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Biochemical analysis of serum mineral and vitamin levels in benign essential blepharospasm

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Abstract

Background/Aim: Benign essential blepharospasm (BEB) is a type of focal dystonia characterized by involuntary periocular muscle spasms, resulting in partial or complete eyelid closure. Though BEB has been linked to a variety of mineral and vitamin D deficiencies, its association with serum vitamin B_{12} has never been studied. We intended to determine the association between vitamin B_{12}, serum calcium, magnesium, phosphorus, and 25-(OH) vitamin D levels, and BEB severity and frequency.

Methods: This retrospective case-control study included 20 BEB patients who were followed-up and treated periodically with botulinum toxin injections in Afyonkarahisar Health Sciences University Department of Ophthalmology's Oculoplastic and Reconstructive Surgery Unit between January 2019 and January 2021. Twenty age- and gender-matched healthy individuals were also included. The Jankovic rating scale was used to determine the severity and frequency of BEB. Acquired data were assessed retrospectively in terms of age, gender, serum minerals, 25 (OH)-Vitamin D and Vitamin B_{12} levels.

Results: In this study, 20 BEB patients (F:M=14:6) and 20 healthy individuals (F:M=12:8) ($P=0.321$) were investigated. The mean ages of BEB patients and healthy individuals were 64.37 (4.21) and 63.83 (3.13) years, respectively ($P=0.239$). Compared to healthy individuals, BEB patients had significantly lower serum levels of 25-(OH) vitamin D ($P=0.037$), vitamin B_{12} ($P=0.014$) and calcium ($P=0.011$). In BEB patients, serum levels of 25-(OH) vitamin D (r=-0.375, $P=0.043$), calcium (r=-0.319, $P=0.039$), and vitamin B_{12} (r=-0.408, $P=0.027$) were all strongly negatively correlated with the Jankovic severity score.

Conclusion: A strong negative correlation between disease severity and decreased vitamin B_{12}, 25-(OH) vitamin D, and calcium in BEB patients indicated that, among other things, BEB may be a rare form of vitamin B_{12} deficiency. In the absence of an obvious cause for BEB, serum vitamin B_{12} testing, in addition to serum minerals and 25-(OH) vitamin D, may be useful.

Keywords: Benign essential blepharospasm, Vitamin B12, Magnesium, Phosphorus, Calcium, 25-(OH) vitamin D, Jankovic rating scale

Introduction

Benign essential blepharospasm (BEB) is a type of focal dystonia characterized by involuntary spasms of the orbicularis oculi, corrugator, and procerus muscles that cause partial or complete closure of the eyelids. Symptoms of this condition include a slightly higher blink rate, involuntary eyelid closure, and functional blindness in some cases. BEB affects between 1.4 and 13.3 individuals per 100,000, with symptoms appearing between the fifth and seventh decades of life [1]. The annual mean incidence of BEB is reported as 0.10 ‰, with female and male incidences of 0.12 ‰ and 0.07 ‰, respectively [2]. Even though it is more common in females than in males, a potential explanation for women's predisposition to this disease is yet to be discovered [3, 4]. BEB patients may also experience mid-facial or lower-facial spasms, a condition known as Meige syndrome [5]. Although BEB etiology has not been fully clarified, potential contributions of basal ganglia (BG), and cortical process abnormalities, as well as genetic, and environmental factors have been reported [6]. Involuntary contractions of the orbicularis oculi muscle may lead to a continuous eyelid closure. A series of involuntary activation of periorbital muscles may also be associated with BEB. Treatment of BEB involves botulinum toxin injection, which is basically applied to periorbital areas to inhibit acetylcholine release at the neuromuscular junction, and thus induces temporary paralysis of the muscles responsible for dystonia [7, 8].

