Tr. J. of Medical Sciences
29 (1999) 125-128

Dilek Koç BAŞER[1]
Asuman SUNGUROĞLU[1]
Işık BÖKESOY[1]
Akın UYSAL[2]

Spontaneous micronuclei in cytokinesis-blocked bone marrow and peripheral blood lymphocytes of CML patients

Received: March 05, 1997

Departments [1]Medical Biology, [2] Hematology, Ankara University, Ankara-Turkey

Abstract: Some genetic diseases may have increased cellular instability. Since chronic myloid leukemia (CML) has a genetic base, we aimed to seek the genetic instability of patients by micronucleus analysis, a mutation screening test which was applied by cytokinesis-blocked (CB) method.

In this study, 10 CML patients at diagnosis, were analysed for their micronuclei (MN) frequencies. The values were evaluated in different culture conditions of blood and bone marrow cells.

The results of CML patients are compared with control' MN values obtained by peripheral blood cultures induced with PHA. Between bone marrow and peripheral blood cultures MN values with or without PHA among patients didn't show any significant difference. However, when these values compared with the control group all of them exhibited higher MN values in a statistically significant manner.

It is suggested that this method is useful in the routine cytogenetic studies of leukemia.

Key Words: *Micronucleus assay, cytochalasine B, bone marrow, CML.*

Introduction

Countryman and Heddle proposed the human micronucleus assay in 1976 as a faster and alternative method to metaphase analysis (1). Micronuclei can arise from chromosomal aberrations in a variety of cell types besides bone marrow. So, it is important to be able to distinguish among the mechanisms that generate micronuclei. There are, in fact, four recognised mechanisms by which micronuclei and micronucleus-like structures can arise; mitotic loss of acentric fragment, a variety of mechanical consequences of chromosomal breakage an exchange, mitotic loss of whole chromosomes and apopitosis. The latter is a form of nuclear destruction in which the nucleus disintegrates and nuclear fragments are formed (2, 3, 4, 5, 6, 7, 8,).

The conventional micronucleus method is simple and rapid but its full potential has not been realised because it can not discriminate between dividing and non-dividing cells. To visualise micronuclei the cells have to go through mitosis. In peripheral lymphocyte cultures it is difficult to distinguish interphase nuclei that have undergone a division from those that have not. The cell cycle kinetics of lymphocyte cultures shows variation due to both culture conditions an differences between individuals (6).

The 1980s heralded the development of methods to overcome this difficulty. The most popular is the use of cytochalasine B to block cytokinesis, which causes the accumulation of binucleate cells at the first mitosis (9).

The micronucleus test has been generally applied to human bone marrow red blood cells of patients treated with anti leukemic agents (10, 11). In this study we applied the cytokinesis-block micronucleus assay to measure the chromosome damage in peripheral blood and bone marrow lymphocytes of 10 CML patients at diagnosis before treatment.

Material and Methods

In this study two female and eight male CML patients, between 20-71 years of age, were examined for the MN frequency in their peripheral blood and bone marrow samples. Control group were constituted from healthy, non-smokers who were age and sex matched with patients group (12, 13). For each patient three different cell cultures were made: bone marrow culture, peripheral blood culture with and without PHA, whereas for control group only peripheral blood cell culture with PHA is carried out.

İn order to evaluate the spontaneous MN frequency, cultures without PHA were performed both from bone marrow and peripheral blood of patients. Their interior control was assessed by determining the MN values from PHA stimulated İymphocytes. Bone marrow and peripheral blood cultures were initiated with 10^6 cell/ml RPMI 1640. Cyt B were added to each culture to a final concentration of 5µg/ml at the last 24^{th} hour (14).

Bone marrow and peripheral blood lymphocytes cultures without PHA were harvested after 48 hours. Peripheral lymphocytes were harvested after 72 hours. Routine harvesting was carried out after the culture period. 1% solution of Trisodium Citrate was used for mild hypotonic effect (11).

At least 200 binuclear cells of each culture, that is, at least 600 binuclear cells for each patient was evaluated for MN. On the other hand, 500 binuclear cells were assessed in each control culture because they were induced with PHA.

Factors that must be taken into consideration in evaluation are; 1-cytoplasm of binuclear cells must be smooth and not be injured. Binuclear cells that their cytoplasms were even became knotted in mild degree were excluded since this type of cellls may be the fusion of two mononuclear cells, 2-binuclear cells must not be overlapped with other cells and with nuclei of binuclear cells, 3-surrounding of MN must be smooth and its diameter must not exceed 1/3 of their nuclei and its colour must be similar to that of nucleus. AII MN's inside of a binuclear cell were counted.

Bone marrow as well as peripheral blood samples w/o PHA cases were statistically considered as dependent variable and there fore Wilcoxon test was used. Samples and control group was also considered as independent variable, and Mann Whitney-U test was also used.

Results

Table 1 shows the MN values of bone marrow, and of peripheral blood samples with and without PHA. As shown in Table 2, no statistical difference was noticed for the results of different culture conditions of patients. When MN values of three cultures of patients were compared with that of control group, they were found to be markedly higher than control (Table 3).

