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Study of Chromosomal Aberrations and Micronucleus Formation in Some Iraqi Patients infected with Acute Myeloid Leukemia (AML)

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Abstract: The effect of acute myeloid leukemia (AML) was studied on some cytogenetic parameters occur in lymphocyte in three cases; before, during and after chemotherapy treatment. The study involved 47 AML Iraqi patients their ages ranged between 2.5-81 years including 20 females and 27 males. Results were compared with 26 healthy individuals and summarized as follows: 1- AML was the most common leukemia in adults as compared with children. It was found that 46.8% AML patient ages were less than 15 years; 90.15% at 30-16 years; 40.9% at individuals at 31-45 years, the percentage increased to 90.5% in those who are over 45%. 2- AML was more common in males than females recording 57.5% and 42.5% respectively; representing 1.35:1.00 male:female ratio. 3- Two cases representing 5.3 of the studied sample exhibited dioploidy after examination under light microscope. The highest mitotic index was 7.498% and occurred in patients before taking therapy compared to 6.784% during therepy and 7.000% after therapy. 4- Micronucleus mean values recorded 0.033, 0.020 and 0.036 MN/1000 cells for AML patients before, during and after therapy respectively, when compared with the control which recorded 0.002 MN/1000cells. Nuclear division index (NDI) means before, during and after therapy of AML patients were 1.658, 1.000 and 1.424 respectively compared with control which recorded 1.282.

Key words: AML, CA, MI, MN, NDI

دراسة التشوهات الكروموسومية وتكوين النويات الدقيقة عند بعض المرضى العراقيين المصابين بإبيضاض الدم النخاعي الحاد

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الخلاصة: دُرس تاثير مرض إبيضاض الدم النخاعي الحاد (AML) في بعض معايير الوراثة الخلوية الحاصلة في كريات الدم البيضاء في ثلاثة حالات للمرضى اشتملت على مرحلة ما قبل العلاج وفي اثناء العلاج وبعد العلاج الكيميائي. شملت الدراسة التشوهات الكروموسومية وتكوين الانوية ايضاً. تكونت عينة الدراسة 47 من المرضى العراقيين المصابين بمرض إبيضاض الدم النخاعي الحاد الذين تراوحت أعمارهم بين 2.5 – 81 سنه إذ تضمنت على 20 انثى و27 ذكر. قورنت النتائج مع عينة مكونه من 26 من الاصحاء ويمكن اختصار النتائج التي تم التوصل اليها وكما يلي: 1- مرض AML أكثر شيوعاً في البالغين مما هو في الاطفال، إذ وجد بان

46.8% من المرضى المصابين هم من أعمار دون 15 سنة، تلتها 90.15% في الاعمار 16-30 سنة وبلغت 40.9% في الاشخاص الذين تراوحت أعمارهم بين 31-45 سنة وارتفعت الى 90.5% في الاعمار التي زادت عن 45 سنة. 2- سجلت الذكور نسبة أكثر من النساء لتسجلا 57.5% و 42.5% على التوالي وبنسبة 1.35ذكور:1.0 اناث. 3- سُجلت حالتين وبما يشكل 5.3% من العينة المدروسة من المرضى ذات خلايا ثنائية المجموعة الكروموسومية (Diploid) بعد فحصها تحت المجهر الضوئي. تحقق أعلى دليل لإنقسام الخلايا (MI) في المرضى قبل تعاطيهم العلاج الذي وصل الى 7.498± 1.7 بينما سجل 6.784±4.5 اثناء العلاج وارتفع الدليل قليلاً ليصل الى 7.00± 2.5 في المرضى بعد تعاطيهم العلاج. 4- سُجلت معدلات قيم للانوية الصغيرة 0.033 ± 0.018 ،0.020 ± 0.00015 و 0.036 ± 0.01 في المرضى قبل واثناء وبعد العلاج على التوالي في الوقت الذي سجلت معاملة المقارنة 0.002 ± 0.00002. وكانت معدلات دليل الانقسام النووي (NDI) قبل واثناء وبعد العلاج 1.658±0.200، 1.000±0.022 و 1.424 ±0.19 على التوالي والتي سجلت إختلافاً معنوياً عن معاملة السيطرة (1.282±0.09).

