

High-dose vitamin B supplementation for persistent visual deficit in multiple sclerosis: a pilot study

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SUMMARY The aim of this study is to investigate the potential neuroprotective effect of high-doses vitamins B1, B6 and B12 in patients with relapsing-remitting multiple sclerosis (RRMS) and persistent visual loss after acute optic neuritis (AON). Sixteen patients (20 eyes) diagnosed with RRMS and visual permanent disability following AON were enrolled for the present open, pilot study. Each patient was treated with oral high-doses 300 mg of vitamin B1, 450 mg of vitamin B6 and 1,500 mcg of vitamin B12, as add-on treatment to concomitant disease-modifying therapies (DMTs) for consecutive 90 days. Outcome measures were to determine changes from baseline to month three in visual acuity (VA) and visual field (VF) testing, with correlations with clinical parameters. Logistical regression was performed to evaluate predictors of final VA. A statistically significant improvement was registered in visual acuity ($p = 0.002$) and foveal sensitivity threshold (FT) ($p = 0.006$) at follow-up compared to baseline. A similar trend was demonstrated for mean deviation (MD) ($p < 0.0001$), and pattern standard deviation (PSD) ($p < 0.0001$). Age at the time of inclusion was positively correlated with latency time ($\rho = 0.47$, $p = 0.03$), while showing a negative correlation with visual acuity ($\rho = -0.45$, $p = 0.04$) and foveal sensitivity threshold ($\rho = -0.6$, $p = 0.005$) at follow up. A statistically significant correlation was demonstrated between foveal sensitivity threshold and visual acuity at baseline ($\rho = 0.79$, $p < 0.0001$). In a linear regression model, the main predictor of visual acuity at follow up was the foveal sensitivity threshold ($B = 1.39$; $p < 0.0001$). Supplemental high-dose vitamins B1, B6 and B12 resulted as effective therapy to improve visual function parameters in MS-related visual persistent disability.

Keywords B Vitamins group, visual function, neuroprotection

1. Introduction

Multiple sclerosis (MS) is an autoimmune neurodegenerative disorder associated with chronic inflammation of the central nervous systems (CNS), resulting in characteristic demyelination and axonal damage. With an increased incidence between 20 and 40 years, it represents the most common acquired disabling neurological disease in young adults, and females appear to be more frequently diagnosed than males by 2-3:1 ratio. MS affects approximately 2.5 million people worldwide, with a tendency of prevalence data to considerably differ from one country to another, and registered highest percentages in North America (140 per 100.000) and Europe (108 per 100.000) (1-3). Although the etiopathogenesis is still unclear, genetic profiles predominantly involving immune response genes (*HLA-DRB1*, *HLA-DPB1*, *HLA-A TNF* genes), as well as inflammatory and environmental factors including

Epstein-Barr virus infection, tobacco exposure, vitamin deficiencies and high saturated fat/carbohydrate diet are currently recognized among the most relevant causative agents (4-9).

The main efforts in MS therapy are primarily directed at developing immunomodulatory and immunosuppressive agents for reducing the frequency of new episodes, however, the persistence of symptoms in most patients, drugs side effects and limited long-term efficacy emphasized the relevance for complementary treatments.

The B vitamins are known to effectively modulate the repair and maintenance of lipids, including myelin, and to protect against axonal hypoxia through enhanced ATP production. Of note, their deficiency appear to have detrimental effects in terms of occurrence and progression of multiple sclerosis (10-14). In recent years, B vitamins supplementation has attracted growing interest as far as incidence, progression and potential

reversal in MS, demonstrating improved neurological conditions even in absence of documented deficiency (15-23). To date, limited clinical studies included sensitive measures of visual function to evaluate vitamin B efficacy at restoring neurological damage in patients with MS (21-23). High-dose vitamin B12 effectively improved visual evoked potentials (VEP) as evaluated in 6 patients with chronic progressive MS on concomitant disease modifying therapies (DMTs) (21). Unlike favorable preliminary results, no significant improvement in visual acuity was indeed observed in a recent randomized controlled trial on high-dose biotin in patients suffering from progressive MS-related visual disability on DMTs (22,23). Thus, the re-myelinating effects of B vitamins are somewhat promising for irreversible optic nerve damage in MS, but currently lack of sufficient insight and decisive results.

Moreover, in spite of the reported neurologic synergistic effects of B1 (thiamine), B6 (pyridoxine), and B12 (cyanocobalamin) (24-27), there is no available data on their concomitant use in MS. Furthermore, there is no literature evaluating visual function in relapsing-remitting MS type (RRMS) on B vitamins supplementation.

