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المجلة العراقية لأمراض الدم

مجلـــة علميـــة محكمـــة تصـــدر مرتيـــن في السنـــة عـــــن المركز الوطني لبحوث وعلاج امراض الدم- الجامعة المستنصرية- بغداد- العراق

تشرين الثاني 2014 المجلد 3 العدد ١

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Title pages

Instructions to Authors

The Iraqi Journal of Hematology is a periodic peer-reviewed journal published biannually by the National Center of Hematology with the cooperation of the Iraqi Society of Hematology. The journal welcomes original articles, case reports and letters to editor in all fields relevant to Hematology. Review articles are usually by invitation only. However, review articles of high standards will be considered. Arabic or English languages could be used.

Papers are accepted on the understanding that the subject matter has not and will not be submitted simultaneously to another journal. The following notes are expected to be considered carefully in writing manuscripts.

1- Manuscripts preparation: the format of the Iraqi Journal of Hematology complies with the by the International Committee of Medical Journal Editors (ICMJE) in

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2- Four clear and complete copies (including figures and tables) should be submitted. Manuscripts and figures will not be returned to the authors irrespective of the editorial decision to accept, revise or reject them.

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- **Correct first name, middle name and family name of all authors in Arabic and English as well as a maximum of two highest academic degrees for each author.**
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5- Abstracts for original articles should contain a structured abstract of no more than 250 words in Arabic and English, Abstract headings include: Background, Objectives, Methods, Results and Conclusions.

Abstracts in English of the review articles and case reports should be unstructured and of not more than 150 words.

6- The main text of the original article should be divided into sections; each section should be started on a new page after the title page:

A. Introduction: should state clearly the purpose and rationale of the study.

B. Methods: should include selection of subjects, identifications of the methods, apparatus and chemicals used and include statistical analysis.

C. Results: presented in a logical sequence preferably with tables and illustrations emphasizing in the text only the important observations.

D. Discussion: emphasizes new findings of the study, implications and reference to other relevant studies.

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F. References: should be in the Vancouver style. They should appear in the text by numbers in the order. List all authors when six or less; when seven or more, list only first six and add et al. Journal titles should be abbreviated in accordance with index Medicus. Examples

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Book chapter: Pen AS. Immunological features of myasthenia gravis. In:Aguayo AJ, Karapti G, editors. Topics in Nerves and Muscle Research. 31st ed. Amsterdam: Experta Medica; 1975; p.123-32.

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8- Measurement is preferably expressed in SI units.

9- Use only standard abbreviations in the title and abstract. The full term for which the abbreviations stand should precede its first use in the text.

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Expression of Aberrant Antigens CD7 and CD19 in Adult Acute Myeloid Leukemia by Flow Cytometry.

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ABSTRACT

Background: Flow cytometric immunophenotyping (FCI) is an indispensable tool for quantitative and qualitative evaluation of antigen expression of hematopoietic cells. From a diagnostic and therapeutic point of view, distinction between acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) and for diagnosis and definition

of particular AML subtypes. Aberrant antigen expression (i.e. expression of an antigen which is inappropriate for a lineage) is rarely seen in normal bone marrow cells and their incidence is varied between various studies.

Objectives : To explore the existence of the aberrant lymphoid antigens (CD7) and (CD19) in adult acute myeloid leukemia patients and their distribution among French-American-British (FAB) classification subtypes and correlate them with hematological parameters including red cell count (RBC) , hemoglobin, white cell count (WBC) , platelets count, blast cell percent and hematological response to the induction chemotherapy.

Patients and Methods: EDTA anticoagulated peripheral blood (PB) sample of 2.5 ml and /or bone marrow (BM) aspirate samples of 0.5 ml from 25 cases of de novo AML including 14 male and 11 female with mean of age of 37.56±18.07 were included from June to November 2012.Hematological parameters including RBC, hemoglobin, WBC, platelets count, blast cell percent were obtained from the case file of the patients where they were done by automated device (Cell-DYN, RUBY list) and diagnosed by cytomorphology by Leishman stain and cytochemistry by Sudan Black B and PAS stain and AML cases were classified according to the FAB criteria. Aberrant lymphoid antigens, (CD7) and (CD19) expression was explored by CyFlow© multiparametric flow cytometry at diagnosis. The patients were evaluated after three weeks of one cycle of chemotherapy for complete remission.

Results: CD7, CD19 and co-expression of CD7 and CD 19 were expressed in 40%, 16% and 12% of AML patients respectively. Statistically significant associations were found between aberrant CD7 expressions and age, low WBC, and early FAB AML (M1, M2) subtypes. Complete remission was achieved in 19 out of 25 patients (76%) with standard chemotherapy whereas six patients did not achieved complete remission; three of them had aberrant CD7 expression (two of them died during induction therapy) and the other one had poor response to induction therapy.CD7 was detected in 7 patients; 6 of them were male. There is no statistically significant association between aberrant CD7 expression and hepatosplenomegaly and treatment response to one cycle of chemotherapy (P value > 0.05).Also there is no relation between CD7 expression and percent of blast cells.

Conclusions:

1. The incidence of aberrant expression of CD7, CD19 and both CD7, and CD19 were 40%, 16%, and12% respectively.
2. CD7 was detected mainly in males whereas CD19 was distributed among males and females.
3. Total WBC count and malignant cell percent were lower in patients harboring aberrant expression compare to those without aberrant expression.
4. CD7 was mainly detected in early FAB classification (M1 and M2).
5. Six out of twenty five AML patients had no response to standard therapeutic regimen; three of them were harboring CD7 and no one had CD19 alone, thus we may propose that CD7 was associated with poor response to induction therapy.

Introduction

Acute myeloid leukemia (AML) represents a group of hematopoietic neoplasms derived from the bone marrow precursors of myeloid lineage. The neoplastic process is the result of clonal proliferation of an aberrant, committed stem cell at the level of CFU-S or later stages of differentiation leading to the accumulation of immature forms without, or with limited, maturation [1].

Flowcytometric immunophenotyping (FCI) had become an indispensable tool for quantitative and qualitative evaluation of antigen expression of hematopoietic cells. From a diagnostic and therapeutic point of view, distinction between AML and acute lymphoblastic leukemia (ALL) is extremely important and Flow Cytometry (FCM) is very instrumental in this matter [2-4].

Neoplastic cells frequently show nonrandom expression of antigens in a manner that deviates from the tightly regulated patterns of antigen expression seen in normal maturation. This is the basic principle that allows for the detection of hematopoietic neoplasia by immunophenotyping [5].

Abnormal antigenic expression in acute leukemia can be grouped into four basic categories [2, 5]:

- Abnormally increased or decreased levels of expression (intensities) of antigens normally expressed by cell type or lineage at a particular stage of maturation, including the complete loss of normal antigens in some instances.
- Asynchronous antigen expression (deviations of the normal differentiation and maturation pathway; i.e., expression of antigens normally expressed by the cell type or lineage but at an inappropriate time during maturation).
- Abnormally homogeneous expression of one or more antigens by a population that normally exhibits more heterogeneous expression.
- Gain of antigens not normally expressed by cell type or lineage (include expression on myeloid blasts of markers usually not present on cells of that particular lineage, e.g. lymphoid markers, such as CD7, CD19 and CD56).

Occurrence of these aberrant phenotype has been reported in both ALL and AML with varying frequencies. In AML, aberrant lymphocyte phenotype in AML (Ly+ AML) has been reported in up to 48% cases. The most frequent lymphoid antigens in AML that have been reported include, CD7 (T-cell marker) and CD19 (B-cell marker) [6].

Immunophenotypic aberrancies also have been explored to predict treatment outcome in AML as they are useful for MRD detection and quantification with the aim of providing prognostic information [2, 3].

Materials and Methods

2.1 Patients:

This cohort study was conducted on twenty five adult AML patients, including 14 male and 11 female, their mean age was 37.56 ± 18.07(mean ±

SD) and 14 of them were male and 11 were female from June to November 2012.

Those patients were admitted to the Hematology Department of Baghdad Teaching Hospital. The Patients 'peripheral blood (PB) and bone marrow aspiration (BMA) samples and their staining's were analyzed in the teaching laboratories of the Medical City in Baghdad. Flowcytometry was done at Al-Rawabi Private Laboratory in Baghdad.

For each patient a questionnaire form was done, hematological parameters including hemoglobin (HB) , packed cell volume (PCV) , WBC count, and blast cells per cent and platelet count were obtained from the case file of the patients where they were done by automated device (Cell-DYN, RUBY list).

2.2 Inclusion Criteria

Criteria for the inclusion of the patients:

1. The patients were randomly collected in relation to sex
2. All AML patients were above 15 years old.
3. All AML patients were newly diagnosed, de novo and they were not receiving any chemotherapy before the time of collecting blood samples and secondary AML cases were excluded from the study.
4. Patients with AML were classified according to the FAB classification criteria (from M0 to M7) are usually diagnosed by cytomorphology [7, 8]. However, cases of AML FAB M0 and M7 categories were diagnosed by Flowcytometry in the Central Medical Laboratories in Sulymania City.

2.3 Therapy and Follow-up

All patients were evaluated for complete remission achievement three weeks after one cycle of chemotherapy. Complete remission (CR) was defined by Cheson et al (< 5% bone marrow blast cells of normal cellularity and restoration of normal peripheral blood values of at least 1500/µL neutrophils and 100,000/µL platelets) [9].

For remission induction , all patients received therapeutic regimens routinely used for AML (i.e. the standard protocol of '3+7' of Doxorubicin 30 mg/m^2 on days 1-3 or daunorubicin 60 mg/m^2 + Cytosine Arabinoside 100 mg/m^2 on days 1-7) . AML-M3 patients received different regimen (ATRA 45 mg/m^2 daily until complete remission plus Doxorubicin 30 mg/m2 for 4 doses) [10].

For all patients with AML, PB and BM aspirate samples were repeated 2 weeks after completion of chemotherapy to assess response or CR, for AML M3 patients bone marrow repeated only after recovery of hematological parameters [11].

Two patients experience early death during early therapy induction.

2.4 Laboratory Tests

2.4.1 Blood sampling

A total venous blood sample of 2.5 ml and /or bone marrow aspirate sample of 0.5 ml were obtained from each patient included in this study by venipuncture from antecubital fossa or bone marrow aspirate from posterior superior iliac crest under aseptic technique respectively, and the samples were collected in EDTA tubes.

Blood sample from suspected patient was examined for complete blood indices in the teaching laboratory department of the Medical City, a blood film was made by taking a drop of blood sample spreads it on a clean dry slide, and staining it by Leishmen, Sudan black B and PAS stain; the slides were examined by a specialist in the teaching laboratory department of Medical City.

Accordingly sample of peripheral blood or bone marrow aspirate were obtained from AML confirmed cases (by FAB cytomorphology or Flowcytometry) for flowcytometry study to investigate the expression of aberrant surface marker antigens CD7, CD19 (both or one of them).

Three weeks after the patients had received induction chemotherapy PB samples was withdrawn from the patients to assess the patients' response to therapy by cytomorphology and complete blood count, for AML M3 patients bone marrow repeated only after recovery of hematological parameters [11].

2.4.2 Staining:

2.5 Flowcytometry Immunophenotyping

In this study immunophenotyping for aberrant lymphoid antigens CD7, CD19 were investigated in those AML patients by using four –color Cyflow® Cube 6 flow cytometry device (PartecCyflow®, Germany) in AL Rawabi private laboratory.

2.5.1 Reagents and Assay Procedure

Cy Lyse® stands for an erythrocyte lysing reagent kit with a complete preservation of the surface proteins and particularly no loss of cells.

2.5.2 Determination of the Aberrant Phenotype

Identification of blast cells was performed using forward scatter (FSC) versus side scatter (SSC) parameters. Antigen expression was considered to be positive when the percentage of positive blast cells was equal or greater than 20%. Similarly, aberrant phenotypes were defined when at least 20% of the blast cells expressed that particular phenotype [12].

(**Figures1-3**)

2.6 Statistical Analysis

Statistical analysis was carried out using SPSS version 18. Categorical variables were presented as frequencies and percentages. Continuous variables were presented as means with their 95% confidence interval (CI).

Independent (t-test) was used to find mean differences between two variables. One way analysis of variance (ANOVA) was used to find the mean differences among more than two variables. Pearson's chi square (X2) test was used to find the association between dependent and independent

variables. A p-value of < 0.05 was considered as statistically significant.

Results

This study included 25 adult patients with de Novo acute myeloid leukemia diagnosed cytomorphologically by Leishmen stain and cytochemichally by Sudan black B and PAS stain on peripheral blood (PB) and bone marrow (BM) aspirate smears.

Immunophenotyping was done by Flowcytometry to detect the aberrant expression of CD7 and CD19 in adult acute myeloid leukaemia patients.

3.1. Clinical parameters

3.1.1 Age Groups

The mean age of all patients included in this study was 37± 18.07* SD, with a median of 33 years old and a range of 15 to 70 years old. Figure 4 Showed that more than half of AML patients (52%) were in the age group of 21-40 years.

3.1.2 Gender

Regarding the gender of patients; AML was observed more in male (14 male (56%)) than female (11 female (44%)) with a male to female ratio of 1.3:1.

Regarding the relation of gender to the age groups; the per cent of male patients in the most common age group (21-40 years) (57.1%) was more than the percent of female (45.4%) **table 1**.

3.1.3 Clinical Features

Clinical features of all AML patients included in the study were shown in Figure 2 which revealed that the two most frequent signs and symptoms for all patients included in this study were easy fatigability and fever, followed by hepatosplenomegaly whereas lymphadenopathy and nausea with vomiting were the least frequent.

3.1.4 Distribution of AML Subtypes according to FAB Classification

In current study 7 out of 25 patients were of M1 sub type, 5 patients were of M2 subtype, 3 patients were of M5, M3 and M0, and 2 patients were of M4 and 1 patient for each M6, M7. Most patients with aberrant antigens expression were of M1 FAB classification. Figure 6

3.2. Aberrant Antigen Expression

The AML patients in this study were divided into two groups according to the presence or absence of aberrant expression of CD7 and CD19 marker by Flowcytometry.

The first group composed of 11 patients who present with aberrant expression. The second group composed of 14 patients with negative expression.

Figure 6 showed that the CD7 was the most predominant aberrant marker, 7 out of 11 patients (63.6%) had only CD7 marker and 3 out of 11 patients (27.3%) had CD7 with CD19; Thus 10 out of 11 patients (90.9%) expressed CD7.CD19 was expressed as a sole marker in one out of 11patients (9%) and 3 patients having CD19 with CD7.Thus the total expression of CD19 was in 4 cases

3.2.1Relationship between the aberrant antigens expression and the Clinical parameters.

Age

The first group compose of 11 patients who present with aberrant antigen expression had mean age of 33.45 ±17.16 years (mean ± SD), seven of them who had CD7 had mean age of 35.85 ±12.18 years (mean ± SD). The age of one patient with CD19 was 67 years and the three patients with both CD7 and CD19 had mean age of 16.66 ± 4.72 years (mean ± SD).

The second group composed of 14 patients who had no aberrant antigen expression and their mean age of 40.78 ± 18.72 years (mean ± SD). **Table 2** showed that there was a significant association between age and aberrant CD7, CD19 expression (P value < 0.05).

Gender

Table 3 showed that there was no significant association between gender and aberrant CD7 and CD19 expression (P value > 0.05). However 6 out of 7 patients with CD7 + were male.

Hepatosplenomegaly

Table 4 showed that there was no significant association between hepatosplenomegaly and aberrant CD7 and CD19 expression (P value > 0.05)

3.2.2. Relationship between the aberrant antigens expression and the haematological parameters.

Table 5 showed that the mean WBC count and the malignant cells percent of patients without aberrant expression were significantly higher than those of patients with aberrant expression (p value < 0.05). Table 3.6 showed that there was a significant relationship between mean of WBC count and the type of aberrant marker expression, so that the lowest mean WBC count was detected in patients with CD 7+, whereas the highest WBC count was detected in those harbouring both markers, however there was no significant relationship with other haematological parameters i.e. malignant cell percent, RBC, HB and platelet count .Table 3.6.

3.2.3 Distribution of Aberrant expression of CD7 and CD19 markers according to FAB classification

By relating the FAB classification to the expression of aberrant markers 4 out of 10 patients of CD7+ were M2, 3 out of 10 were M1, 2 out of 10 were M5 The 4 patients with and 1 out of 10 was M3. CD19+ distributed equally on M0, M1, M2 and M5 subtypes. The 3 patients harbouring CD7 and CD19 present in M1, M2 and M5. Table 7

3.2.4. The Distribution of Aberrant Groups in Relation to FAB Classification

CD7 was detected in the 4 patients with M2 subtype; 3 of them showed CD7 alone and one patient showed CD7 with CD19. CD7 was detected in 3 patients with M1 subtype; 2 of them showed CD7 alone and one showed CD7 with CD19. In M5 sub type two patients had CD7 and one of them had CD19 also. Only one patient with M0 subtype showed CD19 expression.

3.2.6. The relation of expression of aberrant marker to AML induction therapy response.

Complete remission after medical therapy was achieved in 19 patients (76%) with standard chemotherapy. Six patients had no response to standard therapeutic regimen. Figure 3.5 showed that 3 out of 11 with the aberrant expression did not respond to treatment (two patients with CD7 had passed early in study during receiving induction therapy and they were included with the patients who have no response) and 3 out of 14 patients with no aberrant expression have no response; there was no significant relationship between response and not response to treatment and the expression of aberrant Antigen.

Figure 6 Show the distribution of aberrant antigens expression according to responsive and non-responsive to treatment after 3 weeks at the end of induction chemotherapy. 8 patients with aberrant antigen had responded to treatment induction therapy. 5 of them had CD7, one patient had CD19 and 2 patients had both CD7 and CD19. Three patients who had aberrant expression had fail to respond to induction therapy; of them 2 patients had CD7 and one patient had both CD7 and CD19. There was no statistically significant association between aberrant antigen expression and treatment response to one cycle of chemotherapy (P value>0.05).

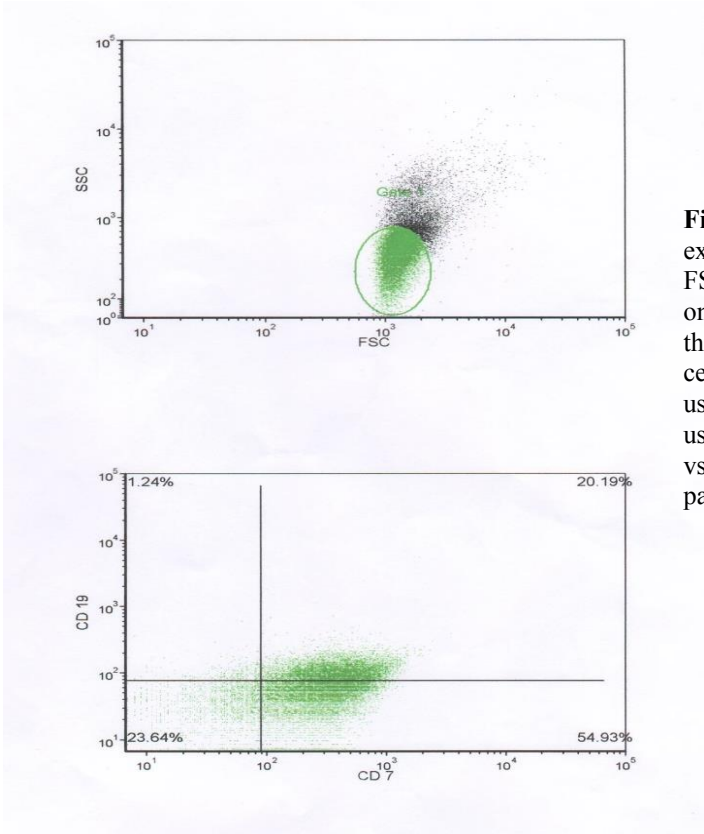


Figure 1: Showed patient with aberrant co-expression of CD7and CD19. The FSC vs SSC plot was used to gate on the blast cell population. This is the older technique to gate on blast cells. The newer technique is by using CD45 vs SSC. However, using the older technique of FSC vs SSC is still useful especially in patients with high blast cell percentage.

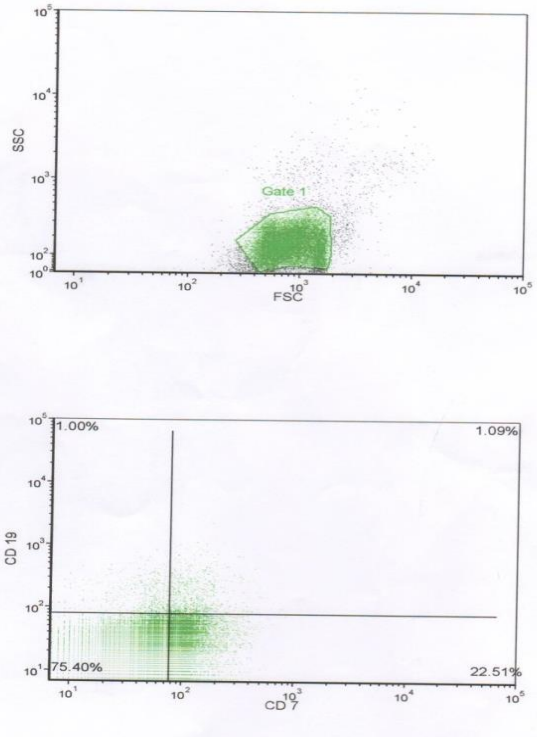


Figure 2 Showed patient with aberrant expression of CD7

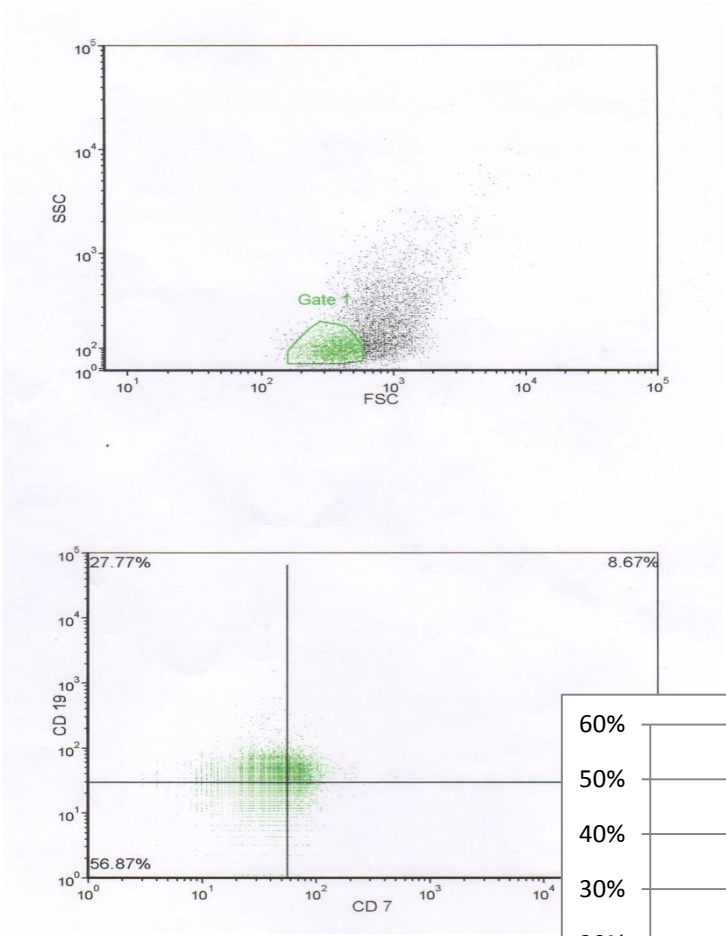
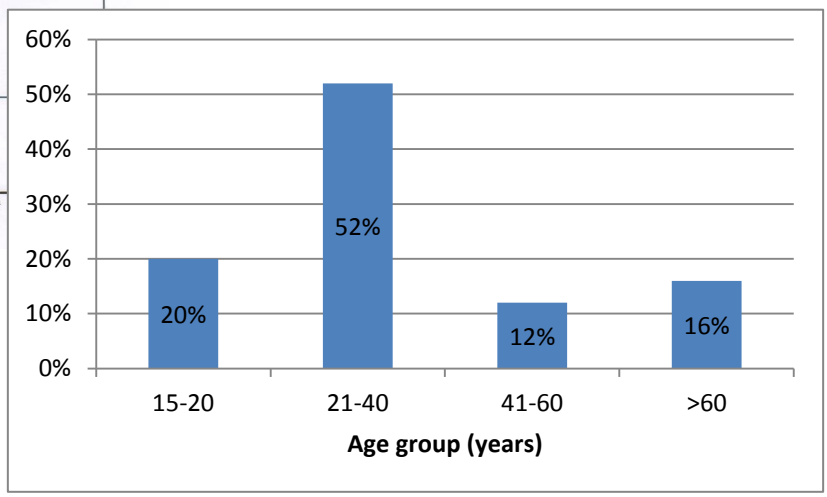


Figure 3 Showed patient with aberrant expression of CD19



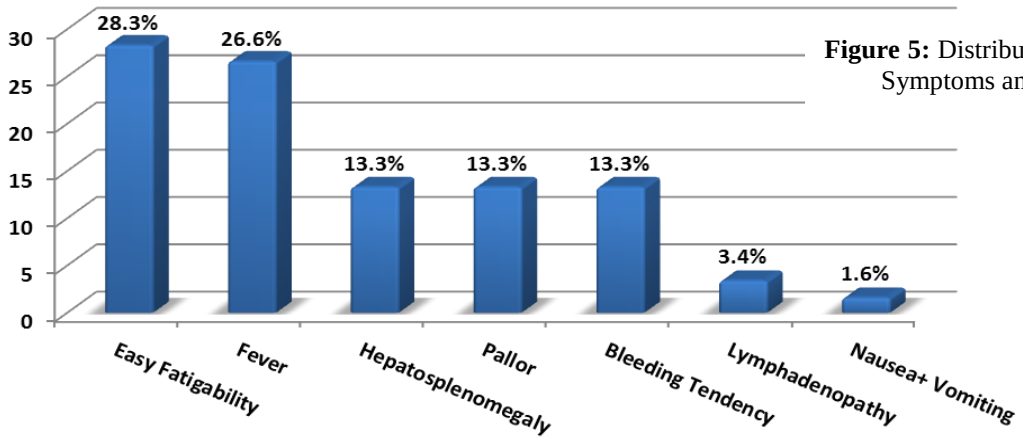


Figure 5: Distribution of Patients according to Symptoms and Physical Signs.

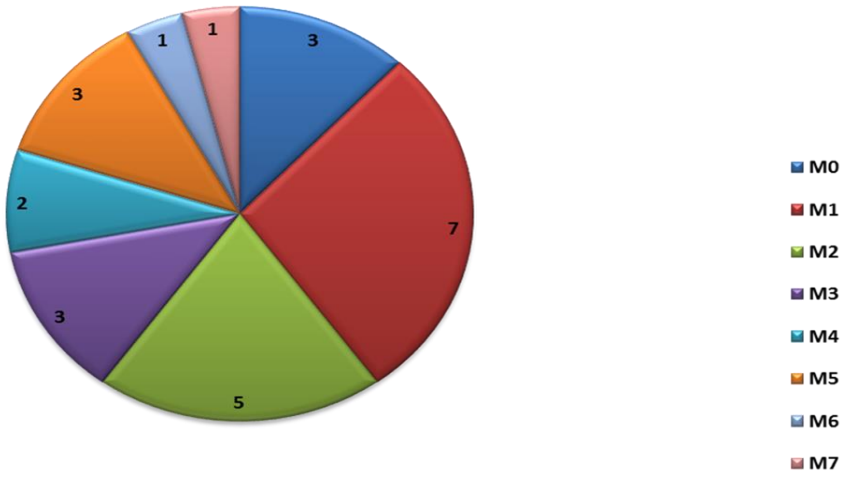


Figure 6: Distribution of patients according to FAB classification.