An involvement of calcium regulation in dystonia has been discussed at the cellular level. In both animal and human models, accompanying mutations have also been identified [9]. Hypocalcemia is associated with neuromuscular overstimulation, while hypercalcemia causes muscle pain, tenderness, weakness, and muscle spasm [10-12]. Extracellular calcium ions (Ca^{+2}) have been shown to be just as critical in central and peripheral neuronal functions as intracellular calcium ions [13]. Muscle strength and serum magnesium ion (Mg^{+2}), which is involved in muscle contraction and tone maintenance, have been related. Involuntary muscle contractions may occur in hypomagnesemia [14]. Hypermagnesemia, however, blocks calcium channels in all smooth, skeletal and/or cardiac muscle cells [15]. Phosphorus regulates a Ca^{+2}/ATPase pump responsible for muscle contraction and relaxation. An insoluble calcium phosphate precipitate formed by inorganic phosphate reduces Ca^{+2} ion release [16]. The 25-hydroxy (OH) vitamin D is essential for immune function, mineral homeostasis, and bone biology. Controlling the concentration of ionized calcium in extracellular and intracellular compartments is crucial. Hypocalcemia has also been related to a vitamin D deficiency [17]. In addition, muscle weakness may be caused by hypophosphatemia or vitamin D deficiency [18].

Vitamin B_{12} is an essential vitamin and must be supplied in the diet, primarily through foods such as meat, fish, eggs, milk, and liver [19]. Usually, the daily requirement for vitamin B_{12} is approximately 2 mg in adults [20]. Adults store 2-3 mg in their bodies, which means that many years of dietary deficiency is actually necessary until the disorder is clinically evident [21-23]. Vitamin B_{12} deficiency has been linked to a variety of neurological symptoms for over 150 years [24]. Myelopathy, large fiber neuropathy, optic atrophy, dementia, chronic seizures, psychosis, and mood disorders constitute the symptoms of vitamin B_{12} deficiency [25, 26]. Moreover, adults with cerebellar ataxia and extrapyramidal symptoms such as dystonia and chorea have been uncommonly reported [25, 27-30]. Apart from the parameters described above, the relationship between BEB and vitamin B_{12} level has never been investigated before, making the current research the first to determine the potential relationship between BEB and vitamin B_{12} level, among other things.

The aim of this study was to analyze serum calcium, magnesium, phosphorus, 25-(OH) vitamin D and vitamin B_{12} levels in BEB patients to determine whether there was a connection between these values and BEB severity and frequency.

Materials and methods

Study participants

This retrospective case-control study included 20 BEB patients who were followed-up and treated periodically with botulinum toxin injections in Afyonkarahisar Health Sciences University Department of Ophthalmology's Oculoplastic and Reconstructive Surgery Unit and Endocrinology clinics between January 2019 and January 2021. Twenty age- and gender-matched healthy individuals were also included for comparison. The study protocol complied with the ethical principles of the Declaration of Helsinki and received full approval from the institutional review board of Afyonkarahisar Health Sciences University Ethics Committee (Date: 05.02.2021, Acceptance code: 2021/139). Written informed consent was obtained prior to the study.

Inclusion and exclusion criteria

To rule out any potential neurological disorders, all patients underwent complete neurological examination. Patients who were followed up in the ophthalmology clinic with a diagnosis of BEB disease but without prior eyelid or intraocular surgery and/or any systemic disease that might affect eyelid movements were included in the study. However, patients with eyelid disorders secondary to any prior ocular trauma, underlying neurological disorders, prior thyroid and parathyroid surgery that may affect calcium metabolism, use of supplements such as vitamin B_{12}, vitamin D and magnesium, calcium and phosphorus, and presence of Meige Syndrome were excluded from the study.

Jankovic rating scale

The Jankovic rating scale, an existing clinical scale commonly used for grading BEB severity and frequency during the initial assessment and measuring therapeutic outcomes during patient follow-up, was used to determine the severity and frequency of BEB. As shown in Table 1, this scale rates severity and frequency separately, assigning a score of 0 to 4 to each [31]. 0 points are given in case there are no symptoms, while 4 points are given when the severity of BEB symptoms is maximum and most frequent. Total score is obtained from the sum of the two sub-scores. The Jankovic rating scale focuses primarily on the objective manifestations of BEB. However, this scale also includes some subjective symptoms, such as whether it neutralizes increased blinking and spasms due to its assessment by the physician. This clinical scale is relatively simple and can easily be used by both patients and physicians. Measurement

tests may be completed without the use of complicated equipment or scoring procedures. The Jankovic rating scale has some drawbacks, such as a lack of evaluation of how the patient's blepharospasm affects everyday activities and a lack of determination of small changes in BEB severity or frequency.