The present study was designed to investigate the efficacy of three months-treatment with high-dose vitamins B1, B6, and B12 in combination, as adjunctive to immunomodulatory therapy in eligible patients diagnosed with RRMS, to improve visual function parameters in persistent visual loss following acute optic neuritis (AON).

2. Materials and Methods

A pilot study was carried out on 16 consecutive patients (20 eyes) (13 F/3 M), mean age 36.31 years \pm 7.34 standard deviation (SD), with a diagnosis of relapsing-remitting (RR) MS according to the 2017 revised McDonald criteria, and presenting with a history of one or more previous episodes of AON and persistent impairment in visual symptoms and visual field testing (28). Patients were enrolled from May 2019 to October 2019 at the University of Rome 'Sapienza', Umberto I Hospital, Italy.

Patients were all stable on disease modifying therapies (interferon- β -1b, glatiramer acetate, fumarate, and teriflunomide) at the time of inclusion, and in course medications were allowed throughout the study.

Exclusion criteria were: (1) previous diagnosis of glaucoma and any other optic neuropathy; (2) poor reliability indices of visual field tests (scores exceeding 20% of fixation losses or false negative/false positive errors); (3) any other previous or coexisting ocular condition that could interfere with functional outcomes including *i.e.* media opacities, amblyopia, uveitis, congenital ocular malformations, retinal and macular diseases, refractive defects greater than ± 6 D

(spherical equivalent); (4) patients who had undergone intraocular surgery, other than cataract surgery, more than the preceding 6 months; (5) bilateral VA \leq 1/20; (6) relapse (among which acute optic neuritis) occurred less than 6 months before inclusion; (7) unstable VA in the 6 months prior to inclusion; (8) treatment for MS introduced $<$ 3 months prior to inclusion.

Each patient underwent a comprehensive ophthalmological examination at baseline and at scheduled 3-months follow-up time, including clinical history, measurement of the best-corrected visual acuity by using the standardized, 70-letter Early Treatment Diabetic Retinopathy Study chart (Chart 'R', Precision Vision, La Salle, IL, USA) at 4m distance, evaluation of intraocular pressure by using the Goldmann applanation tonometry, slit-lamp biomicroscopy, mydriatic indirect fundus biomicroscopy, and automated perimetry with the Humphrey Field Analyzer (Carl Zeiss Meditec, Dublin, CA, USA) and Swedish Interactive Threshold Algorithms standard strategy (program 30-2). The parameters evaluated on visual field testing were mean deviation (MD, dB), pattern standard deviation (PSD, dB) and foveal sensitivity threshold (FS, dB). Two consecutive visual field tests were carried out for each patient, and only the second was considered for the aim of the present study. The examination of patients, along with the interpretation of instrumental results, was performed by two independent experienced ophthalmologists. Acquisition of instrumental data was conducted by certified optometrists. The time elapsing between the last episode of acute optic neuritis to the time of inclusion was calculated for each patient and expressed as 'latency time'.

All patients were treated with oral high-doses of 300 mg vitamin B1 (thiamine), 450 mg vitamin B6 (pyridoxine) and 1500 mcg vitamin B12 (cyanocobalamin) administered as three capsules daily for 90 consecutive days. Employed dosages appear as safe for short-term periods as per current knowledge (29-31). No additional drugs other than ongoing immunomodulatory agents and vitamin B complex were introduced during the period of treatment.

The study was approved by the Ethics Committee of the Sapienza University of Rome. The study followed the tenets of the Declaration of Helsinki and all patients signed informed consent.

3. Results

Data were collected on a Microsoft Office Excel worksheet. The normality of data was assessed by Shapiro-Wilk test. Correlations were assessed by using calculation Spearman's rho. Differences between unpaired variables were assessed through Mann-Whitney test, while differences between paired variables were performed by Wilcoxon test. A linear regression model to evaluate predictors of visual acuity at follow-

up was produced. A value of $p \leq 0.05$ was considered statistically significant. Intraobserver and interobserver agreement was evaluated with the K Cohen Coefficient. The statistical analysis was performed using Graph Pad vers. 8.0.2 and SPSS vers. 24 on the Windows 10 Home edition platform. None of the enrolled patients experienced any serious adverse event during the whole follow-up period. Results from all visual field examinations performed on the affected eye at baseline were abnormal, and most consisted of central visual field defects, according to current literature. In detail, central or cecentral-type scotomas were detected in the majority of patients (12 patients, 14 eyes), whereas non-central scotomas such as paracentral and arcuate defects, were identified in 4 patients and 6 eyes.