Note: all numerical values in this figure mean number of patients.

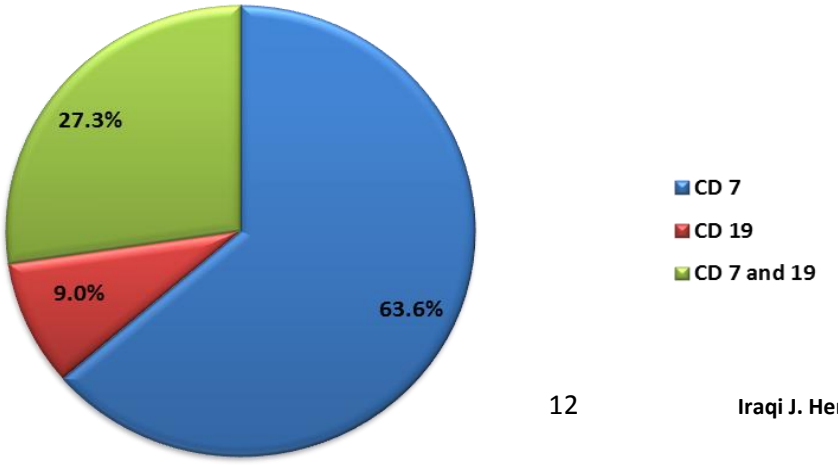


Figure 7: The Percentage of Each Group of Aberrancy from the 11 AML patients having aberrant antigen expression.

Note: all numerical values in figure 3.4 mean percent of patients.

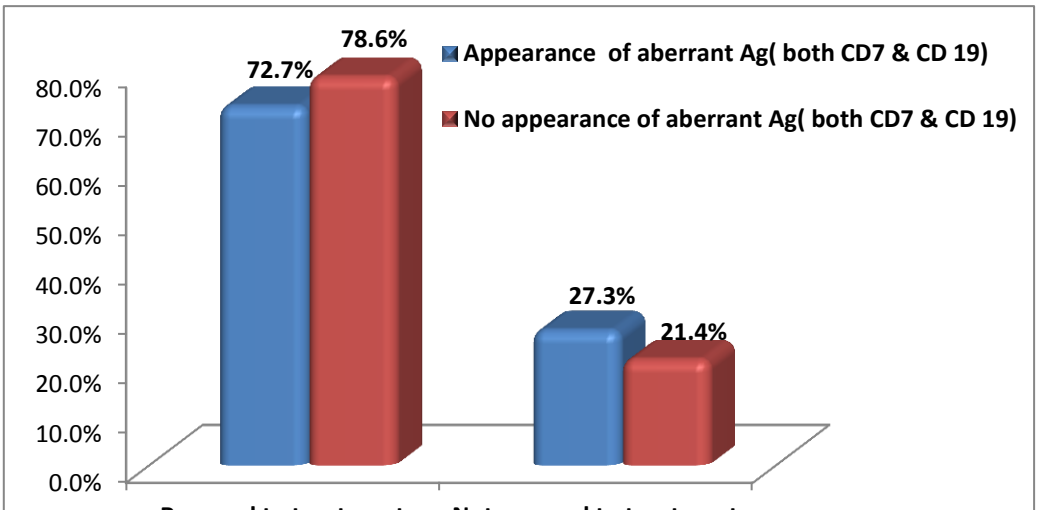


Figure 8: Distribution of aberrant antigens expression of CD7 and CD19 in relation to response and non-response to treatment after 3 weeks of end of induction chemotherapy.

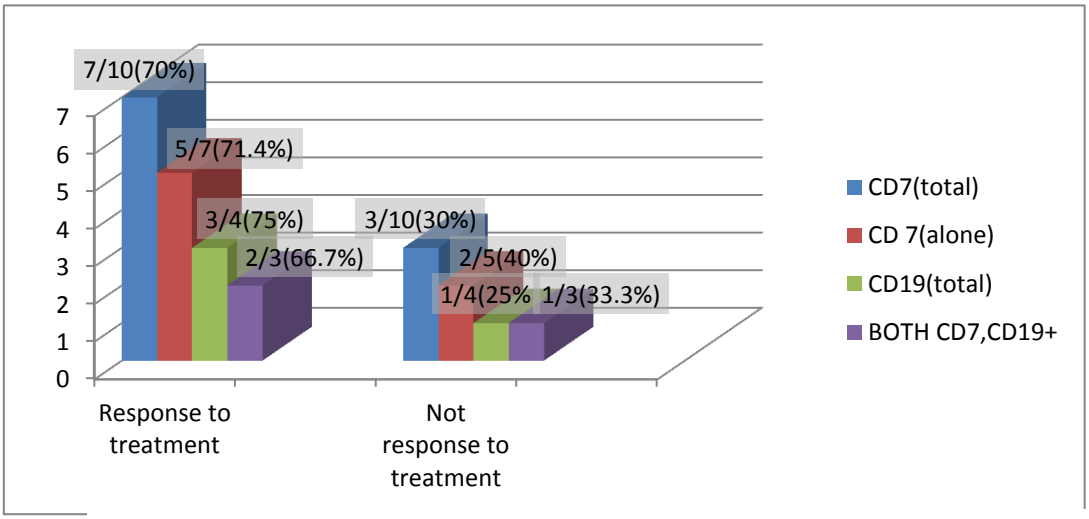


Figure 9: The Distribution of type Aberrant Expression Antigens in Relation to the Responsive and Non Responsive Patients.

Table 1: The Frequency of Gender in Relation to Age Group

Age groups	Gender		Total
	Male	Female	
15- 20 years	1 (7.1%)	4 (36.4%)	5 (20.0%)
21-40 years	8 (57.1%)	5 (45.5%)	13(52.0%)
41-60 years	2 (14.3%)	1 (9.1%)	3 (12.0%)

> 60 years	3 (21.4%)	1 (9.1%)	4 (16.0%)
Total	**14 (100.0%)**	**11 (100.0%)**	**25 (100.0%)**

Table 2: Relationship between age and aberrant antigen expression

Variable	CD marker	No.	Median (year)	Mean (year)	S.D	F test	P value
Age	CD7	7	32	35.85	12.18	8.599	**0.01***
	CD 19	1	67	67.00	------		
	CD 7, 19	3	15	16.66	4.72		
	Total of positive	11	27	33.45	17.16	2.13	0.34
	Not aberrant Ag	14	40	40.78	18.72		

Table 3: Correlation between gender and aberrant antigen expression

Gender		CD EXPRESSION			Total	Not aberrant
		CD 7	CD 19	CD 7, 19		
male	Count	6	1	1	8	6
	% of Total	54.5%	9.1%	9.1%	72.7%	42.9%
female	Count	1	0	2	3	8
	% of Total	9.1%	.0%	18.2%	27.3%	57.1%
Total	Count	7	1	3	11	14
	% of Total	63.6%	9.1%	27.3%	100.0%	100%

Table 4 Correlation between Hepatosplenomegaly and Aberrant Antigen Expression

HEPATOSPLENOMEGALY		CD expression			Total	Not aberrant Ag
		CD 7	CD 19	CD 7, 19		
Splenomegaly	Count	2	1	0	3	3
	% of Total	18.2%	9.1%	.0%	27.3%	21.4%
Hepatomegaly	Count	5	0	3	8	4
	% of Total	45.5%	.0%	27.3%	72.7%	28.6%
No hepatosplenomegaly	Count	0	0	0	0	7
	% of Total	0%	0%	0%	0%	50%
Total	Count	7	1	3	11	14
	% of Total	63.6%	9.1%	27.3%	100.0%	

Table 5 the Relation of Haematological Parameters to the Aberrant Expression

Variable	CD	N	Mean	S.D	t-test	P value
RBC×10^{12}	Negative	14	3.32	0.99	0.201	0.843
	Positive	11	3.40	0.82		
Hb g/dl	Negative	14	8.06	2.61	0.408	0.687
	Positive	11	7.68	1.87		
WBC×10^{9}	Negative	14	31.9	11.2	6.68	**0.002***
	Positive	11	7.8	4.6		

Malignant cells%	Negative	14	67.4	8.4	10.7	**0.001***
	Positive	11	34.2	6.7		
Platelet×10^9	Negative	14	67.98	33.48	0.135	0.894
	Positive	11	66.37	23.27		

Table 6 The Relation of The Three Groups of Aberrancy to Haematological Parameters (at presentation)**.**

Variable	CD marker	No.	Mean	S.D	F test (ANOVA)	P value
Hb g/dl	CD7	7	8.13	2.14	0.508	0.620
	CD 19	1	7.00	-----		
	CD 7, 19	3	6.85	1.34		
**WBC count×10^9	CD7 total	10	8.48	3.1	14.86	0.01*
	CD7 alone	7	6.8	3.9		
	CD19 total	4	9.6	1.3		
	CD19 alone	1	1.3	-----		
	BothCD7, 19+	3	12.4	1.4		
Malignant cells%	CD7 total	10	34.50	7	5.5	0.989
	CD7 alone	7	34.57	8		
	CD19 total	4	33.50	6		
	CD19alone	1	31	------		
	Both CD 7, 19	3	34.33	7		
Platelet count×10^9/l	CD7	7	61.87	11.66	1.106	0.377
	CD 19	1	50.00	------		
	CD 7, 19	3	82.33	41.40		

** There was a significant relation between the 3 parameters (CD7 alone, CD19 alone and both CD7, CD 19) with mean of WBC count.

Table 7 The Incidence of aberrant expression of markers according to FAB classification.

FAB Category	N. of AML Patients	CD7		CD19		Both CD7 andCD19	
		No.	Incidence %	N	Incidence %	N	Incidence %
M_0	3	-	0	1	33.3	-	0
M_1	7	3	42.8	1	14.2	1	14.2
M_2	5	4	80	1	20	1	20
M_3	3	1	33.3	-	0	-	0
M_4	2	-	0	-	0	-	0
M_5	3	2	66.6	1	33.3	1	33.3
M_6	1	-	0	-	0	-	0
M_7	1	-	0	-	0	-	0
Total	25	10	40	4	16	3	12

Table 8 Incidence of CD7 in various studies

Study	Incidence of CD7 Positivity
El-Sissy et al (Saudi study)	11.8%
Bahia et al (Brazilian study)	25.7%
Kita et al (Japanese study	19%
Julius et al (USA study)	32.6%
Chang et al (Canadian study)	37%

Khurram et al (Pakistani study)	37.03%
Auewarakul et al (Thailand study)	27%

Table 9 Incidence of CD19 in various studies.

Study	Incidence of CD19 Positivity
El-Sissy et al (Saudi study)	11.8%
Bahia et al (Brazilian study)	8.6%
Chang et al , 2004 (Canadian study)	5%
Khurram et al (Pakistani study)	11.1%
Auewarakul et al (Thailand study)	4%

Discussion

4.1. Age and Gender:

The mean age of all patients included in this study was 37 ± 18.07, with a median of 33 year old and range of 15-70 years, which was in agreement with Iraqi studies [13-16], Iranian study [17] and Saudi study [18].

In this study 56% of the adult AML patients were male with a male to female ratio1.3 :1 which was in accordance with that reported by the Iraqi ministry of health [19] and other Iraqi studies [16, 18,20] as well as other studies worldwide [17,21,22] .

4.2. Clinical Presentation:

Easy fatigability and fever were the most common presenting symptoms in adult AML patients; 28.3% and 26.6% respectively. The least symptoms were nausea and vomiting, 1.6% for both. Whereas, the most common physical findings were hepatosplenomegaly, pallor and bleeding tendency with frequency of 13.3% for all. The least finding was lymphadenopathy, 3.4%. These results were in agreement with different Iraqi studies [56, 58, 62, and 63] and were similar to the data reported abroad [23].

4.3. Aberrant CD7 and CD19 markers expression:

In this study aberrant expression of lymphoid associated antigens CD7 and CD19 were studied for their biological and clinical significance with comparison to previous studies.

Some studies have shown significant correlation between clinical and laboratory characteristics and aberrant lymphoid antigen expression such as CD7, CD19 and CD56. Most of these studies concerned with the prognostic significance of aberrant CD7 expression.

4.3.1Association with Age, Gender and Hepatosplenomegaly:

Regarding the age, patients with $CD7^+$ and $CD19^+$ AML were younger (mean age 33.45±17.16 SD) than those without aberrant expression (mean age 40.78±18.72 SD). This observation was similar to the studies of Chang et al, [24], Kita et al [25] and Khurram et al [26] but inconsistent with the studies of Saxana et al [27] and Del Poeta et al [28] in which no restricted age distribution was found.

Regarding gender, CD7 was detected mostly in male; however this relation did not reach the level of significance; which was in agreement with Saxana et al [27] and Chang et al [24] and Khurram et al [26] study, but inconsistent with the study of Kita et al [25].

Regarding hepatosplenomegaly, 7 out of 14 patients without aberrant antigens expression showed hepatosplenomegaly. While all the patients with aberrant antigens expression showed hepatosplenomegaly which was in agreement with Saxsana et al al [27] and Chang et al [24] study, but inconsistent with the study of Kita et al[25].

4.3.2Association with Hematological Parameters:

In the current study, the percent of blast cells and WBC count in patients harboring aberrant markers were significantly lower than those without aberrant expression, and the lowest WBC count was detected in those having CD7 and higher count in those having both CD7 and CD19. Most of the studies concerning CD7 and CD19 expression in AML showed significant high WBCs and blast count [13-16]. This disagreement in those results may be due to small sample size and environmental factors.

No significant difference in RBC, HB and platelet count in AML cases with or without CD7 and CD19 aberrant markers expression (**table 5**).

According to the FAB classification 7/25 (28%) of AML patients were of M1 subtype followed by M2 subtype 5/25 (20%) and the least frequent were detected in M6 and M7 (one patient for each) (**figure 8**).

Moreover all M2 subtype 5/5 (100%) and 4/7(57%) M1subtype had CD7 expression; this was explained byEl-Sissy et al [29], Saxana et al [27] ,Kita et al [25] and Juluis et al [30] who proposed that CD7 expression associated with early FAB AML subtypes because CD7 is expressed on early hematopoietic progenitors as the earliest surface antigen of T-cells and on malignant precursor T-cells [4, 31, 32].

In this study, 40% of AML cases expressed CD7; this frequency is comparable to other studies (**Table 8**)

Relation to Induction Therapy

The majority of previous studies showed a significant association of the aberrant CD7 antigen with low remission rate and biological aggressiveness in a significant proportion of AML cases. In this study we follow the AML patients after 2 weeks of completion of the standard induction chemotherapy. Detection of complete remission was assessed by morphological examination of the BM. 3/10(30%) patients harboring CD7+ show no response to induction therapy and two of them died during the induction therapy. This observation was similar to Sexana et al [27], Kita et al [25], Del Poeta et al [38] and Julius et al [30] studies which showed that complete remission rate was significantly lower in CD7+ cases portend poor prognosis. Del Poeta also showed that the overall survival and disease free survival rate of CD7+ AML were lower than those who were CD7-negative cases [28]. Similarly Chang et al [33] emphasized that the Patients expressing CD7 had significant shorter disease free survival (DFS) and post-remission survival (PRS) than patients without CD7.

The poor prognosis referred to CD7 was further emphasized by its presence of poor prognostic marker FLT3-ITD [34]. Rausei-Mills et al [34], stated that there was close association between aberrant CD7 expression and FLT3/ITD mutation in the myoblasts of FLT3/ITD+ AML, thus he suggested that FLT3/ITD- mediated leukemic transformation occurs in the more early stage of myeloid progenitor cells.Ogata et al. [35], stated that the proportion of CD7+ cases increased stepwise from the cases with favorable cytogenetic to the cases with intermediate and unfavorable cytogenetic, and CD7-positivity adversely affected the survival only in cases with unfavorable cytogenetic, therefore they recommended that CD7 expression in AML should be interpreted in association with the cytogenetic.

CD19

CD19 is a transmembrane glycoprotein of the Ig superfamily expressed by B cells from the time of heavy chain rearrangement until plasma cell differentiation [32, 36].

In this study, 16% of AML cases expressed CD19, a frequency higher than that reported in previous studies (**Table 9**).

Table 4.2 revealed that the incidence of CD19+ in AML was comparable to that of the Saudi study, El.Sissy et al [29] and Pakistani study Khurram et al [26] which may be due to racial and environmental factors.

Two of four cases of CD19 were detected in M1 and M2, however no study had revealed CD19+ to early FAB classification, large sample may clarify this argument.

Bain had concluded that CD19 expression is associated with AML that either associated with t (8:21) (good prognosis) [16, 40] or with AML associated with t (9:22) (poor prognosis) [37]. Thus the impact of CD19 expression on prognosis in AML should be further investigated and explored and we may propose that AML patients with CD19+ should be send for cytogenetic study to assess their prognosis.

CD7 and CD19 co-expression

In this study the co-expression of both CD7 and CD19 was detected in 3 out of 25 patients with AML. Khurram et al [30] had found that the paired aberrancy of CD7 and CD19 was found in 1 out of 27 cases of AML which was near to the result of this study.

Conclusion

1. The incidences of aberrant expression of CD7, CD19 and both CD7 andCD19 in 25 AML patients were 40%, 16%, and12% respectively.
2. CD7 expression was detected mainly in males whereas the expression of CD19 was distributed among males and females.
3. Total WBC count and blast cell percent were lower in patients harbouring aberrant expression in comparison to those without aberrant expression.
4. CD7 was detected mainly in early FAB classification (M1 and M2).
5. Six out of twenty five AML patients had no response to standard therapeutic regimen; three of them were harbouring CD7 and no one had CD19 alone, thus we may propose that CD7 is associated with poor response to induction therapy.

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Incidence of Transfusion-Transmitted Viral Infections in the Haematology Department of Baghdad Teaching Hospital

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Abstract

Background: Blood and blood product transfusion is vital for patients with hematological disorders including patients with chronic anemia and those with hematological malignancies but it carries the risk of transmission of infection. Hepatitis B, hepatitis C, and Human Immunodeficiency Virus (HIV) are the most prevalent of these transfusion-transmitted infections and should be screened for before transfusion for these patients.

Objectives: The purpose of this study was to determine the rate of post-transfusion seroconversion of viral hepatitis B, C and HIV in the patients under the study.

Patients and methods: A total of sixty-six consecutive patients (31 males and 35 females) were enrolled into this study between April 2011 and April 2012. These patients were admitted to the Hematology Department of Baghdad Teaching Hospital for management. These include twenty-six patients with acute myeloid leukemia (AML), sixteen patients with Non-Hodgkin's lymphoma (NHL), eleven patients with acute lymphoblastic leukemia, and two patients from each of the following hematological disorders: chronic lymphocytic leukemia (CLL), hairy cell leukemia, multiple myeloma, myelodysplastic syndrome, myelofibrosis and thrombotic thrombocytopenic purpura(TTP) with one patient with idiopathic vitamin K deficiency. Serology for HBsAg, anti-hepatitis C virus antibodies and HIV 1 & 2 was done for them before transfusion of any blood or blood product at diagnosis,3months and 6 months later to determine the rate of seroconversion of hepatitis B, C and HIV

Results: Seroconversion for HBsAg was seen in nine patients with seroconversion rate of 13.6 %, while the serology for both hepatitis C and HIV were negative with sero-prevalence of (0%). The seroconversion for HBsAg was seen in 3 patients (9.4%) of lymphoid malignancies (1 with CLL , and 2 with NHL) , 5 patients (16.1%) of myeloid malignancies (all of them had AML) , and one patient with TTP . There is no statistically significant association between age , gender , type of hematological malignancies (lymphoid vs myeloid) and the type of the blood product transfused with or without HBsAg seroconversion (P value > 0.05). A statistically significant association was seen between clinical jaundice and seroconversion of HBsAg (which was seen in 7 out of nine patients who have seroconversion (77.8%) (and 2 of the patients who have no seroconversion (3.5%)) and biochemically between the mean of SGOT , SGPT , alkaline phosphatase and total serum bilirubin and seroconversion of HBsAg.

Conclusion: This study showed that transfusion-transmitted hepatitis B seroconversion was more prevalent in our patient than hepatitis C and HIV which mandates more stringent preventive measures including appropriate donor selection , improved diagnostic testing methods that detect the virus at early period such as Nuclear Amplification testing (NAT) testing , use of pathogen reduction and inactivation methods to ensure blood safety and use of hepatitis B vaccination especially in immunocompromised patients with hematological malignancies .

Introduction

Blood and blood product transfusion is an integral component of medical practice and is essential in many treatments including hospitalized surgical and medical patients. Attention to safety of blood and blood products is a major concern of all blood systems and to those recipients and potential recipients who rely on them[1] ,While stringent measures are being taken to minimize the risk of transfusion-transmitted viral infection, it may never be possible to guarantee that donor blood is absolutely 'safe' [2].

Of the 150 countries providing data to WHO, 39 were not able to screen all of their donated blood for one or more of the four infections (HIV, HBV, HCV, and syphilis) that are most widely recognized to be transmitted through blood and are recommended by WHO to be screened at donation with 47% donations in low-income countries are tested in laboratories without quality assurance [3, 4].

Most current HBV transfusion transmission cases are attributable to blood donations by asymptomatic donors during the acute infection phase prior to HBsAg appearance [5]. The detection of hepatitis B virus (HBV) in blood donors is achieved by screening for hepatitis B surface antigen (HBsAg) and for antibodies against hepatitis B core antigen (anti-HBc) [6].

Infection with HCV can follow one of the two courses: acute infection followed by resolution of infection or chronic persistent infection [7]. Blood transfusion was a major risk for acute HCV infection in the past, with more than 10% of transfusion recipients acquiring the infection in some studies [5]. Screening assays based on antibody detection have

markedly reduced the risk of transfusion related infection to less than 0.00001% (1:149,000) [8].

The HIV is transmitted parenterally mainly through sexual contact, mother-to-infant transmission and less commonly by blood or intravenous drug use. It is usually screened in the blood using combined HIV antigen–antibody combination assays, specific for HIV-1 and HIV-2 and less commonly by HIV NAT [7].

The current use of nucleic acid testing for detection of the human immunodeficiency virus (HIV) and hepatitis C virus (HCV) RNA and HBV DNA in a single triplex assay may provide additional safety [9-11].

Patients and Methods

A total of a sixty-six consecutive patients : 31 males (mean age 37.3 ± 18.33 years, age range 16-69 years) and 35 females with the following hematological disorders were enrolled into this study between April 2011and April2012.. These patients are inpatients admitted to the hematology department of Baghdad Teaching Hospital for diagnostic evaluation and treatment of their hematological disease.

The study enrolled patients were referred from several governorates in Iraq newly diagnosed patients with the above hematological disorders were enrolled to this study., and attending the Hematology Department of Baghdad Teaching Hospital for further management. After giving informed consent, a questionnaire was completed either by direct interview with the patients or by their family or their relatives. The data requested were concentrated mainly on patient's name , age , sex , marital status , residence , the diagnosis of the hematological disorder , comorbidity, blood group and Rh , whether the patient donate blood before or not , whether he received blood product transfusion before including the type and number of transfusion (packed red cells , platelet transfusion , cryoprecipitate or fresh frozen plasma) and any previous viral infectious status including previous serological evidence of involvement by viral hepatitis A , B , C or HIV in addition to the results of serological and liver evaluation tests obtained.

Concerning inclusion and exclusion criteria, we included all proven new hematological disorders before transfusion of any blood product described above and we exclude any patient with previous serological evidence of hepatitis B, C and HIV) from this study. Patients who died within the study period before completing at least 3 month evaluation period who had negative viral serology were also excluded later from the study.

The number of blood product transfusion for each patient was difficult to be recorded because of difficulties in registration and recording.

Serological Evaluation

Hepatitis B surface antigen (HBs-Ag) , hepatitis C virus antibody(Anti-HCV), and the Human Immunodeficiency Virus antibody (Anti-HIV 1, 2) were all assayed in the virology department of Baghdad Teaching Hospital using a third generation enzyme-linked immunosorbent assay (ELISA; *Plasmatec* Laboratory Products , UK) on three occasions (at the first questionnaire with the patient , 3 months and 6 months thereafter for seroconversion (which defined as a change in Sero-status from negative to positive [12] if any , to occur .

HBsAg TheHBsAg test kit is an enzyme –linked immunosorbent assay (ELISA) for the qualitative determination of Hepatitis B Surface Antigen (HBsAg) in human serum or plasma[13].

HCVthe HCV kit is a third generation ELISA test for the qualitative determination of antibodies to hepatitis C (anti-HCV Abs) in human serum or plasma [14]. It utilizes antigens from the core, NS3, NS4 and NS5regions of the virus [15].

HIVHIV test kit is an ELISA for the qualitative determination of antibodies to human immunodeficiency virus (HIV) type 1 and/or type 2 in human serum or plasma [15].

It has become well established as a screening method for HIV-1. With the identification of new virus types (HIV-2 and HIV variant (HIV-O), commercially available ELISA screening tests have been continuously optimized and improved in terms of their sensitivity [16].

Liver Function and Evaluation Tests

Liver function tests were carried out at the same time as the viral serology and were repeated along with them at 3 and 6 months apart.

Serum alanine aminotransferase (SGOT or ALT), aspartate aminotransferase (SGPT or AST), bilirubin ; *Randox Laboratories* , UK) , and alkaline phosphatase (ALP) ; *Biomerieux* , France) , levels were measured on a 24 factor automated chemical analyzer using standard reagents in the Clinical Biochemical Department of the teaching laboratories and Baghdad Teaching Hospital . The normal ranges are 5-34 IU/L for SGPT, 0-55 for SGOT, 40-150 IU/L for ALP, and 0.2-1.2 mg/dL for bilirubin [17].

The diagnosis of *transfusion-transmitted viral infection* is made if there is positive serology for the virus with or without the appearance of jaundice and/or elevated liver function tests.

The diagnosis of *post-transfusion hepatitis* is established if there is jaundice and/or elevated liver evaluation tests (SGOT, SGPT, Alp, and TSB) along with evidence of seroconversion.

Statistical analysis

The data were analyzed by the computer software programme Statistical Package for Social Sciences (SPSS, version 18\ IBM.US.\2007) which

was used for entering and analysis of data. Descriptive statistics of different variables were performed as mean ± standard deviation (SD), ranges, frequencies and percentages. Comparative statistical tests then had been performed and different contingency tables had been conducted. Yattes corrected, Chi square (X2) was used for comparison among categorical variables for frequencies and percentages.

Fisher exact test (two tailed) was used as alternative to Chi square when it can't be applied.

Odds ratio was calculated to find the correlation of sero-conversion and different parameters (age group, gender and type of disease).

Students' independent t-test was used for comparison of means of continuous variables.

In all statistical analysis, level of significance ≤ 0.05 was assumed and considered statistically significant, and for contingency tables Yates corrected Chi-square and two- tailed P values had been depended. Finally all data and results had been presented in tables (age, liver enzymes, and other investigation, tables and\ or graphs).

Results

The Patients' characteristics

Mean age and gender distribution and male: female ratio is shown in **table 1**. The demographic and clinical characteristics of all patients in the study are shown in **table 2**

Rate of Sero-conversion of Hepatitis B, Hepatitis C and HIV in the Study

Rate of seroconversion of Hepatitis B (HBsAg) , Hepatitis C (anti-HCV Antibodies) and HIV serotype 1 and 2 (anti-HIV 1 & 2) showed that HBsAg was positive in 9 patients with seroconversion rate of 13.6 % , while the serology for both hepatitis C and HIV were negative with sero-prevalence of (0%) as shown in **table 3**

*Distribution of patients by type of the disease and seroconversion for HBsAg in the study*Seroconversion for HBsAg was seen in 3 patients (9.4%) of lymphoid malignancies (1 with CLL, and 2 with NHL), 5 patients (16.1%) of myeloid malignancies (all of them had AML), and one patient with TTP (50%) as shown in **table 4** .

