Association of Osteoprotegerin with Obesity, Insulin Resistance and Non-Alcoholic Fatty Liver Disease in Children

Meltem Erol,¹,² Ozlem Bostan Gayret,¹ Hikmet Tekin Nacaroglu,² Ozgul Yigit,¹ Oguzhan Zengi,³ Mehmet Salih Akkurt,⁴ and Mehmet Tasdemir⁵

¹Bagcilar Training and Research Hospital, Department of Pediatrics, Istanbul, Turkey
²Bagcilar Training and Research Hospital, Department of Pediatric Allergy, Istanbul, Turkey
³Bagcilar Training and Research Hospital, Department of Biochemistry, Istanbul, Turkey
⁴Bagcilar Training and Research Hospital, Department of Radiology, Istanbul, Turkey
⁵Department of Pediatric Nephrology, Koc University Hospital, Istanbul, Turkey

Abstract

Background: Osteoprotegerin (OPG) is a member of the tumor necrosis factor superfamily. Reduced OPG levels are related to obesity, insulin resistance, and non-alcoholic fatty liver disease (NAFLD).

Objectives: The aim of this study was to evaluate the relationship between OPG levels, obesity, insulin resistance, and NAFLD in pediatric patients.

Methods: This was a prospective, cross-sectional, controlled study that was conducted in the department of pediatrics at Bagcilar training and research hospital in Istanbul, Turkey, between April and August 2015. The study was performed on 107 children with obesity and 37 controls aged 5 - 17 years. In the obese subset, 62 patients had NAFLD. Homeostatic model assessment-insulin resistance (HOMA-IR) was used to calculate insulin resistance. Insulin resistance was defined as a HOMA-IR value greater than 2.5. Plasma OPG levels were measured using enzyme-linked immunosorbent assays. NAFLD was diagnosed by hepatic ultrasound.

Results: The mean age was 11.25 ± 3.38 years in the patient group and 10.41 ± 3.15 years in the control group. The OPG level in the obese group with the mean of 55.20 ± 24.55 pg/mL (median = 48.81 pg/mL) was significantly lower than that in the control group with the mean of 70.78 ± 33.41 pg/mL (median = 64.57 pg/mL) (P = 0.0001). The optimal cut-off point (sensitivity, specificity) of the OPG level for the diagnosis of obesity was ≤ 46.19 pg/mL. According to logistic regression analysis, fasting insulin (P = 0.036) and OPG (P = 0.01) levels were most affected by obesity. In the obese patients, who had HOMA-IR < 2.5, the mean level of OPG was 58.91 ± 6.88729 pg/mL (median = 49.55). In the obese patients, who had HOMA-IR ≥ 2.5, the mean level of OPG was 54.19 ± 22.21 pg/mL (median = 48.47). No significant correlations were found between OPG and HOMA-IR (P = 0.791). No statistically significant difference was observed in the mean OPG between patients with hepatosteatosis (mean = 53.55 ± 25.01 pg/mL) (median = 49.46) and those without the disease (56.30 ± 24.02 pg/mL) (mean = 48.34) (P = 0.089).

Conclusions: We confirmed that serum OPG concentrations reduce in obese children. However, no correlation was identified between OPG and insulin resistance. OPG levels are not meaningful in the diagnosis of NAFLD in children with obesity.

Keywords: Osteoprotegerin, Obesity, Insulin Resistance, Non-Alcoholic Fatty Liver Disease, Children

1. Background

Obesity is a serious public health threat that has rapidly increased in prevalence in recent years. In the last 30 - 40 years, obesity has become a serious problem during the periods of childhood and adolescence (1, 2). Insulin resistance and dyslipidemia are early metabolic complications of childhood and adolescent obesity (3). Cardiovascular diseases, type 2 diabetes, and other metabolic problems are expected to develop in individuals with obesity (4). Experimental evidence shows that the number of macrophages in adipose tissue has increased in individuals with obesity. It is clear that these macrophages represent a possible origin of proinflammatory factors affecting adipocyte biology and systemic insulin resistance (5). Various tissues, such as those in the heart, veins, arteries, lungs, kidneys, and bone, as well as immune system cells, generate Osteoprotegerin (OPG), a soluble glycoprotein which is a member of the tumor necrosis factor (TNF) receptor superfamily. OPG was initially found as an inhibitor of bone resorption, and the expression and production of OPG are regulated by different cytokines and hormones.
2. Objectives

The aims of the current study were to measure OPG concentrations in children with obesity and insulin resistance, and to evaluate the potential association of OPG level with NAFLD.

