Journal of Agricultural Research Advances
ISSN: 2582-7227

Research Article
Open Access

Laboratory procedure for detection of Fanconi (Aplastic) Anaemia

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Received on: 13/07/2021 Accepted on: 29/10/2021 Published on: 26/11/2021

ABSTRACT

Fanconi anaemia (FA) is an inherited bone marrow failure syndrome (IBMFS) that is common among children, in a ratio of 1 person out of a population of 181 individuals. Early screening and/or detection of FA among intending couples or families with history of IBMFS is very important, to avoid bearing children with physical deformities and high risk of cancer development. The most significant marker for FA diagnosis is the appearance of recurrent patterns of chromosome breakages. Fractures can only be noticeable if chromosome samples are treated with Diepoxybutane (DEB), Mitomicin C (MMC) or other similar chemical treatments. Therefore, a concise clinical protocol for identification of FA was the sole focus of this article.

Keywords: Aplastic anaemia; Cancer; Diepoxybutane; Fanconi anaemia; Mitomicin C; Pancytopenia; Peripheral blood.

How to cite this article: Etaware PM (2021). Laboratory procedure for detection of Fanconi (Aplastic) Anaemia. J. Agri. Res. Adv., 03(04): 25-27.

Introduction

Fanconi anaemia (FA) is an autosomal recessive disorder associated with progressive bone marrow failure (Kook *et al*., 1998). It is the most commonly inherited aplastic anaemia among children and it is clearly distinct from other forms of inherited bone marrow failure syndromes (IBMFS), such as diamond blackfan anaemia (DBA), dyskeratosis congenita (DC), and shwachman-diamond syndrome (SDS) etc., as it is characterized by genetic malformations, anomalous formation of pancytopenia and a high risk of cancer development in the infected patients (Fargo *et al*., 2014).

The carrier frequency of FA in the general population is about 1 carrier per 181 healthy individuals (Rosenberg *et al*., 2011). It is very important to conduct early screening for FA among IBMFS patients, as an individual with autosomal recessive disorder located on the long (q) arm of chromosome 13 at position 12.3, which happens to be the gene locus for BRCA2 genes, have an increased risk of contracting breast, ovarian, prostate and other forms of cancer (Chen and Parmigiani, 2007).

The most important feature for diagnosing FA in a group of IBMFS patients is the recurrent occurrence of chromosome breakages in blood samples of infected patients. The laboratory procedures for diagnosis of Fanconi anaemia was discussed below:

Clinical protocol

Stage 1: Physical screening

Preliminary screening of potential FA victims among IBMFS patients using phenotypic markers, described by Fargo *et al*. (2014), was adopted to facilitate early and rapid detection of FA among the rising population of IBMFS patients.

A. *Preliminary assessment of potential IBMFS patients*

The major phenotypic markers used as clinical features for detection of Fanconi (Aplastic) Anaemia are:

- Skin café au lait spots: A type of birthmark characterized by irregular margins with flat patches on the skin. It is typically light brown in colour but may further darken on long term exposure to sunlight.
- Hyperpigmentation: Excessive formation of small dark patches on the skin.
- Short stature: A diminutive appearance in height, more than two standard deviations below the mean height for that age i.e. μ (age group height)>Patient's Height<2σ of μ (age group height).

▪ Abnormal thumbs and radii: Abnormal skeletal formation of the phalanges i.e. the carpals and metacarpals of both thumbs and also the narrow skeletal appendage of the forearm (radius) resulting in a skewed appearance of the upper limbs.

Microcephaly: The possession of a smaller skull structure (head) compared to individuals of the same sex and age.

▪ Microphthalmia: The possession of an abnormally small eyes due to restricted growth of the eyeballs caused by genetic dysfunction.

▪ Abnormal ears or hearing: Abnormal development of the pinna (outer ear) or ear anatomy, resulting in a series of complications in hearing.

▪ Abnormal kidneys: kidney malformation, urinary tract malfunctioning, urethra and bladder dysfunction etc. are basic signs or symptoms for identifying FA patients.

B. Virtual classification of potential IBMFS patients

Preemptive characterization of IBMFS patients prior to laboratory analysis will facilitate rapid screening and diagnosis of FA patients, and probably early medical attention for such patients according to the severity of their situation. Esmer *et al*. (2004) established five (5) basic taxa for the classification of prospective IBMFS patients based on the phenotypic disparity observed during the preliminary screening. The patients' classification was listed below:

1. Potential FA patients: Patients with more than half of the clinical features or symptoms described for FA.
2. Aplastic anaemia: Patients with aplastic anaemia not related to those described for FA.
3. VACTERL association: Patients with two (2) or more body malformations such as vertebral or radial ray abnormalities, anal atresia, tracheoesophageal atresia, congenital cardiovascular malformations etc.
4. Radial ray abnormalities, anal atresia, or tracheoesophageal atresia: Patients with only one of these stipulated congenital anomalies i.e. not related to those described for FA.
5. Myeloid abnormalities: patients with myelodysplastic syndrome (myelodysplasia) or myeloid leukemia

Laboratory protocol

1. Mitomicin C (MMC) analysis
2. Chromosome breakage analysis

Mitomicin C (MMC) Test

Materials

▪ Freshly collected heparinized venous blood (5 mL). Collection of blood from patients can be done using sterile syringe with 18½-G needle and kept at room temperature (25oC) for not more than 48hrs.

