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CLINICAL CORRELATION AND CHROMOSOMAL BREAKAGE ANALYSIS IN FANCONI ANEMIA PATIENTS FROM DIFFERENT REGIONS OF PUNJAB AND KHYBER PAKHTUNKHWA, PAKISTAN

Shifa Aziz[1,4], Sana Aziz[2] and Anam Qaiser[3]

[1]*Department of Molecular Biology, Virtual University of Pakistan, Rawalpindi, Pakistan*
[2]*Lady Reading Hospital, Peshawar, Pakistan*
[3]*University of Health Sciences, Lahore, Pakistan*
[4]*Armed Forces Institute of Pathology, Rawalpindi, Pakistan*
Correspondence Phone: +923325163379; **Email:** shifaaziz5@gmail.com / shifa.aziz@mlt.uol.edu.pk

ABSTRACT

The study was designed to confirm the diagnosis of Fanconi Anemia patients by MMC induced chromosomal breakage studies along with the related clinical characteristics at cytogenetic laboratory AFIP, Rwp. Out of 192 patients included in the study, 24 had confirmed diagnosis of Fanconi Anemia (FA); amongst which 14 (58.33%) were male and 10 (41.66 %) were female. The mean age calculated for this disease in our study is 11.18 years. Major characteristics noted in the present study were the anomalies related to weight, height, and skeleton. Leukemia or Myelodysplastic syndrome was also present in some of the cases. Moreover, they had other clinical history of bruises, infections, bleeding gum and fever. Around 30-50% of the metaphases showed breakages in the chromosomes after being subjected to clastogenic stress by mitomycin-C. MMC induced chromosome instability proved to be the gold standard test for the timely diagnosis of FA as clinical features cannot confirm the diagnosis alone. Also, wide-scale national community-based survey with FA registry could help in estimating the size of the problem in our country more accurately and can also help in providing genetic counseling to the patients and their families. as previously no such work has been done or available in the literature.

Keywords: Fanconi anemia, chromosomal breakage, mitomycin C, anomalies, karyotyping.

INTRODUCTION

Fanconi anemia (FA) is a type of rare, inherited blood disorder that usually presents with various clinical features and developmental abnormalities that could eventually lead to bone marrow failure. Studies reveal that around 90% of patients with Fanconi anemia have impaired bone marrow function that may lead to decreased formation of all blood cells (Issa, 2013). It is an autosomal recessive congenital abnormality that may lead to malignancy or bone marrow failure (BMF). The only way to acquire this disease is possible when both the parents have defective gene passed off to the offspring. In this case there is a 25% probability that the child will have FA. Around 2% of FA cases are X-linked recessive (Bhatnagar, 2016). This disorder is associated with an abnormal response to DNA damage. Fanconi anemia results from the inheritance of any of the mutations amongst the different known subtypes of 19 different genes involved in FA (Garaycoechea and Patel, 2014). Fanconi anemia has been reported in individuals of all races. However, due to the rarity of this disorder, FA is very underdiagnosed. The male-to-female proportion in the literature cases is 1.2:1, while equal numbers are expected in a syndrome with over 99% autosomal recessive inheritance (Falci *et al.*, 2011). In childhood this condition usually presents with anemia, having high bleeding tendency, susceptible to infections. However, in adults, the presentation may be with AML, myelodysplastic syndrome, aplastic anemia, gynecological cancers in women less than 50 years of age, head and neck squamous cell carcinomas-HNSCC (Alter, 2007). Physical abnormalities that can be noted in approximately 75% of the patients with Fanconi anemia include hyper pigmented skin with café-au-lait spots, low birth weight and small for age and the face may be triangular in shape, thumbs, fingers, toes and radii can show structural abnormalities (usually aplasia), microcephaly, microphthalmia and deafness occur, cardiac and renal malformations are also encountered. The gonads in older patients tend to be atrophic or dimorphic with a range of associated genitourinary abnormalities, causing reduced fertility. Since Fanconi anemia occurs as a result of mutations in the genes that are involved for the DNA repair, the cells of Fanconi anemia patients will show an increased level of breakages in their chromosomes when exposed to mutagenic agent such as mitomycin C (MMC). There is no available data in literature about the frequency or chromosomal abnormalities noted in Fanconi anemia patients in Pakistan. Also, there is absence of diagnostic tools or drugs/therapies to cure the disease. The aim of current study is to have acquaintance of all the patients suffering from fanconi anemia along with the other bone marrow related

disorders from various regions of Punjab and Khyber Pakhtunkhwa. This study would also help researchers in understanding the prevailing risk factors to develop better interventions for minimizing the associated risks. Also, a country specific data may help formulate developing better follow up for patients and prescribing policies for their families.

