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Assessment of IL-6 Serum Level in Patients With Acute Myeloid Leukemia

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Abstract :

Background: Interleukin-6(IL-6) is a pleiotropic cytokine that plays a major role in response to injury or infections and involved in immune response, inflammation and haematopoiesis. It is produced by a variety of cell types in response to variety of stimulants. Also deregulation impacts to numerous disease states, including many types of cancer. Elevated IL-6 levels have been observed in most types of cancer and it's implicated in the pathogenesis of several disease such as acute myeloid leukemia, multiple myeloma, lymphoma and it may be a prognostic factor for solid tumors, such as prostate cancer.
Objectives: Estimation of the serum levels of IL-6 in patients with AML in different stages of the disease and assess their prognostic value and study its relationship with bacteremia, total leukocyte count ,and hemoglobin concentration.
Materials and Methods: Forty patients of AML in different stages in National centre of hematology-Baghdad were studied immunologically for the detection of serum IL-6 by Enzyme Linked Immune Sorbent Assay(ELISA) kit. Specimen of blood collected from AML in relapse stage with episode of fever, were subjected to well known established microbiological methods for diagnosis and identification of isolates and data were compared with 20 healthy donors.
Results: Newly diagnosed and in relapse stage patients with AML had significantly higher serum levels of IL-6 compared with both control group and leukemic patients in remission stage $P<0.05$,in addition there was a positive correlation of IL-6 with the presence of fever due to bacterial infection .The clinical specimen result in(8) isolates, from which (6) were coagulase negative and (2) were staphylococcus aureus ,all isolates show high sensitivity to erythromycin and high resistant to chloromphenicol .Also IL-6 level correlated positively with total leukocyte count (TLC) and inversely with hemoglobin (Hb) concentration in AML at diagnostic and relapse stage.
Conclusion: Serum levels of Interleukin-6 can be used as prognostic serum markers at diagnosis of adults acute myeloid leukemia and it could be used as follow up parameters for early detection of relapse stage, in addition it seem to be a good markers to detect patients with bacteremia.

Keywords: *Acute myeloid leukemia (AML), interleukin – 6(IL-6).*

Introduction:

Interleukin-6 is a protein of 185 amino acid glycosylated at position 73 and 172. It is synthesized as a precursor protein of 212 amino acid. IL-6 belongs to a family of 10 cytokines, which all act via a receptor complexes containing the cytokine receptor subunit group 130[1,2].
Interleukin-6 exhibits functional pleiotropy and redundancy, induces a variety of responses from many cell types[3] .It's a potent and essential factor for the normal development and function of both T and B lymphocytes and has a broad actions on cells of hematopoietic system [4] .Also it's a central regulator of the acute phase response in the liver

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and regulates fever, in addition to its effects on the central nervous system by regulating glial cell activation[5]. Interleukin -6 is an important factor in many disease including, rheumatoid arthritis, crohn disease, inflammatory bowel disease, sepsis, fever, and cardiovascular disease [6,7,8].

A specific gene polymorphism in the IL-6 promoter region (-174 cc genotype) is associated with an increased risk of developing Alzheimer disease, particularly in women[9].

In cancer, high levels of circulating IL-6 are observed in almost every type of tumor studied and predict a poor outcome.

furthermore, elevated IL-6 levels are associated strongly with several of the striking phenotypic features of cancer[10,11].

In the setting of leukemias , IL-6 appears to have both stimulatory and suppressive effects. IL-6 levels are elevated in patients with AML both inhibitory and stimulatory effects on clonogenic blast growth [12,13].

Aim of this work:

The aim of this study was to determine the value of serum level of interleukin-6 in different stages of acute myeloid leukemic patients, to assess their prognostic effect ,and its relationship with bacteremia.

Subjects and Methods:

Subjects:

A comparative study was conducted from November 2010 to March 2011 on "60" subjects divided into two groups:

Group A: consisted of 40 patients with acute myeloid leukemia attending the National center of hematology in Baghdad.

They are :(groupA1) involved (10) patients diagnosed stage before starting therapy their mean age(47.00±17.13) rang(18-65); (groupA2) had (10) patients in remission but still under therapy, their mean age(37.20±12.25)rang(22-65); (groupA3)consisted of (10) patients in relapse their mean age(55.80±8.30)rang(42-63); and(groupA4)with (10) patients in relapse stage with bactermia their mean age(45.10±13.81)rang(29-65).

Group B: (control group) involved 20 healthy volunteers were used for comparison with AML patients with mean age(33.40±8.06)rang(23.0-45.0) table(1).

