

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

Shaban Alizadeh,¹ Shahab Bohlooli,² Ali Abedi,² Seyed Hadi Mousavi,¹ Hossein Dargahi,¹ Behzad Jafarzadeh,³ Nourooz Hamrang,³ Ali Imani.³

1. Allied medical school, Tehran University of Medical Sciences.

2. Medical school, Ardabil University of Medical Sciences.

3. Imam Khomeini hospital, Ardabil University of Medical Sciences

Corresponding author: Shaban Alizadeh, Allied medical school, Tehran University of Medical Sciences. (Phone/Fax: +98 21 88964009, E-mail: alizadehs@tums.ac.ir)

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 myeloid 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 Serum 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 \pm 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/m^2)	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	$.76 \pm 0.13$	<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 ($10^9/\text{L}$)	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 significantly lower and WBC was higher in CML patients compared to the control group. In the patient group, there was no significant relationship 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- α , 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 these 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

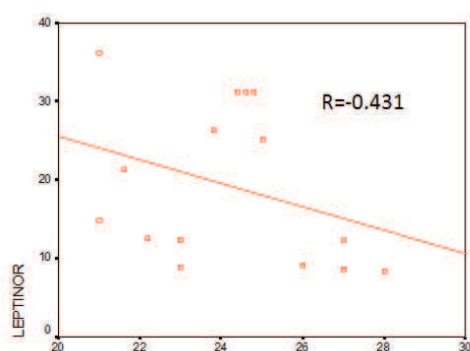


Figure 1. Correlation between BMI and Leptin in the control group.

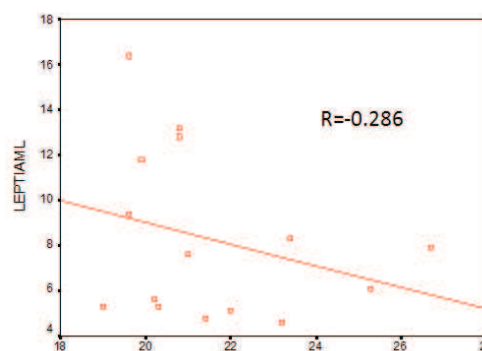
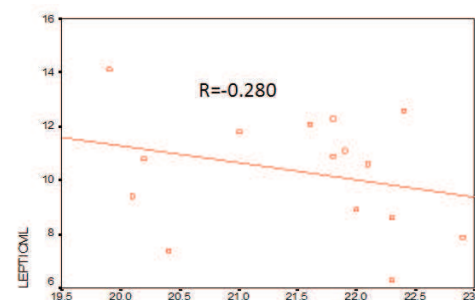


Figure 2. Correlation between BMI and Leptin in the AML group.

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/m ²)	24.2±2.2	24.2±2.2	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.

References

1. Yilmaz M, Kis C, Ceylan NO, Okan V, Pehlivan M, Kucukosmanoglu E, et al. Serum leptin level in acute myeloid leukemia patients. *Hematology*. 2008; 13: 21-3.

2. Ning HM, Zhang Y, Mao N. Leptin and its receptor in acute myeloid leukemia. *Zhongguo shi yan xue ye xue za zhi*. 2010; 18: 234-7.

3. Mikhail AA, Beck EX, Shafer A, Barut B, Gbur JS, Zupancic TJ, et al. Leptin stimulates fetal and adult erythroid and myeloid development. *Blood*. 1997; 89: 1507-12.

4. Nakao T, Hino M, Yamane T, Nishizawa Y, Morii H, Tatsumi N. Expression of the leptin receptor in human leukaemic blast cells. *Br J haematol*. 1998; 102: 740-5.

5. Konopleva M, Mikhail A, Estrov Z, Zhao S, Harris D, Sanchez-Williams G, et al. Expression and function of leptin receptor isoforms in myeloid leukemia and myelodysplastic syndromes: proliferative and anti-apoptotic activities. *Blood*. 1999; 93: 1668-76.

