Original paper

DNA Quantitation in Pediatric Acute Leukemia

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Abstract

Background: Deoxyribose Nucleic Acid (DNA) ploidy, and immunophenotyping are now established as prognostic markers, however provide vulnerable information regarding long term outcome of acute leukemia.
Aims of study: To establish a histochemical quantitation of nuclear DNA content of acute leukemia patients by Microspectrophotometry (MSP).
Patients and methods: Prospective study on (55) patients with newly diagnosed acute leukemia from Children Welfare Teaching Hospital / Medical City Complex during the period from October 2002 to June 2003 and (10) patients with viral lymphocytosis as control group. MSP technique was used to determine the DNA content in leukemia blasts nuclei. Data were tabulated using SPSS (Statistical package for the social sciences) version 18.0 for windows. P-values equal or less than 0.05 were considered significant.
Results: DNA cytochemical quantitation was studied after applying Feulgen stain. The amount of DNA per nucleus were expressed as means optical density × 10^{-3}. The results showed that the mean DNA value was (0.545) while that of control group was (0.418). The ALL group was shown to have significantly higher mean OD compared to other groups.The frequency distribution of the different groups shows that there is homogeneous population of the mean OD of Feulgen stained nuclei for the control, AML, and AUL groups while it shows a heterogeneous population for ALL group. In comparison with the range of OD of nuclei of the control group, leukemia cases can be differentiated into diploid and aneuploidy classes. DNA aneuploidies were identified in (12/55) cases analyzed, thus accounting for (21.8%). For the ALL group, the mean OD readings in (18) patients (60%) were within the diploid region while (12) were outside the range (aneuploidy type) (40%).
Conclusions: DNA quantitation determined by MSP may represent an additional factor to improve the definition of risk groups of acute leukemia, it will continue to be a valuable tool for understanding tumor growth heterogeneity.
Key words: Acute leukemia, DNA ploidy, Microspectrophotometry.

Introduction

Cellular DNA (Deoxyribose Nucleic Acid) contains all of the information required for the synthesis of cellular and extracellular structure and to regulate the cells development in the environment of the whole organisms, which must be faithfully duplicated so that it can be passed from one cell generation to the next and from one organism to the next [1, 2].

The demonstration of DNA is carried through Feulgen method which was first introduced by Feulgen and Rossenbeck in (1924) as one of the cytochemical reaction most widely used in biology and medicine. They discovered that aldehyde groups could be generated in DNA by mild acid hydrolysis, and they could then be demonstrated with Schiffs reagent. An explanation of the reaction mechanism was offered by Stacey in (1946). Element

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containing DNA are stained a red purple color [3]. DNA Ploidy in human tumor cells can be divided into two major groups; Diploid and Non-Diploid (Aneuploidy), the later can be either unimodels with one major cell population having abnormal DNA content or Multimodal or mosaic with several distinct abnormal cell population differing in DNA contents [4]. There are many methods for analysis of cell proliferations. These include mitotic count, Thymidine labelling Index, immunological proliferation markers, measurement of Quantitative cytophotometry, Microspectrophotometry (MSP) and Flowcytometry (FCM) [5,6]. MSP advantages lies in its ability to measure the DNA content of an individual cell in any phase of mitotic cell cycle. It is less expensive than others methods and can measure both fluorescence intensity and optical density [7]. In Acute Lymphoblastic Leukemia of childhood, hyperdiploid (aneuploid) DNA modes correlates with a favorable prognosis. In contrast, patients with Acute Myeloid Leukemia and an abnormal DNA contents may have a more favorable prognosis [4].

Aims of study:

To establish a histochemical quantitation of nuclear DNA content of acute leukemia patients by Microspectro-photometry.

Patients and methods

Patients and control groups: Fifty five newly diagnosed acute leukemia patients admitted to the oncology unit in Children Welfare Teaching Hospital/ Medical city during the period between October, 1st 2002 and June, 30th 2003. Ten pediatric patients with reactive lymphocytosis (presented with viral infections) regarded as external control group, admitted to the same hospital in the same period for different causes. The diagnosis and classification of cases of acute leukemia was based on cytomorphological examination of peripheral blood, bone marrow aspiration and/or biopsy with touch slides, aided by the use of cytochemical stains like Sudan Black and Periodic Acid Schiff stains and classified according to FAB – criteria [4]. Cases which don't fit to Acute Lymphoblastic Leukemia (ALL) or Acute Myeloid Leukemia (AML) were considered as Acute Undifferentiated Leukemia (AUL). Further distinction among Lymphoid (L1 - L3) and Myeloid (M0 – M7) was based on the morphological characters of the cells.

