Evaluation of serum Vitamin B12 level and related nutritional status among apparently healthy obese female individuals

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Abstract

Objective: Obesity is a major public health problem and great risk for not only cardiovascular diseases but also cancer, musculoskeletal, and gynecological diseases. This study was aimed to investigate the association between serum Vitamin B12 (vitB12), body mass index (BMI), and nutritional status among obese women.

Methods: This cross-sectional study enrolled consecutive female subjects. The consumptions of red meat, fish, bovine liver, egg, and mushroom were recorded. According to the Dietary Reference Intakes, the patients were categorized as insufficiency and sufficiency. Three cutoff points were defined for vitB12 status: (1) Deficiency if vitB12 is <200 pg/mL; (2) insufficiency if vitB12 is 250–350 pg/mL, and (3) sufficient if vitB12 is ≥350 pg/mL. According to BMI, the patients were assigned to nonobese and obese groups. BMI, serum vitB12 level, consumptions of red meat, fish, bovine liver, egg, and mushroom were evaluated and compared between two groups.

Results: Mean level of vitB12 was 247.8 ± 10.4 pg/mL and significantly associated with consumption of egg (P = 0.031), bovine liver (P = 0.004), mushroom (P = 0.040), and red meat (P = 0.003). VitB12 was significantly higher in nonobese than obese group (282.5 ± 106.8 vs. 242.5 ± 107.5 pg/mL, P = 0.001). The ratio of vitB12 deficiency was significantly higher in obese than nonobese group (37.6% vs. 24.7%; P = 0.019). VitB12 level was negatively correlated with BMI (r = −0.155; P < 0.001), but not insulin resistance (r = −0.172; P = 0.062).

Conclusion: Obesity was associated with low level of vitB12 in obese women, and more likely to be vitB12 deficient. Consumption of certain types of food contributes to increase vitB12 level.

Key words: Body mass index, insulin resistance, nutrition, obesity, Vitamin B12

Date of Acceptance: 29-Mar-2016

Introduction

Vitamin B12 (VitB12) is an essential nutrient in human nutrition. VitB12 deficiency may affect millions of people worldwide, with significant public health implications. This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

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Access this article online
Quick Response Code:
Website: www.njcponline.com
DOI: 10.4103/1119-3077.181401

consequences including anemia, neurological diseases, and hyperhomocysteinemia with significant public health consequences. There is increasing evidence that subclinical vitB12 deficiency (asymptomatic patients) or a marginal long-term status of vitB12 deficiency may have detrimental health consequence.\textsuperscript{[1,2]} VitB12 deficiency is widely present in the geriatric population, with a prevalence of 5–20%. Age as a factor is associated with poor nutritional status, which may partly explain the poor clinical outcome in older patients.\textsuperscript{[3]}

VitB12 deficiency is well documented in adults with inadequate intake, gut malabsorption, or pernicious anemia. Malabsorption of vitB12 is also associated with metformin therapy, proton pump inhibitors, increasing in weight, insulin resistance, and Helicobacter pylori infection.\textsuperscript{[4–7]} It is well known that obesity is epidemic, and many countries are suffering from obesity.\textsuperscript{[8,9]} Obesity is a great risk for Vitamin D deficiency along with dyslipidemia, hypertension, metabolic syndrome, and diabetes mellitus.\textsuperscript{[10,11]} While obesity itself does not contribute to vitamin deficiency, the consequences of obesity can play a role such as nutritional contents and absorption.\textsuperscript{[12]} Association of H. Pylori with obesity is controversial. It is previously documented that its prevalence was high in obese individuals, but a study reported that it was not increased among obese young individuals in Greece.\textsuperscript{[13–15]}

Pinhas-Hamiel et al.\textsuperscript{[16]} found a >4-fold increased risk of low vitB12 status in obese compared to normal weight children and adolescents in Israeli. Baltaci et al.\textsuperscript{[17]} reported that vitB12 concentration was low in middle-aged obese women and negatively correlated with body mass index (BMI), compared to lean body individuals.

