Journal of Elementology ISSN 1644-2296

Karacaer, C., Bahtiyar, N., Aydemir, B., Küçük Ataman, B., Şekeroğlu, M., Sevinç Afşar, L., Güler Aksoy, E., Nogay, F., Algül, Y., Demir, C., Narinoğlu, T. and Cinemre, F. (2024) 'Circulating trace element status in vitamin B_{12} deficiency: antioxidant properties', *Journal of Elementology*, 29(4), 821-835, available: https://doi.org/10.5601/jelem.2024.29.2.3361

RECEIVED: 11 June 2024
ACCEPTED: 26 September 2024

ORIGINAL PAPER

Circulating trace element status in vitamin B_{12} deficiency: antioxidant properties*

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Abstract

It is known that vitamin B_{12} and certain trace elements possess antioxidant properties; however, the mechanisms underlying relationships between vitamin B_{12} and trace elements have not been fully elucidated. The aim of this study was to evaluate the relationship between serum levels of vitamin B_{12}, selenium (Se), cobalt (Co), copper (Cu), zinc (Zn), iron (Fe), and manganese (Mn) in patients with vitamin B_{12} deficiency compared to healthy controls. The study included a total of 50 patients with vitamin B_{12} deficiency and 40 healthy controls. Serum levels of Se, Co, Cu, Zn, Fe, and Mn were measured using inductively coupled plasma optical emission spectrometry (ICP-OES), while biochemical parameters were assessed using an automated analyzer. Statistical analysis was conducted with SPSS 21.0 statistical software (SPSS, Chicago, IL, USA), with a P-value of <0.05 considered statistically significant. Serum levels of vitamin B_{12}, Zn, Mn, Co, Fe, and iron binding capacity were significantly lower in the vitamin B_{12} deficiency group compared to controls. Positive correlations were observed between vitamin B_{12} and Se, Fe and mean corpuscular volume (MCV), Fe and ferritin, Zn and Cu, and Zn and Se. Conversely, negative correlations were found between vitamin B_{12} and Fe, Se, and Mn in the vitamin B_{12} deficiency group. Our data suggest that the interactions among circulating Zn, Mn, Co, Fe, and vitamin B_{12} are significant in the oxidant/antioxidant balance, and may play a crucial role in the antioxidant properties observed in patients with vitamin B_{12} deficiency.

Keywords: vitamin B_{12}, deficiency, trace elements, antioxidant properties

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* The source of funding – this study was not financed by any fund.

INTRODUCTION

Vitamin B_{12}, also known as cobalamin, is an essential water-soluble vitamin that plays a crucial role in maintaining neuronal health and hematopoiesis. Vitamin B_{12} deficiency can arise from various factors, including environmental influences, nutritional deficiencies, medication use, and genetic factors. Clinical vitamin B_{12} deficiency is rare in developed countries and is primarily attributed to genetic abnormalities. It commonly results in myeloneuropathy or megaloblastic anemia. However, potential consequences of asymptomatic subclinical vitamin B_{12} deficiency have not yet been fully elucidated (Green et al. 2017, van de Lagemaat et al. 2019).

