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Investigation of Leptin, Leukemia Inhibitory Factor (LIF), and IL-6 Serum Levels in Myeloid Leukemia

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Abstract

Background: Leptin has been implicated in the differentiation and proliferation of hematopoietic cells. Leukemia inhibitory factor (LIF) may play an important role, along with Interleukin-6 (IL-6) and granulocyte colony stimulating factor (G- CSF), in the regulation of early hematopoietic stem cells. The aim of the study was to evaluate serum level of leptin, LIF, and IL-6 in myeloid leukemia patients.

Materials and Methods: We investigated serum level of leptin, LIF, and IL-6 levels, body mass index, hemoglobin, and hematocrit in 30 myeloid leukemia patients (15 patients with acute and 15 with chronic myeloid leukemia) before chemotherapy, and compared the results with 15 healthy controls.

Results: Leptin, LIF and IL-6 serum levels, and lipid profile in myeloied leukemic patient was significantly different from the control group ($p<0.05$). The relationship between leptin and BMI was statistically significant in control group, whereas in the patient group, there were no significant relationship between leptin and BMI.

Conclusion: Lipid profile and leptin, LIF, and IL-6 serum levels of leukemic patients were significantly different from normal population.

Keywords: Leptin, Leukemia inhibitory factor, Interleukin-6, Myeloid leukemia.

Introduction

Leptin is a regulator of fat metabolism that is synthesized in adipocytes and released into circulation.[1] This 16 KD hormone, the product of obese gene, is a secretory protein that exerts multiple biological functions by binding to its receptor.[2] These receptors are expressed in primary acute myeloid leukemia (AML) blasts, acute promyelocytic leukemia (APL) CD34+ and CD34- CD33+ promyelocytes, chronic myeloid leukemia CML blasts, several hematopoietic cell lines, and weakly in acute lymphoblastic leukemia (ALL) blasts, but not in chronic lymphocytic leukemia (CLL) cells.[3-6] Leptin alone and in combination with other cytokines has an important role in control of the proliferation and differentiation of normal primitive hematopoietic cells, and also stimulates the growth and viability of leukemic cells.[2, 7] Furthermore, leptin also stimulates normal myeloid and erythroid development and leukemic cell growth in vivo by promoting angiogenesis.[7, 8] It is suggested that unregulated expression of a variety of growth factors and/or their receptors has some roles in the pathogenesis of certain leukemias.[9] Serums leptin level has a direct relation with body mass index (BMI), but might quickly reduce in fasting and inflammatory reaction.[10-12] This hormone is the regulator of body weight in obese individuals.[13]

Leukemia inhibitory factor (LIF) is a pleiotropic cytokine that exhibits multiple functions in various tissues and cell types such as extensive hematopoietic, neuronal, and endocrine actions.[14, 15] An important function of LIF is to activate POMC gene transcription in response to immune signals.[15] It may play an important role, along with interleukin-6 (IL-6) and granulocyte colony stimulating factor (G-CSF), in the regulation of early hematopoietic stem cells.[16, 17] IL-6 is a pleiotropic cytokine produced by various cell types, and has an important role in pathogenesis of different diseases.[18, 19] LIF and IL-6 are associated with IL-6 type cytokines that reduce leptin through a common receptor (gp130).[19, 20] In

this study, we evaluated leptin, LIF, and IL-6 serum levels in patients with myeloid leukemia, and compared them with the healthy control group.

Materials and Methods

Patients

The study group was consisted of 30 patients (mean age 49 ± 3, 16 men and 14 women) with myeloid leukemia (15 with acute and 15 with chronic myeloid leukemia). The control group included 15 healthy individuals (mean age 50 ± 3, 8 men and 7 women). All of the patients were newly diagnosed, and had not received chemotherapy. Informed written consent was obtained from all participants.

Blood samples of the patients were collected on the day when leukemia was diagnosed. Blood samples were centrifuged and serum supernatants were stored at -40°C.

Leptin, LIF, and IL-6 Measurement

A commercial immunoenzymatic kit (Quantikine Human Leptin, R & D, USA) was used to measure leptin, LIF, and IL-6. This test is based on a solid double antibody sandwich ELISA (enzyme-linked immunosorbent assays). Sensitivity of the test was 0.5, 0.15, and 2 ng/l for leptin, LIF, and IL-6, respectively. After a 15-minute incubation, stop solution was added and the enzymatic activity of peroxidase was measured at 450 nm with the use of automated reading system Stat Fax 2100. Standards were provided to draw semi-logarithmic reference curve, which all the results were referred to it. A laboratory technician performed tests blindly.

