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Expression of Cytokine of Chronic Lymphocyte Leukemia from Baghdad / Iraq Population

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Abstract:

Aim of the current study is to investigate correlation proinflammatory cytokines (Interleukin 6, 8, 10, and TNF α) with Chronic Lymphocyte Leukemia. In this study, serum was collected from (100) sample, a representative of 80 samples of chronic lymphocyte leukemia who were newly diagnosed by the consultant medical staff at the National Center of Hematology and 20 healthy sample on the other hand. IL-6, IL8, IL10 and TNF α were studied using the enzyme linked immunosorbent assay methods from (R and D system/USA). This study have shown that IL6 levels increased about two fold compared with healthy groups and TNF α levels was three times higher in CLL patient compared with healthy and IL10 levels was 3.5 fold that healthy. The conclusion IL 6, 10, and TNF α levels have significantly increased in patients compared with healthy control. However, IL8 was the same production in CLL compared with control.

Key words: *CLL. ELISA, and TNF α.*

Introduction:

Leukemia is a progressive , malignant disease of the blood forming organs characterized by distorted proliferation and development of leukocytes and their precursors in the blood and bone marrow and can be classified clinically on the basis of duration and character of the disease into acute and chronic leukemia (1). In Iraq leukemia ranks second among ten types of cancer in 2004 (2), while in 1989 , it ranked seven among the ten types of cancer (3). One important type of leukemia is chronic lymphocytic leukemia (CLL), is a disease in which mature lymphocytes become cancerous and gradually replace normal cells in lymph nods. More than three fourths of the people who have chronic lymphatic leukemia (CLL) are older than 60. This type of leukemia affects men 2 to 3 times more than the women. CLL is the most common type of leukemia in North America and Europe. It's rare in Japan and South east Asia, which indicates that heredity play role in its development (4). Cytokines are multifunctional polypeptides synthesized from various cell types of human body they have important roles in many processes, such as the development of humoral and cellular immune responses , triggering of the inflammatory response, regulation of hematopoiesis suppression of cellular differentiation and reproduction and wound healing (5). Interleukin-6 (IL6) and (IL-10) are a pleiotropic cytokine IL6 produced by a rarity of cells types including fibroblasts, endothelial cells, monocytes normal hematopoietic cells and lymphocytes (6). Several earlier studies suggested a possible role for dysregulation production of IL-6 in malignant lymphomas (7). Interleukin-10 produced by type 2 helper cells (Th2) (8) as well as monocytes and macrophages and normal neoplastic B lymphocytes (9), anther cytokine is TNF-α is multifunctional cytokine that is also normal cachexia. It has a homotrimeric structure and synthesized from various cell types, especially from macrophages, monocytes fibroblasts, endothelial cells, adiposities, B cells and tumor cell types (10), 1L6, 1L10 and TNF- α are cytokines that have important roles in cancer pathogenesis (11). The aim of this study is assess correlation between expression of cytokine (IL6, 8, 10 and TNF- α) and chronic lymphocyte leukemia (CLL) in the Iraqi Population.

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Material and Methods:

Collection of Sample

Serum was collected from (100) a representative sample of 80 sample of chronic lymphocyte leukemia (CLL) (40 males and 40 females, age ranged from 4 to 25 years) and 20 healthy samples from the National Center of Hematology/ Al

Mustanisyria University from 2-2013 to 2-2014. The disease was clinically diagnosed by the consultant medical staff at the centre.

The IL6 and IL8 Detection Assay

The IL6 and IL8 concentration was detected using IL6 or IL8 enzyme linked immuno sorbent assay (ELISA) for in vitro quantitative determination of cytokine in supernatant, buffered solutions, serum and plasma samples. The cytokine kit is a solid phase sandwich ELISA, a monoclonal antibody specific for IL6 or IL8 has been coated onto the wells of the micro titer plate ((R and D system / USA).

Cytokine Detection Assay

A: The IL6 Cytokine Kit, Catalog Number HS600B

Wash Buffer of IL6 and IL 8, If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 100 mL of Wash Buffer Concentrate into deionized water to prepare 1000 mL of Wash Buffer.