MATERIALS AND METHODS

Patients from different regions of Punjab and Khyber Pakhtunkhwa were screened for the diagnosis of fanconi anemia at Armed Forces Institute of Pathology (AFIP), CMH Rawalpindi over the duration of 12 months. The inclusion criteria for our study was that all anemic patients were considered and patients with abnormalities suggestive of fanconi anemia were also tested for the disorder. Patients who had undergone bone marrow transplant were excluded from the study. Consecutive sampling technique was used to collect the data. Permission was taken from Hospital Ethical Committee. Patient's identification number, referred hospital, age and sex were recorded. MMC- induced chromosomal breakage analysis was performed on all the blood and bone marrow samples of patients that were clinically suspected for Fanconi anemia. The test was performed according to the laboratory protocol (Cirkovic *et al*., 2014). The latest blood count reports and transfusion history was also noted down of all possible cases in order to classify the severity of the disorder. 4-5mL of whole blood collected in a sodium heparin tube was cultured in RPMI medium for 72 h. After that 1% 0.2mL colchicine was added to it and was further incubated for 1 h at 37°C. Cells were fixed and cytogenetic analysis of chromosomes was performed according to the known standard protocol. At least 20-25 groups of metaphases were studied and counted for chromosomal aberrations. The breaks, spaces or relocations were noted down on a chart. The gaps among chromosomes were scored separately as less than the width of a single identical strand of its chromosome. Breakages were scored as either greater or equal to the width of its chromatid. Single chromosome/chromatid was recorded as a one break. Tetraradial form was reported as 3 breakages while triradial forms were reported as 2 breakages. Chromosomal breakages were reported as break per abnormal metaphase or as break per single metaphase.

All the relevant data and lab findings such as clinical features, signs and symptoms, physical anomalies and cytogenetic results were calculated using SPSS software version 22.

RESULTS

A total of 219 patients were screened for the diagnosis of fanconi anemia amongst which 27 were donors for pre-transplant assessment for FA. Rest 192 patients were tested for the screening of FA. Out of those 192 patients, 24 were confirmed as having FA which included 14 male and 10 female patients. Mean age of the patients being diagnosed was around 11.18 years. The average count of hemoglobin, white blood cells and platelets being noted in the diagnosed cases of Fanconi anemia are represented in the following Table 1.

Table 1. Mean count of blood parameters in diagnosed cases of fanconi anemia

Blood Parameters	**Mean Count**
Hemoglobin	7.65 mg/dL
Leukocytes	3.5×10^{9}/L
Platelets	27.14×10^{9}/L

The physical anomalies being observed in patients with Fanconi anemia were as follows (Table 2).

Table 2. Different types of physical abnormalities being noted in 24 diagnosed cases of FA.

Physical anomalies	**Patients n (%)**
Microcephaly	6 (25)
Hyperpigmentation on body	3 (12.5)
Dysplastic thumb	3 (12.5)
Hypoplastic thumb	1 (4.2)
Absence of thumb	3 (12.5)
Absent radii	1 (4.2)
Short stature	5 (20.8)
Low birth weight	4 (8.3)

Bone marrow reports were also considered for some clinically serious patients which were later confirmed as having fanconi anemia as this would help to understand the proper status and functioning of marrow among fanconi anemia patients. The picture of bone marrow reports of such patients is represented in the following Table 3.

Table 3. Clinical picture of bone marrow of diagnosed cases of FA

Bone marrow report	**Patients n (%)**
Hypocellular marrow with aplastic anemia	1 (4.2)
Hypoplastic bone marrow	2 (8.3)
Hypoplastic bone marrow with pancytopenia	2 (8.3)
Pancytopenia	3 (12.5)
Hypoplasia of megakaryocytic series.	1 (4.2)

Out of all the FA patients, 37.5% (n = 9) had previous histories of blood transfusion at different intervals of life. Among our group of FA patients, other clinical history of having thrombocytopenia, fever, cough, bruises, progressive paleness, failure-to-thrive (FTT), menorrhagia, Parvo virus, gut history and bleeding gums complain was also noted. The percentage of MMC-induced aberrant cells in FA ranged from 30%-50%. Different types of structural abnormalities being noted in the chromosomes are represented in the Fig. 1.