3. Methods

3.1. Study Population

This single center, prospective, cross-sectional, controlled study was conducted in the department of pediatrics at Bagcilar training and research hospital in Istanbul, Turkey, between April and August 2015. Bagcilar is one of the most crowded districts of Istanbul. Our study population belonged only to the Bagcilar district. Bagcilar training and research hospital is a general tertiary referral government hospital. Department of pediatrics has 40 beds. Outpatient clinic and emergency service of hospital admit 2,000 children a year. Between April and August 2015, 2000 children were admitted in the pediatric outpatient clinic. According to our inclusion and exclusion criteria, 144 out of 300 participants (107 obese children and 37 normal controls aged 5-17 years) were included in the study. The consort flowchart of sample selection is shown in Figure 1.

The children who had any of infection, metabolic or endocrine diseases or used dietary supplementation and refused to give informed consent were excluded. Medical records were evaluated for age, gender, and physical examination findings.

The inclusion criteria for the study consisted of being obese and ages 5-17 years. Healthy children in the same age range, referred to the pediatric polyclinic of the hospital for general medical examination, with BMI below the 95th percentile and without chronic diseases or infection symptoms included in the control group.

3.2. Anthropometric Measurements

Weights and heights of children were measured in the first examination in pediatric outpatient clinic. All anthropometric measurements were performed by the same pediatrician. Weights were measured with subjects in minimal (without shoes and with light clothing) underclothes, using a standard beam balance sensitive to 0.1 kg. Heights were determined to the nearest 1 cm using a portable Seca stadiometer. Body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters squared). Patients with a BMI greater than the 95th percentile for their age and gender were considered obese (18). The patients with the body mass index less than 95th percentile were accepted as non-obese.

3.3. Laboratory Measurements

After overnight fasting, blood samples were taken for determination of glucose, insulin, total cholesterol, triglyceride, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and alanine amino transferase (ALT) levels. All patients underwent a standardized clinical and laboratory evaluation. To measure OPG levels, peripheral venous blood samples were drawn after overnight fasting for 10 hours. These samples were centrifuged at 3000 rpm for 10 minutes, and the isolated plasma was stored at -80°C until assayed. An enzyme linked immunosorbent assay (ELISA) was used to measure OPG (Affymetrix eBioscience, San Diego, CA, USA, Human OPG kit, Cat no: BMS2021). Triglyceride, cholesterol, and ALT levels were studied using an enzymatic colorimetric method on a Roche Cobas 6000 device. The homeostasis model assessment of insulin resistance (HOMA-IR; fasting insulin × fasting glucose/22.5) was used as an index.
of insulin resistance. Insulin resistance was defined as a HOMA-IR value greater than 2.5 (19, 20).

The ultrasonographic (USG) examinations of all the children were performed using a 3.5 MHz convex transducer (TOSHIBA). All children were evaluated by the same radiologist in the department of radiology, Bagcilar training and research hospital. USG evidence of NAFLD was based on the bright hepatic echo pattern, increased echo attenuation, and loss of intrahepatic architecture (21).

The study protocol was approved by the research ethics committee of Bagcilar training and research hospital (approval number of 2015/365) in accordance with the declaration of Helsinki. Informed consent was obtained from all study participants and/or their parents.

3.4. Statistical Analysis

All statistical descriptions and hypothesis tests were analyzed using SPSS version 23 and MedCalc 16.2 software.
4. Results

