LEPTIN AND RESISTIN LEVELS IN SERUM OF PATIENTS WITH HEMATOLOGIC MALIGNANCIES:
CORRELATION WITH CLINICAL CHARACTERISTICS

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Aim: To evaluate leptin and resistin levels in patients with various hematologic malignancies. Methods: We included 21 patients with lymphoma, 14 with multiple myeloma (MM), 14 with acute leukemia, 13 with chronic lymphocytic leukemia (CLL), and 25 healthy control subjects into our study. The subjects’ body mass indexes (BMI) were calculated; hematological and acute phase response parameters, serum lipid were determined; serum leptin and resistin levels were determined by ELISA. Results: Serum leptin level was significantly increased in CLL and MM groups when compared to the control group (p < 0.01). Resistin level was significantly higher in lymphoma patients than in CLL, acute leukemia and control groups (p < 0.01). In the control group, leptin level was negatively correlated with hemoglobin level (r = –0.44, p = 0.047); and in all patients with hematologic malignancies, leptin level was correlated with BMI (r = 0.32, p = 0.02). Leptin in lymphoma subjects correlated with hemoglobin level (r = 0.64, p = 0.005), resistin level correlated with the platelet count in patients with hematologic malignancies (r = 0.26, p = 0.044). In addition, leptin level had negative correlations with international prognostic score (IPS) in Hodgkin lymphoma (r = –0.9, p = 0.002) and with international prognostic index (IPI) in non-Hodgkin lymphoma (r = –0.77, p = 0.03). In CLL patients, leptin level had a correlation with the poor prognostic marker — CD38 level (r = 0.68, p = 0.03). Conclusion: We found higher leptin levels in MM and CLL patients, and higher resistin levels in lymphoma patients: this fact demonstrates that changes in adipose tissue and metabolism occur in these disease states.

Key Words: leptin, resistin, chronic lymphocytic leukemia, multiple myeloma, lymphoma.

Adipocytes secrete highly active biological molecules like leptin, resistin and adiponectin [1]. Leptin is a 16-kD polypeptide which has a role in nutrient intake and in the regulation of metabolism [2]. Some studies showed a significant association between leptin level and blood cell count [2, 3]. Recently, it has been demonstrated that leptin receptors were expressed on CD34+ hematopoietic stem cells [4] and that leptin had influence on the maturation and proliferation of normal hematopoietic cells [5, 6]. In addition, it was stated that leukemic cells in newly diagnosed acute or chronic leukemia patients expressed leptin receptors [2, 4]. It was suggested that leptin receptor expression occurred mainly on immature leukemic cells; and that leptin had a role in leukemogenesis [2].

Another protein of adipocyte origin is resistin which was found to be associated with the development of obesity and insulin resistance in animals [7]. In humans, resistin is mainly expressed on inflammatory cells; and proinflammatory cytokines increase the expression of resistin on monocytes [8]. Resistin was shown role in the relation between inflammation and insulin resistance [7]. It is known that steroids cause resistin expression, thereby increasing insulin resistance [9]. Until now, no study on the levels of resistin in hematological malignancies has been conducted.

In this study, we evaluated leptin and resistin levels in patients with various hematologic malignancies; compared these data with that of the healthy control group; and determined clinical and laboratory parameters with which they were associated.

MATERIALS AND METHODS
This study was conducted in the Department of Hematology, Trakya University Medical Faculty; 21 lymphoma, 14 multiple myeloma (MM), 14 acute leukemia, and 13 chronic lymphocytic leukemia (CLL) patients were included into the study. The control group composed of 25 healthy subjects within the same age range. Of lymphoma patients, 11 had Hodgkin lymphoma (HL) and 10 had non-Hodgkin lymphoma (NHL). Of leukemia patients, 11 had acute nonlymphoblastic leukemia (ANLL) and 3 had acute lymphoblastic leukemia (ALL). Patients were either initially diagnosed or they were relapsed patients. All patients were off treatment for at least 3 months. Patients with documented infection within the last 2 weeks; those with febrile neutropenia, sepsis, any organ failure (kidney, liver, lung, heart); patients with hypertension or diabetes were excluded.