Vitamin B_{12} is an essential vitamin that cannot be synthesized by the human body but is produced primarily by microorganisms. The amount synthesized by bacteria in the human intestine varies depending on the intestinal flora, but it is typically very low or insufficient. Therefore, vitamin B_{12} must be obtained through dietary intake. Inadequate dietary consumption is the primary cause of vitamin B_{12} deficiency (Haddad et al. 1999, Stabler and Allen 2004). Vitamin B_{12} acts as a cofactor in two critical biochemical processes in humans: the cytosolic methionine synthase reaction and the mitochondrial methylmalonyl-CoA mutase reaction. Disruption of either of these reactions can result in vitamin B_{12} deficiency. Although vitamin B_{12} deficiency is more prevalent with advancing age, it can occur across all age groups, particularly in the context of food insecurity, as malabsorption disorders are more common among the elderly. The consequences and severity of vitamin B_{12} deficiency vary based on the extent and duration of the deficiency. Major organ systems affected include the blood, bone marrow, and nervous system (Allen 2012). Megaloblastic anemia is caused by a defect in thymidine and, consequently, deoxyribonucleic acid (DNA) synthesis in rapidly dividing cells. The nervous system involvement is varied and includes issues related to faulty myelin synthesis and repair. Cognitive impairment and psychosis may also occur. Vitamin B_{12} metabolism and deficiency are closely related to another B vitamin, folate, as are involved in key biochemical reactions: the cytosolic methionine synthase reaction and the mitochondrial methylmalonyl-CoA mutase reaction. Disruption of either of these reactions leads to vitamin B_{12} deficiency. In these enzymatic processes, the cobalt (Co) atom cycles between different oxidation states to form reduced cobalamin (Cbl I) and oxidized forms, Cbl II and Cbl III. The cellular processing of cobalamins to form methylcobalamin (MeCbl) for methionine synthase and adenosylcobalamin (AdoCbl) for methylmalonyl-CoA mutase (MCM) relies on adequate levels of the reducing agents nicotinamide adenine dinucleotide phosphate (NADPH) and glutathione (GSH). Therefore, the clinical significance of cellular redox homeostasis and vitamin B_{12} function in the context of vitamin B_{12} deficiency should be considered (Offringa et al. 2021, Green, Miller 2022).

Trace elements (TEs) obtained from the diet are crucial components that contribute to essential bodily functions, including metabolic processes, tissue repair, growth, and development. Since the human body cannot naturally synthesize these elements, it is important to obtain them through diet or supplements. An imbalance, either excess or deficiency, in trace elements can lead to various diseases, such as inflammation, obesity, and essential hypertension (Aydemir et al. 2015, Amin et al. 2020, Serinkan Cinemre et al. 2021). While some trace elements have anti-inflammatory and antioxidant properties, others can be toxic. Recent studies have identified several trace elements, including iron (Fe), copper (Cu), selenium (Se), and zinc (Zn), as being associated with various diseases (Himoto, Masaki 2020, Fanni et al. 2021, Kahvaz et al. 2021, Socha et al. 2021, Vaghari-Tabari et al. 2021). Therefore, it is reasonable to assume that these elements might directly or indirectly influence cellular redox homeostasis. Analyses of trace elements in various diseases have shown altered levels between the acute and chronic stages of these conditions. However, studies that comprehensively explain changes in trace elements associated with vitamin B_{12} deficiency appear to be quite limited (Islam et al. 2023).

Moderate micronutrient deficiencies can lead to significant health problems in older individuals. Due to the inability to compensate for these deficiencies at this stage, age-related diseases may arise with increased oxidative stress. Although much of the existing research focuses on the quantitative availability of vitamin B_{12} from dietary sources in its role as a cofactor, there is comparatively less emphasis on its broader redox-related functions (Hovdenak, Haram 2012, Offringa et al. 2021, Cikim et al. 2023). According to Kwashiorkor's Golden Theory, free radical damage plays a role in the etiopathogenesis of various diseases. This theory requires validation through quantitative measurements of micronutrients in malnourished children, pregnant women, the elderly, and individuals with various chronic diseases (Etukudo et al. 1999, Kamath et al. 2023).

In light of the aforementioned information, there is a limited number of studies in the literature examining certain trace elements in the context of vitamin B_{12} deficiency. Most existing research focuses on the role of vitamin B_{12} in the etiopathogenesis of various diseases (Hovdenak, Haram 2012, Aydemir et al. 2015, Amin et al. 2020, Serinkan Cinemre et al. 2021, Cikim et al. 2023, Islam et al. 2023, Kamath et al. 2023). However, potential changes in the oxidant/antioxidant balance of trace elements in vitamin B_{12} deficiency have not been thoroughly investigated. Therefore, this study aimed to explore the relationship between serum vitamin B_{12} levels, certain biochemical parameters, and trace elements including Se, Co, Cu, Zn, Fe, and manganese (Mn) levels in individuals with and without vitamin B_{12} deficiency.

