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# DNA-Repair Capacity in Down's Syndrome

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## ABSTRACT

**Down's syndrome (DS) is the most common chromosomal abnormality in human. Subjects with DS are known to be peridisposed to develop leukemia. The molecular basis of the association between DS and leukemia is unknown. The unscheduled DNA synthesis (UDS) test measure the ability of DNA-repair in mammalian cells after excision of a stretch of DNA containing the region of damage induced by chemical or physical agents. To get further insight into the cause(s) of leukemia in DS, the induction of UDS, in human peripheral blood lymphocytes was determined using hydroquinone and ethidium bromide, as DNA-damaging agents. Peripheral lymphocytes were obtained from 14 patients (7 males, 7 females) and 14 healthy sex- and age-matched normal subjects. The mean of UDS observed in control lymphocytes when treated with hydroquinone and ethidium bromide were 1.69 + 0.65, and 1.10 + 0.29, respectively, and corresponding values for DS lymphocytes were 1.11 + 0.40 and 0.92 + 0.26, respectively. Our results revealed that the lymphocytes of DS patients, showed a decrease in the UDS level. These data suggest that the low capacity of DNA-repair in conjunction with hypersensitivity of DNA and chromosomes of DS patients after treatment with mutagens may be relevant to the high incidence of leukemia in DS patients.** *Iran. Biomed. J. 2: 123-127, 1998*

*Keywords*: Down's syndrome, Trisomy 21, Unscheduled DNA synthesis, UDS, DNA-repair.

## INTRODUCTION

In addition to a wide spectrum of developmental abnormalities observed in Down's syndrome (DS) patients, they have an increased risk of developing leukemia, with estimates ranging from 14 to 30 times the incidence rate observed for chromosomally normal children [1, 2]. Although the molecular basis of this phenomena is not fully understood, several reports have revealed that the chromosomes and DNA of DS patients are more sensitive to various mutagens than those of normal control group. These mutagens are ionizing radiation [3-9], blue or green fluorescent light [10], viruses [11, 12], and chemicals [13, 14]. It has been reported that the DNA-repair deficiency in a number of clinical syndromes such as xeroderma pigmentosum and ataxia telangiectasia has been related to increased incidence of cancer [15]. There is a relationship between a low DNA-repair efficiency and the emergence of secondary malignancy in patients treated with alkylating agents [16] or other agents [9], and workers exposed to genotoxic agents [17, 19].

Damages induced in DNA can be repaired by repair systems which involve unscheduled DNA synthesis (UDS). In nucleotide excision repair, DNA damage is removed through incision of the damaged strand on both sites of the lesion, followed by repair synthesis or UDS. It should be noted that the DNA-repair is essential for DNA maintenance [20]. Therefore, the quantification of UDS is an indicator of the DNA-repair ability of cells [9, 16- 21]. In order to get more insight into the relationship between the ability of DNA-repair and increase risk of leukemia in DS, we tried to determine the DNA-repair ability of DS cells after treatment with DNA damaging agents and to compare it with that of control cells.

## MATERIALS AND METHODS

***Down's syndrome and control subjects:*** Samples of 5-6 ml of heparinized peripheral blood were obtained from 14 patients (7 males, 7 females) with DS; ranging in age from 12 to 18 years (15.9 ± 2 years, mean age ± SD); and 14 age- and sex-

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matched normal subjects. Both patients and control groups were unrelated Iranian Muslims. None of the individual had confounding factors (e.g., current illness, on chemotherapy, exposure to radiation and chemicals, cigarette smoking). The duration between collection of samples and arrival in the laboratory ranged from 15 to 30 min.

***Unscheduled DNA synthesis assay:*** DNA excision-repair capacity was estimated by measuring UDS induced in vitro after treatment with DNA-damaging agents in peripheral lymphocytes by the method of Martin et al, with a slight modification [21]. In brief, lymphocytes were isolated using Ficoll-Hypaque density centrifugation. Cells were washed in RPMI 1640 (Sigma) and resuspended at $1\times10^6$ cells/ml in complete medium (RPMI 1640 containing 10% heat inactivated fetal calf serum, 20 units/ml penicillin and 30 µg/m1 streptomycin). In order to reduce background of DNA replication, the cell suspension was treated with 5 mM hydroxyurea for 1 h prior to exposure to DNA-damaging agents. Cells ($5 \times 10^5$ in 500 µl) were treated with the DNA-damaging agents in complete medium containing 5 µCi/m1 ($^3$H) thymidine (specific activity = 24.0 Ci/mmol; Amersham Life Science). The cells were incubated for 3h at 37°C. Then the cells were harvested and the amount of radiolabel incorporated into the DNA was quantified using a Pharmacia liquid scintillation counter. The DNA-damaging agents used in this study were hydroquinone (final concentration 500 µM), and ethidium bromide (final concentration 50 µg/ml). Hydroquinone and ethidium bromide induced UDS as reported previously [22, 23]. The experiments were done in duplicate.

***Statistical methods:*** The significant differences of the mean of UDS between DS and control groups were analyzed using one tailed Student's unpaired t-test [24]. A probability of $P<0.05$ was considered statistically significant.

