

# Differentiation of Fanconi and Aplastic Anemia Using Chromosomal Breakage Test in Southern Iran

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## Abstract

**Background:** Fanconi anemia (FA) is a chromosomal breakage disorder characterized by familial aplastic anemia (AA), various congenital anomalies, and a characteristic chromosomal response to clastogenic stress.

**Methods:** In this study, chromosome breakage test was performed for 38 patients suspected of having FA and age-matched controls.

**Results:** According to the results, ten patients were considered as FA cases and 15 patients with no chromosomal breaks were considered as AA.

**Conclusion:** Differentiation of FA from AA is very important because the primary treatment is different. This test should be done in every primary presentation of AA.

**Keywords:** Fanconi anemia; Aplastic anemia; Southern Iran

## Introduction

FA is an autosomal recessive disorder caused by defects in at least eight distinct genes: FANCA, FANCB, FANCC, FANCD1, FANCD2, FANCE, FANCF and FANCG.<sup>1,2</sup> This syndrome is marked by bone marrow hypoplasia, a variety of congenital anomalies involving skin, heart, genitourinary tract, skeletal system, central nervous system, growth and mental retardation.<sup>2,3</sup> The principal cellular phenotype is hypersensitivity to DNA damage, particularly inter-strand DNA crosslinks.<sup>4,5</sup>

Since 1992, two of the suggested genes have been characterized, and molecular analysis of their products has revealed a complex mode of action.<sup>3</sup> Many proteins form a nuclear multi-subunit complex that appears to be involved in the repair of double-strand DNA breaks. Additionally, at least one of the proteins, FANCC, influences apoptotic pathways in response to oxidative damage. Further analysis of the

FANC proteins will provide vital information on normal cell responses to damage and allow therapeutic strategies to be developed that will hopefully supplant bone marrow transplantation.<sup>2-5</sup>

The aim of this study was to develop a procedure in cytogenetic laboratory of Mottahary clinic for the detection of chromosomal breakage and differentiation of FA from AA, using this test.

## Materials and Methods

During a 40 month period, 38 patients were referred to the Cytogenetic laboratory of Mottahari Clinic. Age-matched healthy controls were concurrently analyzed in order to provide a baseline breakage rate.

Three to five milliliter of sodium-heparinized whole blood was collected from each patient and control individual. If the patients' hematocrit was less than 50%, serum was removed in order to obtain cells to serum ratio of 50:50. Afterwards, 0.5cc of each patient and control individual's blood sample was added to 5cc of a complete media containing PRMI 1640, fetal calf serum (10%), PHA (10 µg/ml), L-glutamate (2 mM),

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Penicillin (200 unit/ml), and Gentamycin (50 µg/ml).

Each sample was cultured in triplicates. Mitomycin-C (MMC) solution was added to two of the triplicate cultures with a final concentration of 25, and 50 ng/ml. After 70 hours of incubation in 37°C, colcemide was added (0.2 µg/ml). After 30 minutes, the cells were harvested by centrifugation (130 xg for 10 minutes). Then, 5ml of 0.075 M KCl solution was added and mixed and incubated at 37°C for 15 minutes. After centrifugation (130 xg for 10 minutes), hypotonic supernatant was removed. Then, 5cc cold, fresh fixative solution (3:1 methanol-acetic acid) was added drop-wise to the cell pellet. Centrifugation was done afterwards and the supernatant removed. These two latter steps were repeated until a clear pellet was obtained. Finally, cells obtained from each triplicate were dropped on distinct slides. Solid staining with Giemsa was performed for some of the slides prepared from each patient.

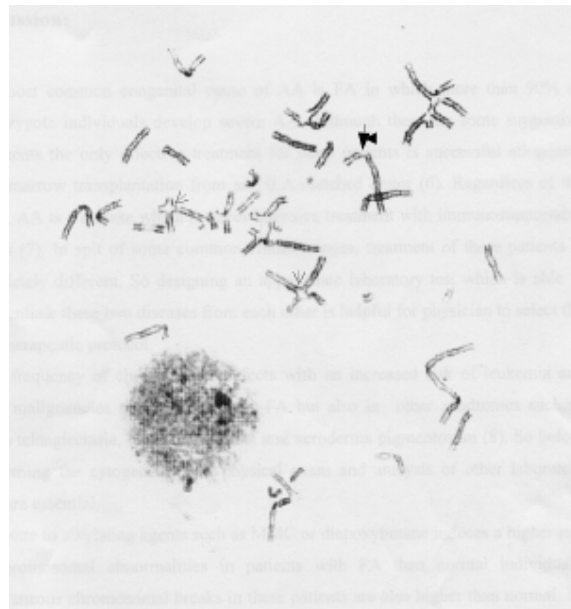
Fifty metaphases, each from the MMC-treated and routinely cultured on both the patients and the normal controls, were analyzed for chromosomal breakage. Metaphases with 40 or fewer chromosomes were excluded. The number of breaks per cell for each culture was calculated. The diagnosis of FA was done when only the MMC-induced breakages exhibited a significant increase in comparison to the controls and the results were in agreement with the hematological and clinical data of FA.

## Results

In this study, 38 patients suspected of FA or AA were referred to cytogenetic laboratory during a 40 month period. The age range of the patients was between 3 and 14 years. Eighteen patients were female and twenty of them were male. The blood cells of 13 patients showed no growth in the cultures. Ten samples showed lots of chromosomal breaks such as gaps and double strand breaks. Several samples showed radial formation pattern (Figure 1). Fifteen samples with no breaks were considered as AA according to hematological indices.

## Discussion

The most common congenital cause of AA is FA in which more than 90% of homozygote individuals develop severe AA. Although there are some supportive treatments, the only effective one for these patients is successful allogeneic bone marrow transplantation



**Fig 1:** Metaphase preparation exposed to MMC of patients with FA. The chromosomes have been stained with Giemsa (→quadriradial→gap)

from an HLA-matched donor.<sup>6</sup> Regardless of the cause, AA is a disease which requires intensive treatment with immunosuppressive agents.<sup>7</sup> In spite of some common clinical signs, and treatment of these patients is completely different. So, designing an appropriate laboratory test which is able to differentiate these two diseases from each other is helpful for physicians to select the best therapeutic protocol.

A high frequency of chromosomal defects with an increased risk of leukemia and other malignancies is not only found in FA but also in other syndromes such as Ataxia telangiectasia, Bloom syndrome, and xeroderma pigmentosum.<sup>8</sup> So, before performing the cytogenetic test, physical exam and analysis of other laboratory tests are essential.

Exposure to alkylating agents such as MMC or diepoxybutane induces a higher rate of chromosomal abnormalities in patients with FA than in normal individuals. Spontaneous chromosomal breaks in these patients are also higher than normal. So, having a sex/age matched normal control as well as a culture without alkylating agent from the patient is necessary for the determination of the baseline breakage rate.

The choice of treatment for FA is Bone Marrow Transplantation (BMT), but in mild to moderate AA, cyclosporine, steroid and anti-lymphocyte globulin are the modalities of treatment even though BMT is used for severe types. Therefore, differentiation of FA

from AA is very important because the primary treatment is different. This test should be done in every primary presentation of AA.

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**Conflict of interest:** None declared.

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