

# Detection of Relationship between Zinc and Multidrug-Resistance in Acute Leukemia Patients

Yi Qin<sup>1</sup>, Qun Qin<sup>2\*</sup>, Zongming Guo<sup>3</sup>

1 Department of Oncology and Hematology, The Second people's Hospital of Hunan province, Changsha 410007, P.R.China

2 Department of Pharmacy, Xiangya Hospital, Centra South University Changsha Hunan 410008 P.R.China

3 Department of Hematology, People's Hospital of Taizhou Zhejiang province Taizhou Zhejiang 317000 P.R. China

**Abstract Objective** To investigate the relationship between AL patients' plasma Zinc concentration change and clinical curative effect. **Methods** The plasma Zinc concentration of 52 patients with acute leukemia was examined by absorption spectrophotometry, the expression of P-gp in mononuclear cells of bone marrow in refractory/relapsed AL patients was observed by immunohistochemistry. **Results** There were 52 AL patients in the experiment, the change of Zinc concentration in the primary cases was significantly lower than that in normal control ( $P<0.01$ ). The Zinc concentration change in the complete remission patients was significantly higher than that in the refractory/relapsed patients ( $P<0.05$ ), Zinc concentration of the complete remission was significantly higher than that in primary patients ( $P<0.01$ ). There were 15 patients with positive P-gp expression in 17 refractory/relapsed AL patients. There was a negative correlation between the plasma Zinc concentration and positive cell rate of P-gp expression ( $r=-0.69$   $P<0.01$ ). **Conclusion** The plasma zinc concentration of AL patients is related to pathogenetic condition and clinical curative effect, and the change of AL patients' plasma zinc concentration can be one of the indicators for judging clinical condition and curative effect and predicting prognostic. The Zinc may take a part in occurrence of multidrug-resistance (MDR) in acute leukemia.

**Key words** Leukemia; Acute; Zinc; Multidrug resistance

Microelement is an important part of human body with crucial physiological effects in metabolism. When its content changes, health would be influenced and diseases would be caused. The relationship between microelement and tumor is one of the most focused tasks in tumor prevention and therapy. In this research, we investigated the relationship between the change of acute leukemia (AL) patients' plasma Zinc concentration and clinical curative effect and MDR, in order to search new clue to the research of tumor drug resistance reversion.

## MATERIALS AND METHODS

### Research objects

All 52 AL patients were in-patients from department of Hematology, Xiangya hospital, Hunan province

of China. Of them, 32 were male and 20 female, their average age was of 30 years old with ranged from 14 to 65 years. And there were 40 patients of acute non-lymphocytic leukemia (ANLL) and 12 acute lymphocytic leukemia (ALL) diagnosed by clinic, bone marrow cell morphology and cytochemistry staining. The diagnosis and the curative effect standard was referred to the reference literature [1]. Of the 52 patients, there were 19 primary cases who had not receive chemotherapy and 33 AL patients who had received two or more courses of treatment by combined chemotherapy. In the latter, 16 cases alleviated completely remission and 17 were refractory/relapsed ones, the standard of which were referred to reference literature [2]. In addition, there were 18 healthy blood donor whose average age was 33 years old, ranged from 29 to 43 years old, and 8 males and 10 females.

### Main reagents and apparatuses

WAY-402 atomic absorption spectrophotometer(U.S.A), JSB-1Mono-Antibody provided by SIGNET lab of U.S.A. Immunohistochemistry S-ABC reagent box provided by JingMei Biological Company, Lymphocyte

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Correspondence to: Qun Qin, female, MD

Tel: +86-731-4328991

Email: xzxchangsha@tom.com

Table 1 Concentration of plasma Zinc in different AL patients.

Group	N	Zinc ( $\mu\text{g/ml}$ )
Control group	18	4.28 $\pm$ 1.68
New treatment group	19	2.62 $\pm$ 1.12
Complete remission group	16	4.66 $\pm$ 2.18
Relapsed group	17	3.34 $\pm$ 0.93

Table 2 Concentration of plasma Zinc before and after chemotherapy.

Group	N	Zinc ( $\mu\text{g/ml}$ )	P
Before chemotherapy group	19	2.62 $\pm$ 1.12	<0.01
After chemotherapy group	33	3.98 $\pm$ 1.78	

separation medium made by The Second Reagent Factory of ShangHai. LXT -B electrical centrifuge was made in Tianjin, China.

### Measurement methods

Plasma zinc determination: Centrifuge plasma after anticoagulation with heparin (50u/ml). Draw 0.2ml and heat up with nitric acid and perchloric acid for assimilation. Then dilute it to 5.00ml by 1%  $\text{HNO}_3$ +1% Sr. 2+solution. Measure plasma zinc content by WAY-402 atomic absorption spectrophotometer.

