old, >3 years old to ≤4 years old (OR 3.09, 95% CI [0.87–10.91], OR 1.87, 95% CI [0.78–4.49], and OR 1.42, 95% CI [0.63–3.22]). An overall trend was demonstrated across quartiles. These results are summarized in Table 3A,3B.

3.5 Sensitivity Analysis Using First Available MCV Value and Last Available MCV Value in Children

The sensitivity analysis using the first available MCV value and last available MCV for the children demonstrated similar findings as the model with the MCV quartiles, that is, an overall increased odds of ASD with increasing MCV values (**Supplementary Table 1**). Similarly, the unadjusted odds of ASD when three groups were utilized (low MCV, normal MCV, and high MCV) demonstrated increased odds of ASD in the >1-year-old to ≤2 years old age group in the high MCV group (**Supplementary Table 2**). Additionally, Lowess plots illustrate the relationship between MCV and ASD and the overall trend (**Supplementary Fig. 1**).

4. Discussion

Based on previous research demonstrating the detrimental effects of vB12 and folate deficiency on neurodevelopment, we hypothesized that maternal and child MCV, a marker of inadequate vB12 and folate, predicted a later diagnosis of ASD in the child. In unadjusted and adjusted analyses, mean MCV in the mother one year before birth was not associated with a later diagnosis of ASD in their children. In children, there was a broader overall increased odds of ASD, consistent across several statistical models. Compared to the referent group (mean MCV 76 fL), a mean MCV of 81 fL (Q2), 84 fL (Q3), and 91 fL (Q4) was asso-

Table 3A. Adjusted odds of ASD by mean child MCV and child factors.

Model 1: Adjusted odds of ASD by mean child MCV quartile and child factors*†													
		Quartile 1			Quartile 2			Quartile 3			Quartile 4		
		Mean MCV 76			Mean MCV 81			Mean MCV 84			Mean MCV 91		
N	Child factors	Odds ratio	95% Confidence interval	*p*	Odds ratio	95% Confidence interval	*p*	Odds ratio	95% Confidence interval	*p*	Odds ratio	95% Confidence interval	*p*
9629	Mean MCV (quartiles) (fL)		Reference		1.26	1.11–1.43	<0.001	1.38	1.22–1.57	<0.001	1.32	1.15–1.51	<0.001
	Mean Hemoglobin (g/dL)				1.04	1.01–1.07	0.01	n/a	n/a	n/a	n/a	n/a	n/a
	Number of MCV labs available				1.06	1.01–1.07	<0.001	n/a	n/a	n/a	n/a	n/a	n/a
	Mean age (years)				1.02	1.01–1.04							

Table 3B. Unadjusted odds of ASD by mean child MCV and child factors.

Model 2: Unadjusted odds of ASD by mean child MCV quartile stratified by child's age at the time of MCV lab study and sex*														
		Quartile 1			Quartile 2			Quartile 3			Quartile 4			
		Mean MCV 76			Mean MCV 81			Mean MCV 84			Mean MCV 91			
N	Age interval	Odds ratio	95% Confidence interval	*p*	Odds ratio	95% Confidence interval	*p*	Odds ratio	95% Confidence interval	*p*	Odds ratio	95% Confidence interval	*p*	Trend *p*††
Males														
957	≤1-year-old		Reference		1.60	0.87–2.92	0.13	1.75	0.94–3.23	0.07	1.83	1.20–2.82	0.005	0.008
1431	> 1-year-old ≤ 2				1.29	0.97–1.72	0.08	2.05	1.48–2.83	<0.001	2.35	1.57–3.51	<0.001	<0.001
833	> 2 years old ≤ 3				1.44	1.03–2.01	0.03	1.43	0.97–2.10	0.07	2.00	1.18–3.38	0.009	0.005
820	> 3 years old ≤ 4				1.14	0.77–1.66	0.51	1.63	1.10–2.44	0.02	1.96	1.24–3.10	0.004	0.001
3635	>4 years old				1.07	0.85–1.33	0.58	1.14	0.93–1.41	0.21	1.17	0.94–1.45	0.17	0.27
Females														
213	≤1-year-old				4.58	1.08–19.38	0.04	1.67	0.35–7.84	0.65	3.09	0.87–10.91	0.08	0.260
389	> 1-year-old ≤ 2				1.39	0.78–2.46	0.26	1.82	0.98–3.36	0.06	4.02	2.03–7.95	<0.001	<0.001
260	> 2 years old ≤ 3				0.85	0.44–1.69	0.66	1.18	0.59–2.34	0.64	1.87	0.78–4.49	0.16	0.15
228	> 3 years old ≤ 4				0.84	0.39–1.81	0.66	1.08	0.51–2.29	0.84	1.42	0.63–3.22	0.40	0.29
867	>4 years old				1.41	0.84–2.37	0.19	1.34	0.82–2.19	0.24	2.05	1.28–3.29	0.003	0.002