Calibrator Diluent RD6-11 (1X) Diluted 10 mL of Calibrator Diluent RD6-11 Concentrate into 10 mL of deionized water to prepare 20 mL of Calibrator Diluent RD6-11 (1X).

IL-6 Standard, Reconstituted the IL-6 Standard with 5.0 mL of the appropriate Calibrator Diluent (Calibrator Diluent RD6-11 concentrate for serum samples). This reconstitution produces a stock solution of 10 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 500 µL of Calibrator Diluent RD6-11 concentrate into each tube.

Substrate Solution, Reconstituted the lyophilized substrate with 6.0 mL of substrate diluent at least 10 minutes before use. Re-stopper and re-cap the vial, and mix thoroughly.

Amplifier Solution, Reconstitute the lyophilized Amplifier with 6.0 mL of amplifier diluent at least 10 minutes before use.

Protocol

Add 100 µL of Assay Diluent RD1-75 to each well, Add 100 µL of standard and sample per well. The Covered plate was incubated for 2h at room temperature 25ºC and shaker at 500 ± 50 rpm. The plate was washed as follow: The washing buffer was aspirated from each well, 0.4ml of washing solution was dispensed into well then the content of well was aspirated (washing was repeated twice). Add 200 µL of IL-6 Conjugate to each well. The Covered plate was incubated for 2h at room temperature 25ºC and shaker at 500 ± 50 rpm then washed as in up step, add 50 µL of substrate solution to each well, the covered plate was incubated for 1h at room temperature 25ºC and shaker at 500 ± 50 rpm. Add 50 µL of Amplifier Solution to each well, incubate for 30 minutes at room temperature. Than add 50 µL of Stop Solution to each well, the absorbance of each well were read on a ELISA microplate reader (Olympus/ Japan) at 490nm.

B: The IL8 Cytokine Kit, Catalog Number D8000C

Substrate Solution of IL8, Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light.

IL-8 Standard Reconstituted the IL-8 Standard with 5.0 mL of Calibrator Diluent RD6Z. This reconstitution produces a stock solution of 2000 pg/mL. Pipette 500 mL of Calibrator Diluent RD6Z into each tube. Use the stock solution to produce a dilution series. Mix each tube thoroughly before the next transfer. The undiluted standard serves as the high standard (2000 pg/mL).

Protocol

Added 100 mL of Assay Diluent RD1-85 to each well, add 50 µL of standard and sample per well. The Covered plate was incubated for 2h at room temperature 25ºC and shaker at 500 ± 50 rpm. The plate was washed as follow: The washing buffer was aspirated from each well, 0.4ml of washing solution was dispensed into well then the content of well was aspirated (washing was repeated twice). Add 200 µL of IL-8 Conjugate to each well. The Covered plate was incubated for 1h at room temperature 25ºC and shaker at 500 ± 50 rpm then washed as in up step, add 200 µL of substrate solution to each well, the covered plate was incubated for 30 minutes at room temperature 25ºC and shaker at 500 ± 50 rpm (Protect from light). Than add 50 µL of stop solution to each well, the absorbance of each well were read on a ELISA microplate reader (Olympus/ Japan) at 450nm.

C: The IL10 Cytokine Kit, Catalog Number 18432-05.

Human IL-10 ELISA kit contain the key components required for the quantitative measurement of natural and/or recombinant HIL-10 in a sandwich ELISA format within the range of 50-1600pg/ml.

Procedure

Practical work was done following the instructions of US Biological IL-10 kit protocol/Biochemical and Biological Reagents, United State Biological. The supernatants of treated lymphocytes with different concentrations of extracted polysaccharide were applied with IL-10 ELISA protocol. Aliquot 100µl from each standard and samples were added (in duplicate) into the antibody pre-coated micro titer plate, then incubated for 1 hour at room temperature, without discarding standards or sample solutions about 50 µl Pab (biotin) was added to each well, incubated for 1 hour at room temperature then the plate was washed to remove any unconjugated antibodies. The Avidin attached with HRP enzyme was added to all wells in quantity of 100 µl the plate was incubated in dark at room temperature for another 1 hour followed by washing step, finally 100 µl substrate mixture was added for 15 minutes stand period in dark at room temperature then to stop their reaction, 100 µl stop solution was added. At the end of experiment a standard curve for different standard concentrations verses their absorbance at 620 nm were plotted, then each IL-10 concentration was calculated and then evaluated statically.