Introduction

Leukemia is a disease characterized by a clonal expansion of malignant blood cells. It evolves from the myeloid/granulocyte cell line, and it is acute myelogenous leukemia (AML) while lymphocytic precursors give rise to acute lymphocytic leukemia (ALL). In contrast, Leukemia accounts for some 300,000 new cases each year (2.8% of all new cancer cases) and 222,000 deaths. This high ratio of death cases (74%) reflects the bad prognosis of leukemia in many parts of the world, where the somewhat complex treatment regime required, are not available (1).

There were 698 cases of leukemia in children less than 14 years registered at Basrah's Ibn Ghazwan pediatric oncology ward from 1993–2007. The number of cases ranged from 15 cases in the first year to 56 cases in the final year and reached a peak of 97 cases in 2006 (2). In 2010, approximately 12330 people were diagnosed with AML and 8950 died (3).

AML is the most common type of acute leukemia in adults, accounting for 80% of new cases, but is uncommon in children. The incidence increases steadily with age, with a sharp increase after the age of 45 years. Inconstant ALL is the most common malignant disease affecting children, accounting for approximately 30% of all childhood cancers (4).

Etiology of AML is largely unknown, but associated factors include ionizing radiation, earlier cytotoxic chemotherapy, exposure to benzene, and smoking (5). In the classical cytogenetic techniques, chromosomes are studied directly by observing and counting aberrations in metaphases (6). The study of DNA damage at the chromosome level is an essential part of genetic toxicology because chromosomal mutation is an important event in carcinogenesis (7). Approximately 200 recurrent chromosomal aberrations have been detected in cytogenetic analyses of AML. The most common cytogenetic changes were trisomy 8, t(8;21), t(15;17), inv(16), t(6;9), and t(8;16). Balanced reciprocal translocations typical for AML usually cause alterations in the function or transcription factors involved in myeloid differentiation or in tyrosine kinases (5).

The *in vitro* micronucleus assay is a genotoxicity test for the detection of

micronuclei (MN) in the cytoplasm of interphase cells. Micronuclei may originate from acentric chromosome fragments (i.e. lacking a centromere), or whole chromosomes that are unable to migrate to the poles during the anaphase stage of cell division. The assay detects the activity of clastogenic and aneugenic chemicals, in cells that have undergone cell division during or after exposure to the test substance (8). Accordingly, and because of the number of cases being affected by AML and the urgent need for better understanding of the mechanisms behind this serious disease. The present paper was designed to evaluate chromosomal aberrations in AML, micronuclei formation and mitotic index in AML patients.

Materials and Methods

A total number of 47 patients, attended to the Baghdad Teaching Hospital and Children Protection Hospital, were diagnosed with AML by complete blood count (CBC) and bone marrow (BM) examination. Patient's ages ranged from 2.5-81 years. All patients were suffering from leukemia symptoms and subjected for cytogenetic essay. Control group consisted of 26 healthy human individuals of different ages (23-57 years). Blood samples (5ml each) were collected from each participating subject, tubes with 0.5ml heparin were prepared for cytogenetic studies.

Chromosomes metaphases were prepared according to (9). Micronucleus and mitotic index were prepared and estimated according to (10, 11, 12).

Chromosomes and micronucleus were examined using the magnification power 1000 (100 x 10). At least 100 metaphase and 1000 cells were scored. For MN frequency, the cells were classified as mononucleate, binonucleates, trinucleates or titranucleates.

The proliferation index was estimated by measuring the nuclear division index according to Lamberti ***et al.*** (11).

NDI= [1(M1%) +2(M2%) +3(M3%) +4(M4%)]/N

MN= [1(MN1) +2(MN2) +3(MN3) +4(MN4)/N]

NDI=Nuclear division index, M, 1, 2, 3, 4=Number of nucleate cells

MN, 1, 2, 3, 4=Number of micronucleus in cells, N= Total number of cells.

The statistical **GenStat** discovery (13) program was used to analyze the effect of AML infection, age, and gender in level of chromosomal aberration and micronucleus formation. Significant differences were obtained according to Duncan (14).

Results and Discussion

The age of all patients ranged from less than 15 to more than 45 years. Table 1 revealed that 22 (46.8%) patients suffer from AML were under 15 years old, while 9 (19.15%) patients their age ranged between 16-30 years; 9 (19.15%) of patients their age were more than 45 years and 7 (14.9%) of patients their age ranged between 31-45 years.

