ORIGINAL RESEARCH PAPER
Volume - 14 | Issue - 11 | November - 2025 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

A PRELIMINARY STUDY ON CHROMOSOMAL BREAKAGE ANALYSIS IN FANCONI ANEMIA

Genetics

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ABSTRACT

Fanconi Anemia (FA) is an autosomal recessive chromosomal instability disorder. It is characterized by defective hematopoiesis and congenital anomalies. The most common features of FA are short stature, hyperpigmentation, low platelet count, low birth weight and abnormal thumbs and radii. This study describes the chromosomal breakage analysis in patients diagnosed with Fanconi Anemia, which is a rare disorder which occurs when the bone marrow fails to produce healthy new blood cells. About 10 case study is discussed in both chromosomal and genetic level. 72 hours standardized peripheral blood cultures with induced Mitomycin C in both control and samples were set-up, following harvesting, slide preparation and chromosomal breakage analysis was carried out. At the end of the study, it was seen that chromosomal breakage studies and mutational analysis can help diagnose the disease which when done early can be used effectively to treat the patients. As the symptoms can be heterogenous for this genetic disease it can be difficult to diagnose, but when diagnosed Chromosome Breakage Studies (CBS) will be one of the confirmatory tests. The whole exome sequence for a panel of genes was outsourced to study the genes such as FANCA, FANCC and FANCG are the major 3 genes responsible for FA. The result of this preliminary study is discussed.

KEYWORDS

INTRODUCTION:

Fanconi anemia is a heterogenous disease caused by defects in genes responsible for DNA damage repair mechanism. Over the last 30 years the Discovery of the disease-causing genes for Fanconi anemia (FA) and its role in protein regulation and DNA repair have identified 23 genes (table 1) that, when mutated, cause FA. Due to this, the patient's cells are unable to repair DNA interstrand crosslinks (ICLs), which are lesions that covalently link two strands of DNA and inhibit the essential cellular processes of DNA replication and transcription. [1,2]. In contrast, chromosome breakages studies (CBS) using cytogenetic clastogenic DEB and mitomycin C (MMC) to cause chromosome-breaking effect also known as cross-linking/ intercalating effect provides a reliable cytogenetic marker for the diagnosis of FA [3,4,5]. FA individuals are at increased risk for acute myelogenous leukemia, myelodysplastic syndrome, and solid tumors of the neck, head, oral cavities, and genitourinary system.

Table 1: List Of Genes Involved In FA [6]

1. FANCA, 2. FANCB, 3. FANCC, 4. FANCD1/BRCA2, 5. FANCD2, 6. FANCE, 7. FANCF 8. FANCG, 9,10 FANCI, 11,12. FANCJ/BRIP1, 13. FANCL, 14-17. FANCM , 18. FANCN/PALB2, 19, 20. FANCO/RAD51C, 21,22. FANCP/SLX4, 23. FANCQ/ERCC4 , 24,25.FANCR/RAD51, 26. FANCS/BRCA1, 27-29.FANCT/UBE2T, 30. FANCU/XRCC2, 31. FANCV/REV7, 32.FANCW/RFWD3 , and 33. FANCY/FAP100 .

Aims:

Preliminary analysis to associate gene mutations and Chromosome breakage studies.

Primary Objective:

To identify and investigate the chromosomal breakage studies associated with Fanconi Anemia.

MATERIALS:

Fanconi Anemia cases workup is carried out by analyzing chromosome breakages. The study included 10 individuals from 3 to 18 years of age who have visited the Division of Human Genetics at St. John's Medical College, Bangalore during April 2022 to May 2023. These patients are referred for FA and Aplastic Anemia from the St. John's Medical College and Hospital, Bangalore. Before the sample collection ethical clearance from the Institutional Ethics Committee of St. Johns' Medical College and Informed consent from patients were obtained. Inclusion criteria for control- siblings are parents were excluded. And individuals with no history of anemia or FA with normal karyotypes were included as controls.

Methodology: Cells are cultured in the presence of the Clastogens (Mitomycin C (MMC)). Metaphase chromosomes are prepared from each culture and stained with Giemsa stain. Fifty metaphases are scored from each culture for the presence of breaks/gaps and radial formations. Reagents required: M4287 sigma 2mg Mitomycin C powder Solution A: 2mg is dissolved in 10ml sterile distilled water and stored at 2-8 deg. Solution B: 1 part of solution A into 4 parts of sterile distilled water.

Plain stained slides were analyzed using 100X objective under BX53 Olympus microscope with ASI software version 8.3.1. 50 metaphases for each concentration of MMC were captured for each case and the corresponding control cultures.

Chromosome Breakage Analysis [7]: Both sample and control are analyzed to check for radial formations Quadri-radial and tri-radials only are counted and compared with control. Using the formula if more than 40 radials is considered hypersensitive to MMC and reported as positive to Chromosome Breakage Studies. If the breaks are normal or less than the range, then it is said that it is hyposensitive to MMC and reported as negative for CBS. For every sample there is a control to which the concentration of MMC is same as the sample. In non-induced cultures with zero MMC, the total breaks including chromatid breaks, chromosome breaks, and chromatid gaps and chromosome gaps, single and double minutes, ring chromosomes and dicentric chromosomes are checked in both control and Patient sample.

RESULTS:

Out of 10 cases with FA, one case was culture failure, 6/10(60%) were negative and 3/10(30%) positive.

WES Results: In positive cases, two cases were outsourced (table 1) which showed pathogenic variants in exon 13 of FANCL and exon 4 of FANCG genes.

DISCUSSION:

True FA patients' cells must have hypersensitivity to chromosomal breakage brought on by DNA cross-linking agents like mitomycin C (MMC)[7]. In FA patients, somatic mosaicism is often discovered for the first time during a diagnostic test using a DNA crosslinking agent on a peripheral blood sample [10].

