RESEARCH ARTICLE

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Relationship of Vitamin B12 and Vitamin D with IL-4, IL-10, TNF Beta in Obese Patients

ABSTRACT

Objective: We aimed to investigate the relationship of vitamin B12 and vitamin D with IL-4, IL-10 and TNF-Beta in obese patients.

Method: Serum IL-4, IL-10 and TNF-Beta levels were measured using kits based on the enzyme-linked immunosorbent assay (ELISA) principle.

Results: The IL-10 level was found to be significantly lower in the low vitamin D group (p= 0.039). When vitamin B12 normal, vitamin B12 low and control groups were compared, a statistical difference was found between the groups in terms of IL-10 (p=0.002). As a result of post hoc analysis, the IL-10 level was found to be significantly lower in the vitamin B12 low group than in the vitamin B12 normal group (0.04). At the same time, vitamin B12 was statistically higher in the normal group (obesity positive) than in the control group (non-obese vit B12 normal) (p=0.001). A positive correlation was found between vitamin B12 and IL-10 (r= 0.203 p= 0.058).

Conclusion: It has been shown that low levels of vitamin D and vitamin B12 in obese patients cause low levels of IL-10. It was also found that obesity caused an increase in IL-10 levels. No relationship was found between IL-4 and TNF-Beta and vitamin D and vitamin B12. Longer follow-up and studies in larger case populations are needed to better understand the effects of vitamin B12 and vitamin D on IL-4, IL-10 and TNF-Beta levels in obese patients.

Keywords: IL-10, IL-4, Obesity, TNF-Beta, Vitamin B12, Vitamin D.

Obez Hastalarda Vitamin B12 ve Vitamin D' nin IL-4, IL-10, TNF-Beta ile İlişkisi

ÖZET

Amaç: Obez hastalarda vitamin B12 ve vitamin D nin IL-10, IL-4, TNF-Beta ile ilişkisini incelemeyi amaçladık.

Gereç ve Yöntem: Serum IL-10, IL-4 ve TNF-Beta seviyeleri ELISA (Enzyme Linked Immunosorbent Assay) prensibine dayalı kitler kullanılarak ölçülmüştür.

Bulgular: IL-10 düzeyi, vitamin D düşük olan grupta anlamlı olarak daha düşük bulunmuştur (p= 0.039). Vitamin B12 normal, vitamin B12 düşük ve kontrol grupları karşılaştırıldığında IL-10 açısından gruplar arasında istatistiksel düzeyde fark bulunmuştur (p=0.002). Post hoc analizi sonucunda IL-10 düzeyi, vitamin B12 düşük grubunda vitamin B12 normal grubuna göre anlamlı olarak daha düşük olarak tespit edilmiştir (0.04). Aynı zamanda vitamin B12 normal grupta (obezite pozitif) kontrol grubuna (non-obez vit B12 normal) göre daha yüksek olarak belirlenmiştir (p=0.001). IL-10 ile vitamin B12 ve Vitamin D arasında korelasyon bakıldığında vitamin B12 ile IL-10 arasında pozitif korelasyon tespit edilmiştir (r=0.203 p= 0.058).

Sonuç: Obez hastalarda vitamin D ve vitamin B12 düşük seviyelerinin IL-10 düzeyinde düşüklüğe neden olduğu gösterilmiştir. Ayrıca obezitenin de IL-10 seviyesinde artışa neden olduğu belirlenmiştir. IL-4 ve TNF-Beta ile vitamin D ve vitamin B12 arasında ilişki bulunamamıştır. Obez hastalarda vitamin B12 ve vitamin D nin IL-4, IL-10, TNF-Beta düzeylerine etkilerini daha iyi görebilmek için daha uzun süreli takip ve daha geniş vaka popülasyonlarında yapılacak çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: IL-4, IL-10 Obezite, TNF-Beta, Vitamin B12, Vitamin D.

INTRODUCTION

Cytokines are small glycoproteins that are made by immune and non-immune cells. They play a key role in causing or stopping inflammation, angiogenesis or angiostasis, tissue damage or repair, and other things (1). Cytokines regulate inflammation, cell growth, tissue healing and systemic response to injury, immune and inflammatory events (2). Cytokines can be classified as proinflammatory [interleukin (IL)-1β, IL-2, IL-6, IL-12, IL-18, tumor necrosis factor-alpha (TNFα), interferon (IFN)-γ]; anti-inflammatory (IL-4, IL-10, IL-17) and regulatory cytokines (TGF-β, IL-27 and IL-6) (3-7).