MATERIALS AND METHODS

Study groups

Our study included 90 individuals who visited the Internal Medicine outpatient clinic at Sakarya University Training and Research Hospital. These individuals were between the ages of 20 and 60 and had no clinical diagnoses other than deviations in serum vitamin B_{12} levels. Participants with serum vitamin B_{12} levels within the normal range (180-550 pg mL^{-1}) were classified as the control group (*N*=40), while those with vitamin B_{12} deficiency (levels <180 pg/mL) were classified as the patient group (*N*=50). Exclusion criteria included Crohn's disease, previous gastric or ileal resection, acute or chronic disorders, substance abuse, family history of inflammatory bowel disease, acute chronic metabolic, endocrine, cardiovascular, autoimmune, rheumatological diseases, iron deficiency anemia, coagulation disorder, obesity, pregnancy, concurrent use of metformin, the use of vitamins and minerals supplements, a completely vegan and vegetarian diet, or serum creatinine levels greater than 1.1 mg dL^{-1} for women and greater than 1.3 mg dL^{-1} for men. All participants were informed about the survey and freely signed and dated the consent form. The protocol was approved by the Ethics Committee of the Medical Faculty of Sakarya University (Approval number: E-71522473-050.01.04-39908-365), and was conducted in accordance with the Helsinki Declaration, 1975, amended 2000.

Sample collection

Blood samples were collected into tubes with and without anticoagulant after an overnight fast. After centrifugation at 1500×g for 20 min at room temperature, serum samples were separated in eppendorf tubes and frozen at -20°C until analysis.

Measurement of biochemical parameters

The iron binding capacity, glucose, urea, creatinine, serum glutamic--pyruvic transaminase (SGPT) and serum glutamic-oxaloacetic transaminase (SGOT) were measured by automated colorimetric methods using commercially available kits (Cobas 8000 modular analyser; Roche Diagnostics, Indianapolis, IN). Serum vitamin B_{12}, folate, and ferritin levels were also determined using an automated ABBOTT ARCHITECT I-2000 (Abbott Park-IL, 60064, USA). In addition, complete blood cell count was analysed with a CELL-DYN 3700 fully automatic hematological analyser (Abbott Park-IL, 60064, USA).

Measurement of the trace elements

The trace element levels were measured using an inductively coupled plasma optical emission spectrophotometer (ICP-OES, Thermo iCAP 6000, Cambridge, UK). Serum samples were diluted (1:9) with distilled water

(Millipore, Bedford, MA, USA) containing 0.3% HNO_3 (Merck, Darmstadt, Germany). Calibration standards were prepared using the stock solution at a concentration of 1,000 mg L^{-1} (Chem-Lab, Belgium). Elemental solutions of 0.0025, 0.005, 0.010, 0.025, 0.05, 0.25, and 0.50 ppm concentrations were prepared by using stock solution and distilled water containing 0.3% HNO_3. The Se, Zn, Mn, Cu, Co, and Fe element levels were determined at wavelengths of 196.026, 213.800, 257.610, 324.754, 228.616, and 259.940 nm, respectively. Results were presented as µg dL^{-1}.

Statistical analysis

Statistical analysis was performed using SPSS 21.0 statistical software for Windows (SPSS, Chicago, IL, USA). Results were reported as mean ± standard deviation (SD). Distributions of the data were tested by the Kolmogorov-Smirnov test. The means for normally distributed continuous variables were compared using Student's *T*-test. Non-normally distributed continuous variables were compared using the Mann-Whitney *U* test. Correlations between biochemical parameters and trace element levels were assessed using Pearson's correlation coefficient. A value of $P<0.05$ was considered the minimum statistical significance.

