# IJH Iraqi Journal of Hematology

**IJH is a Peer Reviewed Scientific biannual Medical Journal published by the National Center of Hematology Al-Mustansiriya University, Baghdad-Iraq.**

**November 2013** **Volume 2,**

**Issue 1**

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# Dear doctors

**We would like to congratulate all colleagues specially those who work in the hematology field clinical and laboratory for publishing the second issue of the Iraqi Journal of Hematology we sincerely hope from the authors to continue sending original articles, scientific comments and criticism to the editors in order to keep the journal going and to keep raising its standards.**

**Kindest regards**

**Editorial Board**

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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.**

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**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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**Abstract**

**Background:** The non-Hodgkin lymphomas (NHL) of childhood are a diverse collection of diseases originating in the cells and organs of immune system.

**Objectives:** To determine epidemiology and clinicopathological presentations of children with NHL admitted to Child's Central Teaching Hospital.

**Methods:** A retrospective study was done from 1st of January 2004 to the 31st of December 2009; the patients with newly diagnosed NHL, age less than 15 years, who were admitted to the pediatric oncology unit in the Child's Central Teaching Hospital.

**Results:** The total number of patients was 84; the mean age at diagnosis was 6.3years, with a male to female ratio of 2:1. Most of patients were presented in stage III&IV (88%).Most common presenting features were abdominal distension or a mass in 51%.Burkitt lymphoma and Burkitt like lymphoma were the most common histological subtype (58.33%).

**Conclusions:** The majority of cases were between 5-9 year age group, and the mean age at presentation was 6.3 years old, with a male to female ratio of 2:1. The most common presenting site was the abdomen. The majority of cases were fallen in advanced stages (III&IV). Histopathologically Burkitt's lymphoma was the commonest subtype.

**Key word:** Non-Hodgkin Lymphoma

## Introduction

The NHLs in children and adolescents are a diverse group of aggressive neoplasms in humans .The onset of clinical manifestations may be explosive and characteristically the duration of symptoms is short, and most patients present with advanced disease [1,2,3].

Initial symptoms including cough, sore throat, abdominal pain, vomiting and lymphadenopathy are non-specific and may be indistinguishable from those of common childhood illnesses [1].

NHL can arise virtually in any site of lymphoid tissue and the manifestations are related to the sites primarily involved. Rarely children have widely disseminated disease for which the site of origin can't be determined [1].

## Objective

The goal of this study is to study the epidemiological and clinicopathological features of the children with NHL who were treated at" Child's Central Teaching Hospital".

## Material and Methods

A retrospective study was done from 1st of January 2004 to the 31st of December 2009; with the last day of review of files was 31st of December 2010; 84 patients with newly diagnosed NHL ,age less than 15 years ,who were admitted to the pediatric oncology unit in the Child's Central Teaching Hospital in Baghdad.

The information all were taken from the records of the oncology consultation clinic.

Clinical staging was based on *St. Jude Staging System* [4]. Histological classification was done according to International Working Formulation (IWF) as defined by National Cancer Institution (NCI) [1, 5].

Pretreatment studies included: patient' history ,physical examination, CBC, serum electrolytes, liver and renal functions profile, BM aspirate or biopsy, CSF analysis (including cytospin since 2008), tissue biopsy (excisional or incisional) or cytology (FNA), radiological studies, (including chest X ray, ultrasound, CT scan or MRI ) .

The patient is considered lost to follow up, if he or she didn't attend to the center for at least 6 weeks from the last visit.

The qualitative data were expressed as frequency and percentage; the quantitative data were expressed as mean and median. The *P* value ≤ 0.05 was considered significant.

**Results**

The total number of patients was 84 patients, the mean age at diagnosis was 6.3 years and range from 1-15 year, with age group (5-9) year is the most common 39 patients 46.6%. The age distribution is shown in table (1).

**Table (1) Age distribution of patients**

| Age (year) | NO. | % |
|---|---|---|
| **<5** | 29 | 34.4 |
| **5-9** | 39 | 46.6 |
| **10-15** | 16 | 19 |
| **Total** | 84 | 100 |

The male to female ratio was 2:1

Regarding the duration of illness prior to diagnosis, the mean duration was 5.2 weeks, and median of 4 weeks, and range from 1-32 weeks, with 52 patients 61.9% presenting with less than 6 weeks.

Abdomen was the most common primary site involved in 57 patients 67.8%, followed by peripheral lymphadenopathy in 23 patients 23.3%, details are shown in table (2).

**Table (2) shows the primary site involved**

| Primary site* | No. | % |
|---|---|---|
| **Abdomen** | 57 | 67.8 |
| **LAP**** | 23 | 27.3 |
| **BM** | 14 | 16.6 |
| **Thorax***** | 9 | 10.7 |
| **Jaw** | 5 | 5.9 |
| **Testes** | 5 | 5.9 |
| **CNS** | 2 | 2.3 |
| **Tonsils** | 2 | 2.3 |
| **Pelvis** | 1 | 1.1 |
| **Scalp** | 1 | 1.1 |

*some patients had more than one primary site involvement

**The number of patients who presented with only LAP was 11 (13%).

***Mediastinal or hila mass or pleural effusion.

***NB:*** One patient died before BM aspirate is performed, also one patient died before CSF aspiration is done.

Cervical LAP was most commonly involved in 14 patients 60.9 % followed by generalized LAP in 7 patients 30.4% as shown in table(3).

**Table (3) Pattern of peripheral lymph node groups involvement in 23 patients.**

| Lymph node group* | No. | % |
|---|---|---|
| **Generalized** | 7 | 30.43 |
| **Cervical** | 14 | 60.9 |
| **Axillary** | 3 | 13.0 |
| **Submandibular** | 2 | 8.7 |
| **Inguinal** | 1 | 4.3 |

***Some patients had more than one group involvement.

The presenting clinical features were as shown in table (4)

**Table (4) clinical features at presentation**

| Clinical feature | Positive (%) | Negative (%) | Not recorded (%) |
|---|---|---|---|
| **Fever** | 34 (40%) | 30 (35.7%) | 20 (23.8%) |
| **Pallor** | 39 (46%) | 22 (26%) | 23 (27.3%) |
| **Abdominal distension/mass** | 43 (51) | 24 (28.5%) | 17 (20.2%) |
| **Lymphadenopathy** | 23 (27.3) | 40 (47.6%) | 21 (25%) |
| **Splenomegaly** | 19 (22.6%) | 49 (58.3%) | 16 (19%) |
| **Hepatomegaly** | 26 (30.9%) | 42 (50%) | 16 (19%) |
| **Ecchymosis/ bruises** | 3 (3.57%) | 66 (78.5%) | 15 (17.8%) |

Depending on St. Jude Staging System, 10 patients 11.9% were in stage I&II, 74 patients 88.1% were in stage III &IV.

**Table (5) Diagnostic procedure for 84 patients.**

| Diagnostic procedure | No. | % |
|---|---|---|
| **Biopsy** | 36 | 42.85 |
| ***-Excisional*** | 31 | 36.9 |
| *-Inc**isional*** | 5 | 5.95 |
| **FNA*** | 37 | 44 |
| **B.M# Aspirate&/ or biopsy** | 1 | 1.16 |
| **Not recorded** | 10 | 11.99 |

* Fine needle aspirate, #bone marrow

**Table (6) Histopathological classification of 84 patients.**

| Histo-pathological subtype | No. | % |
|---|---|---|
| **BL&BLL** | **49** | **58.33** |
| **DLBCL** | **13** | **15.47** |
| **LL** | **12** | **14.28** |
| **Unclassified*** | **10** | **11.92** |
| **Total** | **84** | **100** |

BL (Burkitt lymphoma).
BLL (Burkitt like lymphoma).
DLBCL (Diffuse large B cell lymphoma).
LL (Lymphoblastic lymphoma).
*the histopathologist could not detect the subtype of NHL.

**Table (7) results of B.M & CSF examination of the 84 patients.**

| Test * | positive | Negative |
|---|---|---|
| **B.M aspirate&/ or biopsy** | **14 (16.6%)** | **69 (82%)** |
| **CSF analysis** | **2 (2.38%)** | **81 (96.42%)** |
| ***One patient died before BM examination, and another died before CSF aspiration was performed. | | |

The chest x ray was normal in 65 patients 77.38%, and abnormal in 9 patients 10.7%, and no reported findings in 10 patients 11.9%; details are shown in table (8).

**Table (8) Chest x ray findings of 9 patients and percentage from total patients.**

| Chest X Ray finding* | No. | % |
|---|---|---|
| Mediastinal mass | 5 | 5.95 |
| Hilar mass | 1 | 1.19 |
| Pleural effusion | 5 | 5.95 |
| * Some patients had more than one finding. | | |

**Table (9) Initial serum creatinine &uric acid levels in 84 patients.**

| | Serum creatinine No. ( %) | Serum uric acid No. ( %) |
|---|---|---|
| Normal | 34 (40.47) | 47 (55.9) |
| Elevate | 35 (41.66) | 25 (29.7) |
| Not recorded | 15 (17.87) | 12 (14.4 |
| Total | 84 (100) | 84 (100) |

The initial hematological data of the patients were as following: Hb level was more than 10 gm/dl in 60% of patients with a range of 6.6 -13.3 gm/dl , and a mean of 10.6 gm/dl and median of 11gm/dl .The initial WBC count was normal in 70.2% and low in 10.7%[3] ,with a range of 8-140 $\times 10^9$/L , and a mean of 11.6 $\times 10^9$/L ,and median $8\times 10^9$/L .The absolute lymphocyte count was normal in 61.8% of patients , low in 27.3% [3]; with a range of 0.2-42 $\times 10^9$/L , and a mean of $3.4\times 10^9$/L , and median of $2.3\times 10^9$/L .The platelet count was normal in majority of patients 85.7% , low in 5.9% , and elevated in only 1.1% [3]; with a range of 10-520x$10^3$/mm$^3$ , and a mean of 247.5x$10^3$/mm$^3$ , and median of 250x$10^3$/mm$^3$ .

Regarding the significance of low presenting ALC (absolute lymphocyte count) with CCR (continuous complete remission); there was no significant relationship between presenting ALC and CCR ($P$ = 0.903) as shown in table (10)

**Table (10) the relationship of low ALC at presentation and the CCR**

| ALC | NO. | Patients with CCR | *P* value |
|---|---|---|---|
| Low | 23 | 6 | 0.903 |
| Normal | 52 | 18 | |

## DISCUSSION

The majority of cases in this study belong to the age group (5-9) year (46.4%), this result is different from other studies done in Iraq which revealed that majority of cases were below 5 years of age as found in AL-Haddad study[6]; however this result goes with a study done by Murphy [7].

The mean age of patients at presentation was 6.3 years which is near to what was found by Mohammed in Iraq[8], Klumb et al in Brazil [9] Saud study[10]; however this is lower to mean age found by Marky et al [11]. The male to female ratio was 2:1; other studies also found that male to female ratio was 2:1 as found in Al-Haddad study(6) , Klumb et al in Brazil [9], and Marky et al [11]. The mean duration of illness before diagnosis was 5.2 weeks which is less than what was found by Mohammed 6.2 weeks [8]. These two results show that most patients are presenting late in the disease.

The most common primary site involved was the abdomen in 67.8% of patients , other studies also found that abdomen was the most common site involved as by AL-Haddad 85% [6],and Klumb et al 75% [9]. Bone marrow involvement was found in 16.6% of patients which is higher than study done by Mohammed which was 10.6 %[8], and similar to Klumb which was 17 %[9]. Chest involvement (Mediastinal or hilar mass or pleural effusion) occurs in 10.7% of patients which is lesser than result obtained by Mohammed study [8] and Murphy[7].This difference in results may be due to that the findings were registered depending mainly on CXR and not on CT scan. CNS involvement was present in 2.3% of patients which is clearly lower than results obtained by Mohammed study 8.8% [8] and by Al Haddad study in 9.6% [6].

This may be due to a technicopersonal factors in evaluating the CSF and the lack of the cytospin machine in our center.

Fever at presentation was present in 40% of patients which is lower than Saud study

in 50.7% of patients [10]. Pallor was present in 46% of patients which is similar to result obtained by Saud study 47.8% [10]. Splenomegaly was present in 22.6% which is higher than results obtained by Saud study who found it in only 3 %[10]. Hepatomegaly was present at diagnosis in 30.9%of patients while in Saud study in 14.9% of patients [10]. This difference in the above 2 findings may be due to variable personal efficiency in detecting hepatosplenomegaly or probably inadequate file records, as both studies were retrospective.

The majority of patients were in advanced stages i.e. stage III&IV in 88% of patients which is near to results obtained by Mohammed study 91.2% [8] , and higher than AL Haddad study 71.5% [6], and Marky et al[11],and Pillon et al [12] who found 69% and 68% of the patients were stage III&IV respectively. This high percentage of patients who presented in advanced stages is due to many factors which led to the long mean duration of illness prior to diagnosis.

Burkitt lymphoma was the most common histological subtype in 58.33% which is higher than Murphy et al which was 40%[7] and nearly similar to AL Haddad study[6] which was 57%,but lower than Klumb et al study72%[9]. Diffuse large B cell lymphoma (DLBCL) was present in 15.47% of patients. Klumb et al found DLBCL in 11% of cases [9]. Lymphoblastic lymphoma was present in 14.28%, this is nearly similar to what was found by Saud study16.4 %[10] and lower to Murphy et al which was 35 %[7]. The unclassified NHL was present in 11.9% of patients, which is lower than result obtained by Mohammed study which was 36 %[8], but nearly similar to Klumb et al which was 11 %[9].

This percentage of unclassified NHL is due to lack facilities for the pathologists as histochemical staining ,immunophenotyping, and cytogenetic studies.

Serum uric acid at presentation was elevated in 29.7% of patients, and the serum creatinine was elevated in 41.6% of patients, this reflects the higher percentage of patients at risk of developing *tumor lysis syndrome* (TLS), also it reflects the aggressiveness of the disease in pediatric age group and even a delay in the diagnosis. In a study done by Cheg et al in Hong Kong 2009[13], it was found that 12% of patients developed laboratory evidence of TLS, and another 12% developed clinical TLS, these results by no means indicate that our patients are at higher risk for developing TLS.

The initial Hb level was less than 10 gm/dl in 20.2% of patients; while in Saud [10] 76.1% of patients had this low level of Hb. Nevertheless it is well known that the limited extent of BM infiltration in NHL usually does not lead to anemia [14].

The initial platelet count was low in only 5.95% of patients, while in a study done by Saud [10] found that 16.4% had a platelet count of less than $100x10^3/mm^3$ .The initial WBC count was normal in majority of the cases 70%, and low in 10.7% of patients, and elevated in only 1.19%, this also goes with common findings in NHL [14].

The initial absolute lymphocyte count was normal in 61.8% of patients and low in 27.3% of patients, this similar to results obtained by Isaballe et al who found that lymphopenia is present in 27% of cases [15].

The low presenting ALC was not shown to be adversely affecting the outcome of patients regarding CCR in comparison to patients who had normal ALC at presentation ($P = 0.903$).

## Conclusions

The majority of cases were between 5-9 year age group, with a male to female ratio of 2:1.The most common presenting site was the abdomen. The majority of cases were fallen in advanced stages (III&IV), Burkitt lymphoma was the commonest subtype.

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# Assessment of GM-CSF Level in the Serum of Patients with Different Stages of Chronic Myeloid Leukemia Befor and After Imatinb Mesylate Therapy

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**Abstract**

**Background:** Chronic myeloid leukemia (CML) is a clonal myeloproliferative disorder of primitive haemopoietic progenitor cells. The cytogenetic hallmark of CML is the Philadelphia chromosome, created by a reciprocal translocation between chromosomes 9&22 (t [9;22][q34;q11]). Survival and amplification of hematopoietic progenitors are controlled by a number of regulatory molecules (hematopoietic growth factors). The role of Granulocyte-macrophage colony-stimulating factor (GM-CSF) and its receptor in pathology arises largely as a result of abnormal signaling leading to deregulated myelopoiesis. The development of the BCR-ABL-targeted Imatinib mesylate represents a paradigm shift in the treatment of CML. Data indicate that the level of immune responses against CML is low before Imatinib, rises as treatment is administered and declines again when the BCR-ABL transcript numbers fall to low levels.