* Odds ratios are compared to the referent group with mean MCV of 76.

† Odds ratios adjusted for other independent variables in the table.

†† Model for trend *p* included categorical mean MCV as a continuous variable.

ciated with 26%, 38%, and 32% increased odds of ASD; when stratified by sex and age, compared to the referent group, the odds increased in males and females in some age groups.

Folate and vB12 are essential nutrients for myelination during in-utero and post-natal neurologic development [11,37,38]. Brain development begins prenatally, whereas central nervous system myelination begins as early as mid-gestation and continues through school age, with the most rapid period of brain growth during the first two years of life [11]. Micronutrients, specifically vB12, are needed to form myelin, the protective covering of nerve cells; brain myelination, which is greatest from mid-gestation to age two, can be affected by vB12 deficiency. Disruptions in myelination slow down nerve conduction, such as in the auditory and visual systems, which can lead to learning and social interaction difficulties. Furthermore, retardation of brain myelination in infancy can lead to delayed acquisition of cognitive skills and brain atrophy leading to regression of skills [11,39].

Even though studies have demonstrated the potential benefits of prenatal folic acid supplementation to reduce autism risk, a modifiable risk factor for autism [40,41], the fortification of foods with folic acid beginning in 1998 has not thus far been associated with a population-level decrease in ASD. On the contrary, as mentioned earlier, the incidence of ASD has increased in the post-fortification era. VB12 and folate have a close metabolic relationship in the complex interdependent folate-methylation cycle processes [40,42]. VB12, along with folate, is an important cofactor for reactions in the folate and methylation cycle; in the methionine synthase reaction, which catalyzes the methylation of homocysteine (HCY) to methionine, and in the demethylation of methyltetrahydrofolate (MTHF) producing tetrahydrofolate (THF) in the one-carbon metabolism [40–43]. The impairment of the MTHF demethylation in vB12 deficiency leads to a folate trap; that is, MTHF is no longer able to convert to the active form, THF, a transporter of the one-carbon unit to the site of DNA synthesis. In addition, the attenuation of MTHFR enzyme activity due to the 677CT polymorphism, which is strongly associated with ASD [20] and may be associated with severe ASD symptoms [21], further impairs the efficiency of this one-carbon cycle, leading to impaired DNA synthesis and gene expression [40]. Urine samples of children with ASD, showing simultaneous vB12, vitamin B6, and B9 deficiencies, also demonstrated increased concentrations of 5-MTHF, which leads to lower availability of methyl donors, justifying the well-known reduction of protein and DNA methylation reported in children with ASD [44].

In this study, in children, there was an overall increased odds of ASD with increasing MCV values compared to the referent group, and somewhat notable in the younger children. These findings may represent early vB12 levels dropping exponentially during the greatest period of myelination. Newborns who are breastfed by deficient mothers are at risk of developing a vB12 deficiency, usually becoming symptomatic between 4–6 months of life but diagnosed in the later portion of the first year of life [18]. Perhaps these hematologic changes demonstrate the slow depletion of their vB12 as they move into the second year of life. These children, may represent a subset of children born from vB12 deficient mothers leaving the infants with inadequate liver stores from not being adequately supported in utero [45] or breastfeeding and born to either vB12 deficient mothers or replete mothers with the MTHFR polymorphism who cannot keep up the micronutrient levels required after birth during the rapid myelination and growth period immediately after birth and through the first year of life. It is interesting to note that the median age of ASD diagnosis in the children was 3.38 years old, possibly indicating the slowing of neurodevelopment the two years prior.