Discussion

It is well known that CML is a genetic disorder and has a mutational base, as in all cancers(15). The aim of this study is to determine the extent of spontaneous mutation rate in this disease by using MN test, an index of genetic instability, and therefore used for mutational screening (3, 5, 6, 16, 17, 18).In addition to blood cultures with PHA, cultures of bone marrow as well as peripheral blood without PHA were prepared to observe this tendency of blastic and mature cells of cases, and stabilities of normal and transformed cells were tried to evaluate.

A statistically significant difference was not observed in MN values of bone marrow and two different blood cultures protocol of CML patients. MN values of bone marrow were somewhat lower than that of peripheral blood without PHA. This discrepancy, when the blastic cells are taken into consideration, may due to age of blasts. That is, the blasts in peripheral circulation are in more mature stage than that of bone marrow, their genetic instability may be higher. Stability of bone marrow blasts may be higher because they are younger than that of peripheral blood. Although mathematical mean of MN values in two different milieus were different, there was no statistical difference between them.

Our results show that MN values of bone marrow and peripheral blood with and without PHA were significantly higher than that of control group (Table 3).

A few studies of MN were carried out in patient with leukemia (10, 19, 20). One of them was realised in blood samples with PHA of ALL patients (11). The aim of the study was to investigate the treatment-dependent MN values, but not the spontaneous MN values of leukemia. It is difficult to compare our results with the mentioned study, because of the differences of age, type of leukemia and size of groups. The value of spontaneous MN before treatment of six patients they obtained was 5.05% for blood culture with PHA.

Another study about the MN values in leukemia was carried out in bone marrow erythoblasts and MN values of 41 cases with ours because o the difference of cells (19) as well as study carried on cell lines by Slavotinek at al (20).

Mean MN value of our control group was 1.30%. In the study of Channarayappa et. al. spontaneous MN values of peripheral blood in a healthy man who is 24 years old was found to be 0.2 (6). Migliore et al. (11) and Scarfi et al. (21) gave 0.36 and 1.02% for the age group of 21-33 and 1.39% for the age group of 41,54, respectively. The MN percentages of our laboratory are 0.885±0.118 for the age group 19.8±0.66 and 1.76±0.21 for the age group 49.3±0.83.

Table 1. Bone marrow and peripheral blood MN values of CML patients and control group:

Patient	Bone marrow			PB without PHA			PB with PHA			CG–PB with PHA		
	Bi N	MN	MN(%)	Bi N	MN	MN(%)	Bi N	MN	MN(%)	Bi N	MN	MN(%)
1	256	13	5.07	217	10	4.60	101	9	8.91	203	1	0.49
2	262	10	3.8	209	7	3.34	206	8	3.88	520	10	1.92
3	209	1	0.47	205	17	8.2	209	7	3.34	216	4	1.85
4	205	8	3.90	209	4	1.91	204	15	7.35	710	7	0.98
5	203	5	2.46	214	8	3.73	223	6	2.69	530	8	1.50
6	201	11	5.47	252	9	3.57	255	11	4.31	511	5	0.97
7	209	9	4.30	206	7	3.39	225	8	3.55	252	5	1.98
8	209	12	5.74	216	10	4.62	210	10	4.76	472	5	1.05
9	216	7	3.24	209	17	8.13	213	9	4.22	203	1	0.49
10	210	8	3.80	212	11	5.18	203	11	5.41	580	10	1.72
Mean±SD	218±22.5	8.4±3.53	3.83±1.55	214.9±13.63	10.00±4.19	4.67±2.05	204.9±39.65	9.4±2.55	4.84±1.92	419.7±184.63	5.6±3.2	1.30±0.57

Bi N: Binuclear cell, MN: Micronucleus, PB: Peripheral blood, BM: Bone marrow, CG: Control group.

Table 2. Statistical differences in comparing MN values of patients.

Patients	MN values (% MN)	Statistical differences
BM	PB without PHA	P>0.05
3.83 ± 1.55	4.67 ± 2.05	
BM	PB with PHA	P>0.05
3.83 ± 1.55	4.84 ± 1.92	
PB without PHA	PB with PHA	P>0.05
4.67 ± 2.05	4.84 ± 1.92	

Table 3. Statistical differences in comparing MN values of patients and control groups.

Patients	Control group (%MN)	Statistical differences
BM	PB without PHA	P<0.01
3.83 ± 1.55	1.30±0.57	
PB without PHA	PB with PHA	P<0.001
4.67±2.05	1.30±0.57	
PB with PHA	PB with PHA	P<0.001
4.84±1.92	1.30±0.57	

We are not aware of similar study on CML as ours therefore these results should be tested by other researchers and with more patients. Our results suggest that suggested that; MN values of blastic and mature cells in cases of CML elevated significantly than that of peripheral blood cells in control group. These can be taken as evidence of genetic instability in CML patients, not only present in transformed cells but also in mature cells of CML patients.

It can be concluded that MN analysis using CB methodolgy sets an alternative to other mutation screening tests like chromosomal analysis and sister chromatid exchange. It is relatively less expensive, easy to apply, and gives rapid results on the evaluation of higher number of cells in a reliable way. These features render this method useful in the routine cytogenetic studies of leukemia.

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