There was a statistically significant improvement of visual acuity (44.8 ± 11.7 vs. 50.3 ± 12.4 ; $p = 0.002$) and foveal sensitivity threshold (FT) (28.2 ± 7.4 vs. 30.6 ± 6.2 ; $p = 0.006$) at follow-up (T1) if compared to baseline (Figures 1 and 2). Similarly, there was a statistical significant reduction of mean deviation (MD) (-7.12 ± 4.9 vs. -5.45 ± 4.3 ; $p < 0.0001$), and PSD (5.78 ± 3.5 vs. 3.88 ± 2.5 ; $p < 0.0001$) at follow-up in comparison to baseline (Figures 3 and 4). Age at the time of inclusion showed a statistically significant correlation with the latency time ($\rho = 0.47$, $p = 0.03$), with visual acuity ($\rho = -0.45$, $p = 0.04$) and with foveal sensitivity threshold ($\rho = -0.6$, $p = 0.005$) at follow up. A positive correlation was demonstrated between foveal sensitivity threshold and VA at baseline ($\rho = 0.79$, $p < 0.0001$).

The results of the bivariate correlations are shown in

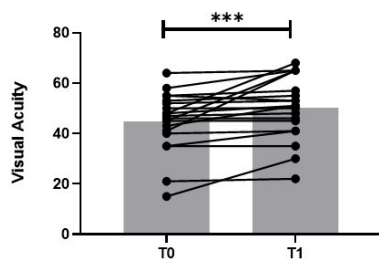


Figure 1. Comparison between visual acuity of enrolled subjects at baseline and follow-up. *** $p = 0.002$.

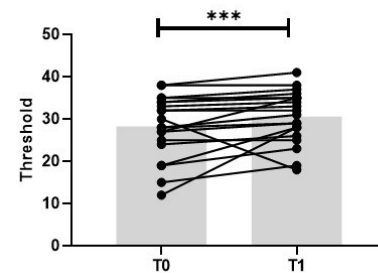


Figure 2. Comparison between foveal threshold of enrolled subjects at baseline and follow-up. *** $p = 0.006$.



Figure 3. Comparison between mean deviation at baseline and follow-up. *** $p < 0.001$.

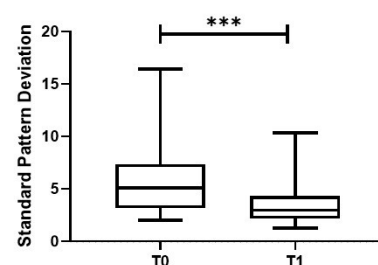


Figure 4. Comparison between pattern standard deviation at baseline and follow-up. *** $p < 0.001$.

Table 1. In a linear regression model, the main predictor of visual acuity at follow up was the foveal sensitivity threshold, which retained a statistically significant positive association ($B = 1.39$; $CI (95\%) = 0.93-1.85$; $p < 0.0001$). The intraobserver and interobserver agreement in clinical and instrumental assessment was excellent ($Kappa = 0.92$).

4. Discussion

Our results, based on clinical and automated perimetry data, demonstrated that daily administration of high doses vitamin B1 (thiamine), vitamin B6 (pyridoxine) and vitamin B12 (cyanocobalamin) for consecutive 90 days significantly improved visual acuity, FT, MD and PSD in patients with RRMS and visual permanent disability following documented episodes of AON. The disease generally manifests itself thorough time-varying attacks of neurological dysfunction with sensory, motor, autonomic, cognitive, or neuropsychiatric symptoms, followed by partial or complete recovery (32,33). AON is an inflammatory and demyelinating disorder of the optic nerve that occurs in up to 50% of patients with MS, and distinctively presents itself as an acute monocular loss of vision, though it can possibly show bilateral simultaneous or sequential involvement (34-36).

