RESEARCH ARTICLE

Micronucleus Expression and Acute Leukemia Prognosis

Run-Chao Wang, Lei Yang, Yang Tang, Ou Bai*

Abstract

The micronucleus frequency (MNF) in peripheral blood lymphocytes (PBL) is a biomarker of chromosomal damage and genome instability in human populations.The relationship of micronucleus frequency with prognosis of patients with acute leukemia is not clear. We therefore investigated MNF in mitogen-activated peripheral blood lymphocytes from patients with hematologic diseases and solid tumours. Patients included 50 with acute leukemia, 49 diagnosed with myelodysplastic syndrome (MDS), 54 with benign blood diseases, and 45 with solid tumours, examined with 50 healthy controls. The mean MNF was significantly higher in cases of hematologic diseases and solid tumor patients than in healthy controls ($P<0.001$). There was no evident difference between MNF in the acute leukemia (7.15±2.18) and solid tumor groups (7.11±1.47), but both were higher than in the MDS group (5.12±1.29) and benign blood diseases group (3.08±1.08). Taking 7.15‰, the average MNF of the acute leukemia group as standard, and dividing 50 cases of acute leukemia patients into high MNF group (MNF≥7.15‰) and low MNF group (MNF<7.15‰). The overall response (complete remission + partial remission) rates of the low MNF group were significantly higher than in the high MNF group ($P=0.001$). The high MNF group further showed lower overall survival rates than the low MNF group. MNF expression and progression-free survival seemed to have a opposite relationship, with a correlation coefficient of -0.702. These data indicate that MNF in peripheral blood lymphocytes is important for evaluation of prognosis of acute leukemia patients, and it can reflect progression of disease to a certain degree.

Keywords: Micronucleus - karyotype - prognosis

Asian Pac J Cancer Prev, **14 (9)**, 5257-5261

Introduction

Micronucleus (MN) is a form of chromosome aberration in karyocyte. In the interphase stage of mitosis, chromosome fragments, or acentric fragments, may result from centromere loss or spindle damage. Some fragments stay in the cytoplasm of daughter cells after mitosis. They form one or more secondary nucleus, and known as micronucleus. The micronucleus embeds in cytoplasm,the diameter is 1/20 to 1/3 of the nucleus. The micronucleus is round or oval shaped and separate from the nucleus. The dye, or color, is similar to that of the nucleus (Countryman and Heddle, 1976). The frequency of a micronucleus occurrence has positive correlation with chromosome aberration (Kuramoto et al., 2002; Smith et al., 2003; Fenech, 2006; Hamza et al., 2009; Juchimiuk-Kwasniewska et al., 2011). This can reflect chromosome damage at a certain degree. Micronucleus testing in drug research began in the 1970s (Friedman et al., 1977). At present, the MN test in peripheral blood lymphocytes used as a biomarker of chromosomal damage both in vivo and in vitro (Fenech et al., 1999; Mateuca et al., 2006). Much theoretical evidence has accumulated supporting the causal role of MN induction in cancer development.

Leukemia is a serious malignant disease in which the hematopoietic progenitor cells are out of control. The cloning leukemia cells lose their ability to grow causing stagnation in the different stages of cell growth.

Several studies (Bonassi et al., 2007; Hamurcu et al., 2008) show that peripheral lymphocyte micronucleus expression is relevant to degree of disease. Patients with more micronucleus expression will have more malignancy. There are few studies about micronucleus testing and prognosis of Leukemia or MDS.

The purpose of this study was to explore the correlation between micronucleus expression and prognosis of hematopoietic malignancies, according to clinical data.