This study demonstrated an interaction between sex and mean MCV on ASD status. For males with a mean MCV of 91 fL (Q4), we observed increased odds of ASD in all age groups $\leq$4 years old, whereas for the females, the increased odds were specifically in the $>$1-year-old to 2 years old and the $>$4 years old; these findings underscore the possible etiological differences between sexes. While the etiology of ASD remains unknown, existing epidemiologic evidence suggests ASD prevalence varies with sex. In a recent study, moderate folate supplementation of pregnant and lactating mice led to hyperactivity in both male and female pups; however, the folate supplementation led to a downregulation of MTHFR, producing a methyl metabolism disruption in the brain, particularly in the male pups [46]. Females less than one year old and with a mean MCV of 81 fL (Q2) had 4.58 times higher odds of having ASD as compared to the reference group; however, this was not observed in males. The disparities between males and females may also have a genetic component. Females with ASD have a 300% increase in harmful copy-number variants than males with ASD, suggesting that females may require a greater genetic hit than males [47]. In addition, normal values for hematologic parameters change dramatically in the first 6 months of life; therefore, it is difficult to interpret these findings, albeit it is feasible that these females had early lab work due to other significant developmental disabilities observed shortly after birth. The increased odds of ASD, in females with a mean MCV of 91 fL (Q4) greater than 4 years of age, compared to the referent group (mean MCV of 76), may be reverse causation; restrictive eating patterns commonly seen in children with ASD [48] with possible sex differences noted in previous studies, particularly pronounced in females [49,50]. Finally, these differences may reflect true variability in susceptibility but also could be, in part, an artifact due to the likelihood of diagnosis due to the lower sensitivity of screening tools for ASD in girls compared to boys. The sensitivity of the screening tools that use the current diagnostic criteria used to diag-

nose ASD may be missing females because of differences in the clinical presentation in females [51].

One would have expected our analysis to also demonstrate MCV changes in the mothers be associated with increased odds of ASD in their children, however, it had been well described that vB12 levels in the low normal range alone don't exclude functional deficiency [18] and perhaps this also extends to lack of significant hematologic changes. The utility of MCV during pregnancy remains ambiguous. It has been suggested that the increase in MCV during pregnancy is not suggestive of vB12 and/or folate deficiencies but rather a measurement of increased red blood cell production to meet the demands of pregnancy (immature RBCs are larger) [52]. Conversely, a study of women in the first trimester of pregnancy reported that first-trimester folate-vB12 status interactions were associated with plasma methylmalonic acid (MMA) and MCV throughout pregnancy. They found a greater difference in MCV between elevated and non-elevated folate status in those in the top quartile of MMA values compared to those in the lower quartile of MMA values. The same study also reported that women with elevated folate had higher MMA levels and sub-optimal vB12 levels [53]. Perhaps the abnormal MMA range is representative of sub-clinical vB12 deficiency, which is rapidly brought on by the increasing demand for micronutrients during pregnancy [43], with one study reporting higher levels of MMA in the third trimester [54]. Lastly, even though we adjusted for hemoglobin in our analysis, women with macrocytic anemia, coexisting with other deficiencies such as iron deficiency, commonly seen in pregnancy, may have hematological changes caused by vB12 or folate deficiency, however, masked by the hematological changes in iron deficiency (microcytic anemia) [18].

Inconsistent with previous research [55], increased maternal age was associated with statistically significantly decreased odds of having a child with ASD. This finding may be a surrogate for socioeconomic status. In the military, older age may be a representation of higher rank and, therefore, income, which may translate to better access to and compliance with prenatal care. This finding was also similar to the one reported in the primary retrospective matched case-cohort study [16], making it less likely to be an indicator of selection bias due to breaking the match in this secondary analysis. Furthermore, to control for selection bias in the secondary analysis, we controlled for match variables, date of birth, and sex in the models.