RESULTS AND DISCUSSION

This study included data from a total of 90 subjects (64 females and 26 males) aged between 20 and 60 years. The subjects were divided into two groups: 50 individuals with vitamin B_{12} deficiency (patient group) and 40 healthy individuals (control group). The median age did not differ significantly between the patient and control groups ($P>0.05$).

Previous studies have shown that vitamin B_{12} deficiency is common in various populations. Vitamin B_{12} is vital as it plays a role of a coenzyme in cellular processes and the activity of some enzymes. Therefore, its deficiency causes many temporary or permanent health problems. Although hematological symptoms improve after treatment for vitamin B_{12} deficiency, neurological symptoms cannot be reversed, which is an important situation for public health (Romain et al. 2016, Green et al. 2017, Green, Miller 2022, Kiran et al. 2023).

Table 1 presents the characteristics of the biochemical parameters, including the levels of vitamin B_{12}, iron binding capacity, ferritin, glucose, urea, creatinine, SGOT, SGPT, folic acid, erythrocyte count, leucocyte count, platelet count, hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), platelet distribution width (PDW), red cell distribution width (RDW), mean platelet volume (MPV), and plateletcrit (PCT) values in the vitamin B_{12} deficient and

Table 1

Comparison of some biochemical parameters between vitamin B_{12} deficient and control groups

	Vitamin B_{12} deficient (N=50)	Control (N=40)	P
Vitamine B_{12} (pg mL^{-1})	152.3±25.12	321.3±96.12	**< 0.001**
Iron binding capacity (μg dL^{-1})	289.9±91.42	342.6±66.18	**0.005**
Ferritin (ng mL^{-1})	48.67±44.89	57.24±61.42	ns
Glucose (mg dL^{-1})	92.64±11.89	91.06±12.24	ns
Urea (mg dL^{-1})	29.12±10.64	28.95±6.73	ns
Creatinine (mg dL^{-1})	0.692±0.122	0.691±0.182	ns
SGOT (U L^{-1})	20.81±6.200	22.26±12.74	ns
SGPT (U L^{-1})	21.89±16.26	23.12±14.92	ns
Folic Acid (ng mL^{-1})	7.240±2.726	6.998±2.426	ns
Erythrocyte (M Ul^{-1})	4.912±0.768	4.956±0.428	ns
Leucocyte (K Ul^{-1})	6.586±1.848	6.948±1.436	ns
Platelet (K Ul^{-1})	241.1±62.23	259.26±79.17	ns
Hb (g dL^{-1})	13.76±1.624	13.74±1.843	ns
Hct (%)	40.32±4.324	41.08±5.120	ns
MCV (fL)	83.94±6.350	83.03±8.164	ns
MCHC (g dL^{-1})	32.74±0.792	33.04±0.853	ns
PDW (%)	18.24±0.962	17.94±0.846	ns
RDW (%)	16.43±2.154	16.78±2.426	ns
MPV (fL)	8.684±3.298	7.712±0.976	ns
PCT (%)	0.211±0.062	0.200±0.059	ns

Data are presented as mean ± SD. Bold values indicate statistical significance. SGOT – serum glutamic oxaloacetic transaminase, SGPT – serum glutamate pyruvate transaminase, Hb – hemoglobin, Hct – hematocrit, MCV – mean corpuscular volume, MCHC – mean corpuscular hemoglobin concentration, PDW – platelet distribution width, RDW – red cell distribution width, MPV – mean platelet volume, PCT – plateletcrit, ns – no significant

control groups. Serum levels of vitamin B_{12} and iron binding capacity were decreased in the vitamin B_{12} deficient group compared to the control group (P=0.001 and P=0.005, respectively). There were no significant differences in other markers between the vitamin B_{12} deficient group and healthy subjects (P>0.05, for all).