Based on the literature, it was hypothesized that there will be an inverse correlation between BMI and low vitB12 levels in serum. The aim of this study is to investigate the association of vitB12 with BMI and nutritional status among obese individuals.

**Methods**

**The study design**

The study was designed as cross-sectional survey and conducted between January 2014 and June 2014 by the Department of Family Medicine at Medical Faculty in Duzce University, Duzce, Turkey. The consecutive subjects who were admitted to out-patient clinic of family medicine for periodic examination and tested for vitB12 level were enrolled. The study survey included information about basic sociodemographic data, anthropometric measurements, and basic nutritional status regarding consumption of red meat, fish, bovine liver, egg, and mushroom were applied to subjects in face to face interview.

**Sample size**

Based on the prevalence of vitB12 deficiency reported a study with large Turkish population, it was accepted as 29.3\%\textsuperscript{[18]} Confidence level was as %90 and $\alpha$ as %3. Estimated sample size the study was about 621.

**The inclusion and exclusion criteria**

The nonpregnant subjects aged between 18- and 65-year-old were included. Informed consent was inclusion criteria. The subjects who were able to understand and fill the study survey were enrolled. The subjects with hypertension, mild depression-anxiety disorders, and postmenopausal women were not excluded.

The subjects who have used proton pump inhibitors, metformin, corticosteroids and colchicine over 3-month periods were excluded. The subjects who have used vitamin replacement within last 6 months were excluded. The data of the patients previously diagnosed with diabetes mellitus, chronic renal and hepatic diseases, cancer, and underweight (BMI <18.5 kg/m\(^2\)) were dropped to be analyzed.

**Ethics**

This study was conducted following the guidelines outlined in the Declaration of Helsinki. All procedures involving human patients were approved by the Ethic Committee of Medical Faculty, Duzce University, Duzce, Turkey (Ethic No: 2014/51). Informed consent was obtained from all patients.

**Blood sample**

Blood sampling was performed for every subject on admission and assayed on visit day. Venous blood samples were drawn into a tube with ethylenediaminetetraacetic acid for complete blood count (CBC), and a standard biochemical tube for biochemical assay from all patients in the morning time, at least, an 8-h overnight fasting. The tubes were gently shaken and then separated by centrifugation at 3200 rpm for 10–15 min. Lipid profiles were assayed using the colorimetric method (Cobas 6000 C 501, Roche Diagnostics GmbH, and Mannheim, Germany) Insulin, folic acid and vitB12 levels were assayed using electrochemiluminescence immunoassay method (Cobas 6000 C 601, Roche Diagnostics GmbH, and Mannheim, Germany). Homeostasis model assessment of insulin resistance (HOMAIR) was calculated with the formula: Fasting serum glucose (mg/dL) × fasting plasma insulin level (μU/mL)/405.\textsuperscript{[19]}

**Nutritional status**

A study survey was applied for nutritional status of subjects in face to face interview. According to Dietary Reference Intakes (DRI) recommendation, consumptions of red meat, fish, bovine liver, egg, and mushroom were recorded, and the subjects were categorized as sufficiency DRI group and insufficiency DRI group.\textsuperscript{[20]} Red meat
Anthropometric measurements

The anthropometric values of subjects were measured: Body weight, height, and waist circumference (WC) and hip circumference, according to standard procedures. Weight and height were measured with the patients wearing light clothing and without shoes. Height was recorded with accuracy of 0.1 cm with a stadiometer. Weight was recorded to the nearest 0.1 kg using a balance-beam scale. WC was measured with accuracy of 0.1 cm at the midpoint between the last rib and the top of the iliac crest, at the end of exhalation, using a waistline measurer employed with patients standing without clothing covering the waist area.

Bioelectric impedance fat analysis

Bioelectric impedance (BEI) visceral and total body fat composition was measured with biochemical impedance analyzer with 50 kHz bioimpedance meter without shoes in light indoor clothes using a hand-to-foot single frequency (Omron BF 510; Omron Corp. Kyoto, Japan). After entering the patients’ data such as height, age, and gender in the BIA, electrodes were placed on hand and foot. The subjects were fasting and wearing barefoot and light clothing. All metallic accessories were removed. The subjects with pregnancy and cardiac pacemaker were avoided from BIA measurement according to manufacturer’s instructions.

