TÜBİTAK

Turkish Journal of Medical Sciences

http://journals.tubitak.gov.tr/medical/

Research Article

Turk J Med Sci
(2015) 45: 1280-1284
© TÜBİTAK
doi:10.3906/sag-1407-81

Evaluation of oxidant and antioxidant status in patients with vitamin B12 deficiency

Tekin GÜNEY[1,*], Murat ALIŞIK[2], Sema AKINCI[1], Salim NEŞELİOĞLU[2], İmdat DİLEK[1], Özcan EREL[2]

[1]Department of Hematology, Atatürk Research and Training Hospital, Ankara, Turkey

[2]Department of Medical Biochemistry, Atatürk Research and Training Hospital, Ankara, Turkey

Received: 16.07.2014 • **Accepted/Published Online:** 27.10.2014 • **Printed:** 31.12.2015

Background/aim: The aim of this study was to examine the theory that oxidative stress might have an important mediating effect on the deleterious results of vitamin B12 metabolism deficiency seen throughout the body.

Materials and methods: Forty patients with vitamin B12 deficiency and 40 healthy controls were included in the study. Venous blood samples were collected from all participants to evaluate serum vitamin B12, homocysteine, methylmalonic acid, total antioxidant status (TAS), and total oxidant status (TOS) levels in the pre- and posttreatment periods.

Results: There were no significant differences in TAS, TOS, or oxidative stress index (OSI) levels between the pretreatment patient and control groups, and there were no significant differences in TAS or TOS levels between the posttreatment and control groups. In addition, there were no significant differences between the pre- and posttreatment TAS, TOS, and OSI levels of the patients.

Conclusion: These findings are remarkable in that cyanocobalamin treatment had no direct effect on oxidant and antioxidant status in patients with vitamin B12 deficiency. The fact that there were no differences in oxidant and antioxidant status between the patients and the controls might suggest that oxidative stress does not play a role in the systemic negative effects of vitamin B12 deficiency.

Key words: Vitamin B12, homocysteine, oxidative stress, total antioxidant status, total oxidant status

1. Introduction

Vitamin B12 is an essential vitamin that plays a crucial role in many chemical reactions and affects many mechanisms in the body. Nucleic acid metabolism, transference of methyl groups, synthesis and repair of myelin sheaths, and formation of red blood cells are all functions in which vitamin B12 plays a role (1–4). Vitamin B12 deficiency can lead to a great deal of dysfunction, mainly hematological and neuropsychic symptoms. The activity of the methylmalonyl-coenzyme A mutase enzyme is interrupted by vitamin B12 deficiency, and, as a result, serum methylmalonic acid (MMA) levels and urine MMA excretion increase. Vitamin B12 deficiency also interrupts methionine synthase activity, resulting in elevated serum homocysteine levels. Homocysteine and MMA levels increase progressively through the process of vitamin B12 deficiency. For this reason, even in the early stages, it is important to measure MMA and homocysteine levels; in particular, a concurrent increase in both serum MMA and homocysteine levels is accepted as a laboratory marker of vitamin B12 deficiency (5).

In vitamin B12 deficiency, regulation of the citric acid cycle deteriorates due to increasing MMA levels, and fatty-acid degradation decreases while fatty-acid synthesis increases; thus, ATP generation is interrupted, which means that the number of oxidative reactive species increases in the mitochondria. On the other hand, the levels of propionic acid, succinate dehydrogenase, cytochrome C activity, propionyl-CoA, amino acids, cell metabolism, protein synthesis, fatty-acid synthesis enzymes (ATP citrate lyase), homocysteine, and MMA increase in cases of vitamin B12 deficiency (2). Hence, vitamin B12 deficiency hypothetically results in an effect that favors oxidant status in the oxidant/antioxidant ratio. Increased levels of homocysteine, which has a prooxidant capacity, have been closely related to oxidative stress (6). It has been claimed that homocysteine triggers oxidative stress by generating reactive oxygen species, which affects developing disulfide bonds (7). In addition, the trans-sulfation of homocysteine to glutathione, which is a major intracellular antioxidant, is an important metabolic pathway related to homocysteine. Thus, a decrease in glutathione levels is closely related to oxidative stress (8).

* Correspondence: tekin-guney@hotmail.com

Oxidant status can be defined as the total burden that results from many reactions at a molecular level and has a negative effect, whereas the protective response that develops to correspond to the prooxidant molecules is called the antioxidant effect. Total oxidant status (TOS) has been identified as the in vivo marker of a shift developing in an oxidative/antioxidative ratio in favor of the oxidative side. Total antioxidant status (TAS), on the other hand, reflects all total plasma antioxidant substrates. Oxidative stress index (OSI) is an indicator of the degree of oxidative stress; it is the percentage of the TOS level/TAS level ratio (9–11).