Materials and Methods

Patients and controls

The study was performed in phytohemagglutinin stimulated peripheral blood lymphocytes from 50 patients with acute leukemia (AL), 49 patients with MDS, 54 patients with benign blood diseases, 45 patients with malignant solid tumor, and 50 healthy controls. All cases meet criteria of the World Health Organization (WHO) without chemotherapy and/or large doses of glucocorticoid-therapy before specimen collection. The specimens were collected from December 2009 to

*Tumour Center, Jilin University First Hospital, Changchun, China *For correspondence: oubai16@yahoo.com*

Table 1. Group Situation of Patients and Controls

Groups	Subgroups	N=248	n(male) 132	n(female) 116	Age	Median age
Acute leukemia		50	26	24	13~73	42
AML		39	20	19		
	AML-M1	2	1	1		
	AML-M2	14	11	3		
	AML-M3	13	6	7		
	AML-M4	6	0	6		
	AML-M5	4	2	2		
ALL		11	6	5		
Benign blood diseases		54	18	36	14~79	48
MDS		49	31	18	15~84	54
Malignant solid tumor		45	36	9	30~86	58
Healthy controls		50	19	31	19~76	42

AML, acute myeloid leukemia; ALL, acute lymphoid leukemia

September 2010 at Tumor Center, First Hospital of Jilin University. The diagnoses for every patient is confirmed by pathological, or cellular morphology, and karyotype. The healthy controls were healthy staff of First Hospital of Jilin University. The control cohort consisted of 19 males and 31 female. Table 1 details information on the group situation.

Some patients received chemotherapy. We observed curative effect and adverse reactions of these patients. After the last inpatient therapy, a follow-up study were held until July 2013 and progression-free survival (PFS) and overall survival (OS) of some AL patients were obtained.

The local ethics committee approved the study protocol. The study was conducted in accordance with the Declaration of Helsinki or local laws, whichever afforded greater protection to the patients.

Whole blood cultures for human lymphocytes

Heparinized 3 mL blood samples followed informed consent documentation from all patients and healthy controls. Whole blood (0.2 ml-1ml according to blood routine test) incubated for 72 hours at 37°C in 5 mL of culture medium supplemented with 80% RPMI1640, 10% fetal calf serum, 10% phytohemagglutinin, 100 U/mL penicillin, and 100 μg/mL streptomycin.

Micronucleus assays

After 72 hours of incubation, the cultures were stopped, treated with hypotonic solution (0.075 mol /L KCl) for 15 minutes, and fixed in two changes of methanol-acetic acid (3:1) (Hamurcu et al., 2005). The fixed cells, spread onto glass slides, and stained with 10% Giemsa for a set time of 30 minutes. All slides were coded and read blind. To determine intra-individual differences, two parallel cultures of each person, and different slides of the two parallel cultures were prepared. Scoring of the micronucleus with criteria set by Countryman, et al (Countryman and Heddle, 1976), For each case,1,000 lymphocytes were analyzed.

Statistical analysis

The data expressed as mean ± SD. Kruskal-Wallis assessed the significance of differences among the groups. Corrected inspection standard compared two groups. Corrected *P* values (α') =2α/k (k-1, group count (k) =5, *P* values (α) =0.05, or so by calculating α'=0.005. Enumeration data was assessed using a one-way analysis of variance (ANOVA) test. *P* values < 0.05 are significant. All the data management is by SPSS 17.0 software.

Table 2. Micronucleus Expression Level of Different Groups

Groups	MNF‰	*P* value
(1) The healthy controls	0.76±0.52	(1)&(2) <0.001
(2) benign blood diseases	3.08±1.08	(2)&(3) <0.001
(3) MDS	5.12±1.29	(3)&(4) <0.001
		(3)&(5) <0.001
(4) acute leukemia	7.15±2.18	(4)&(5) 0.943
(5) malignant solid tumor	7.11±1.47	

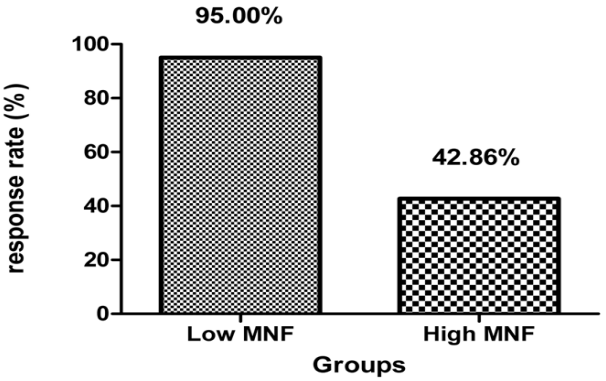

Figure 1. Responses Rate of Different Groups of Acute Leukemia Patients According to MNF Expression