The pivotal field defect in AON is a widespread depression of sensitivity, and visual field testing typically reveals a central scotoma, although bitemporal hemianopia, paracentral scotoma, arcuate and altitudinal deficits have also been reported. Vision

Table 1. Spearman's rho correlation

Items	Age	Latency Time	VA0	VA1	FT0	FT1	MD0	MD1	PSD0	PSD1
Spearman's rho										
Age										
Correlation Coefficient	1.000	0.473*	-0.250	-0.452*	-0.522*	-0.600**	0.121	-0.126	0.112	0.320
Sig.2 (2-tailed)		0.035	0.289	0.045	0.018	0.005	0.612	0.596	0.639	0.169
N	20	20	20	20	20	20	20	20	20	20
Latency Time										
Correlation Coefficient	0.473*	1.000	-0.234	-0.350	-0.343	-0.543*	0.005	-0.212	0.277	0.460*
Sig.2 (2-tailed)	0.035		0.320	0.130	0.130	0.013	0.982	0.371	0.237	0.041
N	20	20	20	20	20	20	20	20	20	20
VA0										
Correlation Coefficient	-0.250	-0.234	1.000	0.706**	0.791**	0.495*	0.248	0.210	-0.340	-0.370
Sig.2 (2-tailed)	0.289	0.320		0.001	0.000	0.026	0.291	0.374	0.164	0.109
N	20	20	20	20	20	20	20	20	20	20
VA1										
Correlation Coefficient	-0.452*	-0.350	0.706**	1.000	0.791**	0.756**	0.413	0.540*	-0.288	-0.573**
Sig.2 (2-tailed)	0.045	0.130	0.001		0.000	0.000	0.070	0.014	0.218	0.008
N	20	20	20	20	20	20	20	20	20	20
FT0										
Correlation Coefficient	-0.522*	-0.343	0.791**	0.791**	1.000	0.838**	0.271	0.374	-0.405	-0.648**
Sig.2 (2-tailed)	0.018	0.130	0.000	0.000		0.000	0.247	0.104	0.077	0.002
N	20	20	20	20	20	20	20	20	20	20
FT1										
Correlation Coefficient	-0.600**	-0.543*	0.495*	0.756**	0.838**	1.000	0.122	0.344	-0.325	-0.707**
Sig.2 (2-tailed)	0.005	0.013	0.026	0.000	0.000		0.608	0.138	0.162	0.000
N	20	20	20	20	20	20	20	20	20	20
MD0										
Correlation Coefficient	0.121	0.005	0.248	0.413	0.271	0.122	1.000	0.851**	-0.558*	-0.432
Sig.2 (2-tailed)	0.612	0.982	0.291	0.070	0.247	0.608		0.000	0.011	0.057
N	20	20	20	20	20	20	20	20	20	20
MD1										
Correlation Coefficient	-0.126	-0.212	0.210	0.540*	0.374	0.344	0.851**	1.000	-0.463*	-0.551*
Sig.2 (2-tailed)	0.596	0.371	0.374	0.014	0.104	0.138	0.000		0.040	0.012
N	20	20	20	20	20	20	20	20	20	20
PSD0										
Correlation Coefficient	0.112	0.277	-0.340	-0.288	-0.405	-0.325	-0.558*	-0.463*	1.000	0.752**
Sig.2 (2-tailed)	0.639	0.237	0.164	0.218	0.077	0.162	0.011	0.040		0.000
N	20	20	20	20	20	20	20	20	20	20
PSD1										
Correlation Coefficient	0.320	0.460*	-0.370	-0.573**	-0.648**	-0.707**	-0.432	-0.551*	0.752**	1.000
Sig.2 (2-tailed)	0.169	0.041	0.109	0.008	0.002	0.000	0.057	0.012	0.000	
N	20	20	20	20	20	20	20	20	20	20

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed). VA: Visual Acuity, FT: Foveal Sensitivity Threshold, MD: Mean Deviation, PSD: Pattern Standard Deviation.

loss typically extends over hours or days, and peaks within 1 or 2 weeks. The maximum recovery after the peak is usually reached within the first 3 months and rarely continues beyond 6 months following a relapse (34,35,37). Currently, MS treatment only relies on immunomodulatory therapeutics aimed at reducing the frequency of new episodes, and the introduction of complementary neuroprotective approaches directly acting on neuronal damage still remains challenging. In recent years, vitamins and dietary supplements have attracted growing interest as far as incidence, progression and potential reversal in MS (15-23,38-40).

The B vitamins refer to a biochemically heterogeneous group of water soluble molecules: B1 (thiamine), B2 (riboflavin), B3 (nicotinamide), B5 (pantothenic acid), B6 (pyridoxine), B7 (biotin), B9 (folic acid), B12 (cyanocobalamin), required as

cofactors for the metabolism of fatty acids, amino acids, neurotransmitters, myelin, and nucleic acids, and for providing energy to support neurons in such mechanisms. In particular, vitamin B1, B6, and B12 have demonstrated to considerably contribute to neuro-immune homeostasis and appeared to be firmly involved in MS pathogenesis (41). Intriguingly, vitamin B12 deficiency and MS share similarities in terms of clinical presentation, including megaloblastic anemia or macrocytosis, and MRI findings (38, 42).