Our study had several limitations, which may have restricted our ability to study the utility of the MCV value during pregnancy and in early childhood. First, the stratification of our data resulted in smaller strata across the age groups, which may have over-exaggerated the odds ratios or reduced the power to detect significant associations in the smaller strata. Second, the limited number of labs available may have introduced some sort of information bias. It had been reported that there may be MCV differences throughout pregnancy [53]; however, due to the limited lab values available, we were not able to assess differences across trimesters. If mothers received care in civilian facilities, we did not have access to that laboratory data, and if pregnancy and birth occurred before 2009, laboratory data were not available. Of note, the mothers of children later diagnosed with ASD and children with ASD had a slightly higher mean number of labs, which may be a marker of the mother's and child's health status. This may also represent confounding by indication bias; that is, the differences in the mean MCV observed could be attributed to eating preference, restrictive eating patterns, and issues with feeding in infancy that are common for children with ASD. To date, one case of vB12 induced megaloblastic anemia had been reported in a pediatric patient with ASD with a chronically unbalanced diet [56]. Future studies can inform this by assessing the first lab for those under 6 months of age. Lastly, we did not have data on parity or socioeconomic status.

Future studies should assess if the predictive MCV value differs throughout the pregnancy. In addition, assessing pre-pregnancy MCV values can help further understand if a change in the MCV from the mother's baseline is associated with greater odds of having a child with ASD. Further consideration should be given to studying pregnant women with the MTHFR allele polymorphisms to identify an algorithm for screening women who may be at risk for vB12 or folate abnormalities. This analysis should also be extended to the children. Future studies of children with a larger population are warranted to further delineate any differences in predictive MCV values across the first few years of life. Additionally, consideration should be given to studying children with the MTHFR allele polymorphisms to identify an algorithm for screening children who may be at risk for inadequate levels of vB12 and folate and evaluating MCV in the children during the most significant time of myelination (birth to age 2), as this would also be a period for the greatest need for vB12. Lastly, if future studies also identify that a child's increase in MCV is associated with increased odds of ASD diagnosis, further investigation of the underlying mechanisms should be explored since research had suggested that children with ASD often have significantly lower MCV levels compared to typically developing children attributed to possible iron deficiency however not all studies agree with this finding [57,58].

5. Conclusion

This study reported data that is suggestive of an association between vB12 and folate levels in children, as represented by the association of increasing MCV values within the first few years of life and ASD. There exist promising studies suggesting that the treatment of vB12 at an early age may ameliorate clinical ASD symptoms [59]. If further studies confirm the association between inadequate levels of vB12 and ASD, this may inform public health clini-

cal practice guidelines for vB12 and folate supplementation. Perhaps the MCV, a globally accessible, inexpensive biomarker can be utilized to trigger further evaluation of vB12 or folate deficiencies, to identify a subset of children at risk for ASD or other developmental disorders and lead to early treatment; this exploratory study can inform future larger studies to determine the clinical utility of MCV in early ASD detection, a possible pathway to early intervention to decrease ASD morbidity in the population.

Availability of Data and Materials

A data sharing agreement must be executed with the original data owner to obtain the data with appropriate permissions.

Author Contributions

ÜSF conceptualized and designed the study, carried out the analyses, drafted and revised the manuscript, and critically reviewed the analysis, results, and manuscript for important intellectual content; AIS, CO, EH-G, WY, and AS assisted with the analysis and design of the study and critically reviewed the analyses, results, and manuscript for important intellectual content; WY and AS contributed to data interpretation. AS additionally secured the data.

All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The Uniformed Services University institutional review board approved this study (Risk Factors and Co-Morbid Conditions, #20-10602).

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Conflict of Interest

The contents of this article are solely the responsibility of the authors and do not necessarily represent the official views of the Uniformed Services University, the U.S. Air Force, the U.S. Public Health Service Commissioned Corps, the United States Department of Health and Human Services, the U.S. Department of Defense or the U.S. Government. None of the authors have any conflicts of interest to disclose.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/IJVNR26726.