It is concluded that AML is mostly common in adults (over 15 old) and less common in children. The results agree with Mittal and Meehan (15) who reported that AML is most common leukemia in adults and it is uncommon in children. This may due to the exposure of adults to environmental mutagens such as ionizing radiation, benzene, and smoking. It is estimated

that approximately one-fifth of AML cases are caused by smoking (16). Scientists have suggested that a mutation in the gene responsible for the deactivation of certain toxic metabolites may have the ability to increase the risk of acute myeloid leukemia in adults (17).

Table 1. Distribution of AML patients and control groups according to age and Gender

Age range	Patient group (47)			Control (26)		
	Female	Male	Total and (%)	Female	Male	Total and (%)
≤15	5	17	22 (46.80%)	5	3	8 (30.8%)
16-30	3	6	9 (19.15%)	7	4	11 (42.3%)
31-45	6	1	7 (14.90%)	3	3	6 (23.1%)
≥45	6	3	9 (19.15%)	1	0	1 (3.8%)
	20	27	47 (100%)	16	10	26 (100%)

Gender distribution data for both control and patient groups are shown in table 1. The total and percentages of females and males were 20 (42.5)% and 27 (57.5)% respectively. These results are in agreement with Scheinberg *et al.* (16); (18) who stated that AML is more common in males than females. The ratio M/F 1.35:1.0 is again similar to that of Ahmed *et al.* (19) who found that M/F ratio is 1.4:1. The little differences between the two ratios may be attributed to the small number of cases involved in the current study because of the difficulties in attending Baghdad specialized hospitals by patients from different governorates as a result of the present unstable security situation. Males are commonly exposed to environmental risks of mutagens more than females resulting in cytogenetic abnormities and mutations producing abnormal proteins and thus stimulating leukemic cells. Blood cell cultures taken from AML patients succeeded in 38 out of 47 cultures, failure in the 9 cultures may be due to the outgrowth of leukemic cells and could be as a result of poor blood samples handling led to disturbance of cells undergoing mitosis.

Two AML patients (5.3)% out of 38 displayed tetraploid cells as it is shown in figure 4.1.Three tetraploid cells were found out of 50 metaphases when subjected to microscopic examination, and thus they ought to be M3.The diploid metaphases in this study are in accordance with a diagnosis conducted by Licht *et al.* (20), who stated that patients with M3 have diploid cells, and those patients do not respond to All Trans Retinoic Acid (ATRA) as a chemotherapy treatment. Means of MI in all patients and control groups under the current investigation are illustrated in table 2 and figure 2, which indicate that the highest MI (7.498%) occurred in patients before taking treatment. During and after the treatment although, they recorded fewer values than before treatment while no significant differences ($P \leq 0.05$) between the three groups were recorded. All three groups significantly differed compared with the control which recorded the least MI

value (2.816%). High rate of MI in AML patients before treatment may be resulted from the over expression and high proliferation of leukemic cells. During chemotherapy treatment, means were the lowest among groups because of chemotherapy effect while began to increase after treatment but still higher than control. This result agrees with Al-Sudany (21); Altaaie (22) who proved that high doses of chemotherapy decrease MI in Leukemia patients.

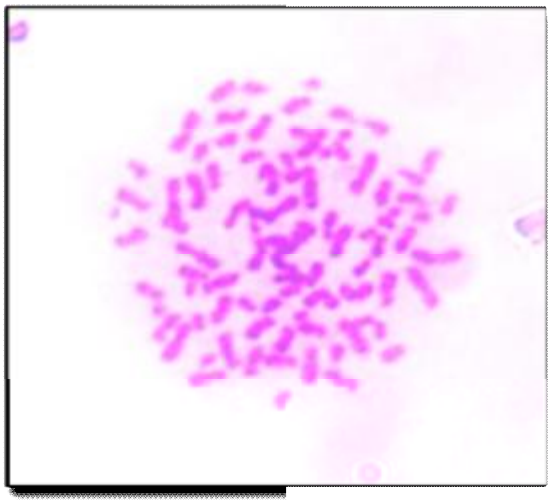

Figure 1: Tetraploidy cells found in 5.3% of AML (M3) patients (100X)

MN and other nuclear anomalies such as nucleoplasmic bridges (NPBs) and nuclear buds (NBUDs) are biomarkers of genotoxic events and manifestations of chromosomal instability that are often seen in cancer as shown in figure 3 which is in accordance with Bonassi *et al.* (23) who stated similar results.

