

Original Research Article

Evaluation of Leukemia Inhibitory Factor, Interleukin6 and Leptin in Acute and Chronic Myeloid Leukemia in Babylon Province

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Accepted 29 May, 2016

Abstract

Leukemias are a group of diseases described by augmented numbers of white cells in the blood and bone marrow as a result of unregulated clonal proliferation of immature malignant cells. These abnormal white blood cells that not completely developed are called (blasts), or (leukemia cells). The diagnosis of Leukemia was confirmed by blood investigation and by bone marrow biopsy. Leukemia inhibitory factor may play an important role, along with Interleukin-6 and granulocyte colony stimulating factor, in the regulation of early hematopoietic stem cells. Leptin has been implicated in the differentiation and proliferation of hematopoietic cells. In this case control study, we evaluate, the levels of LIF, IL6, Leptin in serum of patients with myeloid leukemia and assess the relationship between leptin level & body mass index in myeloid leukemic patients, in the current study involved 96 individuals; 48 patients with myeloid leukemia and 48 controls. Leukemia inhibitory factor, Interleukin 6 and Leptin levels are measured by ELISA assay in both patient and control groups. The mean LIF, IL6 in the AML and CML groups were significantly higher than control group ($p < 0.05$). while Leptin in the AML and CML groups was significantly lower than control group ($p < 0.05$). And the mean (BMI) in the AML and CML groups were significantly lower than control group ($p < 0.05$). In this study, we conclude that LIF, IL6 and Leptin play an important role in pathogenesis of Leukemia, LIF, IL6 levels in myeloid leukemic patients more than controls while Leptin level in myeloid leukemic patients less than controls and There is significant relationship between Leptin and body mass index.

Key words: Leukemia inhibitory factor, Interleukin 6, Leptin and myeloid leukemia.

تقييم العوامل المثبطة لايبيضاض الدم ، انترلوكين 6 و اللبتين في مرض ابيضاض الدم النقوي الحاد والمزمن في محافظة بابل

الخلاصة

سرطان الدم هو مجموعة من الأمراض التي تتميز بزيادة في عدد الخلايا البيضاء في الدم ونخاع العظام ويحدث بسبب تكاثر غير مسيطر عليه لخلايا سرطانية غير ناضجة. كريات الدم البيضاء التي لم تتطور بصورة طبيعية تدعى (خلايا الارومة) (خلايا ابيضاض الدم). تشخيص المرض يتم عن طريق الفحوصات الدموية و اخذ خزعة من نخاع العظم. العوامل المثبطة لسرطان الدم (LIF) قد تلعب دوراً هاماً، إلى جانب (IL6) و (CSF)، في تنظيم الخلايا الجذعية المبكرة للدم. وكذلك اللبتين (Leptin) ذات تأثير في تمايز وتكاثر الخلايا المكونة للدم. في هذه الدراسة نقوم بمعرفة مستويات العوامل المثبطة لسرطان الدم (LIF) و (IL6) وكذلك اللبتين (Leptin) في مصول المرضى المصابين بسرطان الدم النقوي وهدف آخر تحديد الترابط بين اللبتين مع مؤشر كتلة الجسم. هذه الدراسة هي دراسة مقارنة وشملت هذه الدراسة 48 مريض سرطان الدم النقوي تم تشخيصهم حديثاً بالإضافة إلى 48 شخص سيطرة أدرجت في هذا الموضوع. لقد تم قياس مستوى العوامل المثبطة لسرطان الدم (LIF)، انترلوكين 6 (IL6) واللبتين في المصل باستخدام مقاييس الممتز المرتبط بالانزيم (ELISA) من خلال استخدام Human Elisa kit. لقد بينت النتائج وجود زيادة معنوية بمتوسط مستويات العوامل المثبطة لسرطان الدم (LIF)، انترلوكين 6 (IL6) ($P > 0.05$). اما متوسط اللبتين في مجموعة المرضى كان