Determination of obesity and Vitamin B12 deficiency

Obesity was defined as a BMI value >29.9 kg/m², described by the WHO.[21] The subjects were assigned to two groups of nonobese and obese according to BMI classification. In the study, we used three descriptive cut-off points to define status of vitB12, suggested by Tucker et al.[21] Accordingly, the current clinical cutoff was 200 pg/mL (148 pmol/L), the intermediate point was 250 pg/mL (185 pmol/L), and a point at which individuals may be at risk of deficiency was 350 pg/mL (258 pmol/L).

Data analysis and statistics

Data were analyzed using SPSS 15.0 (SPSS Inc., Chicago, IL, USA). Data were expressed as means ± standard deviations for continues variables, and number and percentages for categorical variables. Age, WC, BMI, BEI total fat, BEI visceral, high-density lipoprotein cholesterol low-density lipoprotein cholesterol (LDL-cho), triglyceride, HOMAIR, and vitB12 were not normally distributed. Logarithmic transformation was applied for continues variables which were not normally distributed, and logarithmically transformed values were presented as adjusted value. Student’s t-test performed between two groups of obese and nonobese patients. Serum level vitB12 in nonobese group and obese groups were shown in error bars graph. Mean level serum vitB12 was also compared between categories of nutritional status (insufficient DRI group and sufficient DRI group) regarding red meat, bovine liver, egg, mushroom, and fish consumption. Nutrition status of subjects was compared between nonobese and obese groups using Fisher’s exact test. Spearman’s coefficient was performed to analyze correlation between vitB12 levels and BMI and HOMAIR. A two-tailed P < 0.05 was considered statistically significant.

Results

The study included 680 female patients between 18- and 65-year-old (36.7 ± 12.3 year). The frequency of patients previously diagnosed with hypertension, depression was 26.8% and 32.5% in nonobese and obese groups, respectively. The ratio of patients who have been treated for depression was slightly higher among obese group (2.9% vs. 1.7%). About 24% of the obese patients were postmenopausal, but 16% of the nonobese patients were postmenopausal. When assigned into two groups, nonobese group was presented with 40.2% (n = 273) and obese group was 58.8% (n = 407). Table 1 presents the comparative and basic features of subjects regarding age, BMI, WC, WHR, BEI total, BEI visceral fat, lipid profile, folic acid, and homa-ir.

Table 1: Basic metabolic and anthropometric features of subjects

<table>
<thead>
<tr>
<th>Metabolic and demographic features</th>
<th>All (mean±SD)</th>
<th>Groups (mean±SD)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>38.1±12.3</td>
<td>36.6±13.3</td>
<td>40.6±11.8</td>
</tr>
<tr>
<td>Folic acid (pg/mL)</td>
<td>9.72±3.6</td>
<td>9.46±3.41</td>
<td>9.79±3.71</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>34.8±11.4</td>
<td>26.8±2.6</td>
<td>37.5±5.9</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>101.3±12.2</td>
<td>88.8±9.7</td>
<td>107.1±13.2</td>
</tr>
<tr>
<td>BEI total fat (%)</td>
<td>44.2±7.4</td>
<td>32.4±8.1</td>
<td>46.6±8.2</td>
</tr>
<tr>
<td>BEI visceral (%)</td>
<td>9.6±3.2</td>
<td>6.7±1.4</td>
<td>11.1±3.7</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>117.2±32.4</td>
<td>110.2±24.2</td>
<td>118.1±44.3</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>51.1±12.0</td>
<td>55.4±14.2</td>
<td>42.3±13.3</td>
</tr>
<tr>
<td>nTG (mg/dL)</td>
<td>129.9±64.3</td>
<td>116.4±76.7</td>
<td>138.4±67.8</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>3.4±2.4</td>
<td>2.2±1.5</td>
<td>3.7±3.5</td>
</tr>
</tbody>
</table>

HOMA-IR = Homeostasis model assessment of insulin resistance; LDL-C = Low-density lipoprotein cholesterol; HDL-C = High-density lipoprotein cholesterol; SD = Standard deviation; BEI = Bioelectric impedance; BMI = Body mass index; TG = Triglycerides
and HOMAIR. Mean serum level of LDL-chol ($P = 0.057$) and folic acid ($P = 0.707$) were not statistically different between two groups.