A synergistic effect of vitamins B1, B6, and B12 for improving neurologic functions has been demonstrated based on overlap in several biochemical pathways (24-27,41). Vitamin B12 operates as a cofactor for methylmalonyl coenzyme A (CoA) mutase (MCM), an enzyme that catalyzes the reversible isomerization of l-methylmalonyl-CoA to succinyl-CoA. If MCM turns

out to be defective, it occurs an upstream accumulation of methylmalonyl CoA and its precursor, propionyl CoA, with subsequent propionyl CoA replacing succinyl CoA in the Krebs cycle. As a result, effective long-chain lipid synthesis is hindered, and aberrant short lipid chains end up constituting the myelin sheath (41). In addition, vitamin B12 exerts a decisive role in the homocysteine remethylation process to methionine within folate metabolism, and its deficiency is thereby associated with reduced synthesis of phospholipids and increased levels of serum homocysteine. In accordance, increased plasma homocysteine levels in MS patients have been reported (43,44). Moreover, homocysteine can be removed by conversion to cysteine (amino-acid having role in myelin formation) with vitamin B6 as a cofactor, this underlying the strict interplay between B6 and B12 vitamins (45). Furthermore, vitamin B6 serves as a cofactor in sphingolipid and neurotransmitter synthesis and in free radicals scavenging activity (46,47). Similarly, studies suggest that vitamin B1 is involved in the synthesis of myelin, nucleic acids, and several types of neurotransmitters, and shows antioxidant activity (41). B12 in addition to interferon-beta improved demyelination and reduced astrogliosis, resulting in near normal motor function in experimental autoimmune encephalomyelitis (EAE) mice (15). Similar results were achieved with B12 supplementation in combination with paclitaxel in EAE animals (16). Intramuscular B12 administration for 24 weeks in 138 patients with MS resulted in improvement by 2 Guy's neurological disability scale (GNDS) points in a randomized placebo controlled, double blind study (19). In a pilot study on 15 patients with MS in remitting phase experiencing fatigue, high doses thiamine resulted in fatigue regression despite normal blood thiamine levels (17). Despite mentioned encouraging results, a few studies investigated visual function parameters as manageable model for evaluating the potential neuroprotective effects of B vitamins in MS. It has been estimated that one-third of MS patients show persistent visual symptoms after AON, with significant reduction in quality of life (48). The effects of massive vitamin B12 supplementation (oral 60 mg/day for 6 months and intravenous 5 mg vitamin B 12 every day for 14 days before oral administration in 3 patients) in 6 patients with progressive MS on either low dose prednisolone or azathioprine, resulted in improved visual and brainstem auditory evoked potentials at 3 and 6 months follow-up, with bilateral re-appearance of P100 wave after treatment in one case (21). None of the patients had low serum vitamin B12 levels when entering the study and no one experienced any adverse effect. An open-label pilot study based on high-dose biotin administration (oral 300 mg/day for a mean duration of 9.2 months) in progressive MS, showed encouraging preliminary results, inclusive of visual function assessment, in terms of reversing disease progression and reducing chronic disability (22). However, in a recent

randomized 6-months controlled trial, high-dose biotin did not significantly improve visual acuity in MS-related chronic visual loss compared to placebo, with 70.7% of patients on concomitant DMTs (23). We reported a statistically significant improvement of visual acuity ($p = 0.002$) and foveal sensitivity threshold ($p = 0.006$) at 3-months follow-up time (T1) compared to baseline (T0). Similarly, a significant reduction was observed for MD ($p < 0.0001$), and PSD ($p < 0.0001$) at follow-up, in comparison to baseline. Interestingly, age at the time of inclusion showed a statistically significant correlation with the latency time ($\rho = 0.47, p = 0.03$) at follow up. This could be explained by the fact that patients younger at onset have demonstrated to experience larger number of relapses before progression, in accordance with literature (49). A positive correlation was demonstrated between foveal sensitivity threshold and VA at baseline ($\rho = 0.79, p < 0.0001$), and it appears as interesting information since there are described cases with FT decline and relatively good visual acuity in the context of subclinical optic neuritis or cases with good FT measurements but very reduced vision as per involvement of factors other than optic neuropathy (50). Linear regression analysis showed that baseline foveal sensitivity threshold was the only variable significantly correlated with final visual acuity, with a statistically significant positive association ($B = 1.39$; $CI (95\%) = 0.93-1.85$; $p < 0.0001$). We speculate that the relevance of foveal threshold for prediction of visual outcome could be related to the frequent involvement of central visual field in MS patients, and accordingly in our sample.

