Association of Attention Deficit Hyperactivity Disorder and Autism Spectrum Disorders with Mean Platelet Volume and Vitamin D

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Background:
The purpose of this study was to assess the values of the mean platelet volume (MPV) in children with attention deficit hyperactivity disorder (ADHD) and with autism spectrum disorders (ASDs) to determine the risk of cardiovascular disease in these 2 disorder groups.

Material/Methods:
The study included a total of 79 patients with ADHD or ASDs and controls in the Van region of Turkey. The control group included subjects of matching age and sex with no ADHD, ASDs, or chronic disease and taking no vitamins. The hematological parameters of the patients, including MPV, vitamin B12, and vitamin D, were assessed.

Results:
The study included a total of 79 children and adolescents aged 2–18 years (32 females and 47 males). Of the patients, 36 were in the ADHD group, 18 in the ASDs group, and 25 in the control group. There was no statistically significant difference in hematological parameters between the groups, but there were significant differences in terms of vitamin D and vitamin B12. The patient groups showed lower levels of vitamin B12 and vitamin D. In the ADHD group, there was a negative correlation between both vitamins and MPV (p<0.05). Partial correlation analysis of the ADHD group showed that MPV in particular was negatively correlated to vitamin D, and not to vitamin B12 (p: 0.03).

Conclusions:
Both ADHD and ASDs may accompany increased risk for cardiovascular disease due to the presence of vitamin B12 and D deficiency and their own characteristics. Therefore, these disorders should be closely followed up.

MeSH Keywords:
Attention Deficit Disorder with Hyperactivity • Autistic Disorder • Mean Platelet Volume • Vitamin B 12 Deficiency • Vitamin D

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Background

Attention deficit hyperactivity disorder (ADHD) and autism spectrum disorders (ASD) are 2 different disorders with important impacts on the lives of children. The frequently seen attention deficit hyperactivity disorder may lead to lack of school success and to disruptive behavior [1]. The pathophysiology of ADHD is complex and not fully clarified. Although there is no specific known cause of this disorder, multiple factors in its pathophysiology have been implicated [2]. Some prenatal and genetic risk factors have been particularly implicated in its etiology [3]. Autism spectrum disorders are a heterogeneous group of biological neuro-developmental disorders. This group is associated with certain risk factors such as mutated or variant genes, advanced father’s age, prematurity, and birth complications [4–6]. Recently, there have been papers on the association of ADHD in children with increased thickness of carotid intima media [7]. Children with ADHD and with ASD have higher body mass index (BMI) than controls, and this increased BMI increases the risk for cardiovascular disease [8]. Furthermore, co-deficiency of vitamin B12 and vitamin D in both ADHD and ASD has recently been reported and claimed as a possible risk factor for both disorders [6]. Deficiency of vitamin B12 and vitamin D has been implicated as a factor increasing the risk for cardiovascular disease [9–14]. When one looks at the situation in terms of all predisposing factors, it is evident that ADHD and ASD are accompanied by an increased risk of cardiovascular disease.

Platelets play a role vital role in the pathophysiology of thrombogenesis and atherogenesis [15]. Platelet adhesion and aggregation cause adverse atherothrombotic events. When platelets are more active, the risk of atherothrombosis is higher, and outcomes are worse. Platelet activity has been related to acute vascular events, and the administration of anti-platelet agents is a Class I recommendation for treatment and secondary prevention of coronary artery disease (CAD) [16]. In addition, many recent studies have correlated platelet activity with development and progression of atherosclerosis [16]. Platelet functions have been determined to be associated with the risk of adverse events in different types of CAD [17]. Tests for measuring platelet function are currently available, but most are expensive and are only used for research, not routine clinical practice [18]. The mean platelet volume (MPV) is a simple indicator of platelet size, which may represent platelet activity and be associated with plaque burden, morphology, progression, and vulnerability in vascular endothelial tissue. Larger platelets are younger, contain more alpha granules, have more expression of adhesion receptors, and are more metabolically and enzymatically active; therefore, they possess more marked thrombogenic features [18]. A recent meta-analysis in adults has strongly suggested an association between MPV and CAD [19].