**Objective:** To assess and evaluate the significance of levels difference in GM-CSF through newly diagnosed patients, different responder groups (optimal, suboptimal and failure cytogenetic response) and advanced stages (acceleration and crisis groups) of CML Iraqi patients whom receiving Imatinib mesylate (TKI), as an indicator to assess the role of growth factor in pathogenesis of CML disease development and response to treatments.

**Patients and methods:** In this study 128 Iraqi CML patients asses before and after receiving imatinib mesylate treatment which categorized by complete blood picture and fluorescent *in situ* hybridization (FISH) analysis in to different response groups and stages, then used an ELISA technique to assess serum level of GM-CSF in each response group and advance stage (acceleration and transformed) of CML patients, in comparison to level in 32 healthy control subjects.

**Results**: Out of 128 patients the mean of GM-CSF serum level (pg/ml) for the newly diagnosed, optimal responded, suboptimal responded, failure cytogenetic and advance stage of CML were 399.53±104.50, 325.23±66.37, 428.90±45.70, 347.12±54.45, 521.56±83.73, respectively. While healthy was 269.25±86.27.

**Conclusion:** Uncontrolled granulopoiesis in newly diagnosed patients with CML may be mediated by increased plasma CSF concentrations caused by BCR-ABL activity which may give an idea regarding the role of colony stimulating factors in the pathogenesis of CML disease.

**Keywords:**Chronic myeloid leukemia, GM-CSF, Imatinib mesylate**.**

## Introduction

CML is defined specifically as a myeloproliferative disease that is characterized by the invariable presence of the Philadelphia chromosome (Ph) or the BCR/ABL fusion gene [(1)]. It is originating in a pluripotent stem cell common to all three hematopoietic lineages, resulting in overproduction of myeloid cells in all stages of maturation [(2)].

The course of the CML is characteristically triphasic: a chronic phase (CP) lasting 3-6 years is followed by transformation to an accelerated phase (AP) and then a terminal blast phase of short duration [(3)], the latter of which resembles an acute myeloid state [(4)].

Transition from CP to more advanced stages of the disease is not well understood, but it is believed to result from genomic instability, BCR-ABL-induced cell proliferation would lead to the acquisition of additional chromosome abnormalities, known as clonal evolution [(5)].

Dramatic progress has been made in treatment over the past several years, so most people with CML are now surviving at least 5 years after diagnosis and initiation of tyrosine kinase inhibitor therapy. But because the highly effective drugs are still fairly new, the average survival of people now being diagnosed with CML is not known [(6)].

The mechanism by which BCR-ABL-positive cells achieve growth factor independence has not yet been fully elucidated [(7)]. Some of the studies showed, BCR-ABL protein contains the active tyrosine kinase region of ABL, producing a cytokine independent, constitutive proliferative signal and affects a variety of downstream pathways. This signal results in continuous cell growth and replication [(8)].

From the other view, the possible mechanisms include the activation of cytokine signal transduction pathways by BCR-ABL and/or aberrant expression of cell cycle control genes, cytokine receptors, and the autocrine production of the growth factor by the cell itself [(7)].

GM-CSF and its receptor may have a role in CML pathology, arises largely as a result of abnormal signaling leading to deregulated myelopoiesis with enhanced proliferation and survival of myeloid precursors, which is a common feature of myeloproliferative disorders and myeloid leukemias [(9)].

## Patients and Methods

This study conducted between Oct.2011 up to July.2012 including 128 Iraqi cases of chronic myeloid leukemia with different stages and different therapy responses, receiving imatinib mesylate 400-800mg per day for at least 18months were evaluated in Baghdad teaching hospital/ hematology department.

Patients of the study were free of fever, diabetes mellitus, hypertension and infection. Laboratory tests including complete blood picture and fluorescent in situ hybridization results were taken from patients records at time of sampling. Patients are categorized according to FISH analysis results to either: CML patients of optimal response (normal CBC and 0% of FISH result) or CML patients of suboptimal and failure response (normal CBC and FISH analysis of 1-35% and >35% respectively) and CML patients in advance stage (abnormal CBC indices regardless of FISH analysis result).

Five ml of vein puncture blood were withdrawn from both patients and controls then dispensed in plain tube and centrifuged for 15 minutes at 3000 rpm after being allowed to clot at room temperature for 30 minutes. The separated sera were stored frozen at -20°C for determination of GM-CSF concentration.

Serum GM-CSF level was determined by ELISA using a quantitative sandwich enzyme immunoassay technique (Abcam, UK). All tests were carried out by vigorously following manufacturer instructions. Serum GM-CSF level was calculated by interpolation from a standard curve that was performed in the same assay as that for the sample.

The data were processed using the SPSS-20, mean GM-CSF level (quantitative data) was compared between groups using independent Student t-test.

## Results

Out of 128 CML patients at different stages were included in the study, 70 (54.68%) were males and 58 (45.31%) were females (M: F ratio1.2:1). and mean age 41.06±12.42 ranging 20-76years while Thirty two samples of apparently healthy volunteers were included and evaluated as control samples with mean age was 36.37±7.65.

All CML cases were categorized according to different stages depending on the presenting signs and symptoms of patients, full blood count with blood film and by FISH for BCR-ABL analysis results as shown in Table (1).

**Table- 1: Distribution of chronic myeloid leukemia cases according to the different stages of the disease.**

| stage of CML | No. of male(%) | No. of female(%) | Total no.(%) |
|---|---|---|---|
| **Newly diagnosis CML** | 14(43.8) | 18(56.3) | 32(25) |
| **Optimal response** | 18(56.3) | 14(43.8) | 32(25) |
| **Suboptimal response** | 16(72.7) | 06(27.3) | 22(17.18) |
| **Failure cytogenetic response** | 10(38.5) | 16(61.5) | 26(20.31) |
| **Advance stage** | 12(75.0) | 04(25.0) | 16(12.5) |

All CML patients apart of newly diagnosed CML (naïve) included in this study were on imatinib mesylate as shown in Table (2).

**Table-2: Distribution of chronic myeloid leukemia cases according to the imatinib therapy doses.**

| Imatinib Dose/mg | Newly | Failure | Advanced | Suboptimal | Optimal |
|---|---|---|---|---|---|
| | % | % | % | % | % |
| **400** | - | 26.9 | 25.0 | 63.6 | 100 |
| **≥600** | - | 73.1 | 75.0 | 36.4 | - |

Patients characteristics according to their disease duration, FISH analysis results and the mean ±SD (pg/ml) of serum GM-CSF level are shown in Table (3).

**Table-3: Distribution of chronic myeloid leukemia cases according to the Mean±SD of disease duration, FISH results and GM-CSF level.**

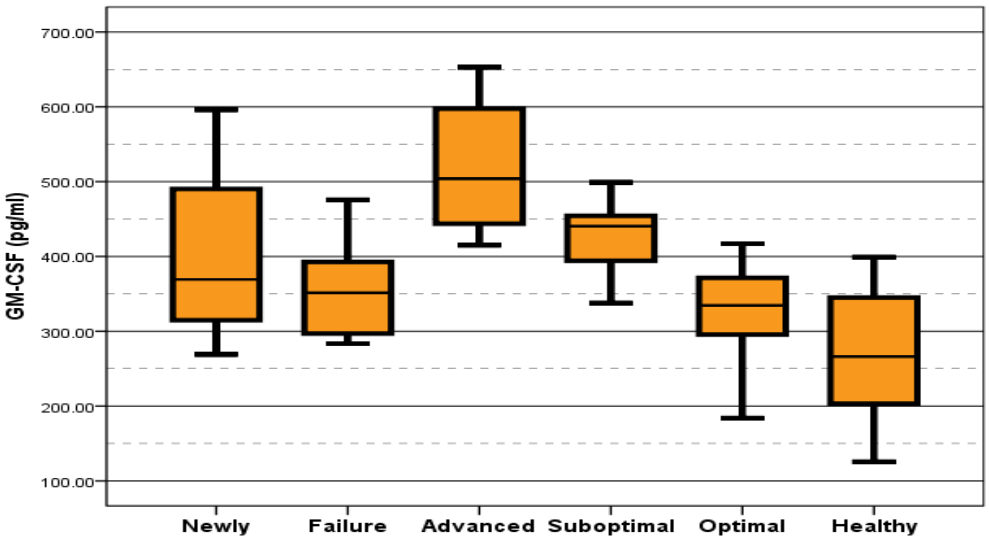
| Disease status | Disease duration(years) Mean±SD (Range) | FISH result (%) Mean±SD(Range) | GM-CSF (pg/ml) Mean±SD |
|---|---|---|---|
| **Newly** | - | 92.94±8.69 (50-98) | 399.53±104.50 |
| **Optimal** | 3.53±1.67 (1-8) | 0 | 325.23±66.37 |
| **Suboptimal** | 4.23±1.63 (2-8) | 12.91±11.94 (1-33) | 428.90±45.70 |
| **Failure** | 5.31±2.56 (1-11) | 61.54±17.46 (9-88) | 347.12±54.45 |
| **Advanced** | 6.63±4.11 (1-13) | 78.19±13.14(58-99) | 521.56±83.73 |
| **Healthy** | - | - | 269.25±86.27 |

The statistical analysis of GM-CSF mean level through different stages of CML patients by using student t-test is shown in Table (4) Figure (1).

**Table-4: Shows P-value of each studied group of CML patients and controls according to the GM-CSF mean level.**

| GM-CSF (pg/ml) | Healthy | Newly | Optimal | Suboptimal | Failure | Advanced |
|---|---|---|---|---|---|---|
| **Mean** | 269.25 | 399.53 | 325.23 | 428.90 | 347.12 | 521.56 |
| **P value compared to healthy** | - | 0.0001* | - | - | - | - |
| **P value compared to Optimal** | - | 0.001* | - | 0.0001* | 0.182 | - |
| **P value compared to Advanced** | - | - | - | - | 0.0001* | - |
| *Significant using t-test for two independent means at 0.05 level of significance. | | | | | | |

**Figure- 1: Distribution of mean GM-CSF level through different groups of CML patients and controls.**




## Discussion

Colony stimulating factors are the principal cytokines in granulopoiesis and differentiation of granulocyte precursors [10, 11].Because of the widespread expression of the GM-CSF receptor in hematopoietic cells, it was assumed that both GM-CSF and its receptor were key players in the regulation of steady state functions [9].

The role of GM-CSF and its receptor in pathology, on the other hand, arises largely as a result of abnormal signaling leading to deregulated myelopoiesis with enhanced proliferation and survival of myeloid precursors, a common feature of myeloproliferative disorders and myeloid leukemias [9].

Imatinib mesylate (Gleevec; Novartis Pharma), a selective inhibitor of BCR-ABL kinase activity, selectively inhibits downstream signaling and the growth of BCR-ABL+ cells, inducing apoptosis of these cells [8].

Imatinib mesylate is the most prominent example for a new generation of anticancer drugs [12]. Despite the rapid success of imatinib as a targeted cancer therapy, there

seems to be some controversy about its influence on immune function [13].

Recent study analysis showed, in newly diagnosed CML patients a statistical significant increase in GM-CSF when compared with healthy control group. Also data showed no evidence of significant correlation between GM-CSF and high WBC count. This may contribute; to the abnormal microenvironment but the abnormalities in progenitors themselves of CML patients, may be mostly a cause for autonoms secretion from the cloned cells. This is similar to [14, 15] who suggested that growth factors may participate in delaying apoptotic cell death of myeloid leukemic cells.

Accordingly this may be contributed to the selective expansion of leukemic progenitors and suppression of normal hematopoiesis in CML. Our results are similar to the findings of Balleari *et al*. [10] study, who concluded that significant amounts of endogenous GM-CSF is detectable in the serum of a substantial percentage of patients with CML in chronic phase. Similarly, mice transplanted with BCR-ABL transduced bone marrow display increased transcripts and serum level of GM-CSF which contribute to the genesis and/or clinical phenotypes of CML [16] this finding disagree with another murine model by Li *et al* [17] concluded that GM-CSF is not required for induction of CML-like disease by BCR-ABL. Also this result disagreed with the result of Lee *et al* [18] were suggest the possibility that patients with CML have less functional GM-CSFr than healthy adult and these findings suggest that lower levels of plasma CSFs concentration and dysfunction of CSFr, especially GM-CSF, in patients with CML are important points in discriminating the pathogenesis of neutrophilia from that of infection, and may play an important role in explaining leukemogenesis in patients with CML.

In imatinib therapy era and through assessment of GM-CSF level in different responses and stages of CML patients, present results showed, CML patients who had achieved optimal (FISH BCR-ABL analysis <1%) cytogenetic response, their GM-CSF level showed statistical significant decreased level when compared them with the newly diagnosed CML patients; suggesting that an imatinib inhibit the BCR-ABL cells proliferation, Beside improvement of bone marrow microenvironment by imatinib. Engler *et al*. [19] also speculate that rapid depletion of mature CML cells by imatinib may deprive CD34+ cells of essential cytokines normally produced by the mature leukemic population. This cytokine-dependent environment may remove the proliferative advantage of leukemic hematopoiesis, facilitating the re-growth of the residual non-leukemic hematopoietic cells that will be reflected in deeper molecular responses.

From another view, those CML patients who had progression in their disease and they lost their complete cytogenetic response (suboptimal and failure cytogenetic response), Serum GM-CSF level analysis showed significant increase in suboptimal responders group when compared with the optimal responders group, as an increasing level of BCR-ABL may lead to increase in the GM-CSF secretion by cloned leukemic cells which may contributed to disease progression. While those with failure cytogenetic response showed non-significant increase in GM-CSF level when compared with the optimal responders (p-value 0.182), in spite of, showed a significant decrease when compared with the suboptimal responders (p-value 0.0001). This may be due to increased dose of imatinib in the failure group, 73.1% of patients were on 600-800mg/day which may cause a partial suppression of BCR-ABL cloned cells in spite of their was no significant correlation between WBC count and different doses of imatinib from one side and GM-CSF level from another side.

In patients with advance disease stage (acceleration and blast transformation), our data showed a significant increase serum

level of GM-CSF when compared with the failure group. This may be due to increasing the cloned BCR-ABL cells which secrete GM-CSF (autocrine process). This is agreed with Wang *et al*. [20] who describe the potential novel role for autocrine GM-CSF secretion as a counter regulatory mechanism of BCR-ABL positive cells to resist imatinib and nilotinib via mediated JAK2/STAT5 pathway activation which are critical antiapoptotic and transforming targets of BCR-ABL.

From present data, imatinib seems to be capable to remodulate the bone marrow microenvironment leading to conditions favorable to immune function restore and activate cells of the immune system. Thus microenvironment of the bone marrow gradually improves till optimal cytogenetic response is achieved where BCR-ABL then will be at a very low level. So the abnormal high level of GM-CSF will be get down due to decreased numbers of cloned BCR-ABL progenitors that causing increase secretion of GM-CSF. This idea is similar to the conclusion by Poggi & Zocchi, [21] who found patients who are responder to imatinib mesylate, the production of the stromal-derived factor-1(SDF-1) and of the B lymphocyte activating factor of the tumor necrosis factor family (BAFF), both involved in normal B cell development and maturation is induced by imatinib, at variance with non-responder patients. Also another study which showed that antileukemia T-cell responses develop in the majority of analyzed CML patients in hematologic and cytogenetic remission under imatinib treatment [22].