All CLL patients fulfilled CLL diagnostic criteria defined by the International Workshop on CLL [10]. The diagnoses of HL and NHL were based on the histopathologic examination of a lymph node or extranodal tissue biopsy specimen. All CLL patients were classified into stages according to the system of Rai et al. [11]. Lymphoma patients were staged clinically according to Ann-Arbor classification [12]. MM patients were classified according to Durie-Salmon staging system [13]. CLL patients with

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Abbreviations used: ALL — acute lymphoblastic leukemia; ANLL — acute nonlymphoblastic leukemia; BMI — body mass index; CLL — chronic lymphocytic leukemia; CRP — C-reactive protein; ELISA — enzyme-linked immunosorbent assay; ESR — erythrocyte sedimentation rate; HL — Hodgkin lymphoma; IPI — international prognostic score; IPS — international prognostic score; MM — multiple myeloma; NHL — non-Hodgkin lymphoma.
Rai stages III, IV were accepted to have advanced-stage disease. Lymphoma patients with Ann-Arbor stages III, IV had advanced-stage disease. MM patients with stage III disease had advanced stage disease. Seventeen lymphoma, 6 CLL and 12 MM patients had advanced stage disease. The International Prognostic Index (IPI) including age, stage, performance status, extranodal involvement, and LDH was calculated in NHL patients. The International Prognostic Score (IPS) was calculated in advanced-stage HL patients.

Data about patients’ primary hematological diseases, demographic and clinical features were recorded down from hospital files. Body mass index (BMI) was calculated by dividing body weight (kg) by square height (m). Blood samples were obtained after an overnight fast when all subjects were still fasting. Whole blood count, serum total cholesterol, HDL, LDL, triglyceride, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) were determined. In addition, flow cytomtery results in CLL patients were recorded down. Serum leptin and resistin (BioVendor Laboratory Medicine, Inc. Brno, Czech Republic) were determined by the method of enzyme-linked immunosorbent assay (ELISA). The minimum detectable value of leptin was 0.5 ng/ml. The intraassay coefficient of variation is 4.8 to 7.4%, and the interassay coefficient of variation is 4.3 to 8.8%. The minimum detectable value of resistin was 0.2 ng/ml. The intraassay coefficient of variation is 3.2 to 4%, and the interassay coefficient of variation is 6.3 to 7.2%.

One-way variance analysis (ANOVA) was used to analyze variances among the groups. Statistically significant differences obtained from these analyses were further tested by the Tukey test for post hoc pairwise comparisons between each group, with p values adjusted downward to roughly 0.01. Correlation was calculated according to Pearson’s coefficient.

### RESULTS

There was no significant difference between the hematological malignancy and the control group in age, sex, and mean BMI (p > 0.05). The clinical features, whole blood counts, and acute phase parameters of the groups are seen in Table 1.

Mean leptin levels in MM and CLL patients were significantly higher than in the control group (p < 0.01). Resistin level was significantly higher in lymphoma patients than in CLL, acute leukemia and the control group (p < 0.01). In both the control group and in patients with hematological malignancies, serum leptin levels were higher in females than in males; however, the difference was significant in only the control group (p = 0.01). Leptin, resistin levels and biochemical values are seen in Table 2.

In the control group, leptin level had a significant negative correlation with the hemoglobin level (r = –0.44, p = 0.047). In patients with hematological malignancies, leptin level correlated with BMI (r = 0.32, p = 0.02); resistin level, on the other hand, correlated with the platelet count (r = 0.26, p = 0.044).

HL and NHL groups were not different in their leptin and resistin levels (p>0.05). As most lymphoma patients had advanced stage disease, differences among stages could not be calculated. In lymphoma patients, leptin level correlated with hemoglobin level (r = 0.64, p = 0.055); resistin level correlated negatively with HDL (r = –0.51, p = 0.04). In addition, it was detected that IPS score in HL patients and IPI score in NHL patients had negative correlations with leptin level (respectively, r = –0.9, p = 0.002 and r = –0.77, p = 0.016).

In CLL group, leptin and resistin levels did not differ between early and advanced stage patients (p > 0.05). In the CLL group, leptin level had a significant negative correlation with triglyceride level (r = –0.71, p = 0.02). In addition, the percentage of CD38-positive lymphocytes

| Table 1. The general clinical features, whole blood counts, and acute phase responses of the studied groups of the patients |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | Lymphoma       | CLL             | Acute leukemia  | Multiple myeloma | Controls        |
| BMI (kg/m²)     | 23.3 ± 3.3      | 25.1 ± 3.3      | 25.8 ± 3.4      | 26.7 ± 3.8      | 25.5 ± 3.4      |
| Age (years)     | 55.1 ± 15.3     | 60.2 ± 5.9      | 60.7 ± 8.5      | 60.7 ± 12       | 54.3 ± 12       |
| Hemoglobin (g/dl)| 10.4 ± 2.4      | 10.7 ± 3.6      | 9.3 ± 2.1       | 9.3 ± 2.1       | 13 ± 1.9b      |
| Leukocytes (10³/l)| 11.4 ± 16.2    | 69.5 ± 64.6a   | 19.2 ± 32.3     | 5.9 ± 2.9       | 6.6 ± 1.2      |
| ESR (mm/hr)     | 53.6 ± 45.1     | 32.3 ± 40       | 70.1 ± 47.1     | 95.2 ± 33.3a    | 11.9 ± 10.7    |
| CRP (mg/dl)     | 3.9 ± 6.5       | 1.3 ± 1.3       | 4.1 ± 5.7       | 1.8 ± 1.5       |                 |