The specific features, frequent lack of nutritional factors such as vitamins B12 and D, and high levels of oxidative stress in ADHD and ASD have led us to think that these disorders may be a risk factor for cardiovascular disease. The purpose of our study was to assess the risk of cardiovascular disease in subjects with ADHD and ASD by determining the values of MPV, a predictor of cardiovascular disease, in both ADHD and ASD patients and in healthy controls.

Material and Methods

The study included patients aged 3–18 years with ADHD and with ASD, and healthy controls, in the Van region of Turkey. The patients were divided into 2 groups based on diagnosis: the ADHD group and the ASD group. The study plan was approved by the Ethics Committee of the Medical School, Yüzüncü Yıl University, and was made according to the ethics principles of the Declaration of Helsinki. After informing each patient or each patient’s family about the study, the patient or the family provided signed informed consent.

The sampling groups of the study included children and adolescents presenting to the Pediatric Psychiatry Outpatient Clinic of the Van Regional Training and Research Hospital, who had received the diagnoses of ADHD or ASD in the period between February and July 2014. The diagnosis of ASD was made according to the DSM-5 and DSM-4 TR diagnostic criteria based on ASD-diagnostic interview and the Childhood Autism Rating Scale (CARS). The diagnosis of ADHD was based on DSM-5 and DSM-4 TR diagnostic interview and on the evaluation of the Scale for Screening/Evaluation for Disruptive Behavior Disorders filled in by the family and the teacher.

After measuring the weight and height of the patients, their blood samples were collected. The criteria for exclusion from the study were: comorbidity, a diagnosed genetic or metabolic disorder, history of head trauma or surgery, use of any vitamins, infection during the time of presentation, another chronic disorder, and long-term use of drugs. We tested the renal and hepatic function of patients and controls, and those with abnormal values were excluded from the study (the values of these tests have not been demonstrated in the study). The control group comprised normal subjects who had presented to the Pediatric Endocrinology Outpatient Clinic for a developmental check-up. The controls matched the study group patients with regard to age and sex, they did not have the diagnostic criteria for ADHD or ASD, did not have a chronic disorder, and were not taking vitamins of any kind. The controls also underwent the tests described above.

Measurements of biochemical parameters, serum electrolyte levels, renal and hepatic functions, calcitriol, and vitamin B12
levels were made at the Central Laboratory of the Medical School using the Architect CI-16200 (Abbott Diagnostics, Abbott Park, IL) and the chemiluminescent method. Ferritin levels were determined in the Biochemistry Laboratory of the Medical School Hospital using the IMMULITE® 2000 (Siemens Healthcare Diagnostics, Los Angeles, CA) and the chemiluminescent method. Hematological tests that measured hemoglobin, platelets, white blood cells (WBC), and MPV were performed using the Cell-Dyn Ruby analyzer (Abbott Diagnostics).

The data obtained were statistically analyzed using the SPSS13 package program. The results obtained in the groups are expressed as mean ± standard deviation, Min – minimum; Max – maximum.

### Results

The study included a total of 79 children and adolescents aged 2–18 years (32 females and 47 males). Of these subjects, 36 were in the ADHD group, 18 in the ASD group, and 25 in the control group. The comparison of the groups in terms of age, weight, and height is shown in Tables 1 and 2. There was no significant difference between the groups in terms of age, sex, weight, or height.

The hematological test results of the groups are displayed in Table 3. There was no significant difference between the groups in terms of any hematological parameters (p>0.05). The comparison of biochemical parameters in the groups is presented in Table 4. A significant difference was present between the groups in terms of vitamin D, vitamin B12, and ferritin levels (Table 5). There was no anemia based on age in any of the patients. The lowest vitamin B12 level was determined in the ASD group, whereas the vitamin B12 level in the ADHD group was markedly lower than that in the control group. The lowest vitamin D level was found in the ASD group. The ferritin level was highest in the ASD group and lowest in the control group. The correlation analyses within each group showed no statistically significant difference between the groups, but in the ADHD group there was a negative correlation between vitamin D, vitamin B12, and MPV (p<0.05). In the control group, a significant negative correlation was present between MPV and serum folate levels (p: 0.003). The partial correlation analysis of the ADHD group showed that MPV in particular was negatively correlated to vitamin D but not to vitamin B12 (p: 0.03, 0.424, respectively) (Table 6).
In this prospective case-control study, the risk of cardiovascular disease was assessed in children with ADHD and with ASD by determining the MPV levels. In the literature, there has been only 1 study assessing the association between MPV and ADHD [20]. Our study may be a contribution to the literature on this subject. There is no study in the literature assessing

