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Functional Vitamin B12 Deficiency Represented by Elevated Urine Methylmalonic Acid Levels in Patients with Migraine

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Aims: Although vitamin B12 is currently used as a treatment option in patients with migraine, a possible cobalamin deficiency in those patients has not yet been investigated. In this study, we aimed to investigate cobalamin metabolism in patients with migraine.

Materials and Methods: Fifty women having migraine without aura were matched with healthy controls (n=6) with the same age distribution. Fasting serum vitamin B12, folate, plasma total homocysteine (tHcy) and first morning urine methylmalonic acid (MMA) levels were determined in patient and control groups. Statistical differences in laboratory parameters between patient and control groups were analyzed by SPSS for Windows software.

Results: Vitamin B12 and folate levels in the patient group were not different from those of the control group. Although mean plasma tHcy and urine MMA levels in the patient group were significantly higher than those in the control group, only a small portion (12%) of patients had plasma tHcy levels higher than the reference range, whereas 70% of patients had increased urine MMA levels.

Conclusions: There was a functional vitamin B12 deficiency represented by elevated urine MMA levels in patients having migraine without aura. We suggest that vitamin B12 treatment for migraine patients should be started thereafter screening for MMA elevations.

Key Words: Migraine, vitamin B12 deficiency, methylmalonic acid, homocysteine

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Migren Hastalarındaki Fonksiyonel Vitamin B12 Eksikliğinin İdrardaki Metilmalonik Asit Yüksekliği ile Değerlendirilmesi

Giriş: Migren tedavisinde vitamin B12 kullanılmasına rağmen bu hastalardaki vitamin B12 eksikliği durumu henüz yeterince araştırılmamıştır.

Amaç: Bu çalışmada migren hastalarındaki cobalamin metabolizmasını araştırdık.

Yöntem ve Gereç: Aurasız 50 migren bayan hasta benzer yaş dağılımına sahip 46 sağlıklı kontrol grubu ile eşleştirildi. Hasta ve kontrol grubundan açlık serum vitamin B12, folat, plazma total homosistein(tHcy) ve sabah ilk idrarlarından metil malonik asit çalışıldı. Hasta ve kontrol grubu arasındaki istatistiksel analizleri SPSS for Windows programı kullanılarak yapıldı.

Bulgular: Hasta ve kontrol grubunun Vitamin B12 ve folat seviyeleri arasında fark yoktu. Hasta grubunun plazma tHcy ve idrar metilmalonik asit ortalamaları kontrol grubundan belirgin olarak farklı olmasına rağmen hasta grubunun çok küçük bir kısmında (% 12) plazma tHcy seviyeleri normal aralığın üzerinde idi. Oysa hasta grubunun % 70'inin idrar MMA seviyeleri yüksekti.

Sonuç: Aurasız migren hastalarında idrar MMA seviyelerindeki yükseliş kendi başına fonksiyonel vitamin B12 eksikliğini gösterir. Biz bu çalışmamızda migren hastalarında kullanılan vitamin B12 tedavisinin idrar MMA seviyeleri ile izlenmesini önermekteyiz.

Anahtar Sözcükler: Migren, vitamin B12 eksikliği, metilmalonik asit, homosistein

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Introduction

Migraine, with and without aura, is a prevalent and complex neurovascular disorder that affects approximately 12% of the Caucasian population. It is characterized by nausea and vomiting, photophobia and phonophobia, neurological disturbances and severe recurrent headache. Pharmaceutical treatments for migraine exhibit variable efficacy among patients and there is no laboratory-based diagnostic test available. At

present, migraine is clinically diagnosed based on criteria specified by the International Headache Society (IHS). The IHS has defined two major classes of migraine: migraine without aura, which accounts for ~70% of all migraine in the population, and migraine with aura, which comprises ~25% of all migraine (1).