Table 1: Jankovic rating scale

Severity	Descriptions
0	No symptoms
1	Only under the influence of external stimuli (e.g. bright light, wind, reading, etc.)
2	Mild, spontaneous blinking (without spasms), clearly visible, sometimes troublesome, but without functional impairment
3	Moderate, clearly visible spasms of the eyelids; moderate deterioration
4	Severe, disruptive spasms of the eyelids, possibly involving other facial muscles
Frequency	Descriptions
0	No symptoms
1	Slightly increased flashing frequency
2	Eye flickering with an individual blink time of less than a second
3	Spasms of the eyelids that last more than a second; eyes open more than 50% of waking time
4	Functional blindness caused by prolonged closing of the eyes for more than 50% of the awakening time

Data from the respective groups were assessed retrospectively in terms of age, gender, serum mineral (calcium, magnesium, and phosphorus), 25-(OH) vitamin D and Vitamin B_{12} levels. In our laboratory, the normal ranges for serum calcium, magnesium, and phosphorus were 8.8 to 10.6 mg/dl, 1.8 to 2.6 mg/dl, and 2.5 to 4.5 mg/dl, respectively. Vitamin D deficiency was described as 25-(OH) vitamin D <20 ng/l and vitamin B12 deficiency was described as vitamin B12 <200 pg/ml.

Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences version 21.0 software package (SPSS Inc., Chicago IL, USA). The distribution of data was analyzed with the Kolmogorov Smirnov test. Continuous variables with normal distribution were presented as mean (SD). Categorical data were reported as number (frequency). Student's t test and Chi-square test were used to compare the data. Pearson's correlation analysis was performed for normally distributed data. $P<0.05$ was statistically significant.

Results

Twenty BEB patients and 20 healthy individuals were included in this study. Female-to-male ratios in BEB patients and healthy individuals were 14:6 and 12:8, respectively ($P=0.321$). The mean age was 64.37 (4.21) years in BEB patients and 63.83 (3.13) years in healthy individuals ($P=0.239$). Compared to healthy individuals, BEB patients were associated with statistically significantly lower levels of 25-(OH) vitamin D ($P=0.037$), vitamin B_{12} ($P=0.014$) and calcium ($P=0.011$), respectively. Besides, BEB patients had lower levels of phosphorus and magnesium than healthy individuals, although the difference was not statistically significant ($P=0.680$ and $P=0.340$, respectively) (Table 2).

Correlation analysis

In BEB patients, serum levels of 25-(OH) vitamin D (r=-0.375, $P=0.043$), calcium (r=-0.319, $P=0.039$), and vitamin B12 (r=-0.408, $P=0.027$) were all negatively correlated with the Jankovic severity score. No correlations were observed between the serum parameters of Group 1 patients and the Jankovic frequency score (Table 3).

Table 2: Vitamin and mineral serum levels in the respective study groups

Parameters	BEB patients mean (SD)	Healthy individuals mean (SD)	P-value
25-(OH) vitamin D (ng/ml)	16.58 (7.23)	19.23 (6.37)	0.037
Vitamin B_{12} (pg/ml)	274.11 (107.22)	380.13 (119.09)	0.014
Magnesium (mg/ml)	1.94 (0.36)	1.96 (0.28)	0.340
Phosphorus (mg/dl)	3.53 (0.98)	3.54 (0.66)	0.680
Calcium (mg/dl)	9.32 (0.38)	9.76 (0.29)	0.011

SD: Standard deviation, $P<0.05$ was considered statistically significant.

Table 3: Illustration of the Jankovic frequency and severity scores in BEB patients

Parameters	Jankovic frequency score Pearson's correlation coefficient (r)	P-value	Jankovic severity score Pearson's correlation coefficient (r)	P-value
25-(OH) vitamin D (ng/ml)	0.198	0.092	-0.371	0.043
Vitamin B_{12} (pg/ml)	0.162	0.103	-0.408	0.027
Magnesium (mg/dl)	0.136	0.53	0.208	0.328
Phosphorus (mg/dl)	0.228	0.384	0.271	0.491
Calcium (mg/dl)	0.201	0.168	-0.319	0.039

BEB: Benign esseantial blepharospasm; Pearson's correlation coefficient was considered significant if within 0.300-0.700; $P<0.05$ was considered statistically significant.