D: The TNF-α Cytokine Kit, Catalog Number T9160-01.

The kit depend on TNF- α enzyme linked immunosorbent assay (ELISA), which is a quantitative sandwich immunoassay allows good determination for concentration of TNF- α in the sample. The micro titer plate provided in this kit has been

pre-coated with a monoclonal antibody specific to TNF- α. Standards or samples are then added to the appropriate micro titer plate wells with a biotin-conjugated polyclonal antibody preparation specific for TNF-α. TNF- α if present, will bind and become immobilized by the antibody pre-coated on the wells and then be "sandwiched" by biotin conjugate. In order to measure the concentration of TNF-α in the samples, this kit includes two calibration diluents. According to the testing system, the provided standard is diluted (2-fold) with the appropriate calibrator diluents and assayed at the same time as the samples. This allows the operator to produce a standard curve of optical density (O.D) versus TNF- α concentration (pg/ml). The concentration of TNF- α in the samples is then determined by standard curve and the straight line equation. The range of standard concentrations is (15.625-1000pg/ml).

Procedure

The work was done following the instruction of US Biological TNF-α kit protocol /Biochemical &Biological Reagents, United State Biological, catalog No (T9160-01). At the end of experiment a standard curve for known concentration with their absorbance at 620nm was plotted, then all test reading were calculated each TNF- α concentration according to straight line equation and all results were evaluated statically

Statistical Analysis

The statistical analysis system-SAS (2010) was used to effect of different factors in study parameters. Least significant difference –LSD test (T-test) was used to significant compare between means in this study (13).

Results:

Cytokine (IL6, IL8, IL10, and TNF α are the most commonly studied cytokines and are associated with various soft tissue tumor, IL6 inhibits proliferation via the induction of apoptosis in cancer cells and thus creates DNA fragmentation, which is a characteristic feature of apoptosis in these cells (14 .(In this study the measured average IL6 plasma levels of the CLL patients and the healthy (1.969 ± 0.19 and 0.409 ± 0.04, respectively), and have significance among the groups (P<0.05) shown as table (1) and figure (1).

Table 1: Comparison of serum IL-6 & IL-8 levels as measured by ELISA in CLL patients (N:80) and in healthy control (N:20), P<0.05.

Group	No.	Mean ± SE	
		IL-6	IL-8
Patients	80	0.409 ± 0.04	0.088 ± 0.01
Healthy	20	1.969 ± 0.19	0.106 ± 0.01
T-test	---	0.289 *	0.013 NS
* (P<0.05).			