Association between sero-conversion of HBsAg with some of Patient's Characteristics The association between sero-conversion of HBsAg and some of the patient's characteristics are shown in **table 5** which revealed that:

- There is no statistically significant association between the age (≤ 37 and > 37) , the gender of patients and the type of the hematological malignancy with HBsAg seroconversion (P value > 0.05)

The association between seroconversion of hepatitis B and the type of Blood Component transfusion

Tables 6 and 7 showed the frequency and percentage of blood component transfusion in the study .There is no statistical significant association between the type of blood component transfused and the rate of seroconversion for HBsAg (P>0.05).

The association between seroconversion for HBsAg and Jaundice

Jaundice was seen in 7 out of nine patients who have seroconversion (77.8%) and 2 of the patients who have no jaundice (3.5%). There is a strong statistically significant association between seroconversion for HBsAg and clinical jaundice (P value < 0.05) as shown in **table 8**

The difference in mean value of SGOT, SGPT, ALP, TSB between patients with and without HBsAg seroconversion

Laboratory evidence of hepatitis is manifested as a rise in liver enzymes with or without elevated total serum bilirubin. There is a statistically significant difference in mean value of SGOT, SGPT, ALP, and TSB between the two groups (P value < 0.05) as shown in **table 9**

Death

Death occurred in 5 patients (7%) on follow up:

- 4 of them may be related to hepatic failure due to hepatitis
- Three of them have relapsed acute myeloid leukemia

Table 1: Mean age of patients and gender distribution.

Gender*	Number (%)	Age	
		Mean ± SD	Range
Male	31 (47%)	37.3 ± 18.33	16 – 69
Female	35 (53%)	37.7 ± 15.7	15 – 75
Total	66 (100%)	57.5 ± 16.8	15 - 75

* Male to Female ratio = 0.88

Table 2: The demographic and clinical characteristics of all patients in the study

	Characteristic	
Number of patients	66	
Sex (Male/Female)	31/35	
Age range (years)	14-75	
Male	16-69	
Female	15-75	
Hematological Disease	**Patient Number**	**Male/ Female**
Lymphoid Malignancies		
NHL	16	1/1
ALL	11	8/3
CLL	2	6/10
MM	2	2/0
HCL	2	2/0
Myeloid Malignancies		
AML	26	13/13
MF	2	1/1
MDS	2	1/1
TTP	2	1/1
Vitamin K deficiency	1	0/1
Total	66	31/35

Table 3: The rate of seroconversion for Hepatitis B , C and HIV in the Study

Viral Serology	Frequency	%
HBsAg	9	13.6%
Anti-HCV Abs	0	0%
Anti-HIV 1,2	0	0%

Table 4: Distribution of patients by type of the disease and seroconversion for HBsAg in the study

Diagnosis	Hepatitis BsAg		
		Positive	Negative
Lymphoid Malignancies	Number	3	29
	Percentage	9.4%	90.6%
Myeloid Malignancies	Number	5	26
	Percentage	16.1%	83.9%
TTP	Number	1	1
	Percentage	50.0%	50.0%
Idiopathic Vit. K Deficiency	Number	0	1
	Percentage	.0%	100.0%
Total	Number	9	57
	Percentage	13.5%	86.5%

Table 5: Association between sero-conversion for HBsAg and Some of Patients' Characteristics

	Age group	HBsAg		Total	P.value
		Positive	Negative		
Age (year) (mean age =37.3)	≤ 37.3	6 (9.1%)	31 (47.0%)	37 (56.1%)	0.74
	> 37.3	3(4.5%)	26 (39.4%)	29 (43.9%)	
Gender	Male	3(4.5%)	28 (42.4%)	31 (47.0%)	0.6
	Female	6(9.1%)	29 (43.9%)	35 (53.0%)	
Type of disease	Lymphoid	3(4.5%)	29 (43.9%)	32 (48.5%)	0.67
	Myeloid	5(7.6%)	26 (39.4%)	31(47.0%)	

Table 6: Frequencies of multiple types of blood products administered to the patients in the study group

Combination	Frequency	Percent
1 product	15	22.7%
2 products	41	62.1%
4 products	10	15.2%
Total	66	100%

Table 7: Association between sero-conversion of HBV and multiplicity of blood component transfused

Combinations of Blood Products	Positive HBsAg		P.value
	Number	%	
1	2	22.2%	0.087
2	5	55.6%	
4	2	22.2%	
Total	9	100%	

Table 8: Association between Jaundice and seroconversion for HBsAg

Jaundice	HbsAg		Total	P.value
	Positive	Negative		
Present	7 (77.8%)	2(3.5%)	9 (13.6%)	0.000001
Absent	2 (22.2%)	55(96.5%)	57 (86.4%)	
Total	9	57	66	

Table 9: Mean SGOT , SGPT , ALP , TSB between the patients with and without HBsAgseroconversion

Liver function test	Negative HBsAg n=57	Positive HBsAg n=9	P.Value
Mean SGOT ± SD (U/L)	17.1 ± 3.04	56.3 ± 33	0.008
Mean SGPT ± SD (U/L)	21.6 ± 6.1	77.33 ± 60.4	0.002
Mean ALP ± SD (U/L)	79.27 ± 27.3	152.9 ± 99.6	0.000135
Mean TSB± SD (U/L)	0.974 ± 1.8	4.22 ± 3.7	0.019

Discussion

To the best of our knowledge, there are several studies concerning transfusion transmitted infections (TTIs) including transfusion-transmitted viral infections (TTVIs) that threaten the safety of blood supply. These studies are either performed on the healthy donors, or in the multi-transfused patients [18, 19].

This study is concerned with the most clinically significant TTVIs that cause post-transfusion hepatitis (HBV, HCV and HIV) and are recommended to be tested for before transfusion [5, 9]

In the last few years, the Government of Iraq has taken the strategic decision to strengthen blood transfusion services (BTS) as part of its commitment to improve the overall efficiency and effectiveness of health care system [20].

WHO has provided Iraq with all types of viral hepatitis kits and full technical support to the viral hepatitis studies conducted in Iraq to know the prevalence and incidence of different types of viral hepatitis [21]

HBV Seroprevalence and Rate of Seroconversion

The prevalence of HBsAg varies between studies [22], from country to another (being more common in low income developing countries in whom blood donations are not routinely tested for TTIs and who did not adopt safe measures for transfusion) [22, 23] , according to the type of the blood disorder[24] (being more common in patients with hemoglobinopathies [25-28], patients on hemodialysis [29], hemophiliacs [30,31] and immunocompromised patients with hematological malignancies [22] and between different provinces in the same country [22, 32] . An example of the latter is in Iran , which enjoy a high standard of hygiene and stringent safe measures in its blood banks with the prevalence of HBsAg is relatively low compared with other developing countries [32,33] , an exceptionally high rate (26.7%) reported from Rasht city in Gilan province [32] . Also, in the Rlbeirgo Preto region, Brazil, multi-transfused patients with thalassemia showed high seroprevalence rates in the markers of HBV infection (25%) [34].

Despite the marked decline in the prevalence of HBsAg among Iraqi normal blood donors and the normal population (<2%) [35], this study showed very high rate of HBsAg seroconversion (13.5 %) among our multi-transfused patients ,the majority of them have hematological malignancies which may represent a serious economic and public health problem in the community. These findings are higher than that reported by Al-Hilli and Ghadhban,[36] where the prevalence of HBsAg was 10% among multi-transfused patients, and 1.6% among blood donors. It was also higher than rates previously reported among patients with different blood disorders in different regions of Iraq [25-27,30]. Comparing reports from developed countries, which enjoy a high standard of hygiene, our findings were higher.[32,37-42)

However, our results were lower than that reported by Kocaba et al,[43] who reported a 47.4% prevalence of Turkish children with cancer (acute leukemia, lymphoma, and solid tumors), with 20% of child control subjects positive for HBsAg , by Omer et al [22] who report a seroprevalence of 30% for HBsAg and by Juneja et al [44]who report rates of seroprevalence of 31% for HBsAg and 84% for anti-HBc Ab in Indian multi-transfused children with hematological diseases . The higher prevalence of HBsAg among patients with haematological malignancies might be explained by the increased exposure to the risk factors for HBV, such as repeated hospitalizations, frequent injections, frequent blood withdrawal for laboratory tests, and may be also due to multifactorial impairments in cellular and humeral immunological integrity in these patients [33 ,45]. Also , it is well known that a minute amount of HBV particles from a needle stick contamination can cause infection due to the high concentration of these particles, in addition to high stability to heat and other conditions compared with HCV [22].

Association with Age

Some studies [22,26,29] showed that the trend of hepatitis B viral infection varies with the age of the patients with hematological malignancies . These studies showed that the rate being higher among young leukemic patients with HBV than adults, in contrast to HCV, EBV, and CMV infections. This was explained as most of the children and young adults are active, have low health education, and more exposed to accidental needle stick injuries. Our findings are inconsistent with these studies but are in agreement with Okan et al who found no differences in the rate of seroconversion of HBV infection in relation to the age of the patients [46].

Association with Gender

Many studies showed an association between the gender of the patient and the rate of HBV seroconversion .Most of these studies showed that that males were more infected by HBV than females [33,38,47], while other workers reported increased prevalence of HBV infection in female multi-transfused patients among different provinces of Iraq [26, 48 , 49]. However, this study showed no association between the gender of the patient and HBV seroconversion.

Association with Haematological Malignancies

Several epidemiological studies showed significant association between the seroprevalence and rate of seroconversion of HBV among patients with lymphoid or myeloid malignancies [50-58]. This study was inconsistent with these studies but was compatible with the study of Anderson et al[59] , which include >61,000 elderly patients with hematopoietic malignancy and found that HBV infection was not associated with any lymphoid or myeloid malignancies in contrast to HCV which was significantly associated with both lymphoid and myeloid malignancies. HCV was associated with 1.5-5 fold increased risk of lymphoid malignancies by chronic immune or antigenic stimulation which is the mechanism different from that of HBV lymphogenesis [53,57 ,60-63]. Within the types of lymphoid malignancies, Omer et al showed a significantly higher prevalence of HBsAg among patients with ALL than other types of acute leukemia [22].

Association with Clinical and Laboratory Evidence of Hepatitis

This study showed significant association between HBsAg seroconversion with clinical and laboratory evidence of post-transfusion hepatitis.

Association with the Type and multiplicity of Blood Component Transfused

This study showed no significant association between HBsAg seroconversion and type and multiplicity of blood component transfusion. This is in agreement with the fact that the risk of transmitting human immunodeficiency virus, hepatitis B, or hepatitis C from FFP, platelet transfusion and cryoprecipitate is similar to that of whole blood or packed red cell transfusion [64, 65].

HCV

None of the patients in the study showed seroconversion for HCV, which is in contrast to other reports from different regions in Iraq[22,26,27,30] , and from reports of different countries. In Turkey, Kocaba et al [66] found that 5.8% of Turkish children with acute leukemia and lymphoma were positive for anti-HCV antibodies and in Brazil, a high prevalence of HCV infection (46.8%) was detected in a population of multi-transfused patients [34]and in Malaysia this rate was 22.3%.[67], while in Japan it was 7.3%, [51] and in Egypt, it was 4.2% [68].The absence of cases of HCV seroconversion in the study was probably because of the time limit of the study (maximum 6 months) as hepatitis C virus infection is usually chronic infection in the majority of cases , lack of NAT for HCV and because our aim is to determine the rate of seroconversion rather than the prevalence of HCV .

HIV

In the present study, none of the patients in the study were positive for HIV serological markers. Similar findings have been reported in Iraqi studies, such as that of Omer et al [22]and in other developing countries [32, 69, 70] .This finding may indicate that Iraq is considered an area of low prevalence of HIV/AIDS (<0.01%) among the Iraqi population and blood donors [22,71,72].

Conclusion: This study showed that transfusion-transmitted hepatitis B seroconversion was more prevalent in our patient than hepatitis C and HIV which mandates more stringent preventive measures including appropriate donor selection , improved diagnostic testing methods that detect the virus at early period such as Nuclear Amplification testing (NAT) testing , use of pathogen reduction and inactivation methods to ensure blood safety and use of hepatitis B vaccination especially in immunocompromised patients with hematological malignancies

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Estimation of Interleukin-10 Levels as a Predictive Factor in Iraqi Non-Hodgkin's Lymphoma Patients

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Abstract

Background: Interleukin-10 (IL-10) is a pleiotropic cytokine produced by type 2 helper cells (TH2), monocytes , macrophages, and neoplastic B lymphocytes IL-10 production has a strong immunosuppressive effect, and it acts as an effective stimulator for B-cells .IL-10 level increase in Non-Hodgkin lymphoma (NHL) patients ;moreover , this was associated with poor prognosis .

Objectives: to assess the level of interleukin-10 in patients with non-Hodgkin lymphoma and to correlate its level with prognosis.

Materials and Methods: The present study was carried out to evaluate IL-10 level in the sera of 46 NHL Iraqi patients, in addition to a control group involving 46 matched apparently healthy subjects using serological method the enzyme-linked immunosorbent assay (ELISA).This study was carried out at the National center of hematology from January to June 2013.

Results: there were 31 (67%) patients with age group ≥50 years and 15 (33%) patients with age group <50 years. The mean age of NHL patients was 52.74 years ranging from 17 to 80 years? Moreover, the study noticed a male predominance and male to female ratio was 1.7:1.When the patients of NHL compared with control, the levels of interleukin 10 were markedly increased in patients with NHL (18.67 ± 4.12pg/ml vs. 3.4 ± 0.73pg/ml) ($p<0.05$). Higher levels of IL-10 were noticed in the advanced stage patients of compared to early stages (33.1 ± 4.5 pg. /ml vs. 13.4 ± 2.7 pg. /ml) ($P<0.05$).

Conclusion: In the highest age of NHL patients was noticed and it might indicate the increasing rates of NHL patients among older population. in addition increasing level of IL10 is associated with increasing age range and also correlated positively with aggressive NHL cases that might indicates the predictive pivotal role of this cytokine.

Keyword: Non-Hodgkin's lymphoma, Interleukin10.

Introduction

Lymphoma is a cancer of the white blood cells, namely lymphocytes. The two main types of lymphoma are Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL). Lymphoma is the most common blood cancer and the third most common cancer of childhood. Although both cell types B lymphocytes, or B-cells, and T lymphocytes, or T-cells can develop into lymphomas, B-cell lymphomas are more common. Like normal lymphocytes, those that turn malignant can grow in many parts of the body, including the lymph nodes, spleen, bone marrow, blood or other organs [1].

Interleukin 10 (IL10): Cytokines are a diverse group of secreted proteins that are produced by and regulate cells of the immune system [2]. Their biological activities are both pleiotropic and redundant, in part due to overlapping features of their receptors and signal transduction pathways. Disruption of the cytokine signaling network is a key pathophysiologic feature of human immunodeficiency virus (HIV) infection and the acquired immunodeficiency syndrome (AIDS) [3].

Serum concentration of the cytokines may be utilized as a marker of immunity status and/or prognosis in cancer [4].

More than 20 years ago, the cytokine IL-10 was identified as a "cytokine synthesis inhibitory factor," a product of TH2 clones following protein or antigen stimulation that blocked cytokine production from TH1 clones [5]. IL-10 achieved this effect by inhibiting the ability of myeloid cells such as macrophages and dendritic cells (DCs) to activate TH1 cells [6]. We now know that IL-10 is not just made by Th2 cells, but can be produced by most if not all CD4 + T-cell subsets, including TH1 and TH17 cells, B cells, neutrophils, macrophages, and some DC subsets [7]. Regulatory T cells (Tregs), in the context of infectious disease, also serve as a major source of immunoregulatory IL-10 and, depending on their developmental origin, may come in several forms [8].

Interleukin 10 (IL-10) is a potent anti-inflammatory cytokine that plays a crucial, and often essential, role in preventing inflammatory and autoimmune pathologies [9].IL-10 also plays a role in proliferation and differentiation of B cells, T cells and mast cells. Based on its immunomodulating functions, IL-10 has been considered an attractive candidate for therapeutic applications for treatment of acute and chronic inflammation, autoimmunity, cancer and infectious disease [10].Initiation of the signal transduction occurs when IL-10 binds to two receptor chains, IL-10R1 [11] and IL-10R2 [12]. Both chains consist of extracellular, trans-membrane and intracellular/ cytoplasmic domains (the cytoplasmic domain of receptor chain 1 is much longer than that of chain 2), and belong to the class II or interferon receptor family [13].

IL-10 is produced by both myeloid cells and T cells. In myeloid cells, IL-10 is induced following Toll-like receptor (TLR) ligation in response to a plethora of pathogen products. The magnitude of IL-10 induction within different myeloid cell types has been linked to the relative strength of extracellular signal-related kinases 1 and 2 (ERK1 / 2) activation. [14] However, cytokine production by plasmacytoid DCs in response to TLR ligation can be significantly

inhibited when exogenous IL-10 is added to cell cultures. [15].

IL-10 expression or signaling can enhance clearance of pathogens during an acute infection, but also exaggerate inflammatory response, resulting in exacerbated immunopathology and tissue damage. [16, 17]Conversely, some pathogens can harness the immunosuppressive capacity of IL-10 to limit host immune response, leading to persistent infection. [18].

Lymphoma and interleukin 10

The high concentrations of IL-10 found in patients with NHL and possibly present in tumor sites could conceivably exert an inhibitory effect on macrophage and antigen-specific T cell response at the tumor site and thus contribute to lymphoma progression in vivo [19].

Tumor cells from B, T, and NK cell lymphoma are able to produce biologically active IL-10 [20, 21]. As early as 1993, Favrot and coauthors [22].investigated IL-10 serum levels using an ELISA, which detects both viral and human IL-10 in patients with active non-Hodgkin's lymphoma (NHL) and healthy volunteers. They described the detection of IL-10 in serum from about 50% of these patients but none of the control blood donors. IL-10 was detectable with a similar frequency in all subtypes of NHL and in all clinical stages, as well as in both EBV-seropositive and EBV-seronegative patients [22]. In the following years these observations were extended to Hodgkin's disease and other lymphoma species, and due to improved sensitivity of ELISA systems, it was possible to demonstrate that lymphoma patients had significantly higher serum levels of IL-10 than healthy volunteers [23, 24, 25, 27, and 28]. An elevated local expression of IL-10 was detected in various cutaneous T cell lymphoma entities (CTCL) [29].showed IL-10 production by malignant T cells in Se´zary syndrome, a leukemic type of cutaneous T cell lymphoma. It has been demonstrated cutaneous IL-10 mRNA overexpression in mycosis fungoides (MF) lesions [30]. It has been recently observed a stage-dependent decrease in T cell activation of antigen expression suggesting impairment of tumor surveillance in advanced MF stages [41].

Prognostic Value of Interleukin-10 Overexpression.

In different lymphomas, increased IL-10 production has been reported and a negative prognostic meaning of increased IL-10 plasma levels is being discussed [23]. Elevated IL-10 serum levels have been also described as a negative prognostic factor for responsiveness toward treatment, as well as the disease-free and overall survival by patients with melanoma and solid tumors, particularly with lung, gastrointestinal, and renal cell cancer. Several groups reported on increased circulating IL-10 serum levels in gastric, colon, and renal-cell cancer patients [32]. IL-10 serum levels commonly returned to normal in radically resected patients. Persistently elevated IL-10 serum levels after surgery predicted tumor recurrence [33, 34].

Subject, materials and methods:

The study was conducted on 46 Iraqi Non-Hodgkin's lymphoma patients (29 males and 17 females) and 46 matched apparently healthy controls. The age range of the patients was within 17-80 years .This study was done in the National center of hematology & Department of biology at AL-Mustansiriya University from January to June 2013. Clinical data of the patients were recorded.

IL 10 was detected in patients and controls sera using serological method the enzyme-linked immunosorbent assay (ELISA.

Detection Interleukin 10

Preparation of Solutions

Poly propylene tubes were used in this procedure.

Washing solution: An aliquot (20 ml) of washing concentrate was diluted up to 500 ml with distilled water.

Substrate solution: Color reagent A and color reagent B were mixed together in equal volumes within 15 minutes of use. The mixture was protected from light.

IL-10 standard: The lyophilized IL-10 standard was reconstituted with 1.0 mL of distilled water .This reconstitution produces a stock solution of 5000 pg. /mL. The standard was left at least 15 minutes with gentle agitation before making dilutions.

Assay Procedure *as manufacturer instruction: (R&D system .USA)*

Five µl Assay Diluent RD1W was added to each well. Serial concentrations (0, 7.8, 15.6, 31, 2, 62.5, 125, 250and 500 pg. /ml) of the standard were made using the assay diluent after reconstitution of standard vial with 1 ml of diluent water. An aliquot (200 µl) of the standard or sample was added to the well. After that, the well was mixed and the plate was covered and incubated for 120 minutes at room temperature. The wells were washed with four cycles of washing using the washing solution, with the aid of a microtiter plate washer.

An aliquot (200 µl) of conjugate solution was added to each well, and the plate was covered and incubated for 120 minutes at room temperature. The wells were washed with four cycles of washing using the washing solution, with the aid of a microtiter plate washer.

An aliquot (200 µl) of substrate reagent was added to each well, and the plate was covered and incubated in dark for 30 minutes at room

temperature. An aliquot (50 µl) of stop solution was added to each well, and the absorbance was read at a wave length of 450-630 nm using ELISA reader within 30 minutes after stopping reaction.

Calculations:
The sample results were calculated by interpolation from a standard curve that was performed in the same assay as that for the sample Figure (2-1), using a curve fitting equation.

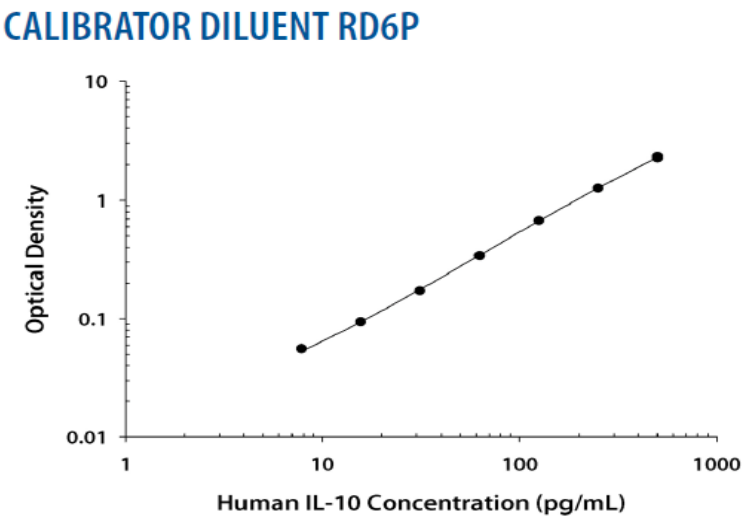

Fig.(1) Standard curve of IL-10.

Statistical Analysis

The Statistical Analysis System- SAS (2010) was used to effect of different actors in study parameters. The LSD test or T-test the comparative between means in this study. Correlation coefficient was used as a qualitative data to express the relation between variables and to measure the dependence of one variable on the other.

Probability values of $p<0.05$ and 0.01 were considered statically significant.

Results:

Distribution of patients with non-Hodgkin's lymphoma and healthy control group according to their age:

Forty six non-hodgkin's lymphoma patients were investigated.,31 out of 46 (67%) patients were ≥50 years and 15 out of 46(33%) patients were<50 years (Table 1). The mean age of NHL patients was 52.74 years ranging from 17 to 80 years

Table (1): Age group of patients & control

Item	age≥50	Age<50	Total
patients	31(67%)	15(33%)	46(100%)
Control	27(55%)	19(45%)	46(100%)

2 .Distribution of patients with non-Hodgkins lymphoma and healthy control group according to the gender:

In this study, the number of total males with NHL was higher (29 cases;**63%**)than total females with NHL(17cases;**37%**).

Table (2): Gender distribution among Patients & Control groups

Item	Male	Female	Total
patients	29 (63%)	17(37%)	46
control	20(43%)	26 (57%)	46

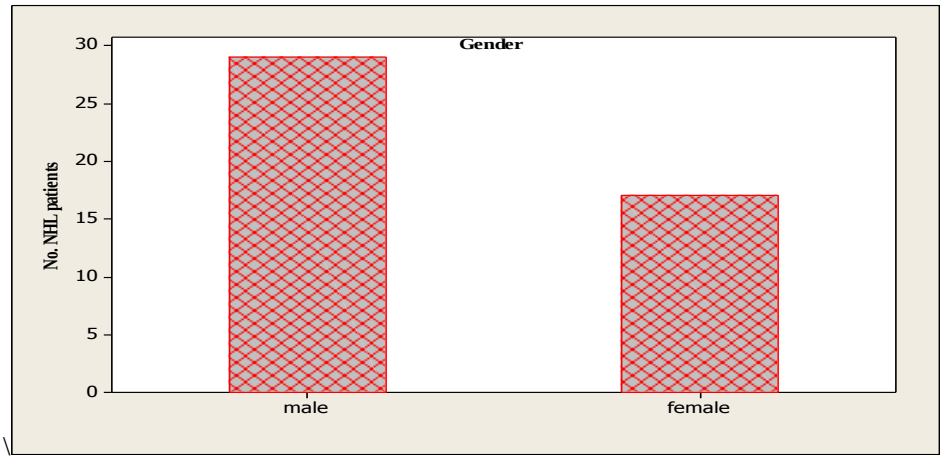

Fig.(2) Distribution of patients with non-Hodgkin lymphoma according to their gender

Effect of patient's age versus IL-10 level.

Forty six NHL patients were divided into two groups according to age (age<50 years; age≥50 years).The mean of IL-10 serum levels did not show a significant difference between the two age groups (p>0.05)(Table 3).

(Table 3) Comparison Interleukin-10 levels between two age groups.

Parameter	Mean ± SE		T-test value
	< 50 years	≥ 50 years	
Interleukin -10 pg/ml	18.17 10.47	18.92 ± 3.61	2.167 NS
* (P<0.05), NS: Non-significant.			

Effect of patient's gender versus IL-10.

Reference to gender the IL-10 level in serum was significantly different (23.77pg/ml ± 6.58 male, 12.06pg/ml ± 3.77 female) (P<0.05)(Table 4).

(Table 4) Interleukin-10 serum level comparison between male and female

Parameters	Mean ± SE		T-test value
	Male	Female	
Interleukin -10pg/ml	23.77 ± 6.58	12.06 ± 3.77	6.745 *
* (P<0.05)			

Estimation of Interleukin 10 serum levels in NHL patients: The levels of interleukin 10 were markedly increased in patients with NHL compared with healthy subjects (18.67 ± 4.12pg/ml *vs.*3.4 ± 0.73pg/ml) (p<0.05). thirty patients(65%) of NHL had high level of IL-10 (Table 4,Fig 3) .

(Table 4). Comparison between patients & healthy group in IL-10 level in serum

Parameter	NHL Patient n=46		Control n=46	T-value
IL-10 Serum Level pg/ml	Mean± SE	18.67 ± 4.12	3.44 ± 0.73	13.281**
	Out of normal range (%)	30(67%)	0%	
* (P<0.05), NS: Non-significant.				