The mean age was $11.25 \pm 3.38$ years in the patient group and $10.41 \pm 3.15$ years in the control group, while the male/female ratio was $64/43$ in the patient group and $21/16$ in the control group. No statistically significant difference was observed in the mean age and gender distribution between the obese children and control group ($P > 0.05$). The mean HDL in the obese group ($52.15 \pm 16.23$ mg/dL) was significantly lower than that of the control group ($59.73 \pm 13.43$ mg/dL) ($P = 0.012$), whereas the mean triglyceride level was significantly higher in the obese group ($112.47 \pm 51.74$ mg/dL) compared to the control group ($82.81 \pm 36.51$ mg/dL) ($P = 0.002$). The mean ALT level was significantly higher in the obese group ($25.93 \pm 14.67$ U/L) compared to the control group ($14.92 \pm 4.77$ U/L) ($P = 0.0001$). The mean fasting insulin level was significantly higher in the obese group ($21.81 \pm 16.65$ mU/mL) compared to the control group ($10.12 \pm 9.54$) ($P = 0.0001$). The mean HOMA-IR level was significantly higher in the obese group ($4.94 \pm 4.2$) compared to the control group ($2.29 \pm 2.36$) ($P = 0.0001$). These results are consistent with obesity (Table 1).

The strength of the study was performed only for statistically significant comparisons. 80% strength is the minimum acceptable strength value (Table 1).

4.1. Osteoprotegerin Level in the Obese and Control Groups

The Mann-Whitney U test was employed to determine whether there was a difference between two groups in terms of Osteoprotegerin level. According to this test, the mean OPG was $55.207 \pm 24.55$ pg/mL in the obese group and $70.782 \pm 33.41$ pg/mL in the control group. Moreover, the median IQR value was $48.806$ pg/mL in the obese group and $64.577$ pg/mL in the control group, that showed a statistically significant difference between the groups in terms of the median values of Osteoprotegerin level ($P < 0.001$) (Table 1).

For the definitive diagnosis of obesity, the area under the ROC curve was calculated as $0.696$, that within $95\%$ confidence interval ($0.613 - 0.796$), was significant for OPG ($P < 0.0001$). This led to a cutoff OPG value of $\leq 46.19$ pg/mL. For this OPG cut-off point, Youden index $J$ was $0.3759$, $95\%$ CI was $\leq 0.29 - \leq 0.50$, the sensitivity was $42.99$, and the specificity was $94.59$ (Figure 2).

To determine the factors most affected by obesity, logistic regression analysis was performed using HDL, triglyceride, fasting insulin, HOMA-IR, and OPG levels as variables. Therefore, fasting insulin ($P = 0.036$) and OPG ($P = 0.01$) levels were determined as the factors most affected by obesity (Table 2).

4.2. Relationship of Osteoprotegerin and HOMA-IR

Since HOMA-IR and osteoprotegerin data did not follow the normal distribution, the Mann-Whitney U test was used. Those with HOMA-IR $\geq 2.5$ in the obese group were consistent with insulin resistance. The mean of OPG level in those with HOMA-IR $\geq 2.5$ in the obese group was $54.19 \pm 22.21$. No significant relationship was found between the level of HOMA-IR (below or above 2.5) and OPG level ($P = 0.791$) (Table 3).

4.3. Relationship of Osteoprotegerin and Hepatosteatosis in Obese Group

According to the results of the Mann-Whitney U test in the obese group, the mean Osteoprotegerin level in those with hepatosteatosis was $54.55 \pm 25.01$ pg/mL (median 49.46 pg/mL), while the mean in those without hepatosteatosis was $56.30 \pm 24.02$ pg/mL (median 48.34 pg/mL), which showed no statistically significant difference between these groups in terms of the median values ($P = 0.098$) (ROC analysis $P > 0.05$). Hence, osteoprotegerin cannot be used as an indicator in the determination of hepatosteatosis.

The mean fasting insulin level was significantly higher in those with hepatosteatosis ($25.08 \pm 17.94$ mU/mL) than those without hepatosteatosis ($17.29 \pm 13.61$ mU/mL) ($P = 0.016$). The mean HOMA-IR level was significantly higher...
Table 1. Comparison of the Data in the Obese and Control Groups

