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Differentiation of Fanconi anemia and aplastic anemia using mitomycin C test in Tunisia

Investigation cytogénétique de l'anémie de Fanconi en Tunisie par le test à la mitomycine C

Faten Talmoudi [a,b], Olfa Kilani [a,b], Wiem Ayed [a,b], Nizar Ben Halim [b], Fethi Mellouli [c], Lamia Torjmane [d], Lamia Aissaoui [e], Yosra Ben Youssef [f], Lobna Kammoun [g], Tarek Ben Othmane [d], Mohamed Bejaoui [c], Neila Ben Romdhane [h], Moez Elloumi [g], Sondes Hadiji [g], Sofiene Hentati [a], Imene Chemkhi [a], Nabila Abidli [a], Helmi Guermani [a], Sonia Abdelhak [b], Ahlem Amouri [a,*,b]

[a] *Laboratory of Histology and Cytogenetics, Institut Pasteur de Tunis, Tunis, Tunisia*
[b] *Laboratory of Biomedical Genomics and Oncogenetics, Institut Pasteur de Tunis, Tunis, Tunisia*
[c] *Department of Peadiatric Immuno-Haematology, National Bone Marrow Transplantation, Tunis, Tunisia*
[d] *Department of Haematology and Transplantation, National Bone Marrow Transplantation Centre, Tunis, Tunisia*
[e] *Haematology Department, Aziza Othmana Hospital, Tunis, Tunisia*
[f] *Haematology Department, Farhat Hached Hospital, Sousse, Tunisia*
[g] *Paediatric Department, Hedi Chaker Hospital, Sfax, Tunisia*
[h] *Haematology Department, La Rabta Hospital, Tunis, Tunisia*

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ABSTRACT

Fanconi anemia (FA) is a recessive chromosomal instability syndrome that is clinically characterized by multiple symptoms. Chromosome breakage hypersensitivity to alkylating agents is the gold standard test for FA diagnosis. In this study, we provide a detailed laboratory protocol for accurate assessment of FA diagnosis based on mitomycin C (MMC) test. Induced chromosomal breakage study was successful in 171 out of 205 aplastic anemia (AA) patients. According to the sensitivity of MMC at 50 ng/ml, 38 patients (22.22%) were diagnosed as affected and 132 patients (77.17%) as unaffected. Somatic mosaicism was suspected in an 11-year-old patient with a FA phenotype. Twenty-six siblings of FA patients were also evaluated and five of them (19.23%) were diagnosed as FA. From this study, a standard protocol for diagnosis of FA was developed. It is routinely used as a diagnostic test of FA in Tunisia.

Mots clés :
Anémie de Fanconi
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Cytogénétique
Test d'instabilité chromosomique
Mitomycine C

RÉSUMÉ

L'anémie de Fanconi (AF) fait partie d'un groupe de pathologies associées à une instabilité chromosomique. Ce phénomène est amplifié après traitement par des agents pontant l'ADN tels que la mitomycine C (MMC). L'exploration cytogénétique constitue une méthode de référence pour le diagnostic de l'AF. Pour cela, un protocole standard clairement établi est nécessaire pour le diagnostic. Dans ce travail, nous avons exploré

* Corresponding author.
E-mail address: amouri.ahlem@pasteur.rns.tn (A. Amouri).

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205 patients atteints d'AA et suspects d'AF en utilisant la MMC à 50 ng/mL. Nous avons confirmé le diagnostic d'AF chez 22,22 % des patients. En outre, le diagnostic d'AF a été retenu chez cinq autres cas parmi les 26 frères et sœurs testés. Par ailleurs, un mosaïcisme somatique a été suspecté chez un patient ayant un phénotype évocateur d'AF. Enfin, le diagnostic d'AF a été exclu chez 77,19 % des patients. Grâce à cette étude, un protocole standard pour le diagnostic de l'AF a été développé. Ce dernier est utilisé en routine comme test de diagnostic de l'AF à l'échelle nationale.

