Serum Vitamin B12 and thyroid hormone levels in Saudi patients with multiple sclerosis

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INTRODUCTION

Vitamin B12, also known as cobalamin, is produced by bacteria in the large intestine of humans. The most important sources of Vitamin B12 for humans are animal proteins. The recommended daily amount of Vitamin B12 for children up to 18 years old, adult nonpregnant women, and men is ~2 μg, and for pregnant women and lactating women is ~3 μg. It has been calculated that one requires 3–5 years to deplete Vitamin B12 stores after not taking any Vitamin B12.

Increased homocysteine is associated with decreased quality of many cognitive functions including attention, executive function, recall memory, and overall cognitive

OBJECTIVES: To determine the relationship between Vitamin B12 levels and thyroid hormones in patients with multiple sclerosis (MS). MATERIALS AND METHODS: One hundred and ten patients with MS were recruited for this study after Institutional Review Board approval. All patients signed a written informed consent form and donated a single blood sample. Plasma Vitamin B12 levels, triiodothyronine (T3), and thyroxine (T4) hormone levels were measured. Data were analyzed using the Statistical Package for Social Sciences (SPSS) software. RESULTS: Analysis of Vitamin B12 levels in 110 patients with MS revealed that 65% had normal levels of Vitamin B12 (200–900 pg/ml), 30% had low levels of Vitamin B12 (<200 pg/ml), and 5% high levels of Vitamin B12 (higher than 900 pg/ml). Further analysis of patients with low levels of Vitamin B12 revealed that this cohort exhibited a significantly high number of patients with low levels of the thyroid hormones triiodothyronine (T3) and thyroxine (T4) (P < 0.005). CONCLUSION: This study suggests a relationship between Vitamin B12 levels and thyroid hormones. This opens the possibility that the use of therapies that increase triiodothyronine (T3) and thyroxine (T4) levels might be beneficial to patients with MS.

KEY WORDS: Multiple sclerosis, thyroxine, triiodothyronine, Vitamin B12
functioning. It is also associated with the increased risk of cardiovascular disease which is a risk factor for dementia by causing silent brain infarctions and atherosclerosis. In addition, increased homocysteine can cause dementia by two other mechanisms; (1) by producing neuronal cell damage via activation of N-methyl-D-aspartate receptors or (2) by creating white matter lesions and hippocampal atrophy. Therefore, confirming a direct association between increased homocysteine levels and dementia may be of benefit.\[^3\]

There is a strong correlation between Vitamin B12 deficiency and cognitive deterioration including slow mentation, memory impairment, attention deficiencies, and dementia.\[^1\] A new finding demonstrates that the poor function of Vitamin B12 is not related to a decrease in intrinsic factors and is detectable by the following biomarkers, including homocysteine, methylmalonic acid, 2-methylcitric acid, and β-cystathionine levels in the blood. The use of these markers has shown that low Vitamin B12, i.e. below 148 pmol/L is not sensitive enough to define poor function of Vitamin B12. This can be called biochemical Vitamin B12 deficiency which does not present with the symptoms of pernicious anemia or megaloblastic red blood cells or spinal cord affection, but can only occur as a result of food-cobalamin malabsorption and cause dementia.\[^2\]

Since 1998, United States Federal Law has required all cereal grain products to be fortified with folic acid to reduce the prevalence of neural tube defects at birth. A standard level of ~145 μg of folic acid per 100 g of grain is now mandatory. However, some studies have raised concern that the benefit of increased folic acid fortification of all cereal grain products may not reach all populations and may even be detrimental to subgroups. The argument is that high levels of folic acid might mask possible Vitamin B12 deficiency. Meeuwsen et al. demonstrated that the prolonged deficiency of Vitamin B12 is associated with cognitive impairment and depending on the severity and period of deficiency might be irreversible.\[^4\]

It is important to note that the serum Vitamin B12-plasma homocysteine concentration–response curve appears curvilinear. This means that as serum Vitamin B12 increases from the lowest detectable level to 950 pg/mL, plasma homocysteine decreases. However, as serum Vitamin B12 further increases to more than 950 pg/mL, plasma homocysteine begins to increase.\[^5\] A study of 22 patients with various degrees of cognitive impairment and 66 patients with dementia by Eastley et al. found that Vitamin B12 treatment improves impairment of cognition, but does not reverse dementia.\[^6\] In contrast, de la Fournièr et al. studied 11 Alzheimer’s disease patients with low serum Vitamin B12 levels (<240 pg/mL) and prepared them to receive injections of either Vitamin B12 (1000 μg) or placebo daily for 5 days followed by one intramuscular injection per month for 5 months. The authors concluded that the two groups did not differ greatly in cognitive outcome.\[^7\]

**MATERIALS AND METHODS**

One hundred and ten patients with MS were recruited in this after approval of the study by the Institutional Review Board. All patients signed a written informed consent form and donated a single blood sample. The status of each patient as an inpatient or outpatient was recorded.

**Inclusion criteria**

Male or female; 18 through 55 years of age; had signed a written informed consent prior to participation in the study; diagnosis of MS as defined by McDonald criteria; neurologically stable with no evidence of relapse or corticosteroid treatment within 30 days prior to recruitment.

