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Detection of DNA Damage Measured by Comet Assay in Pre and Post Chemotherapeutic Acute Lymphoblastic Leukaemia

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ABSTRACT DNA damage measured by comet assay/ SCGE was studied in ten cases of acute lymphoblastic leukemia before treatment and post treatment relapsed cases. The DNA damage of post treatment relapsed cases was more than untreated cases. Further study needs to be done to study DNA damage of post chemotherapy relapsed cases in both relapse and remission.

INTRODUCTION

.The alkaline single cell gel electrophoresis or comet assay is known as a rapid, simple and sensitive method for measuring and analyzing DNA single strand breaks and alkali labile sites (Singh et al.1988; Mekelvey-Meartin et al. 1993; Fairbrain et al. 1995 Collins et al. 1997, Palus et al. 1999).

To study the effects of chemotherapy on DNA damage, we used the comet assay from the ALL patients at pre chemotherapeutic stage as well as at relapsed stage while on maintenance therapy.

MATERIAL AND METHODS

10 diagnosed cases of acute lymphoblastic leukemia were treated according to UK ALL X chemotherapy protocol. Maintenance therapy consisted of vincristine 1.5 mg/m^2 once monthly, wysolone 40mg/m^2 (7 days/month) tab methofranate 20mg/m^2 (weekly) perinethol 50mg/m^2 (21 days in a month). (The consent of all patients and our institute ethical committee clearance were obtained).

Peripheral blood from diagnosed ALL patients prior to induction as well as from relapsed stage after post induction were collected in EDTA. Bone marrow diagnosis was done in both cases.

1 ml heparinized (50 units/mol. Sodium heparin) was taken for comet assay. The comet assay was completed within three days of sample collection. 20 micro litre whole blood was mixed with 1 ml ice cold RPMI 1640 in a microcentrifuge tube and lymphocytes were then isolated by Ficoll-Hypaque density gradient procedure (Singh et.al. 1988).

The comet assay was performed according to Singh et.al (1988) with using lysing solution (2.5 mol NaCl, 100 mM Na2EDTA, freshly added 1 % Triton-X100 and 10 % DMSO), electrophoresis buffer (300 mM NaOH, 1 mM Na2EDTA pH 13.0, 4 C) and 0.6 % normal melting and low melting agarose. Current for electrophoresis was adjusted to 300 mA by raising or lowering the buffer. The slides were stained by 20 ug/ml ethidium bromide.

Observation was made using 40x objective on a fluorescent microscope, (Nikon Microscope - Eclipse, E600 with Y-FL EPI-Fluorescence attachment, Japan) equipped with an excitation filter of 515-560 nm and a barrier filter 590nm. DNA damage was graded as Types 0, I, II, III, IV according to Palus et al. (1999). One hundred cells were analysed from each sample and the DNA damage was scored visually as described by Palus et al. (1999). The cells were graded into five categories by measuring the diameter of the cells with ocular and stage micrometer (McCarthy et al. 1997): no damage (Type 0), low-level damage (Type 1), medium level damage (Type2), high-level damage (Type 3), and complete damage (Type 4). Analysis has performed by one slide reader, thus minimising variability due to subjective scoring.

Haemoglobin (Hb), platelet count, leukocyte count were analysed by cell counter (Sysmex, K.1000,Japan). The liver function test was done by autoanalyser (Pechnicon, RAXT, USA). Bone marrow blast cell was studied by Nikon (Japan)

microscope using 100X objective. Observed data was statistically evaluated by Students "t" test.

RESULTS AND DISCUSSION

Clinical and laboratory parameters before and after chemotherapy were compared in 10 ALL patients. At the relapsed stage substantial decrease of bone marrow blast cell and total leukocyte count were observed (Table 1). SGPT and SGOT were above normal range in post induction (Table 1).

DNA damage was graded by measuring the diameter of the cell with the help of ocular and stage micrometer as Type 0 (range 14 -15.75 um), Type I (range 15.75 - 19.25 um), Type II (range 19.25 - 22.75 um), Type III (range 22.75 -26.25 um), Type IV (range 26.25 - 31.5 um). Percentage of DNA damage was calculated by the percentage of each type (I, II, III, IV) of comet (Table). In relapsed cases DNA damage was more than pre induced patients and most of their cells exhibited type II, III and type IV type of DNA damages.