Vitamin B12 plays a crucial role in DNA, RNA, and protein syntheses, acting as a cofactor. In addition to the direct negative effect of vitamin B12 deficiency on the cell cycle, the increased homocysteine levels that accompany vitamin B12 deficiency contribute to the deterioration of multisystem tissues via various molecular and biochemical pathways. The aim of this study was to examine the theory that oxidative stress might have an important mediation effect on the deleterious results of vitamin B12 metabolism deficiency seen throughout the body by measuring levels of vitamin B12, MMA, homocysteine, TAS, and TOS and determining whether there is any relation among them.

2. Materials and methods

2.1. Patients and controls

Forty patients with vitamin B12 deficiency, aged 19–82 years, and 40 healthy volunteer controls, aged 19–64 years, from the Department of Hematology, Faculty of Medicine of Yıldırım Beyazıt University were included in the study. Venous blood samples (10 mL) were collected from all participants to evaluate serum vitamin B12, homocysteine, MMA, TAS, and TOS levels. After providing cyanocobalamin treatment for 1 month, another round of 10-mL venous samples was obtained to test TAS and TOS levels. Patients with comorbid systemic disorders and patients using any drugs were excluded from the study. The control group consisted of healthy adults who were age- and sex-matched to the vitamin B12 deficiency participants. Written informed consent was obtained from all subjects after they were provided with a complete description of the study. This study was approved by the ethics committee of the Faculty of Medicine of Yıldırım Beyazıt University.

2.2. Blood samples and measurement tools

To avoiding interfering with the study results, the individuals in the patient and control groups were advised not to smoke, drink, or eat prior to undergoing the blood sampling. Venous blood samples were collected from an antecubital vein after an 8-h overnight fasting period; 10 mL of venous blood was drawn from each patient into biochemistry tubes. The biochemistry tubes were centrifuged at 1800 × *g* for 15 min after an incubation period of 30 min. The samples were frozen at –80 °C and stored prior to analysis. TAS and TOS were measured using a Cobas c501 automated analyzer (Roche, Switzerland) in the Biochemistry Laboratory of Atatürk Training and Research Hospital.

2.3. Measurement of serum vitamin B12 levels

Serum vitamin B12 levels were analyzed using the electrochemiluminescence immunoassay method with a Cobas e601 immunological test autoanalyzer and Elecsys vitamin B12 test kits (Roche Diagnostics).

2.4. Measurement of plasma homocysteine levels

Homocysteine levels were measured with an Immulite 2000 autoanalyzer, using the chemiluminescence immunoassay method.

2.5. Measurement of serum MMA levels

MMA levels were measured using liquid chromatography–mass spectrometry.

2.6. Measurement of plasma TOS

Plasma TOS levels were measured using Erel's method for TOS (10). With this method, oxidants present in the sample oxidize the ferrous ion-o-dianisidine complex to a ferric ion. The oxidation reaction is enhanced by glycerol molecules, which are abundantly present in the reaction medium. The ferric ion forms a colored complex with xylenol orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide, and the results are expressed in terms of micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 eqv./L).

2.7. Measurement of plasma TAS

Plasma TAS levels were measured using the method described by Erel (9). The principle of the assay is to incubate ABTS (2,2'-azino-di-[3-ethylbenzthiazoline sulfonate]) with H_2O_2 to produce the radical cation ABTS+, which has a relatively stable blue-green color and is measured at 600 nm. Antioxidants in the added sera cause bleaching of this color to a degree that is proportional to their concentrations. The TAS value is expressed as μmol Trolox eqv./L.

2.8. Determination of OSI

The ratio of TOS to TAS was accepted as the OSI in this study. To carry out this calculation, the resulting TAS unit was changed to μmol/L, and the OSI value was calculated according to the following formula: OSI (arbitrary unit) TOS (μmol H_2O_2 eqv./L) / TAS (μmol Trolox eqv./L) (11).

2.9. Statistical analysis

Data analysis was performed using SPSS 11.5 (SPSS Inc., Chicago, IL). The Kolmogorov–Smirnov test was used to determine whether the distribution of continuous variables was normal. Data were shown as mean ± SD

or median (min–max), as applicable. Mean differences between groups were compared using Student's t-test, and the Mann–Whitney U test was used for comparisons of the median values. The Wilcoxon signed rank test was used to determine whether the differences (in median values) between pre- and posttreatment measurements were statistically significant. Nominal data were analyzed by Pearson's chi-square test. Degrees of association between continuous variables were evaluated by Spearman's rank correlation analysis. $P < 0.05$ was considered statistically significant.