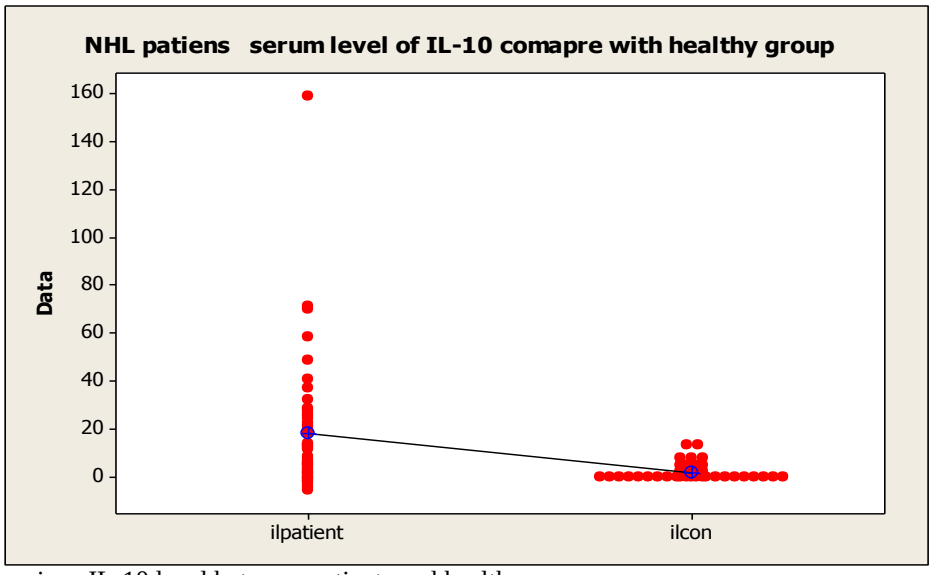

Fig.(3): Comparison IL-10 level between patients and health group

Comparison between NHL advanced stage (aggressive stage) and early stages.

NHL patients were divided into two groups according to the stage of lymphoma, 11 patients (23.9%) advanced stage (aggressive stage) patients and 35(76.1%) early stage patients. we found markedly increased IL-10 levels in the advanced stage patients of NHL compared with early stages patients of NHL (33.1 ± 4.5 pg/ml *vs*. 13.4 ± 2.7 pg/ml) (P<0.05). These findings are in agreement with [38,53,62,63]. Table (5).

(Table 5): Comparison between NHL IL-10 levels early and advanced stages.

NHL stage	No. cases	%	Mean ± SE	T.test
Early stages of IL-10 pg/ml	35	76.1%	13.4±27	-3.77*
Advanced stage IL-10 pg/ml	11	23.9%	33.1 ±4.5	

* (P<0.05)significant.

Discussion

As the number of cases included in this study was 46 and this fact is noticed on reviewing other Iraqi studies[35,36] (Ridda 2004;55 cases; Al-Obaidi 2012; 40cases) do not really reflect the actual age and sex of patients with NHL, since the sample size of these cases was small, too.

Our findings were in agreement with another studies [37,38,39,40,41]. However, this finding is in disagreement with previous Iraqi studies [19,36,42]. Our study is slightly convergence with Pakistani studies which found that the median age of their NHL patients was 48.0±13.3 years, respectively[43]. Moreover, it was also in disagreement with other western studies [44].

Age strongly influenced survival as older persons typically experienced poorer survival, and even within stage, older persons had lower survival rates[45]. In the elderly, the production of TH2 cytokines, IL-4 and IL-10, is greater than it is in the young, whereas production of the TH1 cytokine, IFN, is less, suggesting an age-related shift from a TH1 to a TH2 response [46] that may contribute to reduced cellular immunity in older adults.

Nevertheless age structure of Iraqi Population characterized with increasing young ages 15-64 years (59.3%), whereas ≥65 years were (3%) [47].

A predominance of male gender was noticed in our sires since the studied ratio of males to females patients (M:F) with NHL was 1.7:1 (i.e. 63%; 29 out of 46 males versus 37%; 17 out of 46 females (Table 2 & Figure 2).

The present result of M:F ratio is consistent with [42] in Jordan who found males to females ratio 1.4:1.

The present result are in concordance with results reported by[48] Hashemi and Parwaresh (2001) in Iran (M:F ratio was 1.5:1).

Also, the present M:F predominance result is consistent with two other studies done in Pakistan[43,49] who found M:F ratio of 2:1 and 2.6:1, respectively) and a study done in India by [50]who found M:F ratio of 3:1 .

Moreover, this study is similar to that study done by [51]in USA who found the ratio of males to females in their studied cancer surveillance series of NHL in the period from 1978 through 1995 was 1.5:1 for total NHL group where 1.4:1 was reported in their white population Versus 1.7:1 for black citizens.

The predominance of male gender as documented in our study and many other series of studies is contrasted by the predominance of females in relation to males which reported by another researchers in Germany[52].

However, still there is need to generate more data regarding variation in gender predominance in our population for better studies.

There was no evidence about interleukin-10 levels depends on the age. A similar finding was reported previously by other investigators [19,53,54]. although in other study it has been suggested that,IL-10production is increases with aging because an age-related increase in memory T cells of the TH2 response increases production and secretion of IL-10 which, in turn, can inhibit the production and release of IL-12 and interferon-γ (IFN-γ), thereby suppressing the cellular inflammatory response [38]. While another study found that advancing age is associated with remodeling of the immune system include include reduced levels of circulating IL10 with raised levels of proinflammatory cytokines[55,56].

Although immunosenescence affects both men and women, it does not affect them equally. Our result is in concordance with previous studies [57,58]. Men (all ages) and postmenopausal women exhibit diminished T cell immunity compared to premenopausal women[57].

The decrease in androgens in men with aging may contribute to their immunosenescence; however, the loss of T cell function in men with aging is significantly less dramatic than that observed in women[58].

IL-10 is a cytokine produced by monocytes, macrophages, and B- and T-cells. It has immunosuppressive properties inhibiting macrophage activity, antigen-specific T-cell activation, and production of IFN-γ by NK cells. Various studies reported that IL-10 levels were increase in NHL [27,59] and that were associated with poor prognosis[27]. Recently, it has been reported that there was a relationship between presence of high risk as defined by International Prognostic Index (IPI) in newly-diagnosed NHL patients and high serum IL-10 Significant results were recorded in the current study, but they should be interpreted cautiously, because our patient population was older than the average population of patients with lymphoma and had an atypical distribution of histologic types. With aging, the TH1 response may also include increased production of the pro-inflammatory cytokine, IL-12, which can inhibit the TH2 (antibody-mediated) response. However, an age-related increase in memory T cells of the TH2 response increases production and secretion of interleukin-10 (IL-10) which, in turn, can inhibit the production and release of IL-12 and interferon-γ (IFN-γ), thereby suppressing the cellular inflammatory response [38].

Present results also revealed a significant elevation in IL-10 serum levels of NHL patients in comparison to healthycontrol group, similar results were reported by Iraqi and Arabian investigators [19,28,60]also our result was in agreement with western studies[54,61].

The most acceptable explanation forsuch increase in the level of IL-10 might be due to its production by malignant cells or by different cells of theimmune system, including T and B lymphocytes, macrophages and monocytes[19].

Conclusion :

IL10 level in NHL is associated with increasing age and also correlated positively with advanced stage NHL cases .

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Evaluation of Leukemic Patient`s Compliance with Oral Chemotherapy in Baghdad City

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Abstract:

Background: Noncompliance is a major obstacle to the effective delivery of health care. Estimates from the World Health Organization (WHO) indicate that only about 50% of patients with chronic diseases living in developed countries follow treatment recommendations

Objectives: To evaluate the leukemic patient`s compliance with oral chemotherapy in Baghdad city.

Material and methods: A descriptive study was carried out at Baghdad hospitals (Baghdad teaching hospital, and nursing home hospital). Started from 11 of December 2012 to 27 of June 2013. A non-probability (purposive) sample of 60 patients with leukemia and they were on oral chemotherapy. The questionnaire was designed and constructed by the researcher according to review of literatures and related studies. The content validity of the instrument was established through penal of (11) experts. Reliability of the patient compliance was determined by test-retest method which was estimated as average (r=0.851).Data was gathered by interview technique using the questionnaire format and data was analyzed by application of descriptive and inferential statistical methods.

Results: Regarding age group (20-29) years was the larger group (31.7%). Nearly equal percent of male to female (48.3% and 51.7% respectively). Larger group (38.3%) of sample was primary school in educational level and (65%) of sample has less than one year illness duration. The result indicates the patient compliance with oral chemotherapy in which (55%) of patients was compliance and (45%) was not compliance with oral chemotherapy. The result also shows that there are no significant relationship at $P>0.05$ between (age groups, gender and educational level), and patient compliance to chemotherapy, which there are significant correlation at $P<0.05$ between patients duration of illness and patient`s compliance to chemotherapy.

Conclusion: The results reveals that the majorities of study group were (20-29) years old, nearly equal percent of male to female, and primary school in educational level. The result indicated that (45%) of patients with leukemia are not compliance to oral chemotherapy.

Key words: Factors, leukemic patients, compliance, oral chemotherapy

Introduction

Noncompliance is a major obstacle to the effective delivery of health care. Estimates from the World Health Organization (WHO) indicate that only about 50% of patients with chronic diseases living in developed countries follow treatment recommendations [1].

Major barriers to compliance are thought to include the complexity of modern medication regimens, poor health literacy and lack of comprehension of treatment benefits, the occurrence of undiscussed side effects, the cost of prescription medicine, and poor communication or lack of trust between the patient and his or her health care provider [2]. Noncompliance has also been associated with an increase in physician visits, higher hospitalization rates, and longer hospital stays [3].

Efforts to improve compliance have been aimed at simplifying medication packaging, providing effective medication reminders, improving patient education, and limiting the number of medications prescribed simultaneously [4].

Dosing schedule is an important factor in the effectiveness of some chemotherapeutic agents, and taking drugs more or less frequently than prescribed may affect therapeutic efficacy. Furthermore, the degree of compliance required to achieve the desired treatment goal is likely to vary from one regimen to another [5]. The **aim of this study was** to evaluate the leukemic patient`s compliance with oral chemotherapy in Baghdad city.

Materials and methods

A descriptive study was carried out at Baghdad hospitals (Baghdad teaching hospital, and nursing home hospital). Started from 11 of December 2012 to 27 of June 2013. A non-probability (purposive) sample of 60 patients with leukemia and they were on oral chemotherapy.

The questionnaire was designed and constructed by the researcher according to review of literatures and related studies. The content validity of the instrument was established through penal of (11) experts.

Reliability of the patient compliance was determined by test-retest method which was estimated as average (r=0.851).Data was gathered by interview technique using the questionnaire format and data was analyzed by application of descriptive and inferential statistical methods. The Morisky and Green Test are used to evaluate attitudes regarding treatment. It is made up of four questions, with Yes-No answers. Yes stands for 0 and No stands for 1. The patient compliance to treatment is considered for a score of four points. With three or fewer points, the patient is non-compliant.

Result:

Table 1: Observed Frequencies, Percents and Cumulative Percents of Patient's Demographical Characteristics.

Dem. characteristics	Groups	Freq.	Percent	Cum. Percent
Age Groups	10 - 19	11	18.3	18.3
	20 - 29	19	31.7	50
	30 - 39	8	13.3	63.3
	40 - 49	10	16.7	80
	50 - 59	9	15	95
	60 - 69	1	1.7	96.7
	70 ≥	2	3.3	100
Gender	Male	29	48.3	48.3
	Female	31	51.7	100
Educational level	Illiterate	10	16.7	16.7
	Reads and writes	3	5	21.7
	Primary school	23	38.3	60
	Intermediate School	17	28.3	88.3
	Secondary School	4	6.7	95
	College and more	3	5	100
Duration of illness	Less than 1 year	39	65	65
	1 – 5 years	16	26.7	91.7
	5 – 10 years	3	5	96.7
	More than 10 years	2	3.3	100

Freq: Frequency, Cum. Percent: Cumulative percent.

Table number (1) indicates that the observed frequencies, percents and cumulative percents of demographical characteristics variables in the sample which age group (20-29) years were the larger group (31.7%). Nearly equal percent of male to female (48.3% and 51.7% respectively). Larger group (38.3%) of sample was primary school in educational level and (65%) of sample has less than one year illness duration.

Table 2: Distribution of the Patients Compliance with Chemotherapy.

Parameters	Compliance		Not compliance		Total	
	Frequency	Percent	Frequency	Percent	Frequency	Percent
Patient compliance to chemotherapy	33	55	27	45	60	100

Table (2) indicates the patient compliance with chemotherapy in which (55%) of patients was compliance and (45%) was not compliance with chemotherapy.

Table 3: Association between the Patient's Demographical Data and their Compliance to Chemotherapy

Patient's Demographical Data	Chi-Square test			
	Value	df	Approx. Sig.	C.S.
Age Groups	2.011	6	0.919	NS
Gender	0.133	1	0.715	NS
Education levels	10.315	5	0.067	NS
Duration of illness	10.809	3	0.013	S

NS: Non Significant at P>0.05, S: Significant at P<0.05, C.S.: Computed Significant

This table shows that there are no significant relationship at P>0.05 between (age groups and gender), and patient compliance to chemotherapy, which there are no significant correlation at P>0.05 between patient's educational level and their compliance to chemotherapy while according to the actual P-value is more informative to be reported [6]. in other words we had a confidence within not less than 92.5% of a meaningful differences presented would be. and there are significant correlation at P<0.05 between patients duration of illness and their compliance to chemotherapy.

Discussion:

Throughout the course of data analysis table number (1) indicates that the majority of the samples were (20-29) years old who were a counted (31.7%). This finding comes along with result obtained from study done by (Hartigan, 2003) which indicated that majority of the patient's age were (20-30) years old[7].

In relation to gender, nearly equal percent of male to female (48.3% and 51.7% respectively). This result is compatible with (Marques and Pierin, 2008) which indicated that equal percent of male and female in his study [8].

Relative to educational level larger group (38.3%) of patients was primary school. This finding agrees with results obtain from study done by (Atassi, et al., 2011) which indicated that the majority of the patients in their study was low educational level [9].

Regarding to duration of illness (65%) of patients has less than one year illness duration. This result is compatible with (Selen, et al., 2003) which indicated that most of sample has less than one year's illness duration [10].

Throughout the course of data analysis table number (2) indicates that the (45%) of patients are not compliance to chemotherapy. This finding comes along with result obtained from study done by (Selen, et al., 2003) which indicated that (41.9%) of samples are not compliance with chemotherapy [10].

In this study the results in table (3) reflect that there is no relation between patient's age and their compliance to chemotherapy. This result is compatible with (Patricia, and Angela;2008) which indicated that there is no relation between patient's age and their compliance to chemotherapy [11].

Regarding gender, there is no relation between patient's gender and their compliance to chemotherapy. This result is compatible with (Atassi, et al., 2011) which indicated that there is no relation between patient's sex and their compliance to chemotherapy [9].

In regard to educational level, the result of study indicated that educational level play role in compliance of patients to chemotherapy but this was not statistically significant, while the actual P-value is more informative to be reported. This finding agrees with results obtain from other studies done by (Carolyn, 2006) which indicated that there are good relation between patient's educational level and their compliance to chemotherapy [12].

Regarding duration of illness there is significant relation between patient's years of illness and their compliance to chemotherapy. This result is compatible with (Hartigan, 2003) which indicated that there was positive relation between the duration of patient's illness and the compliance to chemotherapy [7].

Conclusion:

1. The samples are nearly equal in gender (male and female), and larger age group was (20-29) years old.
2. Most of the patients have primary school in educational level.
3. The majority of patients have less than 1 year illness duration.
4. Most of patients were not compliance to chemotherapy.
5. there are no significant relationship at $P>0.05$ between (age groups and gender), and patient compliance to chemotherapy, which there are no significant correlation at $P>0.05$ between patient's educational level and their compliance to chemotherapy while according to the actual P-value is more informative to be reported and there are significant correlation at $P<0.05$ between patients duration of illness and their compliance to chemotherapy.

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Changes of immunological, Cytogenetic & Hematological Profiles in Chronic Myeloid Leukemia Treated with Imatinib Mesylate

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Abstract

Background: Chronic myeloid leukemia (CML) (also known as chronic myelogenous leukemia, chronic granulocytic leukemia) is a clonal disease that results from an acquired genetic change in a pluripotential hemopoietic stem cell .Imatinib mesylate, is an inhibitor of the family of ABL kinases, these proteins serves a complex role in cell cycling and is important in lymphopoiesis, and has been shown to have impressive clinical activity in chronic myeloid leukemia

Objectives: to assess the immunological, cytogenetic, and hematological parameters in patients with chronic myeloid leukemia on imatinib therapy

Material and methods: Thirty one CML patients treated with imatinib mesylate at standard dosage were enrolled in this prospective study. All patients were seen in the Department of hematology at the national center of hematology /Almustansiriya University for six months from February to July 2012.Data on blood cell counts and blood film, serum levels of IgG, IgA ,IgM ,C3 and C4;assessed by radial immundiffusion method,

Results: Twenty four out of thirty one CML patients treated with the imatinib regimen (those had done FISH study) reached a complete cytogenetic response. Reduction percent were 19.35% for IgG, 16.13% for IgM and 9.68% for IgA, six patients had IgG inferior to normal laboratory range, five patients presented a reduction of IgM and three patients had IgA lower than normal range. No significant correlation of immunoglobulin levels compared to duration of treatment

Conclusion: Imatinib mesylate can induce complete cytogenetic response in a high percent of CML patients. Cytogenetic response correlates well with duration of treatment .there is insignificant reduction of immunoglobulins level specially IgM. There is disturbance in the serum level of complement components (C3 &C4).There is insignificant reduction in the absolute count of lymphocytes in correlation with duration of treatment

Keywords: immunological response, imatinib, CML

Introduction

Chronic myeloid leukemia (CML) (also known as chronic myelogenous leukemia, chronic granulocytic leukemia) is a clonal disease that results from an acquired genetic change in pluripotential haemopoietic stem cell.

This altered stem cell proliferates and generates a population of differentiated cells that gradually displaces normal hemopoiesis and leads to greatly expanded total myeloid mass. One important landmark in the study of CML was the discovery of the Philadelphia (Ph) chromosome in 1960; another was the characterization in the1980s of the *BCR–ABL* chimeric gene and associated oncoprotein and a third was the demonstration that introducing the *BCR–ABL* gene into murine stem cells in experimental animals caused a disease simulating human CML [(1)]

Imantinib mesylate, is an inhibitor of the family of ABL kinases, these proteins serves a complex role in cell cycling and is important in lymphopoiesis, and has been shown to have impressive clinical activity in chronic myeloid leukemia (CML).[(2)]

Imatinib has been rationally developed as a selective inhibitor of target proteins involved in cellular transformation including BCR-ABL, ABL, c-KIT, ARG and PDGFR-α and β. However, tyrosine kinases are involved in various intracellular signaling pathways and several *in vitro* experimental studies demonstrated that imatinib affects immune response. Imatinib induces antiproliferative effects in CML-derived transformed cell-lines and in native CML myeloid colonies by blocking the tyrosine kinase activity of BCR-ABL proteins in all phases of CML.[(3)]

Its use in stromal-derived gastrointestinal tumors is based on its inhibitory effect on c-kit, and PDGFR inhibition has been a target for a putative role in the treatment of myelofibrosis .Little is known about the consequences of its blocking effect on the normal c-abl proto-oncogene. [(4)]

Differentiation, cytokine production and ability to elicit T cell responses of dendritic cells were impaired by imatinib treatment *in vitro* and in animal models. Moreover, imatinib reduces T-cell proliferation by arresting the cells in G0/G1 and inhibits T-cell effector functions affecting T-cell receptor signal transduction. The inhibitory effect of imatinib on memory cytotoxic T-cell expansion, B-cell proliferation as well as IgM production in response to lipopolysaccharide (LPS) stimulation may be therapeutically useful in treatment of autoimmune diseases. Physiologically, tyrosine kinases play a prominent role in both T-cell and B-cell receptor signal transduction: c-ABL and ARG tyrosine kinases are necessary for TCR dependent transcriptional activation. Primary T-cells lacking functional ABL showed decreased interleukin-2 production and cell proliferation in response to TCR stimulation. Moreover, ABL phosphorylates the B-cell receptor (BCR) co-receptor CD19, suggesting a role for ABL also in regulation of B-cell proliferation. According to this observation, ABL knocked-out mice display several defects in T- and B-cell development.In spite of *in vitro* experimental evidence of imatinib-related immunity impairment,

several years after its introduction in clinical practice no significant major incidence of infection has been reported. In the present study, we report a noteworthy reduction of immunoglobulin (Ig) levels in 31 CML patients treated with imatinib at standard dosage suggesting the direct role of imatinib in a significant alteration in humeral immunity [5]

The aims of this study are to assess the basic immunologic parameters in CML patients treated with imatinib; this aspect can be of interest since patients with these characteristics may be exposed to this treatment for long periods.

Materials and Methods

Thirty one CML patients treated with imatinib mesylate at standard dosage were enrolled in this prospective study. All patients were seen in the Department of hematology at the national center of hematology /Almustansiriya University for six months from February to July 2012.CML patients at the time of data collection were on average 44 years old (range 20-65), 15 were males and 16 females. Patients received imatinib as first line treatment, whereas 29 CML patients received a prior cytoreductive therapy. In particular, 18CML patients received interferon-a (IFN-a), 4 patients hydroxyurea (HU), 5 patients both IFN-a and HU.

The daily median dosage of imatinib was 400 mg/d (range 300-600 mg/d), for a median of 46 months of therapy. In addition, we collected data on immunoglobulin serum levels of 31 CML patients.

Data on blood cell counts and blood film, serum levels of IgG, IgA ,IgM ,C3 and C4;assessed by radial immundiffusion method, were collected for once time after imatinib treatment with variable durations of treatment. In CML patients, we obtained absolute counts of lymphocyte after imatinib treatment.

Results

Table-1: results of immunoglobulins (IgA, IgM, IgG)

Immunoglobulin	Mean	SD	Minimum	Maximum
IGA	223.40	18.78231	60.5	466
IGM	113.18	11.2363	11.6	256.2
IGG	1136.30	70.24294	520.4	2005.2

Table-2: Pearson correlation between duration of treatment and (immunoglobulins and complement)

	IgA	IgM	IgG	C3	C4
R	0.168	-0.040	0.051	0.119	-0.012
P	0.368	0.829	0.784	0.523	0.950

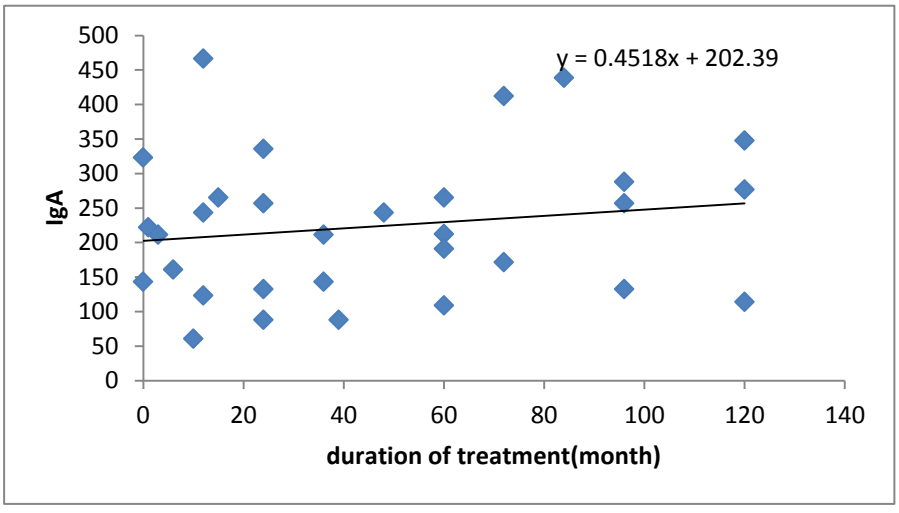

Figure -1: correlation between duration of treatment and IgA

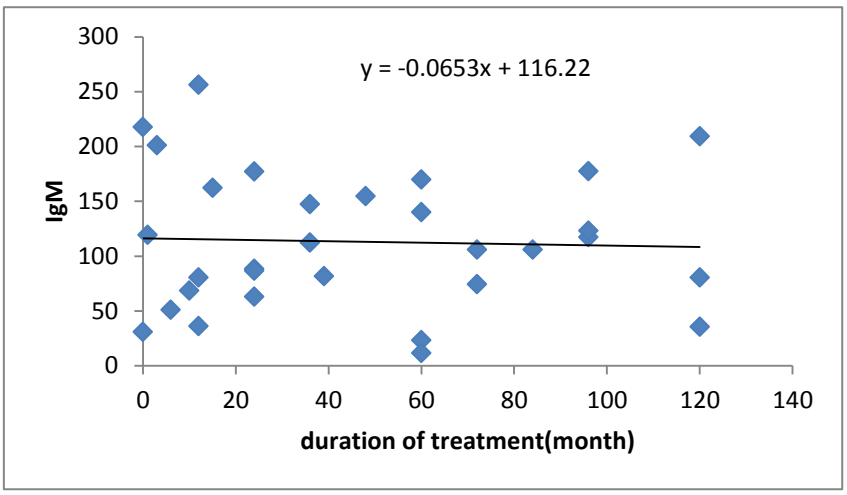

Figure-2: correlation between duration of treatment and IgM

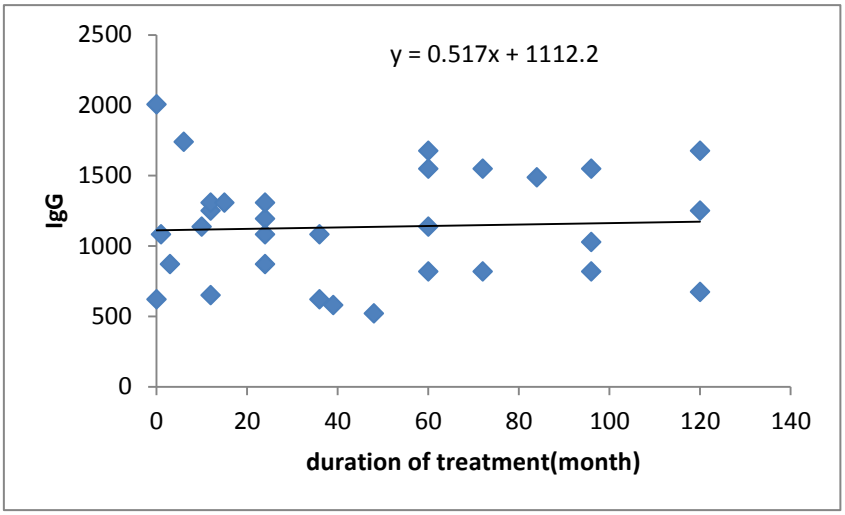

Figure-3: correlation between duration of treatment and IgG

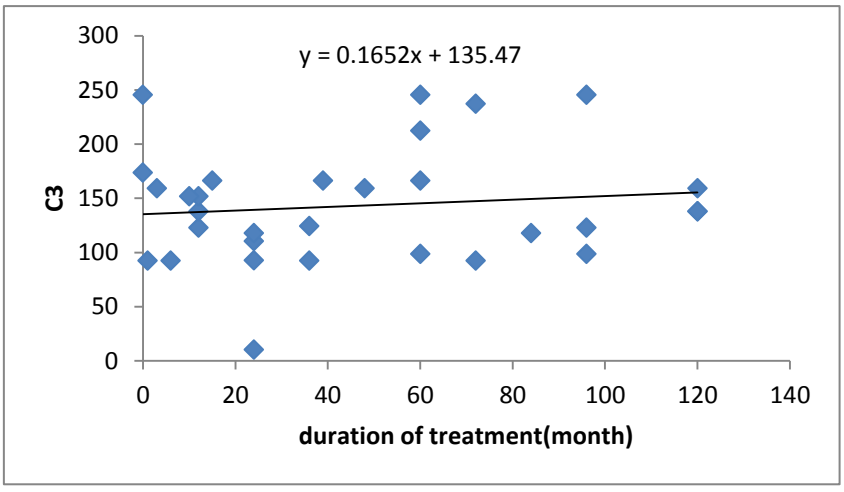

Figure-4: correlation between duration of treatment and C3

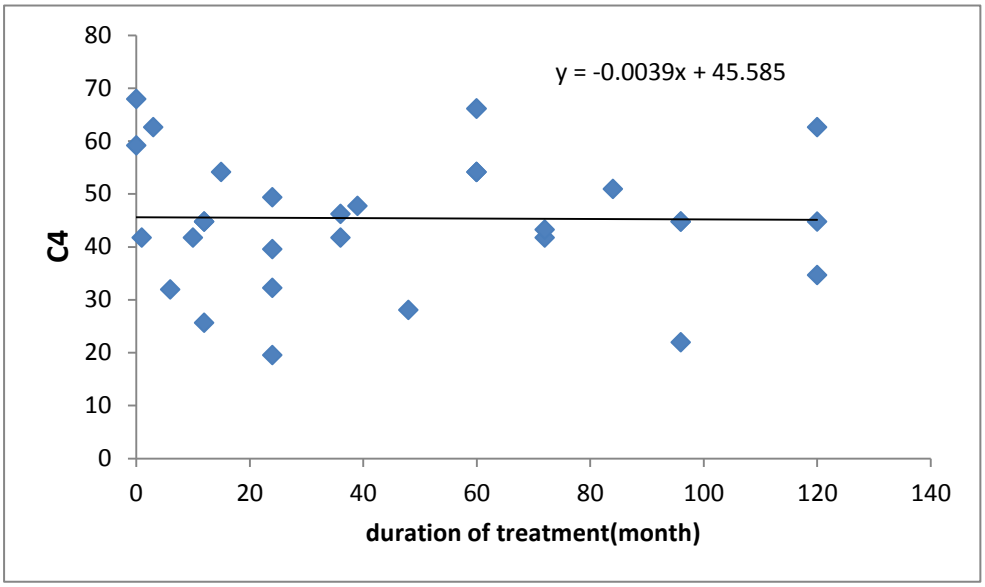

Figure-5: correlation between duration of treatment and C4

Table-3: Pearson correlation between FISH and (duration of treatment, immunoglobulin and complement