References

[1] American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders: Diagnostic and Statistical Manual of Mental Disorders. 5th Edition ed. American Psychiatric Association Publishing. Arlington, VA. 2013.

[2] Shaw KA, Williams S, Patrick ME, Valencia-Prado M, Durkin MS, Howerton EM, *et al*. Prevalence and Early Identification of Autism Spectrum Disorder Among Children Aged 4 and 8 Years - Autism and Developmental Disabilities Monitoring Network, 16 Sites, United States, 2022. Morbidity and Mortality Weekly Report. Surveillance Summaries. 2025; 74: 1-22. https://doi.org/10.15585/mmwr.ss7402a1.

[3] Maenner MJ, Shaw KA, Bakian AV, Bilder DA, Durkin MS, Esler A, *et al*. Prevalence and Characteristics of Autism Spectrum Disorder Among Children Aged 8 Years - Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2018. Morbidity and Mortality Weekly Report. Surveillance Summaries. 2021; 70: 1–16. https://doi.org/10.15585/mmwr.ss7011a1.

[4] Lord C, Risi S, DiLavore PS, Shulman C, Thurm A, Pickles A. Autism from 2 to 9 years of age. Archives of General Psychiatry. 2006; 63: 694–701. https://doi.org/10.1001/archpsyc.63.6.694.

[5] Okoye C, Obialo-Ibeawuchi CM, Obajeun OA, Sarwar S, Tawfik C, Waleed MS, *et al*. Early Diagnosis of Autism Spectrum Disorder: A Review and Analysis of the Risks and Benefits. Cureus. 2023; 15: e43226. https://doi.org/10.7759/cureus.43226.

[6] Liu X, Lin J, Zhang H, Khan NU, Zhang J, Tang X, *et al*. Oxidative Stress in Autism Spectrum Disorder-Current Progress of Mechanisms and Biomarkers. Frontiers in Psychiatry. 2022; 13: 813304. https://doi.org/10.3389/fpsyt.2022.813304.

[7] Hertz-Picciotto I, Schmidt RJ, Krakowiak P. Understanding environmental contributions to autism: Causal concepts and the state of science. Autism Research. 2018; 11: 554–586. https://doi.org/10.1002/aur.1938.

[8] Alam R, Abdolmaleky HM, Zhou JR. Microbiome, inflammation, epigenetic alterations, and mental diseases. American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics. 2017; 174: 651–660. https://doi.org/10.1002/ajmg.b.32567.

[9] Waye MMY, Cheng HY. Genetics and epigenetics of autism: A Review. Psychiatry and Clinical Neurosciences. 2018; 72: 228–244. https://doi.org/10.1111/pcn.12606.

[10] Gardener H, Spiegelman D, Buka SL. Perinatal and neonatal risk factors for autism: a comprehensive meta-analysis. Pediatrics. 2011; 128: 344–355. https://doi.org/10.1542/peds.2010-1036.

[11] Black MM. Effects of vitamin B12 and folate deficiency on brain development in children. Food and Nutrition Bulletin. 2008; 29: S126–S131. https://doi.org/10.1177/15648265080292S117.

[12] Sadowitz PD, Livingston A, Cavanaugh RM. Developmental regression as an early manifestation of vitamin B12 deficiency. Clinical Pediatrics. 1986; 25: 369–371. https://doi.org/10.1177/000992288602500708.

[13] Agrawal S, Nathani S. Neuro-regression in vitamin B12 deficiency. BMJ Case Reports. 2009; 2009: bcr06.2008.0235. https://doi.org/10.1136/bcr.06.2008.0235.

[14] Jadhav M, Webb JK, Vaishnava S, Baker SJ. Vitamin B12 deficiency in Indian infants. A clinical syndrome. Lancet. 1962; 2: 903–907. https://doi.org/10.1016/s0140-6736(62)90682-7.

[15] Baxter AJ, Brugha TS, Erskine HE, Scheurer RW, Vos T, Scott JG. The epidemiology and global burden of autism spectrum disorders. Psychological Medicine. 2015; 45: 601–613. https://doi.org/10.1017/S003329171400172X.