▪ Complete RPMI 1640 or Ham's F10 culture medium (fortified with 15% FBS, streptomycin, penicillin, and Phytohemagglutinin, as utilized in standard cytogenetic whole-blood cultures).

▪ Mitomicin C (stored at 4°C).

▪ Additional materials for the preparation of metaphase spreads.

Procedure

The protocol employed for the treatment of autosomal chromosomes using Mitomicin C (MMC) chemical reagent was a slightly modified procedure adapted from the research of Oostra *et al*. (2012).

1. Prepare a stock solution of MMC at 1.5mM (0.5 mg/mL) by adding 4mL sterile distilled water per vial (This solution can be kept fresh for 3 months if it is stored at 4°C). Note: storage of the solution below 4°C can give rise to the formation of insoluble crystals when thawing the frozen MMC.
2. Prepare whole-blood cultures from suspected FA patients and standard control from healthy blood samples.
3. As usual for a standard cytogenetic analysis, you need four (4) cultures from each suspected FA patients and four (4) from normal blood samples, to be used as standards or control for the experiment. The standards (Healthy blood samples) should not be collected from relatives to the patient(s). Initiate the cultures by adding 0.5mL of blood to 4.5 mL of complete medium.
4. Add, at the time of culture initiation, to each set of 4 cultures: 0, 50, 150, and 300nM of freshly prepared MMC solution.
5. Harvest at 72hrs subsequent to culture initiation, after Colcemid treatment during the last 40mins of incubation.
6. Prepare metaphase spreads on clean slides.

Chromosome Breakage Test

After treatment of the chromosomes using MMC, the following steps are required to complete the analysis.

1. Harvest the treated chromosome samples at the metaphase stage only
2. Prepare between 50 and 100 slides containing well stained and evenly distributed chromosome samples. Make sure that during preparation and

mount of the microscope slides, the chromosomes are not manually damaged or distorted to avoid "False" diagnostic results.
3. Prepare up to 50 slides each from normal patients (Positive control) and blood bank sample of a confirmed case of FA (Negative control), if confirmed FA blood samples are available and accessible by the laboratory analyst.
4. Observe each slide under an inverted microscope at a very high resolution. Prior to the observation of the chromosome strands under a high power inverted microscope, the laboratory technician should be acquainted with the morphology of normal chromosome strands. He/She should be able to distinguish between a chromosome with missing fragments and that which is abnormally formed.
5. Score each slide based on the appearance of cracks, gaps or lines of breakages present on each chromosome.
6. Chromosome aberrations should be scored separately, as this does not indicate breakages or gaps on the chromosome strands that can be used to diagnose fanconi or other aplastic anemic situations.
7. Each observation made should be cross checked with that of a normal chromosome from the control slides.
8. Statistical analyses should be carried out to determine the extent of damage caused to the chromosomes.
9. If necessary, further molecular tests can be conducted to ascertain the type of anaemia the patient has, the degree of loss of genetic materials and the location or loci of genes deleted or inserted into the chromosome.
10. Finally, medical attention should be given to such patient as an early diagnosis and possible treatment might be the key factor to the survival of such patient(s).

Conclusion

Early detection and/or diagnosis of Fanconi anaemia can save an intending couple from a life-time of misery, it can also increase the chances of survival of the patient, since he/she will be placed under medical surveillance and treatment. Since, there is no rapid immunodiagnostic test kits available at the moment for screening of FA, parents and guardians should pay close attention to the physical features described in this article, as this might be a possible option for them to identify a potential IBMFS or FA affected individual.

Glossary

1. Aplastic anaemia: This is a rare condition where the body stops producing enough red blood cells due to acute bone marrow damage caused by genetic disorders or exposure to radiation, or toxic chemicals or infections etc.
2. Myeloid: This is simply defined as relating to the spinal cord or the bone marrow
3. Pancytopenia: This is a medical situation where there is decrease in the cell count of the three (3) blood cell types i.e. erythrocyte, leukocyte and thrombocyte.

References

Chen S and Parmigiani G (2007). Meta-analysis of BRCA1 and BRCA2 penetrance. Journal of Clinical Oncology, 25: 1329–1333.

Esmer C, Sa´nchez S, Ramos S, Molina B, Frias S and Carnevale A (2004). DEB test for fanconi anemia detection in patients with atypical phenotypes. American Journal of Medical Genetics 124A: 35–39.

Fargo JH, Rochowski A, Giri N, Savage SA, Olson SB and Alter BP (2014). Comparison of chromosome breakage in non-mosaic and mosaic patients with fanconi anemia, relatives, and patients with other inherited bone marrow failure syndromes. Cytogenetic Genome Resource 144: 15–27. doi: 10.1159/000366251.

Kook H, Cho D, Cho SH, et al. (1998). Fanconi anemia screening by diepoxybutane and mitomicin c tests in Korean children with bone marrow failure syndromes. *VVJ Korean Medical Science* 13, 623-628. ISSN 1011-8934.

Oostra AB, Nieuwint AWM, Joenje H, de Winter JP (2012). Diagnosis of fanconi anemia: chromosomal breakage analysis. *Anemia*. Accessed: 12/04/2021 at 18:13 GMT. 9pg. https://doi.org/10.1155/2012/238731.

Rosenberg PS, Tamary H and Alter BP (2011). How high are carrier frequencies of rare recessive syndromes? Contemporary estimates for fanconi anemia in the United States and Israel. American Journal of Medical Genetics A-155A: 1877–1883.

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