Results

Micronucleus expression among different groups

The micronucleus frequency (MNF) obtained from the patients and the healthy controls are in Table 2. No significant difference was evident between malignant solid tumor group and acute leukemia group (*P*=0.943), The MNF of the patients with acute leukemia or solid tumor were significantly higher than MDS patients (*P*<0.001). The MNF of MDS patients were significantly higher than benign blood diseases (*P*<0.001), The MNF of healthy controls were significantly lower than any group of patients (*P*<0.001). There was no significant difference between the MNF of AML and the MNF of ALL (*P*=0.831).

Micronucleus expression and curative effect of acute leukemia patients

When the study terminated with 50 cases of acute leukemia patients, 27 patients received chemotherapy. Among them, 13 patients achieved complete remission, and two patients achieved partial remission. One patient had recurrence, five patients died, and five patients never achieved remission. We took 7.15‰, the average MNF of acute leukemia group as standard, and divided 50 cases of acute leukemia patients into High MNF group (MNF≥7.15‰) and Low MNF group (MNF<7.15‰). The overall response (complete remission + partial remission) rates of low MNF group are significantly higher than the high MNF group (*P*=0.001). The results are in shown in Table 3.

Table 3. Micronucleus Expression Level of Different Adverse Reactions Groups

Adverse Groups	Reaction	0 Stage	I-Stage	III-IV Stage	Total	Incidence rate of adverse reactions	X^2	*P*
WBC	High MNF	3(16.7)	2(11.1)	13(72.2)	18	83.33	3.782	0.124
	Low MNF	0(0.0)	1(5.3)	18(94.7)	19	100		
Total		3	3	31	37	91.85		
PLT	High MNF	0(0.0)	1(5.6)	17(94.4)	18	100	0.002	1
	Low MNF	0(0.0)	1(5.6)	18(94.7)	19	100		
Total		0	2	35	37	100		
HGB	High MNF	0(0.0)	2(11.1)	16(88.9)	18	100	0.672	0.66
	Low MNF	0(0.0)	4(21.1)	15(78.9)	19	100		
Total		0	6	31	37	100		
Alopecia	High MNF	7(38.9)	9(50.0)	2(11.1)	18	61.11	2.55	0.246
	Low MNF	11(57.9)	8(42.1)	0(0.0)	19	42.11		
Total		18	17	2	37	51.35		
ECG	High MNF	15(83.3)	3(16.7)	0(0.0)	18	16.67	1.293	0.34
	Low MNF	18(94.7)	1(5.3)	0(0.0)	19	5.26		
Total		33	4	0	37	10.81		
Nausea vomit	High MNF	8(44.4)	6(33.3)	4(22.3)	18	55.56	2.293	0.295
	Low MNF	13(68.4)	3(15.8)	3(15.8)	19	31.58		
Total		21	9	7	37	43.24		
liver function	High MNF	7(38.9)	7(38.9)	4(22.2)	18	61.11	6.307	0.034
	Low MNF	14(73.7)	5(26.3)	0(0.0)	19	26.32		
Total		21	12	4	37	43.24		

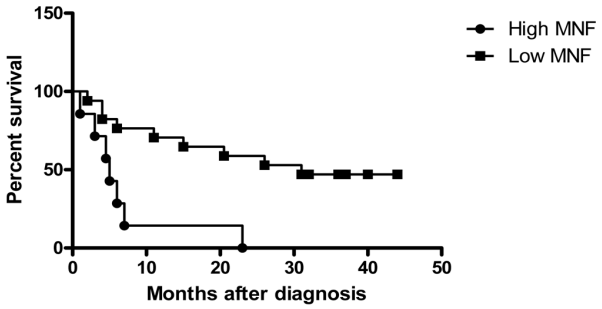

Figure 2. Overall Survival(OS) of Different Groups of Acute Leukemia Patients According to MNF Expression