|                      | Control Group 37 | Obese Group 107 | P       | Power |
|----------------------|------------------|-----------------|---------|-------|
| **Age (year)**       | 10.41 ± 3.15     | 11.25 ± 3.38    | 0.384   |       |
| **Gender**           |                  |                 |         |       |
| Female               | 16 (43.24%)      | 43 (40.93%)     | 0.745   |       |
| Male                 | 21 (56.76%)      | 64 (59.07%)     |         |       |
| **Cholesterol (mg/dL)** |               |                 |         |       |
|                      | 164.08 ± 30.52   | 163.48 ± 25.63  | 0.908   |       |
| **LDL (mg/dL)**      | 87.76 ± 29.72    | 92.77 ± 26.24   | 0.337   |       |
| **HDL (mg/dL)**      | 59.73 ± 13.43    | 52.35 ± 16.21   | 0.012   | 399   |
| **Triglycerides (mg/dL)** |          |                 |         |       |
|                      | 82.81 ± 36.51    | 112.475 ± 51.74 | 0.002   | 399   |
| **Fasting glucose (mU/mL)** |          |                 |         |       |
|                      | 90.43 ± 8.34     | 90.4 ± 8.49     | 0.984   |       |
| **Fasting insulin (mU/mL)** |          |                 |         |       |
|                      | 10.12 ± 9.54     | 21.81 ± 16.65   | 0.0000  | 399   |
| **HOMA-IR**          | 2.29 ± 2.36      | 4.94 ± 4.2      | 0.0001  | 399   |
| **ALT (U/L)**        | 14.92 ± 7.47     | 25.93 ± 14.67   | 0.0000  | 399   |
| **OPG (pg/mL)**      |                  |                 |         |       |
| Mean ± SD            | 70.78 ± 33.41    | 55.20 ± 24.55   |         |       |
| Median (IQR)         | 64.57 (51.63 - 74.50) | 48.81 (40.91 - 66.26) | 0.0000  | 399   |

Abbreviations: OPG, Osteoprotegerin; LDL, Low density lipoprotein; HDL, High density lipoprotein; HOMA-IR, Homeostasis model assessment-insulin resistance; ALT, Alanine aminotransferase.

*Data are given as mean ± SD or n (%), data are given as mean ± SD and median for Osteoprotegerin.

in those with hepatosteatosis (5.74 ± 4.56) than those without hepatosteatosis (3.82 ± 3.38) (P = 0.019). In addition, ALT level was significantly higher in those with hepatosteatosis (28.91 ± 17.13 U/L) than those without hepatosteatosis (21.83 ± 9.04 U/L) (P = 0.013) (Table 4).

When the correlation analysis was performed in all individuals, a correlation was found only between osteoprotegerin and age (r = -0.221) (P < 0.05).

5. Discussion

The main objective of this study was to determine whether a relationship exists between OPG level and insulin resistance in children with obesity. An additional objective was to determine whether OPG is a significant indicator of hepatosteatosis in children with obesity, as there is a paucity of published studies regarding OPG levels in children with NAFDL and obesity. Our main finding is that individuals with obesity have lower circulating OPG levels than their normal-weight counterparts. Previous studies have reported that decreased insulin sensitivity leads to increased insulin production, causing a predisposition to several metabolic disorders, including early atherosclerosis, progressive obesity, acanthosis nigricans, skin tags, hypertension, dyslipidemia, and fatty liver (22). In the present study, as expected, fasting insulin and HOMA-IR values were high in children with obesity, which led to insulin sensitivity. Furthermore, in these patients, HDL levels were low, triglyceride levels were high, deteriorated lipid profiles were observed, ALT levels were high, and hepatosteatosis was identified on abdominal ultrasonography. These findings are consistent with NAFLD. Furthermore, the children with obesity had significantly lower OPG levels compared to the control group. It is not known what role obesity plays in the regulation of circulating OPG. A decrease in OPG levels in subjects with obesity in comparison with lean controls has been reported in a number of studies (13, 23), whereas no relationship has been determined between OPG and BMI in other studies (24, 25). Obesity is accompanied by a number of metabolic changes, including increased insulin resistance. Ugur-Altan et al. (13) classified young patients with obesity into 3 groups based on HOMA-IR index. The OPG levels in their patients with obesity were found to be significantly lower when compared to a control group. Additionally, they reported that the lowest OPG levels were found in the group with the highest HOMA-IR values, and there were negative correlations between serum OPG level and HOMA-IR, fasting insulin, and glucose. In this present study, although the OPG level was determined to be low in the obese group, neither a negative nor a positive correlation was found between OPG level and HOMA-IR values. The number of stud-
Table 2. Logistic Regression Analysis of Factors Associated with Obesity

|                      | B     | S.E.  | P       | OR    | OR 95% CI |
|----------------------|-------|-------|---------|-------|-----------|
|                      |       |       |         | Lower | Upper     |
| HDL                  | -0.02 | 0.02  | 0.291   | 0.98  | 0.95      |
| Triglycerides        | 0.01  | 0.01  | 0.435   | 1.01  | 0.99      |
| Fasting insulin      | 0.35  | 0.17  | 0.036   | 1.42  | 1.02      |
| HOMA-IR              | -1.06 | 0.67  | 0.013   | 0.35  | 0.09      |
| OPG                  | -0.02 | 0.01  | 0.01    | 0.98  | 0.96      |