	Duration Rx	IGA	IGM	IGG	C3	C4
r	0.061	0.054	-0.190	0.096	-0.111	0.273
P	0.792	0.816	0.409	0.680	0.631	0.231

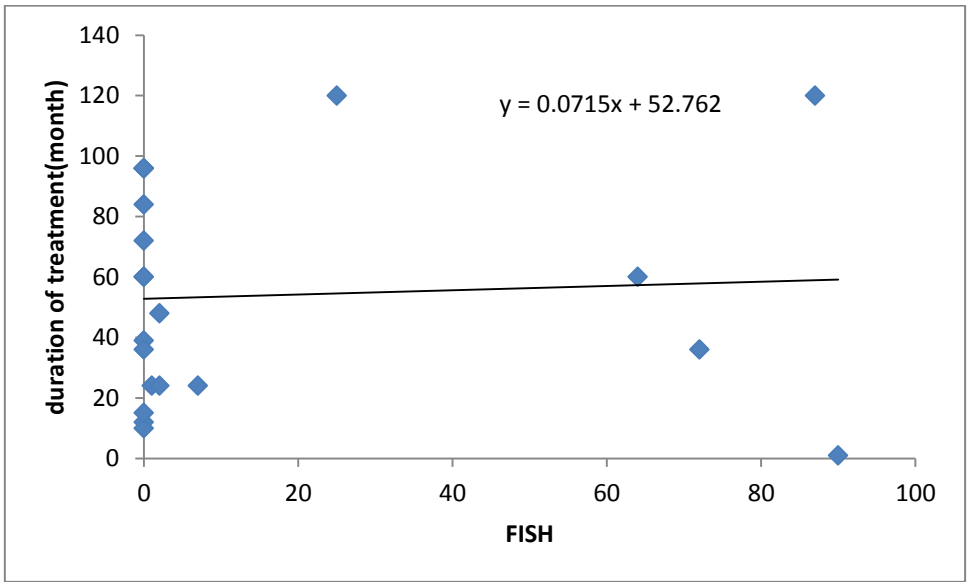

Figure -6: correlation between duration of treatment and cytogenetic study (FISH)

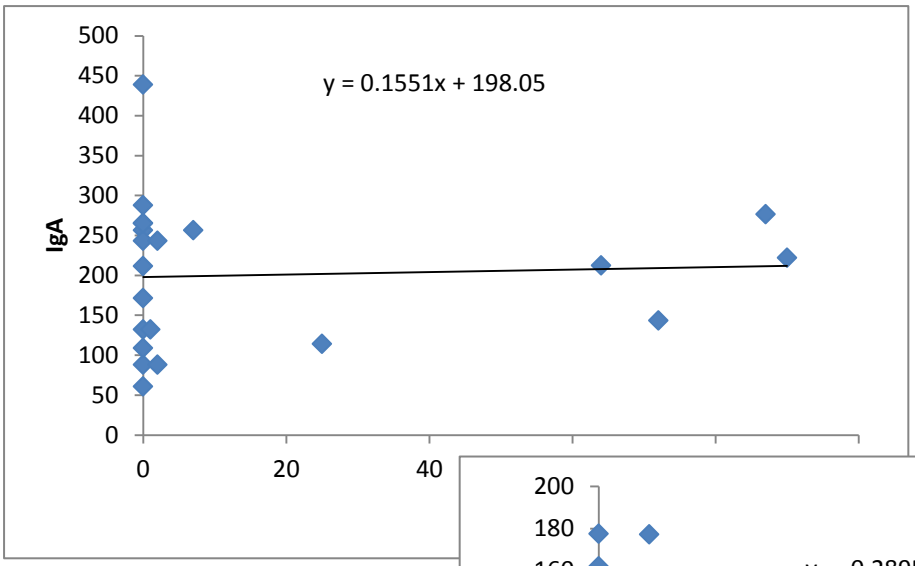

Figure-7 : correlation between IgA and cytogenetic study(FISH)

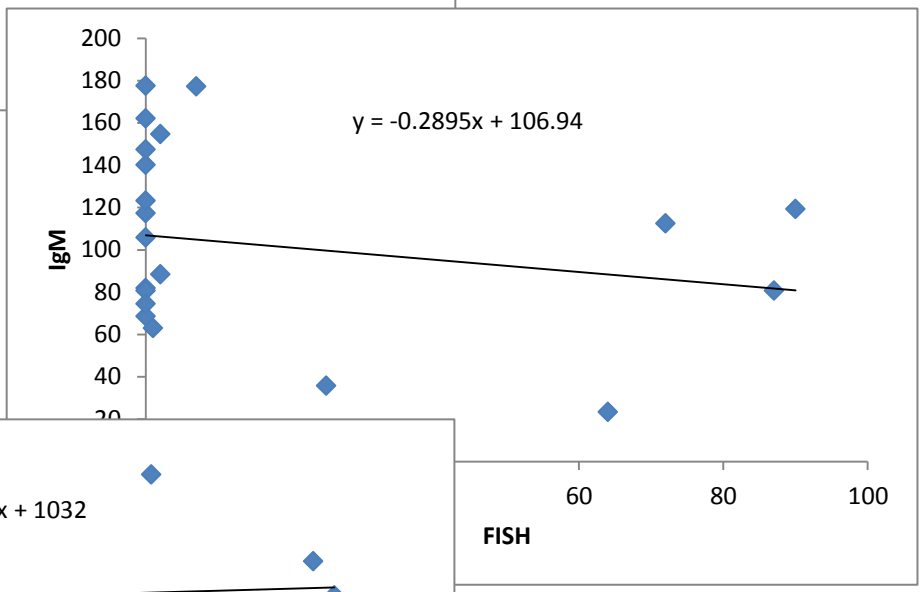

Figure-8: correlation between IgM and cytogenetic study(FISH)

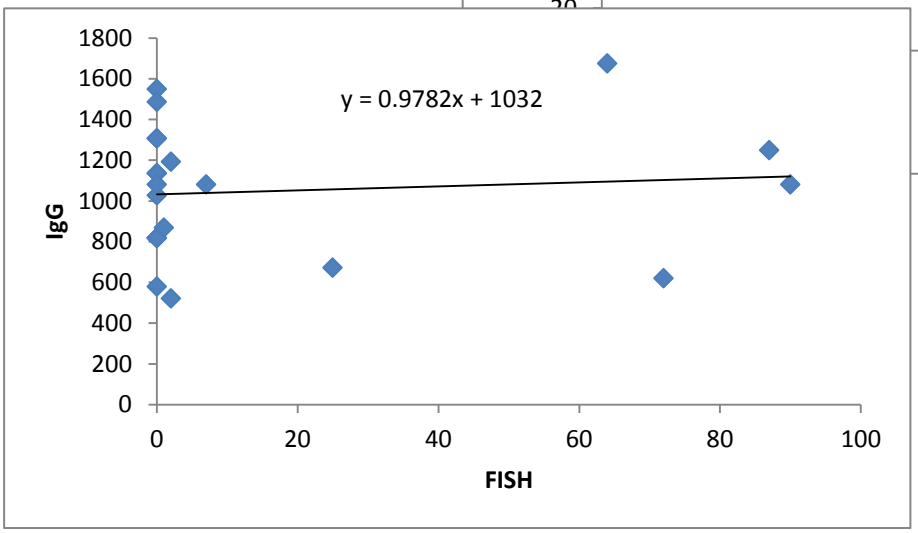

Figure-9: correlation between IgG and cytogenetic study(FISH).

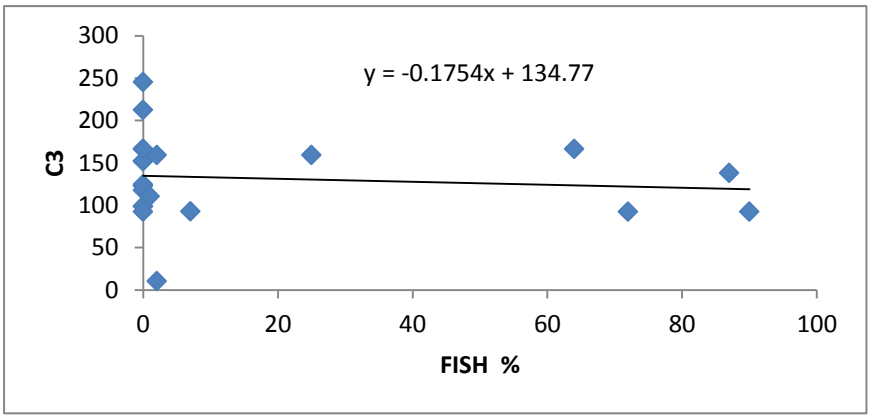

Figure-10: correlation between C3 and cytogenetic study(FISH).

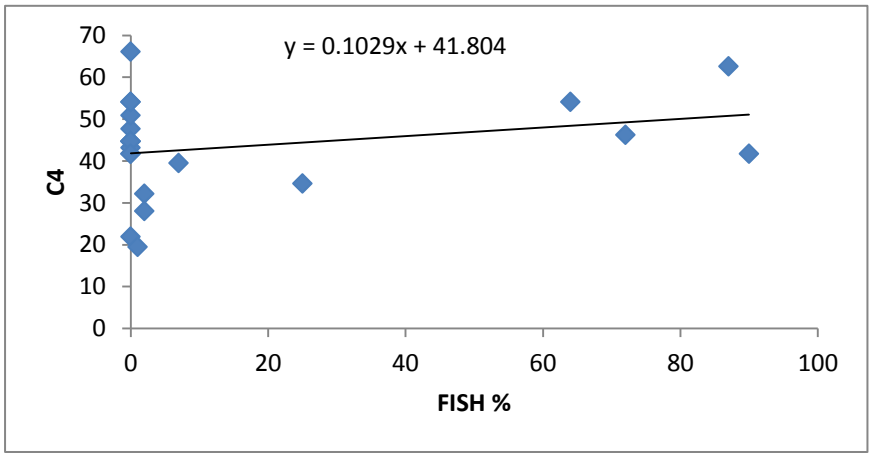

Figure -11 : correlation between C4 and cytogenetic study(FISH).

Table-4: frequency of values of serum level of immunoglobulin and complements those lower than normal range

Parameter	No.	%
IgA	3	9.68
IgM	5	16.13
IgG	6	19.35
C3	1	3.23
C4	1	3.23

Table-5: frequency of values of serum level of complements those upper than normal range

Parameter	No.	%
C3	5	16.13
C4	23	74.19

Table -6: Correlations between hematologic parameters and (duration of treatment and cytogenetic study)

		WBC	LYM	MID	GRAN	RBC	HGB	HCT	MCV	MCH	MCHC	RDW	PLT	MPV
Duration TX	R	.024	-.104-	.074	.033	.195	.140	.102	-.227-	-.170-	.095	-.171-	.272	.201
	P	.897	.576	.693	.862	.294	.453	.585	.220	.359	.610	.358	.139	.278
FISH	R	.383	.347	.387	.377	-.202-	-.314-	-.381-	-.236-	-.117-	.333	.668**	.371	-.115-
	P	.087	.123	.083	.092	.380	.166	.088	.302	.614	.140	.001	.098	.621

Discussion

Twenty four out of thirty one CML patients treated with the imatinib regimen(those had done FISH study) reached a complete cytogenetic response, four patients reached a major cytogenetic response and only three did not reach a partial cytogenetic response similar to the result of Santachiara R. et al. [5]

Reduction of G, M and A immunoglobulin level was documented. Reduction percent were 19.35% for IgG, 16.13% for IgM and 9.68% for IgA, six patients had IgG inferior to normal laboratory range, five patients presented a reduction of IgM and three patients had IgA lower than normal range similar to the result of Santachiara R. et a. [5]

We did not observe significant correlation of immunoglobulin levels compared to duration of treatment. Whoever there is proportional increase of serum levels of IgG and IgA with the duration of treatment while IgM level inversely correlated with the duration of treatment, similar to result of Zuzana Humlová et al. [6]

There is proportional correlation between IgA &IgG levels with the cytogenetic response, while IgM inversely correlated with it.

Regarding C3& C4 they correlate differently with the duration of treatment, where C3 values proportionally related to duration of treatment (DOT), whereas C4 inversely correlated with DOT nearby to the result of Zuzana Humlová et al. whoever in this study about 75% of cases presents with C4 values upper than normal range. Regarding cytogenetic response, C3 inversely correlated with

FISH test results nearby to the result of Zuzana Humlováet al. [6]

Absolute lymphocyte count inversely correlated with DOT, but there is no statistically significant reduction of absolute lymphocyte count, that is similar to the data of Santachiara R.et al. [5]

About the hematological indices; MCV and MCH are inversely correlated with DOT with picture of hypochromic microcytic red cells may be due to chronic illness.

Conclusions:

Imatinib mesylate can induce complete cytogenetic response in a high percent of CML patients. Cytogenetic response correlates well with duration of treatment, there is insignificant reduction of immunoglobulins level specially IgM. There is disturbance in the serum level of complement components (C3 &C4).There is insignificant reduction in the absolute count of lymphocytes in correlation with duration of treatment.

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H.Pylori Associated with Immune Thrombocytopenic Purpura.

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Abstract:
Background: Idiopathic thrombocytopenic purpura (ITP) is a hematological disorder characterized by sensitization of platelets by autoantibodies leading to platelet destruction.). Recently, H. pylori has been found to be associated with ITP and its eradication has shown improvement in platelet count
Objective: To determine the association of *Helicobacter pylori* infection in patients presenting with idiopathic thrombocytopenic purpura (ITP).
Materials and Methods: From October 2012 to March 2013, fifty adult patients with ITP and fifty age and sex matched healthy controls were investigated for the presence of *H. pylori* infection by *Helicobacter pylori* detection IgG and IgM antibody using ELISA method.
Results: *H. pylori* IgM and *H. pylori* IgG: twelve patient (22%) and thirty nine patients (78%), of ITP patients had a positive expression *H. pylori* IgM and *H. pylori* IgG, respectively compared with three (6%) and thirty one (62%) patient in the control group**.** There was statistically significant difference between the mean of ITP patients and healthy control groups.
Conclusion: The study confirms the existence of an association between *H. pylori* infection and ITP.
Keywords: H.pylori, ITP

Introduction:

Idiopathic thrombocytopenic purpura (ITP) is a hematological disorder characterized by sensitization of platelets by autoantibodies leading to platelet destruction. Although its cause remains unclear, ITP is associated with several diseases, including infections [1].

Helicobacter pylori is a Gram-negative microaerophilic bacterium that colonizes the human stomach of more than 50% of the world population. It is recognized as the causative agent of active chronic gastritis and is the predominant cause of peptic ulceration, i.e., gastric and duodenal ulcers [2]. Additionally, *H pylori* is a cofactor in the development of both adenocarcinoma and mucosa-associated lymphoid tissue (MALT) lymphomas, and therefore has been designated as a class I carcinogen by the World Health Organization [3]. Recently, H. pylori has been found to be associated with ITP and its eradication has shown improvement in platelet count [4].

Many hypotheses have been advanced about the mechanisms by which *H pylori* may cause ITP [3]. One of them is molecular mimicry; according to which *H pylori* could induce antibody production in response to antigens that cross react against various platelet glycoprotein antigens [5]. The possible role of CagA-positive strains as a pathogenic candidate for ITP was recognized in two molecular studies. The first showed a decline in platelet-associated immunoglobulin G in ITP patients after the eradication of *H. pylori* infection as well as the existence of a molecular mimicry between those antibodies and the CagA protein. The second study demonstrated that CagA antibodies cross-react with a peptide specifically expressed by platelets of patients with ITP.This study, as well as supporting an association between CagA and ITP, also proposed a possible explanation for the fact that ITP may occur in only a small subset of patients infected by CagA-positive strains [3].

The recent data of an Italian group, showing that the prevalence of the *H pylori* Cag A gene was significantly higher in patients with ITP than in a control group [6]. Other putative targets of molecular mimicry are Lewis (Le) antigens, which are expressed by *H pylori* in a strain-specific manner. Le antigens adsorb to platelets and might serve as targets for anti-Le antibodies in patients with an appropriate genetic background[7], another hypothesis suggests that molecular mimicry of *CagA* or Lewis antigens and platelet antigens may initiate the development of ITP, but with time continued platelet destruction and epitope spreading may lead to the development of chronic thrombocytopenia refractory to eradication of *H pylori* infection [8] .This model is reminiscent of the role played by *H pylori* in the development of MALT lymphomas, which initially may respond to bacterium eradication but may subsequently develop new mutations leading to autonomous disease [9].

Recently, Semple and colleagues demonstrated that in the presence of antiplatelet antibodies, the LPS of Gram negative bacteria can significantly enhance Fc-dependent platelet phagocytosis [10, 11]. These results suggest that infectious agents in combination with antiplatelet antibodies could affect platelet destruction *in vivo*, which may be at least one explanation for why thrombocytopenia worsens in some patients with ITP during infections and, alternatively, resolves in other patients with ITP who are treated with bacterial eradication therapy [12].

Other studies have shown that some strains of *H pylori* bind von Willebrand factor (VWF) and induce glycoprotein Ib (GPIb) – and FcRIIa-dependent platelet aggregation in the presence of *H pylori* antibodies [13]. Activation may promote platelet clearance and antigen presentation, which augments production of antibacterial antibodies. Somatic

mutation may lead to the development of antibodies that either recognizes bacterially derived factors that bind to platelets or cross-react with platelet antigens [14].

Both *H pylori* infection and ITP are associated with a polarized Th1-type phenotype [15, 16]. Accordingly, it may be speculated that *H pylori* infection creates an immunological environment that facilitates the onset and/or persistence of ITP [17].

They document induction of platelet aggregation by *H pylori in vitro* and show that this effect is strain-dependent. Using both the proaggregatory strain (Hp49503) and non aggregatory strains (Hp42504, Hp51932), they demonstrate an essential role for P-selectin and Hp IgG antibody (Hp IGs) in *H pylori*–induced platelet aggregation. This reaction was completely inhibited by anti –P-selectin antibodies. The presence of *H pylori* was shown by demonstration of Hp-specific urease gene fragment in the aggregates. They propose that binding of bacteria/Hp IGs to platelet FcγRIIA receptor activates platelets to release granules and to induce surface P-selectin and von Willebrand factor, leading to aggregation. They also looked into platelet apoptosis evidenced by annexin V binding and membrane blebbing, and observed that both proaggregatory and non aggregatory strains induced apoptosis. Taken together, they conclude that platelet aggregation and apoptosis induced by certain strains of *H pylori* leads to thrombocytopenia [18].

Materials and methods:

This study included 100 subjects, 50 of them were apparently healthy volunteers who considered as a control group, while 50 serum samples were collected from ITP patients. The sampling and work conducted at National Center of Hematology / Al-Mustansiriya University from October /2012 to March /2013.

The average age of ITP patients was 17 – 69 years and gender were 14 males and 36 females, which matched to controls group.

All patients with ITP, defined according to the criteria set forth in the American Society of Hematology (ASH) Guidelines [19].

Complete blood count and examination of peripheral blood smear were done to confirm the diagnosis of ITP.

H. Pylori infection was detected *H.pylori* (IgG & IgM) antibody in patients and controls sera using serological method the enzyme-linked immunosorbent assay (ELISA) (Novotech/germany) for detecting IgG and Monbind /USA) for detecting IgM.

Detection H.pylori IgG antibody.

Reagent and sample preparation:

All reagents are brought in room temperature (15 – 25˚C) before use.

Wash solution is diluted (1 + 20) with fresh deionized water.

Stability up to 60 days at (15 – 25˚C)

The patient's sera were diluted by mixing with vortex 10 µ sera +1000µ dilution buffer and incubated at least 5 min.

Assay procedure:

Dispend 100µ of standards, and diluted samples were added to the appropriate wells, mixed carefully. Covered with foil and incubated 1hour at 37˚C.After incubation the microtiter plate was washed 3 times by adding 300µ washing buffer to each wells.100µ of *H.pylori* anti- IgG conjugate was added to each well. Covered with foil and incubated 30 min at 17 - 25˚C. After incubation the microtiter plate was washed 3 times by adding 300µ washing buffer to each wells. 100µ of substrate reagent was added to each well. Covered and incubated in dark 15 min at room temperature. 100µ of stop solution was added to each well. Mixed carefully. The optical density (O.D) was read at 450 nm as the primary wavelength and 620 nm as the reference wavelength using a microtiter reader.

Calculation of the result:

The standard curve was generated by plotting the O.D (450) obtained for each of the standard concentration on (linear/linear) graph paper in a system of coordinates against their corresponding concentration (0,15,75,and 150 NTU/ml) and draw a standard calibration curve (absorbance value on the vertical y – axis , concentration on the horizontal x – axis).

Read result from this standard curve employing the absorbance value of each patient specimen Finger (1).

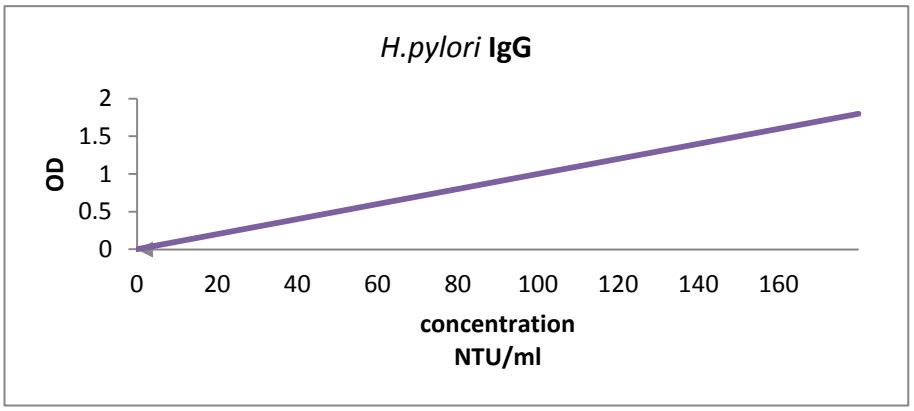

Figure 1: *H.pylori* IgG calibration curve

Detection H.pylori IgM:

Reagent preparation:

All reagents should be allowed to reach room temperature (25 ˚C) before used. Diluted 1 volume of wash buffer (20X) with 19 volume of distilled water.

The patient's sera, negative control, positive control, and calibrator were diluted by adding 10 µ+ 400µ dilution buffer mixed well with vortex and incubated diluted sample at least 5 min .

Assay procedure:

Hundred µl of diluted samples, calibrator, and controls were added to the appropriate wells, mixed carefully. Covered and incubated 30 min. at room temperature. After incubation the microtiter plate was washed 4 times by adding 300µl washing buffer to each wells and one time with distilled water. 100µl of *Helicobacter pylori* anti- IgM conjugate was added to each well. Covered and incubated 30 min at room temperature. After incubation the microtiter plate was washed 4 times by adding 300µl washing buffer to each wells and one time with distilled water. 100µl of substrate reagent was added to each well. Covered and incubated in dark 20 min at room temperature. 100µ of stop solution was added to each well. Mixed carefully. The optical density (O.D) was read at 450 nm as the primary wavelength and 620 nm as the reference wavelength using a microtiter reader.

Calculation of result

Calculate the *H. pylori* IgM EIA Index of each determination by dividing the mean values of each sample by calibrator means value.

Results

The comparison of these two categories (the ITP group fifty patients mean age (40±2.1 years) with mean Platelet count (79.1±10.1) x 10^9/L with the control group fifty healthy control with a mean age (40.2 ± 2.0years) and mean platelet count (253±72) x10^9/L showed substantial variations in the expression of *H. pylori* IgM and *H. pylori* IgG : twelve patient (22%) with mean platelet count (69.17±14.7) x10^9/L and thirty nine patients (78%) with mean platelet count (73.92 ±9. 18) x10^9/L, ITP patients had a positive expression *H. pylori* IgM and *H. pylori* IgG, respectively compared with three (6%) and thirty one (62%) patient in the control group**.** There was statistically significant difference (p=0. 02) between the mean of ITP Patients and healthy control groups In case of detection acute infection of H. *pylori* IgM Table (1).

Table (1): Comparison of acute infection *H.pylori* IgM between ITP patient and healthy control.

Parameter	**ITP Patient n=50**		**Control n=50**	**p-value**
***H. pylori* IgM**	95% Confidence interval (%)	0.84 – 0.95	0.73 – 0.86	0.02*
	Mean± SE	0.89 ± 0.027	0.79 ± 0.03	
* (P<0.05), ** (P<0.01), NS: Non-significant.				

In case of *H. pylori* IgG, there was statistically highly significant difference ($p < 0.001$) between ITP group and healthy control group Table (2) .

Table (2): Comparison of infection *H.pylori* IgG infection between ITP patient and healthy control.

Parameter	**ITP Patient n=50**		**Control n=50**	**p-value**
***H. pylori* IgG**	95% Confidence interval (%)	51.4 – 76.1	40.1 – 67.5	<0.001**
	Mean± SE	63.7 ± 6.14	53.8 ± 6.8	
* (P<0.05), ** (P<0.01), NS: Non-significant.				

Discussion:

The detection method in these studies was serological method detection antibodies IgG and IgM in ITP patient and control.

The prevalence of *H.pylori* – IgG positivity in the ITP group was 78% was highly significant when compared with control group 62% (P <0.001), while the prevalence of *H. pylori* - IgM positivity in the ITP group was 22% was significant when compared with control group 6%(P=0.02) .

The current result was supported by reports described by other investigators [4, 20] which found *H. pylori* infection should be considered in the differential diagnosis of all cases of thrombocytopenia, and should be eradicated in all H. pylori-positive patients with thrombocytopenia.