|                | Mean        | SD  | Min  | Max  | p     |
|----------------|-------------|-----|------|------|-------|
| Leucocytes     |             |     |      |      |       |
| (×10^3 mm⁻³)   |             |     |      |      |       |
| ADHD           | 7.95        | 2.20| 5.3  | 15.3 | >0.05 |
| ASD            | 8.61        | 1.74| 6.3  | 13.1 |       |
| Control        | 7.88        | 2.27| 4.8  | 15.3 |       |
| Erythrocytes   |             |     |      |      |       |
| (×10^6 mm⁻³)   |             |     |      |      |       |
| ADHD           | 5.09        | 0.39| 4.4  | 5.7  | >0.05 |
| ASD            | 4.94        | 0.46| 4.2  | 5.7  |       |
| Control        | 4.90        | 0.34| 4.4  | 6.0  |       |
| Hemoglobin     |             |     |      |      |       |
| (g/dL)         |             |     |      |      |       |
| ADHD           | 13.72       | 0.94| 11.5 | 16.0 | >0.05 |
| ASD            | 13.72       | 1.41| 11.3 | 15.8 |       |
| Control        | 13.29       | 1.16| 11.7 | 16.0 |       |
| Hematocrit     |             |     |      |      |       |
| (%)            |             |     |      |      |       |
| ADHD           | 41.17       | 2.82| 34.4 | 48.0 | >0.05 |
| ASD            | 41.17       | 4.23| 34.0 | 47.4 |       |
| Control        | 39.88       | 3.49| 35.0 | 48.0 |       |
| Mean erythrocyte volume (fL) | |     |      |      |       |
| ADHD           | 81.01       | 5.45| 63.0 | 90.0 | >0.05 |
| ASD            | 83.10       | 6.22| 72.0 | 92.0 |       |
| Control        | 81.33       | 4.84| 69.0 | 91.0 |       |
| Mean erythrocyte hemoglobin | |     |      |      |       |
| ADHD           | 26.93       | 2.04| 20.0 | 30.0 | >0.05 |
| ASD            | 27.41       | 2.18| 23.4 | 31.0 |       |
| Control        | 27.21       | 1.67| 22.0 | 30.0 |       |
| Mean platelet volume (fL) | |     |      |      |       |
| ADHD           | 8.03        | 0.75| 6.8  | 9.5  | >0.05 |
| ASD            | 8.35        | 1.11| 7.3  | 11.2 |       |
| Control        | 8.42        | 0.92| 6.8  | 10.6 |       |
| Erythrocyte volume distribution (%) | |     |      |      |       |
| ADHD           | 13.61       | 0.93| 12.0 | 16.0 | >0.05 |
| ASD            | 13.60       | 1.28| 12.5 | 17.7 |       |
| Control        | 13.12       | 1.03| 12.0 | 16.0 |       |
| Platelets     |             |     |      |      |       |
| (×10^9 mm⁻³)   |             |     |      |      |       |
| ADHD           | 308.39      | 93.23| 143.0| 631.0| >0.05 |
| ASD            | 318.07      | 100.94| 120.0| 459.0|       |
| Control        | 277.04      | 65.29| 187.0| 435.0|       |
| Platelet volume distribution (%) | |     |      |      |       |
| ADHD           | 15.69       | 2.18| 9.3  | 17.5 | >0.05 |
| ASD            | 15.91       | 0.70| 14.0 | 16.7 |       |
| Control        | 15.28       | 2.26| 10.0 | 17.0 |       |

ADHD – Attention Deficit Hyperactivity Disorder; ASD – Autism Spectrum Disorders; SD – standard deviation; Min – minimum; Max – maximum.