Vitamin B12 (cobalamin) is a water-soluble vitamin that contains cobalt, cannot be synthesized in the human body and must be taken from outside. It exists in four forms in the body: cobalamin, methylcobalamin, adenosylcobalamin, hydroxocobalamin, and cyanocobalamin (8). Vitamin B12 is an essential micronutrient required for optimal haemopoietic, neurological and cardiometabolic function (9,10). Oztaş et al. found that vitamin B12 level associated with inflammatory markers and stated that can be used as an inflammatory marker (8). In the literature, vitamin B12 has a regulatory effect on inflammation and proinflammatory cytokines increase in vitamin B12 deficiency has been reported (11). As it is known, especially vitamins A, C, D, E, B2, B6 and B12, folic acid, iron, selenium, zinc and glutamine, arginine, taurine and various amino acids such as sulfur-containing amino acids have an immunomodulatory effect (12).

Vitamin D is formed mostly by the skin through exposure to sunlight and very little of it is taken in through diet. It is converted to 25 OH vitamin D in the liver and to the active form of 25-hydroxyvitamin D (1,25 dihydroxy vitamin D) in the kidney (13,14) The classical roles of vitamin D are regulation of calcium and phosphate metabolism and homeostasis, bone metabolism, and cell growth and division (14). Reported in publications that vitamin D increases the production of some anti-inflammatory cytokines and reduces the release of pro-inflammatory cytokines (15). Experimental studies have shown that 1,25-dihydroxyvitamin D (calcitriol) produces immunological activities on the innate and adaptive immune system and endothelial membrane stability. It has been reported in publications that low serum 25-hydroxyvitamin D [25(OH) D] levels are associated with an increased risk of developing immune-related diseases such as psoriasis, type 1 diabetes, multiple sclerosis, and autoimmune diseases (16).

In this study, we aimed to investigate the relationship between vitamin B12 and vitamin D levels, which have been shown in various studies to be related to inflammation and the immune system in the body, and the anti-inflammatory cytokines IL-4, IL-10 and the regulatory cytokine TNF-Beta in obese patients.

MATERIAL AND METHODS

Patient Selection and Study Design: Fifty obese patients and twenty nine control groups who applied to the Ministry of Health-Ordu University Education and Research Hospital Internal Medicine outpatient clinic were included in the study. The control group consisted of non-obese patients with normal vitamin B12. Vitamin D control group could not be established since the patient who was not obese and had a normal vitamin D level meeting the inclusion and exclusion criteria could not be found sufficiently. Patients with chronic liver disease, malignancy, autoimmune disease, acute and chronic infections, patients taking vitamin therapy, using drugs that have an effect on inflammation, and patients with immune deficiency were not included in the study. In addition, only white-skinned individuals were included in the study.

Sociodemographic characteristics, height and weight, last checked vitamin D and vitamin B12 levels, other hemogram and biochemical parameters, CRP and TSH values of the patients were recorded. BMIs were calculated [BMI = weight (kg)/height^2 (m^2)] (17). Venous blood samples were taken from the patients who accepted the study and gave consent.

Anti-inflammatory cytokines IL-4 and IL-10, regulatory cytokine TNF-Beta biomarkers were studied from these blood samples in Ordu University Medical Faculty Medical Biology Laboratory. Obese patients to be evaluated for vitamin B12 were grouped as vitamin B12 normal (≥200) and vitamin B12 low (<200) (18). A control group was also formed from non-obese vitamin B12 normal patients. Patients to be evaluated for vitamin D were divided into groups as vitamin D normal (≥20) and vitamin D low (<20) (19). Vitamin D control group could not be formed because the patient who was not obese, had normal vitamin D level and met the exclusion criteria, could not be found sufficiently.

Laboratory Analyzes: Serum IL-4, IL-10 and TNF-Beta levels were measured using kits based on the enzyme-linked immunosorbent assay (ELISA) principle. Serum IL-4, IL-10 and TNF-Beta was measured using the Cloud-Clone Corp ELISA Kit (Cloud-Clone, USA). The protocols of the relevant kits were followed. Absorbance (450 nm) was measured using the Biotek Epoch 2 microplate reader and Gen5 software. The amounts of IL-4, IL-10 and TNF-Beta in the serum were calculated from the standard curve and the results were expressed as pg/ml. The range of detection was 15.6 to 1.000 pg/ml for IL-4 and TNF-Beta, and 7.8-500 pg/ml for IL-10.