## Conclusion

This study tested CML patients in different cytogenetic responses and the elevated level of GM-CSF in the newly diagnosed of CML cases and decreasing after initiation of TKI therapy may give us a view about the role of colony stimulating factor in the pathogenesis of CML and the mechanism of an increase proliferation of granulopoiesis in CML patients which open a new view regarding the role of TKI in arrangement of the microenvironment of bone marrow in CML patients.

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# Gender Disparity in Clinical Presentation, Immunophenotyping, and Early Steroid Response in Pediatric Acute Lymphoblastic Leukemia Patients

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**[2]Dr. Sajed Saad Mohammed (M.B.Ch.B, M.Sc. Pathology)**

**Abstract**

**Background:** Acute leukemia is the most common type of childhood cancer, of which acute lymphocytic leukemia (ALL) comprises 78% of cases. Incidence rate, prognosis and survival of childhood ALL patients differ according to gender. Despite overall improvements in survival of children with ALL, male children still experience poorer survival.

**Objective** is to explore differences between male and female pediatric patients with newly diagnosed ALL regarding their presenting clinical features, ALL immunophenotype, and early steroid response.

**Methods:** This study was prospectively designed to include 60 newly diagnosed pediatric ALL patients from April 2011 to March 2013. Each patient was assessed clinically at admission and at the end of a 7-day prednisone prophase to be classified as a prednisone-good responder (<1000/µL peripheral blood blasts on day 8) or a prednisone-poor responder (>1000/µL). Immunophenotype was determined by immunocytochemical staining of bone marrow aspirates for cCD79a (specific for B-cells) and cCD3 (specific for T-cells).

**Results:** The study group consisted of 38 males and 22 females. The median age was 62.5 months for males and 41.5 months for females. Splenomegaly was found in 71% of males versus 63.6% of females, hepatomegaly in 68.4% of males versus 45.5% of females, mediastinal masses were detected in 6 males and 3 females, and CNS disease affected 5 patients, 3 males and 2 females ($p$>0.05). WBC mean count was 63.78±15.98 x$10^9$/L in males and 49.2±21.87 x$10^9$/L in females, the mean Hb was 8.75±0.53 g/dl in males and 7.91±0.34 g/dl in females ($p$>0.05). 75.8% of male patients were B-ALL and 24.3% were T-ALL, and 76.5% of females were B-ALL and 23.5% were T-ALL. 86.8% of male patients and 86.4% of female patients were good steroid responders ($p$ > 0.05).

**Conclusions:** Pediatric male patients were more frequent and older than females, and presented with clinical and hematological features considered to be of poor prognosis more than females. No significant difference was observed regarding ALL immunophenotypes and early steroid response.

**Key words:** Acute lymphoblastic leukemia, gender, immunophenotype, steroid response.

## Introduction

Acute leukemia is the most common type of childhood cancer. It accounts for 30% of all cancers diagnosed in children younger than 15 years. Within this population, acute lymphocytic leukemia (ALL) accounts for approximately 78% of all childhood leukemia diagnoses [(1, 2)].

In Iraq, according to the Iraqi Cancer Registry 2008, leukemia ranks the first among the commonest ten cancers in children, constituting 32.59% of all childhood cancers. According to gender, leukemia is the most common cancer in males and females comprising 33.76% and 30.9% of all cancers, respectively [(3)].

In childhood cancer, the variation of incidence according to gender is well-established worldwide; the incidence of pediatric ALL is consistently higher among males (approximately 20%) relative to females, with a male to female age-adjusted incidence of 1.3. This gender difference is not only observed regarding incidence, but also in regard to prognosis and survival of childhood ALL patients [(4)].

Despite overall improvements in survival of children with ALL with

expected cure rates now exceeding 85% [5], one segment of those children still experience poorer survival, mainly male children [6, 7, 8]. The observed sex differentiation in survival may be related to occurrence of testicular relapses among boys, increased risk for marrow and CNS relapse, lower DNA index, and the greater incidence of T-cell ALL among boys [6, 8, 9].

Several other prognostic factors have been identified in childhood ALL including age, basic laboratory studies (presenting white blood cell count and presence of leukemia in cerebral spinal fluid), characteristics of the leukemic blasts (immunophenotype, cytogenetics, and molecular abnormalities), and response to initial treatment [10, 11, 12].

## Aim of study

This present study is designed to explore differences between male and female pediatric patients regarding their presenting clinical features, ALL immunophenotype, and early steroid response.

## Methods

This study was prospectively designed to include 60 newly diagnosed pediatric ALL patients from the Central Child Teaching Hospital and Child Welfare Teaching Hospital, Baghdad, Iraq, for the period from April 2011 to March 2013. The study protocol was approved by the Ministry of Health in Iraq and an informed consent was taken from one or both parents.

Inclusion criteria for ALL patients were age less than or equal to 15 years, random gender. All patients were newly diagnosed with ALL with no history of any other malignant disease, any anti-cancer drug or a previous blood transfusion.

The diagnosis of ALL was made on bone marrow aspirate (BMA) smears stained with Leishman, Periodic acid Schiff, and Sudan Black B stains according to modified French-American-British (FAB) morphologic criteria. For purpose of immunophenotyping, two additional BMA smears were taken from each patient, fixed in 10% formalin for 10 minutes, air-dried, wrapped with Aluminum foil, and were stored at -20°C until time of immunocytochemical (ICC) staining for cCD79a and cCD3, which was conducted in Al-Yarmook Teaching Hospital, Baghdad, Iraq.

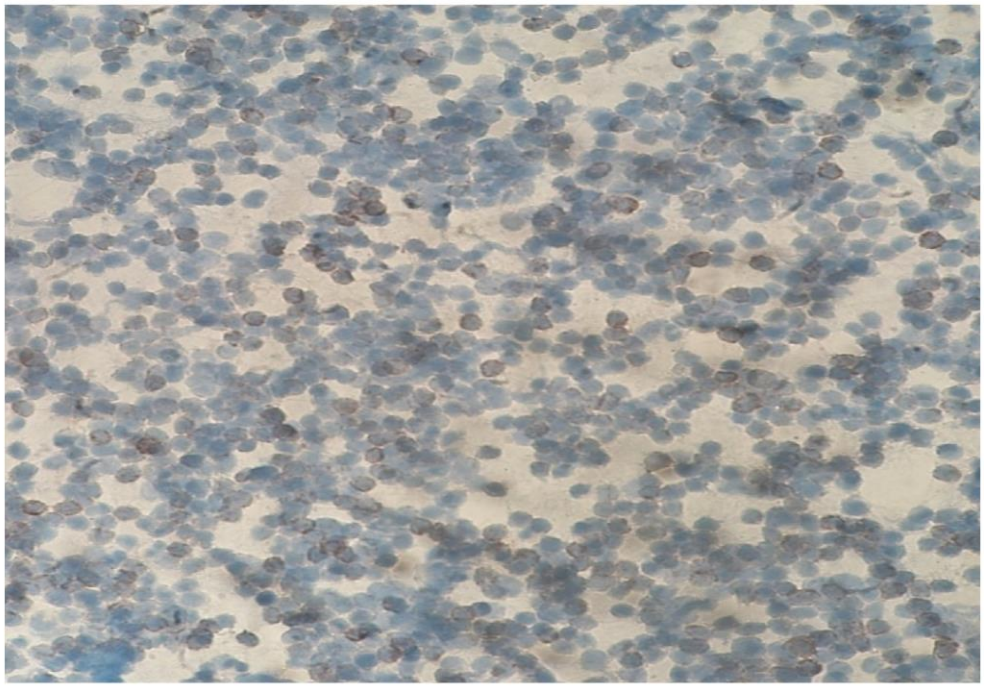
Clinically, each patient was assessed twice, the first assessment was at time of admission, and the second was after a 7 day prednisone course (40 mg/m$^2$). The first assessment included a history taking and physical examination, in addition to relevant laboratory investigations, X-ray and CSF examination if indicated. The second assessment was at the end of a 7-day prednisone prophase to classify the patient as a prednisone-good responder (< 1000/µL peripheral blood blasts on day 8) or a prednisone-poor responder (> 1000/µL peripheral blood blasts) [13]. The patients' clinical data of both assessments was obtained from patient hospital records and clinical monitoring charts.

BMA smears were stained immunocytochemically using three steps-indirect streptavidin method for Monoclonal Mouse Anti-Human cytoplasmic CD79a (cCD79a), clone JCB117, manufactured by DAKO, Denmark and Polyclonal Rabbit Anti-Human cytoplasmic CD3 (cCD3), code no A0452, manufactured by DAKO, Denmark. Brown cytoplasmic staining for cCD79a and cCD3 in at least 20% of blast cells was considered positive reactions in BMA smears [14]. (Figures 1,2). Positive controls for cCD79a and cCD3 were considered from tonsil and colon tissues, respectively. Technical negative controls were obtained by omission of primary antibody.

Data were analyzed using SPSS (Statistical Package foe Social Sciences) version 16 and Microsoft Office Excel 2007. Numeric variables were expressed as **mean±SE** while nominal variables were expressed as frequency and percentage. Comparison of numeric variables between two groups was performed using independent samples student **T-test**, while

among more than two groups, was performed using one way **ANOVA** and post hoc **LSD** multiple comparison test. Comparison of frequency among various groups was done using **Chi-Square** test or **Fischer exact** test whenever is needed. Correlation coefficient was calculated using **Kindall's tau-b** test for one nominal and one numeric variable. ***p*-value** less than 0.05 was considered significant.

**(A)**



**(B)**

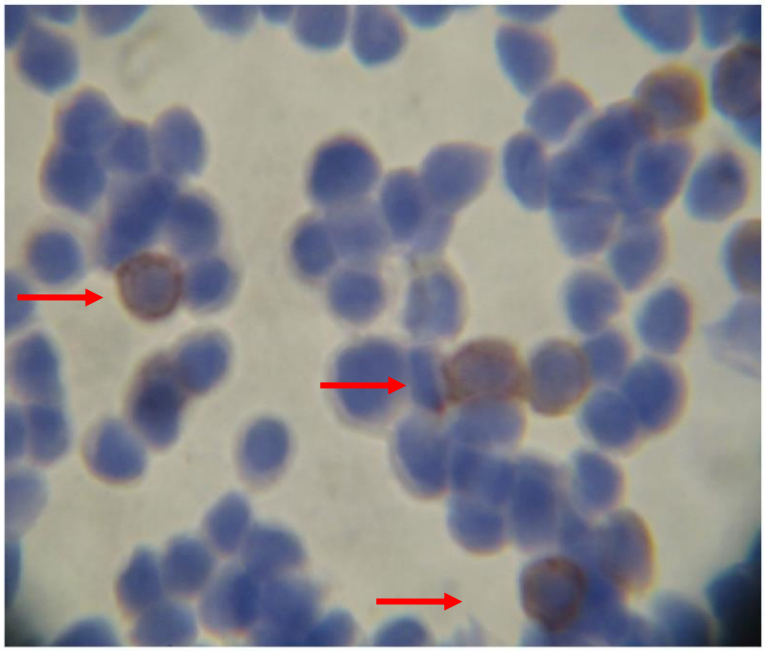
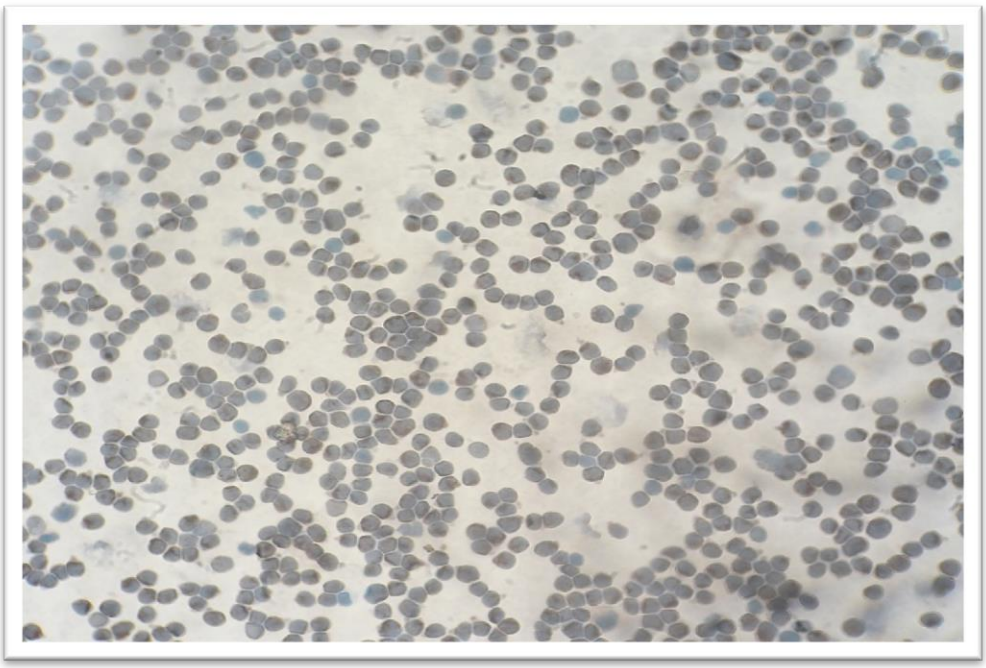


**Figure 1**
**cCD79a immunocytochemical expression in pediatric ALL**
**A. Bone marrow aspirate sample showing cCD79a positive brown cytoplasmic staining (40X).**
**B. Bone marrow aspirate sample showing cCD79a positive brown cytoplasmic staining, arrows (100X).**

(A)



(B)

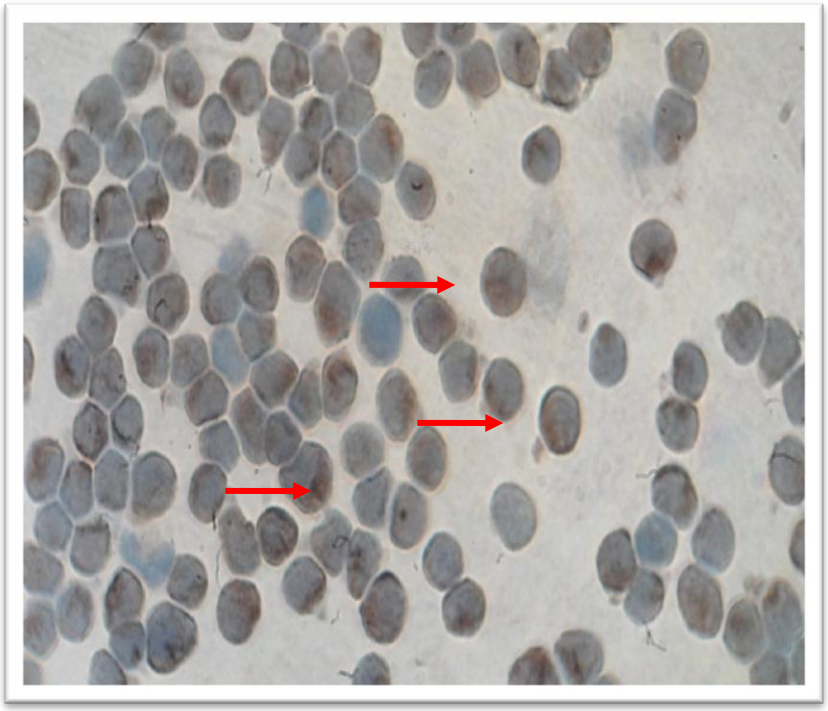


**Figure 2**
**cCD3 Immunocytochemical expression in pediatric ALL.**
**A. Bone marrow aspirate sample showing cCD3 positive brown cytoplasmic staining (40X).**
**B. Bone marrow aspirate sample showing cCD3 positive brown cytoplasmic staining, arrows (100X).**

**Results**

In this study, Pui CH grouping scheme for presenting clinical and lab features was adopted [15]. Accordingly, the patients` ages at diagnosis and their presenting white cell counts, Hb levels, platelet counts, and BM blast percentages were categorized into discrete groups (Table 1).