In conclusion, the current work indicates the possible effectiveness of high-dose B1, B6 and B12 vitamins in patients with MS-related visual permanent disability. The favorable findings from our open, pilot study might open new perspectives for reaching placebo-controlled randomized trials based on B vitamins, and particularly B1, B6 and B12 in combination, as complementary therapeutic against RRMS visual impairment. The results so far presented on vitamin B12 and biotin in progressive MS then, could allow for extending the investigation to progressive forms of disease. An insight on biochemical pathways through which B vitamins promote re-myelination and inhibit inflammation in neurodegenerative diseases including MS, clearly indicates their potential to influence the course of disease by improving and even reversing neurological fixed and disabling damage. Therefore, novel neuroprotective approaches are expected to support existing relapse-preventative strategies in MS, with regard to optic nerve axonal and neuronal degeneration and related impairment in visual quality of life.

Ethics Approval and Consent to Participate:

All procedures performed in studies involving human participants were in accordance with the ethical

standards of the institutional research committee of Sapienza University of Rome and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

Consent for publication:

Informed consent to publish personal or clinical details along with any identifying images was obtained from study patients.

Data Availability Statement:

The data used to support the findings of this study are available from the corresponding author upon request.

Funding Statement:

No financial support has been requested for the study.

References

- Browne P, Chandraratna D, Angood C, Tremlett H, Baker C, Taylor BV, Thompson AJ. Atlas of Multiple Sclerosis 2013: A growing global problem with widespread inequity. *Neurology*. 2014; 83:1022-1024.
- Thompson AJ. Multiple sclerosis--a global disorder and still poorly managed. *Lancet Neurol*. 2008; 7:1078-1079.
- Yan K, Balijepalli C, Desai K, Gullapalli L, Druyts E. Epidemiology of pediatric multiple sclerosis: A systematic literature review and meta-analysis. *Mult Scler Relat Disord*. 2020; 44:102260.
- Marrosu MG, Cocco E, Costa G, Murru MR, Mancosu C, Murru R, Lai M, Sardu C, Contu P. Interaction of loci within the HLA region influences multiple sclerosis course in the Sardinian population. *J Neurol*. 2006; 253:208-213.
- Patsopoulos NA, Barcellos LF, Hintzen RQ, *et al*. Fine-mapping the genetic association of the major histocompatibility complex in multiple sclerosis: HLA and non-HLA effects. *PLoS Genet*. 2013; 9:e1003926.
- Armon-Omer A, Waldman C, Simaan N, Neuman H, Tamir S, Shahien R. New insights on the nutrition status and antioxidant capacity in multiple sclerosis patients. *Nutrients*. 2019; 11:427.
- Nebbioso M, Scarsella G, Tafani M, Pescosolido N. Mechanisms of ocular neuroprotection by antioxidant molecules in animal models. *J Biol Regul Homeost Agents*. 2013; 27:197-209.
- Silva JP, Coutinho OP. Free radicals in the regulation of damage and cell death - basic mechanisms and prevention. *Drug Discov Ther*. 2010; 4:144-167.
- Pescosolido N, Barbato A, Pascarella A, Giannotti R, Genzano M, Nebbioso M. Role of protease-inhibitors in ocular diseases. *Molecules*. 2014; 19:20557-20569.