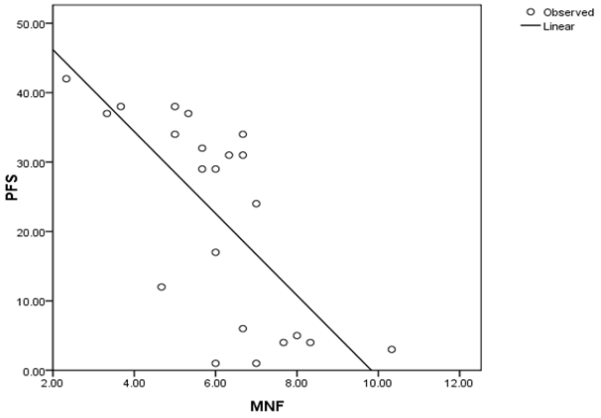

Figure 3. Progression-free Survival (PFS) of Different Groups of Acute Leukemia Patients According to MNF Expression

Micronucleus expression and adverse reactions of acute leukemia patients

Fifty acute leukemia patients divided into high MNF group, and low MNF group, according to the average MNF (7.15‰). Adverse reactions include influence on liver function, bone marrow toxicity, gastrointestinal toxicity, and more. Severe liver toxicity was evident in the high MNF group. There was no significant difference between high MNF group and low MNF group in hematopoietic toxicity, gastrointestinal toxicity, and ECG changes.

Micronucleus expression and prognosis of acute leukemia patients

After 2 years of observation and follow-up visit, some long-term evaluation index for prognosis of AL patients, such as progression-free survival (PFS) and overall survival (OS) of some patients were obtained. Kaplan-Meier survival curve were described according to OS and different levels of MNF. High MNF group showed lower OS rates than Low MNF group.

Two year survival rate of the former group was 0 (0/7), comparaed to that,the two year survival rate of the later one was 58.82% (10/17), the 3 year survival rate was 29.41 (5/17) (Figure 2). Figure 3 shows Scatter diagram and regression line of MNF and PFS. Correlation coefficient is -0.702.

Discussion

We observed several groups with varying malignancy degrees; including healthy controls, benign blood diseases, MDS, acute leukemia and malignant solid tumor. As Table 1 exhibits, micronucleus expression varies in the normal control group as well as groups of different diseases. The MNF of all patient groups was significantly higher than that of healthy control group ($P<0.001$), The results indicate MNF increased from healthy controls to malignant diseases. The trends are similar to former studies (Bonassi et al., 2007; Hamurcu et al., 2008; Narin and Tuncay, 2012).

It recognizes that acute leukemia has close relations to DNA damage and chromosome instability yet it will take years of chromosome instability for cancer to

development. Early detection and treatment will result in better long-term treatment outcomes and reduce long-term impairment to patients. Since a micronucleus results from numerical and structural chromosome aberrations, it can reflect a degree of genetic instability, to a certain extent. This can also provide evidence for early diagnosis, therapy, supervision, and prognosis.

In the present study, we divided the patients into four groups according to the malignant degree of disease. The results show that increased micronucleus expression corresponded to the malignant degree positively. In the acute leukemia group, no difference of MN frequency was between AML and ALL, which was consistent with the report from Hamurcu Z (Hamurcu et al., 2008).

Detection of micronucleus can definitely improve the effect of chemotherapy and reduce adverse effects (Jagetia et al., 2001). We divided the newly diagnosed acute leukemia patients into high MNF group and low MNF group, according to the average micronucleus frequency (7.15‰). On the evaluation of therapeutic side effects, the overall response (CR+PR) rates of low MNF group were significantly higher than high MNF group. With respect to the adverse reactions, high MNF group has more severe liver toxicity (a rise in ALT, AST). There was no significant difference between high MNF group, and low MNF group, in hematopoietic toxicity, gastrointestinal toxicity, and ECG changes in rate of adverse effect.

There exist many difficulties in detecting micronucleus continuously in peripheral blood lymphocytes of patients. Difficulties include patients refusing to cooperate, differences in treatment regimens, and individual variations. All the differences block evaluations of the micronucleus expression for long-term prognosis. After 2 years of observation and follow-up visit, PFS and OS of some AL patients were obtained (22 patients with PFS, 17 patients with OS). From Figure 2 and Figure 3, we found that there was a link between between PFS/OS and micronucleus expression.Patients with lower MNF seemed to have higher PFS and OS, which can be representative of long-term prognosis of AL patients. More tests need to be done because of less sample size of our tests.