Table(1):Descriptive data of patients with Acut Myeloid Leukemia and control groups.

Cases age	AML Diagnostic stage		AML Remission stage		AML Relapse without bacteremia		AML Relapse with bacteremia		Control	
	No	%	No	%	No	%	No	%	No	%
Age (years) <30	2	20.0	2	20.0	-	-	2	20.0	7	35.0
30--39	1	10.0	5	50.0	-	-	2	20.0	9	45.0
40--49	2	20.0	2	20.0	2	20.0	2	20.0	4	20.0
50--59	-	-	-	-	2	20.0	2	20.0	-	-
=>60 years	5	50.0	1	10.0	6	60.0	2	20.0	-	-
Age Mean ±SD(Range)	47.00±17.13 (18.0-65.0)		37.20±12.25 (22.0-65.0)		55.80±8.30 (42.0-63.0)		45.10±13.81 (29.0-65.0)		33.40±8.06 (23.0-45.0)	
Sex Male e	5	50.0	5	50.0	4	40.0	4	40.0	10	50.0
Female	5	50.0	5	50.0	6	60.0	6	60.0	10	50.0

Methods:-

1- Serum samples were collected from all patients groups in two separate appendrof tubes and stored at -70 C which were thawed immediately before the estimation of IL-6 using ELISA kit
(serotech,UK.); direct type method.
The instructions of the procedure for the kit was followed, as it was recommended by the manufacturer.

2- Five ml of blood collected from (GroupA4) patients with episodes of fever aseptically and inoculated into (45) ml of brain heart infusion broth for 72h. at (37co) . The blood culture was inoculated on MacConkey and blood agar plates .The plates were incubated at (37) over night and examined on the following day for growth. All selected colonies examined microscobically using Gram stain than Analytic profile index (API) system was followed for dif

ferentiation detween the isolates and"Kirby-Baur" technique was applied for detection the susceptibility of the isolates to antimicrobial agent.The results of susceptibility of an isolates were compared to a standard zone of growth inhibition (table2).

Statistical analysis:-

Statistical analysis was performed using t. tests compare among patients and control group. P value of <0.05 was considered statistically significant.

Table(2): Interpretation of zone inhibition using Kirby and Bauer method (disc diffusion method.)

Antimicrobial agent	Code	Disc potency Mcg/Disc	Diameter of zone inhibition		
			Resistant	Intermediate	Sensitive
Ampicillin	AM	10	<=11	12-13	>=20
Cefotaxim	CTX	30	<=14	15-22	>=23
Cephalexin	KF	30	<=14	15-17	>=18
Chloramphenicol	C	30	<=12	13-17	>=18
Ciprofloxcin	C IP	10	<=15	16-20	>=21
Clindamycin	CN	2	<=12	13-17	>=18
Tobremycin	TM	10	<=13	13-14	>=15
Erythromycin	E	15	<=13	14-17	>=18
Ampiclox	AMP	30	<=14	15-16	>=17
Gentamycin	GN	10	<=12	13-14	>=15
Nalidixic acid	NAL	30	<=13	14-18	>=19
Pencillin-G	PG	6	<=20	21-28	>=29
Rifampicin	RA	5	<=16	17-19	>=20
Co-Trimoxazole	SXT	25	<=18	19-2	24-32
Amoxicillin	AMX	10	<=19	-	>=29
Amikacin	AN	30	<=14	15-16	>=17

Results:

This study showed that (group A1) the newly diagnosed patients have the following value of IL-6 (mean ±SD:18.85±0.54 Pg/ml); (group A3 ,A4) relapsed patients with and without bacteremia have (mean ±SD:17.83±0.90; 42.29±0.23 pg/ml)which show significantly high serum levels of IL-6 compared to both(group B) control group (mean ±SD:10.85±0.83 Pg/ml) and (group A2) patients in remission stage (mean ±SD:11.14±0.62 Pg/ml) (table3).

Also there was a significant different in serum levels of IL-6 between relapse patients with bacteremia and relapse patient without bacteremia and newly diagnosed patient but there was no significant different between newly diagnosed and relapse patients without bacteremia table (3).

The current study showed no significant different in serum levels of IL-6 ($p>0.05$) between AML patient in remission stage and control group table (3).

There was positive correlation between IL-6 serum levels and TLC in patients with AML (at diagnosis and relapse) but inverse correlation with HB concentration table(4).