- Nijst TQ, Wevers RA, Schoonderwaldt HC, Hommes OR, de Haan AF. Vitamin B12 and folate concentrations in serum and cerebrospinal fluid of neurological patients with special reference to multiple sclerosis and dementia. *J Neurol Neurosurg Psychiatry*. 1990; 53:951-954.
- Ji Z, Fan Z, Zhang Y, Yu R, Yang H, Zhou C, Luo J, Ke ZJ. Thiamine deficiency promotes T cell infiltration in experimental autoimmune encephalomyelitis: the involvement of CCL2. *J Immunol*. 2014; 193:2157-2167.
- Lanska DJ. Chapter 30: historical aspects of the major neurological vitamin deficiency disorders: the water-soluble B vitamins. *Handb Clin Neurol*. 2010; 95:445-476.
- Rawji KS, Young AMH, Ghosh T, *et al*. Correction to: Niacin-mediated rejuvenation of macrophage/microglia enhances remyelination of the aging central nervous system. *Acta Neuropathol*. 2020; 139:911.
- Bubshait DK, Rashid A, Al-Owain MA, Sulaiman RA. Depression in adult patients with biotin responsive basal ganglia disease. *Drug Discov Ther*. 2016; 10:223-225.
- Mastronardi FG, Min W, Wang H, Winer S, Dosch M, Boggs JM, Moscarello MA. Attenuation of experimental autoimmune encephalomyelitis and nonimmune demyelination by IFN-beta plus vitamin B12: treatment to modify notch-1/sonic hedgehog balance. *J Immunol*. 2004; 172:6418-6426.
- Mastronardi FG, Tsui H, Winer S, Wood DD, Selvanantham T, Galligan C, Fish EN, Dosch HM, Moscarello MA. Synergy between paclitaxel plus an exogenous methyl donor in the suppression of murine demyelinating diseases. *Mult Scler*. 2007; 13:596-609.
- Costantini A, Nappo A, Pala MI, Zappone A. High dose thiamine improves fatigue in multiple sclerosis. *BMJ Case Rep*. 2013; 2013:bcr2013009144.
- Birnbaum G, Stulc J. High dose biotin as treatment for progressive multiple sclerosis. *Mult Scler Relat Disord*. 2017; 18:141-143.
- Wade DT, Young CA, Chaudhuri KR, Davidson DL. A randomised placebo controlled exploratory study of vitamin B-12, lofepramine, and L-phenylalanine (the "Cari Loder regime") in the treatment of multiple sclerosis. *J Neurol Neurosurg Psychiatry*. 2002; 73:246-249.
- Miller A, Korem M, Almog R, Galboiz Y. Vitamin B12, demyelination, remyelination and repair in multiple sclerosis. *J Neurol Sci*. 2005; 233:93-97.
- Kira J, Tobimatsu S, Goto I. Vitamin B12 metabolism and massive-dose methyl vitamin B12 therapy in Japanese patients with multiple sclerosis. *Intern Med*. 1994; 33:82-86.
- Sedel F, Papeix C, Bellanger A, Touitou V, Lebrun-Frenay C, Galanaud D, Gout O, Lyon-Caen O, Tourbah A. High doses of biotin in chronic progressive multiple sclerosis: a pilot study. *Mult Scler Relat Disord*. 2015; 4:159-169.
- Tourbah A, Gout O, Vighetto A, *et al*. MD1003 (High-dose pharmaceutical-grade biotin) for the treatment of chronic visual loss related to optic neuritis in multiple sclerosis: A randomized, double-blind, placebo-controlled study. *CNS Drugs*. 2018; 32:661-672.
- Wang ZB, Gan Q, Rupert RL, Zeng YM, Song XJ. Thiamine, pyridoxine, cyanocobalamin and their combination inhibit thermal, but not mechanical hyperalgesia in rats with primary sensory neuron injury. *Pain*. 2005; 114:266-277.
- Bonke D, Nickel B. Improvement of fine motoric movement control by elevated dosages of vitamin B1, B6, and B12 in target shooting. *Int J Vitam Nutr Res Suppl*. 1989; 30:198-204.
- Leuschner J. Antinociceptive properties of thiamine, pyridoxine and cyanocobalamin following repeated oral administration to mice. *Arzneimittelforschung*. 1992; 42:114-115.
- Hakim M, Kurniani N, Pinzon R, Tugaworo D, Basuki M, Haddani H, Pambudi P, Fithrie A, Wuysang A.