**Exclusion criteria**

A manifestation of MS other than relapsing remitting MS; a history of chronic disease of the immune system other than MS or a known immunodeficiency syndrome; a history or presence of malignancy (except for successfully treated basal or squamous cell carcinoma of skin); a known or “new” diagnosis of diabetes mellitus (if screening blood glucose is suspicious for diabetes [≥126 mg/dL or ≥7 mmol/L if fasting and ≥200 mg/dL or 11.1 mmol/L if random testing], a patient should be further evaluated for diabetes mellitus); active systemic bacterial, viral or fungal infections, or diagnosis of AIDS, hepatitis B, hepatitis C; infection defined as a positive HIV antibody, hepatitis B surface antigen, or hepatitis C antibody tests, respectively.

Fasting blood samples from each participant were collected into 7 ml tubes with ethylenediaminetetraacetic acid (EDTA), and 3 ml tubes containing no anticoagulants (Vacutainer; Becton Dickinson). Coagulated blood clots (blood clots) were prepared by allowing the blood tubes, containing no anticoagulant, to stand at room temperature for 1–2 h. Sera were separated by centrifugation at 2000 ×g for 15 minutes at 4°C. After the sera were removed, the blood clots were frozen and stored at −20°C. Uncoagulated blood cell pellets (EDTA-blood cell pellets) were prepared within 1 h of collection by centrifuging the EDTA blood tubes at 2000 ×g for 15 min at 4°C. After the plasma was removed, the uncoagulated EDTA-blood cell pellet was frozen at −20°C.

Plasma Vitamin B12 was measured in duplicate samples by using the Quantaphase II radioassay
(Bio-Rad Laboratories). Subjects were characterized as Vitamin B12-deficient (plasma Vitamin B12 < 200 pg/ml) or Vitamin B12-replete (plasma Vitamin B12 200–900 pg/ml) or Vitamin B12-excessive (plasma Vitamin B12 >900 pg/ml).

The concentrations of triiodothyronine (T3) and thyroxine (T4) thyroid hormones were measured using a competitive immunoassay with an enhanced chemiluminescence-end point (Immulite model 1000, DPC, USA). The Immulite analyzer, an instrument for solid-phase two-site chemiluminescent assay (DPC), was used to measure the hormone. The assay sensitivity was previously determined as >1 µg/dL.

Statistical Package for Social Sciences (SPSS) software version 16 (IL, USA) was used to analyze the data. Statistical significance of the difference in the mean values was tested using Student’s t-test; p <0.05 was considered statistically significant.

RESULTS

A high number of MS patients (65%) presented with normal levels of Vitamin B12 (200–900 pg/ml), 30% presented with low levels of Vitamin B12 (<200 pg/ml), and 5% presented with excessive level of Vitamin B12 (higher than 900 pg/ml) [Figure 1]. Further analysis of patients with low levels of Vitamin B12 revealed that this cohort exhibited a significantly high number of patients with low levels of the thyroid hormones triiodothyronine (T3) levels (P < 0.05) [Figure 2] as well as low level of thyroxine (T4) level (P < 0.005) [Figure 3].

DISCUSSION

The aim of this study was to determine the relationship between Vitamin B12 levels and thyroid hormones in patients with MS. The study analyzed Vitamin B12 levels in 110 MS patients and revealed that 65% presented with normal levels of Vitamin B12 (200–900 pg/ml), 30% presented with low levels of Vitamin B12 (<200 pg/ml), and 5% presented with excessive level of Vitamin B12 (higher than 900 pg/ml). Further analysis of patients with low levels of Vitamin B12 revealed that a significantly high number of them exhibited low levels of the thyroid hormones triiodothyronine (T3) and thyroxine (T4) (p < 0.005).

Vitamin B12, an important vitamin in the metabolism of the body, has a strong association with homocysteine levels. Current studies support the hypothesis that increased homocysteine is associated with decreased quality of many cognitive functions including attention, executive function, recall memory, and overall cognitive functioning. Most studies support the findings of a strong correlation between Vitamin B12 deficiency and hypothyroidism and cognitive deterioration including slow mentation, memory impairment, attention deficiencies, and dementia and that disturbed Vitamin B12 status is common in MS. Advances in neuro-imaging including magnetic resonance imaging and computerized axial tomographic scans of the brains of elderly subjects and patients with dementia and Alzheimer’s disease strongly point to adverse effects of Vitamin B12 deficiency on the brain. The question whether Vitamin B12 improves cognition is yet to be answered because the studies...
performed to date have been for short periods with a small number of participants. In conclusion, the results of this study suggest that serum vitamin B12 and thyroid hormone levels were low in the study population. Though our study was done in small number of patients with MS, it has revealed a trend towards low vitamin B12 and thyroid hormones levels in Saudi MS patients. We need more studies, to confirm this trend and to look at beneficial role of appropriate supplementations of Vitamin B12 and thyroid hormone on symptomatology as well as disease course of MS.

Figure 3: Exhibition of various levels of thyroxine (T4) hormones by a high percentage of multiple sclerosis patients with low levels of Vitamin B12. Thyroxine (T4) assay was conducted according to the protocol described in the materials and methods section and subdivided into low thyroxine (T4) levels (filled bars), normal levels (gray bars), and high levels (open bars)

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Conflicts of interest
There are no conflicts of interest.

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