1. Introduction

Aplastic anemia (AA) is a rare and a heterogeneous disorder characterized by the inability of the bone marrow (BM) to produce an adequate number of blood cells. The majority (70%) of these cases are categorized as idiopathic because their primary aetiology is unknown. In a subset of cases, a drug or infection can induce the bone marrow failure and in approximately 15–20% of patients, the disease is constitutional/inherited [1]. Fanconi anemia (FA) is the most frequent inherited cause of AA [2]. Affected individuals may have one or more somatic abnormalities such as short stature, microcephaly, microphthalmia, thumb and radius deformities, skin hyperpigmentation such as ''café-au-lait spots'', cardiac, renal, genitourinary, and/or other malformations. A subset of FA patients (approximately a third) have no overt physical/somatic abnormalities [3].

There is considerable genetic heterogeneity in FA with 15 different FA genes (*FANCA*, *FANCB*, *FANCC*, *FANCD1/BRCA2*, *FANCD2*, *FANCE*, *FANCF*, *FANCG*, *FANCI*, *FANCJ/BRIP1*, *FANCL*, *FANCM*, *FANCN/PALB2*, *FANCO/RAD51C*, and *FANCP/SLX4*) [3]. The most prevalent being *FANCA*, *FANCC*, *FANCG*, and *FANCD2* [4]. Except for the very rare *FANCB*, which is located on X chromosome [5], all other *FANC* genes are autosomic and the disease is recessive.

FA cells characteristically show an abnormally high frequency of spontaneous chromosomal breakage and hypersensitivity to DNA cross-linking agents such as diepoxybutane (DEB) [6] and mitomycin C (MMC) [7]. Chromosomal breakage test with these agents is the technique of reference to distinguish FA from other AA [8].

In the majority of cases, a precise diagnosis can be made with careful history, physical examination, and a positive chromosomal breakage blood test.

An accurate diagnosis will influence the choice of therapy. Since FA patients are hypersensitive to all DNA cross-linking agents, they require a modified pre-transplantation conditioning regimen, with a lower than usual dose of cyclosphamide or lower doses of chemotherapeutic agents [9].

The main objective of the present research was to determine rational criteria for correctly and unambiguously diagnose patients with FA using MMC-test.

2. Patients and methodes

2.1. Patients and samples

Between 2004 and 2011, a total of 205 AA patients suspected for FA were referred to the cytogenetic laboratory at the Institut Pasteur de Tunis for chromosomal fragility evaluation.

Patients were selected for MMC-induced chromosomal breakage studies on the basis of AA, congenital abnormalities, and other hematological indicators known to be connected with FA, as well as family screening. Sensitivity and chromosomal instability of peripheral blood lymphocyte cultures, induced by MMC, were the main factors for dividing patients into two groups: MMC-sensitive (MMC+, FA) and MMC-insensitive (MMC−, non-FA) patients.

2.2. Chromosome fragility test

A cytogenetic study was carried out on peripheral blood cultures stimulated with phytohemagglutinin. Two cultures were set up for each patient: (i) cultures without alkylating agents and (ii) MMC-induced cultures. MMC-treated and untreated peripheral lymphocyte cultures of patients were prepared in the same conditions and at the same time as their counterpart culture of control subjects. MMC-test was carried out according to methods described in the literature with minor modifications [7,10]. In summary, the culture unit consisted of 1.6 ml of heparinized blood added to 5 ml of RPMI 1640 medium (GIBCO) supplemented with 20% fetal bovine serum (GIBCO), 1% penicillin-streptomycin solution (GIBCO), 1% L-glutamin (GIBCO) and 1% phytohemagglutinin (EUROBIO).

At initiation, MMC solution (SIGMA) was added at a final concentration of 50 ng/ml and incubated for 72 hours at 37 °C in a 5% CO_2 atmosphere at high humidity.

This MMC concentration was optimized in order to not affect the mitotic index and to induce multiple chromosomal breaks in cells from FA patients while having little clastogenic effect on normal cells. Breakage analysis was evaluated on unbanded metaphases (stained with Giemsa). For chromosome fragility evaluation, at least 50 metaphases selected randomly were analyzed from each culture.

Each cell was scored for the numbers and types of structural abnormalities according to methods previously described [6]. Gaps were not counted as chromosome breaks, whereas single chromatid breaks, isochromatid breaks and acentric fragments were scored as one break. The other rearrangements and radials were scored as two breaks.

Chromosomal breakage score was expressed as percentage of aberrant cells, breaks per cell and breaks per aberrant cell. Results were compared with healthy controls.