Correlation between Serum B12 Levels and Lipid Peroxidation in B12 Deficiency Patients

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Abstract

Background: Diet is a potential factor contributing to the development of vitamins deficiencies and their clinical manifestations. Vitamin B12 deficiency has been linked to hematological, neuropsychiatric and metabolic disturbances. The role of vitamin B12 deficiency on lipid per oxidation has not been thoroughly explored. Therefore, the aim of this study was to assess the impact of vitamin B12 deficiency on the levels of lipid per oxidation marker Malondialdehyde and serum total antioxidant capacity in patients with symptoms and signs of B12 deficiency.

Methods: Serum levels of vitamin B12, malondialdehyde as a marker for lipid per oxidation and total antioxidant capacity were determined in blood samples taken from twenty five patients admitted to outpatient clinics. All patients had vitamin B12 deficiency symptoms and serologically confirmed to have low ≤ 200 Pg. /ml or borderline 201-300 Pg. /ml levels. None of the patients was diabetic or vegetarian.

Results: Serum levels of vitamin B12 for the studied patients were ranged between 57 and 291 pg. /ml. A significant inverse correlation was observed between lipid per oxidation profile measured as malondialdehyde and serum B12 levels. The relationship between serum total antioxidant capacity levels and vitamin B12 levels was significantly positive linear in both males and females subjects.

Conclusion: Serum total antioxidant capacity was significantly decreased with lowering serum B12 level. There is a strong correlation between vitamin B12 deficiency and lipid peroxidation which might be the initiator of the clinical manifestations of vitamin B12 deficiency.

INTRODUCTION

Vitamin B12 is a key micronutrient associated with one carbon metabolism and is an essential participant in methylation of nucleic acids and protein [1]. In sufficient intake or disrupted absorption of vitamin B12 results in deficiency which leads to several clinical manifestations. These include hematologic, metabolic and neuropsychiatric disorders [2]. There are multiple path ways in which vitamin B12 play a role in the pathogenesis of various cognitive disorders including dementia, neuro inflammatory, oxidative stress and methylation abnormalities [3]. Moreover; recent study shows the association of low vitamin B12 in type 2 diabetes patients with adverse lipid parameters and higher risk of coronary artery disease [4,5]. Oxidative stress including lipid per oxidation plays an important role in the pathophysiology of several age-related chronic diseases especially those linked with vitamin B12 deficiency [6]. Lipid per oxidation status is typically assessed by several methods one of the widely used method is the measurement of thiorbarbituric acid reactive substances (TARS) which are thought to reflect the production of malondialdehyde (MDA) [7]. The association of plasma levels of vitamin B12 with lipid per oxidation has not been studied in details, therefore; in the present study, we evaluated the relationship between serum levels of malondialdehyde (MDA) and total antioxidant capacity (TAC) with B12 levels in a group of patients with symptoms of vitamin B12 deficiency.

METHODS

Subjects

A total of twenty four patients fifteen females and nine males were selected for this study from outpatients clinics of two
local hospitals based in the Karak governorate in southern part of Jordan. All enrolled patients in this study were diagnosed by specialized pathologist to have vitamin B12 symptoms and signs. Their age range was 36-76 years and their mean ages were 53 Years. None of the patients had a history of gastric surgery or renal and liver diseases. Patients who took medications which are known to lower vitamin B12 level and diabetic as well as vegetarian patients were excluded. The study was approved by the research ethical committee of Mutah University.

**Samples**

Blood samples 5 ml each were drawn from the antecubital vein of 12 h fasting patients in the standard way and immediately stored at 4°C. The blood samples were centrifuged at 2500 rpm for 15 minutes. Serum samples were used immediately for measurements of all hematological parameters.

**Analytical measurements**

**Serum MDA as biomarker for lipid per oxidation:** MDA was estimated spectro photometrically by the double heating method of Draper and Hadley method [8]. For this purpose 2.5 ml of 10% trichloroacetic acid solution were added to 0.5ml plasma sample in each centrifuge tube and the tubes were placed in a boiling water bath for 15 min. After cooling in tap water, the tubes were centrifuged at 1000 rpm for 10 min and 2ml of the supernatants were added to 1 ml of 0.67% Thiobarbituric Acid (TBA) solution in a test tube. The tubes were then placed in a boiling water bath for 15min. The solutions were then cooled in tap water and their absorbance were measured at 532 nm. Control experiment was processed using the same experimental protocol except the TBA solution was used instead of distilled water. The concentration of MDA was calculated by the absorbance coefficient of the MDA-TBA complex ($E=1.56X10^5$) and is expressed as $\mu$mol/g of Hb.

**Total antioxidant capacity (TAC):** Serum TCA was determined using commercial kit manufactured by (Randox Laboratories, UK), according to the instructions provided by the manufacturer. 6-Hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid was provided by the same company was used as a standard antioxidant and the results were calculated and expressed as mmol/l/serum.