TEs such as Se, Mn, Zn, Cu, and Fe play critical roles in regulating various metabolic and signaling pathways. Disruptions in the levels of these essential trace elements, as well as the accumulation of potentially toxic or non-essential trace elements, may be linked to various diseases (Kiziler et al. 2010). In this study, the levels of some TEs and biochemical parameters in patients with vitamin B_{12} deficient and healthy controls were determined to understand their roles in the oxidant/antioxidant system of vitamin

B_{12} deficiency. To the best of our knowledge, our study is the first to examine the changes in Se, Co, Mn, Zn, Cu, and Fe levels in the case of vitamin B_{12} deficiency regarding its antioxidant properties.

In our study, we found that serum Zn, Mn, Co, and Fe levels were decreased in the vitamin B_{12} deficient group compared with the control group (P=0.001, P=0.031, P=0.047, and P=0.027, respectively – Figures 1-4). There

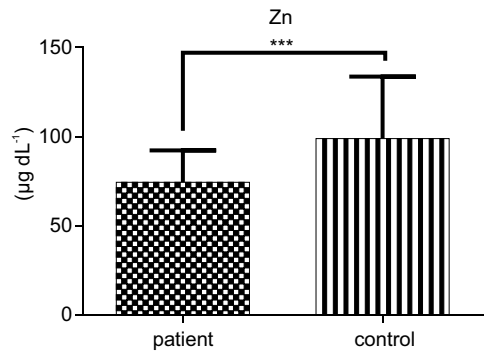

Fig. 1. The levels of zinc in vitamin B_{12} deficient and control groups.
Values are presented as the mean ± the standard deviation.*** P<0.001

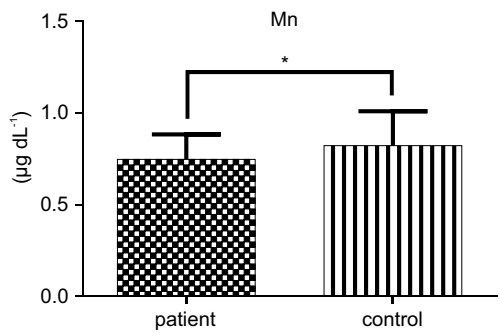

Fig. 2. The levels of manganese in vitamin B_{12} deficient and control groups.
Values are presented as the mean ± the standard deviation.* P<0.05

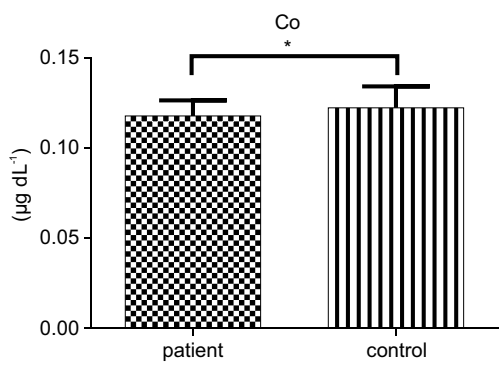

Fig. 3. The levels of cobalt in vitamin B_{12} deficient and control groups.
Values are presented as the mean ± the standard deviation.* P<0.05

were no significant differences between the vitamin B_{12} deficient group and healthy subjects regarding Se and Cu levels (P>0.05, for each – Figures 5, 6).

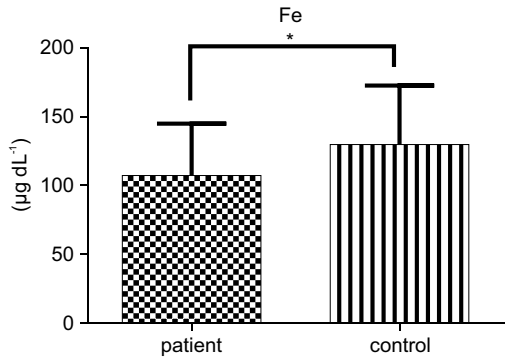

Fig. 4. The levels of iron in vitamin B_{12} deficient and control groups. Values are presented as the mean ± the standard deviation. * P<0.05

