

Sibling Test - A Distinct Diagnostic Approach for Fanconi Anemia in a Patient with Negative Chromosomal Breakage Analysis

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ABSTRACT

Fanconi anemia (FA) is a rare form of an inherited disorder that mainly results in aplastic anemia. In our case, a three-year-old female child presented with recurrent episodes of fever and persistent pancytopenia refractory to any treatment. The chromosomal breakage analysis (CBA) with mitomycin C and solid staining was done, which showed no chromosomal breakage. Considering negative results due to mosaicism, her younger brother's CBA was performed, which showed a positive result. Therefore, based on clinical features, persistent cytopenia, and the younger siblings' CBA, both children were diagnosed with FA.

Keywords: *Fanconi anemia, chromosomal breakage analysis, diagnosis, inherited disorder, case report.*

INTRODUCTION

Inherited bone marrow failure syndromes are a diverse set of genetic disorders characterized by hematopoietic aplasia and cancer predisposition. A Swiss pediatrician, Guido Fanconi, first reported Fanconi Anemia (FA), a rare genetically inherited disease, in 1927 [1]. He reported both visible malformations and blood disorders. The first sign of a hematological problem is usually petechiae and bruises, with later onset of pale appearance, tiredness, and recurrent infections. Even though a conventional set of features such as thumb and radial absence, defective facial features and malformation, typically characterize these patients, FA children commonly present in the first decade of existence with aplastic anemia [2]. Additionally, there is an increased incidence of leukemia and myelodysplasia, along with cellular hypersensitivity as FA itself is caused by a mutation in one of the FA genes [3]. Confirmatory diagnosis is made by lymphocyte chromosomal breakage analysis (CBA) [4]. However, 10 to 15 percent of patients show somatic mosaicism, and their lymphocytes may not show the characteristic high level of chromosomal fragility because of a mixed population of somatic cells, in which case skin fibroblast test is performed [5]. Somatic mosaicism in FA is due to mutations in the stem cells and is linked with reduced responsiveness to clastogens, which is a diagnostic attribute of FA. Herein, we present a case of FA in a three-year-old female child with a negative CBA due to mosaicism.

CASE PRESENTATION

A three-year-old female child weighing 8.5 kg, vaccinated up to age, presented with pallor and generalized weakness for three months along with recurrent episodes of fever associated with respiratory and gastrointestinal symptoms. She was born *via* spontaneous vaginal delivery, full-term at home without any pre and post-natal complications. She was the second product of consanguineous marriage. Family history revealed the death of her elder sibling at the age of two years due to gastroenteritis and an abortion of a twin pregnancy. Her younger one-year-old brother was alive.

On examination (O/E): a well oriented, pale, mildly dehydrated child with global developmental delay presented with respiratory rate (RR) = 34/min, temperature = 98°F, heart rate (HR) = 166 beats/min, blood pressure (BP) = 80/50 (at 50th percentile), length = 82 cm (<3rd percentile), weight = 8.5kg (< 3rd centile), occipitofrontal circumference (OFC) = 44cm (< 3rd centile). Multiple bruises were noted on both lower limbs and trunk, but there was no visceromegaly and lymphadenopathy. Additionally, the child had no facial dysmorphism or skeletal abnormalities

Her initial blood count reported: Hemoglobin (Hb) = 4.2 gm/dl, total leukocyte count (TLC) = 3000/ml, neutrophils = 40%, lymphocytes = 57%, platelet = 9000/ml, serum iron = 56 mcg/dl (decreased), Vitamin B12 = 85pg/ml (decreased) and serum folate = 14ng/ml (normal). Her peripheral smear showed macrocytic hypochromic anemia, leucopenia, and thrombocytopenia. Imaging analysis included chest radiographs, echocardiogram, and abdominal ultrasounds which did not reveal any concern. Initial supportive management was given along with transfusion of 100 ml packed cell volume (PCV) slowly over 2 hours, transfusion of 100 ml platelets BD.,

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Received: October 19, 2021; Revised: November 03, 2021; Accepted: November 03, 2021
DOI: <https://doi.org/10.37184/lnjpc.2707-3521.3.21>

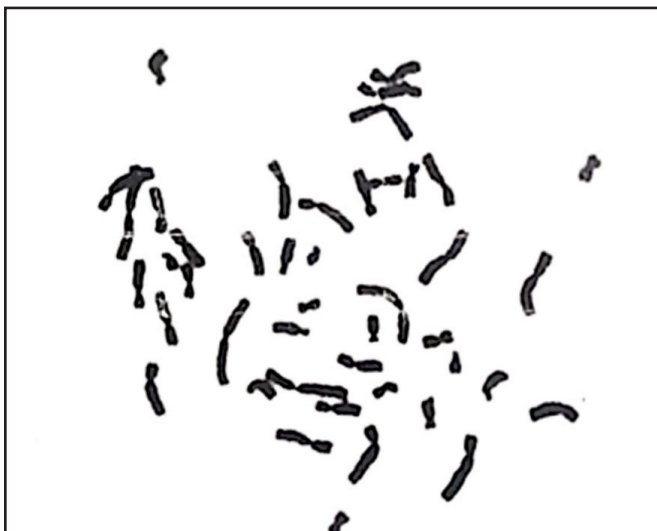


Fig. (1): First chromosomal breakage analysis (CBA) of the patient with Mitomycin C which showed no chromosomal breakage.