منخفض معنوياً عنه في مجموعة السيطرة ($P > 0.05$). وكان هناك ارتباط معنوي بين متوسط مستوى اللبتين ومؤشر كتلة الجسم ($P > 0.05$). في هذه الدراسة نحن استنتجنا بأن العوامل المثبطة لسرطان الدم (LIF), (IL6) وكذلك اللبتين تلعب دوراً هاماً في عملية تطور سرطان الدم بمستويات العوامل المثبطة لسرطان الدم (LIF), (IL6) في مرضى سرطان الدم النقوي أكبر منه من مجموعة السيطرة بينما مستوى اللبتين في مرضى سرطان الدم النقوي أقل منه من مجموعة السيطرة. ويوجد علاقة معنوية ملحوظة بين اللبتين ومؤشر كتلة الجسم.

الكلمات المفتاحية: العوامل المثبطة لسرطان الدم, انترلوكين 6, اللبتين وسرطان الدم النقوي.

Introduction

Acute myeloid leukaemia is a malignant clonal disorder of immature cells in the haemopoietic hierarchical system. Leukemic transformation is understood to occur at, or close to, the stage of the haemopoietic stem cell before it has been embarked on any lineage commitment. a few cases may initiate at a somewhat later stage in cells that are committed to a lineage differentiation. The proliferative benefit of the leukemic stem cell together with impairments in differentiation and inhibition of apoptosis cause growth of immature or blast cells in the BM. The blasts finally suppress normal haematopoiesis leading to marrow failure and gain access to other organs and tissues. While it was not curable previously [1].

Chronic myeloid leukemia is hematopoietic proliferative disorder with particular gene defect and a very distinctive blood picture. There is a clear neutrophil leukocytosis with some circulation of immature neutrophils and an increase in basophils. The translocation between chromosome 9 and chromosome 22 (t9:22) is The gene defect, which is positive in 90% to 95% of the cases. This gene defect is called the Philadelphia chromosome [2].

Cytogenetic investigation is a means element in the assessment of patients with recently diagnosed or suspected acute myeloid leukemia (AML). Particular cytogenetic abnormalities are intimately, and occasionally exclusively, associated with clinically and morphologically distinctive subsets of the disease [3]. The variant/complex translocation is found around 5-8% CML patients, in these cases an additional third, fourth or fifth chromosomes are involved with 9 and

22 chromosomes [4]. The correct diagnosis in myeloid leukemia is necessary for treatment correlative biology study, and evaluation of prognosis. Early assessment requires (family history, medication history, work and exposure history) physical examination, complete blood count with peripheral blood smear review, and bone marrow examination, in addition to usual morphologic evaluation, flow cytometry, cytogenetics and special molecular genetic analysis [5]. Many studies refer to the cytokines (LIF, IL, LEPTIN) play very important role in pathogenesis of leukemia. (LIF) is a member of the interleukin-6 cytokine super family. The activation of LIF, binds with LIF receptor (LIFR) of a plasma membrane with Gp130 glycoprotein (the common signaling receptor for IL-6 family cytokines) to form a high affinity receptor through which LIF signaling is triggered. This direct to activation of the JAK/STAT (stat 3) and MAPK (mitogen activated protein kinase) cascade [6].

Interleukin 6, It's an effective and important factor for the typical development and function of both T and B lymphocyte and has a great actions on cells of hematopoietic system [7]. In addition it's an essential regulator of the acute phase response in the liver and regulate the fever, additionally to its effect on the central nervous system via regulating glial cells activation [8]. In the leukemia, IL-6 shows to have equally stimulatory and suppressive effect. IL-6 level is increased in patient with AML both inhibitory and stimulatory effect on clonogenic blast cell growth It carries a stimulatory factor in multiple myeloma [9]. Numerous studies which confirmed that serum level of IL-6 is a potent prognostic factor in large cell