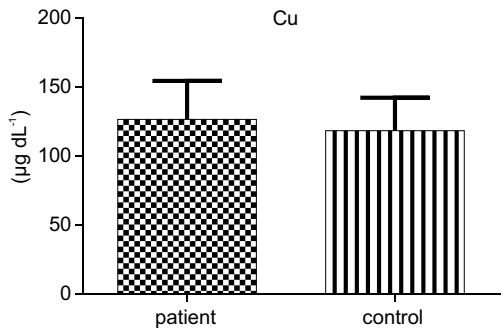

Fig. 5. The levels of copper in vitamin B_{12} deficient and control groups. Values are presented as the mean ± the standard deviation

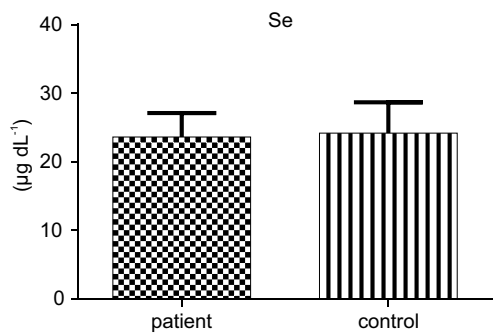

Fig. 6. The levels of selenium in vitamin B_{12} deficient and control groups. Values are presented as the mean ± the standard deviation

When the correlation of vitamin B_{12}, Se, Zn, Mn, Cu, Co, Fe levels and biochemical parameters in the vitamin B_{12} deficient group were analysed, it was seen that there was a positive correlation between vitamin B_{12} and

urea, vitamin B_{12} and Se, Fe and MCV, Fe and ferritin, Zn and glucose, Se and SGPT, Cu and MPV, Cu and glucose, Co and RDW, Zn and Cu, Zn and Se (r=0.352, r=0.319, r=0.469, r=0.359, r=0.408, r=0.473, r=0.353, r=0.321, r=0.306, r=0.380, and r=0.356 respectively), but there was a negative correlation between vitamin B_{12} and Fe, vitamin B_{12} and Hct, vitamin B_{12} and MPV, Fe and RDW, Fe and iron binding capacity, Zn and leucocyte count, Zn and platelet count, Cu and Hb, Cu and Hct, Se and Mn (r=-0.337, r=-0.304, r=-0.362, r=-0.440, r=-0.479, r=-0.404, r=-0335, r=-0.420, r=-0.416, and r=-0.344, respectively) – Table 2.

Table 2

Correlation of vitamin B_{12}, Fe, Zn, Se, Cu, Co, Mn levels and some biochemical parameters of vitamin B_{12} deficient group

Parameters	Correlation
Vitamine B_{12} vs. Se	P = 0.047 r = 0.319
Vitamine B_{12} vs. Fe	P = 0.033 r = -0.337
Vitamine B_{12} vs. Hct	P = 0.045 r = -0.304
Vitamine B_{12} vs. MPV	P = 0.019 r = -0.362
Vitamine B_{12} vs. urea	P = 0.026 r = 0.352
Fe vs. MCV	P = 0.001 r = 0.469
Fe vs. RDW	P = 0.003 r = -0.440
Fe vs. iron binding capacity	P = 0.002 r = -0.479
Fe vs. ferritin	P = 0.025 r = 0.359
Zn vs. Cu	P = 0.015 r = 0.380
Zn vs. Se	P = 0.026 r = 0.356
Zn vs. leucocyte	P = 0.006 r = -0.404
Zn vs. platelet	P = 0.026 r = -0.335
Zn vs. glucose	P = 0.006 r = 0.408
Se vs. SGPT	P = 0.001 r = 0.473
Se vs. Mn	P = 0.030 r = -0.344
Cu vs. Hb	P = 0.004 r = -0.420
Cu vs. Htc	P = 0.005 r = -0.416
Cu vs. MPV	P = 0.018 r = 0.353
Cu vs. glucose	P = 0.036 r = 0.321
Co vs. RDW	P = 0.042 r = 0.306

r – correlation coefficient; P – significance level, Hct – hematocrit, Hb – hemoglobin, MPV – mean platelet volume, MCV – mean corpuscular volume, RDW – red cell distribution width, SGPT – serum glutamate pyruvate transaminase, SGOT – serum glutamic oxaloacetic transaminase