Over expression of micronucleus appears ahead of chromosome aberration in PBL,and it is a predictor of cancer risk (Hagmar et al., 1998; Bonassi et al., 2000; Hagmar et al., 2004; Rossner et al., 2005; Sudha et al., 2011; Balamuralikrishnan et al., 2012). This situation can forecast a degree of genetic material damage, detected in high-risk group, and allow for early detection of cancer. Thus, micronucleus can be a symbol of cancer at an early stage. Patients who have more micronucleus expression have poor prognosis and treatment effects. Therefore, the most important meaning of micronucleus testing is prevention and monitor for cancer, especially for staff who work in specified hazardous environments. MNF examination can also be a useful tool for monitoring hazardous substance of environment,such as radioactive substance and chemical wastes (Liu et al., 2013; Toufexi et al., 2013). There are important implications for cancer prevention before paroxysm or exacerbation with MN test (Bonassi et al., 2007; Sellappa et al., 2010; Rickes et al., 2010; Sellappa et al., 2010; Basso et al., 2011). Especially for MDS patients, observation of MNF in MDS patients ought to be continuous. There are important implications for MDS patients to foresee risk progression to acute leukemia.

MNF in peripheral blood lymphocytes is important for us to evaluate prognosis of acute leukemia patients, There is a need to observe MNF continuously and widely for AL patients and high risk group to progress to AL.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (31070759). The author (s) declare that they have no competing interests.

References

Balamuralikrishnan B, Balachandar V, Kumar SS, et al (2012). Evaluation of chromosomal alteration in electrical workers occupationally exposed to low frequency of electro magnetic field (EMFs) in Coimbatore population, India. *Asian Pac J Cancer Prev*, **13**, 2961-6.

Basso E, Cevoli C and Papacchini M (2011). Cytogenetic biomonitoring on a group of petroleum refinery workers. *Environ Mol Mutagen*, **52**, 440-7.

Bonassi S, Hagmar L, Strömberg U, et al (2000). Chromosomal aberrations in lymphocytes predict human cancer independently from exposure to carcinogens. *Cancer Res,* **60**, 1619-25.

Bonassi S, Znaor A, Ceppi M, et al (2007). An increased micronucleus frequency in peripheral blood lymphocytes predicts the risk of cancer in humans. *Carcinogenesis*, **28,** 625-31

Celik A, Cavas T, Ergene-Gozukara S (2003). Cytogenetic biomonitoring in petrol station attendants: micronucleus test in exfoliated buccal. *Mutagenesis*, **18**, 417-21.

Countryman PI, Heddle JA (1976). The production of micronuclei from chromosome aberrations in irradiated cultures of human lymphocytes. *Mutat Res*, **41**, 321-32.

Fenech M, Holland N, Chang WP, Zeiger E, Bonassi S (1999). The HUman MicroNucleus Project:an international collaborative study on the use of micronucleus technique for measuring DNA damage in humans. Mutat Res, 428, 271-83.

Fenech M (2006). Cytokinesis-block micronucleus assay evolves into a "cytome" assay of chromosomal instability, mitotic dysfunction and cell death. *Mutat Res*, **600**, 58-66.

Friedman MA, Staub J (1977). Induction of micronuclei in mouse and hamster bone marrow by chemical carcinogens. *Mutat Res*, **43**, 255-61.

Hagmar L, Bonassi S, Strömberg U, et al (1998). Chromosomal aberrations in lymphocytes predict human cancer-a report from the European Study Group on Cytogenetic Biomarkers and Health (ESCH). *Cancer Res*, **58**, 4117-21.

Hagmar L, Strömberg U, Bonassi S, et al (2004). Impact of types of lymphocyte chromosomal aberrations on human cancer risk: results from Nordic and Italian cohorts. *Cancer Res,* **64**, 2258-63.