**Table 1: Groups used by the study to present patients` clinical and lab features**

| | Variables | | | | |
|---|---|---|---|---|---|
| | **Age (years)** | **WBC count x10$^9$/L** | **Hb level g/dl** | **Platelet count x10$^9$/L** | **Leukemic blasts in BM (%)** |
| **Groups** | **<1**<br>**1-9**<br>**≥10** | **<10**<br>**10-49**<br>**50-99**<br>**>100** | **<8**<br>**8-10**<br>**>10** | **<50**<br>**50-100**<br>**>100** | **<90**<br>**>90** |

The mean age of the study group was 63.68±5.66 months (mean ± SE), their median age was 50 months, ranging between two months and thirteen years at diagnosis, consisting of 38 (63%) males and 22 (37%) females, with a male to female ratio of 1.73:1 (Figure 3).

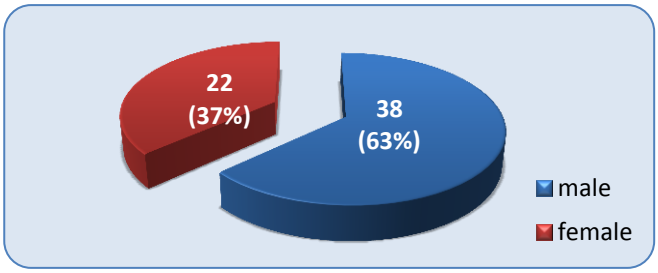



**Figure 3 Gender distribution of ALL patients**

The presenting age of male patients ranged between 2 months and 13 years, the mean age was 69.16±7.10 months (mean ± SE), and the median age was 62.5 months while the age of female patients ranged between 3 months and 12 years, the mean age was 54.23±8.98 months (mean ± SE), and the median age was 41.5 months. The majority of male and female patients at diagnosis fell within the age group of 1-9 years, and the least presenting age group was less than one year (Figure 4).

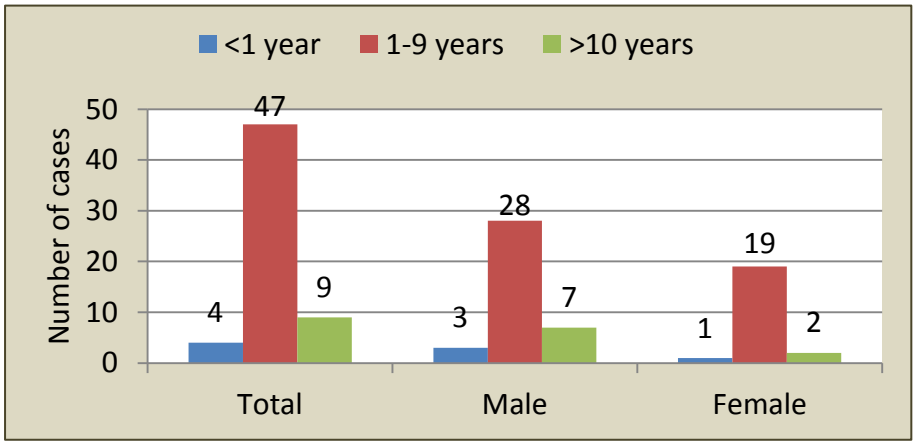



**Figure 4 Age distribution of ALL patients**

The results pertaining to clinical features are shown in Table 2. Fever and splenomegaly were the most common presenting features in both genders. Mediastinal mass was present in 6/38 (15.8%) males and in 3/22 (13.6%) female patients. CNS involvement was found in 3 male patients (two of them had CNS2 and one had CNS3) and in two female patients (CNS2 in one of them and CNS3 in the second) ($p > 0.05$).

**Table 2 Clinical features of ALL patients**

| Character | Total No. (%) (n=60) | Male (%) (n= 38) | Female (%) (n=22) | *p*-value |
|---|---|---|---|---|
| Fever | 48 (80.0) | 32 (84.2) | 16 (72.7) | 0.327 |
| Pallor | 32 (53.33) | 20 (52.6) | 12 (54.5) | 1.000 |
| Bone pain | 18 (30.0) | 12 (31.6) | 6 (27.3) | 0.375 |
| Mucocutaneous bleeding | 14 (23.33) | 7 (18.4) | 7 (31.8) | 0.237 |
| Fatigue | 11 (18.33) | 8 (21.1) | 3 (13.6) | 0.731 |
| Splenomegaly | 41 (68.33) | 27 (71.1) | 14 (63.6) | 0.552 |
| Hepatomegaly | 36 (60.0) | 26 (68.4) | 10 (45.5) | 0.080 |
| LAP | 36 (60.0) | 24 (63.2) | 12 (54.5) | 0.512 |
| Mediastinal mass | 9 (15.0) | 6 (15.8) | 3 (13.6) | 1.000 |
| CNS involvement | 5 (8.33) | 3 (7.9) | 2 (9) | 1.000 |
| Testicular mass | 0 | 0 | 0 | ---- |

Results concerning peripheral blood counts are shown in Table 3.

**Table 3 Hematological parameters of ALL patients**

| Parameter | Total patients n=60 | Male patients n=38 | Female patients n=22 | *p* value |
|---|---|---|---|---|
| **WBC count x$10^9$/L (mean ± SE)** | 58.49±12.84 | 63.87±15.98 | 49.20±21.87 | 0.586 |
| <10 x$10^9$/L | 18 | 12 | 6 | Not valid |
| 10-49 x$10^9$/L | 22 | 11 | 11 | |
| 50-99 x$10^9$/L | 13 | 10 | 3 | |
| >100 x$10^9$/L | 7 | 5 | 2 | |
| **Hemoglobin (g/dl) (mean ± SE)** | 8.44±0.36 | 8.75±0.53 | 7.91±0.34 | 0.264 |
| <8 g/dl | 25 | 15 | 10 | 0.137 |
| 8-10 g/dl | 25 | 14 | 11 | |
| >10g/dl | 10 | 9 | 1 | |
| **Platelet x$10^9$/L (mean ± SE)** | 59.28±6.48 | 50.96±6.62 | 73.64±13.15 | 0.092 |
| <50 | 31 | 21 | 10 | 0.245 |
| 50-100 | 19 | 13 | 6 | |
| >100 | 10 | 4 | 6 | |
| **BM blast% (Mean ± SE)** | 86.88±1.3 | 86.13±1.86 | 88.18±1.46 | 0.451 |
| <90% | 32 | 20 | 12 | 0.886 |
| >90% | 28 | 18 | 10 | |

The WBC count was less than 10 x$10^9$/L in 27.3% of females versus 31.6% of males, was 10-49 x$10^9$/L in 50% of females versus 28.9% of males, ranged between 50-99 x$10^9$/L in 13.6% of females and 26% of males, and exceeded 100 x$10^9$/L in 9.1% of female versus 13.15% of male patients (*p* value was not valid) (Figure 5).

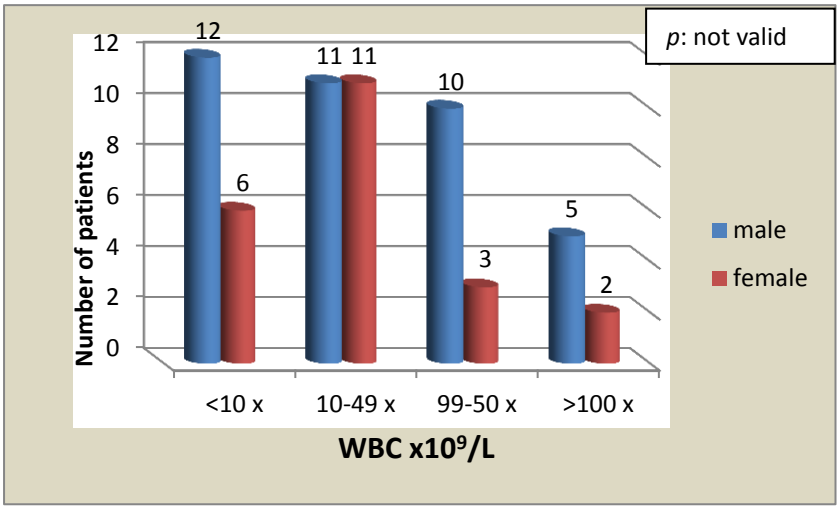



**Figure 5 WBC count distribution according to gender**

As shown in Figure 6, there was a correlation between WBC counts and gender, with decreasing counts in female patients. However, this correlation was statistically not significant (*p* = 0.58).

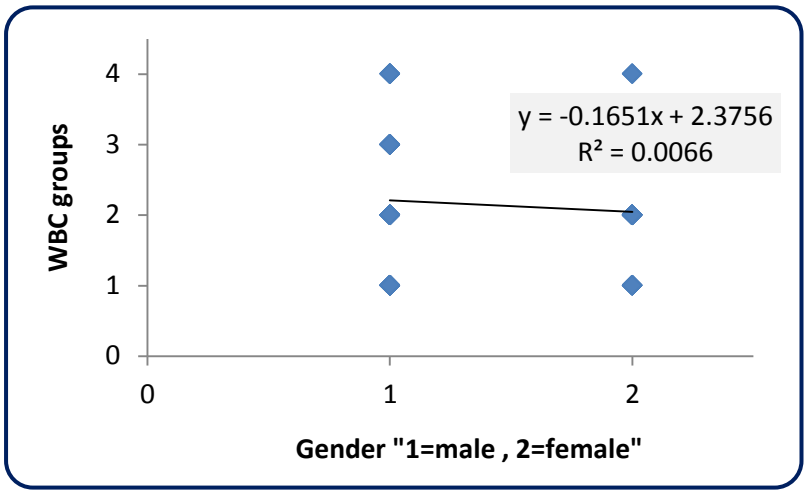



**Figure 6 Correlation between WBC counts and**

Results of ICC staining revealed that 41 out of 60 BMA smears (68%) were cCD79a positive and cCD3 negative and thus considered B-ALL, and 13 (22%) were cCD79a negative and cCD3 positive and thus were classified as T-ALL subtype. No result was obtained in 10% of BMA samples (Figure 7).

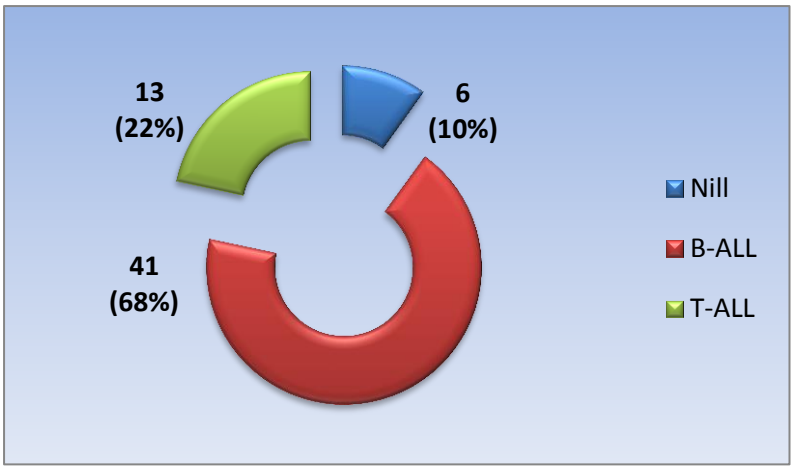



**Figure 7 Distribution of ALL cases according to immunophenotype**

The ICC staining was successful in 37 male and 17 female patients. 28 out of 37 (75.8%) male patients were B-ALL and 9/37 (24.3%) were T-ALL. Whereas in the 17 female patients, 13 (76.5%) were B-ALL and 4 (23.5%) were T-ALL. Male to female ratio was slightly higher in T-ALL than in B-ALL patients (2.3:1 vs 2.2:1) ($p$ = 1.000). (Table 4)

**Table 4 Distribution of gender according to immunophenotypic classification**

| Gender | B-ALL | T-ALL | *p* value |
|---|---|---|---|
| **Male (n=37)** | 28 (75.75) | 9 (24.3%) | 1.000 |
| **Female (n=17)** | 13 (76.5%) | 4 (23.5%) | |
| **male:female** | 2.2:1 | 2.3:1 | |

Assessment of steroid response showed that 52 out of 60 ALL patients (87%) were good steroid responders and 8 (13%) were poor steroid responders (Figure 8). Good steroid response was documented in 33/38 male patients (86.8%) and in 19/22 female patients (86.4%) ($p > 0.05$).

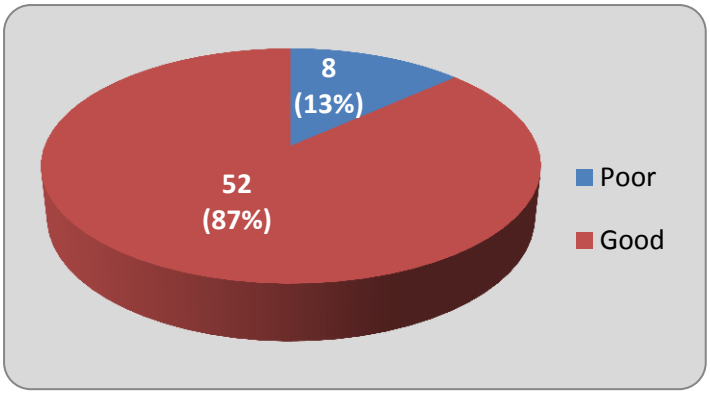



**Figure 8 Frequency of steroid response in ALL patients**

## Discussion

Presenting clinical features of children with ALL such as age and white cell count are continuous variables and such variables are almost always used in prognostic models after categorization [16]. For this reason the presenting clinical features of patients in this study were categorized into discrete groups on the basis of groups proposed by Pui CH [15].

Gender is one of the features that have consistently shown to be associated with outcome of leukemia in children. In the present study males comprised 63% of total cases of pediatric ALL and females 37%, with a male to female ratio of 1.7:1. This ratio is very closely matched to that obtained by Ameen's study [17] where it was (1.6:1), and was similar to other literature which cited a male predominance in pediatric ALL [18,19,20,21,22].

Age at diagnosis has a strong independent prognostic effect. Patients more than 10 years or less than 1 year old fare much worse than children in the intermediate age group [23, 24]. In the present study the incidence of pediatric ALL peaked at 1-9 years of age, with a median age of 50 months (4.2 years). These figures were comparable with other Iraqi studies and other literature on non Iraqi populations [17,19,25,26,27,28]. However other literature reported an older age at presentation, ranging between 7-9 years [20,21,22,29,30].

In this study boys were presented with a higher median age (62.5 months) than that of girls (41.5 months). Concerning age groups, the peak age was at the prognostically favorable 1-9 years age group in both male and female patients. However, the proportion of female patients within this age group was 86.4% which was higher than the 73.7% of male patients, whereas a higher proportion of male patients was observed at both extremes of age, i.e. below 1 year and above 10.

Clinical features associated with high tumor burden, including hepatosplenomegaly and mediastinal mass, are poor prognostic features and associated with a greater risk of relapse [31, 32]. In this study, enlargements of spleen and liver were reported in 68.3% and 60% of patients, respectively. Male preponderance was observed among those patients, suggesting a higher tumor burden in this group. Mediastinal masses were detected in 15% of patients, 6 males and 3 females. This observation is in accordance with other Iraqi studies [19,33] that cited a male predominance of mediastinal involvement.

The presence of CNS disease at diagnosis is an adverse prognostic factor. The 5-year event-free survival (EFS) of CNS-2 and CNS-3 patients is 71.8% and 70.1%, respectively, compared with 79.9% for CNS-1 [31, 34]. In the present work, CNS involvement was detected in 5 (8.3%) patients, 3 males and 2 females. Two had CNS3 (a male and a female) and three had CNS2 disease (2 males and a one female).

In this study no testicular mass was detected in any of the enrolled patients, in line with Pui CH who stated that overt testicular disease is relatively rare at diagnosis, occurring in only 2% of patients [15].