| Table 2. Leptin, resistin levels and other laboratory parameters of the groups |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | Lymphoma       | CLL             | Acute leukemia  | Multiple myeloma | Controls        |
| Leptin (ng/ml)  | 16.4 ± 10.4     | 19.4 ± 12.4a   | 13.2 ± 10.6     | 22.6 ± 14.7a    | 10.3 ± 7.6      |
| Resistin (ng/ml)| 3.94 ± 3.4      | 1.97 ± 0.8     | 2.04 ± 1.2      | 3.33 ± 0.3      | 1.97 ± 0.8      |
| Glucose (mg/dl)| 96.9 ± 10.8     | 106.9 ± 16.6   | 106.8 ± 15.2    | 98.3 ± 20.3     | 98.2 ± 14.9     |
| Uric acid (mg/dl)| 4.5 ± 1.4       | 4.6 ± 1.5      | 5.3 ± 3.1       | 5.6 ± 2.4       | 4.6 ± 1.3       |
| Triglyceride (mg/dl)| 93.4 ± 47.9 | 121.8 ± 41.2   | 122.7 ± 54.4    | 109.4 ± 55.6    | 127 ± 94       |
| T. Cholesterol (mg/dl)| 164.2 ± 44.1 | 158.4 ± 38     | 146.9 ± 38     | 152.2 ± 44     | 182 ± 32       |
| HDL (mg/dl)     | 48.6 ± 5.9      | 41.8 ± 9.2c    | 51.8 ± 7.5      | 51 ± 10.3       | 49.3 ± 6.8      |
| LDL (mg/dl)     | 90.9 ± 36.2     | 86 ± 36.1      | 77.6 ± 33.4     | 86.8 ± 42.9     | 107.3 ± 30.6    |

(P): p < 0.001; Controls different from all other groups.
(P): p < 0.001; CLL different from all other groups.
(P): p < 0.001, acute leukemia is different from all other groups.
(P): p < 0.001, controls different from lymphoma, MM and acute leukemia.
(P): p < 0.001, MM different from lymphoma, CLL.
in CLL group had a correlation with leptin level \( r = 0.68, p = 0.03 \). In the acute leukemia group, resistin level had a negative correlation with BMI \( r = -0.59, p = 0.03 \).

27 of 62 (43.5%) patients with hematological malignancies had used a chemotherapy regimen including steroids. The serum leptin level in the patients who used previously steroids did not differ significantly from those who did not use steroids \( p > 0.05 \). There was a trend towards higher resistin levels in patients who used steroids previously when compared to patients who did not use steroids; however, the difference was not significant \((3.5 \pm 3 \text{ vs. } 2.5 \pm 2.4 \text{ ng/ml}, p = 0.06)\). There was no difference in BMI and other laboratory parameters between steroid-users and steroid-nonusers among patients \( p > 0.05 \). In each hematological malignancy group, serum leptin and resistin levels were not found to differ between patients who previously used steroids and or did not use them \( p > 0.05 \).

**DISCUSSION**

In our study, leptin levels in MM and CLL patients were higher than in the control group, and resistin level in lymphoma patients was higher than in CLL, acute leukemia and control groups. It was reported that leptin receptors were expressed on leukemic cells, especially on immature ones [2]. In our study, we did not find any increase in serum leptin levels in acute leukemia subjects. Nevertheless, we detected higher leptin levels in CLL. One study reported increased levels of leptin in AML, ALL and CML; but, contrary to our results, there was no increase in CLL [14]. Another study demonstrated that leptin level was lower in untreated AML patients, and that it did not vary with chemotherapy or with the development of febrile neutropenia [15]. We found no change in leptin levels in our acute leukemia patients.

In our study, leptin level had a negative correlation with hemoglobin in the control group. Similar to our study, a negative correlation between hemoglobin and leptin level has been reported in healthy Japanese adults [16]. On the contrary, another study found a positive correlation between leptin level and both the erythrocyte and leukocyte counts in Japanese adolescents [17]. One study from France observed just a weak relationship between leptin level and leukocyte count in hospitalized patients [6]. It was reported that fat mass in humans was associated with leukocyte count and leptin level [3]. As a result, we might suggest that leptin might have different relationships with hematopoiesis according to differences in age and sex.

It was interesting that in our lymphoma patients, contrary to controls, there was a positive correlation between leptin and hemoglobin levels. Although it was shown that leptin stimulated macrophage and granulocyte colonies by itself, and improved erythroid development synergistically with eritropoietin [18]; there is not sufficient data in literature about the role of leptin in erythropoiesis in lymphoma patients.