Hamurcu Z, Dönmez-Altuntas H, Borlu M, Demirtas H, Asçioslu O (2005). Micronucleus frequency in the oral mucosa and lymphocytes of patients with Behcet's disease. *Clin Exp Dermatol*, **30**, 565-9.

Hamurcu Z, Dönmez-Altuntas H, Patiroglu T (2008). Basal level micronucleus frequency in stimulated lymphocytes of untreated patients with leukemia. *Cancer Genetics*

Cytogenetics, **180**, 140-4.
Hamza VZ, Mohankumar MN (2009). Cytogenetic damage in human blood lymphocytes exposed n vitro to radon. *Mutat Res*, **661**, 1-9.
Jagetia GC, Jayakrishnan A, Fernandes D, Vidyasagar MS (2001). Evaluation of micronuclei frequency in the cultured peripheral blood lymphocytes of cancer patients before and after radiation treatment. *Mutat Res*, **491**, 9-16.
Juchimiuk-Kwasniewska J, Brodziak L, Maluszynska J (2011). FISH in analysis of gamma ray-induced micronuclei formation in barley. *J Appl Genet* , **52**, 23-9.
Kuramoto K, Ban S, Oda K, et al (2002). Chromosomal instability and radiosensitivity in yelodysplastic syndrome cells. *Leukemia*, **16**, 2253-58.
Li L, Liu XP, Nie L, et al (2009). Unique cytogenetic features of primary myelodysplastic syndromes in Chinese patients. *Leuk Res*, **33**, 1194-8.
Liu QJ, Lu X, Zhao H, et al (2013). Cytogenetic analysis in 16-year follow-up study of a mother and fetus exposed in a radiation accident in Xinzhou, China. *Mutat Res*, **755**, 68-72.
Mateuca R, Lombaert N, Aka PV, Decordier I, Kirsch-Volders M (2006). Chromosomal hanges: induction, detection methods and applicability in human biomonitoring. *Biochimie*, **88**, 1515-31.
Narin A, Tuncay O (2012). Relationships between malignant melanoma and chromosome damage in human peripheral blood lymphocytes. *Asian Pac J Cancer Prev*, **13**, 5229-32.
Rickes LN, Alvarengo MC, Souza TM, et al (2010). Increasedmicronucleus frequency in exfoliated cells of the buccal mucosa in hair dressers. *Genet Mol Res*, **9**, 1921-8.
Rossner P, Boffetta P, Ceppi M, et al (2005). Chromosomal aberrations in lymphocytes of healthy subjects and risk of cancer. *Environ Health Perspect*, **113**, 517-20.
Sellappa S, Prathyumnan S, Balachandar V (2010). DNA damage induction and repair inhibition among building construction workers in South India. *Asian Pac J Cancer Prev,* **11**, 875-80.
Sellappa S, Sadhanandhan B, Francis A, Vasudevan SG (2010). Evaluation of genotoxicity in petrol station workers in South India using micronucleus assay. *Ind Health*, **48,** 852-6.
Smith LE, Nagar S, Kim GJ, Morgan WF (2003). Radiation induced genomicin stability: radiation quality and dose response. *Health Phys*, **85**, 23-9.
Solé F, Espinet B, Sanz GF, et al (2000). Incidence, characterization and prognostic significance of chromosomal abnormalities in 640 patients with primary myelodysplastic syndromes. *Br J Haematol*, **108**, 346-56.
Strick R, Zhang Y, Emmanuel N, Strissel PL (2006). Common chromatin structures at breakpoint cluster regions may lead to chromosomal translocations found in chronic and acute leukemias. *Hum Genet*, **119**, 479-95.
Sudha S, Kripa SK, Shibily P, Joseph S, Balachandar V (2011). Biomonitoring of genotoxic effects among shielded manual metal arc welders. *Asian Pac J Cancer Prev*, **12**, 1041-4.
Toufexi E, Tsarpali V, Efthimiou I, et al (2013). Environmental and human risk assessment of landfill leachate: An integrated approach with the use of cytotoxic and genotoxic stress indices in mussel and human cells. *J Hazard Mater*, **260**, 593-601.