In summary, this study found that pediatric male patients presented with clinical features considered being of poor prognosis more than females. This supports the conclusion of Pui *et al* and Shuster *et al* that male gender is associated with an inferior outcome [6, 7].

Regarding hematological features, leukocyte count is a well-known continuous prognostic variable, whereby increasing counts confer a poorer outcome [24]. A WBC count of 50 $x10^9$/L is generally used as an operational cut point between better and poorer prognoses [35].

In this study, 50% of female patients had a WBC count within the good prognostic group of 10-49 $x10^9$/L versus 28.9% of male patients, whereas hyperleukocytosis (> 100 $x10^9$/L) was measured in 9.1% of females versus 13.2% of male patients. WBC counts were found to correlate, although statistically not significant, with gender with decreasing counts in female patients. These figures of lower counts in females strengthen the concept of favorable prognosis of female patients in pediatric ALL.

The mean Hb of ALL patients was 8.44 ± 0.36 g/dl, with higher values in males than in females (8.75±0.53 versus 7.91±0.34 g/dl). The majority of patients (83.4%) were presented with moderate to

severe anemia (Hb < 10 g/dl) including 95.5% of females and 76.3% of males whereas only 16.7% of patients had Hb levels higher than 10 g/dl, including 23.7% of males and only one (4.5%) female. Thus lower Hb levels less than 10g/dl were more often diagnosed in female patients.

These results were close to those reported by Al-Barazanchi who cited a higher proportion (30%) of male patients versus 9.5% of female patients presented with Hb level >10g/dl [(33)].

Anemia in ALL mainly results from suppression of normal hematopoiesis in the bone marrow by infiltrating blasts. Several studies have demonstrated a converse correlation between degree of anemia and survival [(16, 36)]. Whereas other contradictory evidence suggest that lower Hb levels at diagnosis are linked to a lower risk of relapse and a higher EFS compared to higher Hb levels, which may reflect conditions with a high proliferation rate of an aggressive leukemic cell clone [(37)].

Differences in platelet counts with regard to gender showed a higher mean platelet count in females than in males. The percentage of female patients with platelet counts >100 $x10^9$/L was higher than that of males, and the very low platelet count group (<50 $x10^9$/L) included 55.3% of male patients versus 45.5% of females. Even though, mucocutaneous bleeding was more frequent in female than in male patients. The cause for which is unknown and entails further verification.

Platelet count is not considered a risk criterion. Miller *et al* stated that platelet count is not a significant predictor of disease-free survival [(38)] and Settin *et al* found no significant correlation between platelet counts above or below 50 x $10^9$/L and remission rates in ALL patients [(39)].

Higher percentages of BM blasts give a rough estimate of tumor burden. In the present study, a slightly higher proportion of male patients (47.4%) versus (45.5%) of female patients, had > 90% BM blasts at diagnosis.

The present study did not show a significant difference in ALL immunophenotype between male and female patients, the percentage of male patients with T-ALL subtype was slightly higher than that of females. The same finding was observed regarding early steroid response in which no significant difference was found between male and female ALL patients.

A larger sample of patients is required to clearly determine the effect of gender on prognosis of pediatric ALL patients. In this study, the *p* value was not valid in some instances because of the low number of the enrolled patients.

The impact of gender on the prognosis of pediatric ALL was assessed by Pui *et al* in a large study involving 2055 children. In agreement with this current study, Pui *et al* cited that boys, compared with girls, were more likely to have unfavorable presenting features, including a T-cell immunophenotype, high leukocyte count, and a presenting age of 10 years or older. Additionally, boys were less likely to have a favorable DNA index and more likely to have a poor early response to remission induction therapy than girls [(6)]. Studies also revealed that girls have a superior EFS compared to boys, even when they are treated with less therapy [(23, 31, 40)].

## Conclusions

1. Male patients were more frequent and older than female patients.
2. At the prognostically favorable 1-9 years age group, the proportion of female patients was higher than male patients. Whereas a higher proportion of male patients was observed at both extremes of age below 1 year and above 10 years.
3. Pediatric male patients presented with clinical and hematological features considered to be of poor prognosis more than females, including hepatosplenomegaly, mediastinal mass, high WBC counts, and high Hb levels.

4. No significant difference was observed between male and female patients regarding ALL immunophenotypes and early steroid response.

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# Expression of CD7, CD13, CD14, CD33 and CD34 on Myeloblasts in Bone Marrow Aspirate of Patients with Newly Diagnosed Acute Myeloid Leukemia Using Multicolor Flow Cytometry

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**Abstract:**

**Background:** An important part of diagnosis of acute leukemia is to decide on the lineage whether it is of myeloid or lymphoid. Familiarity with expression of various surface antigen markers is essential to diagnose difficult cases and to follow up for minimum residual disease.

**Aim of the study:** To evaluate the expression of CD7, CD13, CD14, CD33 and CD34 in bone marrow aspirate of patients with newly diagnosed AML using multicolor multiparametric flow cytometry.

**Patients, materials and methods:** This is a prospective study which includes 21 newly diagnosed adult patients with AML from April 2012 to March 2013. Flow cytometry analysis for CD7, CD13, CD14, CD33 and CD34 was carried out on bone marrow aspirate samples using 2-laser, 4-color PARTEC Cube6 and using De Novo FCS Express 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. Only samples with blasts that contain Auer rods, positive for SBB cytochemical stain (≥3%) and/or MPO+ by FC (≥10%) are included.

**Results:** The median, range and interquartile range percentages for myeloblasts, CD7, CD13, CD14, CD33 and CD34 are 48, 22-84 and 24; 30, 20-36 and 7; 62, 34-98 and 28; 53, 21-86 and 6; 56, 30-91 and 17; and 73, 25-94 and 34respectively.

**Conclusions:** The use of FC at diagnosis of AML can be useful to add objective confirmation especially in cases where Auer rods are not present and when SBB stain did not add towards the diagnosis. Moreover, if the aim is to follow up for MRD then the use of FC becomes essential.

## Introduction:

According to the 2008 WHO classification of acute leukemia, the diagnosis of acute leukemia requires morphological demonstration of the presence of 20% or more blast cells in peripheral blood and/or bone marrow, except in cases with certain cytogenetics abnormalities or erythroleukemia, and then to decide on the lineage whether it is of myeloid or lymphoid origin. The main marker to classify cases as acute myeloid leukemia (AML) is positivity for myeloperoxidase (MPO) by cytochemistry, flow cytometry (FC) or immunohistochemistry[(1)].

Conventionally, positivity for Sudan Black B (SBB) stain is considered equal to myeloperoxidase (MPO) positivity[(2)], although itis now more than 30 years since first reporting that in very rare cases lymphoblasts may show SBB positivity [(3)]; however, characters like being of less intensity than the "internal control" remnant normal cells of the myeloid series, non-granular and diffuse reaction help to indicate that these are not myeloblasts. Therefore, in practice the choice between SBB and MPO cytochemical stains is based on availability and local experience[(4)].

In Iraq, acute leukemia being of myeloid lineage is decided by morphology if Auer rods are present; otherwise, positivity for SBBby cytochemistry is used which is a subjective test that in many occasions requires a careful examination by an experienced hematopathologist.

More recently testing for myeloperoxidase by flow cytometry (FC) has been performed on several samples of acute leukemia and this is mainly because morphologyissufficient to fulfill the criteria of blast percentage and evidence of myeloid

lineage inmany but not all cases of AML and morphology is not enough to diagnose cases of acute lymphoblastic leukemia (ALL) and mixed phenotypic acute leukemia (MPAL).However, although MPO highly specific to diagnose AML, it is not sensitive enough and alone is not adequate to follow up patients for minimum residual disease (MRD).

**Aim of the study:** To evaluate the expression of CD7, CD13, CD14, CD33 and CD34 in bone marrow aspirate of patients with newly diagnosed AML using multicolor multiparametric flow cytometry.

**Patients, materials and methods:** This is a prospective study which includes 21 patients with AML who had bone marrow aspirate samples submitted for evaluation by acute leukemia panel by flow cytometry in Al-Rawabi Laboratory during April 2012 to March 2013. The patients were admitted to the National Center for Hematology and to Baghdad Medical City.In this study, all patients are adults and their identification details are 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-suspend 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. Only samples with blasts that contain Auer rods, positive for SBB cytochemical stain (≥3%) and/or MPO+ by FC (≥10%)[6] are included and they were tested for the following protocols:

| Tube number | Blue laser | | | Red laser |
|---|---|---|---|---|
| | FITC | PE | PE-DY$^{647}$ | APC |
| 1 | CD34 | CD13 | CD45 | CD33 |
| 2 | CD14 | BLANK | CD45 | BLANK |
| 3 | CD7 | BLANK | CD45 | BLANK |
| 4 | MPO | BLANK | CD45 | BLANK |

The first 3 tubes were used for all patients; however, the last tube was used only if there was a suspicion of AML with absence of Auer rods and SBB stain not available or inconclusive.

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, thengating on CD34+ myeloblasts using CD34 versus SSC plot. Thendrawing different plots to study the expression of MPO (if required), CD13, CD14and CD33 on CD34+ myeloblasts.However, in samples where CD34- myeloblasts and/or promyelocytes are present, then the expression of the above mentioned markers were studied in

the blast region of the CD45 versus SSC plot.

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

SPSS version 18.0.0was used for statistical analysis of data.

**Results:**In this study, 21 adult patients with AML were included, 12 males and 9 females with male:female ratio of 1.3:1, with median age of 51 years and range of 16-63 years.

The diagnosis of patients with AML according to FAB classification is presented in table 1, and the descriptive results for this study are summarized in table 2 and figure 1.

**Table 1: Diagnosis of patients with AML according to FAB classification:**

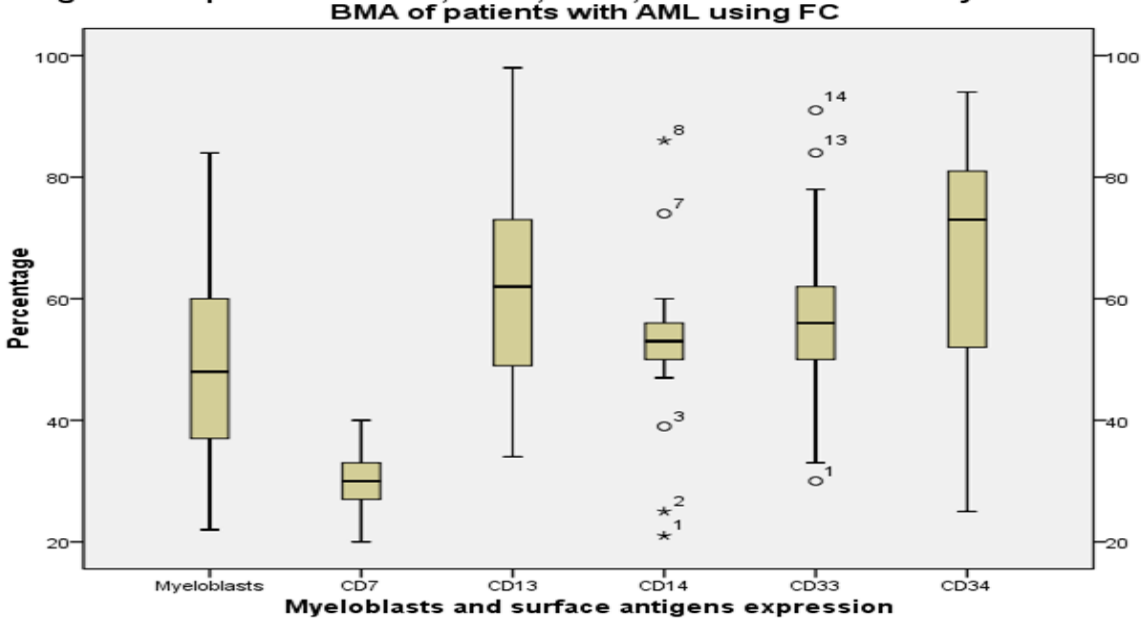
| FAB classification | No. | % |
|---|---|---|
| M0 | 1 | 5 |
| M1 | 6 | 28 |
| M2 | 8 | 38 |
| M3v | 1 | 5 |
| M4 | 3 | 14 |
| M5a | 1 | 5 |
| M5b | 1 | 5 |
| Total | 21 | 100 |

**Table 2: Expression of CD7, CD13, CD14, CD33 and CD34 on myeloblasts in BMA of patients with AML using FC:**

| Parameter | Myeloblasts (%) | Percentage of antigen expression on surface of myeloblasts * | | | | |
|---|---|---|---|---|---|---|
| | | CD7 (n=6) | CD13 (n=19) | CD14 (n=8) | CD33 (n=15) | CD34 (n=20) |
| **Median** | 48 | 30 | 62 | 53 | 56 | 73 |
| **Range** | 22-84 | 20-36 | 34-98 | 21-86 | 30-91 | 25-94 |
| **Interquartile range** | 24 | 7 | 28 | 6 | 17 | 34 |
| **Mean ± SD** | 49.2 ± 17.7 | 29.7 ± 5 | 61.7 ± 17.5 | 52.4 ± 13.6 | 56.7 ± 15.6 | 66.2 ± 20.9 |

Surface antigen expressionby FC of <20% is considered negative[7] and, therefore,is not included within this table, "n" represents number of samples with positive expression of CD marker.

Figure 1: Expression of CD7, CD13, CD14, CD33 and CD34 on myeloblasts in BMA of patients with AML using FC



**Discussion:**

The available combination of CD markers for diagnosis of AML is large and increasing, however, in developing countries the cost is an important limiting factor. One good policy is to choose antigen markers that clearly serve a diagnostic, follow up or therapeutic aim.

Laboratory diagnosis of acute leukemia in Iraq is conventionally based on morphology with/without SBB stain. More recently FC has been used to support diagnosis aiming to identify the lineage and to design a panel for follow up for MRD.

Forward scatter (FSC) versus side scatter (SSC) plot is used for all samples to get rid of the debris. Then CD45 versus SSC is used to define cell populations. The use of CD34 in flow cytometry in the majority of cases of acute leukemia is important to define the blast population which is found in the blast gate and/or monocyte/monoblasts gate. The use of CD13 and CD33 is mainly as surrogate markers to define cells of myeloid origin and this will be useful for follow up of MRD and also as aberrant markers on lymphoblasts which is beyond the interest of this study. CD13 is the most sensitive marker for AML. The use of CD7 in AML is as an aberrant marker to better define leukemic myeloblasts at follow up for MRD and to differentiate it from remnant/regenerating normal myeloblasts. CD14 is used as a marker for cells of monocytic lineage [7, 8, 9].

From this study, if the decision was to use FC for diagnosis, the use of CD13, CD33 and CD34 appear to be essential for objective confirmation of lineage (CD13+ and CD33+) and nature of cells as blasts (CD34+) as this was the case in the majority of samples, 19/21, 15/21 and 20/21 for CD13, CD33 and CD34 respectively.

The use of CD14 alone cannot be recommended from the available data in the present study as althoughthe use of CD14 did change the diagnosis in 2/21 samples and confirmed the morphological diagnosis in 6/21 samples; however, there is the possibility of use of cytochemical stain, as non-specific esterase (NSE) which, although not tested in this study, may represent a reasonable and cheaper replacement to the use of monocyte markers by FC. Moreover, the 2008 WHO classification recommends the finding of at least 2 positive monocyte markers, including CD11c, CD14, CD64 and/or lysozyme, to assign the lineage.

The use of CD7 did not add to the diagnosis; however, if the aim is to build a follow up template for MRD then there is good potential of being helpful as it is positive in slightly less than 29% of samples in this study.

Finally, the current study represents a humble trial to formulate a logical and cost-effective combination of CD markers to be used locally in Iraq; we tried our best to keep expenses to the minimum and we are aware that there is a large potential to add more CD markers when testing AML samples with FC at diagnosis and for follow up of MRD.