Statistics Analysis: The data were tested in terms of normality with Kolmogorov-Smirnov test. Normally distributed data were analyzed with One-Way Anova test and Student's-t test. Non-normally distributed data were compared with Kruskal Wallis test and Mann-Whitney U test. Categorical data were compared using the Chi-Square test. In the chi-square tests, if a cell had an expected frequency below 5, likelihood ratio chi-square value was used instead of Pearson chi-square value. The numeric variables as mean ± SD and median (min-max), the categorical variables as percentage were expressed. Pearson correlation test was used for normally distributed data and Spearman correlation test was used for non-normally distributed data. SPSS v25 (IBM Inc., Chicago, IL, USA) statistical software was used. Results were evaluated at 95% confidence interval and the significance level was $p < 0.05$.

RESULTS

When the vitamin B12 normal group (obese Vitamin B12 normal), vitamin B12 low group (obese vitamin B12 low) and control groups (non-obese vitamin B12 normal) were compared; there was no difference between the groups in terms of gender, smoking or chronic diseases (Table 1).

Table 1. Comparison of gender and chronic diseases of vitamin B12 groups

	Vit B12 normal (n=33)	Vit B12 low (n=17)	Control group (n=29)	P value
Gender (%)				
Male	10 (29.4)	5 (30.3)	12 (41.4)	0.580
Female	23 (70.6)	12 (69.7)	17 (58.6)	
Hypertension (%)	22 (66.7)	11 (64.7)	13 (44.8)	0.183
Thyroid diseases (%)	7 (21.2)	2 (11.8)	9 (31)	0.294
Coronary Artery Disease (%)	2 (6.1)	0	1 (3.4)	0.423
Heart failure (%)	1 (3)	0	0	0.414
Cigarette (%)	10 (17.6)	3 (30.3)	8 (27.6)	0.606

When the hemogram, biochemical parameters, CRP, TSH, HbA1c, IL-4, IL-10 and TNF-Beta were compared, a difference was found between the groups in terms of blood urea nitrogen (BUN) and IL-10 (respectively p= 0.013, p= 0.002). As a result of post hoc analysis, BUN was found to be significantly lower in the low vitamin B12 group than in the control group (p= 0.009). As a result of post hoc analysis, IL-10 was higher in the vitamin B12 normal group than in the control group (p= 0.001). IL-10 was significantly higher in the vitamin B12 normal group than the vitamin B12 low group (0.04). There was no difference between the groups in terms of IL-4, TNF-Beta (Table 2).

Table 2. Comparison of age and laboratory tests of vitamin B12 groups

	Vit B12 normal (n=33)	Vit B12 low (n=17)	Control group (n=29)	P value
Age (years)	51.27±12.31	49.29±11.72	50.72±15.68	0.665
Hemoglobin (g/dl)	13.66±1.65	13.32±1.78	14.14±1.49	0.233
White blood cell (10^3 uL)	7.63±2.17	6.56±1.94	7.28±1.74	0.171
Platelet (10^3 Ul)	252±67	269±59	257±64	0.706
Fasting blood glucose (mg/dl)	157±73.60	142±65.04	138.63±60.80	0.281
Creatinine (mg/dl)	0.78±0.21	0.68±0.14	0.84±0.34	0.161
Blood urea nitrogen (mg/dl)	14.16±4.67	10.56±2.90	15.96±7.85	***0.013***^{b}
Aspartat aminotransferase (IU/l)	24±15.87	19.41±9.57	17.77±6.32	0.341
Alanine aminotransferase (IU/l)	26.89±17.65	21.41±15.01	18.68±10.11	0.126
Total bilirubin (mg/dl)	0.47±0.21	0.49±0.34	0.51±0.21	0.385
CRP (mg/dl)	0.52±1.32	0.50±0.48	0.69±1.18	0.464
Total cholesterol (mg/dl)	205.4±49.6	196.8±39.1	196.20±44.19	0.837
LDL-cholesterol (mg/dl)	118.8±44.6	165.07±21.6	117.2±38.24	0.979
HDL-cholesterol (mg/dl)	46.07±10.67	45.31±14.10	49.98±12.9	0.262
Triglyceride (mg/dl)	233.35±148.69	196.94±122.77	179.20±205	0.437
TSH (mU/L)	2.64±1.24	2±1.33	2.04±1.43	0.189
HBA1c	7.63±1.99	6.96±1.73	6.98±1.97	0.124
IL-4 (pg/mL)	25±22.26	22.8±29.69	19.15±18.78	0.160
IL-10 (pg/mL)	9.66±0.99	9.44±1.74	8.96±0.88	***0.002***^{a,c}
TNF-Beta (pg/mL)	20.06±1.63	19.72±2.67	18.65±2.94	0.124