## RESULTS

The results of the UDS response to hydroquinone and ethidium bromide for DS and control lymphocytes are summarized in Table 1. Data are presented as the ratio of cpm/$5 \times 10^5$ cells after treatment with the mutagens to that of before treatment. This ratio gives information about increased incorporation of the radiolabeled ($^3$H) thymidine as a consequence of DNA-damage. This ratio has been termed the UDS or "repair index". A value of 1.00 indicated there was no detectable repair synthesis.

Since no statistically differences were observed between sex groups for the mean of induced UDS in lymphocytes after treatments with hydroquinone (control group tdf=12=0.17, P>0.05; patient group tdf=12=0.31, P>0.05) and ethidium bromide (control group tdf=12=1.38, P>0.05; patient group tdf=12=0.01, P>0.05), the sex groups were pooled.

The mean of UDS observed in control lymphocytes when treated with hydroquinone and ethidium bromide were 1.69 + 0.65 and 1.10 + 0.29, respectively; and corresponding values for DS lymphocytes were 1.11 + 0.4 and 0.92 + 0.26, respectively. There are significant decreases in the mean values of UDS in DS patients compared with those of control group when induced by hydroquinone (tdf=26= -2.92; P< 0.001) and ethidium bromide (tdf=26= -1.73; P< 0.05; one tailed) treatments.

## DISCUSSION

It is now well established that the DS patients have an increase in the risk of leukemia, with unknown mechanism(s) [1, 2]. A number of reports suggest that cells from DS subjects are more sensitive to various mutagens than those of control group [3-14]. These observations, that are consistent with our results, could be related to a decreased ability of DS cells to repair of genetic damage. However, contrasting results on the ability of DNA-repair in DS subjects have also been reported [25, 26]. We know that these controversial results are difficult to interpret. At least in part these inconsistent results are due to the fact that different cell types, different DNA damaging agents, and different methods for assay of DNA-repair ability were used.

Recently, the DNA-repair markers including the levels of induced UDS and activities of DNA polymerases were assessed in the lymphocytes of DS patients, and the DS group showed lower DNA-repair efficiency when compared with control group [27], confirming our results.

It is reported that in the trisomic cells from a mosaic case of DS, the frequency of sister chromatid exchange was twice as high as in his normal cells [28], indicating the trisomic cells are more sensitive than normal cells.



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Frequencies of chromosomal damage and the distribution of breakpoints in the chromosomes in the peripheral leukocytes of patients with DS, on exposure of gamma rays [29] or ethyl methane sulphonate [30], were reported. It is of particular interest that those breakpoints bands are the locations of oncogenes, cancer breakpoints, or fragile sites [29, 30]. Probably the site-specific chromosome rearrangement in proliferating cells in DS individuals after exposure to mutagens predisposes them to develop leukemia [29].

Although cells from DS patients have an increased susceptibility to the formation of chromosomal aberrations and frequency of sister chromatid exchanges when exposed to ionizing radiations [3-9, 29, 30], viruses [11, 12], and chemical mutagens [13, 14, 29], the present results demonstrate that lymphocytes from DS patients when treated with chemical mutagens show low level of DNA-repair efficiency compared to normal control (see Table 1). Taken together, it is suggested that the low capacity of DNA-repair with non-random occurrence of chromosomal breakpoints and hypersensitivity of DNA of DS cells after treatment with mutagens may have relevance to the high incidence of leukemia in DS patients.

**Table 1.** Comparison of repair index induced by Ethidium bromide and Hydroquinone in peripheral lymphocytes from control group and Down's syndrome patients.

| Individual | Sex[&] | Ethidium bromide[b] | | Hydroquinone` | |
|---|---|---|---|---|---|
| | | Control | Patient | Control | Patient |
| 1 | F | 1.52[d] | 0.86 | 1.81 | 1.50 |
| 2 | F | 0.99 | 0.74 | 1.58 | 1.19 |
| 3 | F | 0.91 | 0.97 | 1.10 | 0.84 |
| 4 | F | 0.80 | 1.03 | 2.78 | 0.92 |
| 5 | F | 1.87 | 1.42 | 2.25 | 1.33 |
| 6 | F | 1.30 | 0.67 | 1.28 | 1.04 |
| 7 | F | 1.07 | 0.74 | 1.24 | 1.17 |
| 8 | M | 1.10 | 1.21 | 3.28 | 0.70 |
| 9 | M | 0.85 | 0.91 | 1.48 | 1.76 |
| 10 | M | 1.03 | 0.70 | 1.16 | 1.05 |
| 11 | M | 0.94 | 1.02 | 1.57 | 0.93 |
| 12 | M | 1.18 | 0.88 | 1.48 | 1.15 |
| 13 | M | 0.79 | 1.26 | 0.99 | 1.59 |
| **14** | **M** | 1.10 | 0.45 | 1.67 | 0.36 |
| | Mean (± SD) | 1.10 ± 0.29 | 0.92 ± 0.26 | 1.69 ± 0.65 | 1.11 ± 0.40 |

[a]F and M are females and males respectively; [b]ethidium bromide used at concentration of 50 µg/m1; [C] hydroquinone used at concentration of 500 µM; [d] the values are the mean of 2 replicates.

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