Procedure: Two milliliters (2 ml) of venous blood delivered into EDTA – tube for complete blood picture including blood film from both patients and control groups. Air-dried smears from each control sample were stained by Feulgen stain for DNA quantitation. From the patients, one milliliter (1 ml) of bone marrow blood were aspirated and delivered into EDTA - tube for bone marrow aspiration smears then stained by Leishman stain for diagnosis and Feulgen stain for DNA quantitation study. The DNA Cytophotometric study included slide preparation, staining procedure using Schiff reagent, and spectrophotometric analysis [8]. The work on MSP (measurement and calibration) was done in the department of anatomy, medical college, Al-Nahrain University. DNA contents of the individual cell nuclei was determined by the absorption measurement using MSP in each slide preparation where the leukemic blasts were measured randomly using lymphocytes as external controls of the normal diploid DNA content. Measurement was expressed in Optical Density (OD), which was assessed by measurement of the light transmission within the Feulgen stained nuclei. Numerical values were assigned to each measurement according to the formula (OD=2–Log(T)) where T means transmittance and Log means Logarithm.

Statistical analysis: Data were tabulated and processed using SPSS (Statistical package for the social sciences) version 18.0 for windows. P-values equal or less than 0.05 were considered significant [9].

Results

A total number of (55) patients were classified according to FAB-classification into ALL (30), AML (20), and AUL (5). The age incidence among patients ranged from (45) days to (14) years, male to female ratio was (1.6:1). According to FAB-classification, all ALL cases were of (L2) subtype while for AML (6) patients were labelled as M1, another (6) as M3, (4) as M5, (2) as M2 and another (2) as M6. This distinction between Lymphoid and Myeloid morphology is mainly based on the morphological characters of the cells (FAB classification). DNA cytochemical quantitation was studied after applying Feulgen stain that produce purple color of the nuclei of the leukemic blasts for the patients and mature lymphocytes for the control. Thirty field from each group (ALL, AML, AUL and control) were selected for means of OD. For each case, at least (10 - 20) Feulgen stained nuclei were examined. The amount of DNA per nucleus were expressed as means optical density × 10^{-3}. The result of DNA measurement in nuclei showed that the mean DNA value was (0.545 × 10^{-3} of Feulgen stained nuclei) while that of control group was (0.418 × 10^{-3} of Feulgen stained nuclei) as shown in table (1) and figure (1).

In comparison between the study groups and depending on the mean OD of Feulgen stained nuclei, the ALL group was shown to have significantly higher mean OD compared to the control, AML and AUL groups as shown in table (2).

The frequency distribution of the different groups shows that there is homogeneous population of the mean OD of Feulgen stained nuclei for the control, AML, and AUL groups while it shows a heterogeneous population for ALL group (three peaks, one at the diploid region, the others at aneuploid region) as shown in figure (2).

Table 1. The amount of DNA by MSP in the nuclei of the study groups (mean OD × 10^{-3} of Feulgen stained nuclei).

Group	No.	No. of nuclei	Mean ± SD	Range
Control	10	100	0.418±0.041	0.226-0.555
ALL	30	600	0.545±0.146	0.221-0.893
AML	20	400	0.407±0.041	0.199-0.601
AUL	5	100	0.460±0.01	0.229-0.628

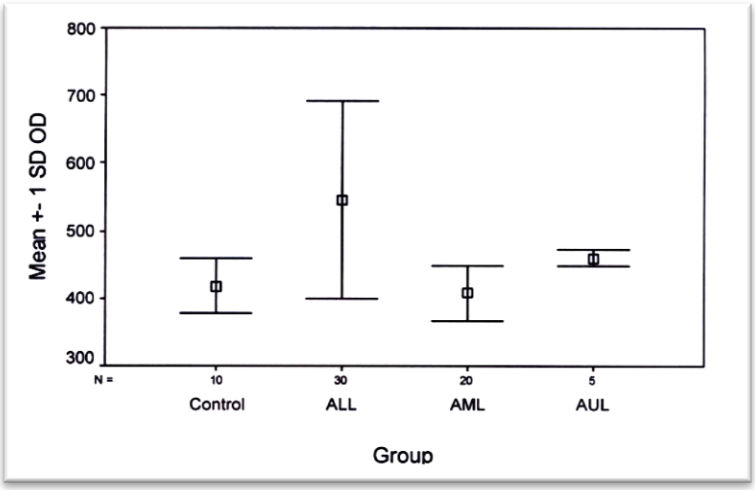

Figure 1. Chart showing the mean and standard deviation for study groups

Table 2. Comparison between the groups dependent variable mean O.D $x10^{-3}$.

Compared groups	P-value
ALL x control	0.001
ALL x AML	0.0001
ALL x AUL	0.02
AML x control	0.99
AUL x control	0.06
AML x AUL	0.0001