Table 2 lists the status of nutrition which has effect on vitB12 storage in the human body among all individuals and between the groups. The vast majority of patients (66%) consumed sufficiently one egg 2–3 times per week. Of all subjects, 11.4% ($n = 59$) were not consuming red meat. It was detected that only 19.8% of subjects consumed sufficiently red meat. Among the patients, the least consumed food was fish and mushrooms-one portion of sea foods, mainly fish, 2–3 times per week and one serving of mushroom 2 or more times per week (5.7% and 3.2% respectively). Only one-fifth of subjects (19.8%) consumed sufficiently bovine liver. There was no significant difference for consumption of red meat, bovine liver, egg, mushroom, and fish between obese and nonobese patients ($P = 0.675$, $P = 0.716$, $P = 0.532$, $P = 0.533$ and $P = 0.306$, respectively).

Table 3 shows that mean level of vitB12 in serum was compared between insufficient DRI group and sufficient DRI group in terms of consumption status regarding to egg, fish, red meat, bovine liver, and mushroom. Accordingly, it was observed that mean level of vitB12 was significantly higher among individuals who consumed sufficiently egg ($P = 0.031$), bovine liver ($P = 0.004$), mushroom ($P = 0.040$) and red meat ($P = 0.003$) than those who consumed insufficiently. However, no significant difference was observed between individuals who consumed sufficiently and insufficiently fish ($P = 0.449$).

Table 4 presents the comparison of CBC index between two groups. Mean serum hemoglobin level ($13.2 \pm 1.3$ g/dL vs. $13.1 \pm 1.3$ g/dL), hematocrit ($39.2 \pm 3.5\%$ vs. $39.4 \pm 3.8\%$) and mean corpuscular volume ($86.5 \pm 6.2 \mu m^3$ vs. $86.6 \pm 6.3 \mu m^3$) were not statistically different between groups ($P = 0.675$, $P = 0.716$, $P = 0.533$, $P = 0.532$ and $P = 0.306$, respectively).

**Table 2: Evaluation and comparison of nutritional status among subjects and between groups**

<table>
<thead>
<tr>
<th>Nutritional commodities</th>
<th>All (%)</th>
<th>Group 1 (%)</th>
<th>Group 2 (%)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red meat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insufficient</td>
<td>80.2</td>
<td>77.5</td>
<td>80.9</td>
<td>0.675</td>
</tr>
<tr>
<td>Sufficient</td>
<td>19.8</td>
<td>22.5</td>
<td>19.1</td>
<td></td>
</tr>
<tr>
<td>Bovine liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insufficient</td>
<td>81.2</td>
<td>79.7</td>
<td>81.3</td>
<td>0.716</td>
</tr>
<tr>
<td>Sufficient</td>
<td>19.8</td>
<td>20.3</td>
<td>18.8</td>
<td></td>
</tr>
<tr>
<td>Egg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insufficient</td>
<td>34</td>
<td>34.1</td>
<td>33.8</td>
<td>0.533</td>
</tr>
<tr>
<td>Sufficient</td>
<td>66</td>
<td>65.9</td>
<td>66.2</td>
<td></td>
</tr>
<tr>
<td>Mushroom</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insufficient</td>
<td>96.8</td>
<td>97.1</td>
<td>96.7</td>
<td>0.532</td>
</tr>
<tr>
<td>Sufficient</td>
<td>3.2</td>
<td>2.9</td>
<td>3.3</td>
<td></td>
</tr>
<tr>
<td>Fish</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insufficient</td>
<td>94.3</td>
<td>93.8</td>
<td>96.4</td>
<td>0.306</td>
</tr>
<tr>
<td>Sufficient</td>
<td>5.7</td>
<td>6.3</td>
<td>3.6</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 1:** Demonstration of Vitamin B12 status among subjects. The frequency of Vitamin B12 deficiency among all was 35.2%. The ratio of individuals who suffered from Vitamin B12 deficiency was significantly higher among obese ($n = 273$) than nonobese individuals ($n = 407$) ($P = 0.019$).