6. Tabe Y, Konopleva M, Munsell MF, Marini FC, Zompetta C, McQueen T, et al. PML-RARalpha is associated with leptin-receptor induction: the role of mesenchymal stem cell-derived adipocytes in APL cell survival. *Blood*. 2004; 103: 1815-22.

7. Hamed NA, Sharaki OA, Zeidan MM. Leptin in acute leukaemias: relationship to interleukin-6 and vascular endothelial growth factor. *Egypt J Immunol*. 2003; 10: 57-66.

8. Hino M, Nakao T, Yamane T, Ohta K, Takubo T, Tatsumi N. Leptin receptor and leukemia. *Leuk Lymphoma*. 2000; 36: 457-61.

9. Tsiotra PC, Pappa V, Koukourava A, Economopoulos T, Tsigos C, Raptis SA. Expression of leptin receptors in mononuclear cells from myelodysplastic syndromes and acute myeloid

leukemias. *Acta Haematol.* 2005; 114: 71-7.

10. Maffei M, Halaas J, Ravussin E, Pratley RE, Lee GH, Zhang Y, et al. Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. *Nat Med.* 1995; 1: 1155-61.

11. Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, et al. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med.* 1996; 334: 292-5.

12. Russell CD, Ricci MR, Brolin RE, Magill E, Fried SK. Regulation of the leptin content of obese human adipose tissue. *Am J Physiol Endocrinol Metab.* 2001; 280: E399-404.

13. Muszynska-Roslan K, Krawczuk-Rybak M, Topczewska M, Sawicka-Zukowska M. Relationship between body mass index and leptin levels in children treated for acute lymphoblastic leukemia during and after maintenance therapy. *Endokrynol Diabetol Chor Przemiany Materii Wieku Rozw.* 2006; 12: 91-5.

14. Ren SG, Seliktar J, Li X, Braunstein GD, Melmed S. Measurement of leukemia inhibitory factor in biological fluids by radioimmunoassay. *J Clin Endocrinol Metab.* 1998; 83: 1275-83.

15. Auernhammer CJ, Melmed S. Leukemia-inhibitory factor-neuroimmune modulator of endocrine function. *Endocr Rev.* 2000; 21: 313-45.

16. Leary AG, Wong GG, Clark SC, Smith AG, Ogawa M. Leukemia inhibitory factor differentiation-inhibiting activity/human interleukin for DA cells augments proliferation of human hematopoietic stem cells. *Blood.* 1990; 75: 1960-4.

17. Faderl S, Kantarjian HM, Talpaz M, Estrov Z. Clinical significance of cytogenetic abnormalities in adult acute lymphoblastic leukemia. *Blood.* 1998; 91: 3995-4019.

18. Gearing DP, Thut CJ, VandeBos T, Gimpel SD, Delaney PB, King J, et al. Leukemia inhibitory factor receptor is structurally related to the IL-6 signal transducer, gp130. *Embo J.* 1991; 10: 2839-48.

19. Inoue K, Sugiyama H, Ogawa H, Yamagami T, Azuma T, Oka Y, et al. Expression of the interleukin-6 (IL-6), IL-6 receptor, and gp130 genes in acute leukemia. *Blood.* 1994; 84: 2672-80.

20. Okamoto H, Yamamura M, Morita Y, Harada S, Makino H, Ota Z. The synovial expression and serum levels of interleukin-6, interleukin-11,

leukemia inhibitory factor, and oncostatin M in rheumatoid arthritis. *Arthritis Rheum.* 1997; 40: 1096-105.

21. Rafet M, Ymdat D, Ekrem A, Reha E, Hayati A, Cevat T, et al. Relationship between leptin levels and body indexes in patients with haematologic malignancy. *Turk J Haematol.* 2001; 18: 185-9.

22. Bruserud O, Huang TS, Glenjen N, Gjertsen BT, Foss B. Leptin in human acute myelogenous leukemia: studies of in vivo levels and in vitro effects on native functional leukemia blasts. *Haematologica.* 2002; 87: 584-95.