Lipid parameter

Serum total cholesterol, HDL, LDL, and triglyceride were determined by an enzymatic kit (Pars azmoon) in Imam Khomeini hospital, Ardabil university of medical science.

Statistical analysis

Results of serum leptin, LIF and IL-6 level are expressed as means ±SD. Results were compared by the U Mann-Whitney test. Statistical significance was considered at $P<0.05$. The Pearson test was used for the analysis of the correlations between BMI and leptin concentration.

Results

AML Patients

The serum level of leptin, LIF, and IL-6 in leukemic patients were lower than those in control group and this difference was statistically significant. (Table 1) Statistically significant difference was observed in lipid profile between patients and control group, except triglyceride level. (Table 1) Serum cholesterol level of the control group was higher than the patient group. Hb and HCT were lower, and white blood cell (WBC) count was higher in the patient group than the control group. The correlation between leptin and BMI was statistically

Table 1. Clinical and biochemical features in AML patients and the control group.

Parameters	AML patients	Control group	P value
N (M/F)	(8/7)	(8/7)	
Age (years)	(45±3)	(50±3)	
BMI (Kg/m²)	21.5±2.2	24.2±2.2	0.001
Leptin (ng/ml)	8.3±3.7	19.3±10	0.002
LIF (ng/ml)	0.5±0.08	.76±0.13	<0.0001
IL-6 (ng/ml)	40.6±8.1	53.2±13.2	0.016
Cholesterol (mg/dl)	109.7±47	172.2±64.1	.0120
Triglyceride (mg/dl)	174.7±108.9	192.3±92.4	0.66
HDL (mg/dl)	12.6±1.2	40.9±13.5	<0.0001
LDL (mg/dl)	12.6±1.2	116.3±43.2	0.001
Leukocyte (10^9/L)	92066.7±36054.4	7090±1598.2	<0.0001
Hemoglobin (g/dl)	9.1±1	14.3±1.1	<0.0001
Hematocrit (%)	27±2.1	42.9±3.2	<0.0001

significant in the control group (figure 1), whereas in the patient group, there were no significant correlation between leptin and BMI (figure 2).

CML Patients

Serum level of leptin, LIF, and IL-6 of the patient group were significantly different from the control group. (Table 2) Serum cholesterol level, but not triglyceride, of the patient group was significantly lower than the control group. (Table 2) The same as AML patients, level of Hb and HCT were signifaicantly lower and WBC was higher in CML patients compared to the control group. In the patient group, there was no significant correlationship between leptin and BMI (figure 3), whereas the correlation between leptin and BMI was statistically significant in the control group (figure 1).

Discussion

In humans serum leptin concentration reflects the amount of adipose tissue in the body.[21] In our study, serum level of leptin in AML and CML patients was lower than the control group. These results are in accordance with the data of Qystein et al, that reported lower serum leptin level in AML patients.[22] Another study demonstrated that leptin level was lower in untreated AML patients.[23] Pamuk et al also reported decrease in serum leptin level in leukemic patients.[24] Findings of studies by Wasik et al and Gaja et al were also compatible with our data.[25, 26] On contrary, another study by Konopleva et al found that leptin level in AML patients was normal.[5]

In the control group of our study, there was a significant correlation between BMI and serum leptin level. Several previous studies reported similar findings.[27-32] This correlation was not seen in leukemic patients.[33, 34] This could suggest that there are different mechanisms for controlling leptin level in leukemic and non-leukemic persons.

In patients with a malignant disease, increased cytokine production leads to a decrease in appetite, weight loss, and finally cachexia.33 Cachexia is a leading feature in more than half of the cancer patients. It is characterized with loss of the adipose tissue and skeletal muscle mass. These patients usually have decreased calorie intake and increased basal energy expenditure.[35] A number of cytokines including tumor necrosis factor-alpha, interleukins 1 and 6, IFN-α, leukemia inhibitory factor, and ciliary neurotrophic factor, which have been proposed as mediators of cachectic process, may play a pivotal role in long-term inhibition of appetite by mimicking hypothalamic effect of strong negative feedback signal of leptin.[36]

The serum LIF concentration is dependent not only on the amount of LIF production and secretion but also on the amount bound to cells and extracellular matrix.[37] Human myeloid leukemic cell lines could be suppressed by combination of LIF, and GCSF or GM-CSF.[38] In this study LIF level in leukemia patients was lower than control group. Song Guang et al reported similar findings.[14] But another study by Ahmet et al did not confirm theses results.[37]