**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 screening and confirming the diagnosis of acute leukemia and its subtypes and to avoid technical and cellular contamination start with serial gating using FSC versus SSC plot to get rid of the technical debris, then CD45 versus SSC plot to gate on the possible blast cells which are usually CD45dim versus low SSC, then CD34 versus SSC plots to better gate on the leukemic blast cells which are usually CD34+. Further gating can be used when a specific diagnosis of certain subtype of acute leukemia is being investigated.
3. For screening of AML, after proper serial gating to isolate the possible leukemic

blast population, using a combination of surface markers that ensure high sensitivity, namely CD13 and CD33.

4. For confirmation of provisional diagnosis of AML, after proper serial gating to isolate the possible leukemic blast population, then confirming the nature of cells as being myeloblasts by testing for cMPO expression.
6. The use of FC at diagnosis of AML can be useful to add objective confirmation especially in cases where Auer rods are not present and when SBB stain did not add towards the diagnosis. Moreover, if the aim is to follow up for MRD then the use of FC becomes essential.

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# Type 2 Diabetes Mellitus is causing red blood cell hemolysis

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**Abstract:**

**Background:** Diabetes mellitus is a metabolic disease and its complications are interlinked and usually have a common soil. The clinical effect of diabetes on erythropoiesis is always interesting as the production of red cells is always at high rate and continuous, yet the red cell mean life span of 120 days is of an appropriate length, making it an excellent candidate for tests to detect the effect of diabetes and its complications and monitor for the response to treatment.

**Aim of the study:** To determine the level of reticulocyte percent in patients with uncomplicated type 2 Diabetes Mellitus and to evaluate the effect of folic acid treatment on this percent.

**Patients, controls and methods:** 140 patients with type 2 Diabetes Mellitus were included in this study. The exclusion criteria from this study were anemia, pregnancy, personal and family history of hemolytic anemia, overt microvascular complications of DM (retinopathy, neuropathy and nephropathy), acute infection and/or inflammation and history of chronic disease other than diabetes. The patient medical record was reviewed and peripheral blood specimen withdrawn for determination of hemoglobin concentration, PCV %, reticulocyte % and $HbA_1c$. A subgroup of (82) patients (45 males and 37 females) were chosen who consented on not to change their treatment for the next coming one month except for the addition of daily one 5mg tablet of folic acid. PCV %, hemoglobin concentration, reticulocytes % were tested for using manual techniques. $HbA_1c$ % was measured using automated HPLC machine.

**Results:** This study revealed increased red cell destruction in type 2 diabetics in comparison to healthy control subjects of the same sex and age. Also the reticulocyte increment was more in those with higher $HbA_1c$ %, although it was not in linear relationship with it. These findings are suggesting that the initiating event for red cell hemolysis is the increased blood glucose level.

**Conclusion:** Type 2 diabetes patients are subject to oxidative stress as a result of hyperglycemia. This study suggests that the addition of folic acid treatment to the regime of type 2 diabetic patients can be useful.

## Introduction:

Diabetes mellitus (DM) is a syndrome of altered carbohydrate, fat, and protein metabolism resulting from an absolute or relative deficiency of insulin resulting in a group of common metabolic disorders that share the phenotype of hyperglycemia, the two broad categories of DM are type 1 and type 2 [(1)].

The burden of this disease is best demonstrated by the accompanying risk for microvascular and macrovascular complications and death [(2)], and the fact that the prevalence of hyperglycemia in Iraq is 10.4% [(3)]; and it is noticeably increasing [(4)].

The diagnosis of Diabetes is based on the intention to identify the fasting blood glucose concentration that best predicted the risk of developing specific diabetic

microvascular complications (nephropathy, retinopathy and neuropathy) [5].

$HbA_1c$ is a term used to describe stable minor hemoglobin components formed slowly non-enzymatically from post-translational modification of HbA by glucose. The rate of formation of $HbA_1c$ is directly proportional to the ambient glucose concentration as erythrocytes are freely permeable to glucose; practically there is a strong relationship between levels of $HbA_1c$ and the average blood glucose levels over the previous 3 months [6]. $HbA_1c$ is the gold standard test of blood glucose control [7].

Hemolysis is defined as a reduction of the red cells life span, this usually results in anemia. While in compensated hemolysis a compensatory increase in erythropoiesis may be adequate to prevent the development of anemia. An elevated reticulocyte count is a reflection of the compensatory increase in erythropoiesis by the bone marrow, and it is, therefore, an indirect indication of shortened red cells life span [8].

Diabetes mellitus is a metabolic disease and its complications are interlinked and usually have a common soil. The precise effect of diabetes on red blood cells is incompletely studied.

The clinical effect of diabetes on erythropoiesis is always interesting as the production of red cells is always at high rate and continuous, yet the red cell mean life span of 120 days is of an appropriate length, making it an excellent candidate for tests to detect the effect of diabetes and its complications and monitor for the improvement and response to treatment.

**Aim of the study:**

To determine the level of reticulocyte percent in patients with uncomplicated type 2 Diabetes Mellitus and to evaluate the effect of folic acid treatment on this percent.

**Patients, controls and methods:**

140 patients, 70 males and 70 females, aged 30-62 years, with type 2 Diabetes Mellitus attending the national center for Diabetes Mellitus at Almustansiriya University in Baghdad-Iraq, during the period from August-November 2010 were included in this study.

The exclusion criteria from this study were anemia, pregnancy, personal and family history of hemolytic anemia, overt microvascular complications of DM (retinopathy, neuropathy and nephropathy), acute infection and/or inflammation and history of chronic disease other than diabetes.

The patient medical record was reviewed and peripheral blood specimen withdrawn for determination of hemoglobin concentration, PCV %, reticulocyte % and $HbA_1c$.

A subgroup of (82) patients (45 males and 37 females) were chosen who accepted to consent on not to change their treatment for the next coming one month except for the addition of daily one 5mg tablet of folic acid.

A total of 140 healthy controls, 70 males and 70 females, were included in this study; they were selected in a way to be age (range) and sex (ratio) matched for the patients group.

PCV % was measured using the microhematocrit technique. Hemoglobin concentration was measured using cyanmethemoglobin method. Reticulocytes % were counted using peripheral blood smears stained with new methylene blue. $HbA_1c$ % was measured using automated HPLC machine.

Statistical analyses were performed by using Statistical Package for Social Sciences (SPSS) version 18.0 software, with p-value of less than 0.05 considered significant.

**Results:**

There was a highly statistical significant difference between hemoglobin concentration, PCV % and reticulocyte % between type 2 diabetic patients and healthy control subjects (Table 1).

Also there was a significant difference between the different groups of type 2 diabetic patients that were stratified according to $HbA_1c$ % using ANOVA test. However, there was no linear correlation between each patient $HbA_1c$ % and its correspondent reticulocyte % (Table 2).

The addition of folic acid treatment would considerably improve the reticulocyte percent (Table 3).

**Table 1: Differences in mean Hb concentration,anbbbd PCV% and reticulocytes% between healthy subjects and type 2 diabetic patients:**

| Parameter | | Healthy subjects | Type 2 diabetic patients | P-value |
|---|---|---|---|---|
| Hb (g/dl) * | Males | 14.6 (13-16.9) | 14.1(13-15.2) | < 0.001 |
| | Females | 12.9 (12-14.6) | 12.4 (12-13.1) | < 0.001 |
| PCV % ** | Males | 46 (40-50) | 42 (40-46) | < 0.001 |
| | Females | 39 (36-45) | 37 (36-40) | < 0.001 |
| Reticulocytes % | | 1 (0.3-2) | 3.3 (1.8-4.6) | < 0.001 |

* Normal Hemoglobin concentration for males 13-17 g/dl and females 12-15 g/dl [9].
** Normal PCV % for males 40-50 % and females36-46 % [9].

**Table 2: Relation between $HbA_1c$ % and reticulocytes %:**

| $HbA_1c$ % | Reticulocytes % mean and range | P-value |
|---|---|---|
| < 6 | 2.2 (1.8-2.5) | 0.02 |
| 6.1-7 | 3.2 (2-3.8) | |
| > 7 | 3.8 (2.9-4.6) | |

**Table 3: Effect of folic acid treatment on reticulocyte %:**

| Reticulocytes % mean and range | | P-value |
|---|---|---|
| Before folic acid treatment | After folic acid treatment | |
| 3.5 (2.2-4.3) | 2.4 (1.6-3.7) | < 0.01 |

**Discussion:**

This study revealed increased red cell destruction in type 2 diabetics in comparison to healthy control subjects of the same sex and age. Also the reticulocyte increment was more in those with higher $HbA_1c$ %, although it was not in linear relationship with it. These findings are in consistence with Hudson et al [10], suggesting also that the initiating event for red cell hemolysis is the increased blood glucose level.

Type 2 diabetes patients are subject to oxidative stress as a result of hyperglycemia. Folate supplementation improves markers of oxidative stress [11]. This study suggest that addition of folic acid treatment to the regime of type 2

diabetic patients can be useful, but the prospect of applying these data to the overall diabetes management and follow up for improvement in diabetes control with reticulocyte% (or preferably absolute reticulocyte count if available) necessitate studying this subject even more.

There should be more detailed investigations to answers the questions about the exact mechanism of red cell destruction in type 2 diabetes mellitus, as whether it is due to the oxidative stress imposed by the hyperglycemia.

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# Incidence of Von Willebrand Disease Among Patients presenting with Various Bleeding Tendency to Out-Patient Clinic of the National Center of Hematology

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**Abstract**

**Background**: Von Willebrand disease is frequent hereditary bleeding disorders with an incidence of about 1% in asymptomatic people. Previous Studies available around the Middle East displayed a prevalence ranged from 3 % to 34 % of Von Willebrand disease within the hereditary bleeding disorders. People with mucocutaneous bleeding represent a major subtype of hematologic clinical presentations but simultaneously present a substantial diagnostic challenge. On the other hand, bleeding symptoms are frequent in the general population, but their clinical relevance may be difficult to assess The aim of this study was to estimate the incidence of Von Willebrand disease in patients presenting with various bleeding tendencies to out-patient clinic of the national center of hematology

**Methods:** A total of 146 sequential patients referred to the national center of hematology between January 2011 and April 2012 were investigated .Tests performed for the diagnosis of Von Willebrand disease included complete blood count and blood film including platelet count, bleeding time, prothrombin time, activated partial thromboplastin time, Factor VIII:C assay, and von Willebrand Factor Antigen assay.

**Results**: Amongst 146 patients, 29 (19.8%) had Von Willebrand disease. Patients' age ranged from 1 year to 65 years, with 35 males and 111 females. Menorrhagia was the most common presentation. Amongst vWD patients, there were 7 male and 22 female. Positive family history in patients with VWD was found in 21 out of 29 patients (72.4 %) while positive family history in bleeding tendency other than VWD was found in 47 patients (40%). Statistical significant differences were found in prothrombin time, activated partial thromboplastin time, Factor VIII: C assay, and von Willebrand Factor Antigen assay between the studied groups.

**Conclusions:** Von Willebrand disease still among the most common cause of inherited bleeding tendency in patients presented with mucocutaneous or menorrhagia, yet many cases of vWD remain undiagnosed due to wide range of clinical presentations and lack in lab diagnosis.

**Keywords:** incidence, vWD, bleeding tendency

## Introduction

Von Willebrand disease (vWD) is the most common, primarily autosomal dominant inherited bleeding disorder, which affects both male and female at equal level. It is estimated that about 1% of general population had vWD. [1,2] It is caused either by quantitative deficiency (Type 1 and Type 3) or qualitative defect (Type 2) of von Willebrand factor (vWF). The vWF is produced in the endothelial cells and megakaryocytes. It is kept in Weibel Palade bodies in the endothelial cells and alfa granules of thrombocytes.

The plasma level of vWF is equal to 10μg/ml. In the plasma it presents into the multimeric dimer configuration with different sizes ranging from small (500 kD) to very large (>10,000 kD) high molecular weight multimeric (HMWM) forms.

The larger molecules have greater sticky ability because of high number of individual adhesion positions. The vWF flows in the plasma in association with factor VIII: coagulant protein (FVIII: C) as vWF: FVIII: C complex. The two most significant roles of vWF are (i) it assists the adhesion of platelets to subendothelium at the position of damage, therefore contributing in primary hemostasis and (ii) it stabilizes FVIII:C in circulation and increases its half-life by five to 10 folds,

therefore it as well takes part in secondary hemostasis. vWD is actually a heterogenous disorder since it has different molecular mutations and variable penetrance. Within certain group of people there can be variety of phenotypes with different bleeding manifestations that may alter after a period of time. [3] There are many types of assays to be done by the laboratory specialists undertaking the investigations for vWD. Because of restrictions of each laboratory test, no single test technique is enough to allow detection of all types of vWD. [4] For all the reasons mentioned above, vWD continues to be an under diagnosed entity. Very few studies are available from Iraq or form Middle East pointing to prevalence of about 14% of vWD amongst all inherited bleeding disorders. [5, 6] that's why this study was designed to estimate the incidence and of vWD in our center.

**Material and method**

The study was carried on at the national center of hematology/ Almustansiriya University, Baghdad, Iraq. Patients attended out-patient clinic or referred form other hospitals because of bleeding tendency like prolonged bleeding from injuries, following tooth extraction, epistaxis, menorrhagia, gum bleeding, ecchymosis, hemarthrosis, etc., were tested for vWD. There were two groups in this study. Group 1 included 146 sequential patients, while group 2 includes 60 apparently healthy persons. The study period was between January 2011 and April 2012. The study was approved by the Institutional Ethics Committee.

Oral informed consent was obtained from the patients or parents for participation in the study prior to collection of blood samples. A detailed questionnaire containing the nature of the bleeding episodes, age at the onset, frequency of bleeding, family history, mode of inheritance and history of prior medication including blood transfusion was filled along with detailed physical examination.

Complete blood count and platelets were analyzed on whole blood containing EDTA by Cell counter, (Cell Dyne,USA).Coagulation tests were examined on fresh blood containing 3.2% sodium citrate anticoagulant centrifuged with 2500 G rpm for 15 minutes.[7]

Then coagulation tests were performed on platelet poor plasma. Bleeding time was performed by a laboratory technician using IVY technique. In this method, the blood pressure cuff is placed on the upper arm and inflated to 40 mmHg and after making an incision with appropriate size and depth on the forearm, the bleeding time was measured with a filter paper. PT (Prothrombin time) was measured with STAGO kit and the STA compact analyzer with clotting method and 3 units above control PT was considered abnormal. Also, aPTT was measured with STAGO kit and the STA compact analyzer with clotting method and 5 units above control PTT was considered abnormal. vWF: antigen levels were estimated by ELISA using commercial kits (Diagnostica Stago, France).

**Results**

A total of 146 patients were examined for abnormal bleeding manifestations. Out of 146 patients, 29 (19.8%) patients were diagnosed as von Willebrand disease by appropriate tests done for them. The age of overall patients ranged from 1 year to 65 years with median age of 33.7 years. There were 35 males and 111 females with M: F ratio of 0.31. Amongst vWD patients, there were 7 male and 22 female. Positive family history in patients with VWD was found in 21 out of 29 patients (72.4 %) while positive family history in bleeding tendency other than VWD was found in 47 patients (40%).

Table (1) shows that mean age± SD of patient with bleeding tendency and control group were 18.26±14.23 years , 23.11±14.1

years respectively .There was slight significant difference in the age of the studied groups($p < 0.05$).Statistical significant differences were also found in PT, PTT,BT, VonWillebrand factor and factor VIII between the studied groups.

Table (2) shows the percent of the clinical manifestations in 146 patients in which menorrhagia (33.5 %) was the most common presentation followed by epistaxis (31.5%), and ecchymosis (28%) in patient with or without von Willebrand disease.

Table (3) shows no significant statistical difference in age and PT between patients with VonWillebrand disease with bleeding tendency and those without ($p>0.05$).while Significant statistical differences found in PTT, BT, factor V111 and vWF between the two groups.