It is thought that increased oxidative stress may lead to health problems due to neglected vitamin B_{12} and trace element deficiencies. Many studies have reported that there is an imbalance between the production of reactive oxygen species (ROS) and antioxidant repair processes. Oxidative stress, which is characterized by an elevation in the steady-state concentrations of ROS, is involved in a wide range of biological and pathological conditions. The effects of ROS on lipids, proteins, and DNA are controlled by enzymatic and non-enzymatic antioxidants. ROS cause lipid peroxidation of polyunsaturated fatty acids in membranes and modification of biomolecules in biological systems.

It is believed that increased oxidative stress may lead to health problems due to deficiencies in vitamin B_{12} and trace elements. Numerous studies have reported an imbalance between the production of reactive oxygen species (ROS) and antioxidant repair mechanisms. Oxidative stress, characterized by elevated steady-state concentrations of ROS, is implicated in a wide range of biological and pathological conditions. The effects of ROS on lipids, proteins, and DNA are modulated by both enzymatic and non-enzymatic antioxidants. ROS induce lipid peroxidation of polyunsaturated fatty acids in membranes and modification of biomolecules within biological systems (Dalle-Donne et al. 2006, Bhattacharyya et al. 2014, Tian et al. 2017, Gimm et al. 2020, Karacaer et al. 2020).

Cu and Fe can promote the formation of ROS and catalyse the reaction between superoxide anion and hydrogen peroxide to produce hydroxyl radical. Additionally, Cu can directly bind to the free thiol groups of cysteines, leading to oxidation and the formation of cross-links between proteins, which can inactivate enzymes or degrade structural proteins. Zn may function as an antioxidant through two mechanisms. It can increase the availability of Fe and Cu by competing for binding proteins, and it can also protect against oxidative damage by binding to the sulfhydryl groups of proteins. Se is an important trace element that plays many fundamental roles in human health at both the cellular and organismal levels. The biological effects of Se are primarily mediated by selenoproteins, which are involved in antioxidant/redox and anti-inflammatory processes. Mn is an essential nutrient for intracellular activities, functioning as a cofactor for various enzymes, including manganese superoxide dismutase (Mn-SOD). Through these metalloproteins, Mn plays critically important roles in the regulation of development, digestion, reproduction, antioxidant defense, energy production, immune response, and neuronal activities (Hovdenak, Haram 2012, Cikim et al. 2023, Islam et al. 2023, Kamath et al. 2023). The International Agency for Research on Cancer (IARC) has classified cobalt (Co) as a probable carcinogen. However, Co is an essential trace element and a component of vitamin B_{12}, also known as cobalamin, which is crucial for the normal functioning of the brain and nervous system, as well as the process of erythropoiesis. Co is involved in the metabolism of every cell in the human body; it affects DNA synthesis, fatty

acid metabolism, and energy production. Additionally, it plays a role in the activation of certain enzymes and the stabilization of molecules and compounds that contribute to antioxidant defense mechanisms. Nevertheless, overexposure to Co can lead to adverse health effects (Halliwell, Gutteridge 1990, Aydemir et al. 2006, Chen et al. 2018, Qazi et al. 2019, Rizzo, Laganà 2019, Karagas et al. 2022, Tiszler et al. 2024).