The LIF receptor shares the glycoprotein 130 signal-mediating receptor subunit with several members of the interleukin-6 (IL-6) cytokine receptor super-family.[39] In our study, IL-6 serum level in the patient group was lower than control group. IL-6 is a proinflammatory cytokine that increases in inflammatory diseases, such as

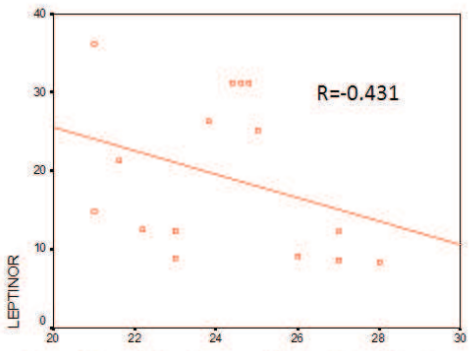

Figure 1. Correlation between BMI and Leptin in the control group.

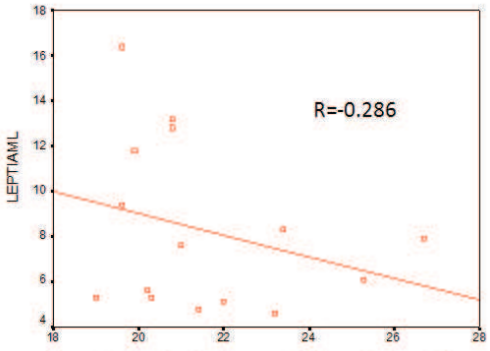

Figure 2. Correlation between BMI and Leptin in the AML group.

Table 2. Clinical and biochemical features in CML patients and the control group.

Parameters	CML patients	Control group	P Value
N (M/F)	(8/7)	(8/7)	
Age (years)	(54±3)	(50±3)	
BMI (kg/m²)	24.2±2.2	24.2±22	0.001
Leptin (ng/ml)	10.3±2.2	19.3±10	0.004
LIF (ng/ml)	0.47±0.07	0.76±0.13	<0.0001
IL-6 (ng/ml)	41.6±5.8	53.2±13.2	0.015
Cholesterol (mg/dl)	121.5±25.4	172.2±64.1	0.019
Triglyceride (mg/dl)	160.8±81.6	192.3±92.4	0.313
HDL (mg/dl)	20.6±4.8	40.9±13.5	<0.0001
LDL (mg/dl)	65.8±21.3	116.3±43.2	0.003
Leukocyte (10^9/L)	181666.7±48232.6	7090±1598.2	<0.0001
Hemoglobin (g/dl)	9.8±1.2	14.3±1.1	<0.0001
Hematocrit (%)	29.4±3.3	42.9±3.2	<0.0001

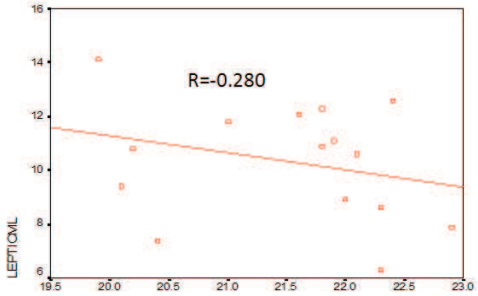

Figure 3. Relation between BMI and Leptin in CML patients.

rheumatoid arthritis, and does not increase in non-inflammatory diseases such as leukemia.[40]

We observed significant increase in leukocyte count and decrease in Hb and HCT in all patients compared to the control group. Increased leukocyte count is caused by abnormal proliferation of leukocytes, which is a feature of leukemia. Decrease of Hb and HCT is a marker for anemia, and could be related to the blast cells proliferation in leukemic patients. Blast cells occupy the majority of bone marrow space and do not allow other cells to increase. Also, in leukemic patient, stem cell disorder causes abnormality in erythroid lineage and contributes in anemia development.

Serum lipid levels of both patient groups were lower than the control group. These findings were also reported by Pamuk et al.[24] This could be related to the high metabolic rate of malignant cells, along with fever and body weight loss.

Conclusion

In conclusion, Hb, HCT, and serum level of lipids, leptin, LIF, and IL-6 of leukemia patients are significantly different from normal population. These results suggest that the mentioned cytokines may have an important role in leukemia pathogenesis.

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