**Discussion**

In this prospective case-control study, the risk of cardiovascular disease was assessed in children with ADHD and with ASD by determining the MPV levels. In the literature, there has been only 1 study assessing the association between MPV and ADHD [20]. Our study may be a contribution to the literature on this subject. There is no study in the literature assessing

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**Garipardic M. et al.: MPV in ADHD and ASDs**
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the association between ASD and MPV. Furthermore, in our study, the association between vitamins B12 and D and MPV was assessed for the first time. We found no significant difference in MPV values between ASD, ADHD, and the control groups, but we determined that in the ASD and ADHD groups, the levels of vitamins B12 and D were significantly lower than those in the control group. In the ADHD group, there was a significantly negative correlation between vitamins B12 and

| Table 4. Comparison of biochemical parameters in the groups. |
|---------------------------------------------------------------|
| **Mean** | **SD** | **Min** | **Max** | **p**  |
| Glucose (mg/dL)                                            |
| ADHD            | 90.93  | 7.97   | 76.0    | 110.0 | >0.05  |
| ASD             | 90.71  | 17.11  | 68.0    | 109.0 |        |
| Control         | 92.48  | 9.42   | 79.0    | 116.0 |        |
| Creatinine (mg/dL)                                        |
| ADHD            | 0.51   | 0.19   | 0.3     | 1.0   | >0.05  |
| ASD             | 0.38   | 0.20   | 0.2     | 0.7   |        |
| Control         | 0.53   | 0.11   | 0.3     | 0.7   |        |
| Vitamin B12 (pg/mL)                                       |
| ADHD            | 371.03 | 155.74 | 153.0   | 915   | <0.001 |
| ASD             | 232.06 | 65.17  | 115.0   | 410   |        |
| Control         | 428.50 | 173.86 | 189.0   | 903   |        |
| Folate (ng/mL)                                            |
| ADHD            | 10.21  | 2.85   | 4.0     | 15.0  | >0.05  |
| ASD             | 9.09   | 3.91   | 4.0     | 16.0  |        |
| Control         | 8.43   | 3.85   | 3.0     | 20.0  |        |
| Vitamin D (ng/mL)                                         |
| ADHD            | 18.5   | 8.53   | 7.2     | 35.3  | <0.001 |
| ASD             | 14.3   | 7.25   | 3.5     | 24.5  |        |
| Control         | 29.42  | 9.07   | 11.7    | 45.8  |        |
| Ferritin (ng/mL)                                          |
| ADHD            | 40.53  | 15.9   | 10.0    | 77.0  | 0.01   |
| ASD             | 50.98  | 30.51  | 19.0    | 140.0 |        |
| Control         | 27.50  | 16.94  | 2.5     | 68.0  |        |

ADHD – Attention Deficit Hyperactivity Disorder; ASD – Autism Spectrum Disorders; SD – standard deviation; Min – minimum; Max – maximum.

| Table 5. The correlation curve between Mean Platelet Volume (MPV) and vitamin B12, vitamin D, ferritin, and folate in the groups. |
|-------------------------------------------------------------------------------------------------------------------------------------|
| **Correlation Coefficient (CC)** |
| **p**  |
| ADHD | ASD | Control |
| MPV  | MPV | MPV  |
| Vitamin B12 | -0.438 | 0.289 | 0.016 |
| P     | 0.043 | 0.296 | 0.942 |
| Vitamin D | -0.383(*) | -0.1 | 0.063 |
| P     | 0.026 | 0.873 | 0.776 |
| Ferritin | 0.054 | -0.261 | -0.273 |
| P     | 0.812 | 0.498 | 0.306 |
| Folate | 0.183 | 0.39  | -0.605(**) |
| P     | 0.325 | 0.168 | 0.003 |

CC – correlation coefficient; ADHD – Attention Deficit Hyperactivity Disorder; ASD – Autism Spectrum Disorders.
D and MPV. The partial correlation analysis showed that vitamin D particularly had an effect on MPV. The only study in the literature assessing the association between ADHD and MPV is by Yorbik et al. [20], who found increased levels of MPV in children aged 6–16 years and adolescents with ADHD, but concluded that the reason for and the importance of increased MPV should be clarified by further studies. In our study, we found no association between MPV and ADHD or ASD. This finding may be due to the limited number of patients studied. In order to verify this finding, further studies with larger patient groups are needed. One important finding in our study was the impact of vitamin D on MPV, particularly in the ADHD group. We showed that there was a negative correlation between MPV and vitamins D and B12. In the partial correlation analysis indicating that when vitamin B12 was taken as the controlling factor there was a significant negative correlation between MPV and vitamin D, but when vitamin D was taken as the controlling factor there was no significant correlation between vitamin B12 and MPV. Thus, vitamin D in particular affected MPV.