CRP: C Reactive protein. TSH: Thyroid stimulating hormone. HBA1c: Hemoglobin A1c. IL-4: Interleukin 4. IL-10: Interleukin 10. TNF-Beta: Tumor Necrosis Factor Beta

a: There is a difference between the control group and the vit B12 normal group

b: There is a difference between the control group and the vit B12 low group

c: There is a difference between vit B12 normal group and vit B12 low group

When the vitamin D low group and vitamin D normal group were compared; there was no difference between the groups in terms of gender, smoking and chronic diseases (Table 3). When compared in terms of hemogram, biochemical parameters, CRP, TSH, HbA1c, IL-4, IL-10 and TNF-Beta; BUN was higher in the vitamin D normal group (p= 0.011). IL-10 was found to be significantly higher in the vitamin D normal group (p= 0.039). There was no difference between the groups in terms of IL-4, TNF-Beta (Table 4).

Table 3. Comparison of gender and chronic diseases of vitamin D groups

	Vit D normal (n=34)	**Vit D low (n=19)**	**P value**
Gender (%)			
Male	11 (32.4)	5 (26.3)	0.646
Female	23 (67.6)	14 (73.7)	
Hypertension (%)	22 (64.7)	12 (63.2)	0.910
Thyroid diseases (%)	8 (23.5)	2 (10.5)	0.229
Coronary Artery Disease (%)	2 (5.9)	0	0.149
Heart failure (%)	0	1 (5.3)	0.177
Cigarette (%)	11 (32.4)	3 (15.8)	0.177

Table 4. Comparison of age and laboratory tests of vitamin D groups

	Vit D normal (n=34)	**Vit D low (n=19)**	**P value**
Age (years)	50.53±12.09	50.05±11.42	0.889**
Hemoglobin (g/dl)	13.73±1.73	13.1±1.73	0.280**
White blood cell (10^3 uL)	7.14 (3.08-12.6)	6.38 (4.01-11.42)	0.105*
Platelet (10^3 Ul)	262.70±80.79	264.78±59.14	0.922**
Fasting blood glucose (mg/dl)	132 (87-278)	114.5 (90-305)	0.303*
Creatinine (mg/dl)	0.71 (0.51-1.24)	0.65 (0.46-0.94)	0.810*
Blood urea nitrogen (mg/dl)	13.6 (6.9-23.2)	10.25 (7.2-15.8)	***0.011****
Aspartat aminotransferase I(U/l)	19 (11-74)	19 (6-50)	0.543*
Alanine aminotransferase (IU/l)	22 (11-77)	16 (8-64)	0.102*
Total bilirubin (mg/dl)	0.44 (0.23-1.18)	0.42 (0.27-1.69)	0.710*
CRP (mg/dl)	0.3 (0.08-5.8)	0.33 (0.07-3.37)	0.578*
Total cholesterol (mg/dl)	206.73±48.95	199.0±38.48	0.556**
LDL-cholesterol (mg/dl)	109.2 (52.5-231.1)	117.3 (62.5-995.7)	0.783*
HDL-cholesterol (mg/dl)	45.7±10.7	44.4±13.5	0.697**
Triglyceride (mg/dl)	154 (83-646)	167 (77-594)	0.591*
TSH (mU/L)	2.47 (0.79-5.24)	1.72 (0.47-4.88)	0.368*
HBA1c	7.2 (5-10.6)	6.05 (5.6-11)	0.106*
IL-4 (pg/mL)	15.6 (15.6-105)	15.6 (15.6-138.02)	0.226*
IL-10 (pg/mL)	9.35 (8.59-12.34)	8.78 (8.21-15.6)	***0.039****
TNF-Beta (pg/mL)	19.83 (17.44-24.36)	19.02 (17.06-29.05)	0.140*

CRP: C Reactive protein. TSH: Thyroid stimulating hormone. HBA1c: Hemoglobin A1c. IL-4: Interleukin 4. IL-10: Interleukin 10. TNF-Beta: Tumor Necrosis Factor Beta

*Mann-Whitney U test, **Student's-t test

When the correlation between IL-10 and vitamin B12 and vitamin D was examined, a positive correlation was found between vitamin B12 and IL-10 (r=0.203 p= 0.058). No correlation was found between vitamin D and IL-10 (Table 5).

