Clastogen-Induced Chromosomal Breakage Analysis of Suspected Fanconi’s Anemia Cases of Kashmir, North India

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Abstract
Clastogen induced chromosome breakage analysis is widely used for the differential diagnosis of Fanconi’s anemia. Mitomycin-C (MMC) induced chromosome fragility test was performed on the cultured lymphocytes of 50 children with clinical suspicion of Fanconi’s anemia. According to the results of the MMC test, the patients were divided into two subgroups: FA displaying typical sensitivity to MMC and non FA. The present study revealed 7(14%) of examined patients to have a FA cellular phenotype with increased MMC-induced chromosome fragility. The percentage of MMC-induced aberrant cells was increased more than 36 times in FA patients (Mean=67.14%) when compared to non FA patients (Mean=1.82). The number of MMC-induced breaks/cells was more than 09 times higher in FA patients (Mean=2.42 breaks/cell) when compared to non FA patients (Mean=0.25 breaks/cells). Our results indicate that the clastogen induced sensitivity test is a reliable in vitro method for verification of the FA cellular phenotype. The study being the first of its kind from Kashmir (North India) lays the basis for further studies on patients of this region with a clinical suspicion of FA.

Keywords: Fanconi’s anemia (FA); Chromosome breakage; Mitomycin-C (MMC)

Introduction
Fanconi’s anemia (MIM ID #227650) (FA) is a rare autosomal recessive and a rarely X-linked recessive chromosomal breakage disorder, found in 20 to 30% of children with inherited aplastic anemias (AA) [1,2]. The anemia is caused by progressive bone marrow failure.

The phenotypic heterogeneity of FA also presents an appearance of developmental abnormalities, short stature and increased predisposition to cancer [2]. Fanconi’s anemia is a genetically heterogeneous disease and about 15 different FA complementary groups and corresponding genes have been currently identified [3-6].

Many cellular phenotypes have been revealed in FA but the most reliable and accepted of these is the hypersensitivity to clastogens such as mitomycin C (MMC) which produces interstrand DNA cross-links [7].

Hypersensitivity of FA cells to cytotoxic and clastogenic effects of interstrand DNA cross-linking agents, provides a unique cellular marker that is used to distinguish FA from other progressive bone marrow failures and chromosomal breakage syndromes [7]. In the present study we used the MMC-induced chromosome breakage test for differential diagnosis of FA in Kashmiri children with clinical suspicion of FA.

Material and Methods

Patients and samples
Fifty cases (26 males and 24 females, aged between 1 to 13 years) suspected of having FA and treated at Department of Clinical Hematology, Sher-i-Kashmir Institute of Medical Sciences, Srinagar, J&K, India were investigated from January 2013 to June 2015. Blood samples from patients with clinical suspicion of FA and sex and age-matched controls were collected for stress cytogenetic analysis after an informed consent. Clinical details of the patients were obtained from their clinicians, including age of onset of hematological disease and family screening results. According to the results of the MMC-induced stress cytogenetic test, the patients were divided into two subgroups: FA displaying typical MMC-sensitive cellular response and non FA.

MMC-induced stress cytogenetic test
The MMC-induced stress cytogenetic test on peripheral blood lymphocytes was performed using standard procedure [8-10] with minor modifications. Two lymphocyte cultures were set up for each patient. Forty-eight hours after the culture set-up, the cultures were treated with MMC at a final concentration of 0.1 mg/mL [9].

Cells were harvested 72 hours after initiation with the presence of colcemid during the last 2 hours (2.5 mg/mL). Staining with Giemsa solution was applied [9].

A total of 100 metaphase cells per subject were scored at random and analyzed for chromosome and chromatid aberrations, according to the International System for Human Cytogenetic Nomenclature (ISCN) [11].
Chromatid and chromosome breaks, and acentric fragments were scored as one break. Dicentric and ring chromosomes were scored as two breaks. Numbers of breaks in the radial configurations were scored as the number of chromosomes involved in the configuration [10,12]. For each patient, the chromosome damage was scored as number of breaks per cell.

Statistical analyses

Chi square test was used to evaluate the significance of difference between examined cultures of patients [10] and healthy controls. The FA and non FA groups were distinguished by Mann-Whitney test.

Results

In the present study 7 (14%) out of 50 examined patients were found to have a FA cellular phenotype with increased number of MMC-induced chromatid and chromosome breaks and a variety of chromatid and chromosome interchanges (Figure 1 and Table 1).

<table>
<thead>
<tr>
<th>FA Patient</th>
<th>Breaks/Cell</th>
<th>Aberrant (%)</th>
<th>Breaks/Aberrant Cell</th>
<th>Breaks/Cell</th>
<th>Aberrant (%)</th>
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Table 1: Spontaneous and mitomycin-induced chromosome fragility in 07 patients with Fanconi’s anemia.

The percentage of MMC-induced aberrant cells was increased more than 36 times in FA patients (Mean=67.14%) when compared to non FA patients (Mean=1.82). The number of MMC-induced breaks/cells was more than 09 times higher in FA patients (Mean=2.42 breaks/cell) when compared to non FA patients (Mean=0.25 breaks/cells) (Table 2). Patient No. 2 reached the maximal percentage of MMC-induced aberrant cells of 82.00%, while the minimal value was 48.00% in patient No. 4 (Table 1). Statistical analysis showed significant difference between FA and non FA groups (Mann-Whitney test: p<0.0001).