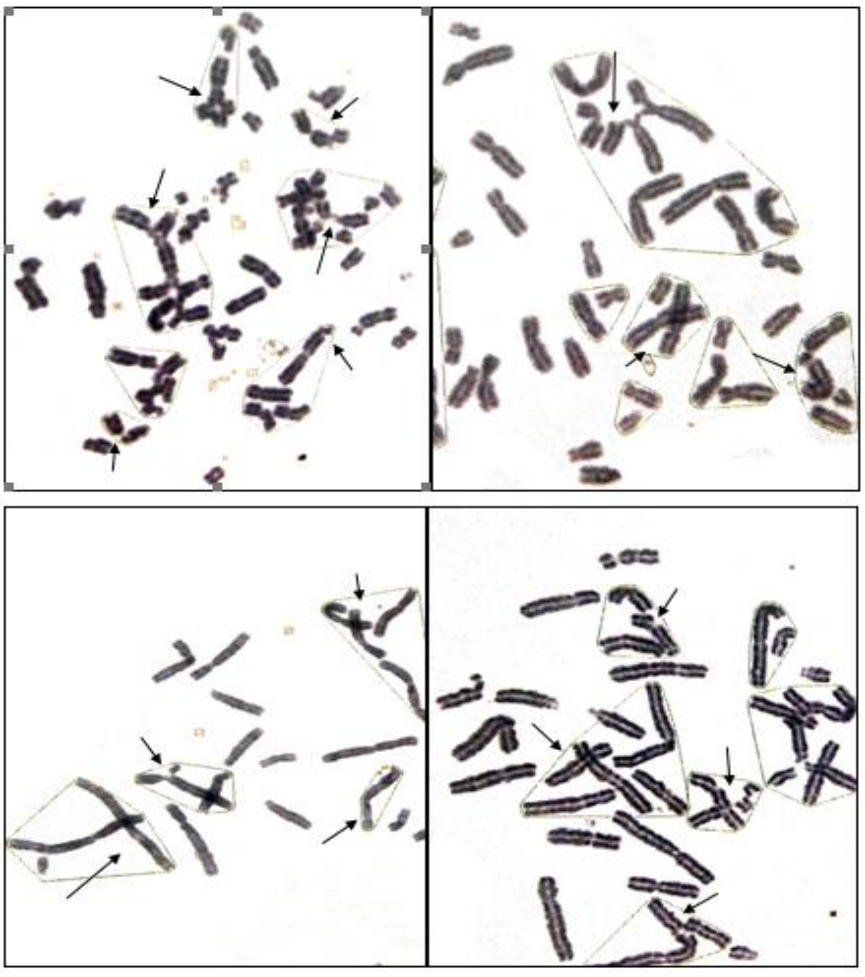

Fig. 1. Images as seen from microscope with arrows showing MMC induced chromosomal breaks, quadriradial chromosome, triradial chromosome, chromatid gap and breaks in patients with Fanconi anemia.

DISCUSSION

Developing a better treatment plan for the patients suffering from genetic disorders specifically like fanconi anemia has been a goal for the medical professionals as it could improve the quality of their life. However, the outlook of fanconi anemia is not very promising. The most commonly performed lab test in routine for the differential diagnosis of Fanconi anemia is karyotyping. A minimum of 20-25 metaphases are examined. Upon exposure to certain chemicals, the chromosomes of Fanconi anemic patients will immediately break down easily. The pattern of chromosomal breakages and their types are scored in each metaphase. The extent to which chromosomes show their sensitivity to DNA- cross linking agents are independent of the severity of disorder as well as its presenting phenotype. As it is an inherited X-linked disorder or autosomal recessive disease, a variety of genes are responsible for causing this disorder. But one thing is common that the mutated genes do not allow the repair mechanism of DNA to function

In our present study, 24 patients (12.5%) out of 192 were diagnosed as having FA during the year 2018. The incidence rate somehow correlates with the study of Mohit Chowdry conducted in India showing 13.1% patients with Fanconi's anemia. Moreover, the different types of structural abnormalities being noted in the metaphase of Fanconi anemia patients were exactly the same as those noticed by (Chowdhry *et al*., 2014). Another study showed disease incidence to be 41.17% in Brazil that deviates from our result (Zen *et al.*, 2011). Among these patients, 14 were male patients and 10 were female patients among them making the ratio of males to females be 1.7:1.2 which means that males are affected 1.4 times more than females. This result deviates from the literature according to which male to female ratio of the affected individual is 1:1. Also, our results differ from those by Akiko Shimamura. According to them, the ratio was1.2:1 (Shimamura and Alter, 2010). The range of ages of the patients being diagnosed with FA in our study was from 3-27 years. However, 83.4% patients were ≥18 years of age while 16.6% were being diagnosed after the age of 18 years. Our study somehow correlates with the study of Carmen who said that limit of age for the diagnosis of this disorder varies between 0-38 years and 10% were diagnosed after the age of 16 years (Esmer *et al*., 2004).