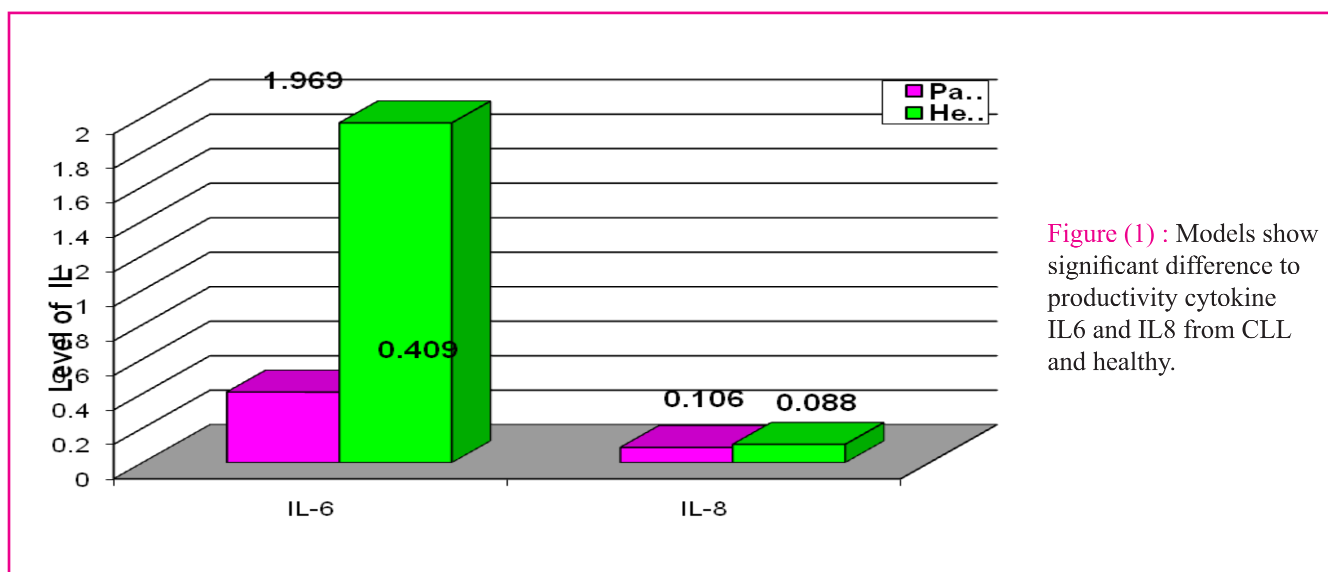

Figure (1) : Models show significant difference to productivity cytokine IL6 and IL8 from CLL and healthy.

In this study appeared higher serum TNF α levels (0.346 ± 0.04) compared with healthy (0.099 ± 0.01) and have significantly than control groups (P<0.05) shown as Table (2) and figure (2).

Table 2: Comparison of serum TNF α and IL-10 levels as measured by ELISA in CLL patients (N:80) and in healthy control (N:20), P<0.05.

Group	No.	Mean ± SE	
		TNF	IL-10
Patients	80	0.346 ± 0.04	0.172 ± 0.01
Healthy	20	0.099 ± 0.01	0.061 ± 0.02
T-test	---	0.155 *	0.048 *
* (P<0.05).			