lymphoma and chronic lymphocytic leukemia and diver effect on the growth of AML blast cell, as well as stimulation and preservation of their growth throughout the IL-6/IL-6 receptor signaling system[10,11]. In CML, multipotent progenitors (MPPs) show an irregular B-lymphoid potential but are transmit to the myeloid lineage via the action of the IL-6 cytokine. The BCR/ABL activity effect IL-6 expression thereby creating a paracrine feedback loop that maintains CML development[12]. leptin and leptin receptors may play a vital role in the control of the extension and differentiation of primitive hematopoietic cells during paracrine interaction in the bone marrow and concerned in the development of hematopoietic malignancies[13]. The fat cell substance of the bone marrow may consequently reveal the requirement for leptin in hematopoietic development. Leptin receptors are also found in leukemic cells from patients affect by CML disease, acute myeloblastic leukemia and acute lymphoblastic leukemia. mostly, in CML cases elevated expression of leptin receptors has been identified through blast crisis not in the chronic phase. Leptin with or without combination with other cytokines, demonstrates anti-apoptotic and proliferative property on leukemia blast cells, signifying that leptin may play a key role in the pathology of leukemia disease[14].

The aims of this Study was To measure levels of LIF, IL6 and Leptin, (Hb, PCV, WBC) and evaluate lipid profile (TC, TG, HDL, LDL) in serum of patients with myeloid leukemia; to assess the relationship between leptin level & body mass index in myeloid leukemic patients; and to assess the relationship between LIF, IL6 & (Hb, PCV, WBC) in myeloid leukemic patients.

Materials and Methods

This is a case-control study . It included 96 individuals; 48 patients with myeloid leukemia and 48 controls. Leukemia inhibitory factor, Interleukin 6 and Leptin levels are measured by ELISA assay in

both patient and control groups. Hematological investigation including (Hb, PCV, WBC), blood film, body mass index and lipid profile were measured and contrast between these two groups. The patients separated into two subgroups:

Subgroup (1):30 patients with AML and age mean(44.43 ± 10.75) years (mean $\pm$ SD).

Subgroup (2): 18 patients with CML and age mean (59.27 ± 6.01)years (mean $\pm$ SD).

Seven (ml) of blood be collected .Five milliliters were reserved in a plane tube without anticoagulant and two (ml) in EDTA tube. Following that, the plane tube was left for 30 minutes at room temperature. Once coagulation occur, tubes were centrifuged at 1000 xg for about 10 minutes, the sera were aspirated and separated into three fractions ,and keep at (-20°C) until time of utilize. The EDTA tube was used to measured Hb, PCV, WBC count and blood film. The ELABSCIENCE BIOTECH CO ELISA kit utilized Sandwich-ELISA as the method. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to (LIF,IL6,LEPTIN). Standards or samples are added to the appropriate micro ELISA plate wells and bound by the specific antibody A monoclonal antibody.At first 100 μL of standard or sample was inserted to every well. And incubated for 90 minutes at 37°C .Then aspiration and washing was for three times. After these steps inserted 100 μL (HRP Conjugate) and incubated for 60 minutes on 37°C .Then the aspiration and washing for five times. After the step of washing, inserted 90 μL Substrate Reagent and incubated for 15 minutes on 37°C and then Inserted 50 μL Stop Solution and Read optical density at 450nm directly. Finally the results were estimated.

BMI (body mass index)

Person can fit in one of the next weight groups:

- Underweight person (BMI less than 18.5).
- normal weight person (BMI between 18.5 & 24.9).

- overweight person (BMI between 25.0 & 29.9).
- obese person (BMI 30.0 and above).

Statistical Analysis

The data were analyzed by using computerized SPSS (Statistical Package of Social Science) program; Independent t-test was used to estimate differences between two groups in continuous variables. A p-value <0.05 is considered to be statistically significant (Daniel, 1999).

Results

Age and Sex distribution in the patients and control groups.

The study included two groups; first group consisted of 48 patients with myeloid leukemia and second control group consisted of 48 controls. In the patient group, there were 30 (62.5%) patients diagnosis with AML. and 18 (37.5%)

patients with CML. The mean age of the AML patient group(44.4).The mean age of the CML patient group(59.2).Majority of AML patients (66.6%) and controls (63.3%) were males. And majority of CML patients (66.6%) and controls (55.5%) were also males.