The actual pathogenetic mechanisms between *H. pylori* and ITP have not been clarified yet. There are many speculations about pathogenesis of ITP in patients with *H. pylori* infection. It is suggested that active *H. pylori* infection triggers humeral and cellular immune responses, which probably induce *H. pylori*-associated diseases including ITP through molecular mimicry [21]. Furthermore, many data indicate that the effect of eradication treatment for *H. pylori* probably depends on genetic factors of the host, strains of the bacterium or existing ethnic factors. According to these data, *H. pylori* infection influences the pathophysiology of ITP through various mechanisms [22, 23].

The Cag A antigen of *H.pylori* could be responsible for cross – mimicry between *H. pylori* and platelets glycoproteins[3].This hypothesis was not confirmed by Michel and his colleges who tested platelet elutes from three *H. pylori* positive patients with ITP for *H. pylori* antibodies, but none was found to be positive[24]. Conversely Takashashi and his colleagues showed that eluted platelets- associated immunoglobulin G from 12 out of 18 ITP patients' recognized *H. pylori* Cag A protein and that crass reactive antibody level decreased following *H. pylori* eradication in patient who shoed complete platelet recovery [25].

Another factor responsible for molecular mimicry may be the babA gene (blood group antigen – binding adhesion gene) expressed by some *H.pylori* strains, which codifies for antigenic epitopes which recognize sequences of Lewis blood group (Le antigens): these antigens are adsorbed by platelets and could become the target for anti – Le antibodies produced by some patient with susceptible background [7].

The development of thrombocytopenia in *H.pylori* patient infection may also depending on genetic influence .In fact, observed a difference in HLA class II allele patterns ITP patient with or without *H. pylori* infection [26].

Overproduction of TNF-α can lead to autoimmune disease. Hence, *H. pylori*–induced alterations in cytokine profiles might promote development of immune thrombocytopenia [20].

Conclusion:

The study confirms the existence of an association between *H. pylori* infection and idiopathic thrombocytopenic purpura. Further in vestigations on a larger number of patients might allow a better definition of the true prevalence of *H. pylori* infection in patients with ITP.

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Expression of CD10, CD13,CD19, CD20, CD22, CD33, CD34 And CD45 on B Lymphoblasts in Bone Marrow Aspirate of Adult Patients with Newly Diagnosed Acute Lymphoblastic leukemia Using Multicolor Flow Cytometry

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Abstract

Background: According to the 2008 WHO classification, the diagnosis of ALL relies on morphological demonstration of presence of 25% or more blast cells in bone marrow in ALL, and to consciously decide on the B lymphoid lineage of the blast cell population.

Objectives: To evaluate the expression of CD10, CD13, CD19, CD20, CD22, CD33, CD34 and CD45 on B lymphoblasts in bone marrow aspirate of adult patients with newly diagnosed acute lymphoblastic leukemia using multicolor flow cytometry.

Materials and methods: This is a cohort study which included 14 newly diagnosed adult patients with B ALL from April 2012 to May 2013. Flow cytometry analysis for CD10, CD13, CD19, CD20, CD22, CD33, CD34 and CD45 was carried out in a private laboratory on bone marrow aspirate samples using 2-laser, 4-color PARTEC Cube6 and using De Novo FCS Express version 4 Flow Cytometry software. The sensitivity of fluorescent detectors was monitored using standard beads according to the manufacturer's recommendations and normal lymphoid cells within specimens served as internal positive and negative controls. Samples with blasts that aberrantly expressed CD13 or CD33 were tested with SBB cytochemical stain.

Results: The range and median percentages for B lymphoblasts, cells in the CD45dim versus low SSC gate, CD10, CD19, CD20, CD22, CD34, CD13 and CD33were68-99 and 85, 74-99 and 86, 41-88 and 68, 60-99 and 80, 48-65 and 57, 48-99 and 75, 71-98 and 83, 20-51 and 37, and 20-43 and 30% respectively.

Conclusions: FC testing should be accompanied by proper morphological evaluation. For diagnosis of B ALL, start with serial gating using FSC versus SSC, then CD45 versus SSC, then CD34 versus SSC, then using CD19, CD10 and CD22 to confirm the lineage of blasts as B lymphoid.

Introduction:

The 2008 WHO classification of acute lymphoblastic leukemia (ALL) aims for both diagnosis and risk stratification. For diagnosis, the WHO classification relies on the clinical presentation, morphological assessment and immunophenotyping, and for risk stratification, it is based on cytogenetic with/without molecular studies [1].

Traditionally, in the FAB classification the diagnosis of ALL is largely that of exclusion, i.e., absence of evidence of myeloid differentiation and maturation is ALL diagnosis [2].

According to the 2008 WHO classification, the diagnosis of ALL relies mainly on two findings; First, morphological demonstration of presence of 25% or more blast cells in bone marrow in ALL; however, in patients with t(5;14) combined with reactive eosinophilia, B ALL can be diagnosed with any percentage of blast cells. Second, is to consciously decide on the B lymphoid lineage of the blast cell population. This can be evident from finding pathognomonic immunophenotyping with flow cytometry (FC) and/or immunohistochemistry (IHC), which usually include CD19 and either CD10, CD22 and/or CD79a[1].

Moreover, demonstration of aberrant marker expression by B lymphoblast cells, as CD13 and/or CD33 can indicate the malignant/leukemic nature of this population and is very useful with the follow-up for remission and MRD [3].

The use of CD34 in flow cytometry in the majority of cases of acute leukemia is important to better define the blast population which are found in the blast gate [4].

The aim of this study is to evaluate the expression of CD10, CD13, CD19, CD20, CD22, CD33, CD34 and CD45 on B lymphoblasts in bone marrow aspirate of adult patients with newly diagnosed acute lymphoblastic leukemia using multicolor flow cytometry.

Patients, materials and methods:

This is a prospective cohort study which included 14 patients with B ALL who had bone marrow aspirate samples submitted for evaluation by acute leukemia panel by flow cytometry in a private Laboratory during April 2012 to May 2013. The patients were admitted to the National Center for Hematology, to Baghdad Medical City and to Alkadhimiya Teaching Hospital. In this study, all patients were adults and their identification details were rendered anonymous. Data included in this study were extracted from diagnostic database and sample collection procedure was in consistence with the FDA guidelines for in vitro diagnostic device studies using leftover human specimens that are not individually identifiable that would save the need for signed or verbal informed consent [5].

FC analysis was carried out on EDTA-anticoagulated BMA samples using 2-laser, 4-color PARTEC Cube6 and using De Novo FCS Express 4 Flow Cytometry software.

Sample processing for Partec Cube6 flow cytometer was performed using the technique of Stain–Lyse–No Wash, and it involved addition of surface antibodies only. 10µ of each surface

antibody was added to 100µ of well-mixed EDTA-anticoagulated blood into labeled tubes and incubated in the dark at room temperature for 15 minutes. Then 100µ of solution A (for fixation of leucocytes) was added, and incubated in the dark at room temperature for 10 minutes. Then 2.5ml of solution B, for lysis of erythrocytes, was added and incubated in the dark for 20 minutes. Then the solution was re-suspended by vigorous mixing and shaking and data were acquired on the flow cytometer.

The sensitivity of fluorescent detectors was monitored using standard beads according to the manufacturer's recommendations and normal lymphoid cells within specimens served as internal positive and negative controls for various antigens tested. The samples were tested for the following protocols:

Tube number	Blue laser			Red laser
	FITC	PE	PE-DY^{647}	APC
1	CD34	CD19	CD45	CD20
2	CD10	BLANK	CD45	BLANK
3	CD34	BLANK	CD22	BLANK
4	BLANK	CD13	CD45	CD33

The serial gating used in this study involved in the first plot gating on viable cells using forward scatter (FSC) versus side scatter (SSC) plot, then gating on blast region using CD45 versus SSC plot, then gating on CD34+ blasts using CD34 versus SSC plot, then gating on CD19+ versus SSC plot, then drawing different plots to study the expression of other markers.

In patients with aberrant expression of CD13 or CD33, Sudan Black B (SBB) cytochemistry was used to exclude possibility of mixed phenotypic acute leukemia.

In many samples, electronic gating was used to ensure consistency of plots across different tubes of the same patient. Back gating was used when necessary to ensure appropriateness of forward gating.

In this study, arbitrary cut-off points for positive results by flow cytometry were taken as 20% for all the surface markers [6].

SPSS version 18.0.0 was used for statistical analysis of data.

Results:

A total of 14 adult patients, 9 males and 5 females with male: female ratio of 1.8:1, with newly diagnosed B ALL was enrolled in this study. The median age for patients with B ALL was 32 years with range from 16 to 84 years.

B Lymphoblasts range was 68-99%. The proportion of cells in the blast gate, as assessed by CD45^{dim} versus low SSC, was increased in all patients with B ALL (table 1 and figure 1).

CD19 was universally expressed on lymphoblasts of patients with B ALL (table 1 and figure 1).

CD10, CD20, CD22 and CD34 were expressed on B lymphoblasts in 71.4%, 42.8%, 85.7%, 92.9% and 92.9% patients with B ALL respectively(table 1 and figure 1).

CD13 and CD33 were aberrantly expressed on B lymphoblasts in 21.4% and 14.3% patients with B ALL respectively (table 1 and figure 1).

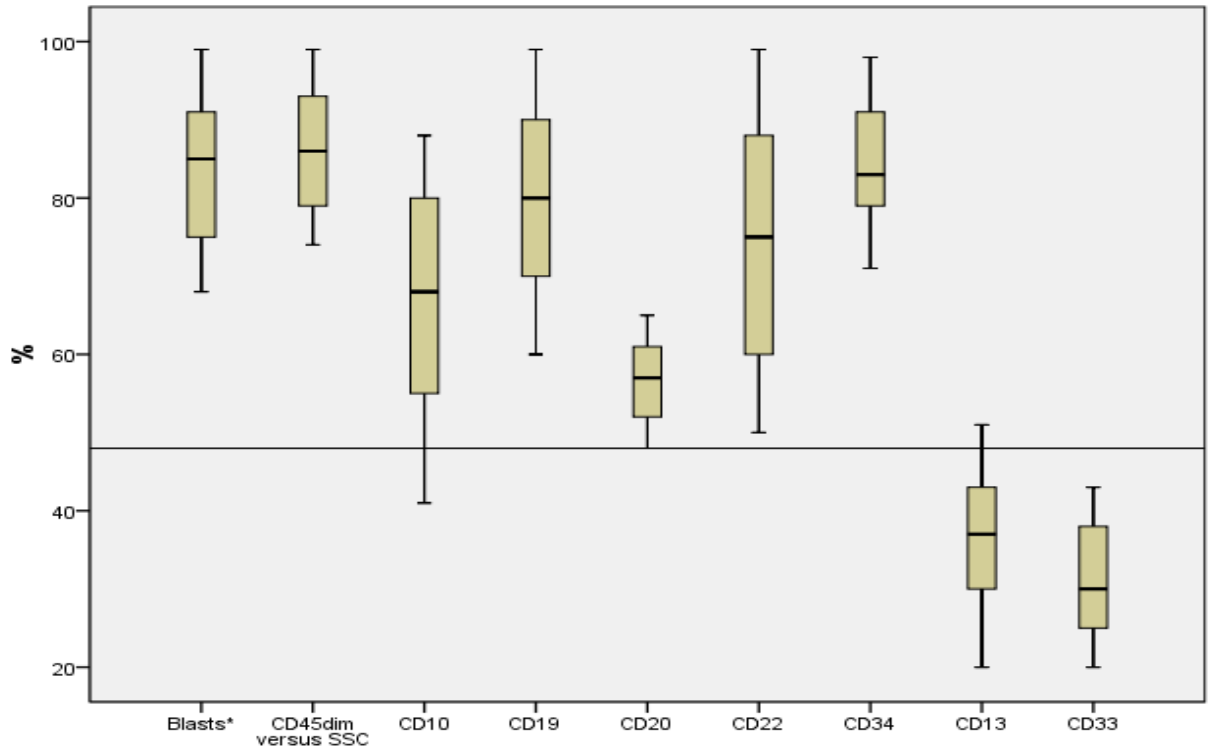
Table 1: Percentage of blasts, proportion of cells in the CD45^{dim} versus low SSC gate, and expression of CD10, CD19, CD20, CD22, CD34, CD13 and CD33 on blast cells of patients with B ALL

Parameter	Blasts*	CD45^{dim}/ low SSC (n=14/14)	Antigen % expression						
			CD10 (n=10/14)	CD19 (n=14/14)	CD20 (n=6/14)	CD22 (n=12/14)	CD34 (n=14/14)	CD13 (n=3/14)	CD33 (n=2/14)
Range	68-99	74-99	41-88	60-99	48-65	48-99	71-98	20-51	20-43
Median	85	86	68	80	57	75	83	37	30
IQR	18	15	27	22	17	30	13	17	14
Mean ± SD	84.14 ± 9.84	86.1 ± 8.1	66.81 ± 14.7	80.43 ± 11.88	56.57 ± 5.23	74.43 ± 15.6	84.29 ± 8.2	36.19 ± 9.64	31.14 ± 7.38

"n" represents number of samples with positive expression of CD marker per number of experiments.

* Blasts' percentage is assessed by morphology.

Figure 1: Range, inter-quartile range (IQR) and median percentage of blast cells, proportion of cells in the CD45dim versus low SSC gate, and expression of CD10, CD19, CD20, CD22, CD34, CD13 and CD33 on blast cells of patients with B ALL.



Discussion:

Morphologically, the presence of blasts has a central role in diagnosis of ALL. In the 2008 WHO classification, a 25% cut-off point has been suggested to arbitrarily define ALL[1].In this study, no such difficulty was faced as the lowest blast cell percentage as assessed by morphology was 68% for B ALL.

When using flow cytometry, proper gating is the most important step in making the correct diagnosis. The homogeneous gating based on physical properties of cells, i.e., forward and sideway light scatter which is most useful with modern flow cytometers to gate on viable cells and to get rid of cellular and technical debris. The second step should involve heterogeneous gating by using fluorescence antibody versus SSC as this is more specific for cell subset analysis. Leukocyte gating uses CD45, B-cell gating uses CD19, and gating on blast cell uses CD34 [7]. When comparing heterogeneous with homogeneous gating, the percentage of tumor cells isolated by the former technique is consistently higher than that isolated by the latter "conventional" technique, no matter whether it is lymphoma or leukemia or what kind of specimen is involved. The rationales behind using heterogeneous gating are to get rid of the excess of non-leukocytes exhibiting leukocyte-like scatter characteristics leading to a lower fraction of recovered leukocytes, and because in the leukocyte populations, the cell size (represented by FSC) and cytoplasmic complexity (represented by SSC) may overlap, causing some difficulty in their separation[8].

There is no standard routine that is universally agreed upon when trying to analyze the FC results' plots; however, a rational approach can start with the use of FSC versus SSC to get rid of technical debris, then the use of CD45 versus SSC to separate cell populations into lymphocyte, monocyte, basophil, neutrophil and immature cells up to myelocytes, eosinophils and blast gate. Later on a step of "back-gating" using population specific marker can be useful in certain occasions to check for the correctness of the "forward" gated populations.

In this study, the use of CD45 versus SSC plot to examine for increased proportion of cells in the blast gate proved to be essential as there were universal increase of blast cells in this gate in patients with ALL.

Detection of aberrant expression of antigen on surface of leukemic cells can be useful to indicate the malignant nature of cells and in follow up for MRD; however, to be useful in practice, the percentage expression of the aberrant marker should be as high as possible, otherwise, it can be genuinely absent in patient with residual/relapse leukemia. Aberrant expression of CD13 and CD33 on surface of lymphoblasts were detected in small percentage

of patients with ALL, the percentage of expression was significantly lower for both markers in comparison to its expression on myeloblasts. An important point was that the aberrant expression was either of CD13 or CD33 but not both in the same patient with ALL.

The use of CD34 is critical in patients with ALL, as it was expressed in very high percentage, and is the best-known defining markers for acute leukemia as it confirms the nature of the cell population as being immature (precursor).

Incorporating CD10, CD19 and CD22 is essential for diagnosis of B ALL according to the 2008 WHO classification, as positivity for CD19 plus at least one more marker is a condition for assignment of acute leukemia to B lymphoid lineage.

Conclusions:

1. FC testing should be always accompanied by proper morphological evaluation, to provide a comprehensive indication, guide the choice of the right immunophenotyping panel and to properly interpret the results.
2. For diagnosis of B ALL, start with serial gating using FSC versus SSC to get rid of the technical debris, then CD45 versus SSC plot to gate on the possible blast cells which are usually $CD45^{dim}$with low SSC, then CD34 versus SSC to identify the precursor (immature) nature of blast cells. Then using CD19 that is universally sensitive for B ALL, and confirming the nature of CD19+ blasts as being B lymphoblasts by testing for CD10 and CD22.
3. The use of CD20 is not essential for diagnosis of B ALL. However, this optional marker can help to decide on adding a therapeutic option.

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Assessment of Soluble Fas in Patients with Chronic Myeloid Leukemia

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ABSTRACT

Background: Soluble Fas was produced from the cell surface of malignant cells in a form lacking the 21 amino acid residues containing the transmembrane domain by alternative splicing. Trimerization of Fas receptor can be inhibited by soluble Fas (sFas) that act as decoys, binding FasL and preventing association with transmembrane Fas. So it is supposed to act as a FasL inhibitor to bind Fas and prevent Fas-mediated apoptosis.

Objective: to evaluate serum soluble Fas (sFas) level in patients with chronic myeloid leukemia

Materials and Method: Serum levels of sFas were measured by ELISA method after venous blood was collected from 56 CML patients (newly diagnosed and optimally treated) and 28 healthy subjects as control group. Absorbance was read at a wave length of 450nm using ELISA reader. Soluble Fas level was then calculated by plotting the optical density (O.D.) of each sample against the concentration in the standard curve.

Results: There were no significant increases in serum sFas patient compared to healthy control with P=0.09. When the mean sFas concentration was obviously highest in newly diagnosed (1163.6pg/ml) followed by optimally treated (1021.7 pg/ml) and lowest in healthy control (970.1pg/ml).

Conclusion: Production of sFas in tumor patients may be a key mechanism to inhibit Fas-mediated apoptosis. The identification of sFas levels as a predictor of outcome in malignant disease further establishes a connection between Fas loss-of-function and tumor progression.

Keywords: Chronic myeloid leukemia (CML), Soluble Fas (sFas), chronic phase (CP), Philadelphia chromosome.

Introduction

Chronic myeloid leukemia (CML) is a myeloproliferative disorder that consequent from the acquisition of a characteristic genetic rearrangement, the Philadelphia chromosome (t9:22), by a hemopoietic stem cell [1, 2]. So it is a malignant clonal disorder of hematopoietic stem cells that results in increases, not only of myeloid cells but also erythroid cells and platelets in peripheral blood with marked myeloid hyperplasia in the bone marrow [3, 4]. The overall incidence of the disease is 1-2 cases per 100,000 populations [5, 6]. It accounts for about 15% of all adult leukemia [7, 8], and although it is uncommon before the age of 10 years, it can strike at any age, with the median age at diagnosis is 45 to 55 years [9]. There are 12 - 30 percent of patients are 60 years of age or older [10], but most cases are identified in the fifth and sixth decades of life, with higher incidence in males than females [11]. It is a triphasic disorder, where the majority of patients present in chronic phase (CP), a stage characterized by <10% peripheral or bone marrow blasts, <20%peripheral or bone marrow basophils and Clonal evolution at the time of diagnosis. If left untreated, the initial CP lasts for approximately 3-6 years, before progressing to accelerated phase (AP). Then the disease progress with additional cytogenetic abnormalities arise and, eventually terminate in blast crisis (BC), with overproduction of blast cells within the BM compartment, where prognosis is generally poor [10, 12, 13, 14, 15].

There are two major forms of the Fas molecule, membranous Fas (CD95) and soluble Fas (sFas) [16]. Molecular cloning and nucleotide sequence analysis revealed a human Fas messenger RNA variant capable of encoding a soluble Fas molecule lacking the transmembrane domain because of the deletion of an exon encoding this region [17].

Trimerization of Fas receptor can be inhibited by soluble Fas (sFas) that act as decoys, binding FasL and preventing association with transmembrane Fas [18, 19]. Soluble Fas was produced from the cell surface of malignant cells in a form lacking the 21 amino acid residues containing the transmembrane domain by alternative splicing. And it is supposed to act as a FasL inhibitor to bind Fas and prevent Fas-mediated apoptosis [20].

Production of sFas in tumor patients may be a key mechanism to inhibit Fas-mediated apoptosis. The identification of sFas levels as a predictor of outcome in malignant disease further establishes a connection between Fas loss-of-function and tumor progression [21].

Aim of study

The aim of this study was to evaluate serum level of sFas in patients with chronic myeloid leukemia at time of diagnosis (newly diagnosed), then after receiving imatinib mesylate treatment, with good response (optimal response) and compare them with healthy control and to evaluate the role of these factors in apoptosis according to the response to treatment of chronic myeloid leukemia.

Materials and Methods

Sampling

The study was conducted between November 2012 up to June2013; during this period 56 Iraqi patients with CML evaluated at Baghdad teaching hospital/hematology department. Of these CML patients, 28 patients were newly diagnosed CML while another 28 patients were CML on Glivec therapy (400 mg/day) for at least 12 months with optimal response (FISH BCR-ABL1 result < 1%).

All patients were free of fever and other chronic illness such as diabetes mellitus, hypertension and infection; also they had no history of smoking and drinking of alcohol. An evaluation of these cases complete blood count (CBC)&fluorescence *in situ* hybridization BCR-ABL(FISH BCR-ABL) was used in diagnosis and assessment the responses states to Tyrosine kinase inhibitor(TKI).

Method

After taking the agreement from each patients and control, 5 ml of venous blood was taken by using disposable syringe, dispensed in plain tube and left to clot at room temperature (22°C). Then, it was centrifuged at 3000 rpm for 10 min to collect serum. The serum was stored in Eppendorff tube in the freezer in (-20°C) until use for assessment of sFas in Education Laboratories of Medical City. The method is a solid phase sandwich ELISA (Abcam Company, Uk).It utilized a monoclonal antibody (capture antibody) specific for human sFas coated on a 96-well plate. Standards and samples added to the wells, and any human sFas present bind to the immobilized antibody. The wells washed and biotinylated polyclonal anti-human sFas antibody (detection antibody) added. After a second wash, avidin-horseradish peroxidase (avidin-HRP) added, producing an antibody-antigen - antibody sandwich. The wells washed again and a substrate solution added, which produced a blue color in direct proportion to the amount of human sFas present in the initial sample. The stop solution then added to terminate the reaction. This results in a color change from blue to yellow. The wells then read spectrophotometrically at 450 nm.

Statistical Analysis

Statistical analyses were done using Statistical Package for Social Sciences (SPSS) version 20 computer software. Data were analyzed with descriptive statistics (mean ± SD), Effect size (Cohen's d) and multiple liner regression models.

Results

Out of 56 CML patients were included in the study, 25 (44.6%) were males and 31 (55.4%) were females (M: F ratio 0.8:1). Age ranged from 15 years to 78 years (mean age 42.6± 14.0). Twenty eight samples of apparently healthy volunteers were included and evaluated as control samples, 14 (50.0%) were males and 14 (50.0%) were females (M: F ratio 1:1). Age ranged from 19 years to 73 years (mean age 42.9± 14.8). All patients with CML and the healthy control samples evaluated for serum sFas levels. The mean sFas concentration was obviously highest in newly diagnosed (1163.6pg/ml) followed by optimally treated (1021.7 pg/ml) and lowest in healthy control (970.1pg/ml). This means that there was no significant increase in serum sFas patient compared to healthy control (Table 1). The mean± SD (pg/ml) of serum sFas level for the newly diagnosed patients was 1163.6pg/ml±231.5 and for the optimally responder cases was 1021.7pg/ml ±360.6.While the healthy control group was 970.1pg/ml ±361.7. Having a newly diagnosed CML with increase sFas concentration by a mean of 193.5pg/ml compared to healthy controls. This disease effect was evaluated as moderately strong effect (Cohen's d 0.64).Optimally treated CML with increase sFas concentration by a mean of 51.6pg/ml compared to healthy controls. This disease effect was evaluated as very weak effect (Cohen's d 0.14).Optimally treated CML with reduce sFas concentration by a mean of 141.9pg/ml compared to newly diagnosed. This disease effect was evaluated as moderately strong effect (Cohen's d 0.46).

Table 1: The mean level of sFas concentration for CML patients and healthy control.

	Study group			
	Healthy controls	**Newly diagnosed CML patients**	**Optimally treated CML patients**	**P**
sFas (pg/ml)				0.09[NS]
Range	(494.7 - 1660.5)	(714.1 - 1714.9)	(454.1 - 1634.4)	
Mean	970.1	1163.6	1021.7	
SD	361.7	231.5	360.6	
SE	70.9	45.4	68.2	
Number	26	26	28	
Newly diagnosed CML cases x Healthy controls:				
Difference in mean =	193.5			
Effect size (Cohen's d)=	0.64			
Optimally treated CML cases x Healthy controls				
Difference in mean =	51.6			
Effect size (Cohen's d)=	0.14			
Optimally treated CML cases x Newly diagnosed CML cases				
Difference in mean =	-141.9			
Effect size (Cohen's d)=	-0.46			

The effect of study group compared to healthy controls on sFas concentration was evaluated after adjusting for the possible confounding effect of age and gender in a multiple liner regression model. The model was statistically significant and able to explain 28% of observed variation in the response variable (sFas concentration).A newly diagnosed CML is associated with statistically significant increase in sFas concentration by a mean of 179.9pg/ml compared to healthy controls, after adjusting (controlling) for the confounding effect of age and gender .Optimally treated CML on the other hand was associated with a marginal and no significant increase in sFas concentration 68.8pg/ml compared to healthy controls, after adjusting (controlling) for the confounding effect of age and gender. Age had a statistically significant positive association with sFas concentration. For each one year increase in age, sFas concentration was increase by a mean of 10.8pg/ml, after adjusting (controlling) for group membership and gender .Male gender was associated with a small and statistically no significant in cases in sFas concentration by a mean of 66.9pg/ml after adjusting (controlling) for group membership and age, as in (Table 2).

Table 2: sFas concentration/ multiple liner regression model with sFas conc. as the dependent (outcome) variable in (newly diagnosed and optimally treated) compared with healthy in addition to age and gender as explanatory (independent) variable.

	Unstandardized regression coefficients	**P**
(Constant)	483.0	<0.001
Newly diagnosed CML cases compared to Healthy controls-regression	179.9	0.028
Optimally treated CML cases compared to Healthy controls-regression	68.8	0.39[NS]
Age (years)	10.8	<0.001
Male gender compared to female	66.9	0.31[NS]

Discussion

McGahon*et al.*,reported that BCR-ABL1 inhibits the programmed cell death induced by the binding of Fas ligand to its cell surface receptor, Fas (CD95). As they suggest a possible link between the signaling cascades downstream of BCR-ABL1 and Fas which appears to be critically dependent on the activity of the ABL tyrosine kinase [22].

From these observations, this work is done to estimate the level of few apoptotic factors in sera of CML patients (sFas) and their effect on the disease progress and response to treatment. For the newly diagnosed CML patients in this study, the mean sFas concentration was highest in comparison to other groups, of which, the mean± SD of serum sFas level for the newly diagnosed, optimally treated CML cases and healthy control were 1163.6±231.5, 1021.7±360.6 and 970.1±361.7 respectively.

This is due to the fact that, there is a direct correlation between the expression and function of Fas in hematopoietic cells with the rate of proliferation, suggesting a potential role for Fas and its ligand in the regulation of hematopoietic homeostasis [23].