This prospective and retrospective study comprises 20 patients exhibiting Fanconi Anemia and aplastic anemia. Out of the 10 cases there were 6 males and 4 females. Among the 10 cases 2/10 were from

the adult age group and the rest were in pediatric age group. The youngest patient was 2 years old male and the oldest was 24 years old male. The symptoms were heterogenous and included low blood count, blood.

Genetic Testing Is Done In Two Levels For FA: 1st level is the chromosome breakage studies, and 2nd level is at the molecular level. Out of 10, 3 got their molecular testing done. And all the 10 cases were subjected for chromosomal breakage analysis [8,9]. Out of the 10 cases 6 showed negative for CBS and 3 showed the increased radial formation and breaks and gaps in non-induced cultures which is said to be positive for CBS. And in one of the cases, the patient's blood sample showed culture failure repeatedly the reason could be because the pancytopenia/ low cell counts in blood. All the control cases showed negative results compared to patient samples, which clearly states that normal individuals have DNA repair mechanisms whereas in positive cases (30%) with increased radial formations states that the DNA repair mechanism is lost because of gene mutations.

CBS is the preliminary genetic testing for FA; CBS plays an important role and is the first test done to rule out FA or Aplastic anemia. Clinical features also helped in prioritizing and validation using mutational analysis [10]. As the clinical feature of FA is very common Chromosome breakage analysis is one of the most accurate genetic tests used in FA. Further testing can be done using mutational analysis. Treatment such as continuous blood transfusions and bone marrow transplants can be effective way of treating FA.

CONCLUSION:

A definitive diagnosis was sought in 4/10 patients enrolled in this study. For the patients with negative results for CBS were advised for further mutational analysis. As we could observe in this study the gender does not play a vital role in the prevalence of the disease, and the early diagnosis is done in the pediatric age group. Appropriate clinical evaluation and counseling is very important to ensure patient co-operation during the diagnostic odyssey and helps in planning the disease and treatment management. Clinical features also helped in prioritizing and validation using mutational analysis. As the clinical feature of FA is very common Chromosome breakage analysis is one of the most accurate genetic tests used in FA. Further testing can be done using mutational analysis. Treatment such as continuous blood transfusions and bone marrow transplants can be effective way of treating FA.

In the evolving era of genetic testing, it is important to raise awareness regarding the need for and importance of genetic testing, since an exact diagnosis can enable presymptomatic testing and prenatal testing in all the 'at risk' relatives and siblings. A final diagnosis will also provide a psychological and economical closure to the patients and family dealing with FA. It is recommended that all patients exhibiting any congenital malformation known to be associated with FA or AA at any age, or any patient with MDS with complex cytogenetic abnormalities, have a peripheral blood sample tested for cross-linker hypersensitivity [3]. Because of the lack of concordance of FA phenotype among affected siblings, siblings of an FA patient should also be screened.

Acknowledgments:

We thank the patients and their referring physicians from Pediatric Hemat-Onco, St. John's Medical College & Hospital. Mrs Raina MP and Mrs Mary Margaret and Mrs Nisha Fransis are gratefully acknowledged for their technical assistance for chromosome breakage studies starting from sample processing and slide preparation for this study.

List Of Figures: 1a,1b, 2a,2b & 3 showing increased breaks and gaps.

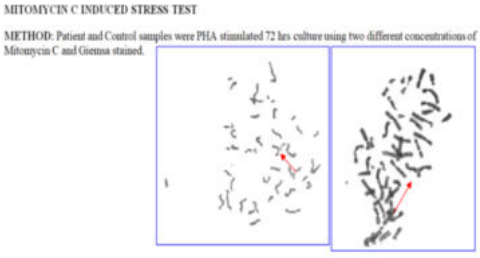

Image1&2: Metaphases showing quadri-radii and tri radial formation in MMC induced culture of Patient sample

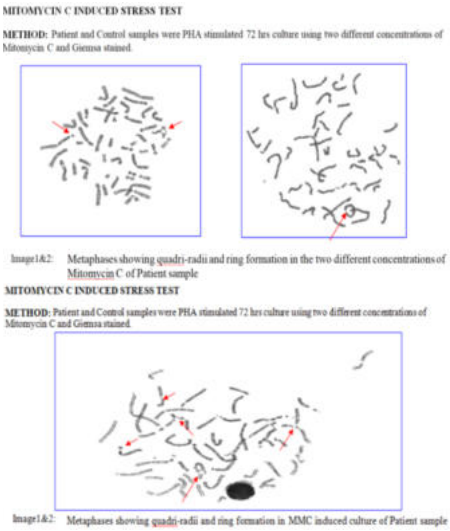

Image1&2: Metaphases showing quadri-radii and ring formation in the two different concentrations of Mitomycin C of Patient sample

Image1&2: Metaphases showing quadri-radii and ring formation in MMC induced culture of Patient sample

Table 2: List Of Age/Sex/Consanguinity And FA Hypersensitivity

SLno	Age (in years)	Sex	Consanguinity	Referral reason	Result
1.	18	F	Consanguineous	FA	Negative
2.	6	F	Non-Consanguineous	FA	Positive
3.	4	M	Non- Consanguineous	FA	Negative
4.	9	M	Non- Consanguineous	FA	Negative
5.	3	M	Consanguineous	FA	Positive
6.	11	F	Consanguineous	FA	Positive
7.	5	F	Consanguineous	FA	CF1 and CF2
8.	2	M	Non- Consanguineous	FA	Negative
9.	24	M	Non- Consanguineous	FA	Negative
10.	11	M	Non- Consanguineous	FA	Negative