Discussion

In this study, BEB patients had significantly lower serum calcium and 25-(OH) vitamin D and insignificantly lower serum magnesium, and phosphorus levels compared to healthy individuals. In addition, the Jankovic severity score was strongly negatively correlated with serum calcium and 25-(OH) vitamin D in BEB patients. Few studies investigating serum calcium, magnesium, phosphorus, and/or 25-(OH) vitamin D levels in BEB patients have been reported with mixed results [32].

Most importantly, in addition to the above parameters, this study included, as far as we understand, the first assessment of serum vitamin B_{12} levels in these patients. In this regard, relative to healthy individuals, BEB patients had significantly lower serum vitamin B_{12} levels. Besides, in BEB patients, the Jankovic severity score was strongly negatively associated with serum vitamin B_{12}.

The precise mechanisms of BEB etiology are yet to be discovered. Involuntary spasms of the orbicularis oculi muscles often involving the corrugator supercilii and procerus, identified as eyelid protractor muscles, have been strongly correlated with BEB features [6]. The primary form of BEB has a gradual onset and occurs most frequently in middle-aged females, while secondary form is associated with BG, brainstem and thalamus lesions. Focal dystonia is one of the important distinctive findings of BEB. Dystonia is defined as an isolated or generalized disproportional contraction of muscles. It is a neurological movement disorder that results in forceful body bending, repetitive movements and/or sometimes painful abnormal postures [9, 33]. Its pathogenesis has been linked to disorders in BG and cerebellar circuits in the motor network with dyshomeostasis in mitochondrial dysfunction and dopamine as well as calcium signaling [8, 33].

Investigation of the relationship between acute dystonic reactions and serum calcium in 17 acute psychotic patients with non-primary dystonia reported no association between acute dystonia and serum calcium [34]. Also, while calcifications in BG, cerebellum, thalamus, and cerebral white matter have been identified in patients with autosomal dominant dystonia, serum calcium levels in these patients were within normal limits [35]. In this study, on the other hand, serum calcium levels in patients with BEB decreased significantly compared to healthy controls.

However, none of them had hypocalcemia and/or associated neuro-radiological findings. In view of this, although these findings demonstrate the relationship among dystonia, BG and Ca^{+2} homeostasis, the exact pathophysiology remains undisclosed.

The role of 25-(OH) vitamin D in muscle strength and contraction has been reported [36]. In 25-(OH) vitamin D deficiency, muscle strength may be maintained by increasing plasma phosphorus levels, supporting the notion that 25-(OH) vitamin D alone does not play a role in the function or activity of the skeletal muscles [37, 38]. However, the association between low 25-(OH) vitamin D and muscle weakness in the elderly has been reported in epidemiological studies [37, 39]. Further, the association of 25-(OH) vitamin D and phosphate metabolism has been known since Nicolaysen demonstrated an improvement in phosphate absorption in response to vitamin D [40]. Vitamin D increases plasma phosphate in low phosphate intake [41]. It is noteworthy that phosphate deficiency affects muscles with a slower metabolism and produces a slower sustainable response [42, 43]. Although little or no effect due to rapid metabolism has been observed in the fast-twitch muscle, the slow-twitch muscles have been associated with a higher concentration of phosphate use for a maximum muscle function. Indirect effect of 25-(OH) vitamin D on skeletal muscle has also been reported in some studies [18]. Vitamin D increases the intestinal absorption of phosphorus. However, a decrease in muscle strength is inevitable as the absorption of phosphorus decreases in 25-(OH) vitamin D deficiency. Although it remains unclear whether 25-(OH) vitamin D or phosphorus is directly responsible for muscle weakness, it is clear that 25-(OH) vitamin D plays a positive role in the metabolism of phosphorus. Similarly, in this study, levels of 25-(OH) vitamin D and phosphorus were lower in BEB patients compared to age- and gender-matched healthy individuals with statistically significant decreases in 25-(OH) vitamin D and non-significant decreases in serum phosphorus. The direct proportional decrease of these two parameters confirms the previous reports on their interrelated metabolism and the subsequent muscle physiology in dystonia or BEB.