3. Results

This study included 40 patients and 40 healthy controls. The mean age was 43.1 ± 15.9 years in the vitamin B12 deficiency group and 40.1 ± 16.9 years in the control group. In the vitamin B12 deficiency group, 10 (25%) of the patients were male and 30 (75%) were female; in the control group, 17 (42.5%) of the participants were male and 23 (57.5%) were female. There were no differences between the groups in terms of mean age ($P = 0.419$) or sex ($P = 0.098$) distribution. The demographic data of the subjects are summarized in Table 1.

The comparisons between serum vitamin B12, homocysteine, MMA, and urine MMA measurements of the controls and patients are summarized in Table 2.

Prior to vitamin B12 treatment, there were no significant differences in TAS ($P = 0.694$), TOS ($P = 0.252$), or OSI ($P = 0.189$) between the patient and control groups. The OSI levels of the patients after vitamin B12 treatment were statistically higher than those of the controls ($P = 0.025$). There were no significant differences in TAS ($P = 0.292$) or TOS ($P = 0.090$) between the patient and control groups. Similar to previous findings, there were no significant differences between the patients' pre- and posttreatment TAS ($P = 0.414$), TOS ($P = 0.505$), and OSI ($P = 0.407$) measurements (Table 3).

No significant correlations were found between age and TAS, TOS, and OSI levels before and after treatment, nor in the changes in posttreatment levels compared to the pretreatment levels ($P > 0.05$). Similarly, there were no sex-related differences in terms of pre- and posttreatment TAS, TOS, and OSI levels, nor any changes after treatment ($P > 0.05$).

Table 1. Demographic features of all participants.

	Cases (n = 40)	Controls (n = 40)	P
Age, years, mean ± SD	43.1 ± 15.9	40.1 ± 16.9	0.419†
Age range, years, min–max	19–82	19–64	
Sex, n (%)			0.098‡
Male	10 (25.0)	17 (42.5)	
Female	30 (75.0)	23 (57.5)	

† Student's t-test, ‡ Pearson's chi-square test.

Table 2. The distribution of serum vitamin B12, homocysteine, methylmalonic acid (MMA) levels, and urine MMA measures of all subjects.

	Pretreatment, median (min–max)	Controls, median (min–max)	P†	Posttreatment, median (min–max)	P‡	P¶
Serum vitamin B12	154 (39–200)	324 (171–1430)	<0.001	706.5 (193–2000)	<0.001	<0.001
Serum homocysteine	13.5 (0–126)	11 (5–21)	<0.021	8 (0–25)	<0.001	<0.001
Serum MMA	1 (0–3)	0 (0–0)	<0.001	0 (0–1)	0.171	<0.001
Urine MMA	2 (0–7)	0 (0–4)	<0.001	0.5 (0–12)	0.702	<0.001

†: Comparison of pretreatment measurements with controls; Mann–Whitney U test.
‡: Comparison of posttreatment levels and controls; Mann–Whitney U test.
¶: Comparison of pre- and posttreatment measurements; Wilcoxon signed rank test.

Table 3. All subjects' serum total oxidant status (TOS), total antioxidant status (TAS), and oxidative stress index (OSI) levels.

	Pretreatment, median (min–max)	Controls, median (min–max)	P†	Posttreatment, median (min–max)	P‡	P¶
TOS	3 (0–6)	2 (1–8)	0.252	3 (1–10)	0.090	0.505
TAS	2 (1–2)	2 (1–2)	0.694	2 (1–2)	0.292	0.414
OSI	15 (0–40)	10 (5–80)	0.189	15 (5–50)	0.025	0.407

†: Comparison of pretreatment measurements with controls; Mann–Whitney U test.
‡:Comparison of posttreatment levels and controls; Mann–Whitney U test.
¶: Comparison of pre- and posttreatment measurements; Wilcoxon signed rank test.

4. Discussion

To our knowledge, this is the first study to investigate serum oxidative metabolism prior to and after vitamin B12 treatment in adults with vitamin B12 deficiency. Studies investigating the association of vitamin B12 deficiency or elevated homocysteine levels with oxidative status have been carried out in patients with different systemic diseases, such as diabetes mellitus or Parkinson disease, as well as in vegetarians. In a study conducted by Herrmann et al., it was found that vegetarians had a reduced TAS and that vitamin B12 was positively correlated with TAS. The researchers suggested that serum vitamin B12, as a marker for functional B12 status, was the variable that influences TAS, and that functional vitamin B12 deficiency might contribute to hyperhomocysteinemia and decreased TAS in vegetarians (12). In another study, low serum levels of glutathione, TAS, and impaired antioxidant enzymatic activities were found to be associated with low serum levels of B12 in adults with type 2 diabetes. It was suggested that low B12 status with low folate was a potential triggering factor for developing hyperhomocysteinemia and oxidative stress in adults with type 2 diabetes (8). In a similar study, it was found that treatment with folate supplementation lowered levels of plasma homocysteine and serum malondialdehyde and improved TAS and B12 serum levels in patients with type 2 diabetes mellitus (13). In another study, Qureshi et al. suggested that vitamin B12 deficiency was associated with neurotoxicity in Parkinson disease cases by enhancing oxidative stress (14).