Severe degree of damages of DNA was observed at the relapsed stage of post induced patients when little or no immature cells are seen in peripheral blood and in bone marrow.

Felix (2001) reported that DNA topoisomerase II causes chromosomal breakage and translocation in leukaemia. Direct testing for drug resistance patterns in DNA directed drug moieties by SCGE/CLSM reveal individual variability of human malignant cell lines warranting comparison with results of MTT testing and in vivo patient response (Ball et al. 1999).

Increased DNA damage was observed in workers exposed to styrene exposure and in cholangio carcinoma cells (Vodicka et al.1999). The exposure of human beings to ionizing radiation is still of great concern in occupational and environmental medicine, and the widespread use of radiotherapy in the treatment of cancer has led to anxiety about the possible hazards to staff who are at risk of such occupational exposure. DNA damage in the peripheral lymphocytes of 30 technicians employed in radiation oncology departments for at least 1 year were examined by the alkaline single cell gel electrophoresis "comet" technique. The results were compared with those of 30 controls with comparable age, sex and smoking habits who were not working in radiation oncology or

Table 1: Evaluation of DNA damage and detailed clinical information regarding pre and post chemotherapeutic ALL patients (n = 10)

Parameter	*Pre-chemotherapy (New cases) n=10*	*Post-chemotherapy (Relapsed cases) n=10*	
Age (year)	4.50 ± 1.50*	5.50 ± 1.25	
Liver size (cm)	5.00 ± 1.50	3.50 ± 0.9	NS
Hb (g/dl)	3.50 ± 1.50	4.00 ± 1.20	NS
Spleen Size (cm)	5.00 ± 0.85	4.00 ± 0.52	NS
Total Leukocyte count/cmm	43.900 ±13.02	7,100 ±12.03	p<0.001
Platelet count/cmm	25.000 ± 5.25	1,25,000 ± 4.49	p<0.001
Blast cell of bone marrow (%)	85.5 ± 2.10	65.0 ± 1.50	
Billirubin (mg/dl)	0.30 ± 0.05	0.30 ± 0.01	NS
Total protein (g/dl)	6.40 ± 0.10	6.60 ± 1.50	NS
Albumin (g/dl)	3.50 ± 0.02	3.70 ± 0.10	NS
Globulin (g/dl)	2.90 ± 1.20	2.90 ± 0.90	NS
SGPT (u/l)	11.0 ± 1.30	39.0 ± 2.10	p<0.001
SGOT(u/l)	39.0 ± 2.50	124.0 ± 3.20	p<0.001
Alkaline Phosphatse (u/l)	46.0 ± 3.00	113.0 ± 2.90	p<0.001
Grade of DNA damage (%)			
Type 0	33.5 ± 1.42	18.2 ± 1.10	p<0.001
Type I	20.0 ± 1.58	19.0 ± 0.79	NS
Type II	15.0 ± 0.31	8.20 ± 0.50	p<0.001
Type III	16.0 ± 1.89	26.4 ± 2.21	p<0.01
Type IV	15.0 ± 0.79	28.2 ± 2.10	p<0.005

*Mean ± SE

chemotherapy services. The DNA damage observed in the lymphocytes of the technician was significantly higher than that in the controls (Undepger et al.1999).

DNA comet assay can be used for documentation of DNA repair phenotype in cancer patient (Alapetite et al. 1999). Halothane and isoflurane anesthetics drugs were capable of increasing DNA migration in a dose-dependent manners (Jalozynski et al. 1999).

The increase in DNA damage at relapsed stage after a long gap of chemotherapy could lead to growth inhibition mediated by alterations of the expression of regulatory genes and ultimately cell death. Further studies are needed to assess the role of cytotoxic drug induced damage in a large number of ALL patients immediately after chemotherapy as well as at relapsed stage with maintenance therapy.

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