Table(3):Serum levels of IL-6,TLC,and HB concentration in different studied groups of acut myeloid leukemia and control group.

Hematological Parameters Measurement	AML Diagnostic stage	AML Remission stage	AML Relapse without bacteremia	AML Relapse with bacteremia	Control
Total WBC Mean±SD (Range)	15.90±1.39^{A} (14.0-17.8)	6.76±1.93^{BC} (4.5-10.1)	15.29±1.81 (12.3-17.2)	14.74±1.79 (11.2-17.2)	5.53±1.07 (4.3-7.1)
Hb Mean±SD (Range)	8.90±1.34^{A} (7.2-11.0)	13.26±1.91^{BC} (10.1-15.5)	9.46±1.34 (6.8-11.0)	9.08±1.74 (6.3-11.0)	13.20±0.99 (11.7-15.1)
IL-6 (pg/ml) Mean±SD (Range)	18.85±0.54^{ACD} (18.0-19.6)	11.41±0.62^{BC} (10.0-12.2)	17.83±0.90^{CD} (16.2-19.6)	42.29±0.23^{D} (41.9-42.6)	10.85±0.83 (9.2-12.3)

A:Significant difference from AML Remission B:Significant difference from AML relapse without bacteremia; C:Significant difference from AML relapse with bacteremia D:Significant difference from Control (The mean difference is significant at the 0.05 level

Table(4):Correlation between IL-6 withTLC and HB concentration in patients with AML.

		IL-6 (pg/ml)				
		AML Diagnostic stage (n=10)	AML Remission stage (n=10)	AML Relapse without bacteremia (n=10)	AML Relapse with bacteremia (n=10)	Control (n=20)
Age (years)	Pearson Correlation (r)	0.274	-0.513	0.0001	-0.177	-0.281
	P value	0.443	0.130	0.999	0.625	0.230
Hb	Pearson Correlation (r)	-0.001	0.289	-0.385	-0.077	0.301
	P value	0.999	0.418	0.272	0.832	0.211
Total WBC	Pearson Correlation (r)	0.465	-0.027	0.409	0.642*	-0.146
	P value	0.176	0.941	0.241	0.046	0.538

**Correlation is significant at the 0.05 level (2-tailed).*

Blood samples of AML in relapse stage with bacteremia revealed (8) isolates, from which (6)isolates were coagulase negative while Staphylococcus aureus were two isolates. Antimicrobial susceptibility test show that S.aureus is highly sensitive to gentamycin(90%) and erythromycin(85%) but it is resistant to cefotaxim(40%),and chloromphenicol(40%) while it is intermediate to vancomycin (50%) and augmentin (55%) .

Coagulase negative Staphylococci showed highly sensitive to vancomycin,erythromycin, and cefotaxim but resistant to gentamycin .These isolates showed moderate sensitivity to chloromphenicol and augmentin (table 5).

Table(5):The susceptibility of the Bacterial isolates to antimicrobial agents in percentage

NO. of isolates Bacterial	GM	E	VA	AMC	CE	C
S.aureus (2)	90%	85%	50%	55%	40%	40%
S.epidermidis (4)	10%	100%	100%	50%	80%	40%
S.chromogenes (2)	20%	90%	85%	50%	90%	50%

GM= gentamycin, E=erythromycin ,VA=vancomycin , AMC= augmentin CE=cefotaxime , C=chloramphenicol .

Discussion:

A significant increase was observed in serum level of IL-6 concentrations detected in newly diagnosed and relapsed patients with AML (with bactermia and without), while in remission, serum IL-6 fall within the normal range when compared with control group (table3).It was reported that IL-6 is a pleiotropic cytokine with many effects including stimulating of acute phase, osteoclast activation, and it originates from a multitude of cell types, including monocular phagocytes, vascular endothelial cells, fibroblasts, hepatocytes, B-cells lymphomas and neoplastic plasma cells of multiple myelomas.

It appears to serve as a stimulatory factor in multiple myeloma, produce by both malignant cells and bone marrow stromal cells [14,15,16].

This study showed that their was increase in serum level of IL-6 in relapse AML with episodes of fever due to bacterial infections when compared with group(A1) and(A3) (table 3) .

Several studies [17,18] found close association of IL-6 to fever and provide that IL-6 an endogenous pyrogens and it's a pleiotropic cytokine involved in many aspects of the inflammatory response .Its produce by monocytes, fibroblasts, and endothelial cells in direct response to microbial stimuli [17].