Abbreviations: OPG, Osteoprotegerin; HDL, High density lipoprotein; HOMA-IR, Homeostasis model assessment-insulin resistance.

Table 3. Comparison of the Serum OPG Levels of the Obese Group According to HOMA-IR Level

| Osteoprotegerin (pg/mL) | HOMA-IR < 2.5, n = 22 | HOMA-IR ≥ 2.5, n = 85 | P Value |
|-------------------------|------------------------|-----------------------|---------|
| Mean ± SD               | 58.91 ± 33.13          | 54.49 ± 22.21         |         |
| Median (IQR)            | 49.55 (44.63-73.20)    | 48.47 (49.45-58.92)   | 0.791   |

Table 4. Characteristics of the Obese Patients with Hepatosteatosis and Without Hepatosteatosis

|                      | Hepatosteatosis (-), n = 45 | Hepatosteatosis (+), n = 62 | P       |
|----------------------|-----------------------------|-----------------------------|---------|
| Cholesterol (mg/dL)  | 166.42 ± 25.7               | 161.35 ± 25.58              | 0.315   |
| LDL (mg/dL)          | 99.05 ± 25.0                | 88.34 ± 25.77               | 0.04    |
| HDL (mg/dL)          | 53.55 ± 16.18               | 51.16 ± 16.32               | 0.464   |
| Triglycerides (mg/dL)| 100.8 ± 45.1                | 120.95 ± 54.76              | 0.046   |
| Fasting glucose (mg/dL) | 89.5 ± 6.48                | 91.04 ± 9.69                | 0.362   |
| Fasting insulin (mu/mL) | 17.29 ± 13.6               | 25.08 ± 17.94               | 0.016   |
| HOMA-IR              | 3.82 ± 3.38                 | 5.74 ± 4.56                 | 0.019   |
| ALT (U/L)            | 21.83 ± 9.04                | 28.91 ± 17.13               | 0.013   |
| OPG (pg/mL)          | Mean ± SD                   | 56.30 ± 24.02               | 54.55 ± 25.01 | 0.089 |
|                      | Median (IQR)                | 48.34 (41.20-74.31)        | 49.46 (39.27-60.73) |

Abbreviations: OPG, Osteoprotegerin; LDL, Low density lipoprotein; HDL, High density lipoprotein; HOMA-IR, Homeostasis model assessment-insulin resistance; ALT, Alanine aminotransferase.

6 Iran Red Crescent Med J. 2016; 18(11):e41873.
A correlation between OPG level and insulin resistance has not been clearly delineated. In the study conducted by Gannage-Yared MH et al. (25), a positive correlation was determined between OPG and HOMA-IR. There are significant beneficial impacts of OPG on vascular tissues since it associates with endothelial dysfunction and insulin resistance. In a previous study in which vascular calcification caused by the treatment with warfarin and supraphysiologic doses of vitamin D in rats was prevented by OPG applied parenterally, the protective effects of OPG on vascular lumen were clearly observed (30). In contrast, recent studies have reported an increase in OPG levels in subjects with type 2 diabetes and coronary artery disease (9, 31). It was put forward that an increase in serum OPG levels can be defined as an inadequate compensatory self-defense reaction for the purpose of inhibition of progressing bone loss and the advancement of atherosclerosis (9, 31-33). In recent times, Schoppet et al. (32) identified apoptotic cells surrounding calcified regions in arteries affected by Monckeberg’s sclerosis and atherosclerosis, with the simultaneous identification of OPG and TRAIL in the apoptotic regions. Vascular endothelial dysfunction during obesity can be enlightened by the association between serum OPG levels and HOMA-IR values.