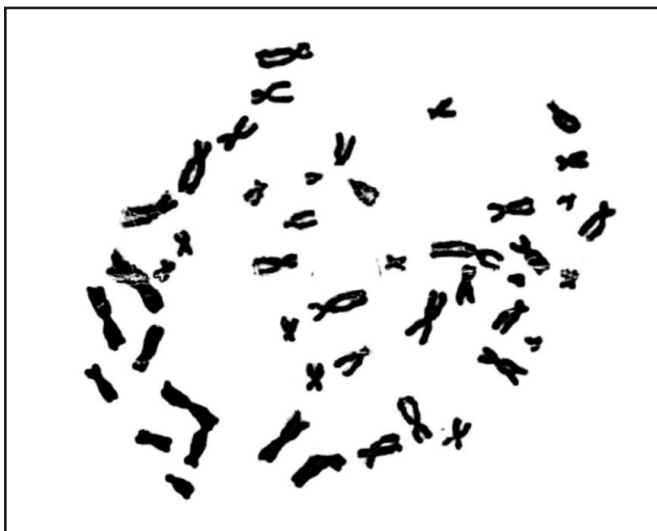


Fig. (2): Second chromosomal breakage analysis (CBA) of the patient without Mitomycin C which showed no chromosomal breakage.

and Inj. Vit. B12 500 microgram I/V diluted slowly over 30 min O.D. On 2nd day, repetition of complete blood count (CBC) showed slight improvement in results: Hb = 6.2gm/dl, TLC = 3300/ml, neutrophils = 44%, lymphocytes = 48%, platelet = 30000/ml. The patient was referred to the hematology department for further clinical assessment of pancytopenia. A trial of hematinic therapy was given with proper nutritional workup for a month to rule out nutritional deficiency and labs were repeated which showed Hb = 8.2gm/dl, TLC = 2900/ml, neutrophils = 36%, lymphocytes = 62% and platelet = 85000/ml, serum iron = 132 mcg/dl, Vitamin B12 = 280 pg/ml and serum folate = 18 ng/ml. Although hematinic therapy initially resulted in improvement of serum iron, cobalamin, folate and cytopenias, further continuation of hematinic therapy did not significantly improve the pancytopenia.

After the failure of hematinic therapy, the hematologist conducted a bone marrow biopsy that showed hypocellular

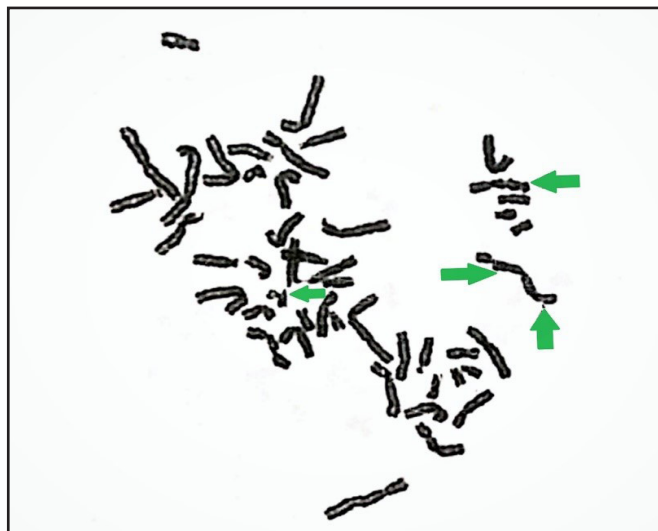


Fig. (3): Chromosomal breakages observed in chromosomal breakage analysis (CBA) of the sibling of the patient.

bone marrow exhibiting subtle dyshematopoietic features, megakaryocytic hypoplasia, and reduced iron stores, and no evidence of hemoparasite, dysplasia, malignancy, or metastatic disease. For confirmation of diagnosis, a CBA with (Fig. 1) and without (Fig. 2) Mitomycin C and solid staining was done which showed no chromosomal breakage, the repetition of the same test with another laboratory by using method 72hrs Phytohemagglutinin (PHA)-stimulated blood samples of suspected FA patient with positive FA and non-FA individual as a positive and negative control respectively has been set up using Mitomycin C which also showed negative results.