Extrapyramidal involvement attributable to vitamin B_{12} deficiency is relatively uncommon in adults, with which the deficiency has been reported to be associated with focal dystonia, chorea, myoclonus, Parkinsonism and even ataxia [26, 28, 44, 45]. No prior reports of BEB in adults associated with vitamin B_{12} deficiency have been reported. In this study, however, in addition to significantly lower serum 25-(OH) vitamin D and calcium levels, BEB patients had significantly lower serum vitamin B_{12} levels compared to age and gender-matched health individuals. Moreover, the correlation analysis revealed a statistically significant negative correlation between vitamin B_{12} along with 25-(OH) vitamin D, calcium and the Jankovic severity score. These findings may indicate a potential collective relationship between serum vitamin B_{12} and BEB in adults, in addition to serum 25-(OH) vitamin D and calcium.

The mechanism for extrapyramidal involvement in serum vitamin B_{12} deficiency is not well comprehended. Vitamin B_{12} deficiency is the most frequent cause of hyperhomocysteinemia. Homocysteine is necessary for methionine methylation. It also possesses N-methyl-D-aspartate

agonistic effect. By acting on the thalamo-cortical pathway, homocysteine may trigger excitatory activity in BG leading to dystonia and chorea [46]. Further, excess methyl level in B_{12} deficiency results in an increased e-methyl tetrahydrofolate level, which is characterized by kainic acid agonistic effect. Kainate has been noted to cause harm comparable to that seen in Huntington's disease in experimental animals [47]. Furthermore, methylmalonic acidemia, an inborn metabolism error, is typically associated with acute extrapyramidal syndrome in infants [48]. In patients with dystonia, increased plasma levels of homocysteine have been reported [19]. It has therefore been suggested that a high level of homocysteine may contribute to the onset and severity of dystonia, and that routine plasma homocysteine testing and treatment of these patients for hyperhomocysteinemia should also be recommended.

The authors recognize the drawbacks of this study. First, the retrospective nature of this study has limited the ability of the authors to discover prospective processes in relation to the association between BEB and serum levels of minerals and vitamins, particularly serum vitamin B_{12}. Second, homocysteine analysis was not performed in this study, which could allow prompt diagnosis of vitamin B12 deficiency. Third, residual influencing factors might have led to an unexplained analytical preference. Moreover, the size of the study population was just not high enough to improve the efficacy of the study.

While there are some drawbacks in this study, there are some strengths as well. As far as we know, in addition to serum calcium, magnesium, phosphate and 25-(OH) vitamin D, this may be the first study in which serum level of vitamin B_{12} was assessed in relation to BEB in a sample of patients and control subjects of the same ethnicity. There is a significant role of ethnicity in the selection of patients and control groups, even though extensive investigation has not yet been undertaken. The high average age of the study participants can foreseeably influence the outcome of BEB from the undiagnosed age-related neuro-degenerative conditions. Fortunately, all study groups were about the same age, which might at some stage alleviate the inherent preference of this study's clinical outcomes.

Conclusion

To conclude, in conjunction with other factors, whether vitamin B12 deficiency and BEB are coincidentally or causally related is not yet determined. The improvement in BEB after only cyanocobalamine supplementation is intriguing, implying a potential causal relationship. Thus, BEB may be a rare manifestation of vitamin B12 deficiency, and early detection is critical for reversing the associated hematological and neurological dysfunction. Significantly lower serum levels of 25-(OH) vitamin D and calcium were also found in BEB patients, as well as a strong negative correlation with disease severity. Moreover, serum magnesium and phosphorus levels were lower in BEB patients as compared to healthy individuals, though the difference was not significant. Long-term prospective studies may yield clinically valuable results in determining the role of calcium regulation and vitamin D involvement in BEB pathophysiology and changes in ion concentrations in BG, cerebrospinal fluid, serum, and orbicularis oculi muscle. In the absence of an apparent cause for BEB, serum vitamin B12 levels,

in addition to serum minerals and 25-(OH) vitamin D, may be worth testing.

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