Table 2: Mitotic index in patients before, during and after receiving chemotherapy

No. patients /group	Age range (year)	MI (means ± S.E.)
Control (26)	23-45	2.816±0.4b
Before treatment (13)	10-81	7.498±1.7a
During treatment (14)	7-69	6.784±4.5a
After treatment (11)	3-72	7.000±2.5a

Different letter: significant ($p \leq 0.005$) differences between means

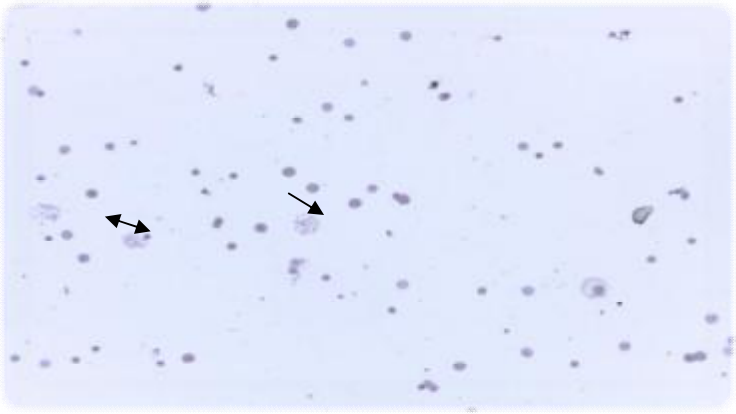

Figure 2:Cells undergoing mitosis in AML patients under light microscope (10X)

The current work investigated 38 AML patients for MNi and NDI before, during and after chemotherapy treatment compared with control.

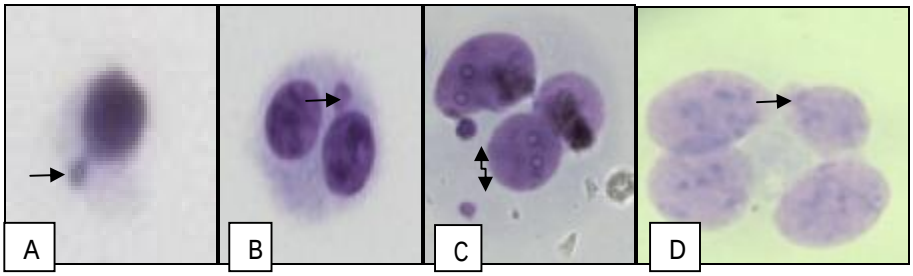

Figure 3: MNi under light microscope (100X) (A) mononucleated; (B) Binucleated; (C) trinucleuated; (D) tetranucleuted. Arrows are indicating on micronuclei

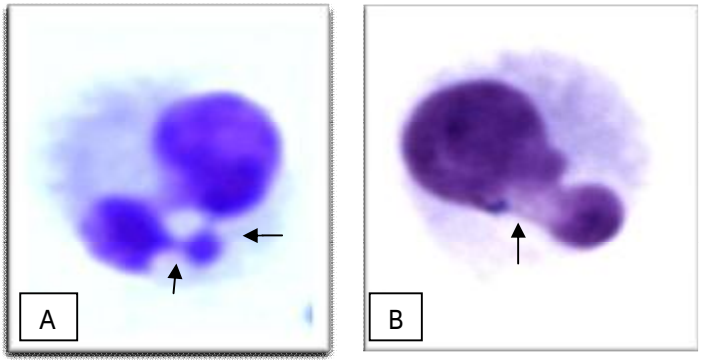

Figure 4: Nucleoplasmic bridges; (A) between binucleases and micronucleus and (B) between nucleuses and MN

Under experimental culture conditions, healthy individuals displayed a micronucleus of 0.002 MN/1000 cells in their peripheral blood lymphocytes and this result was considered as control. Table 3. exhibits that AML caused a significant increase in MNI with mean values 0.033, 0.020 and 0.036 MN/1000 cells for AML patients before, during and after treatment respectively when compared with the control and significant differences between the three groups and with the control were recorded.