- Management of peripheral neuropathy symptoms with a fixed dose combination of high-dose vitamin B1, B6 and B12: A 12-week prospective non-interventional study in Indonesia. *Asian J Med Sci.* 2018; 9:32.
28. Thompson AJ, Banwell BL, Barkhof F, *et al.* Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. *Lancet Neurol.* 2018; 17:162-173.
 29. Smithline HA, Donnino M, Greenblatt DJ. Pharmacokinetics of high-dose oral thiamine hydrochloride in healthy subjects. *BMC Clin Pharmacol.* 2012; 12:4.
 30. Parra M, Stahl S, Hellmann H. Vitamin B₆ and its role in cell metabolism and physiology. *Cells.* 2018; 7:84.
 31. Kennedy DO. B vitamins and the brain: mechanisms, dose and efficacy – A review. *Nutrients.* 2016; 8:68.
 32. Fisniku LK, Brex PA, Altmann DR, Miszkiel KA, Benton CE, Lanyon R, Thompson AJ, Miller DH. Disability and T2 MRI lesions: a 20-year follow-up of patients with relapse onset of multiple sclerosis. *Brain.* 2008; 131:808-817.
 33. Oh J, Vidal-Jordana A, Montalban X. Multiple sclerosis: clinical aspects. *Curr Opin Neurol.* 2018; 31:752-759.
 34. Pirko I, Blauwet LA, Lesnick TG, Weinshenker BG. The natural history of recurrent optic neuritis. *Arch Neurol.* 2004; 61:1401-1405.
 35. Hoorbakht H, Bagherkashi F. Optic neuritis, its differential diagnosis and management. *Open Ophthalmol J.* 2012; 6:65-72.
 36. Nebbioso M, Steigerwalt RD, Pecori-Giraldi J, Vingolo EM. Multifocal and pattern-reversal visual evoked potentials *vs.* automated perimetry frequency-doubling technology matrix in optic neuritis. *Indian J Ophthalmol.* 2013; 61:59-64.
 37. Coret F, Pérez-Miralles FC, Gascón F, Alcalá C, Navarré A, Bernad A, Boscá I, Escutia M, Gil-Perotin S, Casanova B. Onset of secondary progressive multiple sclerosis is not influenced by current relapsing multiple sclerosis therapies. *Mult Scler J Exp Transl Clin.* 2018; 4:2055217318783347.
 38. Najafi MR, Shaygannajad V, Mirpourian M, Gholamrezaei A. Vitamin B(12) deficiency and multiple sclerosis; is there any association? *Int J Prev Med.* 2012; 3:286-289.
 39. McLaughlin L, Clarke L, Khalilidehkordi E, Butzkueven H, Taylor B, Broadley SA. Vitamin D for the treatment of multiple sclerosis: a meta-analysis. *J Neurol.* 2018; 265:2893-2905.
 40. Babri S, Mehrvash F, Mohaddes G, Hatami H, Mirzaie F. Effect of intrahippocampal administration of vitamin C and progesterone on learning in a model of multiple sclerosis in rats. *Adv Pharm Bull.* 2015; 5:83-87.
 41. Calderón-Ospina CA, Nava-Mesa MO. B vitamins in the nervous system: Current knowledge of the biochemical modes of action and synergies of thiamine, pyridoxine, and cobalamin. *CNS Neurosci Ther.* 2020; 26:5-13.
 42. Crellin RF, Bottiglieri T, Reynolds EH. Multiple sclerosis and macrocytosis. *Acta Neurol Scand.* 1990; 81:388-391.
 43. Russo C, Morabito F, Luise F, Piomalli A, Battaglia L, Vinci A, Trapani Lombardo V, de Marco V, Morabito P, Condino F, Quattrone A, Aguglia U. Hyperhomocysteinemia is associated with cognitive impairment in multiple sclerosis. *J Neurol.* 2008; 255:64-69.
 44. Zhu Y, He ZY, Liu HN. Meta-analysis of the relationship between homocysteine, vitamin B₁₂, folate, and multiple sclerosis. *J Clin Neurosci.* 2011; 18:933-938.
 45. Zhang DM, Ye JX, Mu JS, Cui XP. Efficacy of vitamin B supplementation on cognition in elderly patients with cognitive-related diseases. *J Geriatr Psychiatry Neurol.* 2017; 30:50-59.
 46. Bourquin F, Capitani G, Grütter MG. PLP-dependent enzymes as entry and exit gates of sphingolipid metabolism. *Protein Sci.* 2011; 20:1492-1508.
 47. Johnson S. The possible role of gradual accumulation of copper, cadmium, lead and iron and gradual depletion of zinc, magnesium, selenium, vitamins B2, B6, D, and E and essential fatty acids in multiple sclerosis. *Med Hypotheses.* 2000; 55:239-241.
 48. Jasse L, Vukusic S, Durand-Dubief F, Vartin C, Piras C, Bernard M, Pélisson D, Confavreux C, Vighetto A, Tilikete C. Persistent visual impairment in multiple sclerosis: prevalence, mechanisms and resulting disability. *Mult Scler.* 2013; 19:1618-1626.
 49. Scalfari A, Lederer C, Daumer M, Nicholas R, Ebers GC, Muraro PA. The relationship of age with the clinical phenotype in multiple sclerosis. *Mult Scler.* 2016; 22:1750-1758.
 50. Öcek Ö, Gedizlioğlu M, Köşkdereioğlu A, Ortan PK, Yüksel B, Türe M, Gediz F. The value of tests evaluating visual functions in detecting overt or subclinical optic neuritis in multiple sclerosis. *Mult Scler Relat Disord.* 2018; 21:63-68.
- Received May 15, 2020; Revised June 22, 2020; Accepted June 26, 2020.
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