It was reported that leptin level in adults was associated with obesity and BMI [17]. In our study, the groups did not differ in their BMI; however, leptin level had a significant correlation with BMI only in hematologic malignancy patients, there was no such correlation in the control group. We might say that leptin-BMI relationship was preserved in hematologic malignancy patients.

Leptin is a fundamental factor for human T cell proliferation and it induces T helper type I immune reactions [19]. Leptin has also been shown to activate CD4+ T cells [19]. Leptin might also be an “acute phase protein of fat tissue” which supports the immune system during a short-term infectious disease [20]. Leptin-deficient mice exhibit impaired host defences [21] and starvation with low serum leptin levels leads to immunosuppression [22]. Serum levels of leptin are increased during some chronic inflammatory diseases [20]. Increased leptin levels in our MM and CLL patients might be associated with increased tendency to infections and immune dysfunction in these disorders. Nevertheless, more data on this subject are needed.

There is no study on the level of resistin, which is related with insulin resistance and shown to increase with steroid usage, in hematologic malignancies. In our study, we found increased resistin levels in the lymphoma group. Recently, it has been demonstrated that resistin level was correlated with inflammation markers — in addition to insulin resistance — and was predictive of atherosclerotic coronary events [7]. In addition, proinflammatory cytokines like TNF-α and IL-6 were reported to be associated with insulin resistance in adipose tissue and increase resistin level [23]. In our study, we could not detect any significant association between resistin level and any inflammatory parameter (ESR, CRP) in our control and hematologic malignancy groups. Nevertheless, resistin level correlated with only the platelet count in our hematologic malignancy patients.

The increase in leptin and resistin levels in lymphoma and MM patients who used corticosteroids more intensively and more frequently than others points to a role for steroids. Steroid intake affects resistin level; in addition, it has been recently reported that physiologic-dose steroids increase leptin secretion [9]. However, none of our patients were currently using steroids. In addition, when we evaluated all cases in our study we observed that leptin levels in previous steroid-user and -nonuser groups were not different. Although resistin level was higher in previous steroid-users, the difference was not significant.

An interesting result of our study was that leptin level had negative correlations with parameters of poor prognosis like IPS in HL and IPI in NHL. On the contrary, in CLL patients, leptin level had a correlation with the poor prognostic marker CD38 level. As the number of our patients was not high, it is difficult to come to a certain conclusion about the prognostic importance of leptin level. Further studies should be conducted about this subject.

As a result, we found that leptin level was increased in CLL and MM; and resistin level was increased in lymphoma patients who were not currently using any chemotherapeutic agent or steroids. This finding might suggest that leptin in CLL and MM, and resistin in lymphoma might be associated with disease pathogenesis; and immune changes and the inflammatory response which occur during the courses of these diseases.
УРОВЕНЬ ЛЕПТИНА И РЕЗИСТИНА В СЫВОРОТКЕ КРОВИ
БОЛЬНЫХ ОНКОГЕМATOЛОГИЧЕСКИМИ ЗАБОЛЕВАНИЯМИ:
КОРРЕЛЯЦИЯ С КЛИНИЧЕСКИМИ ХАРАКТЕРИСТИКАМИ

Цель: определить уровни содержания лептина и резистина в сыворотке крови больных с различными онкогематологическими заболеваниями. Методы: обследован 21 больной лимфомой, 14 — множественной миеломой (ММ), 13 — острой лейкемией, 13 — хронической лимфоцитарной лейкемией (ХЛЛ), и 25 здоровых доноров. У пациентов определены такие характеристики: уровень лептина и резистина, ЭДА (р = 0,044). При всех группах больных уровень лептина и резистина был значительно выше у больных с ХЛЛ, ММ, чем у здоровых доноров. Уровень лептина при лимфомах коррелировал с уровнем гемоглобина (р = 0,32, р = 0,02), а во всех группах больных уровень лептина коррелировал с уровнем гемоглобина (р = 0,64, р = 0,005), уровень резистина коррелировал с количеством тромбоцитов у больных всех групп (р = 0,26, р = 0,044). При лимфоме Ходжкина выявлена отрицательная корреляция между уровнем лептина и величиной международной прогностической шкалы (р = -0,9, р = 0,002), при неходжкинской лимфоме — величиной международного прогностического индекса (р = -0,77, р = 0,03), у больных ХЛЛ — с уровнем экспрессии CD38 (р = 0,68, р = 0,03). Выводы: у больных ММ и ХЛЛ выявлен высокий уровень лептина, а с лимфомами — высокий уровень резистина: этот факт указывает на то, что у больных указанными онкогематологическими заболеваниями могут возникать изменения в структуре жировой ткани и обмене веществ.

Ключевые слова: лептин, резистин, хроническая лимфоцитарная лейкемия, множественная миелома, лимфома.