**Table 1: Distribution of variables between the two studied groups**

| variable | Bleeding tendency group | Control group | P value |
|---|---|---|---|
| **No.** | 146 | 60 | |
| **Male: female** | 35:111 | 14:46 | |
| **Mean age ± SD (yr.)** | 18.26±14.23 | 23.11±14.1 | 0.02 |
| **PT second (Mean ±SD)** | 13.34±1.2 | 12.32±1.3 | 0.0001 |
| **PTT second(Mean ±SD)** | 37.32±12.44 | 29.12±12.1 | 0.0001 |
| **BT minute(Mean ±SD)** | 5.26±4.0 | 3.2±0.5 | 0.0001 |
| **VWF Ag %** | 78 | 116 | 0.0001 |
| **Factor 8 %** | 61 | 124 | 0.0001 |

P value from student t-test

**Table 2: Bleeding manifestation in vWD**

| Bleeding manifestations | No.of patients | Percent |
|---|---|---|
| **Menorrhagia** | 49 | 33.5 |
| **Epistaxis** | 46 | 31.5 |
| **ecchymosis** | 41 | 28 |
| **Bleeding from minor cut** | 3 | 2 |
| **Bleeding after trauma** | 2 | 1.3 |
| **GIT bleeding** | 2 | 1.3 |
| **Hematuria** | 2 | 1.3 |
| **Gingival bleeding** | 1 | 0.6 |

**Table (3): Distribution of variables between patients with bleeding tendency according to the diagnosis of VonWillebrand disease**

| Variable | Bleeding tendency with VWD | Bleeding tendency without VWD | P value |
|---|---|---|---|
| **No.** | 29 (19.8%) | 117 | |
| **Male:female** | 7:22 | 28:89 | |
| **Mean age±SD (yr)** | 16.25±18.58 | 18.76±12.98 | 0.397 |
| **PT second (Mean±SD)** | 13.07±1.1 | 13.41±1.3 | 0.196 |
| **PTTsecond(Mean±SD)** | 43.59±13.11 | 35.76±11.82 | 0.002 |
| **BT minute(Mean±SD)** | 8±4.6 | 4.58±3.5 | 0.0001 |
| **VWF Ag %** | 4 | 93 | 0.0001 |
| **Factor 8 %** | 10 | 73 | 0.0001 |

P value from unpaired t test

**Discussion**

Von-Willebrand disease is most frequent inherited bleeding disease. It is estimated that about 1% of general population had vWD. [1,2] This study revealed that 19.8% of patients with bleeding tendency had VonWillebrand disease, this high figure is consistent with that of recent studies by Kadir et al [8] and Woo et al[9]. These studies were similar to our study including patients presented with menorrhagia. They excluded patients with uterine pathology. All these raised the importance of screening for Von-Willebrand disease as a part of routine investigation in any patient presented with bleeding tendency in the presence of other normal coagulation profiles.

Other studies stated that women with VonWillebrand disease appear to be at high risk in developing menorrhagia. In this study other bleeding symptoms like bruising, epistaxis, bleeding after surgery and dental extraction were statically significant, this goes with other studies [9, 10].Family history of bleeding was significantly associated with increased incidence of VonWillebrand factor, in patient with bleeding tendency. This may be attributed to the fact that Von-Willebrand disease is inherited as autosomal dominant [10].

Although vWD is a heterogenous disease in which the association between platelets, coagulation factor deficiency, and mucocutaneous bleeding is much more frequent than coagulation type of bleeding like hemarthrosis and hematomas in these patients. In this study Menorrhagia was the most common manifestation and it found in 33.5%. mucocutaneous bleeding like epistaxis, ecchymosis, gum bleeding and bleeding from minor cuts were most common clinical presentation. Females were affected more than males which agree with most of other published studies. [5, 6, 11, 12] Further, it is pertinent to mention that almost all of these studies are hospital based and therefore disease is identified only in those patients who presented themselves for investigation thus giving a higher prevalence of disease. In contrast, most of the studies done in Western countries are epidemiological studies done in general population giving an overall prevalence of vWD ranging from 0.7% to 1.6% with an average prevalence of ≈ 1% and a very high prevalence of Type 1 disease.[13,14,15] Identification of Type 1 vWD (mild form) is very important as these patients are either asymptomatic or suffer

from mild and infrequent bleeding episodes which make them to consider their bleeding tendencies as normal. If they are not identified, they may bleed profusely after getting major hemostatic challenges like surgery and trauma. In Iraq, characterization of vWD is difficult in general population because of financial constraints, inadequate awareness and lack of support for these patients from health care system. The complexities involved in its diagnosis requiring a series of tests which are not widely available even in major hospitals, need of the tests to be repeated for correct diagnosis, make this disease an underestimated entity in many countries. Efforts are needed to develop national registries and to make basic services for diagnosis widely available so that patients of vWD could be managed properly avoiding morbidity and mortality during major hemostatic challenge or after any invasive procedure done on them.

In conclusions Von Willebrand disease still among the most common cause of inherited bleeding tendency in patients presented with mucocutaneous or menorrhagia, yet many cases of vWD remain undiagnosed due to wide range of clinical presentations and lack in lab diagnosis.

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# Case report: Pleural Effusion as A Manifestation of Extramedullary Blastic Crisis in A Patient with Chronic Myeloid Leukaemia

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**Case Report**

Extramedullary blasticcrises in CML is considered an indicator of dismal prognosis which should lead to change in therapy . In chronic myeloid leukaemia ,the pleura is a very rare site of extramedullary involvement. We describe a patient who presented with massive pleural effusion

suggestive of extramedullary blastic crisis which predates and heralds the onset of marrow blastic crises. Cytological examination of the pleural fluid morphology reveals both myeloid and lymphoid blastic crisis. The patient was managed by repeated thoracentesis and was too ill to tolerate systemic chemotherapy and die shortly thereafter.

A 50-year-old women was diagnosed as a case of chronic myelogenous leukaemia 5 years ago and was on imatinib (*Gleevec*©, Novartis ) 400 mg/day . Her first admission to Baghdad Teaching Hospital was in March, 2011 when she presented with progressive pallor, generalized body swelling, intermittent abdominal pain and left shoulder pain. She reported occasional fever but no bleeding episodes. On physical examination she had pallor and splenomegaly (10 cm below left costal margin), no lymphadenopathy and raised jugular venous pressure were seen . Blood pressure was 150/90, PR 100/min and temperature was 36.8 C. She was evaluated for possible disease acceleration.

Laboratory investigations shown in table 1 revealed peripheral blood findings of chronic myeloid leukaemia in addition to hypocalcaemia (which possibly results from poor intake or imatinib-induced osteomalacia)[(1)], hyperuricaemia, hypoalbuminaemia and raised lactate dehydrogenase .

Bone marrow aspiration showed infiltration by blasts forming 9% of nucleated cells with increased granulopoiesis with almost all the cells showing severe dysplasia (Figure 1) . The severe dysplasia seen on bone marrow on first admission was consistent with accelerated phase of chronic myeloid leukaemia.[(2)] The dose of imatinib was increased to 600 mg/day and the patient discharged from the hospital.

One month later, the patient was readmitted with 1 week history of fever and exertionaldyspnea . On examination she looked ill, tachypneoic, chest examination showed signs of right-sided pleural effusion and splenomegaly 14 cm below left costal margin. Chest X-ray showed huge right-sided pleural effusion (Table 2 , Figure 2). The latter was aspirated for diagnostic and therapeutic purposes and a smear of pleural fluid showed large number of heterogenous blasts, small to large with basophilic vacuolated cytoplasm; many granulocytic cells at different stages were also seen. The findings are consistent with extramedullary blastic crises (Table 3, Figure 3). Bone marrow aspiration now shows infiltration by blast cells forming about 85-90%; they were large with granular cytoplasm, many showed cytoplasmic vacuolation and a few show basophilic cytoplasm. The findings were those of blastic crises CML.

Together with thoracentesis and supportive treatment, the decision of treatment with systemic chemotherapy was discussed with the patient. The patient couldn't tolerate treatment with systemic chemotherapy. She had repeated admission for severe dyspnea requiring repeated thoracentesis but unfortunately the fluid rapidly reaccumulate and she die few weeks later.

**Tables**

**Table 1- Results of Laboratory investigations**

| | on first admission | on second admission |
|---|---|---|
| **CBP** | | |
| Haematocrit (%) | 24 | 34 |
| White cell count($\times 10^9$/L) | 46 | 15 |
| Differential count (%) | | |
| Neutrophils | 39 | 40 |
| Lymphocytes | 3 | 5 |

| | | |
|---|---|---|
| Monocytes | 0 | 0 |
| Eosinophils | 2 | 5 |
| Basophils | 4 | 17 |
| Others | Promyelocytes 3% , myelocytes 51% | Myelo 16% , Promyelo 2% , blasts 8% |
| Platelet count ($\times 10^9$/L) | 359 | 434 |
| **Biochemistry** | | |
| Urea (mg/dL) | 44 | 38 |
| Creatinine (mg/dL) | 0.8 | 0.9 |
| Uric acid (mg/dL) | 9.0 | 7.4 |
| Protein (g/dL) | | |
| Total | 8.6.,x | 8.2 |
| Albumin | 2.5 | 2.3 |
| Globulin | 6.1 | 5.9 |
| Calcium (mg/dL) | 5.8 | 5.6 |
| Bilirubin (mg/dL) | 0.5 | 0.6 |
| ALT (unit/L) | 12 | 9 |
| AST (unit/L) | 6 | 11 |
| LDH (unit/L) | 420 | 550 |

AST: Aspartate aminotransferase, ALT: Alanine aminotransferase; LDH: Lactate dehydrogenase

**Table 2- Results of Imaging Studies**

| | First admission | Second admission |
|---|---|---|
| **CXR** | Obliteration of right costophrenic angle | Huge right-sided pleural effusion |
| **Abdominal ultrasound** | Splenomegaly, no ascites or lymphadenopathy | Splenomegaly, pelvic ascites |
| **CT chest** | No bony abnormalities, no masses, small right-sided pleural effusion | |

**Table 3- Results of Pleural fluid analysis**

| Variable | Results |
|---|---|
| **Appearance** | Amber color |
| **Protein (g/dL)** | 2.6 |
| **Sugar (mg/dL)** | 129 |
| **White cell count ($\times 10^9$/L)** | 9.259 |
| **Differential count (%)** | |
| **Neutrophils** | 94 |
| **Lymphocytes** | 6 |

| | |
|---|---|
| **Gram stain and Culture** | No bacteria seen |
| **Acid-Fast Bacilli** | Negative |
| **Cytology** | Hypercellular smear showing many blastic myeloid cells, some were binucleated; **consistent with extramedullary (blastic crises) metastases** |
| **Cytospin** | Showed large number of heterogenous blasts, small to large aggressively looking with blastic vacuolated cytoplasm with many granulocytic cells at different stages are seen |

**Table 4 - Mechanisms of pleural Effusion in CML*** 

| |
|---|
| ▪ Pleural extrameduallaryhaematopoiesis |
| ▪ Leukaemic infiltration of pleura |
| ▪ Pleural reaction secondary to bleeding into the pleura |
| ▪ Non-malignant causes |
| • Infection |
| • Drugs (e.gdasatinib) |
| • Granulocytic sarcoma |
| • Heart failure |
| • Hypoalbuminemia from liver or renal insufficencies |
| *** Adapted from : Alexandrakis M., SteiropoulosP .et al. Pleural effusions and thoracentesis in patients with hematological malignancies. In: Azoulay E. *Pulmonary involvement in patients with haematological malignancies* .Springer 2011; P: 191-200.** |

**Discussion**

Malignant pleural effusion is defined as the presence of cancer cells in the pleural space. It can occur as the initial presentation of cancer, delayed complication in patients with previously diagnosed malignancies or the first manifestation of cancer recurrence after therapy. [3]

Pleural effusions are a troublesome and debilitating complication of advanced malignancies. They are commonly encountered by internists, respiratory physicians and oncologists.

They can present as isolated entity or be associated with paranchymal lung abnormalities. [4]

In haematological malignancies , the incidence of pleural effusions vary between the types of haematological neoplasms being more common in Non-Hodgkin's lymphomas (mainly T-cell) especially when mediastinal involvement is present while it is rarely seen in acute or chronic leukaemias (Table 6) . [5]

*Mechanisms of Pleural Effusion In Haematological Malignancies*

It might be due to the disease itself, drug-related toxicity, infections, extramedullary haematopoiesisor other complications . [5]

*Mechanisms of Pleural Effusion in CML* (Table 4)

1. *Extramedullary blastic crises*. In CML , there are several possible mechanisms of pleural effusion , extramedullary blastic crises is the most common [5]. The termination of CML into blastic crises is defined as having more than 20% blasts in the bone marrow or peripheral blood, the presence of large aggregates and cluster of blasts in the bone marrow biopsy or the development of extramedullary blastic infiltrates. [6]

Extramedullary manifestations occur in about 10% of patients with CML and may involve a variety of organs and tissues. They usually occur during the accelerated phase (10%) and blastic crises and most commonly involve the skin, lymph nodes, central nervous system and rarely the synovia, gastrointestinal tract, kidneys and pleura and may be of myeloid and/or lymphoid lineage. [6, 7].

They can occur during imatinib therapy for accelerated phase disease. All types of blast crisis[8], including promyelocytic blast crisis, can occur during imatinib therapy.[9]

Our patient had been presented with an extramedullary pleural effusion which is usually serous as an uncommon site of extramedullary involvement and she had no extramedullary disease in the skin, lymph nodes or other sites. The pleural fluid showed mixed lineage of acute leukaemia.

2. *Leukaemic infiltration of the pleura*. Is the second most common mechanism. The pleural fluid analysis in this case is usually haemorrhagic and revealed variable stages of granulocytes and leukaemic blasts .[7]
3. *Pleural reactions due to bleeding*. The fluid is usually haemorrhagic and predisposing factors such as leucostasis and platelet dysfunction may play a role. [7]
4. *Infection*. The possibility of infection (including parapneumonic effusion or tuberculous effusion) should be ruled out and the presence of necrotic debris and /or the positive identification of the microorganisms by special stain may suggest an infectious process. [7]
5. *Drug toxicity*. Drug-induced pleuropulomnary disease should be excluded when we face a patient with pleural effusion. Imatinib mesylate is rarely a cause of pleural effusion [5] but dasatinib is a relatively common cause of pleural /pericardial effusion (up to 35% of cases). [1]

**Discussion of Management**

*Treatment of accelerated phase*

When the patient presented with accelerated phase, she was treated by increasing the dose of imatinib.[10] Imatinib and other tyrosine kinase inhibitors (dasatinib, and nilotinib) have been utilized as bridging therapies to permit allogeneic stem cell transplantation in accelerated phase.[11] Combination therapies are being explored in the accelerated phase of disease, including dasatinib plus imatinib.[12]

*Treatment of extramedullary blastic crises*

Extramedullary disease in CML is considered an indicator of dismal prognosis which should lead to change in therapy. [7, 13]The therapeutic options for both marrow and extramedullary disease are:

1. *Tyrosine Kinase Inhibitors*. At the time of blastic crises, imatinib monotherapy has produced complete hematologic remissions in approximately 20% of patients. [14]However, the duration of response was relatively short, with a median estimated time to disease progression of only 2 months and so it is less an option especially if the patient was already on imatinib. [13,15] For patients developing blastic transformation on imatinib, the recent availability of other tyrosine kinase inhibitors (dasatinib and nilotinib) has broadened the potential therapeutic options. However, the responses are rarely durable. [16,17]
2. *Systemic Chemotherapy* .For systemic chemotherapy it is important to determine the lineage of the acute leukemic transformation and treat accordingly :[18]
   - Treatment of myeloid blasticcrises :anthracyclines (e.g. daunorubicine or mitoxantrone) plus etoposide or cytarabine with or without combination with imatinib .
   - Treatment of lymphoid blastic crises : anthracyclines (e.gdaunorubicin) plus

vincristine and prednisolone with or without imatinib Or the HyperCVAD regimen (Hyperfractionated doses of cyclophosphamide , vincristine and dexamethasone) (20)

- Treatment of myeloid/mixed/undifferentiated blast crisis : high-dose cytarabine-based regimens (21)

3. *Haematopoietic stem cell transplantation.*is considered eligible if the patient returned to the chronic phase or a complete remission has complete remission. [13, 18, 20].