It has been suggested that nitric oxide (NO) may be involved in the early phase of spontaneous migraine attacks. Hydroxocobalamin (OH_{B12}) appears to be such a NO-scavenger (2) and is a likely candidate for migraine prophylaxis. Therefore, cobalamin deficiency could also lead to migraine attacks by altering NO levels. Although vitamin B12 was reported as a therapeutic agent in migraine prophylaxis (3-7), there is not sufficient knowledge about the existence or not of vitamin B12 deficiency in patients with migraine. Vitamin B12 must be converted to its isoforms in order to become metabolically active. Methylcobalamin (MeCbl), the coenzyme of methionine synthase enzyme that converts homocysteine to methionine, and adenosylcobalamin (AdoCbl), the coenzyme of methylmalonyl CoA mutase enzyme that catalyzes the conversion of methylmalonyl CoA to succinyl CoA, are active isoforms of vitamin B12. Elevated total homocysteine (tHcy) levels are related to MeCbl deficiency, whereas increase in methylmalonic acid (MMA) levels is related to the AdoCbl deficiency. tHcy levels could also be affected by folate status. Thus, vitamin B12 deficiency could be detected by measuring its metabolic markers, plasma tHcy and urine or serum MMA levels.

Recently, in a large group of migraine patients, tHcy levels were found unchanged (8). This implies that there is no problem in the MeCbl isoform of vitamin B12 in those patients. However, deficiency of AdoCbl may also occur in patients with migraine. To our knowledge, there has been no study defining MMA levels, which reflect the AdoCbl levels, in patients with migraine. In this study, we determined vitamin B12 state by serum vitamin B12, folate, plasma tHcy and urine MMA levels in patients with migraine without aura.

Materials and Methods

Patients

Patients with a history of migraine for more than one year were diagnosed according to the IHS criteria (1).

They suffered from at least two and at most eight migraine attacks per month. Exclusion criteria were the use of any prophylactic drug in the three months prior to inclusion, tension-type headache >5 days per month, age <16 years, (wish for) pregnancy or lactation, anemia (hemoglobin cut-off for women 7.5 mmol/L and for men 8.5 mmol/L), use of vitamin B12-containing supplements, acute or chronic rhinitis, abuse of alcohol or other substances, and abnormal findings on liver or kidney function tests. The protocol was approved by the local Ethics Committee. The patient group consisted of 50 female patients with migraine without aura and the control group included 46 healthy women with the same age distribution as the patient group.

Laboratory Analysis

Blood samples from the patient and control groups were taken between 08:30 and 9:00 am after a 12-hour fast. For homocysteine measurements, blood samples were obtained in ice-cooled EDTA tubes. Plasma was separated by centrifugation at 5 °C and stored at -20 °C. First urine samples in the morning from patient and control groups were used for urine MMA measurements. Blood and urine samples of the patient group were taken in an attack-free period. Urine MMA levels were measured by using spectrophotometric method based on the reaction between MMA and diazotization p-nitroaniline (9). MMA levels were normalized to urine creatinine. tHcy was measured by high performance liquid chromatography (HPLC) method using commercial homocysteine determination kit (Chromosystems, Germany) in HP Agilent 1100 HPLC system. Serum vitamin B12 and folate levels were determined by chemiluminescent microparticle immunoassay (CMIA) method using commercial kits (Abbott Laboratories, Abbott Park, Chicago, IL) in Architech i 2000 analyzer. The normal ranges in our laboratory are <15 µmol/L for tHcy, >2.7 ng/mL for folate, 180-883 pg/mL for vitamin B12 and <6 mmol/mol creatinine for urine MMA.

Statistical Analyses

Data are described as mean and standard deviation (SD) for laboratory variables. Parameters of the two groups were compared using the Student t test. A P value >0.05 was considered statistically significant, and all P values were based upon two-tailed tests. Statistical analysis was performed using SPSS for Windows ver. 11.0 (SPSS Inc., Chicago, IL, USA).

Results

The age of the patients ranged from 18 to 57 years (mean: 37.2 years). The age of the controls ranged from 20 to 78 years (mean: 35.4 years). The duration of migraine ranged from 1 to 30 years (mean: 11.6 years). All patients had migraine without aura. The physical and neurological evaluation and brain computed tomography (CT) in all patients were normal. Plasma tHcy, serum vitamin B12, folate and urine MMA levels were in the reference range in all controls. tHcy was normal in 44 of 50 patients. Vitamin B12 was normal in 48 of 50 patients. Folate was normal in all patients. Urine MMA was normal in 15 of 50 patients.