Hematological and lipid Investigations

The mean (Hb,PCV,WBC) of the AML patient group showed a significant difference when compared with the control group (P<0.05) Table1. The mean(HB,PCV,WBC)of the CML patient group shows a significant difference when compared with the control group (P<0.05) Table 2. The mean Lipid level in the AML group was significantly lower than control group (p<0.05)Table 1. The mean Lipid level in the CML group was significantly lower than control group (p<0.05) Table 2.

Table 1: Hematological and lipid profile assay in control and Acute myeloid leukemia

Parameter	AML patients	Control group	P value
Hb (gm/dl)	7.9±1.4	12.4±1.9	*<0.05
PCV %	22.03 ±1.2	38.4±1.2	*<0.05
WBC (10 ⁹ /L)	60.5±15.7	7.4±1.4	*<0.05
TC (mg/dl)	121.8±14.6	181.6±43.11	*<0.05
TG (mg/dl)	122.3±23.9	168.16±46.5	*<0.05
HDL (mg/dl)	59.9±14.9	73.3±16.07	*<0.05
LDL (mg/dl)	106.7±10.19	130.2±18.3	*<0.05

Table 2: Hematological and lipid profile assay in control and chronic myeloid leukemia

Parameter	CML patients	Control group	P value
Hb (gm/dl)	9±1.04	12.5 ±1.7	*<0.05
PCV %	26.79±3.65	39.06±5.3	*<0.05
WBC (10 ⁹ /L)	150.5±28.7	8.4±1.7	*<0.05
TC (mg/dl)	107.7 ±12.6	160.3 ±33.1	*<0.05
TG (mg/dl)	114.1 ±17.2	149.5 ±38.5	*<0.05
HDL (mg/dl)	54.3 ±14.1	71.7 ±11.3	*<0.05
LDL (mg/dl)	108.2±9.7	147.2±20.8	*<0.05

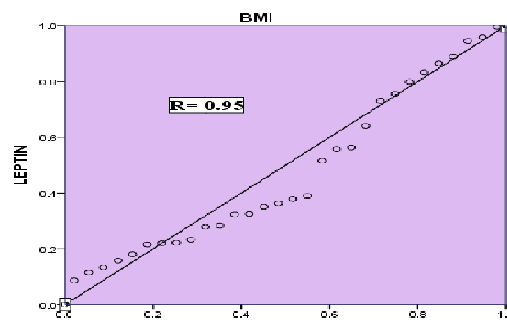
LIF,IL6,Leptin Levels.

The mean LIF,IL6 in the AML and CML groups were significantly higher than control group ($p<0.05$). while Leptin in the AML and CML groups was significantly lower than control group ($p<0.05$). And the mean (BMI) in the AML and CML groups

were significantly lower than control group ($p<0.05$).

The correlation between BMI and Leptin.

The correlation between BMI and Leptin in patients with AML and CML patients were (significant) (Figure1,2). Correlation is significant at the 0.05 level (2-tailed).

**Figure1:** The correlation between leptin and BMI of the AML Groups.

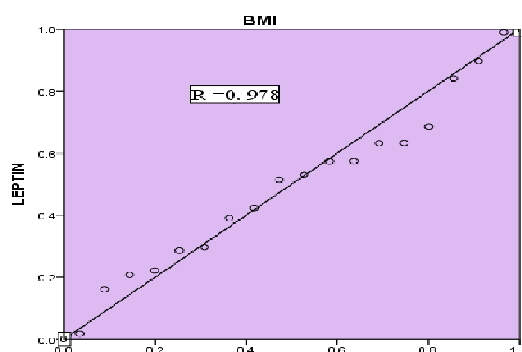


Figure 2: The correlation between leptin and BMI of the CML Group

Discussion

In this study, the mean age (mean $\pm$ SD) of AML patients was (44.43 $\pm$ 10.75) years. These results were comparable to other Iraqi studies in 2009 and 2012[15,16]. The age(mean $\pm$ SD) of CML patients (59.27 $\pm$ 6.01). These results agreed with results about CML that were mentioned by Surveillance Epidemiology and End Results[17]. Also it has been found that AML and CML were more in males (66.6%) than females(33.4). This result is consistent with the study conducted by Alwan et al[18].