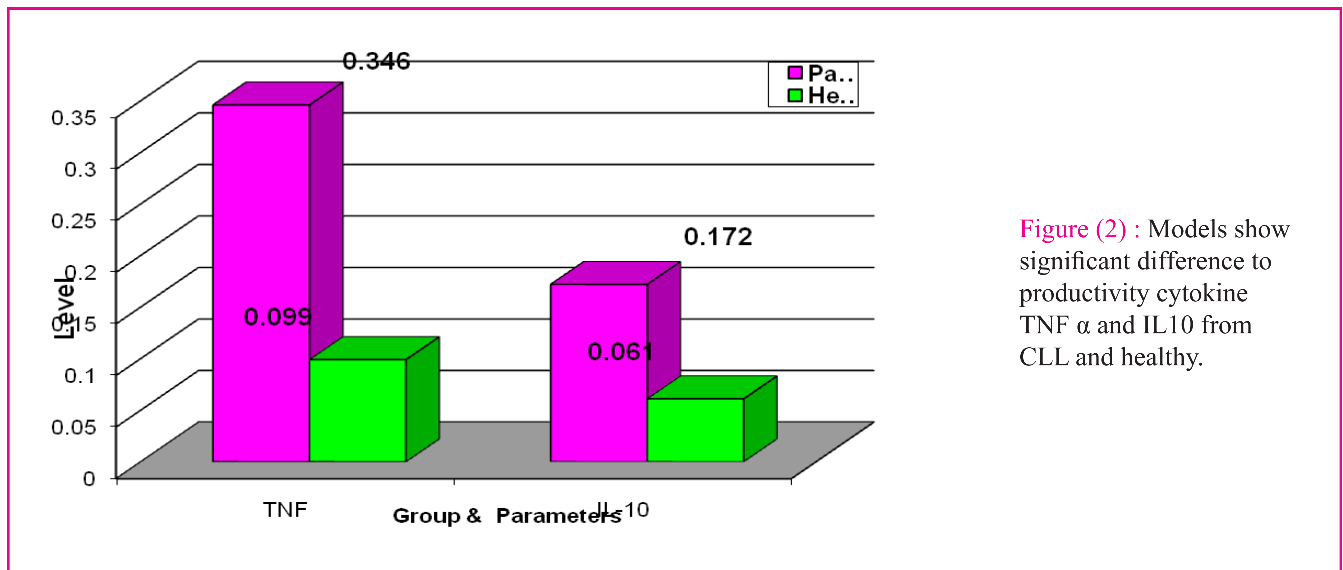

Figure (2) : Models show significant difference to productivity cytokine TNF α and IL10 from CLL and healthy.

This study appeared IL10 levels of CLL patient of Iraq and control groups were 0.172 ± 0.01 and 0.061 ± 0.02, respectively and significantly than control group P<0.05 shown Table (2) and Figure (2). However, serum IL8 level was no statistically significance difference in the measured average IL8 levels of the CLL patients compared with healthy 0.088 ± 0.01 and 0.106 ± 0.01 respectively shown table (1) and figure (1).

Discussions:

In another study, Pluda et al., (15) , referred to serum IL6 levels have been correlated with an increased risk for development of lymphoma in patients with acquired immunodeficiency syndrome (AIDS) and Obata et al., (16) showed that IL6 may enhance tumor growth by affecting the adhesion and migration of the ovarian cancer cells, and many of study, referred to investigators found that the IL6 levels relation to colorectal cancer (17 and 18), gastrointestinal cancer (19), pancreases cancer (20), esophageal cancer (21), and Chronic lymphocytic leukemia (22). Previous studies showed that IL6 is regulator of TNF α activity through enhances the cytotoxic effect of TNF α on human lymphoma cell (23). The TNF α level is correlated with increased tumor stage and expression of the chemokine receptor CXCR4 in biopsy specimens (24), and have important roles in the prognosis of breast cancer (25), and several studies showed that IL6 and TNF α levels increased from two fold to tenfold compared with the levels in control (26 and 27). Serum IL10 levels have been found to be important prognostic factors for Hodgkin lymphoma (28), and (29), referred to that IL10 is related to survival rate of cell lung cancer patients in African Americans, Fayad et al., (22) showed serum IL10 levels were higher in CLL patients. The conclusion, the IL6 , IL10, and TNF α levels of the CLL patients in our study were significantly higher than control healthy, however IL8 levels was not significant when compared with control.

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التعبير الخلوي من مرضى ابيضاض الدم اللمفي المزمن في بغداد / العراق

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مركز بحوث التقنيات الاحيائية / جامعة النهرين – بغداد – العراق

الخلاصة:

الهدف من الدراسة الحالية هو دراسة علاقة السايتوكينات الالتهابيه الاولية (انترلوكين 6، 8، 10، TNF α) مع مرضى ابيضاض الدم اللمفي المزمن. في هذه الدراسة، تم جمع عينات من مصل من (100) عينة، وممثل عن 80 عينات من ابيضاض الدم اللمفي المزمن المشخصين حديثا من قبل الطاقم الطبي استشاري في المركز الوطني للأمراض الدم و 20 عينة سليمة من ناحية أخرى. تمت دراسة IL-6، IL8، IL10 و TNF α وباستخدام انزيم مرتبط المناعي من الشركه (R and D / الولايات المتحدة الأمريكية) وقد أظهرت هذه الدراسة أن مستويات IL6 زادت بمقدار الضعف مقارنة مع الاصحاء وانتاجية TNF كانت ثلاثة أضعاف في المرضى CLL مقارنة مع الاصحاء و 3.5 اضعاف من انتاج IL10 مقارنه مع الاصحاء. الاستنتاج زياده ملحوضه في انتاج السايتوكسنات الالتهابيه الاولية 6 و 10 و TNF α في مرضى ابيضاض الدم اللمفي المزمن بالمقارنه مع الاصحاء ومع ذلك كان انتاج السايتوكينات 8 نفس انتاج الاصحاء.