Increase IL-6 levels in bacteremic patients mainly were the results of increased secretion by endothelial cells. Hemodynamic studies indicate that the basic pathogenic mechanism in sepsis is an increased vasopermeability caused by endothelium damage in combination with vasodilatation[18,19] .Also in patients with sepsis, activation of the complement system results in damage of endothelial cells, which contributes to the disturbances in the microcirculation. The injured endothelium in turn secretes IL-6 as an alarm hormone, it is released due to the synthesis of proteins in the liver (acute phase proteins) that protect the host against inflammatory reactions [17,18,19].

In the current study serum level of IL-6 in patients with AML (at diagnosis and in relapse)correlated inversely with HB% and positively with total leukocyte count TLC (table 4) and that was reported by several studies which demonstrated that serum level of IL-6 are a powerful prognostic factor in large cell lymphoma and chronic lymphocytic leukemia and diver effects on the growth of AML blasts, including stimulation and maintenance of their growth through the IL-6/IL-6 receptor signaling system [20,21,22]. A high IL-6 serum has been shown to be corrected with a poor response to chemotherapy[21] .

Therefore , apart from the predictive value expressed by several cytokines with regard to the duration of progression -free survival ,the evaluation of prognostic factors at the time of diagnosis may allow us to identify in advance a group of patients who will not benefit from first line treatment[14].

In conclusion, our study suggest that serum levels of IL-6 can be used as prognostic serum markers at diagnosis of AML and could be used as a follow up parameters for early detection of relapse and may help to differentiate diagnostic group of patients with bacteremia, so additional studies are necessary to confirm this findings.

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تقييم معدل الانترلوكين 6 في مصول مرضى Acute Myeloid Leukemia

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الخلاصة:

المقدمة: يلعب الانترلوكين 6 دور أساسي في الاستجابة للإصابات والعدوى وكذلك في الاستجابة المناعية والالتهابات وتكون أو تولد الدم .وينتج من قبل مختلف الخلايا نتيجة الاستجابة لمختلف المنبهات.

ارتفاع معدل الانترلوكين 6 لوحظ في معظم السرطانات وله تأثير في أمراضية أنواعا كثيرة منها AML, Multiple myeloma, lymphomaبالإضافة إلى انه قد يلعب دور كعامل منبه لانواعا أخرى مثل سرطان البروستات.

الهدف: تقدير معدلات الانترلوكين 6 في مصول المرضى AMLفي مراحله المختلفة من المرض وتقييم قيمته التنبوئية وكذلك دراسة علاقته مع ارتفاع الحرارة الناتج من تجرثم الدم ومع عدد كريات الدم البيضاء ونسبة الهيموكلوبين في الدم

طريقة البحث:

اخذت أربعون عينة من الدم من مرضى ألAML الوافدين إلى المركزالوطني لبحوث وعلاج امراض الدم في بغداد وفي مراحل مختلفة من المرض, وقد تم قياس معدل الانترلوكين6 باستخدام تقنية أل ELISA, وكذلك أخذت عينات من دم مرضى ألAMLفي مرحلة الانتكاس والذين يعانون من ارتفاع درجة الحرارة لاحتمال إصابتهم بتجرثم الدم حيث تم تشخيص مكونات دمهم الجرثومية بالطرق العلمية المعروفة وقد قورنت نتائج المصول للمرضى بعشرين عينة من مصول الأصحاء.

النتائج: أظهرت النتائج وجود زيادة معنوية في الانترلوكين6 للمرضى في مرحلة التشخيص والانتكاس وكذلك هنالك علاقة مع ارتفاع درجة الحرارة نتيجة تجرثم الدم.تم الحصول على(8) عزلات بكتيرية مشخصة منها(6)عزلات سالبة لصبغة غرام و(2)عزلات للعنقودية الذهبية وكل العزلات اظهرت حساسية شديدة للارثرومايسين ومقاومة عالية للكلورومفينيكول .ايضا وجدت علاقة متبادلة طردية بين معدل الانترلوكين6 وعدد كريات الدم البيضاء في حين تكون علاقة عكسية مع نسبة الهيموغلوبين للمرضى الذين هم في مرحلة التشخيص والانتكاس.

الاستنتاجات: ان المعدلات المصلية للانترلوكين6 ممكن أن تعتبر علامة تنبؤية في مصول البالغين لمرضى AML في مرحلة التشخيص وكذلك ممكن ان تعتبر مؤشرتتبعي للكشف المبكر عن مرحلة الانتكاس بالإضافة إلى انها تبدو كمؤشر جيد للكشف عن تجرثم الدم.