[16] Hisle-Gorman E, Susi A, Stokes T, Gorman G, Erdie-Lalena C, Nylund CM. Prenatal, perinatal, and neonatal risk factors of autism spectrum disorder. Pediatric Research. 2018; 84: 190–

198. https://doi.org/10.1038/pr.2018.23.
[17] Christensen J, Grønborg TK, Sørensen MJ, Schendel D, Parner ET, Pedersen LH, *et al*. Prenatal valproate exposure and risk of autism spectrum disorders and childhood autism. JAMA. 2013; 309: 1696–1703. https://doi.org/10.1001/jama.2013.2270.
[18] Gramer G, Hoffmann GF. Vitamin B_{12} Deficiency in Newborns and their Mothers-Novel Approaches to Early Detection, Treatment and Prevention of a Global Health Issue. Current Medical Science. 2020; 40: 801–809. https://doi.org/10.1007/s11596-020-2260-7.
[19] Moll S, Varga EA. Homocysteine and MTHFR Mutations. Circulation. 2015; 132: e6–e9. https://doi.org/10.1161/CIRCULATIONAHA.114.013311.
[20] Rai V. Association of methylenetetrahydrofolate reductase (MTHFR) gene C677T polymorphism with autism: evidence of genetic susceptibility. Metabolic Brain Disease. 2016; 31: 727–735. https://doi.org/10.1007/s11011-016-9815-0.
[21] El-Baz F, El-Aal MA, Kamal TM, Sadek AA, Othman AA. Study of the C677T and 1298AC polymorphic genotypes of MTHFR Gene in autism spectrum disorder. Electronic Physician. 2017; 9: 5287–5293. https://doi.org/10.19082/5287.
[22] Shaik Mohammad N, Sai Shruti P, Bharathi V, Krishna Prasad C, Hussain T, Alrokayan SA, *et al*. Clinical utility of folate pathway genetic polymorphisms in the diagnosis of autism spectrum disorders. Psychiatric Genetics. 2016; 26: 281–286. https://doi.org/10.1097/YPG.0000000000000152.
[23] Langan RC, Goodbred AJ. Vitamin B12 Deficiency: Recognition and Management. American Family Physician. 2017; 96: 384–389.
[24] Rashid S, Meier V, Patrick H. Review of Vitamin B12 deficiency in pregnancy: a diagnosis not to miss as veganism and vegetarianism become more prevalent. European Journal of Haematology. 2021; 106: 450–455. https://doi.org/10.1111/ejh.13571.
[25] Sukumar N, Rafnsson SB, Kandala NB, Bhopal R, Yajnik CS, Saravanan P. Prevalence of vitamin B-12 insufficiency during pregnancy and its effect on offspring birth weight: a systematic review and meta-analysis. The American Journal of Clinical Nutrition. 2016; 103: 1232–1251. https://doi.org/10.3945/ajcn.115.123083.
[26] McPhee AJ, Davidson GP, Leahy M, Beare T. Vitamin B12 deficiency in a breastfed infant. Archives of Disease in Childhood. 1988; 63: 921–923. https://doi.org/10.1136/adc.63.8.921.
[27] Rasmussen SA, Fernhoff PM, Scanlon KS. Vitamin B12 deficiency in children and adolescents. The Journal of Pediatrics. 2001; 138: 10–17. https://doi.org/10.1067/mpd.2001.112160.
[28] Siu AL, U.S. Preventive Services Task Force. Screening for Iron Deficiency Anemia and Iron Supplementation in Pregnant Women to Improve Maternal Health and Birth Outcomes: U.S. Preventive Services Task Force Recommendation Statement. Annals of Internal Medicine. 2015; 163: 529–536. https://doi.org/10.7326/M15-1707.
[29] Department of Defense Report. Population Representation in the Military Services: Fiscal Year 2021. 2021. Available at: https://prhome.defense.gov/Portals/52/Documents/MRA_Docs/MPP/AP/poprep/2021/PopRep_FY2021_Summary_Report_Suzanne%20Tovar.pdf?ver=c77lymuIGe8D5e8fT4WXeA%3D%3D. (Accessed: 5 March 2024)
[30] Coleman KJ, Lutsky MA, Yau V, Qian Y, Pomichowski ME, Crawford PM, *et al*. Validation of Autism Spectrum Disorder Diagnoses in Large Healthcare Systems with Electronic Medical Records. Journal of Autism and Developmental Disorders. 2015; 45: 1989–1996. https://doi.org/10.1007/s10803-015-2358-0.
[31] Maner BS, Killeen RB, Moosavi L. Mean Corpuscular Volume. StatPearls Publishing: Treasure Island (FL). 2024.