23. Snoussi K, Strosberg AD, Bouaouina N, Ben Ahmed S, Helal AN, Chouchane L. Leptin and leptin receptor polymorphisms are associated with increased risk and poor prognosis of breast carcinoma. *BMC cancer.* 2006; 6: 38.

24. Pamuk GE, Demir M, Harmandar F, Yesil Y, Turgut B, Vural O. Leptin and resistin levels in serum of patients with hematologic malignancies: correlation with clinical characteristics. *Exp Oncol.* 2006; 28: 241-4.

25. Gaja A, Chury Z, Pecan L, Fra kova H, Jandakova E, Hejlova N. Bone marrow and peripheral blood leptin levels in lymphoproliferative diseases-relation to the bone marrow fat and infiltration. *Neoplasma.* 2000; 47: 307-12.

26. Wasik M, Gorska E, Popko K, Pawelec K, Matysiak M, Demkow U. The Gln223Arg polymorphism of the leptin receptor gene and peripheral blood/bone marrow leptin level in leukemic children. *J Physiol Pharmacol.* 2006; 57: 375-83.

27. Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, et al. Effects of the obese gene product on body weight regulation in ob/ob mice. *Science.* 1995; 269: 540-3.

28. Rosenbaum M, Nicolson M, Hirsch J, Heymsfield SB, Gallagher D, Chu F, et al. Effects of gender, body composition, and menopause on plasma concentrations of leptin. *J Clin Endocrinol Metab.* 1996; 81: 3424-7.

29. Falorni A, Bini V, Molinari D, Papi F, Celi F, Di Stefano G, et al. Leptin serum levels in normal weight and obese children and adolescents: relationship with age, sex, pubertal development, body mass index and insulin. *Int J Obes Relat Metab Disord.* 1997; 21: 881-90.

30. Moller N, O'Brien P, Nair KS. Disruption of the relationship between fat content and leptin

levels with aging in humans. *J Clin Endocrinol Metab.* 1998; 83: 931-4.

31. Suzuki K, Ito Y, Ochiai J, Kusuhara Y, Hashimoto S, Tokudome, S et al. Relationship between obesity and serum markers of oxidative stress and inflammation in Japanese. *Asian Pac J Cancer Prev.* 2003; 4: 259-66.

32. Karachaliou F, Vlachopapadopoulou E, Theochari M, Konstandellou E, Michalados S. Leptin levels in patients with thalassemia major. *Minerva Pediatrica.* 2006; 58: 373-8.

33. Matthys P, Billiau A. Cytokines and cachexia. *Nutrition.* 1997; 13: 763-70.

34. Dedoussis GV, Kyrtsonis MC, Andrikopoulos NE, Voskaridou E, Loutradis A. Inverse correlation of plasma leptin and soluble transferrin receptor levels in beta-thalassemia patients. *Ann Hematol.* 2002; 81: 543-7.

35. Caro JF, Sinha MK, Kolaczynski JW, Zhang PL, Considine RV. Leptin: the tale of an obesity gene. *Diabetes.* 1996; 45: 1455-62.

36. Inui A. Cancer anorexia-cachexia syndrome: are neuropeptides the key? *Cancer Res.* 1999; 59: 4493-501.

37. Ahmed K, Osama E, Zakaria, Nehad S. Serum Level of Leukemia Inhibitory Factor (LIF) in Children with Acute Leukemia. *Egypt J Immunol.* 1998; 5: 159-65.

38. Maekawa T, Metcalf D, Gearing DP. Enhanced suppression of human myeloid leukemic cell lines by combinations of IL-6, LIF, GM-CSF and G-CSF. *Int J Cancer.* 1990; 45: 353-8.

39. Baumann H, Morella KK, White DW, Dembski M, Bailon PS, Kim H, et al. The full-length leptin receptor has signaling capabilities of interleukin 6-type cytokine receptors. *Proc Natl Acad Sci USA.* 1996; 93: 8374-8.

40. Robak T, Gladalska A, Stepień H, Robak E. Serum levels of interleukin-6 type cytokines and soluble interleukin-6 receptor in patients with rheumatoid arthritis. *Mediators Inflamm.* 1998; 7: 347-53.