In our study, Hb was significantly lower in patients with (AML,CML) than controls. This finding was consistent with the results of Yamamoto et al[19]. Other results in this study, PCV in AML and CML patients were significantly lower in patients than controls. This finding was consistent with the results of Chessel et al[20]. However in this study, WBC count was significantly higher in patients with (AML,CML) than control. This result agreed with study of Wetzler et al[21]. This can be explained by the fact that in leukemia there is a clonal proliferation of malignant cells that may arise at any stage of maturation in the bone marrow including lymphoid, myeloid or pluripotent stages[22].

Lipid profile levels were significantly lower in patients(AML,CML) than normal control. This result agreed with study Pamuk et al[23]. This could be related to the high metabolic rate of malignant cells, along with fever and body weight loss[24].

In current study, LIF in AML and CML patients were significantly higher in patients than controls. This finding was consistent with the results of Ahmed et al[25]. Leukemia inhibitory factor, its name was derived from its ability to induce the terminal differentiation of myeloid leukemia cells and in order to prevent the growth of leukemia cells stimulating the differentiation of M1 myeloid leukemia cell and macrophage maturation to suppress leukemia proliferation, at what time LIF levels fall the cells will differentiate[26]. In this study, IL6 in AML and CML patients was significantly higher in patients than controls. This finding was consistent with the results of Dawood comparative study which was conducted from November 2010 to March 2011 on “60” subjects[27], Tsimberidou et al[10] and Elmaksoud et al[11]. Interleukin 6, it's an effective and important factor for the typical development and function of both T and B lymphocyte and has a great action on cells of the hematopoietic system[28]. In this study, leptin in AML and CML patients was significantly lower in patients than controls. This finding was consistent with the results of Wasik et al[29]. That reported lower serum leptin level in AML patients. Another study by Gaja et al[30] and Pamuk et al [23], demonstrated that leptin level was lower in untreated myeloid leukemia patients. Leptin is a 16-kDa protein with 146 amino acids. Membrane proteins are members of the cytokine family and gp130, secreted primarily from adipocytes, was initially recognized as a cytokine that controls fat

metabolism and reproduction, unsatisfactory leptin production has been coupled with obesity in humans al [31]. In current study, the mean (BMI) in the AML and CML groups were significantly lower than control group. This finding was consistent with the results of Alizadeh et al[32] where they took 30 patients, 15 patients (AML) and 15 patients (CML). This result could be related to the high metabolic rate of malignant cells, along with fever and body weight loss[24]. In our study, significant correlation between BMI and Leptin in patients with AML and CML. Reduced the level of leptin associated with reduced of body mass index. This finding was against the results of Alizadeh, et al[32] where they demonstrated that no significant Correlation between BMI and Leptin in patients with AML and CML. This difference might be explained by small sample size and short study time. The fat cell substance of the bone marrow may consequently reveal the requirement for leptin in hematopoietic development, signifying that leptin may play an key role in the pathology of leukemia disease. So as result of high metabolic rate of malignant cells, along with fever and body weight loss[24]. That lead to loss of fat cell substance that result in reduce leptin level and body mass index .

Conclusion

1. The LIF, IL6 and Leptin play an important role in pathogenesis of Leukemia
2. The LIF, IL6 levels in myeloid leukemic patients more than controls.
3. The Leptin level in myeloid leukemic patients less than controls.
4. There is significant relationship between Leptin and body mass index in myeloid leukemic patients.
5. There is no significant relationship between LIF, IL6 and hematological parameters (Hb, PCV, WBCs count) in myeloid leukemic patients.

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