In the present study, serum OPG levels were low in the children with obesity, an observation that is concordant with previous studies. Serum OPG levels have been previously reported to be negatively correlated with HOMA-IR values. In our study, the serum OPG concentrations in the children with obesity decreased, while their HOMA-IR values increased, but no significant differences were detected. Less severe complications and metabolic injuries may be related to early obesity in children.

In the present study, no difference was found in OPG levels between children with obesity who also presented with hepatosteatosis and those who did not. With a sudden increase in obesity observed in children for a period of the previous three decades, it has been detected that the prevalence of NAFLD has increased as well. The risk factors for NAFLD are obesity, hyperlipidemia, insulin resistance, and diabetes (34, 35). In this study, LDL cholesterol, triglyceride, fasting insulin, and HOMA-IR levels were significantly higher in the children with obesity and fatty liver compared to the children with obesity who did not have fatty liver. OPG is associated with insulin resistance (13, 29). Furthermore, it has been stated that OPG regulates bone mineral density in subjects having chronic liver disease (36). OPG performs a function of a decoy receptor for TRAIL and at the same time, its biological impacts are neutralized by OPG (37). Additionally, TRAIL activates apoptosis in hepatocytes (38). A decrease in serum levels of OPG in NAFLD can lead to defects in the mechanisms that act to protect hepatocytes from apoptosis (39). Of much interest and importance, OPG accumulation has been shown to correlate with decreased apoptosis in several cell types (37). In the present study, the obese children with NAFLD had higher ALT levels than those without NAFLD. This finding supports the suggestion that high ALT levels are an initial finding in NAFLD (40). As a result of the above-mentioned observations, a general protective impact of OPG has been detected against the pathophysiologic changes that play a role in NAFLD via minimum two independent mechanisms. The first mechanism involves insulin resistance and the second one involves protection of hepatic cells from apoptosis. Nevertheless, a more detailed examination is required for the specific mechanisms causing a decrease in OPG in subjects having NAFLD. Because neither the cellular origin of serum OPG nor the mechanisms of its secretion are clearly known (37), it is not understood whether the important reductions in serum levels of OPG observed in subjects with NAFLD reflect a lack of production or increase in consumption of this molecule.

In a study reported by Yilmaz et al. (10), two groups were formed from the adult patients with obesity, including a serious NAFLD group and a mild NAFLD group. The OPG levels in the serious NAFLD group were determined to be lower when compared to the mild NAFLD group and the control group. In a study conducted by Yang et al. (11), it was stated that OPG level decreased as NAFLD severity increased, implying a negative correlation between them. Liver biopsies were performed in both studies, and the results indicated that OPG could be used as a noninvasive marker of the damage caused by NAFLD in obesity. In another study conducted by Niu et al. (16), low serum OPG levels were determined in subjects with type 2 diabetes who also had NAFLD.

The above-referenced studies examining the relationship between OPG and NAFLD were carried out on groups of adults. In contrast, the patients in the current study were children, and their liver damage was likely not as severe as that observed in adults. This may explain why no significant difference was found in OPG levels between our subjects who did and did not present with hepatosteatosis.

There are some limitations of the current study. First, there is a known relationship between OPG levels and high HOMA-IR values in individuals with obesity. Additionally, vascular endothelial function and carotid artery intima thickness are affected in patients with NAFLD. Thus, in these patients, the carotid artery intima thickness could
Children with obesity who had high fasting insulin levels and high HOMA-IR values had significantly low OPG levels. Although our patients with hepatosteatosis had lower OPG levels than those without hepatosteatosis, no statistically significant differences were found. Therefore, we were unable to reach a clear conclusion on whether OPG could be used as a noninvasive biomarker of NAFLD in childhood obesity and hence, we call for more studies.

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Footnotes

Authors’ Contribution: Study concept and design: Meltem Erol; acquisition of data: Meltem Erol and Ozlem Bostan Gayret; analysis and interpretation of data: Ozlem Bostan Gayret, Hikmet Tekin Nacaroglu, and Mehmet Tasdemir; drafting of the manuscript: Meltem Erol; critical revision of the manuscript for important intellectual content: Ozgul Yigit, Hikmet Tekin Nacaroglu and Ozlem Bostan Gayret; radiological material support: Mehmet Salih Akkurtt; biochemical analysis: Oguzhan Zengi; study supervision: Meltem Erol.

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