The spontaneous chromosome fragility (percentage of aberrant cells and breaks/cell) values in 07 FA patients were overlapping those in non FA patients (Table 2). All the seven patients showed an increased rate of spontaneous breaks (Table 1). Thus, the mean percentage of spontaneous aberrant cells in the FA group was 8.71% and the mean value of spontaneous breaks/ cells was 0.12 (Table 2).

In the non FA group of patients, the mean values of spontaneous breakages were 0.83% for percentage of aberrant cells and 0.01 for breaks/cell (Table 2).

Controls and the non FA group displayed almost similar values of spontaneous chromosome fragility. In controls the mean percentage of aberrant cells was 0.00% and the mean breaks/cell as 0.00 and in non FA group it was 0.83% and 0.1 respectively (Table 2).

Discussion

Nowadays, chromosome fragility induced by interstrand crosslinks inducing agents, such as MMC or DEB is the most widely used test for...
the diagnosis of FA. We report here the results of the MMC-induced chromosome fragility test as screening for FA in Kashmiri children with clinical suspicion of FA. This study revealed 07 (14%) out of 50 examined patients to have a FA cellular phenotype with increased MMC-induced chromosome fragility.

<table>
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</table>

Table 2: Evaluation of spontaneous and MMC-induced chromosome fragility findings in Fanconi's anemia and non- Fanconi's anemia patient groups.

In India, a comparatively large study on 94 aplastic anaemic patients of all age groups by Neelam et al., showed 13.8% patients with Fanconi's anemia [13] whereas another study by Gupta et al., showed 11.3% positive on stress cytogenetics for Fanconi's anemia [14]. However, a larger study was carried out on 300 aplastic anemia patients by Jain et al., in India and they found 9.20% patients with Fanconi's anemia [15]. The differences in the percentage affected by Fanconi's anemia may be because of the different number of cases included in these studies.

In our study, the spontaneous chromosome fragility (percentage of aberrant cells and breaks/cell) values in 07 FA patients were overlapping those in non FA patients. The International Fanconi's Anemia Registry (IFAR) study showed that the range of spontaneous chromosome breaks in FA group of 104 patients (0.02-1.90 breaks/cell with a mean of 0.27) was overlapping with the range found in a non FA group of 224 patients (0.00-0.12 breaks/cell with a mean of 0.02) [12]. In this study, the unreliability of base line of chromosome breakage in differential diagnosis of FA [12,16] was confirmed. Thus, baseline breakage frequency was proven not to be a useful method for discrimination of FA patients.

According to the MMC sensitivity test, the percentage of MMC-induced aberrant cells in the examined groups was increased more than 36 times in FA patients when compared to non FA patients. The number of MMC-induced breaks/cells was more than 09 times higher in FA patients when compared to non FA patients. There was a clear discrimination between FA and non FA subgroups with no overlapping. The IFAR study, also significantly differed the FA group from the non FA group on the basis of DEB-induced chromosome fragility. The percentage of induced aberrant cells in their study was 85.15% in FA patients and 5.12% in the non FA group [12]. Similarly, the mean of DEB induced breaks/cell in FA patients was 8.96, while in the non FA group it was 0.06 [12]. These two groups showed no overlapping. Our results are in the line with IFAR report [12] and other similar studies [16-18] because FA and non FA groups could be distinguished by the mean percentage of MMC-induced aberrant cells (FA 67.14% vs. non FA 1.82%) and mean breaks/cell (FA 3.46 vs. non FA 0.88).

However, the level of variability in MMC-induced aberrant cells between the 07 patients with FA is high with ranges from 48.00 to 82.00% (Tables 1 and 2). Part of this variability in the patients with FA may be due to the existence of subgroup of patients with T-cell mosaicism who have lower values of chromosome fragility parameters. This subgroup corresponds to FA patients, who represent 15-25% of all FA patients [19-21]. Somatic mosaicism is produced when one of the pathogenic mutations is reverted in a hematopoietic precursor cell [21-23]. In the previously published studies, FA patients with <40% of aberrant cells were considered mosaic, while those with a proportion between 40 and 60% were considered as possible mosaics; FA patients with proportion >60% of aberrant cells were considered as non mosaic patients with FA [24]. In the present study, only two of the FA patients were found to have aberrant cells in the range of 40%-60%. All the other FA patients had >60% aberrant cells which depicts that they were the non mosaic ones.

Conclusion

The present study concludes that clastogen induced chromosome breakage analysis is important for the differential diagnosis of Fanconi's anemia suspected cases. Although, hypersensitivity to drug induced DNA interstrand crosslinks like MMC is the hallmark of FA cells, a precise and accurate diagnosis is compromised in some cases, especially in mosaic patients. Therefore, molecular investigation and identification of the FA complementary group for each MMC sensitive FA patient is the next step necessary in establishing the diagnosis of FA, its therapy management and genetic counseling of affected families. The study being the first of its kind in Kashmir population, lays the basis for further studies on suspected Fanconi's anemia patients of this part of the world.

References