In contrast to other studies, we examined the oxidative stress parameters in patients with vitamin B12 deficiency without any other systemic diseases. The TAS, TOS, and OSI levels of patients with vitamin B12 deficiency in the pretreatment phase were found to be similar to those of the controls. There were no significant changes in the TAS, TOS, and OSI levels of the patients with vitamin B12 deficiency before and after treatment. Although there was a significant increase in OSI levels in the patient group after treatment compared to the controls, we do not think this finding solely reflects oxidant status, as there were no significant changes in TAS or TOS levels. These findings are remarkable in that they show that cyanocobalamin treatment had no direct effect on the oxidant and antioxidant statuses of patients with vitamin B12 deficiency and no other systemic diseases. On the other hand, the fact that the patients with vitamin B12 deficiency did not differ from the controls in terms of oxidant and antioxidant status might suggest that oxidative stress might not play a role in the systemic negative effects of vitamin B12 deficiency.

The most prominent limitation of this study is the relatively small sample size. Nevertheless, exclusion of any comorbid systemic disorders in the vitamin B12 deficiency sample increased the reliability of our study. In addition, it is possible that despite the clinical improvement of hematological symptoms, the clinical symptoms of vitamin B12 deficiency tend to improve over the longer term; neurological response to treatment with cyanocobalamin could take 6 months, for instance, while cellular response might occur right after vitamin B12 treatment. Therefore, this study's design may be another limitation, as the posttreatment evaluations were conducted only 1 month after administration. For this reason, there is a need for further evaluation of oxidative status after a longer posttreatment period and with a larger sampling.

Acknowledgment

The authors thank the people participating in the study on a voluntary basis.

References

1. Tefferi A, Pruthi RK. The biochemical basis of cobalamin deficiency. Mayo Clin Proc 1994; 69: 181–186.
2. Allen RH, Stabler SP, Savage DG, Lindenbaum J. Metabolic abnormalities in cobalamin (vitamin B_{12}) and folate deficiency. FASEB J 1993; 7: 1344–1353.
3. Scalabrino G, Veber D, Mutti E. New pathogenesis of the cobalamin-deficient neuropathy. Med Secoli 2007; 19: 9–18.
4. Scalabrino G, Peracchi M. New insights into the pathophysiology of cobalamin deficiency. Trends Mol Med 2006; 12: 247–254.
5. Antony AC. Megaloblastic anemias. In: Hoffman R, Benz E, Silberstein L, Heslop H, Weitz J, Anastasi J, editors. Hematology: Basic Principles and Practice. 6th ed. Philadelphia, PA, USA: Elsevier; 2013. pp. 473–504.
6. Rogers EJ, Chen S, Chan A. Folate deficiency and plasma homocysteine during increased oxidative stress. N Engl J Med 2007; 357: 421–422.
7. Sibrian-Vazquez M, Escobedo JO, Lim S, Samoei GK, Strongin RM. Homocystamides promote free-radical and oxidative damage to proteins. P Natl Acad Sci USA 2010; 107: 551–554.
8. Al-Maskari MY, Waly MI, Ali A, Al-Shuaibi YS, Ouhtit A. Folate and vitamin B12 deficiency and hyperhomocysteinemia promote oxidative stress in adult type 2 diabetes. Nutrition 2012; 28: e23–26.
9. Erel O. A novel automated method to measure total antioxidant response against potent free radical reactions. Clin Biochem 2004; 37: 112–119.
10. Erel O. A new automated colorimetric method for measuring total oxidant status. Clin Biochem 2005; 38: 1103–1111.
11. Guney E, Fatih Ceylan M, Tektas A, Alisik M, Ergin M, Goker Z, Senses Dinc G, Ozturk O, Korkmaz A, Eker S et al. Oxidative stress in children and adolescents with anxiety disorders. J Affect Disorders 2014; 156: 62–66.
12. Herrmann W, Schorr H, Purschwitz K, Rassoul F, Richter V. Total homocysteine, vitamin B_{12}, and total antioxidant status in vegetarians. Clin Chem 2001; 47: 1094–1101.
13. Aghamohammadi V, Gargari BP, Aliasgharzadeh A. Effect of folic acid supplementation on homocysteine, serum total antioxidant capacity, and malondialdehyde in patients with type 2 diabetes mellitus. J Am Coll Nutr 2011; 30: 210–215.
14. Qureshi GA, Qureshi AA, Devrajani BR, Chippa MA, Syed SA. Is the deficiency of vitamin B12 related to oxidative stress and neurotoxicity in Parkinson's patients? CNS Neurol Disord-Dr 2008; 7: 20–27.