**Figure 2:** Demonstration of correlation of serum Vitamin B12 level with body mass index (kg/m$^2$) and total body fat (%). Serum Vitamin B12 level was negatively correlated with body mass index ($r = -0.155$ and $P < 0.001$) and total fat percentage ($r = -0.133$ and $P = 0.003$).
Mean level of serum vitB12 level among all subjects was 247.8 ± 10.4 (20.6–642 pg/mL). VitB12 deficiency was observed in one-third of subjects. Only in 14.7% of all subjects, serum vitB12 level was over value of 350 pg/mL. Mean serum level of vitB12 level was significantly higher in subjects in non-obese group than obese group (282.5 ± 106.8 pg/mL vs. 242.5 ± 107.5 pg/mL, 0.001). Figure 1 demonstrates the comparison of vitB12 between two groups, the frequency of individuals with vitB12 deficiency was significantly higher in obese group than non-obese group (37.6% vs. 24.7%; 0.019).

Table 5 indicates the frequency of patients’ protein intake and association of vitB12 level. The vast majority of subjects (78.9%) has protein intake under recommended levels. The ratio of subjects who consumed sufficient daily protein was higher than those consumed insufficiently, though statistically insignificant (0.414). When mean level of vitB12 was compared between insufficiency and sufficiency DRI groups both in obese and nonobese groups, it was significantly higher among individuals who took sufficient protein per day (0.019 for nonobese group and 0.011 obese group).

Figures 2 and 3 demonstrate the correlation of serum vitB12 with BMI, HOMAIR, and BEI total and visceral fat. Serum vitB12 level was negatively correlated with BMI (r = −0.155 and P < 0.001) and total fat percentage (r = −0.133 and P = 0.003), but not correlated with body visceral fat percentage (r = −0.088 and P = 0.051) and HOMAIR (r = −0.172 and P = 0.062).
Discussion

This study took into account only female patients. We found that low vitB12 level and vitB12 deficiency was higher among obese than nonobese female individuals, with its level seemed to be decreased as BMI increased. We observed that prevalence of vitB12 deficiency was approximately 35% in all cases and 37% in obese cases. In this study, our subjects insufficiently consumed protein enriched nutrition such as red meat, egg, bovine liver and fish, and mushroom. VitB12 deficiency results from an inadequate intake of nutrition, abnormal nutrient absorption, and rare inborn errors of vitB12 metabolism. VitB12 is mainly present in animal protein, particularly red meats, liver, and to a lesser extent, in seafood, milk, and milk products.\textsuperscript{[22–24]} Consistent with previous reports, our results revealed that serum vitB12 level is closely associated with amount of nutrition containing animal protein except fish.

There are some studies which reported the similar results. Guven \textit{et al.}\textsuperscript{[25]} studied on plasma homocysteine, vitB12 and lipoprotein levels in patients with metabolic syndrome and found low levels of vitB12 compared to normal healthy individuals. Goyal \textit{et al.}\textsuperscript{[26]} conducted a retrospective randomized study to assess the prevalence vitB12 level in morbidly obese population and reported that vitB12 deficiency was more prevalent among obese population. A study by Abu-Samak \textit{et al.}\textsuperscript{[27]} revealed that mean serum level of vitB12 was decreased in overweight, but not in obese individuals, and prevalence of vitB12 deficiency was 16%. However, El-Quadah \textit{et al.}\textsuperscript{[28]} carried out a study showing relation of weight status with serum level of vitB12 among healthy Jordanian students and stated that the prevalence of vitB12 deficiency among men and women aged 19–25 years was about 30%, and concentration of vitB12 appeared to increase as BMI increased. In contrary, two studies conducted by Tungtrongchitr \textit{et al.}\textsuperscript{[29]} and Reitman \textit{et al.}\textsuperscript{[30]} on serum vitB12 in overweight and obese subjects showed no statistically significant difference in the vitB12 levels compared with normal-weight subjects. We found that vitB12 deficiency was more frequent among obese than nonobese female individuals, and negatively correlated with BMI. We observed that prevalence of vitB12 deficiency was approximately 35% in all cases and 37% in obese cases. In our study, we compared serum vitB12 level between obese and nonobese individuals, but did not include overweight individuals.