Table 3: Means of micronucleus/1000 cell and nuclear division index for AML compared with control

Studied groups (No.)	Age	NDI/100 cells (Means ±SE)	MN/1000 cells (Means ± SE)
Control (26)	23-45	1.282c±0.097	0.002c±0.00002
AML patients Before treatment (13)	10-81	1.658a±0.2	0.033a±0.018
AML patients Under treatment (14)	7-69	1.000d±0.022	0.020b±0.00015
AML patient After treatment (11)	3-72	1.424b±0.19	0.036a±0.01

Different letter: significant ($p \leq 0.005$) differences between means

NDI was evaluated by scoring at least 1000 cells from the same slides according to the number of nuclei (mononucleated, binucleated, rinucleated and tetranucleated cells) as shown in figure 4. The protocol was conducted according to Khan *et al.* (24). NDI means before, during and after treatment of AML patients were 1.658±0.2, 1.000±0.022 and 1.424±0.19 NDI/1000 cells respectively (Table 3).

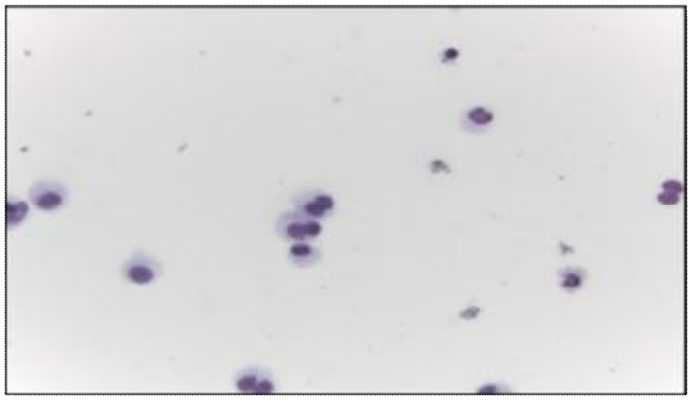

Figure 5: Nuclear division of lemphocitecells from AML patients during counting (10X)

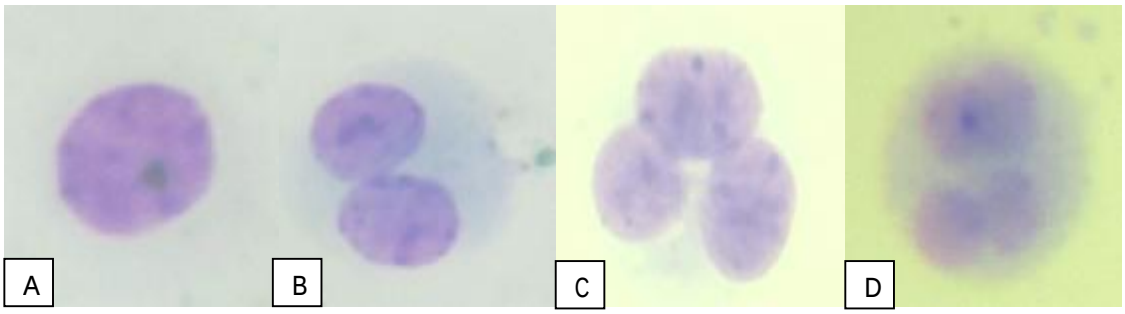

Figure 6: NDI under light microscope (100X). (A) mononucleated; (B) binucleated; (C) trinucleated; (D) tetranucleated

Significant differences among the three groups occurred when these three groups were compared with the control group. A significant increase in NDI was found in AML patients. This result agrees with Alakras (12) who mentioned that NDI is a method commonly used for quantification of the cell proliferation and the cytotoxic or cytostatic activity of the treatment. It ensures that only dividing cells are scored. Treatment with drugs blocks the leukemic cell growth. For example, a targeted therapy may block the action of an abnormal protein that stimulates the growth of leukemia cells.

Conclusions

It has been concluded from the current study the following findings:

1- The Acute Myeloid Leukemia is more common in adult males.

2- Small percentage of AML patients (5.3%) showed diploidy in lymphocyte cells.

3- The highest % of MI occurred in patients before taking treatment.

4- Patients suffer from AML exhibited nuclear anomalies such as nucleoplasmic bridges and nuclear buds.

5- Significant differences between patients before, during and after taking chemotherapy were found in respect to NDI, MI and MNi.

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