ADHD is considered to share the same etiologies with ASD, since ASDs also exist frequently in the states that commonly exist with ADHD [21]. Therefore, factors playing roles in the etiology of ASD also have to be well understood to clarify the etiology of ADHD. Attention deficit hyperactivity disorder is less prevalent in geographical regions with hot climate and longer hours of sunlight [22]. The next question to be answered is whether vitamin D deficiency is frequent in ADHD. Many studies have reported that vitamin D deficiency is frequently seen both in ADHD and ASD, and that in children with these disorders, the levels of vitamins B12 and D are much lower than in controls [23,24]. The relevant studies have also reported that vitamin D is a requirement for normal cerebral homeostasis, cellular differentiation during cerebral development, axonal growth, stimulation of neurotrophic factors, modulation of the production of cerebral reactive oxygen species, and stimulation of glutathione, which is a potent anti-oxidant that plays a role in the synthesis and repair of DNA, whereby vitamin D down-regulates excitotoxicity [25–27]. Hence, failures in these mechanisms caused by vitamin D deficiency may lead to behavior disorders as seen in ADHD and ASD. As a matter of fact, in our study, vitamin D deficiency was frequently determined as a comorbidity as well as an etiologic factor in both ADHD and ASD patients.

After considering all these data, the next question to be answered is whether there is an association between vitamin D and cardiovascular disease. In the cardiovascular system, the endothelial cells, cardiomyocytes, and vascular smooth muscle cells carry the vitamin D receptor gene (VDR), which interacts with vitamin D. It has been reported that when cardiomyocytes are incubated with active vitamin D, cellular proliferation is inhibited, cardiomyocyte formation is increased, the anti-apoptotic effect is induced, and the expression of genes involved in cell cycle is reduced. It has been reported that in cases of vitamin D deficiency, there is an increase of inflammatory cytokines released by active macrophages (IL-1 beta, IL-6, TNF-alpha) induced by interferon-gamma release from Th1 cells, which causes increased oxidation of LDL, and the subsequent destabilization and tearing of the atheroma plaque leads to increased risk of thrombosis [9,10]. A recent comprehensive study has reported that vitamin D deficiency accompanies hypertension, high blood glucose levels, cardiovascular disease, and metabolic syndrome [11]. These studies show that vitamin D deficiency accompanies the risk of cardiovascular disease.

**Table 6.** The partial correlation between mean platelet volume and vitamin B12 and vitamin D in the Attention Deficit Hyperactivity Disorder (ADHD) Group.

| Controlled variable | 25 OH Vitamin D |
|--------------------|-----------------|
|                    | Correlation     | P       |
| Vitamin B12       | Mean platelet volume | -0.52* |
|                    | P               | 0.03    |

- Both ADHD and ASD may accompany increased risk for cardiovascular disease due to the presence of vitamin B12 and D deficiency and their own characteristics. Therefore, these disorders should be closely followed up.
- The lack of a difference in MPV between the patient groups and control group may be due to the limited number of patients studied.
- The vitamin D deficiency, particularly in the ADHD group, may have contributed to the elevated MPV level in the study by Yoruk et al.
- Further studies on larger patient populations are required for verification of the presented data.

**Conclusions**

- Both ADHD and ASD may accompany increased risk for cardiovascular disease due to the presence of vitamin B12 and D deficiency and their own characteristics. Therefore, these disorders should be closely followed up.
- The lack of a difference in MPV between the patient groups and control group may be due to the limited number of patients studied.
- The vitamin D deficiency, particularly in the ADHD group, may have contributed to the elevated MPV level in the study by Yoruk et al.
- Further studies on larger patient populations are required for verification of the presented data.
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Conflict of interest statement

There is no conflict of interest.