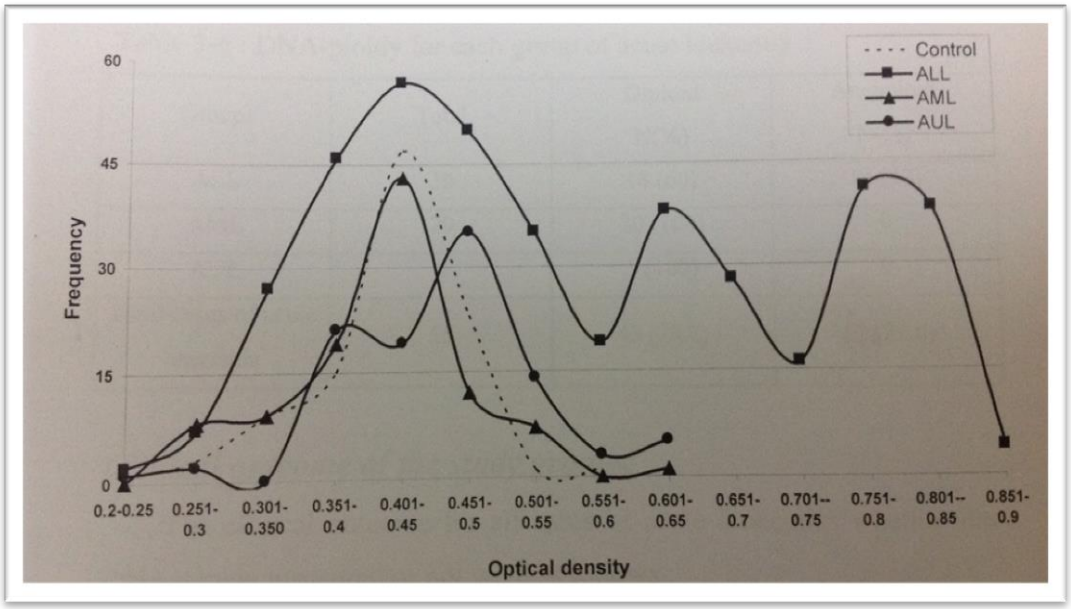

Figure 2. Frequency distribution of OD among study groups

In comparison with the range of OD of Feulgen stained nuclei of the control group which is (0.226-0.555) with the mean OD of each case of study groups, leukemia cases can be differentiated into diploid and aneuploidy classes. DNA aneuploidies were identified in (12) out of (55) cases analyzed, thus accounting an incidence of (21.8%). Among the AML and AUL groups, all cases show mean OD within the range of the control groups, so presumably all are diploid in type. For the ALL group, the mean OD readings in (18) patient (60%) were within the diploid region while (12) patients (40%) were outside the range (aneuploidy type) as shown in table (3).

Table 3. DNA ploidy for each group of acute leukemia.

Groups	Total	Diploid N (%)	Aneuploid N (%)
ALL	30	18 (60)	12 (40)
AML	20	20 (100)	0
AUL	5	5 (100)	0
Total	55	43 (78.2)	12 (21.8)

Discussion

The development of MSP methods for the study of the constituents of the individual cells provides an important tool from cytological research and for the evaluation of many concepts based on purely morphologic hematology [10]. In the current study, MSP determination of Feulgen dye were employed to estimate the relative DNA content of the individual lymphocytes in smears from the blood of normal subjects (as control group) and bone marrow smears from pre-treated patients with acute leukemia. DNA-aneuploidies were identified in 12/55 patients with acute leukemia thus accounting for a frequency of (21.8%), this frequency lies with the range of (8 - 39.2%) reported by various studies using different methodology in quantitation of DNA [11]. The earlier studies showed that aneuploidy is a common finding in ALL and a frequency of 80% and 61% were published by Petrakis and Look et al respectively adopting MSP in the former and FCM in the latter [10,11]. On the contrary, more recent studies using FCM reported that diploid DNA pattern is more commonly encountered with this type of leukemia with a frequency ranging 60 - 82% of the cases [12]. In keeping with these studies, ALL group in this study showed a heterogeneous pattern of DNA-content and (18/30) cases (60%) were found to have a diploid pattern, while (12/30) cases (40%) were aneuploidy. In ALL, the numerical chromosomal abnormalities are more common than translocation, the number of chromosomes in the leukemic cell has been recognized as a distinct cytogenetic feature that can predict clinical outcome. In regard to acute non-lymphoblastic leukemia cases included in the present study (20 AML & 5 AUL), diploid DNA was the ploidy pattern which is encountered in 100% of the cases and this is in agreement with the finding of Powardi et al, using FCM, who reported a diploid DNA content in 100% of the AML & AUL cases included in their study similarly [13] Earlier study done by Look et al, with larger number of AML cases reported, a diploid pattern was observed in 96% of their cases [14]. A higher frequency for aneuploidy has been reported by Hiddemann et al, about (38%) [12].

In AML the frequency of clonal chromosomal abnormalities in children with de novo AML about (70-80%). Chromosomal abnormalities were related to patient's age in AML: structural aberrations (i.e. predominantly translocations) were more common in patients < 50 years than in the older age group, whereas numerical chromosome changes prevailed in patients > 50 years. This possibly may account for the diploid pattern seen in the cases included in this study as all the patients included were < 15 years [15, 16]. In ALL, such high degree DNA hyperdiploid abnormalities were four times more common than AML.

Conclusions

DNA quantitation determined by MSP may represent an additional factor to improve the definition of risk groups of acute leukemia, it will continue to be a valuable tool for understanding tumor growth heterogeneity. The strong correlation between the chromosomal number and DNA ploidy needs cytogenetic analysis and molecular study to be done together with DNA ploidy.

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