Statistical differences in laboratory parameters between the two groups are listed in Table 1. Although mean plasma tHcy and urine MMA levels in the patient group were significantly higher than in the control group, only a small portion of patients (12%) had plasma tHcy levels higher than the reference range, whereas 70% of patients had increased urine MMA levels (Table 1). While urine MMA levels were not correlated with any other laboratory parameter, there was a negative and significant correlation between plasma tHcy and folate levels in the patient group (Table 2).

In receiver operating characteristic (ROC) curves, urine MMA had greater area under the curve, sensitivity and specificity values compared with plasma tHcy (Figure). For migraine patients, sensitivity and specificity for different cut-off values of urine MMA are listed in Table 3. The best sensitivity and specificity values were seen in 4.5 mmol/mol creatinine as a threshold for urine MMA (Table 3). For different urine MMA thresholds, tHcy levels in patients having elevated urine MMA concentrations were not significantly changed compared with those having normal urine MMA levels (Table 4).

Table 2. Pearson correlation coefficients (r) between laboratory parameters in the patient group.

	r	P
Vitamin B12 - Folate	- 0.048	0.742
Vitamin B12 - tHcy	0.249	0.082
Vitamin B12 - Urine MMA	- 0.002	0.990
Folate - tHcy	- 0.494	0.000
Folate - Urine MMA	- 0.111	0.441
tHcy - Urine MMA	- 0.128	0.377

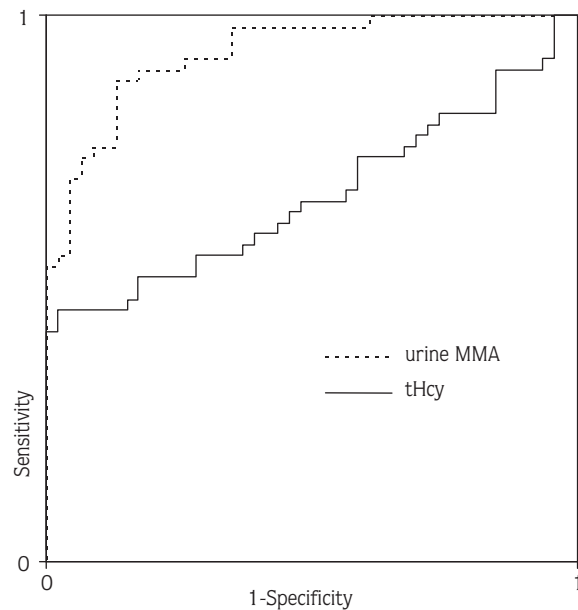


Figure. ROC curves for tHcy and urine MMA parameters for patients with migraine compared with healthy controls (areas under the curve were 0.932 [CI: 0.885-0.979] and 0.669 [CI: 0.559-0.780] for urine MMA and tHcy, respectively).

Table 1. Statistical differences in laboratory parameters between patient and control groups.

	Patient Group (n=50)	Control Group (n=46)	Difference (P value)
Vitamin B12 (pg/mL)	280 ± 86	275 ± 75	0.759
tHcy (µmol/L)	10.96 ± 4.13	8.67 ± 1.99	0.001
Folate (ng/mL)	8.1 ± 2.6	8.1 ± 3.3	0.991
Urine MMA (mmol/mol creatinine)	6.63 ± 1.64	3.38 ± 1.45	0.000
Age (years)	37.2 ± 11.7	35.4 ± 17.5	0.557

Table 3. Sensitivity and specificity values according to different cut-off levels for urine MMA levels.

Cut-off value for urine MMA ($\mu\text{mol/L}$)	Sensitivity (%)	Specificity (%)
4.0	92	66
4.5	90	81
5.0	81	87
5.5	75	91
6.0	62	96
6.5	55	98
7.0	40	100

Table 4. Mean \pm SD values of tHcy, vitamin B12 and folate according to different cut-off values for urinary MMA.