P-gp expression detection: 2ml bone marrow was drawn from AL patients, anti-coagulated by heparin (50u/ml). Mononuclear cells were separated and cell concentration was adjusted to  $1.0-5.0 \times 10^6/\text{ml}$ . They were dripped on slides, and fixed up with 1% paraformaldehyde, the endogenous peroxidase was eliminated with 3%  $\text{H}_2\text{O}_2$ . Dripped normal goat blood serum sealing fluid, then dripped first antibody JSB1, biotin goat anti rat IgG (second antibody), SABC (third antibody) successively for incubation. Stained with aminoethylo carbazole(AEC), washed with distilled water, dehydrated and sealed the slice to observation. There were positive and negative controls in every examination. The cell membrane of p-gp positive cells was stained brown under oil lens. Counted 200 cells and positive cell rate  $\geq 10\%$  was taken as dividing line of pgp positive<sup>[3]</sup>.

### Statistic methods

The statistic processing on the results using statistic software SPSS11.0, the result was expressed by sample

average +standard deviation ( $\pm$ s). *t*-test was used to compare between two groups and straight line correlation analysis to determine the relationship between two indexes.

## RESULTS

### The changes of plasma zinc concentration in AL patients

The plasma Zinc concentration of 18 normal control was average 4.28+1.68 $\mu\text{g/ml}$ , and that of 19 primary patients was 2.62+1.12 $\mu\text{g/ml}$ , which was significantly lower than that of normal control ( $P<0.01$ ). The plasma Zinc average concentration in complete remission patients was 4.66 +2.18 $\mu\text{g/ml}$ , which was significantly higher than that in refractory/relapsed patients( $P<0.05$ ), but was no obvious difference compared with normal control( $P>0.05$ ). Details referred to table 1.

### Changes of plasma zinc concentration before and after chemotherapy in AL patients.

The plasma Zinc concentration of 19 primary cases in 52 AL patients was average 2.62+1.12 $\mu\text{g/ml}$ , which was significantly lower than that of the 33 AL patients who had received chemotherapy ( $P<0.01$ ). Details referred to table 2.

### P-gp expression in AL patients

There were 15 patients whose P-gp expression was positive in 17 refractory/relapsed AL patients, the positive cell rate was 21.3+9.97%. Straight line correlation analysis showed that there was a negative correlation between the plasma Zinc concentration and P-gp expression of mononuclear cells in bone marrow ( $r=-0.69$   $P<0.01$ ).

## DISCUSSION

The changes of microelements correlate closely with growth and development of malignant tumor. For example, zinc participates in synthesis of many enzymes and regulate immune function through effects of these enzymes, it plays an important part in maintaining immune function and resisting tumor<sup>[4,5]</sup>. Zhang Y *et al* re-

ported the changes of plasma cuprum, zinc and the ratio of them in 32 AL patients, the result showed that plasma cuprum, and the ratio of cuprum/zinc in AL patients were significantly higher than those in normal control ( $P < 0.01$ ), while zinc was lower than that in normal control ( $P < 0.05$ ); the plasma cuprum, and the ratio of cuprum/zinc in refractory group were significantly higher than those in remission group ( $P < 0.05$ ) while whose zinc was lower than that in remission group ( $P < 0.05$ ), which explained that changes of plasma cuprum and zinc was correlated to the growth and prognosis of leukemia. The result of this research showed that plasma zinc concentration in primary cases ( $2.62 \pm 1.12 \mu\text{g/ml}$ ) was significantly lower than that in normal control, plasma zinc concentration after chemotherapy was significantly higher than before, especially that plasma zinc concentration in remission group was significantly higher than that in refractory/relapsed group ( $P < 0.05$ ). The above research information showed that detection of plasma zinc concentration in AL not only can indicate that changes of zinc concentration correlate to disease occurrence, but also plays an important part in judging clinical condition and curative effect.

Tumor chemotherapy multidrug-resistance (MDR) research is a hotspot currently, of which over expression of P-glycoprotein coded by *mdr-1* gene was investigated thoroughly. P-gp is trans-membrane glycoprotein, which pumps drugs from inside to outside of cell and decreases drug concentration inside cell, so as to induce drug resistance of tumor cell<sup>[6,7]</sup>. This research found that plasma zinc concentration of 17 refractory/relapsed AL patients was lower than that of remission patients, and there were 15 patients whose P-gp expression was positive. Coincidence rate of the detection result

matched clinic was 88.2%, which is similar to report of Zhu PA *et al*<sup>[8]</sup>. The result also showed that positive cell rate was  $21.3 \pm 9.97\%$  in the 15 patients whose P-gp expression was positive, which was negative correlated to plasma zinc concentration ( $r = -0.69$ ,  $P < 0.01$ ). The above research results indicated that plasma zinc concentration is related to pathogenetic condition and clinical curative effect and the Zinc may take part in occurrence of multidrug-resistance in acute leukemia.

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