Table 5. Correlation between IL-10 and vitamin B12-vitamin D

	r	**P value**
Vit D	0.203	0.058
Vit B12	0.09	0.411

DISCUSSION

In this study, IL-10 levels were found to be significantly lower in the vitamin D low group in obese patients (p= 0.039). IL-10 level was found to be significantly lower in the vitamin B12 low group than in the vitamin B12 normal group (P=0.04). When the correlation between IL-10 and vitamin B12 and vitamin D was examined, a positive correlation was found between vitamin B12 and IL-10 (r=0.203 p= 0.058). There was no difference between the groups in terms of IL-4 or, TNF-Beta.

Vitamin D functions by binding to the nuclear vitamin D receptor (VDR) and retinoid X receptor to regulate gene transcription. VDR has been identified in many other tissues, including the immune system. Vitamin D is considered to be one of the important regulators of the immune system. It has been shown that all cells of the immune

system, including T cells, express VDR (14). After T cells are activated, they induce 1,25(OH)2 vitamin D by expressing VDR and autocrinely. 1,25(OH)2 vitamin D induces IL-4 while inhibiting the proliferation of mouse and human T cells, producing IFN-γ and IL-17 (14) As part of the adaptive immune system, vitamin D interferes with T lymphocyte proliferation and function. Antigen-activated pluripotent Th0 lymphocytes produce a variety of cytokines, including IL-2, IL-4, IL-10 and interferon γ (IFN-γ). Calcitriol directly inhibits the expression of Th1 cytokines (IL-2, IFN-γ, tumor necrosis factor) and stimulates Th2 cytokines (IL-3, IL-4, IL-5, IL-10) (16). Consistent with the literature, we found the IL-10 level, known as an anti-inflammatory cytokine, to be low in the obese vitamin D low group in this study.

It is mentioned in publications that vitamin B12 deficiency increases inflammation (20,21), since vitamin B12 has anti-inflammatory activity (22). Vitamin B12 deficiency causes hyperhomocysteinemia (HHcy), and high homocysteine is known to cause an inflammatory state (23). In the literature, there is no correlation between vitamin B12 level and IL10. In this study, it was found that IL10, an anti-inflammatory cytokine, was low in obese patients with low vitamin B12. The anti-inflammatory activity of vitamin B12 can be explained by this mechanism other than homocysteine.

Interleukin 4 (IL-4) plays a role in the development and upregulation of Th2 cells, is required for the biosynthesis of IgE (24). A number of inflammatory cells including T helper type 2 (Th2) cells, innate lymphoid cells, mast cells and basophils secrete interleukin (IL-) 4 and IL-13 (25,26). IL-4 may have a profibrotic effect on scleroderma dermal fibroblasts. These cells have IL-4 receptors and when cultured in media containing IL-4, collagen production is significantly increased (27). IL-4 shares biological properties and receptor specificity with the related cytokine IL-13. Clinical trial results suggest that blocking IL-4 and IL-13 may benefit SSc skin fibrosis (28). In this study, no relationship was found between vitamin B12, vitamin D and IL-4.

Increased TNF-Beta expression is associated with the formation of lymphocytic aggregates (29). It has been reported in publications that it is one of the inhibitory cytokines such as IL-10 and tumor necrosis factor (TNF)-Beta. (30). In this study, we could not find a relationship between TNF-Beta and vitamin D or vitamin B12.

CONCLUSION

It has been shown that low vitamin D and vitamin B12 levels in obese patients cause low IL-10 levels. At the same time, it was determined that obesity caused an increase in IL-10 level. No relationship was found between IL-4 and TNF-Beta and vitamin D and vitamin B12.

Ethics Statement: This study was approved by the Ordu University, Faculty of Medicine Institutional Review Board Ethics Committee (date: 07.10.2021 number: 2021/ KAEK 232).

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