[32] Hultin S. Mosby's Manual of Diagnostic and Laboratory Tests. Annals of Clinical Biochemistry. 2012; 49: 415. https://doi.org/10.1258/acb.2012.201207.
[33] Janus J, Moerschel SK. Evaluation of anemia in children. American Family Physician. 2010; 81: 1462–1471.
[34] Centers for Disease Control. Laboratory Procedure Manual, Methylmalonic Acid (MMA) 2002 [updated September 15, 2017]. Available at: https://wwwn.cdc.gov/nchs/nhanes/continuousnhanes/manuals.aspx?BeginYear=2001 (Accessed: 1 October 2019).
[35] Fideli ÜS, Scher AI, Olsen C, Hisle-Gorman E. Assessing mean corpuscular volume as a screening tool for gestational vitamin B12 deficiency based on NHANES. International Journal for Vitamin and Nutrition Research. 2024; 94: 198–209. https://doi.org/10.1024/0300-9831/a000788.
[36] Powers JM, Sandoval C. Approach to the child with anemia. 2023. Available at: https://www.uptodate.com/ (Accessed: 1 January 2024).
[37] Campbell CD, Ganesh J, Ficicioglu C. Two newborns with nutritional vitamin B12 deficiency: challenges in newborn screening for vitamin B12 deficiency. Haematologica. 2005; 90: ECR45.
[38] von Schenck U, Bender-Götze C, Koletzko B. Persistence of neurological damage induced by dietary vitamin B-12 deficiency in infancy. Archives of Disease in Childhood. 1997; 77: 137–139. https://doi.org/10.1136/adc.77.2.137.
[39] Thompson RA, Nelson CA. Developmental science and the media. Early brain development. The American Psychologist. 2001; 56: 5–15. https://doi.org/10.1037/0003-066x.56.1.5.
[40] Hoxha B, Hoxha M, Domi E, Gervasoni J, Persichilli S, Malaj V, *et al*. Folic Acid and Autism: A Systematic Review of the Current State of Knowledge. Cells. 2021; 10: 1976. https://doi.org/10.3390/cells10081976.
[41] Raghavan R, Riley AW, Volk H, Caruso D, Hironaka L, Sices L, *et al*. Maternal Multivitamin Intake, Plasma Folate and Vitamin B_{12} Levels and Autism Spectrum Disorder Risk in Offspring. Paediatric and Perinatal Epidemiology. 2018; 32: 100–111. https://doi.org/10.1111/ppe.12414.
[42] Mahmood L. The metabolic processes of folic acid and Vitamin B12 deficiency. Journal of Health Research and Reviews. 2014; 1: 5–9. https://doi.org/10.4103/2394-2010.143318.
[43] Hunt A, Harrington D, Robinson S. Vitamin B12 deficiency. BMJ (Clinical Research Ed.). 2014; 349: g5226. https://doi.org/10.1136/bmj.g5226.
[44] Belardo A, Gevi F, Zolla L. The concomitant lower concentrations of vitamins B6, B9 and B12 may cause methylation deficiency in autistic children. The Journal of Nutritional Biochemistry. 2019; 70: 38–46. https://doi.org/10.1016/j.jnutbio.2019.04.004.
[45] Garewal G, Narang A, Das KC. Infantile tremor syndrome: a vitamin B12 deficiency syndrome in infants. Journal of Tropical Pediatrics. 1988; 34: 174–178. https://doi.org/10.1093/tropej/34.4.174.
[46] Cosín-Tomás M, Luan Y, Leclerc D, Malysheva OV, Lauzon N, Bahous RH, *et al*. Moderate Folic Acid Supplementation in Pregnant Mice Results in Behavioral Alterations in Offspring with Sex-Specific Changes in Methyl Metabolism. Nutrients. 2020; 12: 1716. https://doi.org/10.3390/nu12061716.
[47] Jacquemont S, Coe BP, Hersch M, Duyzend MH, Krumm N, Bergmann S, *et al*. A higher mutational burden in females supports a "female protective model" in neurodevelopmental disorders. American Journal of Human Genetics. 2014; 94: 415–425. https://doi.org/10.1016/j.ajhg.2014.02.001.
[48] Baraskewich J, von Ranson KM, McCrimmon A, McMorris CA. Feeding and eating problems in children and adolescents with autism: A scoping review. Autism. 2021; 25: 1505–1519. https://doi.org/10.1177/1362361321995631.
[49] Lundin Remnélius K, Neufeld J, Isaksson J, Bölte S. Eating Problems in Autistic Females and Males: A Co-twin Control