Vitamin B_{12} is one of the largest and most complex vitamin, playing a crucial role in human cell metabolism and being essential for the proper functioning of the central nervous system and bone marrow. Vitamin B_{12} possesses anti-inflammatory and antioxidant properties and is involved in various important roles related to pathophysiological conditions (Weinberg et al. 2009, Manzanares, Hardy 2010, Romain et al. 2016, van de Lagemaat et al. 2019, Wald et al. 2022, Siddiqua et al. 2024). Previous studies have proposed several explanations for the associations between trace elements, vitamin B_{12} levels, and various diseases (Hovdenak, Haram 2012, Amin et al. 2020, Cikim et al. 2023, Kamath et al. 2023). Kıran et al. demonstrated that Co, Se, Zn, vanadium (V), magnesium (Mg), and vitamin B_{12} levels were significantly lower in individuals with vitamin B_{12} deficiency anemia compared to the control group. They also identified significant linear correlations between Co and vitamin B_{12}, as well as V element and mean corpuscular volume (MCV), but found a negative correlation between vitamin B_{12} and MCV in cases of B_{12} deficiency anemia (Kiran et al. 2023). Kuo et al. reported that the prevalence of mercury (Hg), Fe, vitamin B_{12}, folic acid deficiencies, and hyperhomocysteinemia was significantly higher in patients with Behçet's disease. Another study found that low levels of vitamin B_{12} and Co were more common than other micronutrient deficiencies in children with severe acute malnutrition (Kamath et al. 2023). Halifeoglu et al. found that homocysteine, vitamin B_{12}, Cu concentrations, as well as the Cu/Zn ratio, were significantly increased in patients with cirrhosis compared to control subjects. Conversely, folate and Zn levels were found to be decreased in cirrhotic patients. Positive correlations were observed between plasma concentrations of Cu and vitamin B_{12}, while an inverse correlation was found between homocysteine and Cu in cirrhotic patients (Halifeoglu et al. 2004). Yaikhomba et al. found that vitamin B_{12} deficiency was more prevalent than Fe and folate deficiencies in patients with severe acute malnutrition (Yaikhomba et al. 2015). Cikim et al. reported that levels of vitamin B_{12}, Zn, and Se were statistically significantly lower in female patients with gallstone disease compared to healthy female individuals. However, there was no statistically significant difference in Cu, nickel (Ni), and folate levels between these groups (Cikim et al. 2023).

Based on the results of our study, it can be suggested that a decrease in certain elements associated with vitamin B_{12} deficiency may be related to vitamin-element synergy, potentially leading to a reduction in antioxidant activities. While the levels of trace elements and vitamins have been investi-

gated in various studies, there is a lack of research evaluating their antioxidant properties; most studies focus instead on the etiology of diseases associated with element deficiencies. Our study has some limitations, including the collection of samples from a single center and the inability to assess these samples alongside antioxidant and oxidant markers.

CONCLUSIONS

In this study, we observed decreased serum levels of vitamin B_{12}, Zn, Mn, Fe, Co, and iron binding capacity in the vitamin B_{12} deficient group compared to the control group. The low levels of vitamin B_{12}, Zn, Mn, Co, and Fe in the vitamin B_{12} deficient group may be associated with increased oxidative damage. The reduction in vitamin B_{12}, Fe, Co, Zn, and Mn leads to a decrease in antioxidant status, thereby shifting the oxidant/antioxidant balance in favor of oxidants and resulting in damage to cellular biomolecules due to increased free radicals. A decrease in trace elements with antioxidant properties and vitamin B_{12} may disrupt cellular homeostasis through increased oxidative stress. Further research with larger sample sizes is needed to elucidate the oxidative stress-related mechanisms underlying trace element and vitamin B_{12} deficiencies.

Author contributions

CK, NB, BA, FBC, BKA, LA, MRS, EGA, FBN, YA, CD and TSN – researched literature and conceived the study, CK, NB, BA and FBC – were involved in protocol development, gaining ethical approval, patient recruitment and data analysis, CK, NB, BA, FBC, BKA, LA and MRS – wrote the first draft of the manuscript. All authors reviewed and edited the manuscript and approved the final version of the manuscript.

Conflicts of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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