At last, her only alive sibling was called for an investigation by the hematologist. He was a one-year-old boy on examination who was mildly anemic, height was below 3rd percentile, rest of the systemic examination was normal. His complete blood count (CBC) was done: Hb = 7.1g/dl, TLC = 4200/ml, neutrophils = 58%, lymphocytes = 42% and platelet = 30000/ml. Repeated labs of the younger siblings were also consistent with pancytopenia. The hematologist suggested doing a CBA of other siblings with the opinion that the results of older siblings could be negative due to mosaicism, as the clinical picture was suggestive of FA. Bone marrow biopsy of the sibling showed pancytopenia, reticulocytopenia, and subcortical markedly hypocellular bone marrow biopsy, raising concern for bone marrow aplasia while CBA showed multiple chromosomal breakages with a DNA cross-linking agent as shown in Fig. (3).

Based on clinical features, persistent cytopenia, and younger sibling CBA both children were diagnosed as FA.

DISCUSSION

FA is an autosomal recessive disease 99% of the time except for FANCB, which is X-linked [6]. Patients can be either heterozygous or homozygous for a mutation

in any of the 15 currently identified genes. FA patients with birth defects are diagnosed at a younger age than those without a birth defect. Classic features include abnormal thumbs, absent radii, short stature, skin hyperpigmentation, abnormal facial features (triangular face and microcephaly), abnormal kidneys, and decreased fertility [7]. Due to somatic mosaicism, most of these features were not present in this patient. However, our patient had short stature and decreased OFC for age. Evidence of somatic mosaicism in FA patients because of genotypic reversion in lymphohematopoietic stem cells and iatrogenic mitotic recombination in lymphocytes leads to difficulty in diagnosing with DNA crosslinking agent [6-8]. In such cases, testing of skin fibroblasts is performed [9]. In our patient, skin fibroblasts culture was not performed due to limited resources in our country.

This case was diagnosed based on clinical presentation, blood investigation, bone marrow examination, and the sibling's positive CBA. The patient belonged to a low socioeconomic class, and her lab results showed low levels of iron and Vitamin B12. Initially, as the patient was treated with nutritional deficient anemia, only a slight improvement was observed in her pancytopenia despite proper nutritional treatment. Persistent mild anemia in her reports indicated the need to perform a bone marrow biopsy which revealed hypocellular bone marrow suggestive of FA. It was found that FA with somatic mosaicism was actually masked by the effect of nutritional deficiency anemia in the patient.

In patients with FA and bone marrow failure, oxymetholone, a synthetic anabolic steroid, is often utilized. It promotes erythropoietin production in patients with anemias caused by bone marrow failure. About half of children who are given oxymetholone show a temporary hematologic response, with only a few patients having a long-term response. Another agent, oxandrolone has also been suggested as a potentially useful androgen for treatment in patients with FA [10]. The key to treating FA patients with marrow failure and pancytopenia is HLA identical matched sibling donor stem cell transplantation, it improves the quality of life and also decreases the risk for leukemia [11]. Another increasingly studied treatment modality in the initial stages of clinical application is Gene Therapy [12]. In this patient, a bone marrow transplant was not possible due to financial restraints. The patient and her sibling both were kept on supportive treatment which included nutritional support and three-monthly monitoring of their lab reports. Additional counselling would be required for the patient in terms of prognosis and compliance to treatment along with potential cancer screening and prevention.

Fanconi anemia has a strong link to consanguinity, which is concerning, especially in our Pakistani society. Although the culture of our society cannot be changed, it is important to counsel and educate the families regarding the inheritance pattern of this disease to

prevent future generations from acquiring this disease and to alleviate morbidity and mortality [13].

CONCLUSION

Diagnosing this patient with negative test results for CBA was only possible when cross-referenced with her sibling's CBA. The patient presented with signs of pancytopenia and was initially diagnosed with nutritional anemia. Concomitant nutritional deficiencies may be present in patients with constitutional anemia.

The FA must be considered in all pediatric patients with cytopenias, not responding to hematinics. Ideally, the diagnosis should be based on clinical presentation, blood work, bone marrow biopsy, and a CBA. In a resource-limited setting, where genetic testing is not available for definitive diagnosis, the CBA of siblings could be an important tool for the diagnosis of patients of FA with inconclusive CBA. Management of this patient was only supportive but should ideally be a stem cell transplant for a good prognosis, as it is curative. Other treatment options barring the possibility of a transplant would be transfusions and medication. Proper genetic counselling is also required as is the importance of cancer screening and prevention.

CONSENT FOR PUBLICATION

The child's legal guardian's consent was obtained to publish this Case report.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

None.

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