Study. Journal of Autism and Developmental Disorders. 2022; 52: 3153–3168. https://doi.org/10.1007/s10803-021-05198-z.

[50] Spek AA, van Rijnsoever W, van Laarhoven L, Kiep M. Eating Problems in Men and Women with an Autism Spectrum Disorder. Journal of Autism and Developmental Disorders. 2020; 50: 1748–1755. https://doi.org/10.1007/s10803-019-03931-3.

[51] Rynkiewicz A, Janas-Kozik M, Słopień A. Girls and women with autism. Psychiatria Polska. 2019; 53: 737–752. https://doi.org/10.12740/PP/OnlineFirst/95098.

[52] Mutua DN, Njagi EM, Orinda GO. Hematological Profile of Normal Pregnant Women. Journal of Blood & Lymph. 2018; 8: 2. https://doi.org/10.4172/2165-7831.1000220.

[53] Solé-Navais P, Salat-Batlle J, Cavallé-Busquets P, Fernandez-Ballart J, Ueland PM, Ballesteros M, *et al*. Early pregnancy folate-cobalamin interactions and their effects on cobalamin status and hematologic variables throughout pregnancy. The American Journal of Clinical Nutrition. 2018; 107: 173–182. https://doi.org/10.1093/ajcn/nqx041.

[54] Choi R, Choi S, Lim Y, Cho YY, Kim HJ, Kim SW, *et al*. A Prospective Study on Serum Methylmalonic Acid and Homocysteine in Pregnant Women. Nutrients. 2016; 8: 797. https://doi.org/10.3390/nu8120797.

[55] Quinlan CA, McVeigh KH, Driver CR, Govind P, Karpati A. Parental Age and Autism Spectrum Disorders Among New York City Children 0-36 Months of Age. Maternal and Child Health Journal. 2015; 19: 1783–1790. https://doi.org/10.1007/s10995-015-1692-3.

[56] Sawada Y, Sakamoto K, Tsukamura A, Sawai C. Vitamin B12 deficiency-induced megaloblastic anemia in a pediatric patient with autism spectrum disorder with a chronically unbalanced diet. International Journal of Hematology. 2024; 119: 613–616. https://doi.org/10.1007/s12185-024-03759-3.

[57] Bener A, Khattab AO, Bhugra D, Hoffmann GF. Iron and vitamin D levels among autism spectrum disorders children. Annals of African Medicine. 2017; 16: 186–191. https://doi.org/10.4103/aam.aam_17_17.

[58] Gunes S, Ekinci O, Celik T. Iron deficiency parameters in autism spectrum disorder: clinical correlates and associated factors. Italian Journal of Pediatrics. 2017; 43: 86. https://doi.org/10.1186/s13052-017-0407-3.

[59] Rossignol DA, Frye RE. The Effectiveness of Cobalamin (B12) Treatment for Autism Spectrum Disorder: A Systematic Review and Meta-Analysis. Journal of Personalized Medicine. 2021; 11: 784. https://doi.org/10.3390/jpm11080784.