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Short Communication
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Chromosome Stress Test in Fanconi Anemia

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Abstract

Chromosome instability syndromes or chromosome breakage disorders are a group of inherited human diseases unified by the abnormal behavior of their chromosomes. These single gene disorders mainly have an autosomal recessive mode of inheritance. Fanconi Anemia (FA) belongs to this class of chromosome breakage disorders and is a cause of bone marrow failure due to damage in the DNA repair system. The patients show hypersensitivity to specific chromosome damaging agents which manifest as high levels of breaks and radials. Mitomycin C (MMC) induced chromosome stress test was carried out on blood samples of 89 patients suspected to have a plastic anemia. Out of the 89 cases 9 cases were FA positive, 6 cases were mildly positive and 74 were negative.

Introduction

Fanconi Anemia (FA) is one of the most frequent causes of inherited bone marrow failure due to an impaired response to DNA damage leading to chromosomal instability resulting in a decreased production of all types of blood cells. It is an autosomal recessive disorder and is found in all ethnic groups occurring equally in males and females [1,2]. They show hypersensitivity to specific chromosome damaging agents and manifest as high levels of breaks and radials. The affected individuals have an increased risk of developing cancer. A chromosome breakage test is one of the diagnostic tools of FA, which is established by confirming an increased chromosomal breakage rate in peripheral blood lymphocytes in the presence of alkylating agents such as mitomycin C (MMC) which interfere with the cell's DNA [3]. This is also known as the chromosome stress test and is one of the parameters used to differentiate idiopathic a plastic anemia from Fanconi anemia, since all cases of FA do not exhibit the typical skeletal and related anomalies. Early distinction between syndromes has clear implications in disease management and outcome. If undiagnosed, FA patients who initially present with bone marrow failure can have disastrous side-effects after standard-dose conditioning regimens for hematopoietic stem cell transplantation. In this paper we share our experience of the MMC stress test on patients with a suspicion of a plastic anemia over the past 18 years.

Material and Methods

A total of 89 Mitomycin C (MMC) induced chromosome breakage tests were carried out on heparinized blood samples of patients suspected to have a plastic anemia according to cytogenetic protocol specific for Fanconi Anemia [4]. The MMC stress test was performed in each case by setting up 5 cultures each of the patient, and a sex matched unrelated control. MMC was added at final concentrations of 30, 50, 80 and 120 ng/mL, while the internal control cultures did not have MMC. UnbandedGiemsa stained slides were scored and the percentage of breaks and radials observed in the patient and control were compared. 25 suitable metaphases were scored from each culture and tabulated in groups according to the number of breaks per metaphase. Accordingly, the samples were categorized as negative, positive, or mildly positive. The presence of a few induced breaks in the control is an indicator of a successful technical procedure. In general a positive patient should show a 4-10 fold increase in the percentage of chromosome/chromatid breaks and radials compared to the control. Chromosome analysis was done on a Zeiss Axioskop microscope and metaphases were captured with the Meta system sikaros software.

Results

Out of 89 cases studied, 9 (10.1%) were FA positive, with a 4-7 fold increase in the number of MMC induced breaks/radials and the presence of spontaneous breaks, 6 (6.7%) were mildly positive with a 2-3 fold increase and 74 (83.2%) were negative. Figure 1 shows representative images for radials and breaks in FA positive cases. One of the positive cases showed mosaicism, probably

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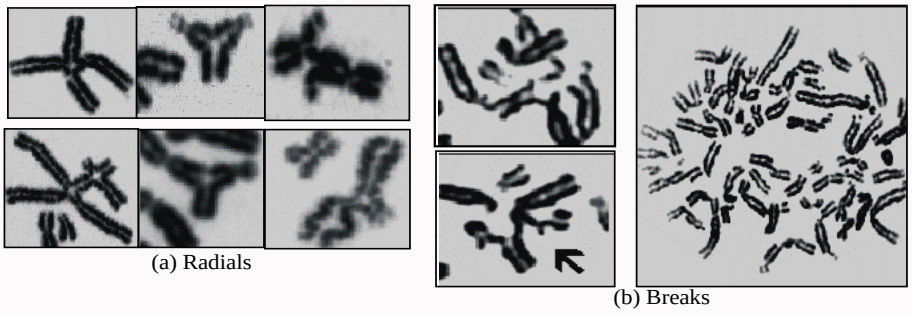

(a) Radials (b) Breaks

Figure 1: Breaks and radials in FA +ve cases.

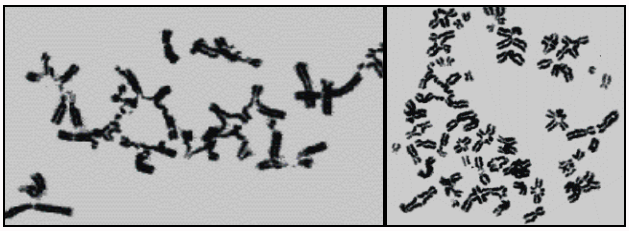

Figure 2: Multiple radials and breaks in a few cells in a case of FA mosaicism.

revertant, as there were few cells with numerous breaks and radials which could not be ignored, while a majority of cells showed no breaks (Figure 2). The test, repeated later in another lab was negative and the patient showed improvement.

Discussion

Fanconi Anemia (FA) is an autosomal recessive disorder that results in bone marrow failure. Many FA genes have now been identified and there is a lot of ongoing research on this. Though FA is associated with skeletal defects such as absent or bifid thumbs, many individuals do not show any clinical symptoms in childhood. The chromosome stress test helps to differentiate between cases of idiopathic a plastic anemia and Fanconi anemia. This information is important as treatment needs to be altered due to hypersensitivity to ionizing radiation and chemotherapeutic agents. Mosaicismis known to occur, where only a few metaphases show multiple breaks and radials. Also, revertant mosaicismis reported, where a positive case can revert back to normal [5]. In revertant mosaicismthe abnormal cell line is known to revert back to the wild type. These could lead to discrepancy in results between laboratories. In order to ensure that the stress test was successful, a few breaks must be visible in the cultures of the control too. We came across one case of revertant mosaicism among our patients.

The percentage of FA positive cases in our series was similar to that of other studies. Though molecular analysis of FANCC gene mutations is an alternative, MMC stress cytogenetic is still needed when variants of uncertain significance are seen on next generation sequencing.

Correlation of laboratory results with clinical findings is important, especially in cases which are equivocal or borderline positive.

References

1. Triemstra J, Pham A, Rhodes L, Waggoner DJ, Onel K. A review of fanconi anemia for the practicing pediatrician. Pediatr Ann. 2015;44(10):444-5.
2. Arthur NBJ, Ganapule AP, Palani D, Viswabandya A, Mathews V, Abraham A, et al. Clinical and molecular characterization of fanconi anemia: An Indian perspective. Blood. 2014;124:2938.
3. Park SN, Kim NH, Im K, Lee JS, Choi S, Lee DS. Diagnosis of fanconi anemia by chromosome breakage tests using 3 different scoring systems and whole genome sequencing among patients with aplastic anemia in Korea. Blood. 2015;126:4792.
4. Rooney DE, Czepulkowski BH, editors. Human cytogenetics: A practical approach. Volume II: Malignancy and acquired abnormalities. 2nd ed. London: IRL Press; 2001.
5. Shukla P, Ghosh K, Vundinti BR. Current and emerging therapeutic strategies for fanconi anemia. HUGO J. 2012;6(1):1.