The complete blood count details from the latest reports showed that 85.71% patients had TLC $<4.5 \times 10^9$/L, 100% patients showed Platelet count$<100 \times 10^9$/L and 71.4% patients showed hb<10g/dL. This approach correlates with the study of Abo-Elwafa *et al.* (2011) who mentioned that Hb, TLC and Platelet count show a significant decrease in FA (Abo-Elwafa *et al.*, 2011).

Our study project totally correlates with the findings of Mozdarani *et al.* (2011) who concluded that FA patients exhibited congenital anomalies on presentation such as abnormalities of skeleton (absence of thumb or radial bone), pigmentation spots on skin, short stature, small sized head and delayed growth leading to difficulties in the survival of individuals. Sometimes thrombocytosis and aplastic anemia may also be present. Some other minute abnormalities may also be noted in some exceptional cases.

Unlike Alter BP's study report whose FA patients had other hematological disorders like acute myeloid leukemia (AML), myelodysplastic syndromes (MDS), liver tumors, solid tumors (Alter, 2003). FA patients of in our study have not shown any of such disorder.

Among the confirmed patients of our study, 29.2% (n = 7) showed 50% chromosomal breakages in their metaphases. 37.5% (n = 9) showed 40% breakages and 33.3% (n = 8) showed breakages in 30% of their metaphases when the culture was subjected to clastogenic stress by mitomycin C. Another study done by Sanja revealed 10 patients with FA whose percentages of uneven mitoses ranged from 32-82% (Cirkovic *et al*., 2014).

In the present study 25% (n = 7) of the patients showed microcephaly, 12.5% (n = 3) showed hyperpigmentation on their body, 29.1% (n = 7) showed abnormalities in their thumb (i.e., 12.5% had dysplastic thumb, 4.2% had hypo-plastic thumb while 12.5% had absence of thumb). 4.2% (n = 1) showed absent radii, 20.8% (n = 5) had short stature, 0.08% (n = 2) had low birth weight and similar percentage i.e., 0.08% (n = 2) had family history of congenital abnormalities. In contrast the study done by Jeffrey M Lipton concluded that birth defects were noted in almost 75% known cases of the disorder. These included pigmented spots on skin and short stature in more than 50% of the cases. Radial or thumb abnormalities were common in 40% of the cases. 25% of the cases presented with microcephaly while 10% of the diseased babies had low birth weight (Lipton, 2013).

Our study not only included patients suffering from fanconi anemia (12.56%) but also comprised of 41.14% (n = 79) patients suffering from AA out of which 4 patients had AA along with FA. Similarly, 9.89% (n = 19) patients presented with pancytopenia out of which only 3 patients had F.A along with pancytopenia. 5.20% (n = 10) patients presented with short stature out of which only 4 had FA as well as short stature. 2.60% (n = 5) patients presented with MDS while 1.56% (n=3) had thrombocytopenia out of which 1 patient thrombocytopenia along with FA. Other research done by Porto b. connects with our study in a way that he also proposed that this population of study not only included patients that presented clinically with the signs and symptoms being suggestive of disorder but also those that had low blood counts or had aplastic anemia (Porto *et al.*, 2011).

Lastly, FA patients were presented with many other clinical histories too like fever, cough, progressive paleness, shortness of breath, infections, gut history, bruises over body, bleeding gums, positive antinuclear antibody (ANA) or menorrhagia. All these complaints are also linked with the study report of Paulo Richardo who said that clinical presentation of bruises, hematomas, being prone to infections, petechiae and adenopathy were common in the group having FA (Zen *et al*., 2011).

CONCLUSION

At present, cytogenetic analysis for detection of mitomycin C (MMC)-induced chromosome instability is the gold standard test for the diagnosis of FA as clinical features cannot confirm the diagnosis alone. Timely diagnosis of this rare disease is very vital for future planning. It is also very important for determining treatments plans of the affected patients and educating their families. MMC induced chromosomal testing should be implemented as a routine screening test in patients presenting with the related clinical features as mentioned in our study to diagnose the disorder at an initial stage. This has become an essential tool for the confirmation of Fanconi anemia. The future research plans of our study may include the identification of genes at a molecular level that are being responsible for causing this disease and identifying the complementation groups being reactive to MMC.

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