*Treatment of Pleural effusion in CML*

Unfortunately there is no effective standard therapy.[7] To the best of our knowledge, there are only few case reports of isolated extramedullary pleural effusion which heralds the onset of blastic transformation of chronic/advanced phase disease described in the literature.

From these, one patient responded to thoracentesis and treatment with hydroxyurea without recurrence[22], one patient respond to imatinib[7], one patient present with pleural lymphoid blastic crises and respond to systemic chemotherapy with vincristin, prednisolone and anthracycline but with little or no effect on the effusion [23] and another patient with pleural myeloid blastic crises responded to systemic chemotherapy with anthracyclines and Ara-C (standard '3-7' regimen for acute myeloid leukaemia)[24].

However, in other patients, like our patient in whom pleural blasts antedated blast transformation, intrapleural infusion of chemotherapeutic agents and systemic chemotherapy were ineffective and all they die within 2months of the onset of blastic transformation.[25] Pleurodesis is contraindicated in the face of her advanced disease with the short life expectancy and its poor prognosis. [3, 26]

In conclusion, in any patient with CML and pleural effusion, the possibility of extramedullary disease and pleural infiltration should always borne in mind after the exclusion of other non-malignant causes. It require change in therapeutic decision and it adversely affects prognosis.

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## Letter to the editor:

# Incidence of hemoglobinopathies among anemic patients visiting National Center of Hematology

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**Abstract**

The hemoglobinopathies encompass heterogeneous group of disorders associated with either single gene mutation in α globin and β globin genes (as in α and β thalassemia).the aim was to estimate the

incidence of hemoglobinopathies in patients with hypochromic microcytic anemia during the period from 1st January 2011 till 31st December 2012 at the National Center of Hematology ion a total of 1103 patients by using high performance liquid chromatography (HPLC) technique. we found these results: 656 patients (58.9928%) shows normal Hb-electrophoreses while 447 patients (40.5258%)shows abnormal Hb-electrophoresis

The inherited diseases of hemoglobin are a heterogeneous group of disorders associated with mutations α-globin & β-globin genes [1,2,3,4].

The commonest are single gene disorders the world health organization estimates that about 7% of the world population are carriers.[2] These conditions are being seen with increasing frequency in many countries in which they had not been recognized previously as a result of migrations of disorders of hemoglobin:-

Genetic disorders of hemoglobin are divided into:-

1. Thalassemia: in which there is reduced rate of production of are or more of globin chains over 200 different mutations were found only hemoglobin β-chain which is present on chromosome 11. [3]
2. Structural changes in globin chain leading to instability of hemoglobin or abnormal oxygen transport.
3. Harmless group of mutations which interfere with the normal switching of fetal to adult hemoglobin known collections as hereditary persistence fetal hemoglobin which can be regarded as well compensated forms of thalassemia.

This way of splitting up the hemoglobin disorders is not entirely satisfactory [4,5,6] as some structural like HbE for example are synthesized in reduced amounts and hence produce a picture like thalassemia.

**The thalassemia & related disorders:**

The thalassemia are the commonest single gene disorders & was first recognized in 1925 in Detroit ,more recently it has become clear that its thalassemia occurs widely throughout the world & that its clinical picture can result from the interaction of many different mutations.[2]

Thalassemia are heterogeneous group of genetic disorders of hemoglobin synthesis which result from reduced rate of productions of one or more of the globin chains of hemoglobin, they are divided into α-,β-,δβ-, 8δβ- thalassemia according to which globin chain is reduced .

β-thalassemias are the most important types of thalassemia because they are so common and usually produce severe anemia in their homozygous and compound heterozygous states.[2,4,7]

**Structural Hb-variants related to thalassemia:**

The unstable Hb-disorders:

These are a rare group of inherited hemolytic anemia's that result from structural changes in the hemoglobin molecule, most of unstable hemoglobin's result from single amino acid substitution, or small deletions.

**High oxygen affinity hemoglobin variants:**

Hereditary persistence HbF which runs in families shows high oxygen affinity with also a family with rare hemoglobin variants that produce same effect.[2]

The study conducted during the period from 1st January 2011 till 31st December 2012. All patients referred to the national center of hematology because of hypochromic microcytic anemia with normal iron profile were subjected to Hb-electrophoresis.

From each patient 2.5 ml of blood was collected in $K_2$EDTA tubes (supplied by AFCO Jordan) CBC was done by using

1. Cell dyne machine (USA).
2. Convergys X5 convergent technologies Germany.

Then blood film was done by using Leishman stain (CDH central drug house biochemical India) to look for cells morphology, patients with hypo chromic microcytic red cells were collected then hemoglobin analysis done by electrophoresis using HPLC Hb-variant from Bio-Rad USA

Then results were collected, categorized and those patients who show abnormal hemoglobin's are gathered and then grouped according to each abnormality found and then grouped according to each abnormality found.

A total of 1103 patients shows hypo chromic microcytic anemia, 656 patients (59.4741%) shows normal Hb-electrophoresis while 447 patients (40.5258%)shows abnormal Hb-electrophoresis which are categorized as below:

| | |
|---|---|
| β-thalassemia minor | 30.4856% |
| α- thalassemia minor | 0.2697% |
| β- thalassemia major | 1.528% |
| α- thalassemia major | 0.8093% |
| Sickle cell trail | 0.5395% |
| Sickle cell disease | 1.8848% |
| Hb-C disease (homozygous) | 0.2697% |
| Hb-C trail (heterozygous) | 0.2967% |
| Hb-D disease (homozygous) | 0.0899% |
| Hb-D trail (heterozygous) | 0.0899% |
| Hb-E disease | 0.3597% |
| Hb sho-E trail | 0.3597% |
| Persistent Hb-F | 3.075% |

From the above results we find that β-thalassemia minor is the commonest hemoglobinopathies seen in patients visiting the national center followed by persistent Hb-F, then sickle cell disease then β-thalassemia major. These findings reflect the epidemiological distributions of these hereditary diseases in this area of Baghdad in which about 1.5 million people inhabit this area therefore incidence of hemoglobinopathies will be 0.0002 which is different or similar from other part of country according to geographical distribution.

As those diseases are genetically determined which cause increase incidence among the community, we see that Legislation strict law about examining newly married couples may be helpful in decreasing the incidence .

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**المجلة العراقية لامراض الدم**

**مجلة علمية محكمة تصدر مرتين في السنة عن المركز الوطني لبحوث وعلاج امراض الدم- الجامعة المستنصرية- بغداد- العراق.**

**المجلد 2** **تشرين الثاني 2013**

**العدد ١**

# المحتويـــات

# بسم الله الرحمن الرحيم

**بمناسبة صدور العدد الثاني من المجلة العراقية لأمراض الدم. تهنئ هيئة التحريرالزملاء الاطباء كافه وخصوصا العاملين منهم في حقل أمراض الدم, السريري والمختبري بهذا الانجاز المتواضع ونأمل منهم الاستمرار في رفد المجلة بالبحوث الرصينة والتواصل في تقديم تعليقاتهم**

**ومقترحاتهم وانتقاداتهم التي من شأنها ان ترفع من مستوى المجلة العلمي وتحفظ ديمومتها لبلدنا العزيز.**

**نسأل الله التوفيق.....**

**هيئة التحرير**

# شكر وتقدير للمقيمين

**تتقدم أسرة تحرير المجلة بوافر الشكر والتقدير إلى الأساتذة المقيمين المدرجة أسمائهم أدناه لما بذلوه من جهدٍ وعناء في تقييم البحوث المنشورة خدمة لمسيرة العلم والتقدم.**

**ا.د. رعد جابر** **ا.د. علي محمد جواد**

**ا.د. نصير علاوي** **ا.د.بان عباس عبد المجيد**

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**ا.م.د.هيثم الربيعي**

**د. نضال كريم**

**ا.م.د.خضير عباس**

**د.بسام فرنسيس**

**ا.م.د.صبح المدلل**

**د.علاءالدين سهام**

**د.وسن ساطع**

**ا.د. سلمى حداد**

*ا.م.د. مازن فيصل*

## الصفات الوبائية والمرضية :سرطان الجهاز اللمفاوي نوع الاهودجكن

**زياد اسماعيل ابراهيم، علاوي حسن، عبد الستار الكبيسي، طارق الشجيري، جعفر الغبان**

**الخلاصة:**

**الخلفية:** سرطانات الجهازاللمفاوي نوع اللاهودجكن في الأطفال تمثل مجموعة متنوعة من الأمراض المتكونة من الخلايا في الجهار المناعي.

**الهدف من الدراسة:** لتحديد الصفات الوبائية والعلامات السريرية والنسيجية لمرض سرطان الجهاز اللمفاوينوع اللاهودجكنفي الأطفال في مستشفى الطفل المركزي التعليمي.

**طريقة البحث:** نوع لاهودجكن، الداخلين الى هي دراسة تراجعية لمرض بسرطان الجهاز اللمفاوي، ممن 2009 ولغاية 2004 مستشفى الطفل المركزي التعليمي في وحدة امراض الدم والأورام لفترة من هم دون سن الخامسة عشرة من العمر.

**النتائج:** سنة، ونسبة 6.3 مريض، متوسط العمر عند التشخيص هو 84 كان العدد الكلي للمرضى هو ) 88% ومعظم المرضى في مراحل متقدمة عند التشخيص (الثالثة والرابعة 1:2 الذكور الى الاناث هي ، ونسيجيا نوع بيركتو نوع مشابه بيركت 51% وكانت انتفاخ البطن او تورمها اكثر العلامات السريرية 58.33% من أكثرالأنواع النسيجية تشخيصا.

**الاستنتاج:** سنوات والذكور اكثر 9-5 اظهرت الدراسة **ان** معظم الحالات كانت ضمن الفئة العمرية اصابة من الإناث، وكان الورم في منطقة البطن من اكثر مناطق الجسم المصابة، كذلك اظهرت **الدراسة** (الثالثة والرابعة) هي الأكثر ونسيجيا نوع بيركت هو أكثر الأنواع **ان** نسبة المراحل المتقدمة من المرض تشخيصيا .

**الكلمات المفتاحية:** سرطان الجهاز اللمفاوي نوع اللاهودجكن.

# التباين بين الجنسين في الأعراض السريرية والنمط الظاهري المناعي والاستجابة المبكرة للستيرويد عند المرضى المصابين بابيضاض الدم اللمفاوي الحاد لدى الأطفال

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**الخلاصة**

**تمهيد**

يعتبر إبيضاض الدم الحاد السرطان الأكثر شيوعا في مرحلة الطفولة، ويشكّل ابيضاض الدم اللمفاوي الحاد 78% من هذه الحالات. يختلف معدل الإصابة والبقاء على قيد الحياة في المرضى المصابين بابيضاض الدم اللمفاوي الحاد لدى الأطفال بين الجنسين. على الرغم من التحسن العام في معدلات البقاء على قيد الحياة عند الاطفال المصابين بهذا المرض الاّ أن معدلات البقاء على قيد الحياة ماتزال ضعيفة المستوى عند المرضى من الذكور.

**الهدف من الدراسة:** الكشف عن الاختلافات بين الأطفال المرضى من الذكور والإناث المصابين بابيضاض الدم اللمفاوي الحاد فيما يتعلق بمميزاتهم السريرية التقديمية، النمط الظاهري المناعي لابيضاض الدم اللمفاوي الحاد، والإستجابة المبكرة للستيرويد.

**الطرائق:** صُمِّمَت هذه الدراسة بأثر مستقبلي لتشمل 60 مريضاً مشَخَّص حديثاً بابيضاض الدم اللمفاوي الحاد لدى الأطفال للفترة من نيسان 2012 الى آذار 2013. تم تقييم كل مريض سريرياً عند دخوله المستشفى وعند نهاية السبعة ايام من الطور الأول من العلاج بالبردنيزون وذلك لكي يتم تصنيف كل مريض كمستجيب جيد (عدد الخلايا الأرومية في الدم المحيطي < 1000\ميكرولتر) او ضعيف الإستجابة (عدد الخلايا الأرومية في الدم المحيطي > 1000\ميكرولتر) لعلاج البردنيزون. تم تحديد النمط الظاهري المناعي بواسطة صبغ الخزع الرشفية لنخاع العظم بطريقة التعبير المناعي الكيميائي الخلوي للمعلمات الجزيئية cCD79a (خاص للخلية B) و cCD3 (خاص للخلية T).

**النتائج:** اشتملت مجموعة الدراسة على 38 ذكر و22 انثى، كان متوسط عمر الذكور 62.5 شهراً ومتوسط عمر الإناث 41.5 شهراً. كان تضخّم الطحال موجوداً عند 71% من الذكور مقابل 63.6% من الإناث، وكان تضخم الكبد موجوداً عند 68.4% من الذكور مقابل 45.5% من الإناث، تم كشف وجود الأورام المنصفية عند 6 ذكور و3 إناث، وأصيب الجهاز العصبي المركزي عند 5 مرضى بضمنهم 3 ذكور واثنين من الإناث. كان معدل عدد خلايا الدم البيض عند الذكور 63.78±15.98×$10^9$\التر وعند الإناث 49.2±21.87×$10^9$\التر، وكان معدل مستوى الهيموغلوبين عند الذكور 8.75±0.35 غم\ديسيلتر وعند الإناث 7.91±0.34 غم\ديسيلتر. 75.8% من الذكور كانوا من النوع B-ALL و24.3% كانوا T-ALL، و76.5% من الإناث كانوا B-ALL و23.5% كانوا من النوع T-ALL. 86.8% من الذكور و86.4% من الإناث كانوا جيدي الإستجابة للستيرويد.

**الإستنتاجات:** الأطفال المرضى من الذكور كانوا أكثر عدداً و أكبر عمراً من الإناث وقَدِموا بخصائص سريرية ودموية تُعتَبَر ذات تكهن سيء للمرض اكثر من الإناث. لم يكن هناك فرق ملحوظ بين الذكور والإناث فيما يتعلق بالنمط الظاهري المناعي لابيضاض الدم اللمفاوي الحاد وكذلك بالنسبة للإستجابة المبكرة للستيرويد.

**الكلمات المفتاحية:** ابيضاض الدم اللمفاوي الحاد، النمط الظاهري المناعي، الجنس، الإستجابة للستيرويد.

## تقرير الحالة السريرية

**عادل العقبي حسنين حسن فرح حسين مصطفى عبد علي**

## الخلاصة

تعد ازمات التحول الى ابيضاض الدم الحاد خارج النخاع لدى مرضى ابيضاض الدم النقياني المزمن الحاد دلالة على سوء التكهن بالمرض ويجب عندها الشروع بتغيير العلاج ، ويعد غشاء الجنب من الاماكن النادرة التعرض لازمات من هذا النوع .

في تقرير الحالة السريرية هذا نصف مريضة أصيبت بأنصباب غشاء الجنب الجسيم الناتج عن أزمة التحول الى ابيضاض الدم الحاد خارج النخاع والذي انذر بحدوث التحول وسبق حدوثه في نخاع العظم، وقد أظهر الفحص الخلوي لسائل الجنب مظهراً مزدوجاً من مظاهر ازمات التحول لكل من ابيضاض الدم اللمفاوي والنقياني الحاد معاً.

عولجت المريضة بالبزل المتكرر لغشاء الجنب ولكنها كانت سقيمة جداً على تحمل العلاج الكيميائي وتوفيت بعدها بفترة وجيزة .