On regard to serum concentration of sFas, although they are statistically non-significant increase, such an increase was noted in other studies for patients with hematopoietic malignancies [20]. Of which, it was noted that, in patients with tumor, the key mechanism to inhibit Fas-mediated apoptosis is through sFas production, so the identification of sFas levels as a predictor of outcome in malignant disease further establishes a connection between Fas loss-of-function and tumor progression [21]. As, Fas on the cell membrane induces apoptosis when it binds Fas-L or sFas-L, however, plasma sFas blocks apoptosis by inhibiting binding between Fas and Fas-L or sFas-L on the cell membrane [24].

All these indicate that, the soluble Fas and FasL system at the interface of tumor cells and the immune system is more complex than expected, with serum soluble Fas is produced from the cell surface of malignant cells (in a form lacking the 21 amino acid residues containing the transmembrane domain by alternative splicing) act as a FasL inhibitor to bind Fas and prevent Fas-mediated apoptosis [17, 20].

The reduction in sFas concentration, in this study was in agreement with Zhao et al., 2008, who indicated that the expression of co-stimulatory molecules was improved significantly while the percentage of Ph+ cells decreased, so the level of Fas on cells was up regulated and the concentration of sFas decreased [25].

So the reduction in the level of sFas concentration in optimally treated patients serum (although still slightly higher than healthy control) could indicate a very good response to Imatinib treatment. Imatinib inhibit BCR-ABL1, so it will decrease the number of malignant cells. So that the sFas will be low when we compared it with the newly diagnosed although non-significant (may be

due to small sample size), but it shows a moderately strong effect (Cohen's d 0.64).

Conclusions

This study demonstrated that newly diagnosed CML patients' shows a statistical non significance increase in sFas when compared with optimally and healthy control groups. The identification of sFas levels as a predictor of outcome in malignant disease further establishes a connection between Fas loss-of-function and tumor progression.

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Assessment of cytogenetic response after treatment with imatinib mesylate in patients with chronic phase chronic myeloid leukemia

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ABSTRACT

Background: Chronic myeloid leukemia (CML) is a myeloproliferative disorder affecting hematopoietic stem cells and affects predominantly granulocyte progenitor line. It is characterized by acquired chromosomal abnormality which called the Philadelphia chromosome (Ph+) in 95% of cases. Imatinib mesylate is a powerful and selective competitive inhibitor of BCR-ABL tyrosine kinase. Imatinib mesylate is the first molecular target therapy for the treatment of CML

Objectives: The aim of this study was to evaluate the cytogenetic response in 58 patients with CML in chronic phase treated with imatinib mesylate.

Materials and methods: A prospective study conducted at the national center of hematology /Almustansiriya University, Baghdad, Iraq from April 2011 to December 2013, fifty-eight patients with CML in chronic phase (32 male and 26 female) were enrolled in this study. All patients were carrying the BCR-ABL fusion gene and treated with Imatinib mesylate (Glivic® Novartis) at 400 mg daily for at least 12 months

Results: There were 32 male and 26 female with male to female ratio (1.1:1).the median age was 36 year (range 14-64 years). The median duration of treatment with imatinib was 18 months (range 12 to 32 months). Complete hematologic responses (CHR) were attained in 54 of 58 (93%) patients treated during the first 3 months of imatinib therapy, where 11 (19%) of patients reached CHR after 1 month, 35 (60%) got CHR within 2 months of treatment with imatinib. Cytogenetic response rates to imatinib therapy at 6 and 12months. : Major cytogenetic response achieved in 35 (70%), 48 (92.3%) patients and minor cytogenetic response attained in 15 (30%), 4 (7.7%) patient at 6, 12 month respectively. Imatinib was usually safe and well tolerated. The vast majority of adverse effects were grade 1 and 2 and bone pain was the most common (86.2%).

Conclusion: After a median follow-up of 18 months, this study confirm that imatinib therapy induced induce durable and sustained hematological and cytogenetic responses in a high proportion of patients with chronic-phase CML. the response rates of imatinib therapy were similar to those reported in other countries. Imatinib was safe and well-tolerated with manageable side effects.

Keywords: cytogenetic response, imatinib, CML

mechanism for this result into fusion of two genes: the ABL gene (cellular homologue of retroviral oncogene v-ABL) located in 9q34 and the gene BCR located in22q11, which generates a hybrid gene called BCR-ABL encoding a chimeric protein 210KDa (P210 BCR-ABL) responsible for dysregulated tyrosine kinase that plays a major role in the development of the disease [(2)] .In 5% of the cases, the BCR-ABL fusion gene may result in either:

1- A variant complex translocation involving a third or more chromosomes,

2- A cryptic insertion of chromosomal material, undetectable by conventional cytogenetic techniques.

In this case, CML is called Ph negative, BCR-ABL positive, and only Fluorescence in Situ Hybridization technique (FISH) and molecular biology technique which is real time polymerase chain reaction (RT-

1- Hematological response: corresponding to the disappearance of splenomegaly and normalization of hematological parameters,

2- Cytogenetic response: corresponding to the decrease in mitosis of Ph +; it is measured as a percentage of residual Ph + cells

3- Molecular response: corresponding to the decrease in BCR-ABL transcript gene.

Imatinib mesylate is a powerful and selective competitive inhibitor of BCR-ABL tyrosine kinase. Imatinib mesylate is the first molecular target therapy for the treatment of CML. it has revealed substantial activity in all phases of Ph positive CML. Daily doses of 400 mg or more of imatinib can induce durable hematologic responses and high rates of cytogenetic responses in approximately all patients

with chronic phase CML, with minimal toxic effects [5-7].

The aim of this study was to evaluate the cytogenetic response in 58 patients with CML in chronic phase treated with imatinib mesylate.

Materials and methods

A prospective study conducted at the national center of hematology /Almustansiriya University, Baghdad, Iraq from April 2011 to December 2013, fifty-eight patients with CML in chronic phase (32 male and 26 female) were enrolled in this study. Informed consent was obtained from all patients. All patients were carrying the BCR-ABL fusion gene and treated with Imatinib mesylate (Glivic® Novartis).The mean age at baseline was 36 years old, range [14-64 years].

The chronic phase was defined according to criteria recommended by WHO [8] .Response criteria were adapted from the National Comprehensive Cancer Networks (NCCN) clinical practice guidelines [9].

Complete hematologic response was defined as a white blood cell count less than 10×10^9/L without immature cells, with <5% basophils, and a platelet count less than 450×10^9/L with no organomegaly. Cytogenetic response was assessed according to FISH analysis and categorized as complete (absence of Ph positive cells), partial (1%-35% Ph-positive cells), minor (36%-65% Ph-positive cells), minimal (66%- 95% Ph-positive cells), or no response (>95% Ph positive cells).

All patients were proved to be Ph-positive through fluorescence in situ hybridization (FISH) analysis, or p210 BCR/ABL transcripts positive done via RT-PCR assay of peripheral blood or bone marrow aspiration samples. FISH analysis was used for evaluating patients' cytogenetic response instead of classical cytogenetic analysis, because of the unavailability of classical cytogenetic analysis in our center.

FISH analysis was done on the interphase cells using a dual color BCR-ABL probe provided by cytocell , Cambridge ,United Kingdom. Slides for FISH analysis used from peripheral blood sample were prepared as follows: 1 ml of peripheral blood was mixed with 9 ml of RBC buffer (Red Blood Lysis Buffer). Cells were kept on ice for 10 minutes and centrifuged for 10 minutes at 1500 rmp. Supernatant was aspirated and cell washed once in 1x PBS buffer and centrifuged for 10 minutes at 1500 rmp. Supernatant was aspirated and cells were incubated in hypotonic KCL solution for 10 minutes at 37 centigrade. Evaluation of FISH signals was done using fluorescent microscope (Zeiss,Germany), a minimum of 200 interphase nuclei were evaluated.

Assessment of clinical outcomes , Complete blood count (CBC) and blood chemistry data were measured twice weekly in the first month, then monthly for the rest of the follow-up. FISH study for ph+ from peripheral blood done at time of diagnosis and after 6 months and 12 months for all patients.

Adverse events

Adverse events were graded according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events [10] and management done according to the NCCN clinical practice guidelines [9]. Imatinib dose was decreased if the patients showed intolerance because of adverse events of imatinib therapy and discontinued if the patient developed grade 3 or 4 toxicity. Therapy was resumed at 300 mg/ day when the toxicity had subsided.

Results

The baseline characteristics of 58 patients enrolled in this study were summarized in Table 1.

There were 32 male and 26 female with male to female ratio (1.1:1).the median age was 36 year (range 14-64 years)

The median duration of treatment with imatinib was 18 months (range 12 to 32 months). Hematological parameters at 6 and 12 months in imatinib treated chronic phase CML patients are detailed in Table 2. Complete hematologic responses (CHR) were attained in 54 of 58 (93%) patients treated during the first 3 months of imatinib therapy, where 11 (19%) of patients reached CHR after 1 month, 35 (60%) got CHR within 2 months of treatment with imatinib. Cytogenetic response rates to imatinib therapy at 6 and 12months are shown in Table 3.

Fifty patients were assessed for cytogenetic response at 6 month of the study period, cytogenetic response rate was: major cytogenetic response achieved in 35 (70%) patients and minor cytogenetic response attained in 15 (30%) patient. Major cytogenetic response comprised of complete cytogenetic response attained in 10 (28.5%) patients and partial cytogenetic response attained in 25(71.5%) patients. Fifty-two patients were available for cytogenetic assessment after 1 year of imatinib therapy. The cytogenetic response rate for those patients was: major cytogenetic response achieved in 48 (92.3%) patients and minor cytogenetic response attained in 4 (7.7%) patient. Major cytogenetic response comprised of complete cytogenetic response attained in 32 (66.7%) patients and partial cytogenetic response attained in 16(33.3%).No patient has advanced to accelerated or blastic phase and all patients are still alive.

Imatinib was usually safe and well tolerated. The incidence of adverse effects related to the drug is summarized in Table 4. The most common non-hematological adverse effects included bone pain (86.2%), muscle cramps (82.7%) and fluid retention

(72.4%), and rash and related events (11.8%). Most adverse effects were grade 1 (mild) or grade 2 (moderate). Regarding hematological side effect, one patient had a grade 3/4, in which platelet count dropped to less than 50 000 per mm^3. Daily dose was reduced to 300 mg because the patient had continued at grade 3/4 for more than two weeks and then treatment interrupted.

Table 1: Baseline characteristics of 58 patients treated with imatinib

Characteristic	Mean ±SD (range)
Age (years)	36±12 (14-64)
White blood cells ($\times 10^9$/L)	146±32 (12-443)
Hematocrit (%)	33±54 (25-44)
Platelets ($\times 10^9$/L)	516±05 (136-1540)
Splenomegaly (cm)	6±17 (0-21)
Hepatomegaly (cm)	2±03 (0-15)
Basophilia (%)	2.8±3 (0-9)
Lactate dehydrogenate (IU/L)	564±54 (247-3133

Table 2: Hematological parameter in 58 patients treated with imatinib

Parameters	6 months mean ±SD (range)	12 month mean ±SD (range)
WBC count × 109/L	7.4±13 (3.4–10)	5.5±05 (3.7–8.3)
Platelet count × 109/L	253±54 (110–354)	171±46 (128–251)
Hemoglobin, g/dL	12.1 ±16 (10.7–13.9)	12.3 (11.1–14.2)

Table 3: Cytogenetic response rate during imatinib mesylate treatment

Cytogenetic response (CyR)	Number of patients (%)	
	6 months	12 months
Major CyR (CCyR+ PCyR)	35/50 (70)	48/52 (92.3)
complete CyR (PCyR)	10/35 (28.5)	32/48 (66.7)
partial CyR	25/35(71.5)	16/48 (33.3)
Minor CyR	15/50 (30)	4 (7.7)
None	0 (0)	0 (0)

Cytogenetic not done	8/58(13.7)	6 /58(10.3)

Table 4. Adverse events related to treatment with imatinib mesylate

Adverse events	n (%)
Bone pain [a]	50(86.2)
Muscle cramps [b]	48 (82.7)
Fluid retention	42 (72.4)
Nausea	34 (58.6)
Skin rash	20 (34.4)
Fever	15 (25.8)
Diarrhea	11 (18.9)
Vomiting	10 (17.2)
Weight gain	8 (13.7)
Stomatitis	2 (3.4)
Anemia	2(3.4)
Neutropenia	1(1.7)
thrombocytopenia	1(1.7)

[a] Includes bone pain and joint pain.
[b] Includes musculoskeletal pain and muscle cramps.

Discussion

Although the clinical and hematological data are mostly very suggestive of CML. precise diagnostic confirmation is needed in the most cases. The current available first line treatments for CML are targeted therapy (anti tyrosine kinase BCR-ABL) imatinib, dasatinib and nilotinib. The choice of one of these targeted treatment depends on many factors the most important getting deeper molecular response, treatment free survival and of course not to forget financial costs. as stated in the literature, CML is art of myeloproliferative neoplasia with Philadelphia chromosome positive in the vast majority and negative in less than 5 %,but still BCR-ABL positive ; thus, the detection of ph+ chromosome is sufficient for diagnosis of CML, whatever the technique [(11)]
Imatinib is a 2-phenylaminopyridine derivative and a BCR-ABL tyrosine kinase signal transduction inhibitor 571 (STI-571). It acts as an inhibitor of the ATP binding site on the protein and prevents its phosphorylation and thus its activity [(12)] .With imatinib, a MCyR can be achieved in more than 80 percent of the patients who are treated in the early chronic phase [(13)].
In the present study, 58 CML patients who were Ph+ received Imatinib 400 mg daily in an early chronic phase. After a median follow up of 13 months, 50 patients were available for evaluation of cytogenetic analysis after 6

months of Imatinib therapy. Out of 50 patients, 35 (70%) patients had MCyR; partial response in 25 (71.5%) and complete response in 10 (28.5%) patients. 15 (30%) patients had minor CR. In a study by Kantarjian et al [14] found that 90% patients attained a MCyR, complete in 72% patients at a dose of 400 mg daily and after a median follow up of 9 months. Likewise, Rajappa etal [15] had shown CCyR in achieved in 56%, partial response in 23%, minor response in 17% and no response in 4% patients after a median follow up of 29.5 months. When the results of this study compared to that of IRIS study; at phase 3 IRIS study with median follow-up of 12 months, the estimated rates of complete hematologic response (CHR) for patients treated with imatinib were 90.48%, major cytogenetic response (MCyR) 92.9% which are mostly similar to result of current study [16]. Goldman et al reported that approximately 40% of the patients achieved a MCyR by 6 months, and 65% achieved a CCyR after 1 year of the therapy which are lower than that of the result of this study[17]. In this study imatinib was well-tolerated, and the most non-hematological and hematological adverse effects were grade 1 or 2 (mild or moderate), however just one patient had stopped imatinib because of thrombocytopenia grade 3/4. These results were comparable to that of Kantarjian et al, who showed that grade 3/4 non-hematologic toxic effects were uncommon, hematologic toxic effects were controllable, and only 2% of the patients stopped the treatment because of drug-related adverse events [18]. Furthermore, the most common any-grade adverse events reported were fluid retention (61.7%), nausea (49.5%), and musculoskeletal pain (47.0%). Any-grade bone pain and muscle cramps occurred in 11.3% and 49.2% of patients, respectively [13]. The most common any-grade adverse events in the current study were bone pain (86.2%), muscle cramps (82.7%), and fluid retention (72.4%). This difference may simply be a reflection of small size of the study population, different social culture, drugs interactions or food interaction or different metabolism in Iraqi patients. No increase in rates of serious adverse events was detected with continuous use of imatinib for follow up periods, compared with earlier time points. These results fully support the widely accepted opinion that the preferred initial treatment for newly diagnosed patients in chronic phase CML should include imatinib, or alternative tyrosine kinase inhibitors such as dasatinib or nilotinib for deeper molecular response.

Conclusion

After a median follow-up of 18 months, this study confirm that an uninterrupted treatment of chronic-phase CML with imatinib as initial therapy was found to induce durable sustained hematological and cytogenetic responses in a high proportion of the assessed patients. The response rates of imatinib therapy were similar to those reported in other countries. Imatinib mesylate was an effective therapeutic option in the treatment of CML with and it was safe and well-tolerated.

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Bone Marrow Involvement as Initial Diagnosis of Metastatic Breast Cancer

Alaadin sahham Naji* **Zakka noory****

Abstract:
Bone marrow examination is commonly used in the evaluation of hemato-oncological disorders and in patients with cancer of solid organs to detect metastases. We present a 68 years old female with anemia, high ESR, minimal axial bone pain, weight loss. These features mimic and raise suspicion of myeloma, however in this case diagnosis was done retrogradely from the bone marrow, despite her aspirate does not show much of that typical cluster of non hemopoietic, her bone marrow biopsy showed extensive fibrosis with cluster of non hemopoietic elements. So bone marrow aspiration and trephine biopsy are an effective and cheap method for evaluating metastatic bone marrow tumors.
Keywords: Bone marrow metastases; breast cancer

Introduction:

Bone marrow has played a prominent role as an indicator organ of occult tumor cell dissemination because it is easily accessible by aspiration, and it represents a relevant site of distant metastases in breast cancer

***Clinical Case*:**

68 years old female referred to Baghdad Teaching Hospital at 28th of February 2014 after she investigated and discovered that she has anemia and high ESR, when dated back to her history ,she gave a history of loss of weight., abdominal pain and for that reason she do it upper and lower scope which yielded duodenal ulcer.

When looking the cause for this ulcer it was found that it is because of abuse of non-steroidal anti-inflammatory drugs to relief bone ache which involved axial spine mainly mid and lower spine and her chest and both shoulder especially at night. She has no past medical history of any chronic illness apart with good performance statutes and do it her daily work at home.

Examination: patient looks healthy, mildly anemic, no peripheral lymphadenopathy, thorough vertebral tenderness without deformity or neurological deficit in upper or lower limb

CBC at January showed the following

Date	Hemoglobin/ g/dl	WBC/m3	Platelet/m3	ESR/hr	
20.01.2014	9.6	9200	380000	120	
05.03.2014	9	11100	378000	142	Leukocytosis lymphocytosis Left shift

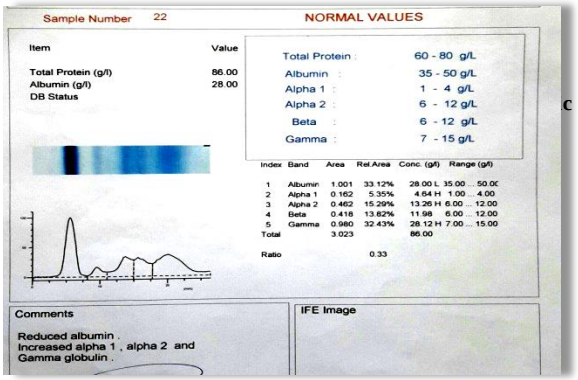
Her anemia, high ESR and leucoerythroblastic blood picture with her underlying bone ache raised suspicion of myeloma

Serum protein electrophoresis: Showed increase in total protein element but in form of polyclonal rather than monoclonal gammopathy as demonstrated below.

Urine for Bence-Jonce protein: was negative

Skeletal survey as part of myeloma: doesn't showed prominent lytic lesions apart from diffuse osteoporosis

Abdominal ultrasound: normal with mild to moderate hydronephrosis of the right kidney



Breast ultrasound: showed fibroglandular tissue with ductal dilatation (BIRADS II)

Complete blood picture and bone marrow:
HB 8.5g/dl, WBC $11.7X10^9$, platelet $385X10^9$
Differential N: 57%, L: 37%, M: 6%
With leukocytosis, lymphocytosis and tendency for rouleaux formation

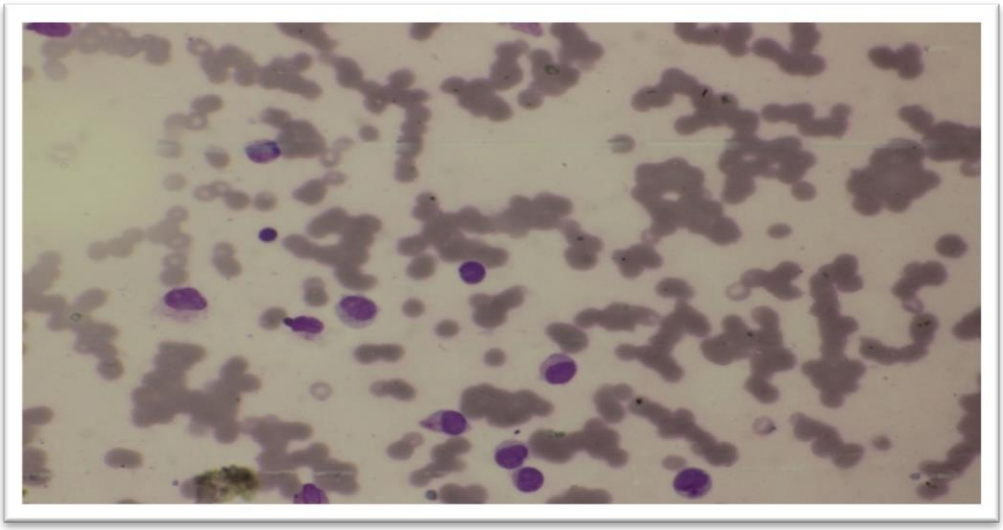
The bone marrow aspirate for the first look, showed scattered cell ***resemble plasma cell*** reaching 60% of all blood element with strange morphology as shown in slides A below making diagnosis of myeloma worrisome as in Figure [1].
when they looking to the **marrow biopsy** was astonishing which showed *extensive bone marrow fibrosis* effaced the whole architecture showing cluster of non hemopoietic elements as shown in Figure [2].
From the feedback of the biopsy and looking more precisely to aspirate showed few clustering as shown in slide B and the report of aspirate come with the Slide A

marrow shows few granulocytic and erythroid precursors, with marrow infiltration *by non hemopoietic elements* in scattered and few cluster
During this time patient come back with confessional state, irritability without focal neurological deficient and the differential at that time: could be either renal, hyperviscosity or hypercalcaemia

CT brain
no abnormality have been detected
MRI brain:
Bilateral white matter lesion in the periventricular area (picture suggest deep white matter lesion)

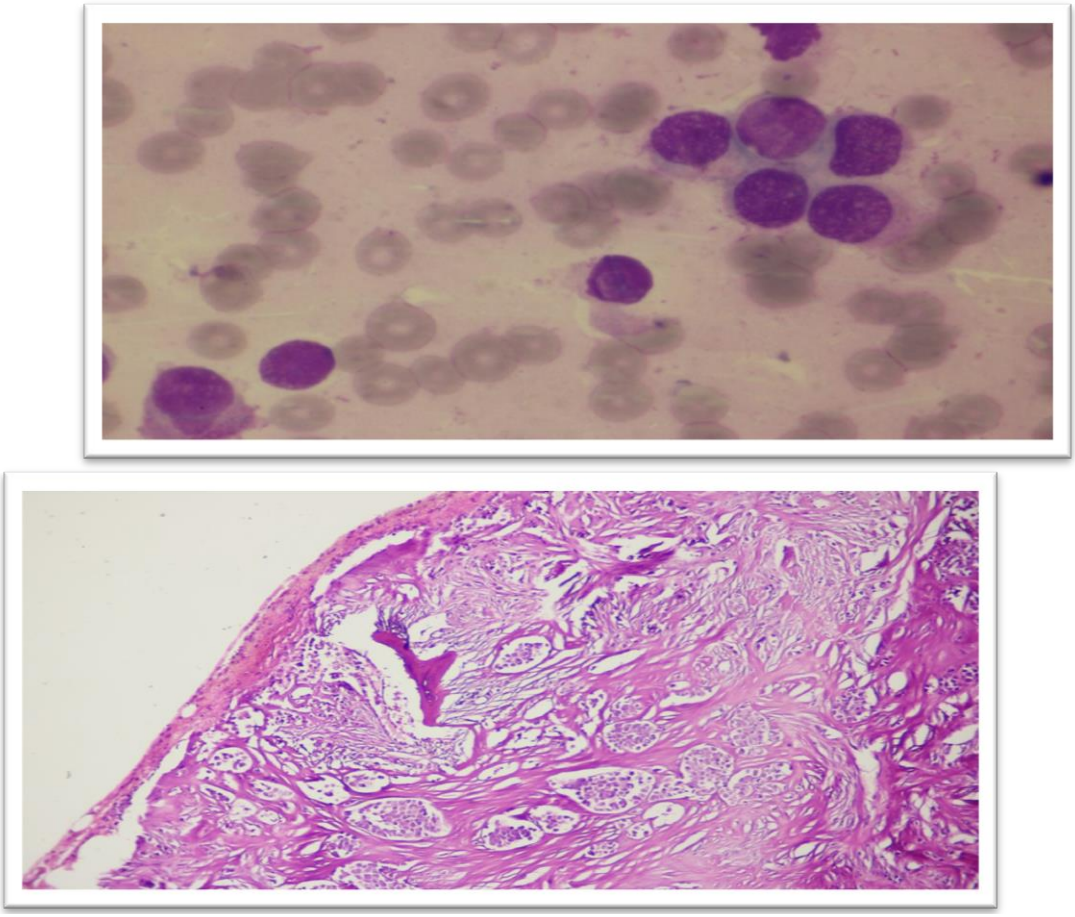
Laboratory investigation:
Random blood sugar 191 mg/dl
Urea 76 mg/dl
Creatinine 1.3 mg/dl
Calcium 13.9 mg/dl (8.5-10.5mg/dl)
Alkaline phosphatase 221 U/l (normal range 40-150)
ALT 104 U/L (0-55)
AST 97U/L(5-34)
Serum uric acid 12.5mg/dl **(2.6-7.2)**

After two days patient regain her consciousness with hydration, steroid therapy and zolendrinic acid



Slide B

Figure (1): Bone marrow aspirate showed showing scattered cell *of non hemopoietic element* rather than clustering morphologically look like plasma cell especially in slide A



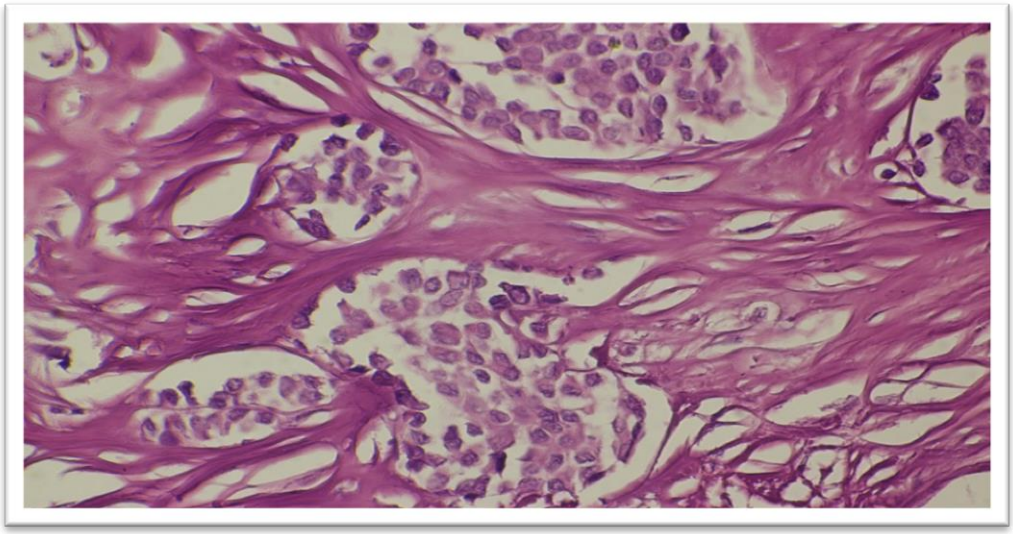


Figure (2): extensive bone marrow fibrosis effaced the whole architecture showing cluster of non hemopoietic elements

Looking for the primary source

Colonoscopy: This was normal up to splenic flexure because patient was not well prepared

CT-chest and abdomen:

Speculated irregular nodule (21X11.5 mm) seen in upper medial part of right breast.