A study conducted by Ho \textit{et al.}\textsuperscript{[31]} revealed that the substantial number of obese adolescents at risk of type 2 diabetes identified with a low or borderline vitB12 status. Also, the present study showed that levels of serum VitB12 in serum were negatively correlated with BMI, but not insulin resistance when Gammon \textit{et al.}\textsuperscript{[32]} reported that no correlation was found between serum vitB12 and insulin resistance. Also we previously found that there was no association between vitB12 and insulin resistance.\textsuperscript{[33]} Another study by Kaya \textit{et al.}\textsuperscript{[34]} studied the relation between insulin resistance and level of vitB12 in serum in women with polycystic ovary syndrome, and they found that level of vitB12 in serum were lower in obese women with insulin resistance compared to those without insulin resistance. It is well known that insulin resistance was significantly related with increasing of BMI, so we can suppose that vitB12 level appeared to mimic BMI.

**Table 5: Mean serum Vitamin B12 level among all subjects and its comparison between groups**

<table>
<thead>
<tr>
<th>Subgroups (reference dietary intake, DRI)</th>
<th>All Mean±SD</th>
<th>Group 1 Mean±SD</th>
<th>Group 2 Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Under DRI (Vitamin B12 pg/mL) (logarithmically transformed)</td>
<td>238.9±101.4 (2.33±0.19)</td>
<td>265.4±94.3 (2.39±0.16)</td>
<td>233.1±101.7 (2.32±0.20)</td>
</tr>
<tr>
<td>Equal to/over DRI (Vitamin B12 pg/mL) (logarithmically transformed)</td>
<td>281.4±115.4 (2.41±0.18)</td>
<td>328.5±127.1 (2.48±0.17)</td>
<td>266.9±107.9 (2.38±0.19)</td>
</tr>
<tr>
<td>p</td>
<td>0.001</td>
<td>0.019</td>
<td>0.011</td>
</tr>
</tbody>
</table>

Raw and logarithmically transformed value of mean serum level of Vitamin B12 was given. For statistical analysis, logarithmically transformed values were used. DRI= Dietary reference intakes; SD= Standard deviation

**The study limitation**

The main limitations of the study are as follows: First, sample size of women in the study was small to generalize. This study is based only on serum vitB12 results without biochemical markers such as methylmalonic acid or homocysteine that might be associated with elevated vitB12 concentrations. Second, major certain nutrition was enlisted in the study, but consumption of milk and milk products was not recorded. They are composed of major nutritional status including protein and some vitamins. Third, only females were included in the study.

**Conclusion**

The findings in our research show that low level of vitB12 in serum was associated with increased BMI. Obese female individuals are likely to suffer from vitB12 deficiency, independently of their nutritional status. Consumption of certain nutrition such as red meat, bovine liver, egg, and mushroom contributed to high level of vitB12 in serum in both obese and nonobese female individuals. Serum vitB12 level seems not to be correlated with insulin resistance and visceral obesity. As a conclusion levels of vitB12 in serum should be closely monitored together with the nutritional status which also impact on serum vitB12 concentrations.

**Acknowledgment**

We would like to thank our staffs for documentation of patients’ records. Also, we thank for kind contribution to Prof. Dr. Handan Ankarali, Biostatistics Department, Medical Faculty, Duzce University.
Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

References