**Vitamin B12 determination:** Serum B12 level was determined in the clinical laboratories of the hospitals by standard ready -for- use electrochemiluminescence Immunoassay kits using Elecsys and Cobas e analyzer (Roche, Germany). Other hematological parameters including hemoglobin and circulating homocysteine were also determined by routine methods used by the hospital medical laboratories.

**Statistical analysis:** The Student’s t- test and ANNOVA correlation study were used to analyze the results. Statistically significant difference was set as $P \leq 0.05$

**RESULTS**

The study included 15 women and 9 men vitamin B12 deficit patients based upon the inclusion criteria mentioned in the methods section. All patients had normal standards hematological parameters. Serum vitamin B12 levels for all patients were determined and found to range between 57 to 291pg/ml for females and between 150 to 271 pg /ml for males. Patients with serum B12 level 200pg/ml or less were considered as having low level where those having 201 and less than 350pg/ml were considered as borderline. The vitamin B12 serum levels (mean ± sd in pg/ml) were 160.5 ± 7.5 and 153.5 ± 6.3 for low level vitamin B12 in female and male subjects. While in the borderline patients the levels were 230.1 ± 12.0 and 232.6 ± 12.3 respectively (Table 1). To gain some insight about the oxidative status of the studied samples we used MDA as an index for lipid peroxidation and TAC for the non-enzymatic antioxidants. The MDA serum levels expressed as (mean ± sd in mmol/L), were 4.98 ± 0.6 and 5.17 ± 0.6 for female and male low levels B12 subjects respectively. In borderline patients these values dropped to 1.42 ± 0.1and 2.28 ± 0.2 in females and males respectively (Table 1). The results of TAC serum levels showed that in female patients with borderline B12 levels, TAC value was 1.42 ± 0.13 mM. This was significantly reduced to 0.88 ± 0.09 (P ≤ 0.05) in low level B12 patients. Similar reduction was also observed in male patients; from 1.34 ± 0.12 to 0.99 ± 0.10 respectively (Table1). Furthermore when the correlation between MDA, TAC serum levels and vitamin B12 levels was studied, a significant inverse linear correlation was observed between serum level MDA and vitamin B12 level in both male and female patients (Figure 1A,B). While appositive and significant correlation was found between

![Figure 1 Correlation between serum vitamin B12 and MDA. A) Correlation between serum vitamin B12 level (Pg/ml) and MDA (µmol/L) of male patients. B) Correlation between serum vitamin B12 level (Pg/ml) and MDA (µmol/L) of female patients.](image-url)
serum TAC and vitamin B12 levels. (Figure 2A,B). Homocysteine levels in vitamin B12 deficiency patients were ranged from 9 to 14.2 (µmol/L) and the mean ± SD was 10.4 ± 2.13. No significance correlation was observed between homocysteine levels and either serum MDA or TAC levels.

DISCUSSION

Vitamin B12 deficiency has emerged as a public health concern in recent years especially in poor nutrition societies. It was estimated that about one third of adult population in Jordan are vitamin B12 deficient [9,10]. It is well known that vitamin B12 is important for DNA synthesis, methionine production for protein synthesis and methylation and preventing the accumulation of homocysteine [11]. Although Vitamin B12 deficiency has been shown to be prevalence in type 2 diabetes patients and was associated with adverse lipid parameters [4,5]. There is a scarcity of published research that addresses the correlation of vitamin B12 deficiency with lipid peroxidation which might be caused by free radicals generation. The measurement of TAC has been widely used to indirectly assess free-radical activity in biological samples, and may offer more relevant information compared to that obtained by the measurement of individual antioxidant components in plasma and body fluids [12]. The results of the present study suggest that low serum level of vitamin B12 is associated with oxidative stress. This is obvious when the two known markers of oxidative stress; TAC and MDA and their correlation with serum levels of vitamin B12 were evaluated. There was a negative correlation between MDA levels and vitamin B12 concentrations and a positive correlation with TAC levels. When serum homocysteine levels were measured for all patients in the present study, higher levels were observed in low and borderline levels vitamin B12 subjects but without significant correlation with either TAC or MDA serum levels. It is known that vitamin B12 is an essential component in the metabolism of homocysteine a possible mediator for lipid peroxidation. Homocysteine is produced as a result of methylation reactions and removed either by its irreversible conversion to cysteine by trans-sulfuration or by remethylation to methionine [11]. Moreover accumulation of circulating homocysteine was demonstrated in vitamin B12- deficient humans [12] and animals [13]. The elevated homocysteine levels reported in the present study might be due to vitamin B12 deficiency and this elevation might promote increased oxidative stress that resulting in the observed lipid peroxidation. In conclusion, our study showed that serum TAC levels are lower and MDA levels are higher in patients with low and borderline serum vitamin B12 levels. These provide a marker for lipid peroxidation, however, the initiator of peroxidative processes is unknown and further studies are needed to verify this notion.

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