Generalized mixed metastatic bony lesion predominantly lytic involved entire skeleton

Bilateral pleural effusion with thickening and dependent atelectasis with right subpleural nodule

Tumors marker:

CEA	more than	200 (up to 10 ng/ml)
CA 125	more than	600 (up to 35 u/ml)
Serum CA 15.3		300 (up to 25 iu/ml)
AFP		1.96 (up to 2u/ml)

Immune histochemical stain of the bone marrow biopsy:

With the looking for the primary source , bone marrow biopsy sent for histochemical stain which yield that neoplastic cell ***showed positive immunostaining*** for ***mammaglobin, cytokeratin 7, ER score(5/7), PR score (7/8), and Her2/neu (score 1+)*** While ***negative for GCDFP-15,and cytokeratin 20 marker, these*** finding go with metastic breast cancer

Discussion:

Marrow aspirates and trephine biopsies are sensitive and cheap techniques for detecting solid tumors metastatic to bone marrow.

Such investigations are indicated when there is significant probability of bone marrow metastases and when knowledge of their presence would affect the choice of primary treatment.

Trephine biopsy is more sensitive than bone marrow aspiration and sensitivity is increased by performing bilateral biopsies or by obtaining a single large biopsy and therefore these two procedures should be regarded as complementary [1].

The detection of tumor cells in a trephine biopsy when none are demonstrable in smears of an aspirate is not uncommon.

However, occasionally tumor cells are seen in aspirate smears when trephine biopsy is normal [2,3].

Marrow infiltration by metastatic tumor may be focal or diffuse. Reticulin and collagen fibrosis are commonly present and are most marked in those cases with greater degrees of marrow infiltration. Marked fibrosis is most common in carcinomas of the breast, stomach, prostate and lung [4,5,6].

The two primary sites whose identification is most important because of their sensitivity to hormonal therapy are breast and prostate. In patients with relapsed or metastatic breast cancer, the finding of bone marrow metastases is reported in 3-52% of patients [7,8,9].

The presence of bone marrow metastases may be helpful in detecting hormonal receptors in patients who have not had the determination done on the primary tumor [10].

By conventional histopathological techniques, the likelihood for the identification of isolated breast cancer cells in bone marrow is as low as 4% [11]..*Redding et al*. used an antiserum against epithelial membrane antigen (EMA) and detected breast cancer cells in bone marrow at the time of primary surgery in 28% of females without overt metastases [12].

Although this marker is known to be rather nonspecific

There was no statistical correlation between the extent of bone marrow involvement and the degree or number of cytopenias or survival after biopsy,

which was a median of 13.5 months. Most of these patients present with bicytopenia or pancytopenia, and most biopsy results showed extensive metastatic involvement of the bone marrow. So many patients have been reported with first presentation as cytopenias, anemia and multiple lytic skeletal lesions, anemia of an unknown cause or isolated low platelet count[13] .

On the basis of these findings, Dr. Zhou recommends that pathologists search even routine bone marrow biopsy specimens for the presence of metastatic carcinoma.

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Case report

Myeloid Sarcoma : A Report of Two Cases

Bassam Francis Matti , FICMS(Haema)* **Hassanain Hani Hassan (FICMS)****

Case 1

A 33 years old male with Relapsed acute myeloid leukaemia on top of MPN with disturbed level of consciousness

Summary

A 33 years old male was diagnosed as acute myeloid leukaemia on top of MPD at July ,2011.He received '3-7' regimen with CR followed by 2 cycles of HiDAC chemotherapy and maintained on Gleevec® daily and stopped at January , 2012 because of frank relapse .He admitted in April ,2012 with disturbed level of consciousness

Lab. Investigations showed:

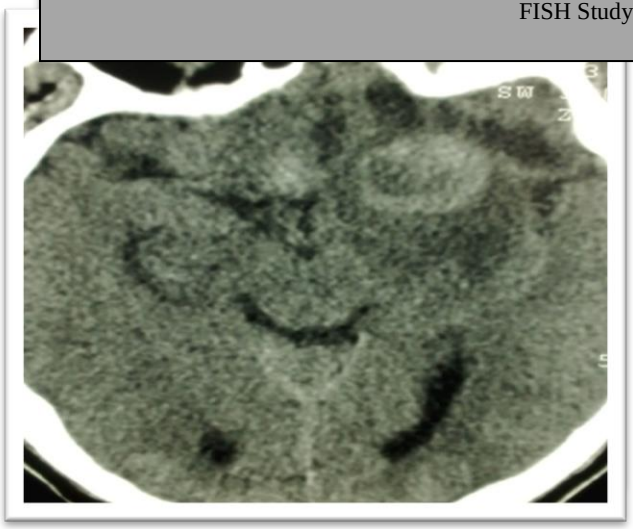
CBP at 26-4-2012 PCV 0.16 WBCs 272 × 109 with 98% blasts Basophilia Platelets 16 × 109

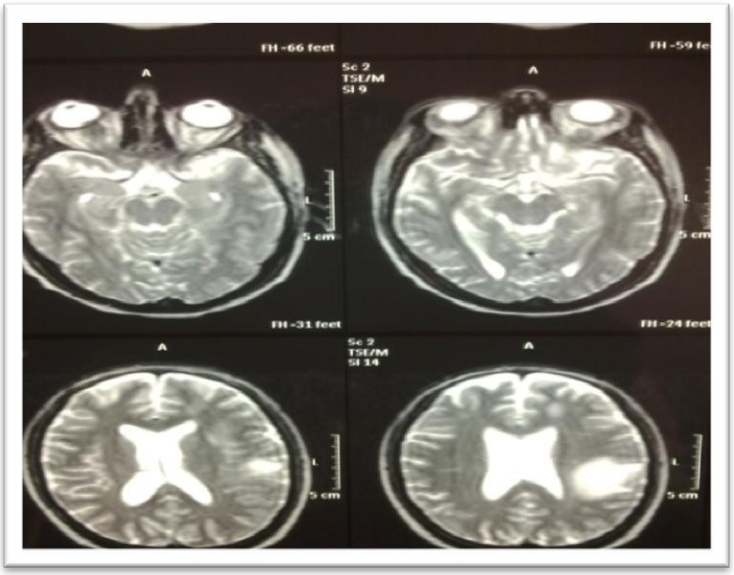
FISH Study for BCR-ABL t(9,22)is detected in 99% of cells

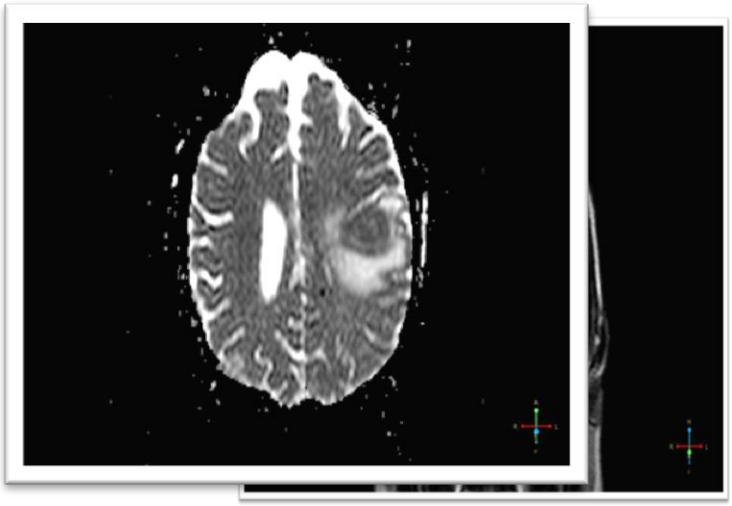
Imaging : CT Brain

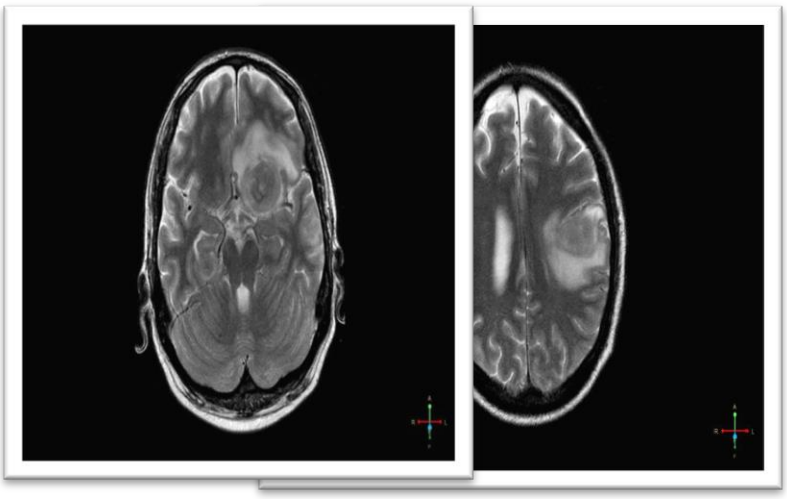
of brain

f Brain







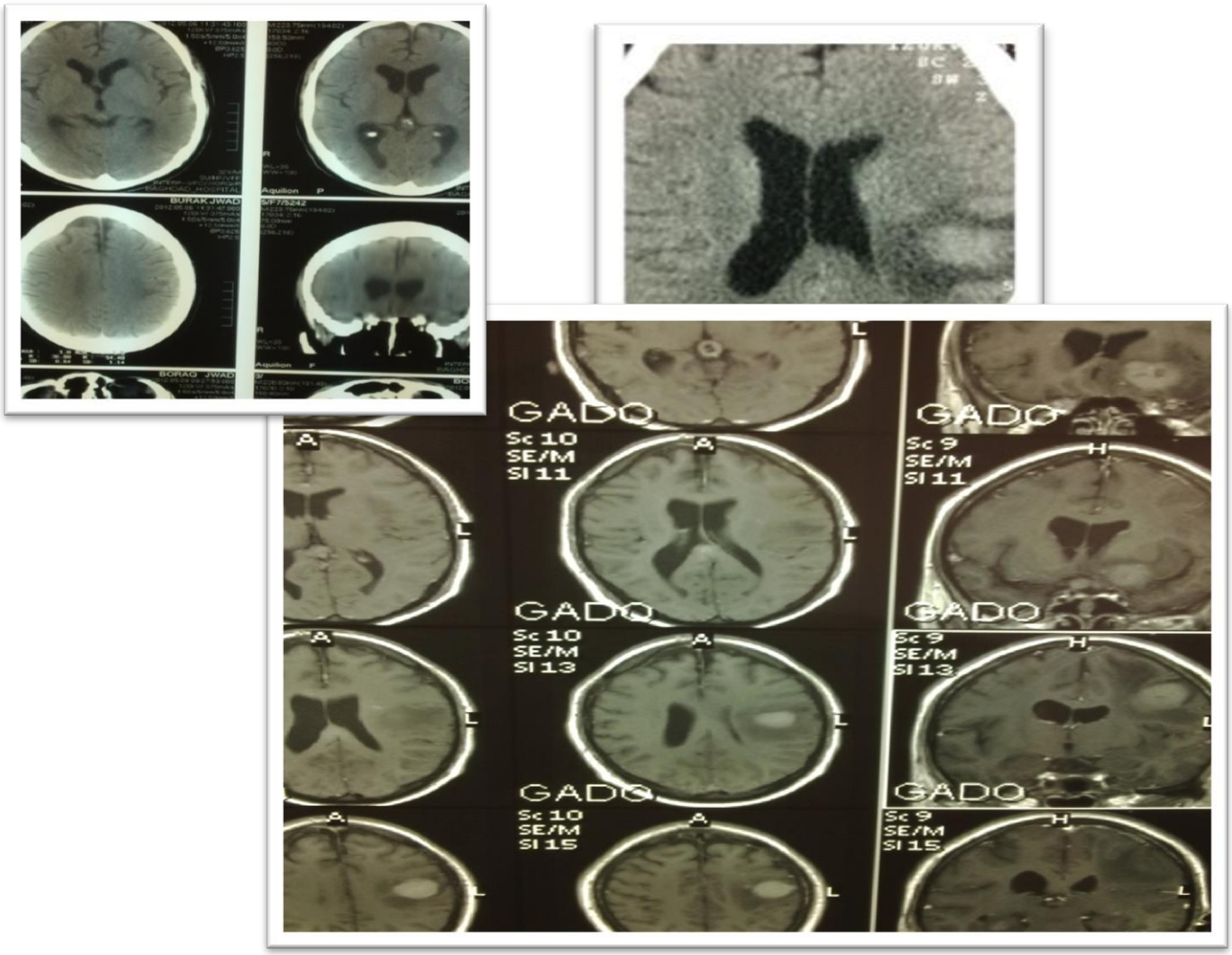


Treatment

The patient is treated by dasatinib 70 mg b.i.d with substantial clinical improvement and gradual resolution of brain lesions on MRI

Last CBP 3-6-2012PCV 0.16WBCs 5.8 × 109/LNO blasts are seenPlatelets-20 × 109/L

Imaging : CT Brain after Treatment with Dasatinib



Case 2 Orbital Swelling with Acute Myeloid Leukemia

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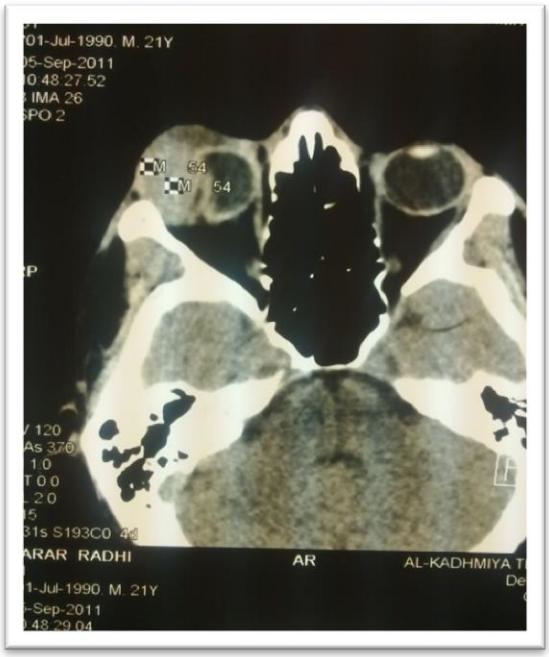
Rapidly Progressive Orbital Swelling in a 22 Years Old Patient with Relapsed Acute Myeloid Leukaemia

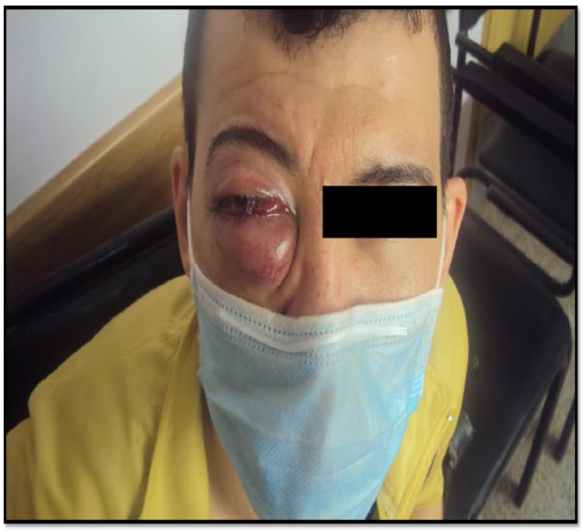
History

A 22 years old male was diagnosed as AML-M2 at July , 2011.He was Refractory to '3-7' regimen and Admitted with rapidly enlarging R.orbital swelling MRI Orbit at September ,2011 showed InferolateralR.orbital soft tissue mass homogenously enhanced after contrast suggestive of epidermoid cyst



Obtained CR after FLAG salvage therapy with disappearance of the mass

Re-admitted to our unit at Feb ,2012 with progressive pallor , fever and pain and proptosis of the eye with lid retraction not responding to broad spectrum antibiotics



CBP in 22-2-2012 PCV 0.15, WBCs 8.2×10^9 No immature cells seen , Platelets 105×10^9

Bone marrow aspiration and biopsy Hypocellular with 3% blasts consistent with remission

First Admission : Imaging Studies

MRI Orbit(fig 1)

Inferolateral right orbital soft tissue mass homogenously enhanced after contrast with intra- and extra-canal compartment invading muscle, measures 30 × 30 × 20 mm invading and deforming the globe suggestive of chloroma

He received second FLAG with moderate decrease in the size of the eye swelling

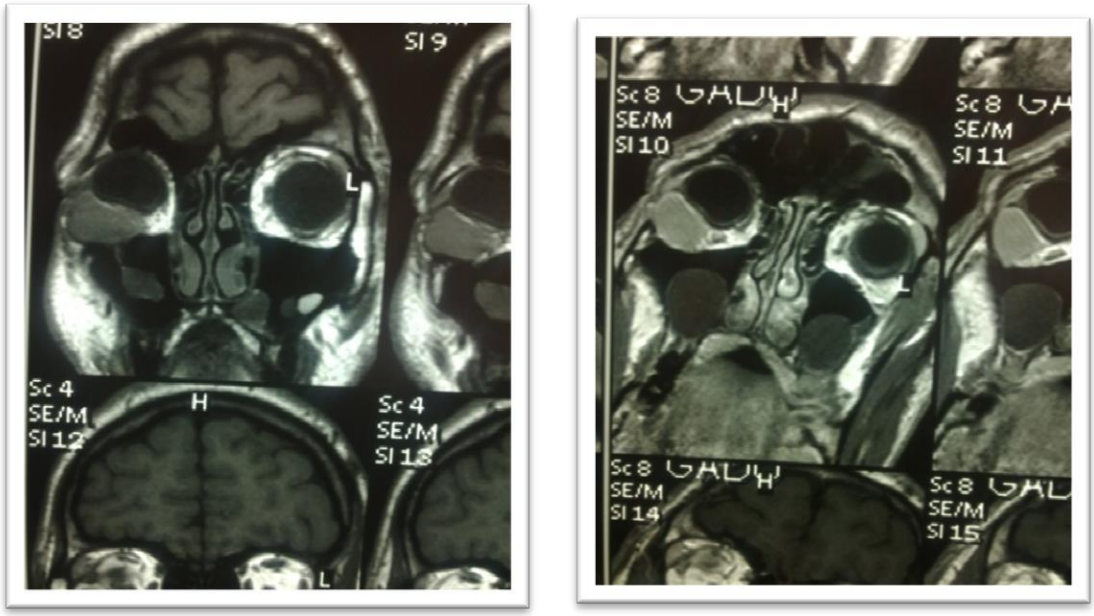


Fig 1: MRI of Orbit

Second Admission in May , 2012 the patient Re-admitted with progressive pain and enlargement of right eye swelling

CBP 17-5-2012

PCV 0.25

WBCs 3.0 × 10^9/L

Blasts 75%

Platelets –moderately reduced

Immunohistochemistry on Parffin-embedded tissue Section CD45 +ve while negative for CD3,CD20 ,CD10 ,CD 5 , tdt consistent with B-cell NHL

Myeloperoxidase stain

Strongly positive myeloperoxidase stain consistent with myeloid sarcoma

Summary & Discussion

Myeloid sarcoma is focal solid mass lesions composed of immature myeloid cells 1

The obsolete term chloroma was derived from the greenish color of the typical tumor due to high level of myeloperoxidase in the immature cell types. 2, 3

However , these tumors can also be gray, white, or brown, depending on the oxidative state of this enzyme

Terms: Chloroma , Granulocyticsarcoma, Myeloblastoma, Myelocytoma, Chloroleukaemia, Myeloid sarcoma, Etramedullary AML

The clinical presentation of myeloid sarcomas varies and is dependent on the site of involvement.

Commonly involved sites: Subperiosteal bone, structures of the skull, paranasal sinuses. Sternum, Ribs, vertebrae, Pelvis, lymph nodes, skin

Rare sites: Pancreas, Heart, Brain, Mouth, Breast. Gastrointestinal and biliary tract, Prostate Urinary bladder , gynecologic tract and Pleura.

Myeloid sarcoma may present in association with different types of myeloid leukemia including

In patients with known acute myeloid leukemia (AML) in the active phase of the disease.

In patients with a myeloproliferative neoplasm (MPN) or a myelodysplastic syndrome (MDS), in whom myeloid sarcoma may be the first manifestation of blastic transformation.

As the first manifestation of relapse in patients previously treated for primary or secondary acute leukemia

De novo in healthy subjects, in whom a typical form of AML (especially M2 , M4 , M5) may occur after an interval of weeks, months or even years and rarely no leukemia develops.

The definitive diagnosis today is usually based on immunohistochemistry.

The best immunohistochemical stains used for this include myeloperoxidase (MPO) and lysozyme.[1]

MPO immunostain is positive in most myeloblastic variants (as well as in some cells myelomonocytic variants) while lysozyme is frequently expressed in monoblastic variants.[2]

Intracranial Myeloid Sarcoma

It is very rare and it caused by spread from the dura along the perivascular adventitial spaces through the arachnoid veins[1,2]

CNS invasion often occurs in meningeal and sometimes in parenchymal form.

Meningeal infiltration may be focal or diffuse.

Dura mater, leptomeninges, or both may be involved [2].When myeloid or granulocytic sarcoma produces focal thickening of leptomeninges, it may mimic:

Dural-based meningioma
Metastatic Carcinoma
Lymphoma
Extramedullary Blastic Crises

WHO definition of blastic crises [1] : Occur in about 10% of patients with chronic myeloid leukaemia and may involve a variety of organs and tissues. [1,2]

They are usually occur during the accelerated phase (10%) and blastic crises

Sites of involvement

Most commonly: skin, lymph nodes and the CNS
Rarely the synovia, GIT, kidneys and pleura

They can occur during imatinib therapy for accelerated phase disease [3]

All types of blast crisis, including promyelocytic blast crisis, can occur during imatinib therapy. [4]

Extramedullary disease in CML is considered an indicator of dismal prognosis which should lead to change in therapy.[2,5]

Discussion of Management: Treatment of Extramedullary Blastic Crises

Tyrosine Kinase inhibitors (TKIs)
Imatinib monotherapy [1]
Other TKIs (e.g dasatinib , nilotinib) [2,3]
Systemic chemotherapy

Myeloid blastic crisis: anthracyclines (e.g. daunorubicine or mitoxantrone) plus etoposide or cytarabine with or without combination with imatinib.[4]

Lymphoid blastic Crisis: anthracyclines (e.gdaunorubicin) plus vincristine and prednisolone with or without imatinib Or the HyperCVAD regimen (Hyperfractionated doses of cyclophosphamide, vincristine and dexamethasone) [5]

Myeloid/mixed/undifferentiated blast crisis: high-dose cytarabine-based regimens [6]

Haematopoietic stem cell transplantation if the patient returned to the chronic phase or a complete remission has been achieved. [5,7]

Myeloid Sacrcoma of the orbit

It is very rare1 and shows considerable geographic variation in incidence [2]

Retina and choroid are more likely to be involved [3]

Usually occurs in the younger age group with definitive male predominance [4]

Presentation may include exophthalmos ,palpepralproptosis , lid oedema , pain and chemosis

OMS : Differential Diagnosis [1-4,9]

Dermoid
Epidemoid Cyst
Orbital cellulitis
Orbital hemangioma
Orbital extension of neuroblastoma
Metastatic rhabdomyosarcoma
Lymphoma
Leukaemia
Mucormycosis

OMS : Imaging

Computed tomography (CT) and magnetic resonance imaging (MRI) are not specific to distinguish granulocytic neoplasms from other tumors. [10]

It may help to distinguish myeloid sarcomas from hematomas and abscesses, which are possible complications of leukemia.

Homogeneous enhancement occurs with either modality. Orbital myeloid sarcoma demonstrating relatively little bony destruction. [11]

Lesions tend to be extraconal, and they preferentially involve the lateral orbital wall [12,13]

The lesion is often fairly confined to orbit with occasional extra-orbital dissemination [14]

50% of patients with OMS had bilateral disease [5]

OMS : Treatment

Chemotherapy with or without radiotherapy [1,2]
Orbital decompression

OMS : Prognosis

The prognosis is related to the course of the underlying hematological malignancy. [1]

Patients with an AML associated with a t(8;21) and presenting myeloid sarcoma have a low rate of complete remission, and overall survival is poor. [2,3]

This appears to be in contrast to the better prognosis generally seen in AML with t(8; 21). [2,3]

OMS : Conclusion

The diagnosis of myeloid sarcoma must be considered in any orbital mass of uncertain origin, particularly if it is bilateral.

It is based on clinical findings, imaging study results and hematological findings.

Special stains and immunohistochemistry play an important role in the diagnosis.

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Haematology Clinico-pathological Exercise

In this new journal feature , we start with real haematology patient' clinical case presentation with haematopathological or histopathological slide image(s) . Questions regarding the image case are followed and the answers are seen in other page of the journal .

Blasts with Bizarre Morphology

Authors

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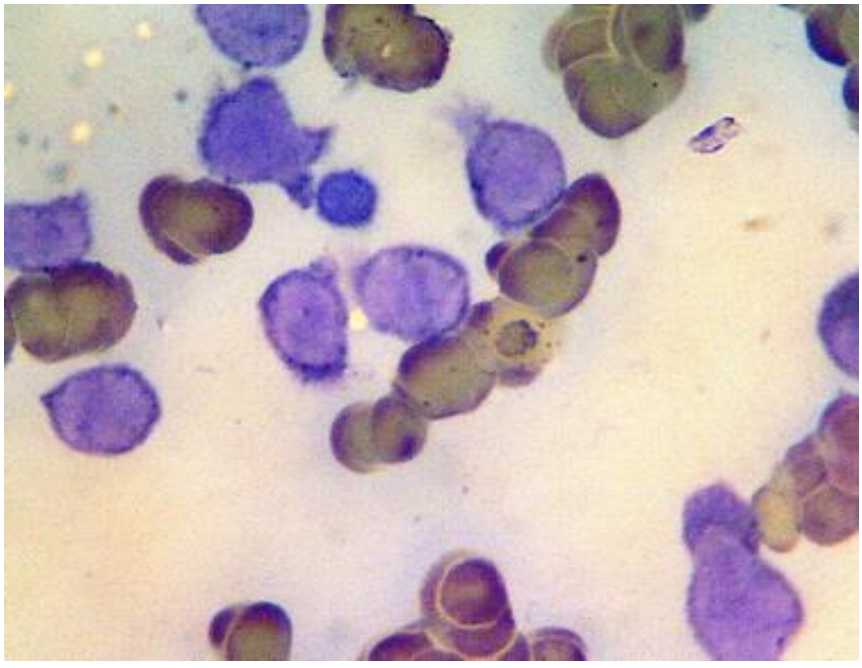
Questions

A 21 years old male was admitted with 2-month history of exertional dyspnea, fever and occasional gum bleeding. CBC reveals anamia and thromboytopnia and circulatory blast cells . Bone Marrow Aspiration findings are illustrated in **Figure**1.

1. What is the diagnosis ?
2. Is there any relationship to prognosis ?



Answers

1. The slide images showed blasts with bizarre morphology resembling " hand mirror" . The presence of Auer rods in blasts points to the diagnosis of "hand-mirror variant acute myeloid leukaemia" . The diagnosis is supported by positive Sudan Black stain (SBB) in blasts (**Figure 2**) and the patient's response to chemotherapy . Hand mirror cells (HMCs) are blast cells with unipolar cytoplasmic extensions that have the appearance of handles. This morphologic variant is not lineage-specific, occurs in acute lymphoblastic leukaemia (ALL) and to a lesser extent in various AML subtypes. [1,2]
2. When compared with patients with acute leukemia in equivalent age and morphologic categories, adults with the HMC variant had a similar, but not better, prognosis, while in children with ALL this variant had a poorer prognosis with a high incidence of CNS relapses despite prophylaxis. [3]

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