Urine MMA	n	tHcy	Vitamin B12	Folate
<4.5	45	10.78 \pm 4.19	284 \pm 86	6.9 \pm 1.4
>4.5	5	12.55 \pm 3.52	247 \pm 84	9.5 \pm 2.9
<5	41	10.59 \pm 4.17	285 \pm 86	7.9 \pm 2.6
>5	9	12.68 \pm 3.69	259 \pm 86	9.1 \pm 2.6
<5.5	38	10.64 \pm 3.95	287 \pm 89	8.0 \pm 2.6
>5.5	12	11.97 \pm 4.70	258 \pm 74	8.4 \pm 2.6
<6	31	11.06 \pm 3.72	285 \pm 96	7.6 \pm 1.0
>6	19	10.80 \pm 4.83	273 \pm 69	8.4 \pm 2.6

Discussion

The existence of functional vitamin B12 deficiency, represented by elevated urine MMA levels with normal blood vitamin B12 levels, in patients with migraine was the main finding of our study. There was also a borderline (but inside the reference range) elevation in tHcy levels in the patient group. MMA and tHcy are the metabolic or tissue level markers of cobalamin deficiency. MMA elevations show reduced conversion of methylmalonyl CoA to succinyl CoA catalyzed by methylmalonyl CoA mutase enzyme using AdoCbl as a cofactor. Thus, there could be either defective or inhibited enzyme or insufficient amount of cofactor leading to elevated urine MMA levels in patients with migraine. This AdoCbl-dependent reaction is important in the catabolism of methionine, valine, threonine, isoleucine, odd chain fatty acids, and cholesterol (10,11).

These metabolites are important in the control of many metabolic pathways. Diminished methylmalonyl CoA mutase activity could lead to metabolic results, which contribute to the migraine pathogenesis.

In the formation of AdoCbl, the 5'-deoxyadenosyl moiety is transferred from ATP and covalently attached to the cobalt(I) by an ATP:cobalamin adenosyltransferase (12) enzyme. In migraine patients, reduced activity of this enzyme could cause diminished AdoCbl demand for methylmalonyl CoA mutase reaction. Therefore, AdoCbl levels, ATP:cobalamin adenosyltransferase and methylmalonyl CoA mutase activities should be further investigated in patients with migraine.

Almost 50 years ago, Urdahl-Aasen observed that the migraine attacks of a patient with anemia perniosa resolved after treatment with vitamin B12 (5). This observation led to the use of vitamin B12 in some people.

However, conflicting results were reported about vitamin B12 treatment in patients with migraine (3-7). In a recent study, 1 mg intranasal hydroxocobalamin daily for 3 months significantly reduced migraine attack frequency in 53% of patients. The observation that not all patients responded to OHB12 may be explained by a different pathophysiology of the migraine in the non-responders (3).

Although investigators explained the differences in response to vitamin B12 treatments by the type of cobalamin isoforms (hydroxocobalamin or cyanocobalamin) given patients (2), this would not sufficiently explain all the differences. The existence of functional vitamin B12 deficiency represented by elevated MMA levels might explain those different results. In migraine patients having raised MMA levels, vitamin B12 treatment might be more successful compared with others. From this perspective, we suggest that vitamin B12 treatment for migraine patients should be started thereafter screening for MMA elevations. Elevated urine MMA levels could either have a role in the disease progression or be the result of hydroxocobalamin deficiency due to NO scavenging mechanisms suggested in the pathophysiology of migraine.

Increased oxidative stress in patients with migraine was previously reported (12). Methylmalonyl-CoA mutase

catalyzes the isomerization of methylmalonyl-CoA to succinyl-CoA, which uses reactive radical intermediates that are susceptible to oxidative inactivation (13). The increased oxidation might damage methylmalonyl CoA mutase and/or ATP:cobalamin adenosyltransferase enzymes. The possible relationship between free radical damage and MMA elevations in patients with migraine should also be further investigated.

Folate metabolism was also thought to be involved in migraine pathogenesis (14). However, neither folate nor tHcy levels were found as changed in migraine patients (7). Our findings in folate and tHcy levels were in agreement with previous results. Furthermore, no association was found between migraine and the MTHFR C677T polymorphism, causing mild hyperhomocysteinemia (15). Thus, these evidences imply that there may not be any folate or methylcobalamin deficiency among migraineurs.

In conclusion, this is the first study showing the existence of functional vitamin B12 deficiency expressed by elevated urine MMA levels in patients with migraine without aura. This defective cobalamin state should be further investigated by using patients having migraine with and without aura. Furthermore, vitamin B12 treatment in migraine should also be reassessed by initial screening of patients for MMA elevations.

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